THE UNIVERSITY OF NEWCASTLE AUSTRALIA

Gac Oil Extraction and Encapsulation by Spray Drying

Tuyen Chan Kha

MPhil. (Food Science)

Thesis submitted for the degree of

DOCTOR OF PHILOSOPHY

STATEMENT OF ORIGINALITY

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by other person, except where due reference has been made in the text.

Tuyen Chan Kha
Date: 26/09/2014

DECLARATION OF AUTHORSHIP

I hereby certify that this thesis is submitted in the form of a series of published papers of which I am a joint author. I have included as part of the thesis a written statement from each co-author, and endorsed by the Faculty Assistant Dean (Research Training), attesting to my contribution to the joint publications.

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Date: 26/09/2014

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III. Tuyen C. Kha, Minh H. Nguyen, Paul D. Roach & Costas Stathopoulos (2014). Ultrasound-Assisted Aqueous Extraction of Oil and Carotenoids from Microwave-dried Gac (*Momordica cochinchinensis* Spreng) Aril. *International Journal of Food Engineering*. Under review (C1).

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IV. Tuyen C. Kha, Minh H. Nguyen, Paul D. Roach & Costas Stathopoulos (2013). Optimisation of Microwave-assisted Extraction of Gac Oil at Different Hydraulic Pressure, Microwave and Steaming Conditions. *International Journal of Food Science and Technology,* 48(7), 1436-1444. (C1).

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VII. Tuyen C. Kha, Minh H. Nguyen, Paul D. Roach & Costas Stathopoulos (2014). Microencapsulation of Gac oil by Spray Drying: Optimisation of Wall Material Concentration and Oil Load Using Response Surface Methodology. *Drying Technology: An International Journal,* 32(4), 385-397. (C1).

VIII. Tuyen C. Kha, Minh H. Nguyen, Paul D. Roach & Costas Stathopoulos (2014). Microencapsulation of Gac Oil: Optimisation of Spray Drying Conditions Using Response Surface Methodology. *Journal of Powder Technology*, 264, 298-309. (C1).

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STATEMENTS OF CONTRIBUTION BY OTHERS

THE UNIVERSITY OF
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27th March 2014

To whom it may concern,

This letter outlines Tuyen Chan Kha' contribution to the series of papers that are submitted as a part of his PhD. All papers that are contributing to his thesis are listed below, with a statement of his contribution for each.

Regards,

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This project was led by Tuyen C. Kha. He conducted all data collection and all analyses, and was primarily responsible for manuscript preparation. Numerically, the contributions from the authors were: Tuyen Kha, 50%; Minh Nguyen & Sophie Parks, 15% each; Paul Roach & Constantinos Stathopoulos, 10% each.

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1. The Research Higher Degree Scholarship granted by the University of Newcastle, Australia (May 2011- July 2014).

2. The Best Poster Presentation Award granted by the Australian Institute of Food Science and Technology at the 47th Annual AIFST Convention in Melbourne, 22 - 25th June, 2014.

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4. The 1st Best Poster Presentation Award granted by International Conference on Food and Biosystems Engineering, Skiathos, Greece, 30 May - 02 June, 2013.

5. Travel grant given by the School of Environmental and Life Sciences for attending an International Conference on Food and Biosystems Engineering, in Skiathos, Greece, 30 May - 02 June, 2013.

6. Travel grant given by the Nong Lam University, Viet Nam for attending the International Conference in Malaysia, 2012.

7. The Best Poster Presentation Award (Nutrition category) granted by the Australian Institute of Food Science and Technology at the 45th Annual AIFST Convention in Adelaide, 15 - 18th July, 2012.

OTHER RELATED ACTIVITIES

1. Constructed a website about research work: http://gacfruit.weebly.com/
2. Co-supervised four Master students who have graduated.
3. Student Membership of Australian Institute of Food Science and Technology (AIFST).

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LIST OF ABBREVIATIONS AND SCIENTIFIC SYMBOLS, AND UNITS OF MEASUREMENT

Abbreviations and Scientific symbols

Aw	water activity
C	chroma
d.w.	dry weight
EE	extraction efficiency or encapsulation efficiency where appropriate
EY	encapsulation yield
GA	gum Arabic
H^o	hue angle
HPLC	high performance liquid chromatography
L	lightness
MC	moisture content
PV	peroxide value
R^2	coefficient of determination
RSM	response surface methodology
SC-CO_2	supercritical carbon dioxide
SEM	scanning electron microscopy
v/v	volume per volume
w.b.	wet basis
WMC	wall material concentration
WP	whey protein
WPC	whey protein concentrate
WSI	water solubility index
w/w	weight per weight
ΔE	total colour difference

Units of measurement

%	percent
^{o}C	degree Celsius
× *g*	g force
bar	bar
g	gram
g/g	gram per gram
kg/cm^{2}	kilogram per square centimeter
kHz	kilohertz
kV	kilovolt
meq/kg	milliequivalents of active oxygen per kg
mg	milligram
mL	milliliter
mL/min	milliliter per minute
min	minute
N	normality
rpm	revolution per minute
W	watt
W/g	watt per gram
µg	microgram

ABSTRACT

Gac fruit (*Momordica cochinchinensis* Spreng) aril contains extraordinarily high levels of β-carotene and lycopene and unsaturated fatty acids, especially oleic and linoleic acids. These bioactive compounds have been proven to be beneficial to human health and are linked with a reduced risk of cardiovascular disease and cancers. Importantly, it has been found that a significant improvement in the absorption of the carotenoids into the human body occurs when they are digested with fat.

The current study hypothesised that optimisation of conditions for several processing steps, including oil extraction, emulsion preparation conditions and spray drying process using response surface methodology (RSM), is expected to maximise the oil yield and increase the retention of bioactive compounds.

The aim of this study was therefore to develop optimal conditions for the extraction of Gac oil enriched in β-carotene and lycopene, and the preparation of the Gac oil rich in β-carotene and lycopene encapsulated powder, for use as nutrient supplement or natural food additive and natural food colourant. The objectives of this study were to investigate the effect of (1) pre-treatments prior to supercritical carbon dioxide extraction, (2) ultrasound-assisted aqueous extraction, (3) microwave-drying followed by pressing on the Gac oil extraction efficiency, and β-carotene and lycopene contents. Moreover, this study was also to optimise the conditions for spray-drying encapsulation of Gac oil using RSM: (1) wall material concentration and ratio of Gac oil to wall material, and (2) spray-drying conditions including air inlet and outlet temperatures. The shelf life and stability of the encapsulated Gac oil powder were also evaluated.

The results showed that high extraction efficiencies in terms of Gac oil, β-carotene and lycopene were obtained using the three different extraction methods. Supercritical carbon dioxide extraction gave the highest oil extraction efficiency (95%), followed by ultrasound-assisted extraction (90%) and microwave-drying followed by pressing (86%). The Gac oil extracts from the different extraction methods contained high concentration of β-carotene and lycopene and had low peroxide value.

For encapsulation, the results indicated that the response surface model was sufficient to describe and predict the response variables with high R^2 value. Under optimal

conditions for wall material (whey protein and gum Arabic, 7/3) concentration (29.5%) and the ratio of Gac oil to wall material (0.2, g/g), the encapsulation efficiencies in terms of Gac oil, β-carotene and lycopene, peroxide value, moisture content and total colour difference were predicted and validated. For spray-drying, using optimal conditions (inlet and outlet temperatures of 154 and 80 ^{o}C, respectively), the encapsulation efficiencies in terms of Gac oil, β-carotene and lycopene, encapsulation yield, moisture content, water solubility and peroxide value were predicted and confirmed. Furthermore, physicochemical (Aw, pH, bulk density, fatty acid composition, and particle morphology), reconstitution and colour properties of the optimally encapsulated Gac oil powder were also evaluated. The results indicated that the encapsulated Gac oil powder could be stored for a long time due to low Aw and good protective structure of particles against light, oxidation, and unwanted release of the oil droplets and carotenoids.

A storage study was conducted to determine preservation of quality in the encapsulated Gac oil powder. Findings from the storage study confirmed that preservation of colour, β-carotene and lycopene in the encapsulated Gac oil powder with a lower peroxide value was more effective when vacuum-packed into laminated aluminum bag and stored at ambient temperature or lower for up to 12 months.

The results also showed that during storage, the incorporated Gac oil in yoghurt, pasteurised milk and cake mix were found to be satisfactory in terms of preserving an attractive colour and β-carotene and lycopene contents, and having a low peroxide value.

In summary, the hypothesis was supported and the aims were achieved in this study. The high extraction efficiency for Gac oil containing high level of β-carotene, lycopene and unsaturated fatty acids was obtained using different extraction methods. The Gac oil rich in those bioactive compounds was effectively encapsulated by spray drying. The encapsulated Gac oil powder was highly stable at ambient temperature or lower. Furthermore, the encapsulated Gac oil powder was found to be easily incorporated into a range of food products. Finally, the Gac oil encapsulated powder is considered suitable for use as natural food colourant, a nutraceutical or an additive in the food industry.

PART 1: OVERVIEW

1.1 Background

1.1.1 Gac fruit

Gac fruit, *Momordica cochinchinensis* Spreng, also known as baby jackfruit, sweet gourd and cochinchin gourd, is one of the traditional fruits in Viet Nam. Many studies have reported that extraordinarily high concentrations of carotenoids, especially β-carotene and lycopene, are found in Gac fruit aril, the brightly red coloured flesh covering the seeds (Aoki et al., 2002; Bauernfeind, 1972; Ishida et al., 2004; Vuong, 2000; Vuong et al., 2006). In comparison with other fruits and vegetables, the lycopene content in the aril is at least five times higher than in the other well-known fruits analysed (grapefruit, tomato, papaya, guava, and watermelon) (Aoki et al., 2002; Rao & Rao, 2007). The β-carotene content in the aril is eight times higher than that in carrots, which are well recognised as being high in β-carotene (Kandlakunta, Rajendran, & Thingnganing, 2008; Singh, Kawatra, & Sehgal, 2001; Vuong, Dueker, & Murphy, 2002). Gac fruit also contains significantly high levels of unsaturated fatty acids (Ishida et al., 2004; Vuong, 2000) and α-tocopherol (vitamin E) (Vuong et al., 2006). Recently, Kubola and Siriamornpun (2011) reported that other bioactive compounds such as phenolic acids and flavonoids were also found in the Gac fruit arils with having the highest content compared to other parts of the fruit, including the yellow pulp and skin. More information about the Gac fruit can be found in the review paper (**Paper I**).

1.1.2 Health benefits of bioactive compounds in Gac fruit

Carotenoids are the most widely distributed compounds in nature with a high level of structural diversity and large variations in biological functions. Intestinal absorption can be affected by the occurrence and properties of carotenoid isomers in plants (Kopsell & Kopsell, 2006). Carotenoids are C_{40} isoprenoid polyene compounds, which are lipid-soluble pigments. They are responsible for the red, orange and yellow colours in fruits and vegetables. Carotenoids are classified into two major groups based on their structures, i.e. *carotenes*, which carotenoids contain only hydrocarbons that are either cyclised such as α-carotene, β-carotene, or linear, such as lycopene, and *xanthophylls*, which carotenoids contain oxygen, including lutein, zeaxanthin and β-cryptoxanthin (Britton, 1995). Some common structures of all-*trans* and *cis* β-carotene and lycopene

isomers found in fruits and vegetables are presented in Fig. 1. To date, among more than 700 carotenoids, there are about 40 dietary carotenoids (such as β-carotene and lycopene) being regularly consumed in the human diet.

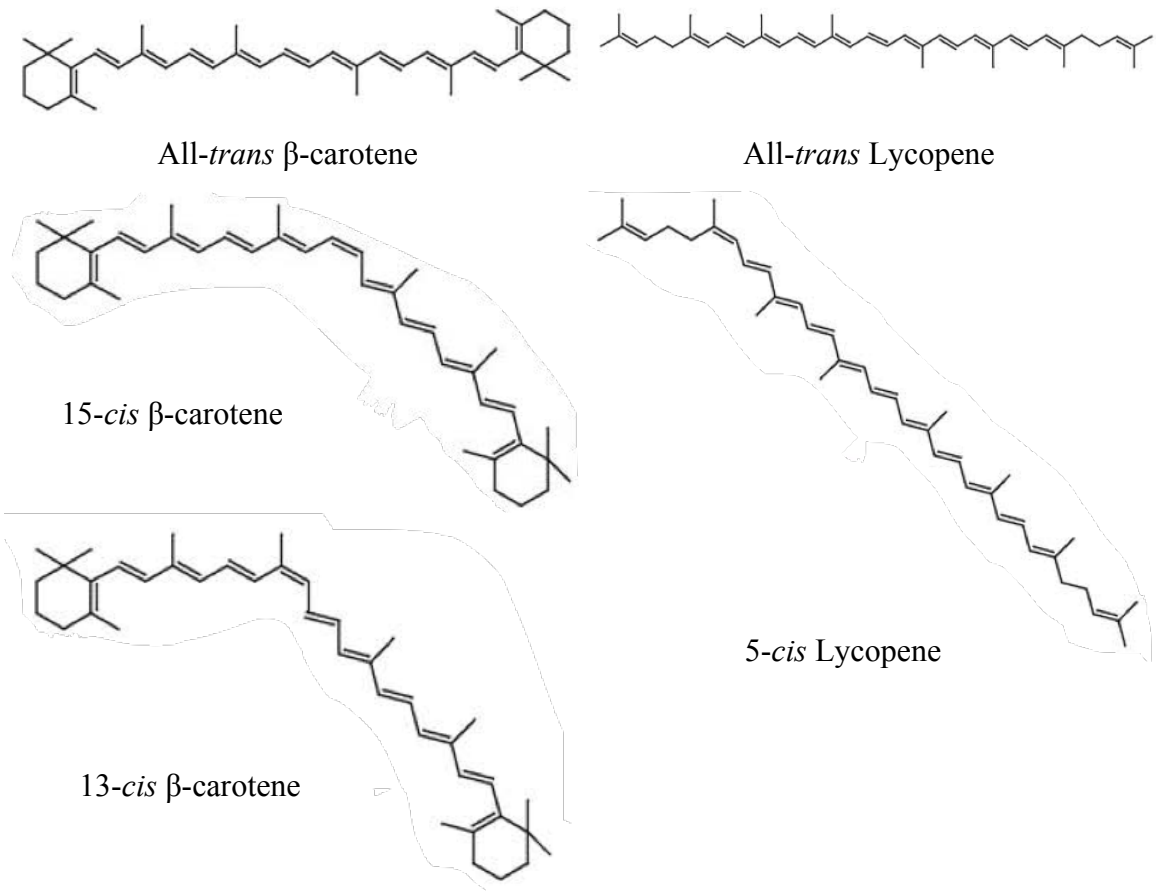

Fig. 1 Structures of some common all-*trans* and *cis* β-carotene and lycopene isomers (Kopsell & Kopsell, 2006)

It is well known that the above bioactive constituents in Gac aril play an important role in human health. For carotenoids, many studies have reported that lycopene-rich diets are linked with a reduced risk of cardiovascular disease and cancers such as lung, breast, stomach, and prostate (Agarwal & Rao, 2000; Lu et al., 2011; Rao & Agarwal, 1999), and also enhance immune system functions (Chew & Park, 2004; Hughes, 1999, 2001). Furthermore, there are a number of other diseases that have been reported as possibly ameliorated by lycopene consumption, for example, ultra violet (UV)-induced sunburn, gingivitis, osteoporosis, mental disorders and asthma (Story et al., 2010).

There are a small number of carotenoids in plants, which are vitamin A precursors, such as α-carotene, β-carotene, and β-cryptoxanthin. Compared to other provitamin A carotenoids, β-carotene is the most potent, of which molecule can be split into two molecules of retinol by a specific 15,15'-dioxygenase. However, α-carotene and β-cryptoxanthin have only approximately half the vitamin A activity of β-carotene (Weber & Grune, 2012). Vitamin A plays an important role in preventing night blindness, proper immune function, growth, development and gastrointestinal function (Grune et al., 2010; Haskell, 2012). Moreover, deficiency of vitamin A is a public health problem in preschool-age children and in pregnant and lactating women in developing countries (Haskell, 2012). Importantly, vitamin A cannot be synthsised in the human body. As a result, it is necessary to consume sufficient amounts of β-carotene as a dietary source of vitamin A (Donhowe & Kong, 2014; Haskell, 2012).

The health benefits of fatty acids in the arils, particularly oleic acid, linoleic acid and α-linolenic acid, are also well known (DeFilippis, Blaha, & Jacobson, 2010; Poudyal et al., 2011; Rodriguez-Leyva, Bassett, McCullough, & Pierce, 2010). Importantly, in foods, it has been found that a significant improvement in the absorption of the carotenoids into the human body occurs when they are digested with fat (Brown et al., 2004; Unlu et al., 2005). Moreover, carotenoids such as β-carotene and lycopene can be used in oils where they can protect unsaturated fatty acids against peroxidation (Steenson & Min, 2000; Warner & Frankel, 1987). In turn, it is interesting that the stability of β-carotene in fortified foods during storage can be greatly enhanced by appropriate addition of high quality soybean oil (Jia, Kim, & Min, 2007). Therefore, it is desirable to produce Gac aril products containing both fatty acids and carotenoids.

Other bioactive compounds found in the arils, including vitamin E (Cordero et al., 2010; Kontush & Schekatolina, 2008; Ye & Song, 2008), phenolic acids (Stevenson & Hurst, 2007) and flavonoids (Yao et al., 2004), have been reported to have beneficial effects on health. Particularly, as a natural antioxidant in foods, vitamin E helps protect polyunsaturated fatty acids, carotenoids and other phytonutrients in the arils from oxidation due to its high antioxidant activity. Therefore, Gac fruit has been known as a super fruit for many years and Gac aril products are now gaining more popularity as health-promoting foods.

1.1.3 Carotenoids as natural colourant

Generally, food colourants are divided into synthetic, nature-identical and natural colourants. The colourants are called synthetic when they are obtained solely by chemical synthesis and are not found in nature. The second group is also produced by chemical synthesis, but they are chemically identical to natural colourants. Natural colourants are extracted from natural edible materials using approved methods, for example lycopene extracted from tomatoes (Henry, 1996).

Colour is the first characteristic that the consumer perceives of a food, and it suggests the quality and flavour. Natural carotenoid extracts are used as food colourants in many processed products such as in oily products (margarines, oils, fats and shortenings), fruit juice, beverages, dry soups, canned soups, dairy products, milk substitutes, coffee whiteners, dessert mixes, preserves, syrups, confectionery, salad dressings, meat products, pasta, egg products, baked goods and others (Delgado-Vargas & Paredes-López, 2003; Francis, 2000). Natural colourants are particularly evident in confectionery, soft drinks, alcoholic beverages, salad dressings and dairy products (Wissgott & Bortlik, 1996).

Unfortunately, gaining approval to use natural colourants as food additives is a complicated task, because it takes time to meet the requirements of governments and organisations (Delgado-Vargas & Paredes-López, 2003). This helps explain why there are only 13 natural colourants approved in the EU and 26 natural colourants certified in the USA (Downham & Collins, 2000). However, in the European Union, the "*Southampton Six"* colours, being Alurra Red (also called Red 40), Ponceau 4R (E124); Tartrazine (Yellow 5) (E102); Sunset Yellow FCF/Orange Yellow S (Yellow 6) (E110); Quinoline Yellow (E104); and Carmoisine (E102)) now must have a specific warning label on food packaging. This increases the demand for natural colourants such as those from Gac fruit.

In addition to use as a nutritional supplement due to their health benefits (section 1.1.2), carotenoids such as β-carotene and lycopene are one of the most popular natural food colourants highly accepted by food industry (Kong et al., 2010). Lycopene is a bioactive red coloured pigment, whereas β-carotene is responsible for orange. It was estimated

total world spending on phytonutrients in 2002 including plant extract sold as food additives or dietary supplements was USD $600 million, and carotenoids used for food colouring and antioxidant properties accounted for more than 60% (USD $375 million) (Pigments, 2002). Furthermore, due to their attractive colourant and health benefits, the demand for carotenoids is still increasing. The worldwide market of carotenoids was worth USD $766 million in 2007, and will increase 2.3% per year, reaching USD $920 million in 2015, β-carotene accounts for 32% of this market (Ribeiro, Barreto, & Coelho, 2011). According to Kong et al. (2010), total world consumption of lycopene was tripled to 15,000 tonnes (worth USD $54 million) in 2004 compared to 5000 tonnes (worth $30 million) in 1995. Currently, tomatoes and carrots are known as the main source of lycopene and β-carotene, which are widely used as a nutritional supplement and a natural food colourant, respectively. Therefore, Gac fruit, which contains very high levels of these carotenoids (refer to section 1.1.1 and Paper I), is considered as an excellent alternative source for their production.

1.1.4 Extraction

As mentioned above, the content of oil rich in essential fatty acids and carotenoids in the Gac arils is high and therefore, it should be of benefit to humans. Consequently, a suitable choice and optimisation of Gac oil extraction, one of the most important steps in oil processing, is needed. Recently, traditional extraction using industrial solvents has been avoided due to health concerns, increased environmental regulations and quality degradation. The hazardous solvent residues in food products are increasingly considered unsuitable and not acceptable by consumers who are concerned about the possible impact of these solvents on their health (Shi et al., 2005). It is important to find a suitable extraction method of Gac oil using non-industrial chemical solvents or food grade solvents.

Regarding the concerns of industrial chemical solvents, water is becoming the solvent of choice for the extraction of vegetable oils and/or bioactive compounds from plant materials. This is because water is readily available, relatively cheap, environmentally friendly, non-flammable, non-toxic and safe for workers. However, water is often less efficient for extracting carotenoids or oils due to low extraction yields compared to industrial chemical solvents such as hexane. Therefore, there is a need to apply novel

extraction methods such as microwave and ultrasound to overcome the low extraction yields to levels resembling that of the industrial solvents while the advantages of water used as extraction solvent are maintained.

Several reports have showed that many plant oil materials can be extracted by alternative methods such as supercritical carbon dioxide (SC-CO_2) extraction, microwave-assisted extraction and aqueous ultrasound-assisted extraction. The main advantages of these novel methods are that they are environmental friendly, they reduce extraction time, increase oil yield, produce high quality oil and are chemical solvent free. The extraction principles, advantages and drawbacks of SC-CO_2 extraction (Azmir et al., 2013; Herrero, Cifuentes, & Ibañez, 2006; Joana Gil-Chávez et al., 2013), microwave-assisted extraction (Desai, Parikh, & Parikh, 2010; Kaufmann & Christen, 2002; Tatke & Jaiswal, 2011) and ultrasound-assisted extraction (Chemat, Zill-E-Huma, & Khan, 2011; Soria & Villamiel, 2010; Vilkhu et al., 2008) in food processing have been reviewed. However, since there is very little information in the literature on Gac oil, further investigations on its extraction using a combination of the mentioned methods is highly recommended in order to increase extraction efficiency.

1.1.4.1 Supercritical carbon dioxide (SC-CO_2) extraction

Extraction mechanisms

The fluid used in this extraction is in its supercritical state, having its pressure and temperature above their critical values. In this state, unique properties of the supercritical fluid such as density, viscosity and diffusivity are intermediate between those of a gas and a liquid. In particular, lower viscosity and higher diffusion coefficient are evident, as compared to that of a liquid. The density of the fluid is similar to that of a liquid. Those properties depend on the pressure, temperature and composition of the fluid (Camel, 2001). Importantly, the density, the dissolving power of the fluid, can be adjusted by changing both temperature and pressure of the fluid. Therefore, these properties offer a number of advantages including shorter extraction times, higher extraction yields and better retention of nutritional and valuable bioactive compounds (Herrero et al., 2006).

It is important to select the most suitable supercritical fluid in this extraction technique. In general, many solvents can be used as supercritical fluids such as ethylene, methane, nitrogen, xenon and fluorocarbons. Among those, carbon dioxide (CO_2) has been a preferred solvent for SFE, known as supercritical carbon dioxide (SC-CO_2) extraction. The main reasons are the low critical temperature of CO_2 (31^oC) and the low critical pressure (74 bar), which enables the extraction process at low temperature and moderate pressures. Due to its low polarity, CO_2 is good for extraction of low or non-polar compounds, but not suitable for polar compounds. To overcome this drawback, the use of a small amount of chemical modifier or co-solvent can significantly enhance the solubility of the polar compounds in SC-CO_2. The co-solvents include hexane, methanol, ethanol, isopropanol, acetonitrile, dichloromethane among others. Of those, ethanol is the most suitable co-solvent because of its lower toxicity and miscibility in CO_2 (Joana Gil-Chávez et al., 2013). In all cases, criteria for selection of the best co-solvent are the properties of sample, desired compounds of interest and results of preliminary experiments (Azmir et al., 2013).

Applications of SC-CO_2 extraction for vegetable oil

For vegetable oils, hydraulic pressing and/or traditional solvent extraction have been commonly used. High extraction efficiency is usually achieved using the hazardous solvent extraction, however, solvent elimination after the extraction is an inconvenient step. Furthermore, the main drawbacks of the traditional solvent extraction are thermal degradation of the bioactive compounds and the incomplete solvent elimination. Since CO_2 is the main solvent used in the SC-CO_2 extraction, it is most effective when the desirable compounds are nonpolar. Importantly, separation of the solute from the CO_2 solvent can be easily obtained by depressurising the SC-CO_2 (Martínez & de Aguiar, 2014). Therefore, the SC-CO_2 extraction can be employed as an alternative to traditional extraction methods with hazardous solvent. In recent years, vegetable oils from plant materials have been extracted using SC-CO_2 extraction technique (Santos et al., 2013; Tomita et al., 2013). According to those studies, material characteristics (particle size and moisture content) and SC-CO_2 extraction conditions (pressure, temperature, time and flow rate) significantly influenced the extraction yield of the vegetable oils. In fact, a faster rate of the CO_2 diffusion is achieved when using a smaller particle size because of increasing surface area to volume ratio of material and

rupturing cell membranes (Del Valle & Uquiche, 2002). As a result, grinding the sample to an appropriate particle size is recommended. This is because there can be a problem with channelling inside the extraction bed if very fine particles are used. Furthermore, it should be noted that when filling the vessel, it is necessary to ensure a homogeneous bed of material to avoid channelling. Moisture content of the material is also an important parameter in the SC-CO_2 extraction. High moisture content can cause mechanical problems such as restrictor clogging due to ice formation. To overcome this, addition of anhydrous Na_2SO_4 and silica gel to the wet plant sample to capture the moisture can be used (Lang & Wai, 2001). However, the preferred pre-treatment is to dry the plant materials to appropriate moisture content before the extraction. It has been reported that the moisture content of the plant materials should not be higher than 12% because water can cause unwanted difficulties such as ice formation in pipelines (Fornari et al., 2012).

There are numerous extraction conditions such as pressure, temperature, time and flow rate influencing the extraction efficiencies. It is generally agreed that applying higher pressure and temperature increases mass transfer and release of bioactive compounds from the plant matrix. However, high pressure and temperature also produces more undesirable compounds in the extract. Generally pressure and temperature can be controlled to optimise the extraction yield of carotenoids. Many studies reported that the extraction pressures between 30 and 40 MPa resulted in the maximal extraction efficiencies of β-carotene and lycopene from different plant matrices (such as tomato and carrot). For extraction temperature, the low temperature of 60^{o}C gave the highest extraction efficiencies of β-carotene and lycopene, which were reported in many studies (Nobre et al., 2009; Şanal et al., 2004). Furthermore, the high extraction temperature of 80^{o}C also favours lycopene extraction from plant materials (Rozzi et al., 2002; Sabio et al., 2003). However, as isomerisation into its *cis* form at temperature higher than 80^{o}C is promoted, the lower temperature is recommended. Therefore, it is necessary to investigate the most important factors affecting the efficiencies and then optimise the conditions.

The use of co-solvents to enhance the extraction efficiencies has been limited due to safety and environmental concerns. An alternative solvent such as vegetable oil, which is relatively cheap and safe, is of growing interest for extracting high molecular weight

compounds such as carotenoids. For example, α- and β-carotene and lutein were extracted using SC-CO_2 extraction at pressure of 27.6 - 41.4 MPa and temperature of 40 - 70^{o}C with canola oil as a co-solvent (Sun & Temelli, 2006). Results indicated that significant improvement in the carotenoid yields was achieved compared with the SC-CO_2 extraction without modifier. Likewise, extraction yield of lycopene from tomato was also significantly increased using hazelnut oil as a modifier (Ciurlia, Bleve, & Rescio, 2009; Vasapollo et al., 2004).

1.1.4.2 Ultrasound assisted extraction (UAE)

Extraction mechanisms

Ultrasound technology is mechanical waves at frequencies above human hearing (16 Hz to 16-20 kHz). The applications of ultrasound in the food industry can be divided basically into two different categories: low-intensity and high-intensity ultrasound. The power levels used in low-intensity ultrasound are very small, typically less than 1 W cm^{-2}. This ultrasonic wave is most commonly applied in the food analysis due to no physical and chemical modifications in the physicochemical properties of the materials, such as firmness, sugar content and acidity (McClements, 1995; Soria & Villamiel, 2010). In contrast, the high-intensity ultrasound uses the large power levels, typically from 10 to 1000 W cm^{-2}. The power ultrasound can alter the properties of food physically and chemically. During the past years the high-intensity ultrasound, which is applied at higher frequencies up to 2.5 MHz, has attracted attention due to strong physical disruption of tissues for extraction purposes.

The main principle of the ultrasound power used in the extraction is the propagation of ultrasound pressure waves, resulting in cavitation. In liquid systems, longitudinal waves are formed via a series of compression and rarefaction waves of elastic materials (Knorr et al., 2011; Soria & Villamiel, 2010). A sufficiently high intensity results in the local pressure waves below the vapour pressure of the liquid, generating a constant growth of gas bubbles being distributed throughout the liquid. These bubbles will reach a critical size, then become unstable and violently collapse (cavitation). High temperature of 5000 ^{o}K and high pressure up to 100 atm are momentarily generated when the bubbles collapse, thus producing very high shear energy waves and turbulence in the cavitation zone (Patist & Bates, 2008). Therefore, it can be seen that the combination of heat,

pressure and turbulence are responsible for a variety of effects of high-intensity ultrasound in the extraction. For instance, the release of extractable compounds is facilitated due to the fast changes in pressure and temperature (cavitation phenomena), which cause shear disruption of cell membranes of the plant matrix. They also enhance the mass transfer by disrupting the plant cell walls (Chemat et al., 2011) and hence extraction rate.

Applications of UAE for bioactive compound extraction

UAE is also known as a novel technique for extraction of bioactive compounds from plants. Reducing extraction time, increasing yield and producing high quality of the plant extracts are the main benefits of this method. In order to achieve an efficient and effective ultrasound-assisted extraction, it is of interest to understand influence of the extraction conditions and the plant characteristics.

For the conditions of ultrasound-assisted extraction, proper choice of the solvent is the key to successful extraction. The important criterion is the extent of ultrasound cavitation in the solvent. For instance, the physical properties such as surface tension, viscosity and vapour pressure of the solvent affect the intensity of cavitation in a liquid medium. In addition, the solubility of the analytes of interest and the interactions between the solvent and plant matrix should also be considered. Other important factors including ultrasound power, temperature and extraction time also need to be taken into account. It is necessary to control the extraction temperature during UAE because ultrasound generates heat. As a result, it is also important to minimise the sonication time to avoid degradation of bioactive compounds.

Carotenoids from plant materials have been successfully extracted using UAE technique (Vilkhu et al., 2008; Xu & Pan, 2013; Ye et al., 2011). Results indicated that UAE conditions such as ultrasound power, temperature and extraction time, type of solvent, ratio of solvent to solid positively affected the extraction yield of the bioactive compounds. Reduction in extraction time and improvement in quality of bioactive components are also the two main advantages of this technique. Thus, it is interesting to note that implementation of UAE may improve throughput in commercial bioactive compound production process.

In addition to the extraction mechanism, it is also desirable to understand the process parameters, which affect extraction rate, to achieve the maximum effect. There are a number of parameters influencing the extent of cavitation phenomena in ultrasound-assisted extraction, including temperature, pressure, frequency and the medium viscosity. For example, more rapid formation of cavitation bubbles occurs at higher temperatures due to increasing vapour pressure and reducing tensile strength (Earnshaw, 1998). In addition, at lower frequencies (e.g. 20 kHz), bigger size of the bubbles is formed when higher energies accumulated are produced because of the implosion of cavitation bubbles. However, at higher frequencies (above 2.5 MHz), bubbles are not formed and cavitation does not occur. The medium viscosity is another important parameter determining the extent of cavitation. Cavitation bubbles form less easily in a highly viscous environment and decrease the effectiveness (Earnshaw, 1998; Patist & Bates, 2008). To overcome this by increasing temperature, which result in reducing viscosity, more violent collapse occurs. Moreover, surface tension, nature and concentration of dissolved gas and presence of solid particles also affect the effectiveness of cavitation (Soria & Villamiel, 2010).

The application of pre-treatment with ultrasound for oil extraction has been successfully reported. Zhang et al. (2009) stated that response surface optimisation of pre-treatment with ultrasound could enhance the oil recovery from autoclaved almond powder. Three variables being extraction time, extraction temperature and a ratio of solvent to solid were investigated. Similarly, many other studies confirmed that increased oil yield and higher quality of oil could be achieved by ultrasound pre-treatment prior to oil extraction from plant materials such as *Jatropha* seed kernels (Shah, Sharma, & Gupta, 2005), soybeans (Li, Pordesimo, & Weiss, 2004), flaxseed (Zhang et al., 2008) and camellia seed (Wu & Li, 2011). From these reports, it can be seen that ultrasound assisted extraction may be applied to obtain high yield of oils from many kinds of plant materials. Therefore, this method should be considered for production of oil from Gac arils.

The application of aqueous ultrasound assisted extraction techniques for plant oil, in which oil separation is based on the insolubility of the oil in water, has been studied widely (Do & Sabatini, 2010; Hou et al., 2013; Naksuk, Sabatini & Tongcumpou,

2009). When using water as the main extraction solvent, there is no energy required for hazardous solvent stripping and no high investment is required for monitoring and controlling the emissions of volatile organic compounds. The advantages of this extraction generally are more environmentally friendly, economical, safer and a higher oil quality than the traditional extraction using hexane. This aqueous extraction process also offers high oil quality without requiring degumming and removal of anti-nutritional factors (Rosenthal, Pyle, & Niranjan, 1996).

1.1.4.3 Microwave assisted extraction (MAE)

Extraction mechanisms

Microwaves are non-ionising electromagnetic waves within the frequency band of 300 MHz to 300 GHz in the electromagnetic spectrum, corresponding to wavelengths between 1 mm to 1 m. Generally, microwave applications are performed at 915 MHz (United States), 896 MHz (United Kingdom) and 2450 MHz (worldwide) for all practical purposes. According to Singh and Heldman (2001), as the velocity of light is 3 x 10^8 m/s, the microwave wavelengths at 915 MHz and 2450 MHz are calculated as 0.328 and 0.122 m, respectively. Therefore, the frequency of 915 MHz is considered as most useful for industrial applications with its greater penetration depth, whereas domestic microwave ovens use the frequency of 2450 MHz.

When microwaves penetrate deeply into a material, the absorption of microwaves by the dielectric component of this material results in release of their thermal energy to it. The two main mechanisms of heating using microwaves are ionic polarisation and dipole rotation (Kaufmann & Christen, 2002). Food materials contain water molecules, which are the most common, and generally have a random orientation. When an electric field is applied, the molecules orient themselves according to the polarity of this field. As the electric field is alternated rapidly, the polar molecules rotate to follow the applied field. This leads to friction with the surrounding medium, resulting in heat being generated. For ionic polarisation, this happens when ions in food solutions move due to their inherent charges as the electric field is applied. Kinetic energy of the moving ions is converted into heat by the resulting collisions between ions; there is a rapid increase in temperature as a consequence (Kadam, Tiwari & O'Donnell, 2013; Zhang, Yang, & Wang, 2011).

Recently, the development of microwave-assisted extraction has been applied to heating the moisture inside a plant cell using microwave radiation, thus evaporation and high pressure on the cell wall are generated. The physical properties of the biological tissues (cell membrane and organelles) are modified by the generated pressure inside the material. The cell membrane is ruptured; the bioactive compounds from the ruptured cells are released into the medium. The penetration of extraction solvent through the porous biological matrix is facilitated. Therefore, the extraction yield of desired compounds could be increased. In addition, the use of this extraction also offers several benefits such as less chemical solvent use, reduced processing time and uniform heating (Azmir et al., 2013; Uquiche, Jeréz, & Ortíz, 2008). These advantages have recently led to microwave-assisted extraction to be considered as one of the most novel techniques for the extraction of bioactive substances from different plant materials.

Applications of MAE for bioactive compounds

Recent interest in microwave-assisted extraction for plant oil has been highlighted owing to their benefits. Effect of pre-treatment with microwave radiation prior to mechanical pressing on oil recovery and quality of oil from Chilean hazelnut was investigated by Uquiche et al. (2008). Different microwave treatment conditions (intensities and times) were studied, and an increase in oil yield was found in comparison to untreated samples. Moreover, the microwave pre-treated oil samples also possess superior quality characteristics in terms of oxidative oil stability, oil composition and other physicochemical properties, as compared to untreated one. Similarly, Azadmard-Damirche et al. (2010) reported that a significantly higher yield of oil from rapeseed could be obtained by pre-treating with microwaves before pressing. The results also indicated that this extraction method could be used to produce the rapeseed oil with high oxidative stability and nutraceutical content. Therefore, future investigation on the microwave pre-treatment conditions prior to pressing oil from Gac arils should be performed. According to Desai at al. (2010), optimisation of microwave assisted extraction conditions has been reported in the literature. Experimental designs such as factorial and central composite have been effectively chosen to optimise the conditions. Several parameters and their levels including solvent type or composition,

solvent volume or solid loading, power applied or extraction temperature, extraction time and material size need to be studied.

Generally, the pre-treatment comprises three main steps being drying, grinding and sieving. Water from raw material can be removed using different drying methods, such as air, vacuum, freeze or microwave drying. Depending on the desirable quality of the extract, a suitable drying method can be chosen. When the conventional and cheap air drying method is applied, the bioactive compounds, which are sensitive to high temperature and oxygen in the air, can easily degrade. Alternatively, the vacuum, freeze and microwave drying methods can be used. Vacuum drying lessens the effect of heat and oxygen on the product. Freeze drying can be used for the samples which are very sensitive to heat and oxygen. However, it takes a lot of time to freeze dry and it is expensive due to high capital and energy costs related to the operation of refrigeration and vacuum system. Therefore, the microwave drying can be applied as an alternative due to decrease in the drying time of the sample without or insignificant nutrient loss (Wojdyło et al., 2014). Furthermore, microwave drying under vacuum, called vacuum-microwave drying technique, has been successfully applied for many natural plant materials (Figiel, 2009). As an example, Regier et al. (2005) reported that both freeze-drying and vacuum-microwave drying techniques improved actual retention of carotenoids in carrot, particularly β-carotene and lycopene. Shorter drying time is also an advantage of vacuum-microwave drying as compared to freeze-drying. Many studies confirmed that drying pre-treatments could have significant effects on yield and quality of bioactives in subsequent extraction (Divya, Puthusseri & Neelwarne, 2012; García-Martínez et al., 2013; Nawirska et al., 2009; Shivanna & Subban, 2013). In all cases, other than the quality of the pre-treated material, the availability of equipment should be also considered.

1.1.4.4 Comparison of different novel extraction methods

The different extraction techniques including SC-CO_2, UAE and MAE have been presented in the previous sections. Depending on the availability of equipment, the target bioactive compounds and the processing cost, a proper choice of the extraction technique or a combination of different extraction methods can be made.

It is well known that the conventional Soxhlet extraction method takes a very long time to complete the extraction process of desired bioactive compounds. Another drawback of the conventional method is the likely degradation of thermolabile compounds due to the high temperature applied. In addition, the large amounts of chemical solvent used and wasted in the solid-liquid extraction incur costs for solvent disposal and environmental control measures, and contribute to the criticism of the conventional extraction methods (Luque de Castro & García-Ayuso, 1998).

To overcome these limitations of the conventional method, the development of innovative technologies for extracting bioactive compounds from plant materials addresses specific human requirements for health and safety. The main advantages of the novel extraction methods including SC-CO_2, UAE and MAE are shorter extraction time, higher extraction yield and better retention of valuable bioactive compounds. The use of SC-CO_2 extraction in the extraction of the bioactive compounds, which can be used as nutraceuticals and pharmaceuticals, has been reported. The extracts containing high bioactive compounds can be used to treat or prevent disease (Henry & Yonker, 2006). SC-CO_2 extraction method uses nil or a small amount of chemical solvent (as a co-solvent) in the extraction, so is considered as more environmentally friendly than the conventional extraction method. However, the main drawbacks of SC-CO_2 extraction technique are economics and onerous operating conditions, thus its use so far is limited to areas such as essential oil extraction and coffee decaffeination (Wang & Weller, 2006).

There have been many studies reporting the benefits of MAE and UAE methods for extracting bioactive compounds. Similar to SC-CO_2, the MAE and UAE methods can operate at low temperature, allowing the extraction of thermolabile compounds from various plants. Between them, UAE device is cheaper and its operating process is easier compared with MAE. Like Soxhlet extraction, UAE can be used with any solvent, in contrast, the extraction solvent used in the MAE must absorb microwave energy. Overall, MAE and UAE techniques are comparable to other innovative extraction techniques such as SC-CO_2 and are considered as strong novel extraction methods in terms of process simplicity, low investment cost and practicality (Wang & Weller, 2006).

Although these methods are very promising as alternatives to the conventional extraction method, they have been used only at laboratory or bench scale, except for several industrial applications of SC-CO_2 extraction (Wang & Weller, 2006). It is important to conduct more research to up-scale these novel extraction methods.

Potentially, from understanding the advantages and drawbacks of different extraction methods as presented above, there are opportunities to combine different extraction methods to overcome the limitations and retain the advantages. Several combined extraction techniques, including ultrasound with microwaves, were successfully applied to extract bioactive compounds from plant materials (Rostagno et al., 2010). For example, Lianfu and Zelong (2008) successfully extracted lycopene from tomato using the combination of ultrasound and microwave. The results confirmed that the shorter extraction time, higher yield of lycopene and less amount of solvent used were obtained in this combined extraction method compared with the ultrasound-assisted extraction on its own.

1.1.5 Encapsulation

In addition to achieving high extraction efficiency of Gac oil, it is also important to preserve the bioactive compounds in the oil extract including carotenoids and polyunsaturated fatty acids. Due to high number of double bonds in the structure of carotenoids and polyunsaturated fatty acids, the oil is susceptible to isomerisation and oxidation during processing and storage. Therefore, it is desirable to find an effective method to preserve the bioactive components in the oil. Encapsulation by spray drying is one of the most effective techniques, which can be employed to protect, stabilise, and release the compounds while also enabling their solubility in an aqueous medium (Rocha, Fávaro-Trindade & Grosso, 2012). During recent years, increasing attention has been given to the application of encapsulation of bioactive compounds, particularly unsaturated fatty acids and carotenoids. The degradation of these compounds can be prevented by applying the encapsulation technique (Arana-Sánchez et al., 2010; Rocha et al., 2012; Shu et al., 2006).

A prerequisite for successful encapsulation is high stability of the emulsion over a certain period of time. As a result, an appropriate choice of wall materials

(encapsulating agent) and ratio of oil to wall material are a very important step to effectively encapsulate Gac oil. Various materials have been reported for effectively encapsulating food oils and carotenoids in terms of good protection against heat, light and oxidation. The encapsulating agents are classified as carbohydrates, cellulose, gum, lipids and protein, which have been thoroughly reviewed (Desai & Park, 2005; Jafari et al., 2008; Jena & Das, 2007; Shahidi & Han, 1993). Several properties of encapsulating agents including viscosity, solubility, stabilisation, reactivity and cost need to be considered and have been reviewed by several authors (Jafari et al., 2008; Shahidi & Han, 1993). The important molecular characteristics of these agents are presented in Table 1. It is important to select the appropriate wall material, a ratio of the material to bioactive compounds, encapsulation technique and desired microcapsule properties. Since there is lack of information on choosing the right wall material for Gac oil encapsulation, more research is therefore needed.

Whey protein (WP), which has nutritional, physicochemical and functional properties, has been used in foods because of its ability form thick and flexible film and preventing coalescence (Stănciuc et al., 2012). It is widely used for microencapsulation of bioactive compounds. WPs are compact and globular proteins, which can interact with polysaccharides to form either soluble or insoluble complexes. Importantly, WP can also be formed into micro or nanoparticles, which are the matrix systems of a dense polymeric netwwork. As a result, the bioactive compounds may be dispersed throughout the matrix. Another advantage of using WP as encapsulating agent in the emulsion is easily to control release of bioactive compounds. This is because they are entirely biodegradable, and hence there is no requirement for any chemical crosslinking agent in the preparation (Gunasekaran, Ko, & Xiao, 2007).

Gum Arabic (GA) is another widely used as encapsulating agent for microencapsulation of bioactive compounds. GA is a branched complex heteropolysaccharide containing D-galactopyranose units linked by β-glycosidic bonds, which has the ability to form a strong protective film around the oil particles (Tatar et al., 2014). According to Garti (1999), GA is considered to be the best gum in use for stabilisation of oil-in-water emulsion systems.

Table 1 Important molecular characteristics of common wall materials (Jafari et al., 2008; Matalanis, Jones & McClements, 2011)

Name	Source	Main structure type	Major monomer
Alginate	Algal	Linear	β-D-Mannuronic acid
Beet pectin	Sugar beet pulp	Branched coil with protein	Glucuronate (backbone)
Carrageenan	Algal	Linear/helical	Sulfated galactan
Gelatin	animal or fish collagen	Linear	Amino acid
Gum arabic	Acacia sap	Branched coil domains on protein scaffold	Galactose
Methyl cellulose	Wool pulp	Linear	Methylated glucose
Pectin	Plant cell walls	Highly branched coil	Glucuronate (backbone)
Xanthan gum	*Xanthomonas campestris* exudate	Linear/helical	β-D-glucose (backbone)
Whey protein	Milk	Globular	β- lactoglobulin and α-lactalbumin

Furthermore, different wall materials have different physical and chemical properties, combinations of encapsulating agents are required to effectively protect and control the bioactive compounds (Sun-Waterhouse et al., 2011). Klein et al. (2010) reported that wall materials containing whey protein (WP) and gum Arabic (GA) could enhance stability of emulsion against significant droplet size increase. In addition to the wall materials, it is also important to optimise the ratio of oil to wall material (Jafari et al., 2008). A matrix with the hydrophilic carbohydrates dissolved in the water phase and the hydrophobic polypeptide chain adsorbed onto the oil water phase can be obtained. As a result, optimisation of the concentration of the wall materials and the ratio could improve encapsulation efficiency.

In general, spray drying is the most widely used method in the food industry. This process can potentially offer many benefits of being economical, flexible and producing

good quality of spray-dried products (Ré, 1998). In order to obtain a good encapsulation efficiency after optimising the conditions for preparation of oil emulsion, spray drying conditions are importantly optimised. The two key parameters need to be optimised include air inlet and outlet temperatures (Gharsallaoui et al., 2007; Jafari et al., 2008). The inlet temperature directly affects the drying rate. In fact, a low inlet temperature results in low evaporation rate, thus high water content and ease of agglomeration. The spray-dried bioactive compounds are also substantially lost when a high inlet temperature is applied. On the other hand, it is difficult to predict and control the outlet temperature as it depends on the inlet temperature and the quantity of the moisture to be removed (feed flow rate). In addition, it is controversial and unclear if the encapsulation efficiency of food flavours and oils is affected by the outlet temperature (Jafari et al., 2008). The high outlet temperature detrimentally affects the quality of the dried powder due to heat damage (Jena & Das, 2007). Hence, optimisation of the inlet and the outlet temperatures needs to be intensively investigated.

1.1.6 Response surface methodology

It is well known that response surface methodology (RSM) is one of the most effective methods to evaluate the relationship between the response and the independent variables and then optimise the process or products (Baş & Boyaci, 2007; Bezerra et al., 2008). This technique also allows the evaluation of the effect of multiple parameters and their interactions on the output variables with a reduced number of trials (Lee et al., 2000). It is a faster and more economical method than other approaches required to optimise a process.

In general, the quality of a product is affected by numerous parameters. It is impossible to control the effects of all variables; hence the major effects of parameters on the process must be identified. It is useful to use RSM to develop, improve and optimise the effects on the processes such as extraction and spray drying processes. According to Baş & Boyaci (2007) and Bezerra et al. (2008), optimisation study using RSM includes three main stages, (1) screening of independent variables and their levels; (2) the selection of experimental design and the prediction and verification of the model equation; and (3) graphical presentation of the model equation and determination of optimal operating conditions.

According to Baş & Boyaci (2007), it is important to screen all variables, which may affect the process. Factorial designs may be used for determining major effects of independent parameters. After this, the levels of key parameters should be identified. This is because the success of the optimisation process is directly related to these levels. To make sure the response is affected more evenly, each variable is coded to range from -1 to 1, and expressed as follows:

$$X = \frac{x - (x_{max} + x_{min})/2}{(x_{max} - x_{min})/2}$$

where x is the natural variable, X is the coded variable and x_{max} and x_{min} are the maximum and minimum values of the natural variable, respectively.

The second stage of RSM is to select the experimental design, predict and verify the model equation. The experimental designs are usually taken from some computer packages, which are based on the special criteria and input. The experimental points, number of runs and blocks will be identified. However, it is very important to choose the appropriate design. If the data show curvature, experimental design for first-order models cannot be used, hence it should use experimental designs for quadratic response surfaces, including a three-level factorial, Box-Behnken, central composite or Doehlert design (Bezerra et al., 2008).

After selection of the design, the model equation is defined and coefficients of the model equation are predicted. The estimated response is easily calculated from the model equation after obtaining the regression coefficients. To verify whether the model fits well to the practically determined experimental data, prediction error sum of squares residuals are normally used. In addition, other techniques such as residual analysis, scaling residuals, or testing of the lack of fits can be used (Baş & Boyaci, 2007).

The optimal operating conditions can be determined by the response surface plot and contour plot. The response surface plot is the three-dimensional plot, which shows relationship between the response and the independent variables, whilst the contour plot is the two-dimensional plot, which helps to visualize the shape of a response surface. Therefore, it is useful to use the plots to evaluate the fit of model (Baş & Boyaci, 2007).

1.1.7 Storage study of encapsulated Gac oil powder and its fortification

Generally, the stability of encapsulated food products during storage also plays an important role in the food industry. The quality of the products changes over time as a result of certain storage conditions. The environmental conditions for storage, such as relative humidity, exposure to light, temperature and oxygen, are well known as affecting the quality of the final products, especially encapsulated oil product. As a result, the need for a storage study of encapsulated Gac oil product is important in establishing the effects of storage conditions on the final product.

The degradation of encapsulated powder during storage is due to physico-chemical and biological changes. Humid atmospheres and elevated storage temperature are the main cause of the physical degradation. For example, the phenomenon of caking or spontaneous agglomeration of powder during storage occurs due to the moisture sorption (Robertson, 2006). For the chemical deterioration, environmental factors such as light, oxygen, temperature and A_w affect the rate of lipid oxidation in foods during storage. Especially, the powder containing high fatty acids and carotenoids will be degraded readily as exposure to light, oxygen or high temperature. The degradation of fatty acids and carotenoids by enzyme or non-enzyme reactions also happens (Robertson, 2006; Singh, 2000). Additionally, the presence and growth of microorganisms reduce the shelf life of powder products. However, the low moisture content of powder can prevent or minimize the growth of microbial organisms. Therefore, it is very important to investigate the environmental factors that influence the degradation of powder product during storage at certain conditions.

The surface oil or non-encapsulated oil of the powder is highly susceptible to oxidation during storage (Velasco, Dobarganes & Márquez-Ruiz, 2000; Velasco et al., 2009). The shelf life of the powder will reduce when the surface oil content increases. It is due to more rapid oxidation process of non-encapsulated oil was observed as compared to encapsulated oil. Several parameters such as high relative humidity conditions, low encapsulation efficiency and temperature over the glass transition temperature were reported as increasing the surface oil content (Velasco et al., 2000). Therefore, certain accelerated storage conditions should be investigated to predict the shelf life of the oil product.

The final step of the encapsulation process is to apply in foods; therefore, it is important to test the stability of the encapsulated product in several kinds of food products. For example, fish oil containing high omega-3 fatty acids has been fortified in several marketed food products such as bakery products, margarines, dairy products, juice and soft drinks worldwide (Borneo et al., 2007). Similarly, carotenoids (lycopene and β-carotene) have been successfully incorporated in various foods such as dairy products, juices, soft drinks, alcoholic beverages and others as a natural colourant and/or nutrient supplement (Delgado-Vargas & Paredes-López, 2003; Francis, 2000; Wissgott & Bortlik, 1996). Therefore, the stability of foods which have been incorporated with encapsulated with encapsulated Gac oil should also be investigated.

1.2 Synopsis of Literature review paper

In this thesis, the review paper entitled ”Gac fruit: Nutrient and Phytochemical Composition, and Options for Processing” reports the overview of Gac fruit including traditional uses, propagation and cultivation, morphology, bioactive compounds and health benefits. Processing options outlined for all parts of Gac fruit such as the skin, the yellow pulp, the arils and the seeds and utilisation of the processed products in a range of foods as nutrient supplements and/or natural colourants are also highlighted. Processing of Gac fruit including different drying methods, extraction methods and encapsulation is also presented.

The Gac fruit remains underutilised. The yellow pulp and the skin containing high carotenoids, and the seeds containing high levels of fatty acids are usually discarded. Therefore, it is also important to utilise these components in order to reduce the environmental problem of waste and to enhance the economic value of the fruit. Those issues are also reported in this paper.

1.3 Literature review paper

Some parts of literature review for this thesis is based on the following review paper:

Paper I: Tuyen C. Kha, Minh H. Nguyen, Paul D. Roach, Sophie E. Parks & Constantinos Stathopoulos (2013). Gac fruit: Nutrient and Phytochemical Composition, and Options for Processing. *Food Reviews International*, 29(1), 92-106.

Paper I

Gac fruit: Nutrient and Phytochemical Composition, and Options for Processing

Tuyen C. Kha, Minh H. Nguyen, Paul D. Roach, Sophie E. Parks & Costas E. Stathopoulos

This review paper was published in *Food Reviews International*, 2013, 29(1), 92-106.
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Gac Fruit: Nutrient and Phytochemical Composition, and Options for Processing

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Gac Fruit: Nutrient and Phytochemical Composition, and Options for Processing

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Momordica cochinchinensis *Spreng or Gac fruits are rich in nutrients, including carotenoids, fatty acids, vitamin E, polyphenol compounds, and flavonoids. Medicinal compounds are also found in the seeds, but the benefits of traditional preparations from these need to be clarified. The plant has the potential to be a high-value crop, particularly as parts of the fruit can be processed into nutrient supplements and/or natural orange and yellow colorants. However, the plant remains underutilized. There is limited information on its requirements in production, and the processing of health products from the fruits is a relatively new area of endeavor. The versatility of the fruit is highlighted through processing options outlined for fruit aril, seeds, pulp, and skin into powders and/or encapsulated oil products. These Gac fruit products will have the potential to be utilized in a range of foods such as pasteurized juice and milk beverages, glutinous rice, yogurt, pasta, and sauces.*

Keywords Antioxidant, Carotenoids, Encapsulation, Fatty acid, Gac fruit, Oil extraction

Introduction

The cucurbit *Momordica cochinchinensis* Spreng, called Gac in Vietnam, is a variable species and is widespread across South East Asia, Malesia, and India.(1) English names for the fruit include baby jackfruit, sweet gourd, and cochinchin gourd. Nutritionally, this fruit is special because the flesh around the seeds (aril) is rich in carotenoids, especially β-carotene and lycopene. Gac fruits also contain relatively high levels of α-tocopherol (vitamin E), polyunsaturated fatty acids,(2–4) and polyphenol compounds and flavonoids.(5) A number of studies highlight the important role these products play in human health. Beyond its natural distribution, Gac aril products are gaining popularity as health-promoting foods. Gac fruit products also have market potential as alternatives to the artificial colorants Tartrazine, Sunset Yellow, and Quinoline Yellow, which are associated with behavioral problems in children.(6)

In addition to the Gac aril having a very high nutritional content, the total carotenoid content (TCC) in the yellow pulp of the Gac fruit (mesocarp) is relatively high as compared

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with many plant foods.[2,7] Furthermore, the yellow pulp represents approximately half of the weight of an entire fresh fruit and is the highest anatomical component.[7] However, whereas the aril is traditionally used for food preparation due to its attractive color and high nutrients, the pulp is often discarded. Similarly, Gac skin, which represents about 17% of the total weigh of the fruit, is not used. Importantly, the seeds containing high levels of fatty acids and other products are not usually used. Therefore, identifying means of utilizing of these components is necessary to reduce the environmental problem of waste and to enhance the economic value of the fruit

This review will focus on the traditional uses and production of Gac fruit, fruit nutrient and phytochemical composition, and the use of Gac products as nutrient supplements and natural food colorants. A potential processing scheme for Gac fruit is proposed to help facilitate greater use of this fruit.

Traditional Uses

Gac fruit is a traditional Southeast Asian fruit. In Vietnam, ripe Gac fruit is most commonly prepared as "Xoi Gac" (the Gac aril cooked in glutinous rice) for Tet (Vietnamese New Year) and wedding celebrations. In India (Assam and Andamans), the fruits are harvested small and green with immature seeds to be consumed as a vegetable.[8] The spiny skin is removed and the fruits are sliced and cooked sometimes with potato or bottle gourd and in some areas the tender leaves and shoots of the plant are also cooked.[9]

Gac fruit seeds are used in traditional Chinese medicine, known as *Mubiezhi*, to treat fluxes, liver and spleen disorders, wounds, hemorrhoids, bruises, boils, sores, scrofula, tinea, swelling, and pus.[10,11] Practically, many people in rural areas in Vietnam use ground Gac seeds mixed with alcohol or vinegar to cure furuncle, swelling, hemorrhoids, and mumps. However, future research needs to clarify the benefits of these preparations.

Propagation and Cultivation

Limited information is available on the requirements in production of the Gac plant for optimum yield and quality of the fruits. The Gac plant is not usually intensively cultivated but can be seen (in Vietnam) growing wild or in domestic settings with the vines growing on lattice in rural homes or in gardens. The plant can be cultivated from seeds or root tubers, and grows as dioecious vines (separate male and female plants). Rooted vine cuttings can also be used for propagation and are more reliable than production from seeds, which can be affected by dormancy and a long lead time into production.[9] Furthermore, several seedlings need to be planted in the one pit so that the male plants can be removed once they are identified as male at flowering, as only a few are needed for pollination.[9] Alternatively, it is possible to graft female scion material onto the main shoot of the unwanted male plant, making it productive.[12]

Hybridization studies using several *Mormordica* species including Gac[13] and studies on the effects of plant growth regulators on Gac[8,14,15] indicate that new varieties with bisexual flowers will be possible, overcoming some of the difficulties currently associated with Gac production.

Approximately 2–3 months after planting, flowering occurs. Pollination is chiefly carried out by insects rather than wind and hand pollination results in a higher fruit set than open pollination.[16] It takes approximately 5 months after flowering before the ripe fruits can be harvested. One plant can produce 30–60 fruits in one season,[17] although this may depend on factors such as climate and plant age, yet to be described.

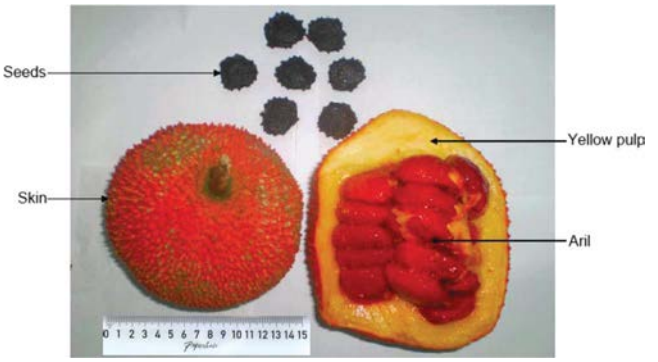

Figure 1. Fresh Gac fruit components (from Kha[19]) (color figure available online).

Fruit Morphology

The fleshy Gac fruit can be botanically described as a pepo. Gac fruits grown in Vietnam are typically round or ovoid in shape but one cultivar grown in India is recorded as oblong-shaped.[18] The exterior skin of Gac is covered in short spines, which can sparsely or densely cover the skin. Its green color becomes red or dark orange when ripe. Gac fruit (Fig. 1)[19] comprises orange/yellow skin containing spines, yellow pulp, and aril (red flesh surrounding the seeds). The highest anatomical component of a Gac fruit is yellow pulp (49%, by weight), whereas the aril, which contains the highest level of carotenoids, accounts for only 18%.[19] The aril weight has also been reported as 10% and 24.6%.[3,7] Storage time and growth stage during which loss of water may contribute to this variation.[3]

Bioactive Compounds of Gac Fruit

Gac fruit is an exceptional fruit whose aril contains excellent sources of carotenoids, α-tocopherol (vitamin E), polyphenol compounds, flavonoids, and essential fatty acids.[2,3,5,7,10,11,20] Depending on the component, these phytochemicals are present in all parts of the fruit so there is the potential to utilize all parts in processed products. Future research will need to focus on the effect of growing, storage, and processing conditions on the phytochemical qualities of fruits such that techniques and varieties are developed to protect and/or enhance the desired bioactive qualities.

Carotenoids

Carotenoids from plant-based foods play a crucial role in human health.[21,22] For example, numerous studies have reported that lycopene-rich diets are linked with reduced risk of cardiovascular disease and cancers such as lung, breast, stomach, and prostate.[23–25] β-Carotene is converted to vitamin A in the body.[26]

Evidence suggests that Gac has promise as a bioavailable source of carotenoids and it has been examined as a food supplement in a study with Vietnamese children. In the study, 185 Vietnamese preschoolers participated in a 30-day supplementation trial and were randomly divided into three groups, one group given Xoi Gac (sticky rice mixed with Gac fruit containing 3.5 mg β-carotene), one group given rice mixed with 5 mg synthetic β-carotene powder, and a control group given rice without fortification. Results indicated that plasma levels of retinol and carotenoids (β-carotene, α-carotene, zeaxanthin, and lycopene)

after supplementation were significantly increased. Moreover, the increase in plasma β-carotene level after supplementation in the fruit group (1.86 μmol/L) was significantly higher than that in the powder group (1.48 μmol/L).[27] Therefore, using Gac fruit as a food-based intervention may be effective for reducing vitamin A deficiency.

The Gac aril, in particular, contains extraordinarily high levels of carotenoids, especially carotenes and lycopene (Table 1), in comparison with other fruits and vegetables. It is claimed that the lycopene concentration in Gac fruit is at least 5 times higher than in other well-known fruits analyzed (grapefruit, tomato, papaya, guava, and watermelon) (Fig. 2).[2,21] It is also shown that Gac aril has the highest known concentration of β-carotene of all fruits and vegetables.[4] For example, it is 8 times higher than the level in carrots, which are recognized as being high in β-carotene (Fig. 3).[27–29]

In addition to the aril, the yellow pulp and skin are good sources of carotenoids and should not be overlooked as carotenoid sources (Table 1). For example, lutein has a

Table 1
Carotenoid content of fresh Gac fruit (mg/100 g)

Carotenoids	Skin	Pulp	Aril
β-Carotene	38.4–141.6[5]*a*	24.0–43.2[5]	160.0[5]
		2.2[2]	63.6–83.6[7]
			10.1[2]
			8.3[20]
Lycopene	38.4–81.6[5]	14.4–49.6[5]	154.6–305.4[7]
		0.1[2]	140.0[5]
			38.0[2]
			40.8[20]
Lutein	189.6–1248[5]	16.0–144.8[5]	na
Zeaxanthin	na	0.2[2]	0.9[2]
β-Cryptoxanthin	na	0.4[2]	0.2[2]

Note. na = not available. [a]Data[5] converted from dry weight to fresh weight using the moisture content of skin, pulp, and aril of 76%, 92%, and 80%, respectively.

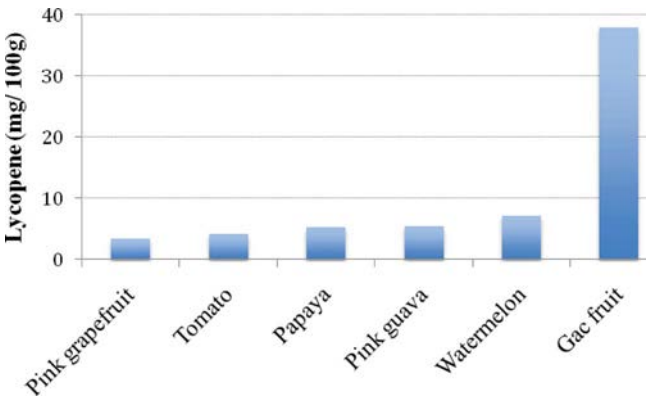

Figure 2. Lycopene content of fruit and vegetables (adapted from Aoki et al.[2] and Rao and Rao[21]) (color figure available online).

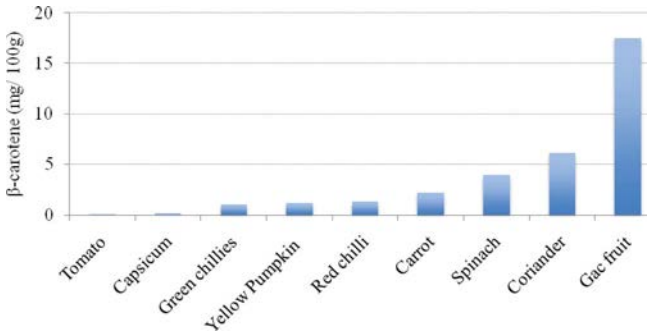

Figure 3. β-Carotene content of fruit and vegetables (adapted from Kandlakunta et al.,[28] Singh et al.,[29] and Vuong et al.[27]) (color figure available online).

higher concentration in the skin than in the aril or the pulp.[5] Many studies have reported that lutein plays an important role in the prevention of age-related macular degradation (AMD).[22,30–32] These components of Gac fruit are usually discarded when the aril is scooped out and used for processing purposes.

Although high, the concentrations of carotenoids content in Gac fruit are variable (Table 1). The factors responsible for this remain to be investigated but may include variety, genotype, season, geographic location, stage of maturity, growing conditions, and storage conditions. For example, one single study investigated concentration changes in carotenoids (lycopene and β-carotene) in Gac fruit as affected by ambient storage conditions and stage of maturity. Fruit maturity was the most important factor, with the content of carotenoids highest in the ripe fruits.[3] Ultimately, the factors that affect the concentration of carotenoids in Gac will need to be actively investigated to allow for production of fruits with a consistently high source of carotenoids.

α-Tocopherol (Vitamin E)

Vitamin E or α-tocopherol is an important fat-soluble antioxidative component in foods and the human body and potentially plays a key role in preventing cardiovascular disease,[33,34] preventing coronary heart disease,[35–37] and delaying Alzheimer's disease.[38,39] The concentration of vitamin E in Gac fruit, at 76 μg/ g of fresh weight, is high compared with other fruits.[20] Vitamin E, as a natural antioxidant, helps protect Gac aril oil from oxidation.[40] In foods, vitamin E could potentially preserve valuable phytonutrients rich in Gac fruit from oxidation.

Polyphenolics and flavonoids

Phenolic acids and flavonoids are found in Gac fruit and potentially have beneficial effects on human health.[41–43] These compounds are in all fruit parts at concentrations between 1.5 to 4.3 mg/g of dry weight. The aril contains the highest concentrations of phenolic acids and flavonoids, 4.3 and 2.1 mg/g, respectively.[5]

Fatty Acids

Primarily, the benefit of Gac-derived fatty acids would be in using these as an alternative to saturated fats in the diet. The benefits of essential fatty acids in human health are well

known. The presence of fat in the Gac fruit aril plays an important role in the absorption of carotenes and other fat-soluble nutrients.[27,44] Similarly, several studies also show that fat ingested with carotenoid compounds in plant foods significantly improves their absorption by the body.[45–47]

Gac fruit (aril and seeds) are rich in fatty acids, particularly monounsaturated and polyunsaturated acids. Unlike the aril, the seeds are usually discarded; therefore, utilization of the seeds contributes to preventing waste disposal problems and maximizing available sources.

The Gac aril contains significant amounts of fatty acids, at 102 mg/g of fresh weight (FW).[27] Seventy percent of total fatty acids in the aril are unsaturated, and 50% of these are polyunsaturated.[4] Unusual for fruits, Gac has a high concentration of linoleic acid and omega-3 fatty acids.[7] The fatty acid composition and total oil content of Gac aril are presented in Table 2.

The total fatty acid content in Gac seeds is between 15.7% and 36.6% of the total weight of the seed.[7] The fatty acid composition includes stearic acid (54.5–71.7% by weight), linoleic acid (11.2–25.0%), and α-linolenic acid (0.5–0.6%). Several other types of fatty acids are found in Gac seeds in smaller amounts.[7]

Gac aril oil contains a high concentration of oleic acid, 34 % of total fatty acids (see Table 2); hence, it can be used in addition to other sources such as sunflower, palm, and soya. However, research on the effects of oleic acid in Gac fruit is still needed to confirm its benefits. Gac aril and seeds also contain α-linolenic acid, which is beneficial to human health. For example, α-linolenic acid has been seen in some studies to play important role in reducing the incidence of cardiovascular disease.[48–50]

Other Components

Gac fruit seeds are used in traditional Chinese medicine and they are rich in beneficial chemical compounds such as oleanodic acid, diterpene columbin, chondrillasterol,

Table 2
Fatty acid composition and total oil content of Gac aril[27]

Fatty acids	Abbreviation	Concentration (mg/g, FW)	% of total fatty acids
Myristic	14:0	0.89	0.87
Palmitic	16:0	22.48	22.04
Palmitoleic	$16:1\Delta^{9}$	0.27	0.26
Stearic	18:0	7.20	7.06
Oleic	$18:1\Delta^{9}$	34.76	34.08
cis-Vaccenic	$18:1\Delta^{11}$	1.15	1.13
Linoleic	$18:2\Delta^{9,12}$	32.06	31.43
α-Linolenic	$18:3\Delta^{9,12,15}$	2.18	2.14
Arachidic	20:0	0.40	0.39
Gadoleic	$20:1\Delta^{9}$	0.15	0.15
Arachidonic	20:4	0.10	0.10
Behenic	22:0	0.19	0.19
Lignoceric	24:0	0.14	0.14
Total		101.98	

Momordica saponins momordins, and pentacyclic triterpenoid ester.[10,11] Some evidence supports the beneficial effect of Gac seed components. Ethanol extract from Gac seed was shown to significantly decrease blood glucose levels and increase insulin in diabetic rats. The presence of saponins, flavonoids, and other compounds in seeds may synergistically or independently contribute to this beneficial effect.[51] Other components in seeds, such as multiple trypsin inhibitors,[52] play an important role in the prevention of human cancer.[53,54]

Processing of Gac Fruit

If the fruit was to be used for all the applications indicated above and more, then appropriate processing would be needed. However, little information is available on how the Gac fruit might be processed to make full use of its components and maintain its quality characteristics. It is envisaged that Gac fruit can be processed in several ways (Fig. 4), including drying, extraction of oil, encapsulation, and incorporation into foods.

Drying Methods

Generally, fruit powders are often used in the food industry, as they are convenient to store, handle, and transport. This is particularly important for fruits such as Gac that are only available fresh for a short season. Powders are also favored when used as natural colorants. Gac fruit, available as a powder, will ensure its supply for use as colorings in food products, including juices and dairy products.

Gac aril. Studies show that the choice of pretreatments and drying treatments plays an important role in effectively maintaining the highest content of carotenoids, color, and antioxidant activity.

In comparing different drying methods, it is clear that freeze-drying processes can substantially preserve the nutritional values of samples, in terms of TCC and total antioxidant activity (TAA). This has been confirmed for Gac powder,[19,55] carrot slices,[56] and paprika powder.[57] However, freeze-drying is generally seen as a very expensive preservation method. For example, freeze-drying costs are 4–8 times higher than that of air-drying.[58]

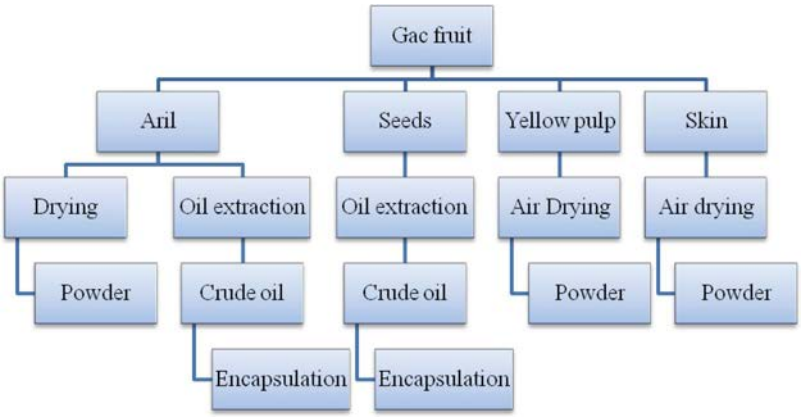

Figure 4. A potential processing scheme of Gac fruit (color figure available online).

Freeze-drying may not always be the superior process, since it did not show any advantage over convective air-drying at below 70 °C in terms of carotenoid retention in carrots. The β-carotene and lycopene contents remained almost constant after the convection air-drying.[56] Similarly, in a comparison of freeze-drying and oven-drying (at 25–75 °C) of tomato pulp solids, the lycopene content was not significantly different.[59] Some research indicates a negative impact of freeze-drying on the content of carotenoids. For example, the amounts of lycopene in two tomato varieties after freeze-drying were reduced to 33–48% of the levels in fresh fruits.[60] In contrast, the lycopene contents after air-drying increased by 152–197% of levels in fresh fruits. In this case, the heating process breaks down the cell walls and the bonding force between lycopene and the tissue matrix. As a result, lycopene is more accessible and increases more *cis*-isomerization.[60]

For Gac, the TCC of samples presoaked in ascorbic solution or bisulfite prior to vacuum-drying at low temperature of 40 °C was highly comparable with the freeze-dried samples.[19] Also, a good-quality Gac powder was obtained, in terms of color, total carotenoids, and antioxidant activity when produced by spray-drying at inlet temperature of 120 °C and adding maltodextrin concentration at 10%.[61] On the basis of these studies, a suitable drying technique has good potential for producing powder from Gac aril.

Gac skin and pulp. Gac skin and pulp may also be suitable for production as powders, since they have a high nutritional value even when dried. For example, air-drying at a temperature of 60 °C was performed to produce powders from Gac skin and pulp.[19] This showed that skin powder is higher in TCC and TAA compared with the pulp powder. Additionally, the TCC of skin and pulp powders is high compared with other fruits and vegetables, including cherry tomatoes, pumpkin, carrot,[62] and several tomato cultivars.[63] This confirms skin and pulp powders as desirable sources of carotenoids and may encourage greater utilization of these by air-drying.

Oil Extraction Methods

Oil rich in essential fatty acids can be extracted from Gac aril and seeds but optimization of Gac oil extraction is needed. Traditional extraction using potentially harmful organic solvents has been abandoned due to health concerns, environmental problems, and quality degradation and it is important to find an alternative extraction method using non-organic solvent or food-grade solvent. Many reports show that plant oil can be extracted by other methods such as supercritical carbon dioxide (SC-CO_2) extraction, aqueous enzymatic extraction, microwave-assisted extraction, and ultrasound-assisted extraction. These methods are environment-friendly and solvent-free. The advantages and drawbacks of ultrasound-assisted pressing extraction[64–66] and microwave-assisted pressing extraction[67–69] in food extraction have been reviewed.

Among the existing methods, SC-CO_2 extraction has been considered as a most promising alternative to traditional solvent extraction and mechanical pressing. It offers a number of advantages, including nonsolvent residues, shorter extraction times, higher extraction yields, and better retention of nutritional and valuable bioactive compounds.[70] In recent years, SC-CO_2 extraction technique has been employed to extract essential oils,[71–73] fatty acids,[74,75] carotenoids,[76–78] and vitamin E[78,79] from fruits and vegetables. However, the SC-CO_2 extraction of fatty acids, carotenoids, and α-tocopherol from Gac aril has not yet been reported.

Encapsulation Process

Encapsulation is the process by which bioactive components (core material) such as food oils are enveloped within a wall. This process is used for protection, stabilization, and slow release of food ingredients. Recently, increased attention has been given to the application of encapsulation of bioactive compounds, particularly unsaturated fatty acids. The degradation of these compounds can be prevented by applying encapsulation techniques. The encapsulation of fatty acids has been successfully reported in numerous studies.[80–82] the process requires agents to protect the oils and emulsifiers to achieve good encapsulation in the spray-drying technique commonly used in the food industry. However, the study of Gac oil encapsulation has not yet been reported.

There are various encapsulating agents (wall materials) effective for encapsulating food oils in providing good protection against heat, light, and oxidation. The agents are classified as carbohydrates, cellulose, gum, lipids, and protein, which are reviewed elsewhere.[83–86] The wall materials have different physical and chemical characteristics, and their properties, including viscosity, solubility, stabilization, reactivity, protective capacity, and cost, have been reviewed by several authors.[84,86] Cyclodextrins are an example of an agent widely used in spray-drying encapsulation of food oil. The monomers of cyclodextrins are connected to each other, giving a ring structure that is relatively rigid and has a hollow cavity with the ability to encapsulate other molecules.[84] Its suitability as an encapsulation agent for Gac fruit is unknown.

The encapsulation process requires an emulsifier, particularly for stabilizing the emulsion used in spray-drying encapsulation. Generally, the choice of emulsifier is determined by its hydrophile-lipophile balance (HLB) value. According to Davis,[87] a high HLB value (8–13), indicates a more hydrophilic surfactant, and is suitable for facilitating oil in water emulsion formation and enhancing its stability. Earlier, Griffin[88] claimed that this range should be about 8–18 for oil-in-water emulsifier. The HLB values of some common emulsifiers can be found elsewhere.[85] Other parameters needing consideration for emulsification include total solids concentration, viscosity, droplet size, and emulsification method.[84]

Among various encapsulation techniques reported,[83,86] spray-drying encapsulation is the most widely used in the food industry.[89,90] This process can potentially offer many benefits such as economics, flexibility, and good quality of encapsulated materials[91] and may be suitable for Gac fruits. However, to achieve good encapsulation efficiency for Gac, the conditions for wall materials, emusifiers, and spray-drying conditions all need optimizing. The key parameters for spray-drying include feed temperature, air inlet and outlet temperatures,[84,92] atomization type and conditions, drying air flow rate and humidity, and powder particle size.[84]

Utilization of Gac Products

Finally, utilization of Gac powder or encapsulated Gac oil can be achieved by incorporating it into foods as a natural colorant and/or nutrient supplements. Natural carotenoid extracts are used as food colorants in many processed products, including oily products (margarines, oils, fats and shortenings), fruit juice, beverages, dry soups, canned soups, dairy products, milk substitutes, coffee whiteners, dessert mixes, preserves, syrups, confectionery, salad dressings, meat products, pasta, egg products, baked goods, and others.[93–95]

Gac aril powders produced by different drying methods such as freeze-drying, vacuum-drying, and spray-drying are easily incorporated into the Vietnamese dish "Xoi Gac,"

pasteurized Gac juice, pasteurized Gac milk beverages, yogurt, fettuccine pasta, and creamy sauce.[19,96] Also, the color, TCC, and TAA of the juice and the milk beverages are maintained after storage for 30 days under refrigeration.[19] Considering these studies and given that Gac aril and Gac oil can be effective natural source of highly bioavailable lycopene and carotenes when cooked,[97] there is great potential to produce high-quality products from processed Gac fruits.

The extraction of natural colorants from Gac would need to follow approved methods, such as those used for extracting lycopene from tomatoes.[98] Unfortunately, gaining approval to use natural colorants as food additives is a complicated task, because it takes time to meet the requirements of governments and organizations.[93] Only 13 natural colorants are approved in the European Union (EU) and 26 natural colorants certificated in the United States.[99] However, in the EU, the "*Southampton Six*" colors, Alurra Red (also called Red 40), Ponceau 4R (E124), Tartrazine (Yellow 5) (E102), Sunset Yellow FCF/Orange Yellow S (Yellow 6) (E110), Quinoline Yellow (E104), and Carmoisine (E102), now must have a specific warning label on food packaging. This increases the demand for natural colorants such as those from Gac fruit.

Drawbacks of developing new colorants are the high costs for manufacturers.[95] Development of Gac products as a natural food colorant needs to consider the many factors affecting its application in a particular food product. These factors include, for example, its solubility and stability in processing, packaging, and storage. It is very important to optimize the factors allowing the stability of natural carotenoids in the final product. For example, the hue of carotenoids is affected by pH.[100]

Conclusions

Gac fruit contains extraordinarily high levels of carotenoids (particularly lycopene and β-carotene), α-tocopherol, and fatty acids in its parts (aril, seeds, yellow pulp, and skin). Other bioactive compounds such as polyphenol compounds and flavonoids are also found in Gac fruit. The seeds are high in fatty acids and are also used as traditional Chinese medicines. Many studies confirm that the valuable compounds in Gac fruit play a crucial role in human health. The proposed processing scheme of all the parts of Gac fruit, including drying, oil extraction, and oil encapsulation, highlights how the utilization of air-dried powder from the pulp and skins prevents environmental pollution from waste disposal problem and enhances the overall value of Gac fruit.

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1.4 Experimental Rationale

As presented in the background section 1.1, Gac arils contain high concentrations of oil and carotenoids. These compounds have been proven to be beneficial to human health and are linked with a reduced risk of cardiovascular disease and cancers. Importantly, it has been found that a significant improvement in the absorption of the carotenoids into the human body occurs when they are digested with fat. Moreover, carotenoids (β-carotene and lycopene) in natural plants can occur in several forms, such as crystalline form inside chromoplasts or carotenoids-protein complexes in chloroplasts (Parada & Aguilera, 2007; Shi & Le Maguer, 2000). This results in a decrease in their relative bioavailability when the fresh fruits and vegetables are consumed. Therefore, it was of interest to effectively extract the Gac oil along with the hydrophobic β-carotene and lycopene.

Since the industrial solvents traditionally used for plant oil extraction, for which extraction efficiency is often high, are to be avoided, the use of novel extraction techniques is strongly recommended. To achieve extraction efficiencies for Gac oil as high as those achieved with the traditional methods, a combination of different novel extraction methods for extracting the oil from Gac arils, such as microwave-drying and pressing, air-drying before SC-CO_2 extraction and microwave-drying followed by aqueous ultrasound-assisted extraction, have been chosen to be studied.

The resultant Gac oil containing unsaturated fatty acids and carotenoids is easily degraded due to isomerisation and oxidation. It is also important to effectively preserve the bioactive compounds in the oil extracts in a convenient oil powder form as nutrient supplements and natural food colourants for ease of consumption, storage and transportation. Microencapsulation by spray drying appeared to be an effective method for preparing these compounds in powder form owing to its advantages. To obtain a successful encapsulation process, oil in water emulsion preparations (wall material concentrations and ratio of oil to wall material) and spray drying conditions (air inlet and outlet temperatures) needed to be optimised.

In the food industry, it is important to know the stability of products during storage. The quality of food products changes over time as a result of specific storage conditions.

Many environmental conditions including light, air and temperature are considered to affect the quality of the final product, particularly the encapsulated Gac oil powder product. Furthermore, it is also important to construct moisture sorption isotherms for calculating the moisture changes and predict the stability of product during storage. Therefore, the need for a storage study of the encapsulated Gac oil powders was desirable in establishing the effects of storage conditions on the Gac oil powders.

Finally, the use of the encapsulated Gac oil-rich in carotenoids powders as nutrient supplements and natural colourants is currently receiving considerable attention from food manufacturers and consumers. Hence, investigation on the stability of the encapsulated oil powders in foods also needed to be carried out in this thesis.

1.5 Hypothesis, Aims and Objectives

The hypothesis was that the characteristics of Gac oil extracts and encapsulated Gac oil powder products, particularly oil yield, fatty acid composition, β-carotene and lycopene, were affected by conditions applied during the extraction, the encapsulation process, the storage conditions and the applications of the encapsulated powder as a nutrient supplement and a natural food colourant. Optimisation of conditions for several processing steps, including oil extraction, emulsion preparation conditions and the spray drying process using response surface methodology, was expected to maximise the oil yield and increase the retention of bioactive compounds.

Overall, the experiments designed for this study aimed (1) to improve extraction efficiencies in terms of Gac oil, β-carotene and lycopene using different extraction methods; (2) to enhance encapsulation efficiencies in terms of retention of Gac oil, β-carotene and lycopene using encapsulation by spray drying; (3) to establish the most suitable storage conditions for the encapsulated Gac oil powder; (4) to test the stability of the encapsulated oil powder in a range of foods.

Importantly, no industrial chemical solvents were used in all the designed experiments. Only water, which is considered a safer, more environmentally friendly and cheaper, was used as extracting solvent for the extraction of oil and carotenoids.

The specific objectives of this study were:

1. To comprehensively investigate the effect of pre-treatment conditions (air-drying temperature, particle size, enzyme concentration, and extraction time) prior to SC-CO_2 extraction on the Gac oil yield, β-carotene and lycopene (Paper II).
2. To comprehensively investigate the effect of ultrasound-assisted aqueous extraction conditions (ultrasound power, extraction time, powder particle size and the ratio of water to powder and the centrifugal force) from microwave-dried Gac aril powder on the extraction efficiency for Gac oil, β-carotene and lycopene (Paper III).
3. To comprehensively investigate the effect of extraction conditions (microwave-drying power, microwave-drying time, steaming time and hydraulic pressure) on the extraction efficiency for Gac oil, β-carotene and lycopene (Paper IV). The most suitable range of the extraction conditions was then used for optimisation process (see paper V).
4. To optimise the Gac oil extraction conditions (microwave-drying time, steaming time and hydraulic pressure) to achieve the maximum Gac oil extraction efficiency and the highest content of β-carotene and lycopene in the Gac oil (Paper V).
5. To compare the extraction efficiencies in terms of the Gac oil, β-carotene and lycopene, and peroxide value of Gac oil extracted by different extraction methods (Paper VI).
6. To optimise the wall material concentration and the ratio of Gac oil to wall material to obtain high encapsulation efficiencies (retentions of Gac oil, β-carotene and lycopene) and minimise peroxide value, moisture content and total colour difference using response surface methodology. The encapsulation yield, colour characteristics, physicochemical properties, and morphology of the encapsulated oil powder were also evaluated (Paper VII).
7. To optimise the air inlet and outlet temperatures to obtain high encapsulation efficiencies (retentions of Gac oil, β-carotene and lycopene), encapsulation yield, moisture content, water solubility index and peroxide value. The physicochemical, reconstitution and colour characteristics of the encapsulated Gac oil powder were also determined (Paper VIII).
8. To examine the shelf life of the encapsulated Gac oil powder, in terms of oil surface, β-carotene and lycopene, peroxide value and colour characteristics,

monitored under a variety of storage conditions (including exposure to light, air and temperature). Kinetic parameters and moisture sorption isotherms were also examined for predicting the shelf life of the powder product. Furthermore, stability of the encapsulated Gac oil powder in a range of foods such as yoghurt, pasteurised milk and cake mix in relation to the colour, β-carotene, lycopene and peroxide value were studied. A storage study of the fortified Gac oil products was also evaluated (Paper IX).

1.6 Format of the thesis

The thesis is arranged in the following way. After this part of overview, **Part 2: Results** contains a synopsis of research result papers and the papers published in the Journals. The **general discussion and conclusions** will be given in the Part 3. The **Bibliography** shows all the references cited in this thesis, including those in all published papers. Finally, some additional information will be given in the **Appendices**.

The first two research papers (II - III) report on the extraction of Gac oil-rich in carotenoids under laboratory conditions.

The next two research papers (IV - V) report on the factorial experiments designed for investigating the effect of the single factor on the extraction of the Gac oil. The optimisation process of the extraction of Gac oil-rich in carotenoids was then carried out using response surface methodology based on the results of the preliminary experiments. The resultant Gac oils were then used for the encapsulation experiments

The next research paper (Paper VI) reports on the comparison of different extraction methods on the extraction efficiencies (oil, β-carotene and lycopene) and other physicochemical properties.

The next two research papers (VII - VIII) report on the optimisation of microencapsulation by spray drying. The optimised encapsulated Gac oil powders were then used for storage study and utilisation in various foods.

The final paper (IX) reports on the storage study and stability of the encapsulated Gac oil powder in foods.

PART 2: RESULTS

2.1 Synopsis of research result papers

In this thesis, the results are presented in a series of eight research papers, including six which are already published, one which is currently under review, one which has been prepared for publication.

The first research paper (**Paper II**) entitled "Effects of pre-treatments on the yield and carotenoid content of Gac oil using supercritical carbon dioxide extraction" investigated the influence of four different pre-treatment factors (air-drying temperature, particle size, enzyme concentration, and extraction time) prior to SC-CO_2 extraction at the pressure of 200 bar and extraction temperature of 50^{o}C on the Gac oil yield, β-carotene and lycopene. Soxhlet extraction was also used for the purpose of comparisons.

The results indicated that the four pre-treatments significantly affected the oil yield and the content of β-carotene and lycopene in the Gac oil extracts. The highest oil yield was obtained when the enzyme concentration of 0.1% (w/w) for pre-treatment before air-drying was used. The air-drying temperature of 50^{o}C and the mean particle size of 0.45 mm were the best pre-treatments prior to SC-CO_2 extraction. Using these conditions, the high Gac oil yield (34%), and the high content of β-carotene (83 mg/100 mL oil) and lycopene 508 mg/ 100 mL oil) after the extraction time of 120 min were achieved. In comparison with Soxhlet extraction, the Gac oil extraction efficiency (95%, w/w) using SC-CO_2 extraction was obtained.

The second research paper (**Paper III**) entitled "Ultrasound-assisted aqueous extraction of oil and carotenoids from microwave-dried Gac (*Momordica cochinchinensis* Spreng) aril" examined the ultrasound-assisted aqueous extraction conditions (ultrasound power, extraction time, powder particle size and the ratio of water to Gac powder) and the centrifugal force on the extraction efficiencies (oil, β-carotene and lycopene) and the peroxide value (PV). Microwave-drying followed by aqueous extraction without ultrasound-assistance and air-drying followed by aqueous extraction with or without ultrasound-assistance were also carried out for comparisons, including observing the

Gac material left behind after the extractions using scanning electron microscopy (SEM).

The results indicated that Gac oil containing high contents of β-carotene and lycopene and having a low peroxide value could be extracted using microwave-drying followed by ultrasound-assisted aqueous extraction. It was found that an ultrasound power of 320 W, an extraction time of 20 min, powder particle sizes of 0.3 - 0.5 mm, a ratio of water to powder of 9 g/g and a centrifugal force of 6750 × *g* gave optimal extraction efficiencies for oil (90%), β-carotene (84%) and lycopene (83%), and the oil had a low PV (2.2 meq/kg). The SEM analysis confirmed that the combination of microwave-drying followed by ultrasound-assisted aqueous extraction caused strong disruption of the Gac aril cellular structures, which was consistent with the high extraction of oil, β-carotene and lycopene achieved with the combination.

The third research paper (**Paper IV**) entitled "Effects of Gac aril microwave processing conditions on oil extraction efficiency, and β-carotene and lycopene contents" investigated the four different extraction conditions including the microwave power, the microwave-drying time, the steaming time and the hydraulic pressure on the oil extraction, efficiency, and the content of β-carotene and lycopene.

The results showed that the microwave power, microwave-drying time, the steaming time and the hydraulic pressure had significantly an impact on the Gac oil extraction efficiency, and β-carotene and lycopene Contents of the oil extracts. The most suitable processing conditions for Gac oil extraction were the microwave power of 630 W, the microwave time of 65 min, the steaming time of 20 min, and the hydraulic pressure of 170 kg/cm^2. Using these conditions, the highest oil extraction efficiency of 93%, and the high content of β-carotene (140 mg/100 mL) and lycopene (414 mg/100 mL) were obtained. The results also indicated that microwave-drying was found to be better than air-drying for pre-treatment prior to pressing. Furthermore, moisture content after microwave-drying and the steaming of 8% and 11% (w/w), respectively was best for pressing.

The fourth research paper (**Paper V**) entitled "Optimisation of microwave-assisted extraction of Gac oil at different hydraulic pressure, microwave and steaming

conditions" was to optimise Gac oil extraction conditions including microwave time, steaming time and hydraulic pressure for maximising oil extraction efficiency and content of β-carotene and lycopene in the oil extract. The levels of the independent variables were based on the results of paper IV, which the most suitable range of the variables was microwave-drying time of 60 - 65 min, steaming time of 15 - 30 min and hydraulic pressure of 160 - 180 kg/cm^2.

The results showed that the quadratic polynomial models were sufficient to describe and predict the response variables of the oil extraction efficiency, and β-carotene and lycopene contents in the oil extract, with high R^2 values of 0.93, 0.85 and 0.86, respectively. It was predicted that the optimal extraction conditions would be microwave-drying time of 62 min, steaming time of 22 min and hydraulic pressure of 175 kg/cm^2. Using those optimal conditions, the maximum oil extraction efficiency (86%), and the highest content of β-carotene (186 mg/100 mL) and lycopene (518 mg/100 mL) were obtained as predicted.

The fifth research paper (**Paper VI**) entitled "Effect of drying pre-treatments on the yield and bioactive content of oil extracted from Gac aril" examined a comparison of different extraction methods of Gac oil in terms of the oil yield, the content of β-carotene and lycopene, and chemical properties.

The results indicated that high oil yields could be obtained when the arils was microwave-dried prior to either Soxhlet extraction (31%) or pressing (27%). This finding was explained by the observation of microstructural changes in the cell walls of the microwave-dried Gac arils using light microscope and SEM techniques. The oil yield obtained from Soxhlet extraction was higher than that of pressing for the air-dried Gac arils. However, the highest quality of the oil extract was achieved by microwave-drying and pressing, in terms of the highest retention of β-carotene (174 mg/100 mL) and lycopene (511 mg/100 mL), and the lowest values for acidity (0.69 mg KOH/g), and peroxides (1.8 meq/kg). Moreover, the desirable oleic acid (48%) and linoleic acid (18%) were found to be the dominant fatty acids in all Gac oil extracts.

The sixth research paper (**Paper VII**) entitled "Microencapsulation of Gac oil by spray drying: optimisation of wall material concentration and oil load using response surface

methodology" was to optimise the two independent variables including wall material concentration and ratio of oil to wall material for microencapsulation of Gac oil using RSM. The response variables included encapsulation efficiencies (Gac oil, β-carotene and lycopene), peroxide value (PV), moisture content (MC), and total colour difference (ΔE). The encapsulation yield (EY), the colour characteristics, physical properties (Aw, pH, bulk density, and water solubility index, WSI) of the optimal encapsulated Gac oil powder were also evaluated. Morphology (inner and outer) of the optimally encapsulated Gac oil powder was also examined using SEM.

The results showed that the data could adequately fit the six quadratic polynomial models for the EEs in terms of Gac oil, β-carotene and lycopene, PV, MC and ΔE with R^2 values of 0.96, 0.95, 0.86, 0.89, 0.88, 0.87, respectively. The optimal wall material concentration (including whey protein concentrate and gum Arabic of 7/3, w/w) and the ratio of Gac oil to the wall material load were predicted as 29.5% and 0.2 (g/g), respectively. Using these optimal conditions, the EEs in terms of the oil, β-carotene and lycopene, PV, MC, ΔE achieved were predicted and confirmed as 92%, 80%, 74%, 3.91 meq/kg, 4.14% and 12.38, respectively.

The resulted also indicated that the EY was about 47%. The physical characteristics of the optimally encapsulated Gac oil powders including Aw, pH, bulk density, and WSI were 0.32, 5.72, 0.36, and 92,6%, respectively. The colour characteristics (lightness, L of 81.3, chroma, C of 48.4, and hue angle, H^o of 71.6) were also determined. The L, C and H^o of the reconstituted Gac oil powder were 61.1, 48.3 and 56.9, respectively.

Furthermore, SEM micrographs confirmed that the outer structure of the encapsulated Gac oil powder particles had rough and smooth surfaces and also had spherical shapes and were free of cracks and pores. It was important characteristics for the encapsulated powders to effectively prevent the oil and carotenoids from oxidation and the undesired release of the oil droplet to the particle surfaces. The inner porous structure had small spherical round, indicating the oil homogeneously distributed in the wall material matrix. Therefore, it was concluded that Gac oil containing β-carotene and lycopene was successfully encapsulated in the protein-polysaccharide matrix. The Gac

oil powder could be then easily incorporated into a range of foods as natural colourants, food supplements or as additives for functional foods.

The seventh research paper (**Paper VIII**) entitled "Microencapsulation of Gac oil: optimisation of spray drying conditions using response surface methodology" was to optimise the spray drying conditions including air inlet and outlet temperatures. The response variables used for optimisation process were encapsulation efficiencies in terms of Gac oil, β-carotene and lycopene, encapsulation yield (EY), moisture content (MC), water solubility index (WSI), and peroxide value (PV). Physicochemical, colour properties, reconstitution, and morphology of the optimally encapsulated Gac oil powder were also evaluated.

The results showed that the data could adequately fit the seven quadratic polynomial models for the EEs in terms of Gac oil, β-carotene and lycopene, EY, MC, WSI and PV with R^2 values of 0.92, 0.91, 0.89, 0.85, 0.89, 0.98 and 0.97, respectively. Using the optimal conditions (inlet and outlet temperatures of 154 and 80 ^oC, respectively), the response variables including the EEs in terms of Gac oil, β-carotene and lycopene, EY, MC, WSI and PV were predicted and validated as 87.2%, 82.8%, 84.3%, 52.8%, 4.9%, 90.3%, and 4.06 meq/kg, respectively.

Compared with the pressed Gac oil before encapsulation, the fatty acid composition in the optimally encapsulated Gac oil powdered was effectively preserved. The physical properties of the optimally encapsulated Gac oil powders including Aw, pH, and bulk density were 0.32, 5.75, and 0.33 g/mL, respectively. The colour characteristics of the optimally encapsulated Gac oil powder determined were L of 74.8, C of 49.3, and H^o of 67.1. The colour characteristics of the reconstituted emulsion of the optimal powder determined were L of 50.8, C of 28.3, and H^o of 46.1, respectively. Compared to the colour characteristics of the infeed emulsion, the total colour difference between the infeed emulsion and the reconstituted was less than 10, indicating the colour of the reconstituted emulsion was just slightly changed.

In addition, the optimal powder particles were micro-sized (< 20 μm), had spherical shape and concave surface, and showed no apparent cracks or fissures, indicating better protection and core retention. The inner structure of the powder

particles had well distributed small holes, indicating the oil droplet containing bioactive compounds were homogeneously distributed in the wall matrix.

Therefore, it was concluded that the encapsulated Gac oil powder containing high contents of β-carotene, lycopene and unsaturated fatty acids could be used as natural food colourants, nutrient supplements or as additives for functional foods.

The eighth research paper (**Paper IX**) entitled "A storage study of encapsulated Gac oil powder and fortification into foods" examined the shelf life of the encapsulated Gac oil powder, in terms of oil surface, β-carotene and lycopene, peroxide value and colour characteristics under various storage conditions. The conditions were the package with laminated and non-laminated, package with and without vacuum sealing, and storage at different temperatures of -20 (for 12 months), 10 (for 12 months), room temperature (12 months), 40 (for 4 months) and 63 ^{o}C (for 1 week). Moisture sorption isotherms and microstructural changes of the Gac oil powders during storage were also determined. Furthermore, the encapsulated Gac oil powders were incorporated into cake mix, yoghurt and pasteurised milk. The Gac oil powder incorporated cake mix product was stored at room temperature for 4 months. The Gac oil powder incorporated yoghurt and pasteurised milk products were stored at 4^{o}C for 4 weeks. Finally, kinetic parameters were also examined for predicting the shelf life of the Gac oil powder and incorporated Gac oil powder products.

The results showed that a significant progressive degradation of colour, β-carotene and lycopene, and a significant increase in oil surface content and PV were found as a result of increasing storage temperature from -20 to 63^{o}C, and with a longer storage period. Significant improvement in all the investigated parameters was achieved when the powders were stored at the low temperature of -20^{o}C, 10^{o}C and room temperature in absence of air and light. Furthermore, the isotherm curves of the powders studied had sigmoid shapes and similar patterns to those which are usually observed in dried food products. Equilibrium moisture content values at a constant temperature increased with increase in equilibrium relative humidity.

For incorporation of the Gac oil powders into various foods, it was possible to incorporate the Gac oil powder into the cake mix. Slight loss of colour, β-carotene and

lycopene and slight increase in PV of the cake mix product were determined when increasing the storage time up to 4 months. It was also possible to incorporate the Gac oil powder to yoghurt and pasteurised milk products. Results showed that no significant difference in the investigated parameters tested was found when the fortified yoghurt and pasteurised milk products were stored at 4 ^{o}C for 1 month.

2.2 Research result papers

The results for this thesis are based on the following eight research result papers, which are referred to in the text by their Roman numerals as follows:

2.1.1 Paper II: Tuyen C. Kha, Huan Phan-Tai, Minh H. Nguyen (2014). Effects of Pre-Treatments on the Yield and Carotenoid Content of Gac Oil Using Supercritical Carbon Dioxide Extraction. *Journal of Food Engineering,* 120(1), 44-49. (C1).

2.1.2 Paper III: Tuyen C. Kha, Minh H. Nguyen, Paul D. Roach & Costas Stathopoulos (2014). Ultrasound-assisted Aqueous Extraction of Oil and Carotenoids from Microwave-dried Gac (*Momordica cochinchinensis* Spreng) Aril. *International Journal of Food Engineering*. Under review. (C1).

2.1.3 Paper IV: Tuyen C. Kha, Minh H. Nguyen, Paul D. Roach & Costas Stathopoulos (2013). Effects of Gac Aril Microwave Processing Conditions on Oil Extraction Efficiency, and β-carotene and Lycopene Contents. *Journal of Food Engineering*, 117(4), 486-491. Special Issue on Extraction and Encapsulation. (C1).

2.1.4 Paper V: Tuyen C. Kha, Minh H. Nguyen, Paul D. Roach & Costas Stathopoulos (2013). Optimisation of Microwave-assisted Extraction of Gac Oil at Different Hydraulic Pressure, Microwave and Steaming Conditions. *International Journal of Food Science and Technology,* 48(7), 1436-1444. (C1).

2.1.5 Paper VI: Tuyen C. Kha, Minh H. Nguyen, Paul D. Roach & Costas Stathopoulos (2014). Effect of Drying Pre-Treatments on the Yield and Bioactive Content of Oil Extracted from Gac Aril. *International Journal of Food Engineering*, 10(1), 103-112. (C1).

2.1.6 Paper VII: Tuyen C. Kha, Minh H. Nguyen, Paul D. Roach & Costas Stathopoulos (2014). Microencapsulation of Gac Oil by Spray Drying: Optimisation of

Wall Material Concentration and Oil Load Using Response Surface Methodology. *Drying Technology: An International Journal*, 32(4), 385-397. (C1).

2.1.7 Paper VIII: Tuyen C. Kha, Minh H. Nguyen, Paul D. Roach & Costas Stathopoulos (2014). Microencapsulation of Gac oil: Optimisation of Spray Drying Conditions Using Response Surface Methodology. *Journal of Powder Technology*, 264, 298-309. (C1).

2.1.8 Paper IX: Tuyen C. Kha, Minh H. Nguyen, Paul D. Roach & Costas Stathopoulos (2014). A Storage Study of Encapsulated Gac (*Momordica cochinchinensis*) Oil Powder and its Fortification into Foods. Under preparation for publication. (C1).

Paper II

2.1.1 Effects of Pre-treatments on the Yield and Carotenoid Content of Gac Oil Using Supercritical Carbon Dioxide Extraction

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Effects of pre-treatments on the yield and carotenoid content of Gac oil using supercritical carbon dioxide extraction

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ABSTRACT

The effects of air-drying temperature, particle size and enzymatic pre-treatment on the oil yield and content of carotenoids in the resultant Gac oil using supercritical CO_2 extraction method were investigated. It was found that the highest oil yield was obtained when using the enzyme concentration at 0.1% (w/w) for pretreatment before air-drying. Furthermore, the content of carotenoids was also enhanced by suitable drying temperature and the particle size. High oil yield (34% g/g) and high content of β-carotene (83 mg/100 mL oil) and lycopene (508 mg/100 mL oil) were obtained using the drying temperature of 50 °C and particle size of 0.45 mm as pre-treatments prior to SC-CO_2 extraction at pressure of 200 bar and extraction temperature of 50 °C. Results also indicated that the most suitable extraction time was 120 min. It was concluded that Gac oil containing high amount of carotenoids could be extracted using the chosen air-drying temperature, particle size, enzymatic pre-treatment and extraction time.

1. Introduction

Several studies showed that high levels of carotenoids and essential fatty acids (Kha et al., 2013b; Vuong, 2000; Vuong et al., 2006) were found in Gac fruit (*Momordica cochinchinensis* Spreng) aril. These bioactive compounds, that play crucial roles in human health, were discussed in a recent review on Gac fruit by Kha et al. (2013a). Moreover, the presence of fat in the Gac aril has an important role in the absorption of carotenes and other fat-soluble nutrients (Kuhnlein, 2004). For example, fat ingested with carotenoid compounds in plant foods significantly improved the absorption in human body (Brown et al., 2004; Unlu et al., 2005; Venket Rao, 2004). Since there are many applications where Gac oil is needed, it is important to preserve these valuable nutrients in the oil.

Supercritical carbon dioxide extraction (SC-CO_2) has been applied for extracting oils and other materials as an alternative to traditional mechanical pressing and extraction with hazardous solvent. It offers a number of advantages including non-solvent residues, shorter extraction times, higher extraction yields and better retention of nutritional and valuable bioactive compounds (Herrero et al., 2006). In recent years, SC-CO_2 extraction technique has been employed to extract fatty acids (Garlapati and Madras, 2010; Toribio et al., 2011) and carotenoids (Mattea et al., 2009; Nobre et al., 2009; Pereira and Meireles, 2010) from plant materials. In these reports, it is generally agreed that pressure, temperature, CO_2 flow rate and extraction time are the major independent parameters that affect extraction efficiency.

In addition to the extraction conditions, the SC-CO_2 extraction of oil-containing plant materials also strongly depends on pre-treatments (Del Valle and Uquiche, 2002). Prior to extraction, the material cells are usually broken by different methods such as drying and grinding. Several studies have shown that drying method as a pre-treatment has significant effects on the oil content of plant materials due to structural changes (Sefidkon et al., 2006; Gutiérrez et al., 2008). Recently, Kha et al. (2011) reported that carotenoid content of Gac powder could be preserved by soaking pre-treatment with ascorbic acid before air-drying at low temperature. Moreover, particle size of oil containing materials may affect the extraction efficiency. There is no diffusion through the unbroken cell walls or larger particles, therefore, oil yield and carotenoid content may be higher when extracting smaller compared to larger particle sizes (Del Valle and Uquiche, 2002).

Generally, enzymatically assisted extraction for edible oil is considered to be an environmentally clean technology. The main advantages of this extraction are high oil yield and improvement of resulting oil quality due to mild operation conditions (Mariano

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et al., 2009). The main principle of the enzymatic process is that the structural polysaccharides, which form the cell and lipid body membrane, are hydrolyzed, thus facilitating oil release from the materials (Domínguez et al., 1994; Rosenthal et al., 1996). For example, the higher oil yield from several materials such as rapeseed, soybean, coconut, avocado and sunflower could be obtained by using enzymatic pre-treatment (Rosenthal et al., 1996). Therefore, investigation of enzymatic pre-treatment prior to SC-CO_2 extraction for Gac oil is highly desirable.

There is a lack of information on pre-treatments, such as air-drying temperature, particle size, enzyme concentration and extraction time, prior to SC-CO_2 extraction in the published literature. Therefore, the objective of this work was to investigate effects of those pre-treatments on the Gac oil yield and content of β-carotene and lycopene in the extracts. Soxhlet extraction was also carried out for comparison purpose.

2. Materials and methods

2.1. Chemicals

All solvents (HPLC grade) used in this research, *n*-hexane, acetone, chloroform, acetonitrile, dichloromethane and methanol, were purchased from Merck Pty. Ltd., Kilsyth, Vic, Australia. Carotenoid standards (β-carotene, C4582, type II synthetic, ⩾95%, HPLC grade and lycopene L9879, ⩾90%, from tomato) and Pectinex® Ultra SPL (P2611), a multi component enzyme with high pectolytic activity made from *Aspergillus aculeatus*, were obtained from Sigma–Aldrich Pty., Ltd., Castle Hill, NSW, Australia. CO_2 (99.99% purity) was supplied by Van Tan Phat Co., Ltd., Viet Nam.

2.2. Materials

Fresh Gac fruits were purchased from a local market in Ho Chi Minh City, Viet Nam. The fruits were put inside an isolated hard plastic container to avoid light and temperature exposure during transport. They were stored under refrigeration until use within 1 day. Different batches of fruit were used for non-consecutive replications of the experiments.

2.3. Treatments

Fresh Gac arils (about 1 kg) were scooped out and soaked in 0.1% ascorbic acid solution (w/w) for 1 h (Kha et al., 2011). The arils including the seeds were then air-dried at different temperatures of 40, 50 and 60 °C to reach a final moisture content of approximately 7% with cross flow air velocity of 1.1 ± 0.1 m/s. The loading of material for air-drying process was 5 kg/m^2. The seeds were removed from the arils and the dried arils were then powdered using an IKA M20 grinding mill (IKA® company, Germany) and the particle size was measured using USA standard test sieve. The mean particle sizes (D50) of 0.49 ± 0.02, 0.45 ± 0.02 and 0.37 ± 0.02 mm, calculated by using the Sautier formula (volume-surface mean diameter) (Reverchon et al., 2000), were used for the SC-CO_2 extraction.

For enzymatic pre-treatment, fresh Gac arils were thoroughly mixed with pectinase at different concentrations of 0.05, 0.1 and 0.15% (w/w). pH of the mixtures was adjusted to the optimum level of 4.5 using HCl 0.1 N and then incubated at temperature of 50 °C for 1 h. The mixture was then air-dried at temperature of 50 °C and powdered using the mill. The mean particle size of 0.45 mm was used for SC-CO_2 extraction.

2.4. Supercritical CO_2 extraction

The supercritical fluid (SCF) system (Spe-ed SFE-4, Applied Separations, Allentown, United States) was employed for Gac oil extraction. The dried powders (about 2.6 g) were placed into a stainless steel column (length 130.75 mm, 14.22 i.d. and 56.90 i.l) used as the actual extraction vessel. Liquefied CO_2 was pumped into the vessel by a high-pressure pump to reach a fixed pressure of 200 bar, the extraction temperature inside the column was gradually increased and maintained at 50 °C by a heating jacket encasing the vessel. The flow rate of CO_2 was fixed at 2 L/min. At the end of the extraction process, the valve was closed and the final part of the lines was depressurized. The oil samples were weighted at different extraction time from 2 to 180 min to determine the oil yield (gravimetrically as g oil/g dried aril) and analyzed for content of β-carotene and lycopene.

2.5. Analytical methods

2.5.1. Determination of β-carotene and lycopene contents

A method of Englberger et al. (2006) was employed for analyzing β-carotene and lycopene in Gac oil samples. HPLC analysis was performed with an Agilent 1200 HPLC equipped with diode array detector system consisting of a Luna C18 (100 × 4.6 mm i.d.: 5 μm) direct-connect guard column coupled to a Jupiter C18 (250 × 4.6 mm i.d.: 5 μm) reversed phase column (Phenomenex). The mobile phase consisted of acetonitrile (ACN), dichloromethane (DCM) and methanol (MeOH) 5: 4: 1 v/v/v, containing 0.1% BHT. The flow rate was 1.0 mL/min, detection was at 450 nm, and the injection volume was 20 μL. The resultant Gac oil was diluted at a ratio of 1:200 with the HPLC solvent. All operations were performed under subdued light to minimize oxidation of the carotenoids. The identification of β-carotene and lycopene were based solely on the retention time of a peak compared with the authentication standards. The amounts of β-carotene and lycopene in Gac oils were expressed as mg/100 mL of crude oil.

2.5.2. Soxhlet extraction

The total oil content of fresh Gac fruit aril was determined using the FOSS Soxtec™ system 2045 extraction unit, Denmark. A procedure of oil extraction was carried out according to the method described by Brkić et al. (2006) with slight modification. Briefly, approximately 3 g of the air-dried sample was weighed in cellulose thimbles and inserted into the extraction system. Then 50 mL petroleum ether (boiling point of 60–90 °C) were filled into weighed aluminum vessels and inserted into the extraction unit as well. The temperature was set at 145 °C and the program of the oil extraction process was: 30 min of boiling, 60 min of rinsing, 15 min of evaporation and finally 20 min of drying the aluminum vessels containing the extracted oil. The sample was then dried in an oven at temperature of 80 °C until constant weight was obtained. The oil content was gravimetrically calculated. The oil content of Gac aril was about 37–42% (g/g of dry weight).

2.6. Statistical analysis

The independent experiments and subsequent measurements were done in duplicate and in triplicate, respectively. A two-way analysis of variance (including extraction time and particle size, extraction time and air-drying temperature, and extraction time and enzyme concentration) and LSD (least significant difference) was used to analyze the data using the SPSS-PASW GradPack 20.0 for Mac (IBM Corp., United States).

3. Results and discussion

3.1. Effects of air-drying temperature

Statistical results indicated that the investigated air-drying temperature significantly affected the oil yield ($P < 0.001$), and content of β-carotene ($P < 0.001$) and lycopene ($P < 0.001$) in the extracts. There was insignificant interaction between air-drying temperature and extraction time on β-carotene and lycopene contents ($P > 0.05$).

Fig. 1 and Table 1 show that higher oil yield, and β-carotene and lycopene contents of the oil extracts were obtained at the drying temperature of 50 °C for 18 h as compared with the lower temperature of 40 °C for 20 h or the higher temperature of 60 °C for 12 h, all at particle size of 0.45 mm. This is due to the fact that air-drying process at the temperature of 50 °C had effected on the microstructure changes of the dried arils by water removal (Gutiérrez et al., 2008), allowing an adequate diffusion of SC-CO_2 through the cell membrane, therefore, the oil and carotenoids were easily extracted. Moreover, the cell walls and the bonding forces between carotenoids and the tissue matrix were broken due to the heating process (Chang et al., 2006). Hence, carotenoids including β-carotene and lycopene were also easily released during the SC-CO_2 extraction. At 60 °C air drying, it is likely that the heating effect was excessive on the cell walls and aril microstructure that reduced the diffusion of SC-CO_2, resulting in lower oil yield.

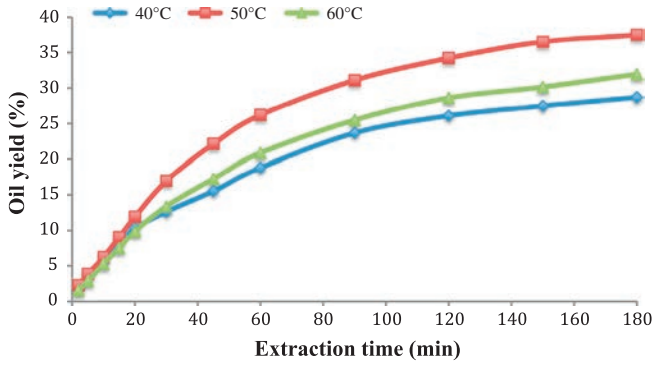

Fig. 1. Effect of air-drying temperature and extraction time on the oil yield obtained by SC-CO_2 extraction.

Table 1
Effects of air-drying temperature and SC-CO_2 extraction time on the content of β-carotene and lycopene (mg/100 mL) of Gac oil extracts.

Parameters		β-Carotene content	Lycopene content
Drying temperature (DT)	40 °C	36.9 ± 21.5[a]	254.9 ± 75.5[a]
	50 °C	51.3 ± 10.2[b]	418.6 ± 92.1[b]
	60 °C	23.4 ± 10.6[c]	182.6 ± 47.1[c]
Extraction time (ET)	60 min	21.4 ± 16.1[a]	205.8 ± 96.1[a]
	120 min	41.8 ± 14.4[b]	306.7 ± 133.3[b]
	180 min	48.4 ± 14.2[b]	343.5 ± 134.2[b]
Significant interaction		Significance	
DT		***	***
ET		***	**
DT × ET		NS	NS

Values are mean ± SD after statistical analyses.
NS, ** and *** indicate *not significant* and *significant* at $P = 0.01$ and 0.001, respectively.
The values in the same column followed by different superscripts (a–c) were significantly different ($P < 0.05$).

As can be seen in Table 1, the contents of β-carotene and lycopene of the oil extracts, when the arils were air-dried at 50 °C, are much higher than those at either lower temperature of 40 °C or higher temperature of 60 °C. The carotenoid content of the dried samples could be degraded at increasing drying temperature due to heat treatment (Kha et al., 2011; Shi et al., 1999). However, since it took a longer time to dry the arils at the lower temperature of 40 °C, the broken tissues were easily exposed to oxygen, resulting in more oxidation, which also caused a substantial loss of carotenoids (Shi et al., 1999). It can be concluded that drying temperature and the length of drying are two major parameters affecting the reduction of carotenoid. Therefore, the air-drying temperature of 50 °C is the most suitable pre-treatment for Gac aril prior to SC-CO_2 extraction.

3.2. Effect of particle size

Statistical results indicated that particle sizes strongly influenced oil yield, and the content of β-carotene and lycopene of the extracts ($P < 0.001$). There was insignificant interaction between particle size and extraction time on the oil yield ($P > 0.05$), however, the interaction between the two factors on the lycopene ($P < 0.5$) was found to be significant.

Fig. 2 and Table 2 show that the oil yield and content of carotenoids are significantly affected by different particle sizes of the dried aril powder, all at 50 °C. Generally, particle size is one of the most important parameters to have an effect on the extraction oil yield and the content of carotenoids. A faster rate of the CO_2 diffusion could be obtained when using a smaller particle size (Del Valle and Uquiche, 2002; Panfili et al., 2003), due not only increasing surface area to volume ratio of materials but also rupturing cell membranes. For the larger size of 0.49 mm, it possibly took a longer solvent diffusion time to reach the highest oil yield as compared with the smaller size of 0.45 mm and 0.37 mm. However, there can be a problem with channeling inside the extraction bed if particle size is small, resulting in lowered oil yield and carotenoid content (Jokić et al., 2012). This is in agreement with several reports investigating effects of different particle sizes of dried tomato materials on the content of carotenoids (Nobre et al., 2012; Sabio et al., 2003; Vági et al., 2007).

As there are some expected overlaps between the mean volume-surface particle sizes of 0.45 mm and 0.49 mm, the significant difference in oil yield and carotenoid content may not be due to the size difference alone. The way the particles were packed within the cross section of the extraction chamber could have some influences. Besides, there was a variation in quality of our collected raw Gac fruit aril as mentioned in Section 2.2. Furthermore, several authors also reported that a big difference in β-carotene and

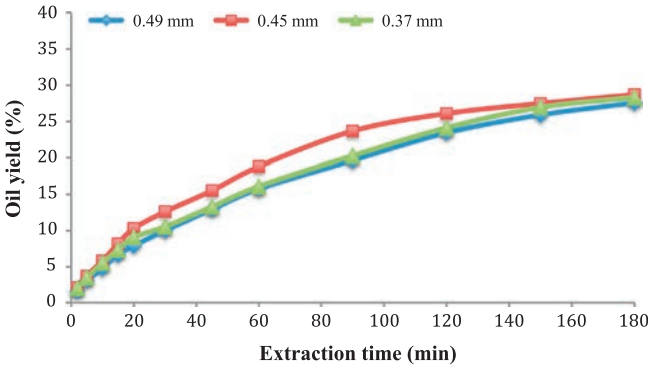

Fig. 2. Effect of particle size and extraction time on the oil yield obtained by SC-CO_2 extraction.

Table 2
Effects of particle size and SC-CO_2 extraction time on the content of β-carotene and lycopene (mg/100 mL) of Gac oil extracts.

Parameters		β-carotene content	Lycopene content
Particle size (PS)	0.37 mm	35.2 ± 19.6[a]	221.3 ± 76.9[a]
	0.45 mm	75.3 ± 22.1[b]	482.6 ± 75.5[b]
	0.49 mm	43.7 ± 15.3[c]	164.8 ± 48.7[c]
Extraction time (ET)	60 min	30.3 ± 18.8[a]	215.6 ± 157.9[a]
	120 min	56.9 ± 22.5[b]	307.3 ± 177.9[b]
	180 min	67.0 ± 22.5[c]	345.7 ± 173.8[c]
Significant interaction		Significance	
PS		***	***
ET		***	**
PS × ET		NS	*

Values are mean ± SD after statistical analyses.
NS, *, ** and *** indicate *not significant* and *significant* at $P = 0.05$, 0.01 and 0.001, respectively.
The values in the same column followed by different superscripts (a–c) were significantly different ($P < 0.05$).

Table 3
Effects of enzyme concentration (w/w) and SC-CO_2 extraction time on the content of β-carotene and lycopene (mg/100 mL) of Gac oil extracts.

Parameters		β-Carotene content	Lycopene content
Enzyme concentration (EC)	0.05%	25.6 ± 6.7[a]	172.1 ± 47.9[a]
	0.1%	14.3 ± 3.7[b]	83.7 ± 15.1[b]
	0.15%	11.6 ± 6.2[b]	119.1 ± 44.3[c]
Extraction time (ET)	60 min	11.0 ± 6.1[a]	87.9 ± 26.4[a]
	120 min	18.8 ± 7.4[b]	128.6 ± 48.5[b]
	180 min	21.7 ± 7.3[b]	158.3 ± 51.6[c]
Significant interaction		Significance	
EC		***	***
ET		***	**
EC × ET		NS	NS

Values are mean ± SD after statistical analyses.
NS, *, ** and *** indicate *not significant* and *significant* at $P = 0.05$, 0.01 and 0.001, respectively.
The values in the same column followed by different superscripts (a–c) were significantly different ($P < 0.05$).

lycopene in fresh Gac fruit was found due to differences in variety, harvesting time and storage conditions (Kha et al., 2013a,b). Accordingly, it could be said that the mean particle size of 0.45 mm gave the best oil yield and carotenoid content in this particular small laboratory unit under the experimental conditions employed but may not be the most suitable size in other situations.

3.3. Effect of enzyme concentration

Statistical analysis showed that the oil yield and the content of β-carotene and lycopene in the extracts were significantly affected by enzyme concentration ($P < 0.001$). There was insignificantly interaction between enzyme concentration and extraction time on β-carotene and lycopene contents ($P > 0.05$).

Fig. 3 indicates that higher content of the oil is obtained over the fractionated extraction time by the enzyme concentration as compared with 50 °C air-dried samples of the same 0.45 mm particle size without enzymatic hydrolysis (Figs. 1 and 2). It can be explained that cell walls are opened up by the use of enzyme through biodegradation; the complex lipoprotein and lipopolysaccharide molecules are also broken up into simpler molecules (Li et al., 2012; Shankar et al., 1997), hence oils were easily released. It can be concluded that the concentration of enzyme had a positive effect on the oil yield which is in agreement with several published results that used enzyme as a pre-treatment prior to extraction for plant materials (Domínguez et al., 1994; Gibbins et al., 2012).

In contrast, β-carotene and lycopene contents in the extracts are much lower than that of those samples without enzymatic hydrolysis (refer to Tables 1–3). Similarly, Tran et al. (2008) reported that retention of carotenoids in Gac powder sample without enzymatic pre-treatment was higher that of the enzymatic one. It could be explained that the contacting surface area of the enzymatic-treated arils with oxygen was larger than the surface of the untreated samples. More cell walls were broken down during the enzymatic hydrolysis and more carotenoids were extracted (Tran et al., 2008). As a result, a degradation of carotenoids occurred substantially due to oxidation during air drying process. In addition, a dilution may take into account for the lower content of carotenoids in the enzyme pre-treated arils due to the higher release of the oils as compared to the untreated samples.

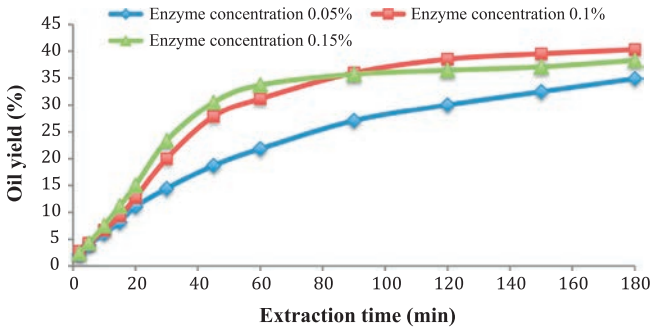

Fig. 3. Effect of enzyme concentration and extraction time on the oil yield obtained by SC-CO_2 extraction.

3.4. Effect of extraction time

As can be seen in Figs. 1–3, the oil yield increased rapidly with the increase of extraction time from 2 to 90 min ($P < 0.001$). Generally, increasing extraction time leads to more complete oil yield (Li et al., 2011; Liu et al., 2009; Zhang et al., 2009). It is important to prolong the contact of the SC-CO_2 solvent with the aril powder in order to maximize the oil yield. Since results indicated that the oil yield slowly increased beyond 120 min, this extraction time could be enough for the extraction process. In addition, SC-CO_2 extraction process was less time consuming than Soxhlet extraction (refer Section 2.5.2), which uses organic solvents. Therefore, SC-CO_2 extraction should be applied in modern industry, being environmental friendly by decreasing toxic residues while producing higher quality foods (Jokić et al., 2012).

Generally, Tables 1–3 showed that the extraction time had a very positive effect on the content of β-carotene ($P < 0.001$) and lycopene ($P < 0.01$) of the oil extracts. The carotenoid content of the extracts increases rapidly in the extraction times of 60–120 min, but the trend becomes less so afterward. It is likely because the carotenoids are mostly already extracted. Moreover, there is insignificant difference between the extraction time of 120 and 180 min for β-carotene and lycopene of the extract when air-dried at different temperatures and different concentrations of enzyme before SC-CO_2 extraction. Therefore, it can be concluded that the extraction time of 120 min could be sufficient for a complete extraction. Several authors reported that longer extraction time might have the negative effect on carotenoid yield due to thermal degradation and isomerisation (Nobre et al., 2012; Zuknik et al., 2012). Moreover, reducing costs related to the extraction procedure and improving energy efficiency could be achieved by decreasing the extraction time (Baysal et al., 2000; Ciurlia et al., 2009).

Table 4
Effect of different extraction methods on the EE, and ö-carotene and lycopene contents.

Oil samples	EE (%)	β-Carotene (mg/100 mL)	Lycopene (mg/100 mL)
Air-dried before SC-CO_2 (this study)	95	83	508
Kha et al. (2013b)			
– Microwave-dried before pressing	93	140	414
– Air-dried before pressing	68	55	240
Vuong and Kinh (2003)	na	271	302
– pressing without treatment			
Soxhlet extraction (this study)	100	35	180

na: not available.

3.5. Comparison of different extraction methods for Gac oil

As mentioned before, pre-treatments play a crucial role in the SC-CO_2 extraction for Gac oil. It is therefore important for this study to compare the quality of Gac oil with other published studies, in terms of extraction efficiency (EE) and content of β-carotene and lycopene. It is also important to compare different extraction methods using the EE due to different oil content in starting materials. The EE was determined as a ratio of mass of extracted oil and mass of oil in starting material using the Soxtec extraction unit. Comparison of these parameters affected by different extraction methods is presented in Table 4.

As can be seen in Table 4, the EE of air-drying prior to SC-CO_2 extraction is slightly higher than that of microwave-drying pre-treatment prior to pressing, about 2%. However, both methods (air-dried samples before SC-CO_2 extraction and microwave-dried samples before pressing) yielded much higher than air-drying before pressing. The differences in β-carotene and lycopene contents between the current and the published reports may be due to the different varieties, growing conditions, stage of maturity and storage conditions of the fresh Gac fruit used as raw material (Kha et al., 2013a). However, it is clear that different pre-treatments produced different results for different extraction methods. Therefore, depending on the extraction method, it is important to find the most suitable pre-treatment to enhance the EE. For SC-CO_2 extraction, air-drying is recommended due to its ease of application and high EE result.

In comparison with Soxhlet method, the amount of β-carotene and lycopene in the SC-CO_2 extracted oil samples is much higher. This indicates that the SC-CO_2 extraction method effectively preserve the content of carotenoids.

4. Conclusion

It can be concluded that pre-treatments of air-drying temperature, particle size, enzyme concentration and extraction time prior to SC-CO_2 extraction strongly influence the oil yield and the content of β-carotene and lycopene of the extracts. Results indicated that the highest oil yield was obtained when using enzyme at 0.1% (w/w) concentration for pre-treatment before air-drying. Furthermore, the drying temperature of 50 °C and mean particle size of 0.45 mm were the most suitable pre-treatments for the laboratory unit employed. Under these conditions, the high Gac oil yield of about 34%, and the high content of β-carotene (83 mg/100 mL oil) and lycopene (508 mg/100 mL oil) after the extraction time of 120 min were obtained.

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Paper III

2.1.2 Ultrasound-assisted Aqueous Extraction of Oil and Carotenoids from Microwave-dried Gac (*Momordica cochinchinensis* Spreng) Aril

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Ultrasound-assisted aqueous extraction of oil and carotenoids from microwave-dried Gac (Momordica cochinchinensis Spreng) aril

Running head: Ultrasound-assisted aqueous extraction of Gac oil

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Classifications:	
Keywords:	Gac aril, Gac oil, microwave, ultrasonication, Carotenoids
Abstract:	The objective was to optimise the ultrasound-assisted aqueous extraction of oil, β-carotene and lycopene from microwave-dried Gac arils ground to a powder. Ultrasound power, extraction time, powder particle size and the ratio of water to Gac powder during the extraction, the centrifugal force used to recover the extracted components and the peroxide value (PV) of the oil were investigated. The analytical Soxhlet extraction and HPLC analysis were used to determine how much oil and carotenoids were available for extraction from the fresh Gac arils, respectively. Microwave-drying followed by aqueous extraction without ultrasound-assistance and air-drying followed by aqueous extraction with or without ultrasound-assistance was also carried out for comparisons, including observing the Gac material left behind after the extractions using scanning electron microscopy (SEM). Ultrasound power of 32 W/g of aril powder, extraction time of 20 min, powder particle sizes of 0.3-0.5 mm, a ratio of water to powder of 9 g/g and a centrifugal force of 6750 × g gave optimal extraction efficiencies for oil (90%), β-carotene (84%) and lycopene (83%), and the oil had a low PV (2.2 meq/kg). The SEM analysis confirmed

	that the combination of microwave-drying followed by ultrasound-assisted aqueous extraction caused strong disruption of the Gac aril cellular structures, which was consistent with the high extraction of oil, β-carotene and lycopene obtained with the combination. It was concluded that Gac oil containing high amounts of β-carotene and lycopene and having a low PV could be extracted using microwave-drying and ultrasound-assisted aqueous extraction.

Ultrasound-assisted aqueous extraction of oil and carotenoids from microwave-dried Gac (*Momordica cochinchinensis* Spreng) aril

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Abstract

The objective was to optimise the ultrasound-assisted aqueous extraction of oil, β-carotene and lycopene from microwave-dried Gac arils ground to a powder. Ultrasound power, extraction time, powder particle size and the ratio of water to Gac powder during the extraction, the centrifugal force used to recover the extracted components and the peroxide value (PV) of the oil were investigated. The analytical Soxhlet extraction and HPLC analysis were used to determine how much oil and carotenoids were available for extraction from the fresh Gac arils, respectively. Microwave-drying followed by aqueous extraction without ultrasound-assistance and air-drying followed by aqueous extraction with or without ultrasound-assistance was also carried out for comparisons, including observing the Gac material left behind after the extractions using scanning electron microscopy (SEM). Ultrasound power of 32 W/g of aril powder, extraction time of 20 min, powder particle sizes of 0.3-0.5 mm, a ratio of water to powder of 9 g/g and a centrifugal force of 6750 × *g* gave optimal extraction efficiencies for oil (90%), β-carotene (84%) and lycopene (83%), and the oil had a low PV (2.2 meq/kg). The SEM analysis confirmed that the combination of microwave-drying followed by ultrasound-assisted aqueous extraction caused strong disruption of the Gac aril cellular structures, which was consistent with the high extraction of oil, β-carotene and lycopene obtained with the combination. It was concluded that Gac oil containing high amounts of β-carotene and lycopene and having a low PV could be extracted using microwave-drying and ultrasound-assisted aqueous extraction.

Key words: Gac aril; Gac oil; microwave; ultrasonication; β-carotene; lycopene.

1. Introduction

Several studies have reported that Gac (*Momordica cochinchinensis* Spreng) aril contains high levels of unsaturated fatty acids, β-carotene and lycopene [1-3]. These bioactive compounds have been proven to be beneficial to human health and are linked with a reduced risk of cardiovascular disease and cancers [4-6]. Importantly, it has been found that a significant improvement in the absorption of the carotenoids into the human body occurs when they are digested with fat [7-9]. Therefore, it is desirable to effectively extract the Gac oil along with the hydrophobic β-carotene and lycopene, in order to increase the likelihood that the carotenoids will be absorbed when Gac aril extracts are prepared for use as nutrient supplements or natural food additives. Due to the attractive red-orange colour of the β-carotene and lycopene, these Gac extracts can also be used as colourants [1, 10].

Traditionally, hexane extraction or a combination of mechanical pressing and hexane extraction has been applied for vegetable oil preparations in the food industry. The main drawbacks of this process are health concerns and increased environmental regulations due to the toxicity of hexane. In addition, the extracted oils can also be of low quality in terms of their content of unwanted free fatty acids, waxes and unsaponifiable matter [11]. Therefore, it is desirable to find a more suitable extraction technique for Gac oil. The application of aqueous extraction techniques for vegetable oil, in which oil separation is based on the insolubility of the oil in water, has been studied widely [11-14]. This type of extraction generally offers the advantages of being more environmentally friendly, economical and safer and yields a higher oil quality than the traditional hexane extraction [15]. Using water as the main extraction solvent, there is no energy required for organic solvent stripping and no high investment is required for monitoring and controlling the emission of volatile organic compounds. Furthermore, high oil quality can be obtained without requiring degumming and anti-nutritional factors are less of a problem in the aqueous process.

A great variety of new methods based on different principles have been developed to extract oil and bioactive compounds from plant materials using water. Recently, the principles, on which these novel extraction techniques are based, have been reviewed by Azmir et al. [16] and they have suggested that the main advantage of these aqueous methods is that they are very environmentally friendly being free of organic chemical solvents. Among the novel techniques for plant oil extraction, microwave-drying followed by ultrasound-assisted aqueous extraction has been highlighted in recent years; a reduction in extraction time, an increase in oil yield and the production of high quality oil are the main benefits of this method [16-18].

The extraction of oil directly from fresh plant material is known not to be very efficient. Therefore, reducing the moisture in the plant material before it is extracted is one of the most

important steps in the aqueous extraction process when it is facilitated by ultrasonication [16]. Compared to other drying techniques, air-drying is well known as the simplest and cheapest drying method. However, the quality of air-dried products is often fairly poor, particularly if the resultant materials are sensitive to heat damage. Recently, microwave-drying has been reported to be an effective technique for removing moisture from Gac arils [19, 20]. According to these reports, a microwave power of 630 W and a drying time of 62 min for a 900 g sample resulted in the highest retention of β-carotene and lycopene in the extracted Gac oil. This is because these microwave-drying conditions provided enough of a driving force to break down the cell matrix but not enough to destroy the oil and the carotenoids.

In the ultrasound-assisted aqueous extraction method, mass transfer of solutes from the plant material into the surrounding water is dramatically enhanced by the ultrasonics, mainly due to cavitation forces and some mechanical effects [17]. Intensive localised pressure, generated by the explosive collapse of bubbles, can result in the effective breakdown of cell walls, and thereby increase the release of intracellular components into the solvent [21]. A greater penetration of the solvent into the material matrix, which is a mechanical effect of the ultrasonics, also occurs and results in an increased contact between the solvent and the compounds to be extracted [22]. Therefore, to achieve a successful extraction of vegetable oil, there are a number of parameters to be considered. Among the most important factors, ultrasound power and the extraction time can affect the cavitation forces [16], and the particle size of the solid material and the ratio of water to the solid can influence the penetration of the solvent into the solid and the mass transfer of the solutes from the solid into the solvent [23, 24]. In addition, some of the disadvantages of traditional extraction techniques, including long processing times and degradation of thermolabile compounds, can be overcome with the ultrasound-assisted aqueous extraction method [16].

Due to their considerable advantages, it is of interest to combine the microwave-drying technique followed by the ultrasound-assisted aqueous extraction method in order to determine whether the extraction efficiency for Gac oil and its content of β-carotene and lycopene can be increased compared to traditional methods. After aqueous extractions, centrifugation is also an attractive method for separating oil from oil-in-water emulsions; it is more effective compared to the gravity settling method, which can be a very slow sedimentation process for the separation of immiscible mixtures [25]. Centrifugation can break down oil-in-water emulsions and effectively separate oil from water if an appropriate centrifugal force is used [26]. Therefore, it is also important to investigate the effect of the centrifugal force on the oil extraction efficiency.

Having only recently been proposed as a process for oil extraction [16-18], there is still a lack of information in the published literature on the use of microwave-drying followed by the ultrasound-assisted aqueous extraction of oil and hydrophobic bioactive compounds from plant

materials. Therefore, the objective of the present study was to optimise the ultrasound-assisted aqueous extraction of oil, β-carotene and lycopene from microwave-dried Gac arils ground to a powder. The effects, on the extraction efficiency (EE) for Gac oil, β-carotene and lycopene, of different conditions including ultrasound power, extraction time, powder particle size and the ratio of water to powder during the ultrasound-assisted aqueous extraction and the centrifugal force used to recover the oil after the extraction, were investigated. Microwave-drying followed by aqueous extraction without ultrasound-assistance and air-drying followed by aqueous extraction with or without the use of ultrasound was also carried out for the purpose of comparisons.

The analytical Soxhlet extraction method was used to determine how much oil was available and HPLC analysis was used to determine how much β-carotene and lycopene were available for extraction from the Gac arils. The peroxide value (PV) of the extracts was measured to determine the extent of lipid peroxidation. Moreover, in order to gain some understanding on the mechanisms involved during the drying and the aqueous extraction processes, scanning electron microscopy (SEM) analysis was employed to study the structure of the Gac aril material left over after it was subjected to various drying and extraction combinations.

2. Materials and Methods

2.1 Chemicals

Carotenoid standards including β-carotene (C4582, type II synthetic, ≥ 95%, HPLC grade) and lycopene (L9879, ≥ 90%, from tomato) were purchased from Sigma-Aldrich Pty. Ltd. (Castle Hill, NSW, Australia). All solvents (HPLC and analytical grades) and BHT (butylated hydroxytoluene) were obtained from Merck Pty. Ltd. (Kilsyth, Vic, Australia).

2.2 Drying and powdering of Gac arils

Gac fruit (of uniform yellow-red colour and size) was purchased from a local market in Ho Chi Minh City, Viet Nam. The red arils including seeds were scooped out and dried using a Panasonic Dimension NN-9853 microwave oven (Panasonic Australia Pty. Ltd., Macquarie Park, NSW, Australia) with cavity dimensions of height (H), width (W) and depth (length, L) of 244 mm, 412 mm, 426 mm, respectively, and operating at a frequency of 2450 MHz. The arils (900 g), of an initial moisture content of 82%, were spread to a thickness of 5 mm onto the turntable plate and microwave-dried at a power setting of 630 W for 62 min, as previously optimised [19]. The temperature of the arils after microwave-drying was recorded as 80 ^{o}C. The dried arils, with a moisture content of 9% on a wet basis (w.b.), were then powdered using a IKA M20 grinding mill (IKA® company, Staufen, Germany) and the particle size distribution of the powder was then determined using a USA standard test sieve (W.S. Tyler, Mentor, OH, USA). Powders of different

particle sizes of >2, 1-2, 0.5-1 and 0.3-0.5 mm were then used for the ultrasound-assisted aqueous extraction.

For comparison, Gac arils (900 g) were spread onto a stainless steel tray (1.2 x 0.8 m) to a thickness of 1.5 cm and air-dried at a temperature of 60 ^{o}C for 12 h with an air velocity of 1.1 m/s, using an air-drying oven (G. T. C Australia Trading Pty. Ltd., Alexandria, NSW, Australia) in order to reach the target moisture content of 9% [27]. The air-drying temperature of 60 ^{o}C was chosen because a significant loss of carotenoid content occurs at higher temperatures [27, 28]. The air-dried arils were then powdered using the grinding mill to produce a powder with particle sizes determined to be the best for the microwave-dried powder described above.

2.3 Aqueous extraction of powdered Gac arils

The microwave-dried Gac aril powder samples (10 g) with the different particle sizes were placed in 40 to 100 mL deionised water in a 200 ml beaker, which was immersed into a WUC-D22H ultrasonic cleaner water bath (DAIHAN Scientific Co., Ltd., Seoul, Korea). The water level in the beaker was lower than that of the ultrasonic cleaner water bath, which had internal dimensions of: L 300 x W 500 x H 150 mm. Different mass ratios (g/g) of water to powder of 4, 5, 6, 7, 8, 9 and 10 were investigated. To test the various ultrasound-assisted aqueous extraction conditions, different ultrasonic output powers (240, 320 and 400 W) and times (10, 20, 30 and 40 min) were carried out with the temperature set at 30 ± 3 ^{o}C and the frequency at 40 kHz. The temperature was maintained using an in-water pipe and the temperature was regulated by the flow ratio between water in and water out.

For the purpose of comparisons, aqueous extractions of microwave and air-dried Gac aril powder samples were also run employing a shaking water bath set at a temperature of 30 ± 3 ^{o}C (Ratek Instruments, Boronia, Vic, Australia). The other extraction conditions, including the extraction time, the powder particle size, the ratio of water to powder and the centrifugal force were those found to be optimal for the ultrasound-assisted aqueous extraction.

2.4 Centrifugation of powdered Gac arils aqueous extracts

Initially, to determine the most suitable ultrasound-assisted aqueous extraction conditions after microwave drying, the samples were poured into a 300 mL centrifuge bottle and centrifuged at 5520 × *g* for 40 min at a temperature of 25 ^{o}C in a J2-MC centrifuge and JA-14 rotor (Beckman Coulter, Brea, CA**,** USA). After determining the most suitable ultrasound-assisted extraction conditions, the suspension was centrifuged at different centrifugal forces for 40 min. The centrifugal forces of 5520, 6750, 8820, 12400 and 15300 × *g*, which correspond to rotational speeds of 6000, 7000, 8000, 9000 and 10000 rpm, respectively, were used to investigate the effect of

centrifugal speed on the EEs, in terms of oil yield and its content of β-carotene and lycopene, as well as on the lipid oxidation index, PV.

After centrifugation, the top oil layer was collected using a pipette, weighed to determine the oil yield and assayed for carotenoid content and PV. After removing the water layer, the pellet, representing the Gac aril tissue left over after the extraction process, was also collected and dried at 40 ^{o}C for 1 h (air drying oven, G. T. C Australia Trading Pty. Ltd., Alexandria, NSW, Australia) for scanning electron microscopy (SEM) analysis. For the Gac aril samples, which were air-dried and/or extracted with water without ultrasonic treatment, the centrifugal force found to be optimal for the microwave-dried and ultrasound-assisted aqueous extraction was used.

2.5 Analytical methods

2.5.1 Determination of total oil content

The FOSS Soxtec^{TM} system 2045 extraction unit (Hilleroed, Denmark) was employed to analyse the total oil content of fresh Gac, microwave-dried and air-dried arils according to the analytical Soxhlet extraction method reported by Brkić et al. [29]. Approximately 3 g of the fresh or dried Gac aril was weighed in cellulose thimbles and put into the extraction system. Petroleum ether (50 mL) with a boiling point of 90 ^{o}C was added into aluminum vessels and inserted into the extraction unit. The running program for the oil extraction was set as follows: the temperature at 145 ^{o}C, boiling for 30 min, rinsing for 60 min, evaporation for 15 min and finally drying the aluminum vessels containing the oil extracts for 20 min. The sample was then dried in an oven set at a temperature of 80 ^{o}C until a constant weight was obtained. The total oil content was gravimetrically calculated as weight of oil extracted per weight of fresh or dried Gac aril (g/g) and expressed as % of dry weight.

2.5.2 Determination of moisture content

The moisture content of the fresh aril, microwave-dried and air-dried samples was determined by drying at a temperature of 105±1 ^{o}C until a constant weight was reached.

According to the AOCS Ca 2c-25 method [30], the moisture content of the extracted Gac oil was determined by weighing the oil in a tared moisture dish and air-drying it at a temperature of 130±1 ^{o}C for 30 min (Air-drying oven, Memmert GmbH + Co.KG, Schwabach, Germany). The dish was then removed and cooled to room temperature in a desiccator and weighed. The process was repeated until the loss in weight did not exceed 0.05% per 30 min drying interval and the moisture content of the oil sample was then calculated.

2.5.3 Determination of β-carotene and lycopene

The method previously described by Kha et al. [19] was used and all operations were carried out under subdued light to minimise oxidation of the carotenoids. For fresh Gac aril or the dried powders, 1 g was placed into 35 mL of a 4:3 (v/v) solution of ethanol and n-hexane, which contained the antioxidant butylated hydroxytoluene (BHT, 0.1% in hexane), and the mixture was blended for 5 min at 5000 rpm using an Omni-mixer homogeniser (Omni International, Kennesaw, GA, USA). The residue was re-extracted with another 35 mL of the 4:3 (v/v) ethanol: n-hexane and then washed twice with ethanol (12.5 mL) and once with hexane (12.5 mL). The combined extracts were washed with deionised water, and filtered through Whatman No. 1 filter (90 mm diameter) paper on a Buchner funnel. The extracts were then diluted with mobile phase solution at an appropriate ratio. For the extracted Gac oil, 10 μL of oil was first dissolved in 2 mL of chloroform and then diluted with the mobile phase at a ratio of 1:200. All the samples were filtered through 0.45 μm cellulose syringe filters (Phenomenex Australia Pty. Ltd, Lane Cove, NSW, Australia) before injecting onto the HPLC system.

The content of β-carotene and lycopene in the Gac arils, the dried powders and oil extracts was determined using an Agilent 1200 HPLC (Santa Clara, CA, USA) equipped with a diode array detector system. A two-column reversed-phase separation system consisting of a Luna C18 column (100 x 4.6 mm i.d; 5 μm) directly coupled to a Jupiter C18 column (250 x 4.6 mm i.d; 5 μm) was used; both columns were purchased from Phenomenex Australia Pty. Ltd. (Lane Cove, NSW, Australia). According to a procedure described by Kha et al. [19], the mobile phase consisted of 50:40:10 v/v/v, acetonitrile, dichloromethane and methanol containing 0.1% BHT. The injection volume was 20 μL, the flow rate was 1.0 mL/ min, and the detection was at 450 nm. The identification of the β-carotene and lycopene peaks was based solely on the retention time of the peaks compared with those of the authentic standards. The amount of β-carotene and lycopene in the samples was expressed as mg/100 g.

2.5.4 Determination of extraction efficiency (EE)

The ratio of the dry mass of extracted oil (after subtracting its moisture content) to the mass of oil measured in the starting Gac aril material using the analytical Soxhlet extraction method [29] was determined as the extraction efficiency of oil (EE-oil) and expressed as % of the oil available in the fresh Gac arils.

Extraction efficiencies in terms of the retention of β-carotene and lycopene in the oil were calculated as the amount of β-carotene and lycopene in the extracted oil to the amount of β-carotene and lycopene in the starting Gac aril material as measured using the HPLC method [19] and expressed as % of the β-carotene and lycopene in the fresh Gac arils.

2.5.5 Determination of peroxide value (PV)

The PV of the extracted Gac oil was measured according to the AOCS Cd 8-53 method [30]. Briefly, 1 g of oil was placed into a 250 mL Erlenmeyer flask and dissolved in 30 mL of a mixture consisting of acetic acid and chloroform (3:2, v/v) and then stirred for a few seconds to ensure thorough mixing. Thereafter, about 0.5 mL of saturated potassium iodide solution was added. Deionised water (30 mL) was added after exactly 1 min, and the solution was titrated with 0.01 N sodium thiosulfate, using a starch solution as an indicator, until the solution became colourless. The mixture was stirred using a magnetic stirrer during the titration procedure. The PV (meq/kg) of the samples was calculated as milliequivalents of active oxygen per kg of sample, as per the following equation:

$$PV_{meq/kg} = \frac{(S-B)\times N\times 1000}{Mass\ of\ sample\ (g)}$$

where S and B are the titration amounts (in mL) of 0.01 N sodium thiosulfate for sample and blank, respectively. N is the normality of the sodium thiosulfate solution.

2.5.6 Scanning electron microscopy (SEM) analysis

In order to investigate the effects, on the external structure of the Gac aril material, of the two drying methods, microwave and air-drying, and the aqueous extractions with and without ultrasonic treatment, the dried Gac aril powders obtained before extraction and the material pelleted after the aqueous extraction mixtures were centrifuged, were analysed using a JSM-7401F SEM (Jeol Co. Ltd., Tokyo, Japan) operating at a voltage of 5 kV.

A small amount of Gac aril material (powder or pellet) was fixed onto the surface of a double-coated metallic adhesive tape adhered to a metallic 320 stub. The stub was then coated with a fine layer of gold and argon in a MSP-1S magnetron sputter coater (Vacuum Device, Tokyo, Japan) under vacuum conditions to avoid charging under the electron beam.

2.6 Statistical analysis

The independent experiments and subsequent measurements were all performed in triplicate. The two-way analysis of variance (ANOVA) was used for experiments with two parameters (the ultrasound power and time experiment and the ratio and particle size experiment for the ultrasound-assisted aqueous extraction and the combined drying technique and aqueous extraction method experiment) and the one-way ANOVA for single factor experiments (the fresh aril and drying technique experiment and the centrifugation speed experiment). When more than one treatment were tested, the least significant difference (LSD) post-hoc test was employed. To analyse the data the SPSS-PASW GradPack 21.0 for Mac (IBM Corp., Armonk, NY, USA) was used and the graphs

of mean values and error bars were created using Excel for Mac 2011 (Microsoft, Seattle, WA, USA).

3. Results and Discussion

3.1 Comparison of microwave-drying and air-drying

The total oil content of Gac arils was found to be 24.9±0.5% (w/w, dry weight, d.w.) using the Soxhlet extraction method (Table 1) and microwave or air-drying followed by grinding into powder had no effect on this measurement. Therefore, the two processes for drying the Gac arils into powder did not cause any loss of oil, prior to its aqueous extraction.

Since the carotenoids may be degraded by drying methods, it was important to determine whether there was any loss of β-carotene and lycopene during the drying of the Gac arils. Table 1 shows the content of β-carotene and lycopene in the microwave-dried and air-dried Gac aril powders compared to the fresh arils. The results showed that there was a very substantial loss ($P < 0.05$) of β-carotene (-50%) and lycopene (-49%) in the air-dried powder compared to the fresh aril. In contrast, compared to the fresh aril, the loss of β-carotene and lycopene in the microwave-dried powder was not significant ($P > 0.05$). Therefore, the microwave could be used for drying the Gac arils followed by grinding to prepare a powder, without loss of β-carotene and lycopene, prior to aqueous extraction.

3.2 Effects of ultrasound power and extraction time

The conditions for the ultrasound-assisted aqueous extraction, which gave the maximum oil extraction efficiency (EE), were then determined using the microwave-dried powder. The EE of Gac oil from the arils was affected by ultrasound power and extraction time as shown in Figure 1A. Statistical analysis showed that both the ultrasound power and the extraction time significantly influenced the EE ($P < 0.001$). In general, higher extraction efficiencies were obtained at the higher power outputs of 320 and 400 W compared to 240 W. However, there was no difference in the EE between 320 and 400 W ($P > 0.05$). An increase in the extraction time also improved the EE ($P < 0.001$). Moreover, there was a significant interaction between the power and the time on the EE ($P < 0.001$). As seen in Figure 1A, the optimal conditions of the ultrasound power at 320 W for an extraction time of 30 min resulted in the highest EE; close to 89% of the oil was extracted from the Gac arils under these ultrasound-assisted aqueous extraction conditions.

Similar to the effect on the EE for the oil, the impact of the ultrasound power and the time used for the extraction on the extraction efficiency, in terms of retention of β-carotene and lycopene in the oil, was also found to be significant ($P < 0.001$) as presented in Figures 1B and C. However, in contrast to the effects on the oil, significant losses of the carotenoids were found when the power

and the time were increased. The lowest retention of the two carotenoids was found for the highest power, 400 W, although there was no difference between the samples sonicated at 240 and 320 W (Figures 1B and C). For lycopene (Figure 1C), but not β-carotene (Figure 1B), its retention in the oil generally decreased as the time for the extraction increased.

In addition, there was a significant interaction between the ultrasound power and the time used for the EE for the carotenoids ($P < 0.01$). The highest retention of β-carotene and lycopene was found at the ultrasound power of 240 W for 20 min and at 320 W for 20 min, respectively. However, taking into account the finding that the optimal conditions of ultrasound power at 320 W for an extraction time of 30 min resulted in the highest EE for the Gac oil (Figure 1A), it was concluded that an ultrasound power of 320 W for an extraction time of 20 min were the best compromise extraction conditions in terms of the extraction of the oil and its content of β-carotene and lycopene. Although the EE for the Gac oil at these extraction conditions is slightly lower than at 320 W for 30 min, the content of lycopene in the oil extracted at 320 W for 20 min was substantially higher than at the longer extraction time of 30 min (Fig 1).

The ultrasound power can alter the properties of plant matrices physically and chemically. In this study (Figure 1), the EE for the oil, which varied from 85 to 89%, was significantly higher at 320 and 400 W compared to 240 W (76 to 85%). This can be explained by the propagation of the ultrasound pressure waves and the resulting cavitation phenomenon. When ultrasound energy is transferred into liquid systems, longitudinal waves are formed via a series of compression and rarefaction waves in elastic materials [31, 32]. A sufficiently high power results in local pressure waves being below the vapour pressure of the liquid, resulting in a constant growth of gas bubbles distributed throughout the liquid. These bubbles reach a critical size, then become unstable and violently collapse, a process called cavitation.

The release of the oil and the carotenoids, is facilitated by the cavitation phenomenon; the formation and collapse of the bubbles causes shear stress within the cells. Essentially, the implosion of the cavitation bubbles generates turbulence, which causes a big difference in the osmotic pressure between the inside and the outside of the cells [33], which in turn causes disruption of the cell membranes of the plant matrix. Disrupting the plant cell walls [17] enhances the mass transfer of components from the inside of the plant cells into the solvent and thereby, increases the extraction rate.

For the oil, the highest EE was obtained at a power of 320 W and an extraction time of 30 min (Figure 1). This appeared to be the optimal time needed for the ultrasound to disrupt the Gac aril cells and for the oil to diffuse into the solvent through the porous structure of the residual solid Gac material [34, 35]. This is consistent with a study by Zhang et al. [34], who reported that an ultrasound time of 30 min was the most effective for oil extraction from flaxseed. However, the

effect of the ultrasound time on retention of the carotenoids in the oil also had to be considered because chemical degradation of bioactive compounds such as β-carotene and lycopene can occur during prolonged ultrasonic irradiation [36]. Consistent with this observation, the retention of β-carotene (Figure 1B) and lycopene (Figure 1C) was found to decrease at 30 and 40 min. Therefore, to optimise the retention of the carotenoids in the oil, 20 min was chosen as the optimal time while a power of 320 W was chosen to optimise the extraction of oil and lycopene. As this was based on a 10 g sample, the specific ultrasonic power used was 32 W/g aril powder.

3.3 Effects of particle size and ratio of water to powder

The conditions for the ultrasound-assisted aqueous extraction at a power of 320 W for 20 min were then further studied using the microwave-dried Gac aril powder to determine the optimal powder particle size and the optimal ratio of water to powder. Fig 2 shows how the EEs for oil, β-carotene and lycopene were influenced by the different particle sizes and ratios of water to powder. The ANOVA indicated that the oil EE was significantly affected by the powder particle size and the water to powder ratio ($P < 0.001$). However, there was no significant interaction between the size and the ratio ($P > 0.05$). For the carotenoids, the EEs for β-carotene and lycopene content were significantly affected by the particle size ($P < 0.001$ and $P < 0.05$, respectively), but not by the ratio ($P > 0.05$) and there was no significant interaction between the two factors ($P > 0.05$).

As seen in Figure 2A, the oil EE generally increased with decreasing particle size at each ratio of water to powder. However, there was no significant difference in the oil EE between the powder with particle sizes of 0.5-1 mm and the powder with sizes of 0.3-0.5 mm. There was a very similar trend for the EE of β-carotene at the different particle sizes for each ratio of water to powder (Figure 2B) but the lycopene content of the oil was not affected by a decrease in the particle size (Figure 2C). Therefore, for the optimum oil and carotenoid extraction, it was desirable to use particle sizes of less than 1 mm. It is also seen in Figure 2A that the EE for oil also significantly increased as the ratio of water to powder increased from 4 to 8 and 9 g/g for each of the particle sizes tested. However, the ratio had no significant effect on the extraction of β-carotene (Figure 2B) or lycopene (Figure 2C). Therefore, mainly based on the extraction of oil, the optimal conditions for the ultrasound-assisted aqueous extraction of the microwave-dried Gac aril powder were chosen to be powder particle sizes of 0.3-0.5 mm at a ratio of water to powder of 9 g/g.

It is well known that particle size is one of the most important parameters in the solid-liquid extraction system. This is because decreasing the particle size increases the surface area available for contact with the solvent and thus the migration rate of components from the solid to the liquid increases from the solid's surface as well as through the pores of the solid material's matrix [37]. The degree of milling of oil-containing materials plays another important role in ultrasound-assisted aqueous extractions; with small particle sizes, the increase in the number of cells directly in contact

with the extracting solvent also increases the number of cells directly exposed to the cavitation process caused by the ultrasound [23, 24].

Similarly, it is generally agreed that compounds can be dissolved more effectively in larger volumes of solvent, resulting in improved extraction efficiencies. Essentially, the large concentration differences, which occur with large ratios of solvent to solid, increase the mass transfer rate of the components of the solid into the solvent [22, 35]. According to Rosenthal et al. [15], it is also desirable to use large quantities of water to obtain less stable emulsions in aqueous extractions of oil from plant material in order to increase the rate of extraction and the yield of oil. However, mass transfer can also reach a limit and even decrease if too much solvent is used [21]. Furthermore, large volumes of water require more equipment and effort during the subsequent oil-recovery steps of filtering and centrifugation. Therefore, the chosen water to powder ratio of 9 g/g for the ultrasound–assisted aqueous extraction of oil and carotenoids from the microwave-dried Gac arils (Figure 2) satisfies the requirements for such extractions [15, 21, 22, 35].

3.4 Effects of centrifugal force

The optimal extraction conditions for the ultrasound-assisted aqueous extraction including the ultrasound power, the time, the powder particle size and the ratio of water to powder were determined to be 320 W, 20 min, 0.3-0.5 mm and 9 g/g, respectively, using centrifugation at 5520 × *g* for 40 min to separate the oil from the water and solid material after the extractions. Figure 3 shows that increasing the centrifugal force could also significantly improve the EE for oil ($P < 0.05$) but not the EEs for β-carotene and lycopene ($P > 0.05$).

As can be seen in Figure 3, a significant increase in the oil EE was found when the force was increased from 5520 to 6750 × *g* but there was no further increase in the oil EE when the force was increased from 6750 to 15,300 × *g* (Figure 3). However, the EEs for β-carotene and lycopene were not affected ($P > 0.05$) meaning that the carotenoids were effectively and equally recovered in the oil at all the centrifugal forces tested.

According to Stoke's law, a faster sedimentation rate is obtained when an increased centrifugal force is generated by acceleration of the centrifuge rotation speed. The effect of centrifugation on the separation of oil from an oil and water emulsion depends on the settling or floating velocity of the oil droplets, which in turn depends on the density of the oil and the diameter of the oil droplets relative to the background density and viscosity of the continuous phase (water) and on the angular velocity of centrifugation and the effective radius of the rotor [11, 26]. Moreover, the zeta potential of an emulsion, which opposes the movement and coalescence of oil droplets, may be decreased by the increasing molecular rotations and movements caused by increasing the centrifugal force. As a result, the oil droplets tend to collide more with each other and

coalesce to form larger oil droplets, which ultimately results in a more efficient separation of oil from water at higher centrifugal forces.

For the separation of Gac oil from water in the present study (Figure 3), Stoke's law and the zeta potential appeared to be optimal at a centrifugal force of 6750 × *g*, although there was no apparent effect on the recovery of the carotenoids. At this force, the EEs for the oil, β-carotene and lycopene were 90%, 84% and 83%, respectively (Figure 3). This meant that 10% of the oil, 16% of the β-carotene and 17% of the lycopene still remained in the water and pelleted solid phases after centrifugation at 6750 × *g*. These constituents in the pellets may be able to be recovered by air-drying, re-grinding into a powder and re-extraction to enhance the final extraction efficiencies. Alternatively, the material could be used as animal feed, before or after re-extraction, in order to reduce the potential environmental pollution problem, which may be caused by disposal of the remaining materials as waste. The waste water can be disposed conventionally after some oil recovery by membrane filtration if required.

3.5 Comparison of microwave and air-drying of Gac arils followed by aqueous extraction with and without ultrasonication

After determining the optimal conditions for the ultrasound-assisted aqueous extraction of oil and carotenoids from the microwave-dried Gac aril (an ultrasound power of 320 W, an extraction time of 20 min, a powder particle size of 0.3-0.5 mm and a ratio of water to powder 9 g/g followed by centrifugation at 6750 × *g* for 40 min to separate the oil and carotenoids from the water and solid material after the extraction), a comparison was made between microwave-drying and air-drying of the Gac arils followed by aqueous extractions with and without ultrasound. For the aqueous extraction without ultrasound assistance, the same extraction and centrifugation conditions were used as for the ultrasound-assisted aqueous extraction. The level of lipid oxidation in the various oil extracts was also evaluated by measuring the PV.

The EEs for oil, β-carotene and lycopene and the PVs, which resulted from this experiment, are presented in Table 2. Statistical analysis showed that the two drying techniques had significantly different influences on the EEs for the oil, β-carotene and lycopene and on the PV ($P < 0.001$). The two extraction methods had significantly different impacts on the EE for the oil ($P < 0.001$) and lycopene ($P < 0.001$) but not for β-carotene and the PV ($P > 0.05$). There was also a significant interaction between the drying techniques and the extraction methods on the EE for lycopene ($P < 0.01$) but not on the EEs for the oil and β-carotene and the PV ($P > 0.05$).

As seen in Table 2, the EEs for the oil, β-carotene and lycopene were all significantly higher for microwave-drying than for air-drying, whether the extraction was ultrasound-assisted or not. This supported the findings described in Table 1 and extended the conclusion in that, microwave-

drying was also the most suitable drying technique when combined with the ultrasound-assisted aqueous extraction. In Table 2, it is also seen that the ultrasound-assisted aqueous extraction yielded higher EEs for the oil, β-carotene and lycopene than the aqueous extraction without ultrasound assistance. As discussed above, the release of the oil and carotenoids from the Gac aril powder was obviously facilitated by the cavitation effects engendered by the ultrasound [17, 38].

Table 2 also shows that the PVs were almost 5-fold higher for the air-dried powder compared with the microwave-dried powder, whether the aqueous extraction was ultrasound-assisted or not. In contrast, the PVs were nearly identical for the oils prepared by ultrasound-assisted aqueous extraction compared to the aqueous extraction without the ultrasound, whether microwave–dried or air-dried Gac aril powder was extracted.

The PV is often used to evaluate the initial stages of the oxidation process. It measures hydroperoxide products, which are formed by the reaction between oxygen and unsaturated fatty acids, hydroperoxides. It took much longer (12 h) to air-dry the Gac arils than it took to microwave-dry (1 h) the arils to the same moisture content (9%) and therefore, the arils were exposed for longer to high temperatures and oxygen during the air-drying process than they were when the microwave was used. Consequently, the conditions during air-drying promoted oxidation and resulted in PV values, which were 5-fold higher than for microwave-drying (Table 2). Therefore, not only was the efficiency of the oil extraction improved using microwave drying, it also yielded much lower PVs for the extracted oil compared to air-drying.

In order to investigate the effects of the two drying techniques, microwave and air-drying, and the aqueous extractions with and without ultrasonic treatment, on the external structure of the Gac aril material, the dried Gac aril powders (before extraction) and the material pelleted after the aqueous extraction mixtures were centrifuged, were analysed using a SEM (Figure 4). Before the aqueous extraction, the cell walls of the microwave-dried powder (Figure 4B) appeared more disintegrated compared to the air-dried powder (Figure 4A), suggesting that microwave-drying disrupted their membrane structures, as seen with other plant material [39]. Figure 4 also shows that the cell walls of the pelleted material recovered by centrifugation after the ultrasound-assisted aqueous extraction of the microwave-dried powder (Figure 4D) were the most disintegrated, compared to all the other extracted samples (Figs. C, E and F). Microfractures were clearly seen in this sample (Figure 4D) and the surface morphology was visibly changed, indicating that the surface morphology of the Gac aril material recovered by centrifugation after microwave drying and ultrasound-assisted aqueous extraction was more broken down than any of the other samples.

Therefore, these visual observations (Figure 4) demonstrated that the combination of microwave-drying the Gac arils followed by the ultrasound-assisted aqueous extraction enhanced the extraction efficiency of the Gac oil, β-carotene and lycopene by causing major disruption of the

Gac aril cells and thereby allowing a better contact between the components inside the cells and the solvent. As discussed above, ultrasound can cause strong shear forces in the vicinity of the cell membranes, due to the formation and collapse of bubbles, and cause the cells to fracture [40]. However, the disruption of cell walls was only apparent when the Gac arils had been dried using the microwave and extracted using ultrasound-assistance. The Gac aril material recovered after air-drying and aqueous extraction with (Figure 4C) or without (Figure 4E) ultrasound showed very little structural cell breakage.

The combination of microwave-drying and ultrasound during the extraction was also seen to be necessary for the observed surface morphology changes (Figure 4D) because microwave-drying followed by the aqueous extraction without ultrasound (Figure 4F) also showed very little structural change. This suggests that microwave-drying may cause enough weakening of the cellular structures (Figure 4B) to lead to substantial breakage of cell membranes during the ultrasound-assisted aqueous extraction but not enough for it to happen during aqueous extraction without ultrasound (Figure 4F). Therefore, it could be concluded that the combination of microwave drying and ultrasound-assisted extraction was more important to obtain higher recoveries of oil and carotenoids, and a lower peroxide value.

4. Conclusions

In conclusion, it was found that an ultrasound power of 320 W, an extraction time of 20 min, powder particle sizes of 0.3-0.5 mm, a ratio of water to powder of 9 g/g and a centrifugal force of 6750 × *g* were the best extraction and centrifugation conditions for the ultrasound-assisted aqueous extraction for microwave-dried Gac powder. Using those conditions, the extraction efficiencies for Gac oil, β-carotene and lycopene, and the PV for the oil were 90%, 84%, 83%, and 2.2 meq/kg, respectively. Moreover, compared with air-drying, it was found that microwave-drying was more suitable prior to ultrasound-assisted aqueous extraction; higher recoveries of oil and carotenoids, and a lower peroxide value were obtained. Scanning electron microscopy confirmed that ultrasound-assisted aqueous extraction in conjunction with microwave-drying caused substantial structural cell breakage in the Gac arils, resulting in increased oil, β-carotene and lycopene recoveries. Therefore, it can be concluded that microwave-drying followed by ultrasound-assisted aqueous extraction is an effective method for the extraction of Gac oil enriched in β-carotene and lycopene and with a low PV. Finally, Gac oil extracted in this way using water as the extraction solvent can be considered to be chemical free and suitable for use as a nutraceutical or an additive in the food industry.

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Figure 1

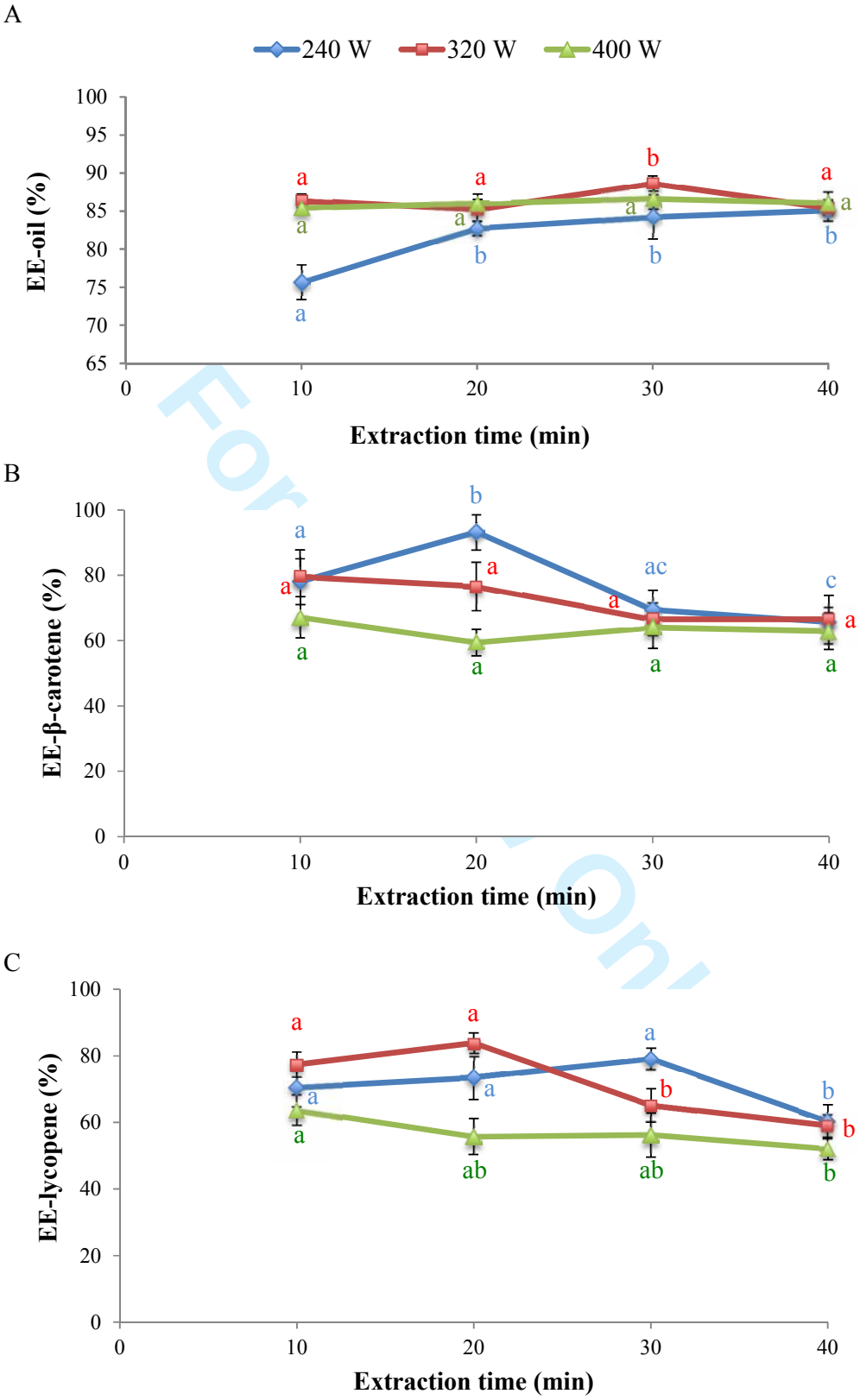

A
240 W
320 W
400 W
EE-oil (%)
Extraction time (min)
B
EE-β-carotene (%)
Extraction time (min)
C
EE-lycopene (%)
Extraction time (min)

Figure 2

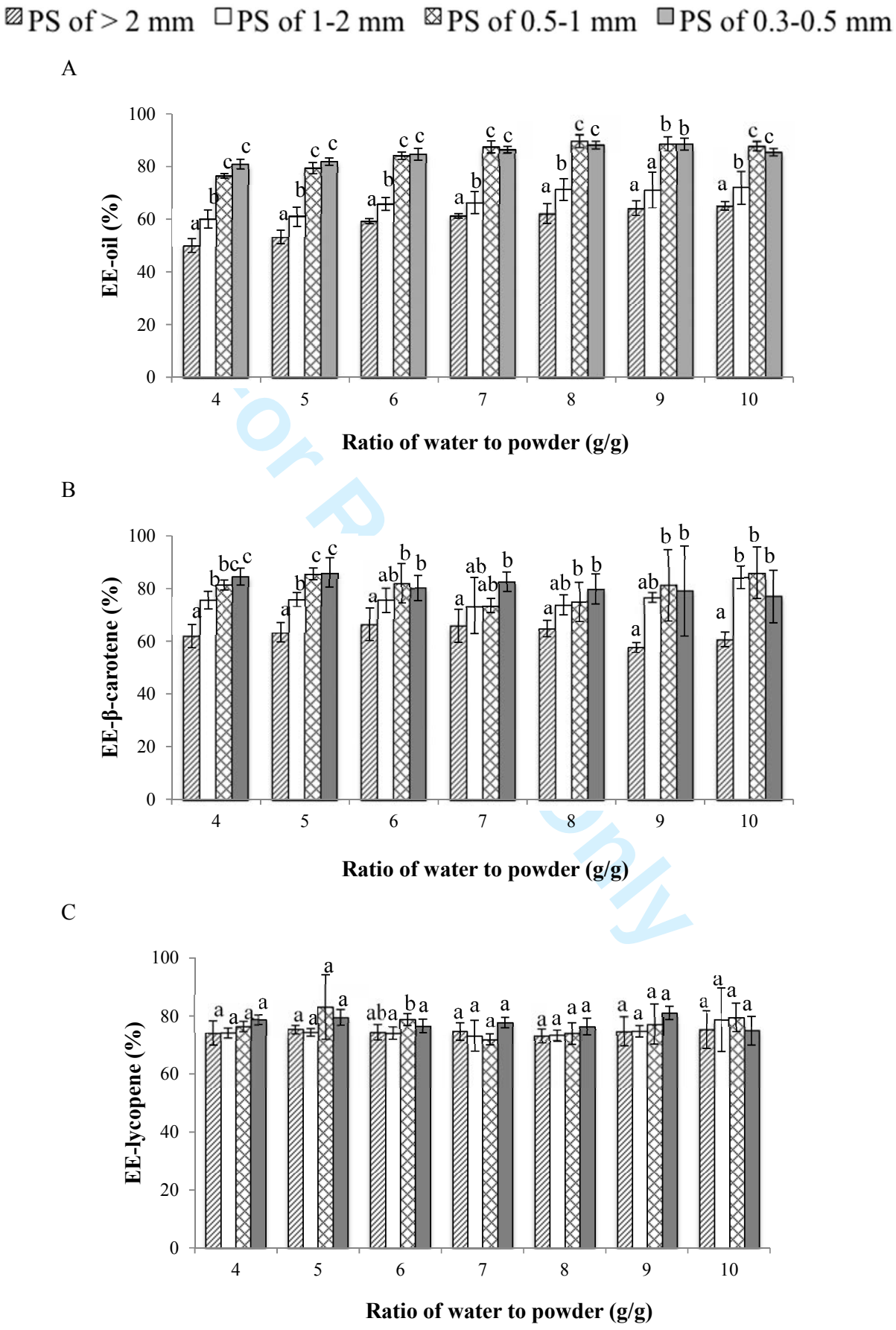

PS of > 2 mm
PS of 1-2 mm
PS of 0.5-1 mm
PS of 0.3-0.5 mm
A
EE-oil (%)
Ratio of water to powder (g/g)
B
EE-β-carotene (%)
Ratio of water to powder (g/g)
C
EE-lycopene (%)
Ratio of water to powder (g/g)

Figure 3

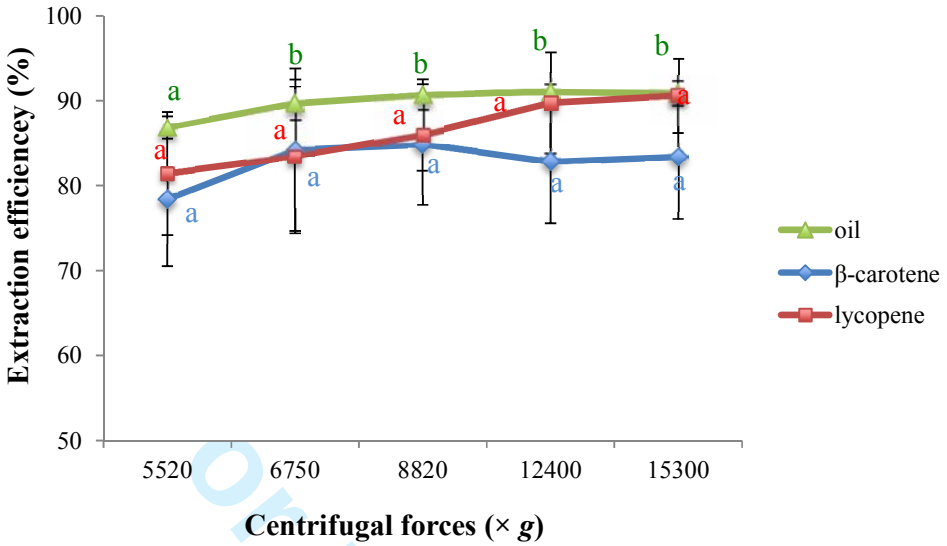
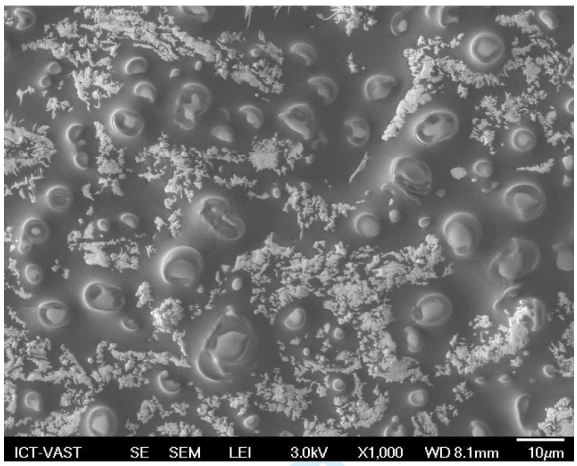
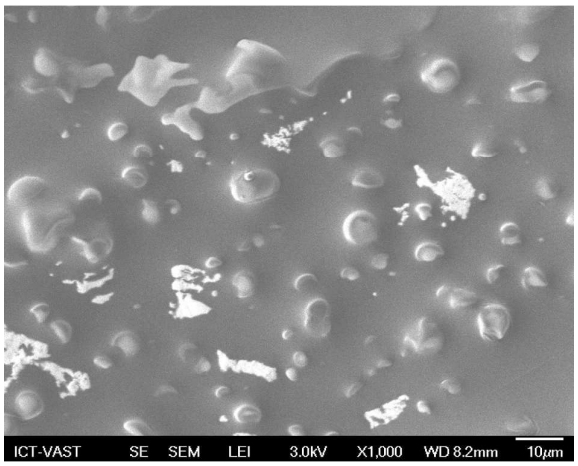


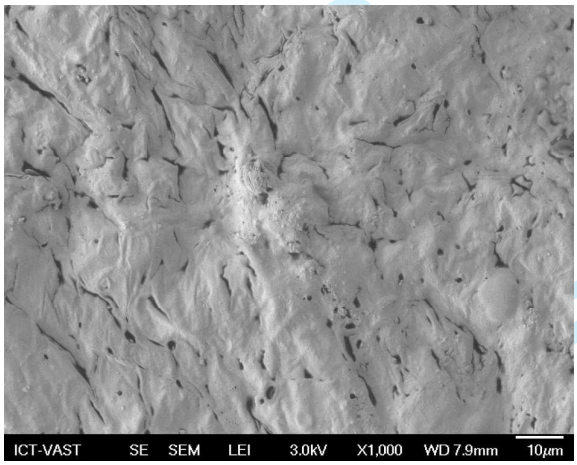
Figure 4



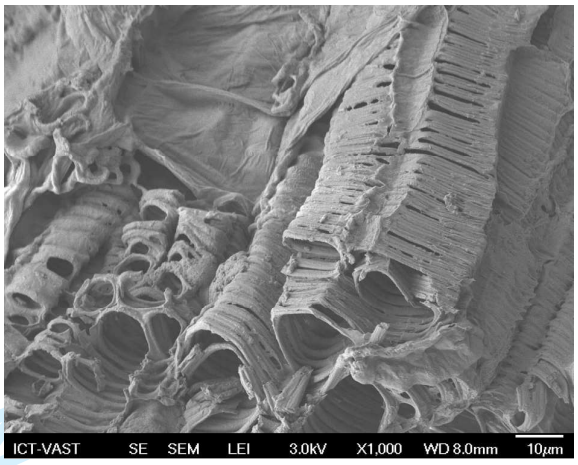
A. Air-dried powder before aqueous extraction



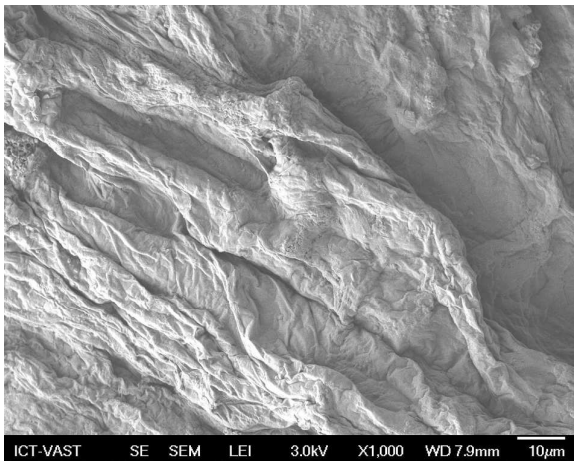
B. Microwave-dried powder before aqueous extraction



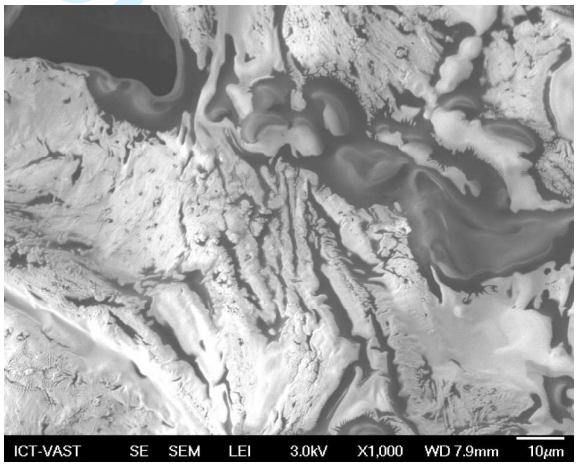
C. Pellet after ultrasound-assisted aqueous extraction of air-dried powder



D. Pellet after ultrasound-assisted aqueous extraction of microwave-dried powder



E. Pellet after aqueous extraction without ultrasound of air-dried powder



F. Pellet after aqueous extraction without ultrasound of microwave-dried powder

List of Tables

Table 1 Effect of microwave and air-drying on the total oil, β-carotene and lycopene content of Gac aril powders

	Fresh aril	Microwave-dried powder	Air-dried powder
Total oil content (% d.w.)	24.9±0.5[a]	24.7±1.1[a]	24.6±0.7[a]
β-carotene (mg/100 g d.w.)	155.7±17.2[a]	138.8±12.1[a]	77.5±7.5[b]
Lycopene (mg/100 g d.w.)	602.8±43.4[a]	551.5±33.2[a]	308.6±33.3[b]

Values in the same row followed by different superscripts (a-b) were significantly different (P <0.05).

Table 2. Comparison of microwave and air-drying before aqueous extraction of Gac oil with and without ultrasound assistance.

Drying	Air	Microwave	Air	Microwave
Aqueous Extraction	With Ultrasound		Without Ultrasound	
EE-oil (%)	67.6±2.7[a]	89.7±2.0[b]	58.4±3.6[c]	76.5±3.9[d]
EE-β-carotene (%)	42.0±3.2[a]	85.0±4.4[b]	39.6±5.48[a]	75.0±5.7[b]
EE-lycopene (%)	43.2±2.8[a]	79.6±4.0[b]	38.5±2.7[c]	62.1±2.5[d]
PV (meq/kg)	10.3±1.3[a]	2.2±0.6[b]	10.0±1.0[a]	2.2±0.8[b]

Values in the same row followed by different superscripts (a-d) were significantly different ($P < 0.05$).

Paper IV

2.1.3 Effects of Gac Aril Microwave Processing Conditions on Oil Extraction Efficiency, and β-carotene and Lycopene Contents

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Effects of Gac aril microwave processing conditions on oil extraction efficiency, and β-carotene and lycopene contents

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ABSTRACT

The effects of Gac oil extraction conditions including microwave power, microwave time, steaming time and hydraulic pressure on extraction efficiency (EE), and β-carotene and lycopene contents were studied. It was found that the EE, and β-carotene and lycopene contents could be enhanced by suitable extraction conditions. Microwave drying was found to be better than air drying for pretreatment. Moisture content after drying and steaming between 8% and 11% (wt/wt) were best for pressing. Results showed that the most suitable conditions for Gac oil extraction from 900 g samples were microwave power of 630 W, microwave time of 65 min, steaming time of 20 min and hydraulic pressure of 170 kg/cm^2. Under these conditions, the highest EE of 93% was achieved while Gac oil contained the highest content of β-carotene and lycopene at 140 and 414 mg/100 mL, respectively.

1. Introduction

Gac fruit (*Momordica cochinchinensis* Spreng) contains high levels of bioactive compounds, especially essential fatty acids, β-carotene and lycopene (Aoki et al., 2002; Kubola and Siriamornpun, 2011; Nhung et al., 2010; Vuong et al., 2006) that are well known to be beneficial in human health. For example, the aril of Gac fruit contains a significant amount of fatty acids, at 102 mg per g of edible portion (Vuong et al., 2002). About 70% of total fatty acids in the aril are unsaturated, and 50% of these are polyunsaturated (Vuong, 2000). The highest concentrations of β-carotene and lycopene are found in the fresh aril, at 101 μg/g and 380 μg/g respectively (Aoki et al., 2002). Furthermore, the presence of fat in the Gac fruit aril plays an important role in the absorption of carotenes and other fat-soluble nutrients (Kuhnlein, 2004). The benefits from Gac nutrients can be obtained from the consumption of Gac powder (Kha et al., 2010, 2011). However there are many applications where just Gac oil is needed. Therefore, it is important to determine a suitable extraction method for Gac oil which yields the maximum extraction efficiency (EE) and bioactive compounds including β-carotene and lycopene.

Generally, mechanical extraction by hydraulic or screw press is one of the most common methods in oil processing industry. However, the EE seldom reaches 70% when a hydraulic press is used (Bargale et al., 1999; Owolarafe et al., 2007). Therefore, attempts have been made to use air-drying or microwave irradiation and steaming prior to pressing to enhance the EE. Recent interest in microwave extraction for plant oils has been highlighted owing to their benefits (Desai et al., 2010). Effect of treatment with microwave radiation before mechanical pressing on oil recovery and quality of oil from Chilean hazelnut was investigated by Uquiche et al. (2008). Different microwave treatment conditions (potencies and times) were studied, and an increase in oil yield was found in comparison with untreated samples. Moreover, the microwave-pre-treated oil samples also possess superior quality characteristics in terms of oxidative oil stability, oil composition and other physicochemical properties, as compared to untreated one. Similarly, Azadmard-Damirchi et al. (2010) reported that a significantly higher yield of oil from rapeseed could be obtained by treatment with microwaves before pressing. Results also indicated that this extraction method could be used to produce rapeseed oil with high oxidative stability and nutraceutical content. Therefore, investigations on the microwave treatment conditions prior to pressing oil from Gac aril are recommended.

Moreover, steaming treatment of the microwave-dried material is one of the most important steps in the oil extraction process. This step can adjust the moisture content of starting materials prior to pressing so it is the most significant factor affecting the oil yield (Mpagalile and Clarke, 2005; Sivala et al., 1991). In addition, under steaming process, the heat can soften and break down the oil containing cells, and also lower oil viscosity (Mpagalile and Clarke, 2005; Ward, 1976). Hence the oil is easily released in sub-

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sequent pressing (Fasina and Ajibola, 1990; Sivakumaran et al., 1985). As a result, to achieve the highest EE, it is important to determine the optimal moisture content of the samples prior to pressing.

There is a lack of information on Gac aril pre-pressing treatments, such as microwave and steaming treatments, prior to hydraulic press in the published literature. Therefore, the objective of this study was to investigate effects of those treatments on the oil EE and the retention of β-carotene and lycopene in comparison with air-drying treatment.

2. Materials and methods

2.1. Chemicals

Solvents and BHT (butylated hydroxytoluene) were purchased from Merck Pty. Ltd. β-Carotene (C4582, type II synthetic, ⩾95%, HPLC grade) and lycopene (L9879, ⩾90%, from tomato) standards were obtained from Sigma–Aldrich Pty. Ltd.

2.2. Gac fruit

Gac fruit, of uniform yellow–red skin and size, was purchased from a local market in Ho Chi Minh city, Vietnam. The fruit was transported in an insulated hard plastic container to avoid light and temperature exposure during transport. The red arils containing seeds were then scooped out and frozen at temperature of −18 °C for further use.

2.3. Equipment

Microwave oven (Panasonic Dimension NN-9853) with cavity dimensions
height × width × depth = 244 mm × 412 mm × 426 mm and operating frequency at 2450 MHz was used in this study.

The laboratory hydraulic press (length:width:-height = 650:850:1900 mm) was designed and made by Tinh Anh company, Vietnam. The press was designed for using cages with different dimensions. A stainless steel press cage used in this study was 210 mm high, 112 mm in internal diameter and 3 mm in thickness. The size of the openings located at the cage was 6 mm in diameter.

2.4. Experiment

The frozen Gac aril including seeds (about 900 g) was thawed at 4 °C prior to microwave drying treatments. The aril (with initial moisture content of about 82 ± 3%) was spread (with a thickness of 5 mm) onto the turntable plate and subjected to heating at different power settings of 900, 630 and 495 W. The treatments were terminated as the samples reached the final moisture content of about 9 ± 1% (wet basis), based on preliminary trials. The preliminary results showed that above this moisture content for microwave drying, the aril would not be pressed adequately. The fixed factors including steaming time and the hydraulic pressure were 20 min and 170 kg/cm^2, respectively. After determining the most suitable power based on the EE and retention of β-carotene and lycopene, different microwave drying times (50, 55, 60, 65 and 70 min) were also investigated. The steaming time (20 min) and the pressure (170 kg/cm^2) were fixed. During the microwave time range, there were no burning or other signs of uneven drying of the sample.

The seeds were removed from the dried aril by hand. Only the aril was then powdered using a laboratory blender. A 5 mm layer of the ground samples were placed inside a stainless-steel steam cooker and steamed for specified times (10, 15, 20, 25 and 30 min) at the steam cooker conditions. Before that, a constant amount of drinking water (2 L) was placed and boiled inside the steam cooker. After the steaming process, the samples were immediately wrapped inside filtration cloths for pressing and the moisture content of samples was also determined. The fixed factors were the microwave power of 630 W, the microwave time of 65 min, and the pressure of 170 kg/cm^2.

After determining the most suitable microwave power, microwave time and steaming time, the samples were pressed at different working hydraulic pressures of 140, 150, 160, 170 and 180 kg/cm^2. The process was performed in two main stages consisting of a compression stage for 0.2 min, where the direct target pressure on the material was achieved, and a relaxation period of 5 min, which were repeated 3 times. The total time for the extraction process was about 15.6 min after which there was no additional oil extraction.

For comparison, air-dried aril was also used instead of the microwave-dried. The fresh aril (about 900 g) including the seeds within, was air-dried at 60 °C for 18 h to reach a final moisture content of approximate 6% (wet basis) based on previous trials. The seeds were then removed by hand and the aril was powdered using the laboratory blender. The Gac oil extraction process using the powdered samples was then performed similar to that for microwave. The fixed processing conditions were steaming time of 20 min and the hydraulic pressure of 170 kg/cm^2.

2.5. Analytical methods

2.5.1. Moisture content determination

The moisture content of microwave-treated and steamed samples were gravimetrically analyzed using an AnD MX-50 moisture analyser (A&D company, Limited, Japan).

The moisture content of oil samples was determined according to Ca 2c-25 (AOCS, 1998). About 5 g of oil sample was placed into a tared moisture dish and air-dried at 130 ± 1 °C for 30 min. After that, the dish was removed and cooled to room temperature in a desiccator and weighed. The moisture content was calculated when the loss in weight did not exceed 0.05% per 30 min drying period.

2.5.2. Soxhlet extraction

The total oil content of microwave-treated Gac samples prior to each press was determined using the FOSS Soxtec™ system 2045 extraction unit, Denmark. A procedure of oil extraction was carried out according to the method described by Brkić et al. (2006) with slight modification. Briefly, approximately 3 g of the sample was weighted in cellulose thimbles and inserted into the extraction system. Then 50 ml petroleum ether (boiling point of 60–90 °C) were filled into weighted aluminum vessels and inserted into the extraction unit as well. The temperature was set at 145 °C and the program of the oil extraction process was: 30 min of boiling, 60 min of rinsing, 15 min of evaporation and finally 20 min of drying the aluminum vessels containing the extracted oil. The sample was then dried in an oven at temperature of 80 °C until constant weight was obtained. The oil content in samples was gravimetrically calculated.

2.5.3. Oil extraction efficiency

The EE was determined as a ratio of mass of extracted oil after subtracting moisture and mass of oil in starting material using the Soxtec extraction unit. The oil content of dried Gac aril was in the range of 18–34% (wt/wt).

2.5.4. Determination of β-carotene and lycopene

A method of Englberger et al. (2006) was employed for analyzing β-carotene and lycopene in Gac oil samples. HPLC analysis was

performed with an Agilent 1200 HPLC equipped with diode array detector system consisting of a Luna C18 (100 × 4.6 mm i.d; 5 μm) direct-connect guard column coupled to a Jupiter C18 (250 × 4.6 mm i.d; 5 μm) reversed phase column (Phenomenex). The mobile phase consisted of acetonitrile, dichloromethane and methanol (ACN:DCM:MeOH) 50:40:10 v/v/v, containing 0.1% BHT. The flow rate was 1.0 mL/min, detection was at 450 nm, and the injection volume was 20 μL. The resultant Gac oil (10 μL) was dissolved in chloroform (2 mL) and then diluted at a ratio of 1:200 with the HPLC solvent. All operations were performed under subdued light to minimize oxidation of the carotenoids. The identification of β-carotene and lycopene were based solely on the retention time of a peak compared with the authentication standards. The amount of β-carotene and lycopene in Gac oils were expressed as mg/100 mL of crude oil.

2.6. Statistical analysis

The independent experiments and subsequent measurements were all done in triplicate. A one-way analysis of variance (including microwave power setting, microwave exposure time, steaming time and hydraulic pressure) and LSD (least significant difference) were employed to analyze the data using SPSS software version 19.0.

3. Results and discussion

3.1. Effect of microwave power setting on extraction efficiency, β-carotene and lycopene

The HPLC system was used to identify the carotenoid content in Gac oil samples. The chromatograms of carotenoid standards (Fig. 1) indicated that retention times of β-carotene and lycopene were 6.081 and 7.212 min, respectively. High content of β-carotene and lycopene in the Gac oil extracted from different processing conditions was found.

The EE and β-carotene and lycopene contents of Gac oil extracted at the different microwave power and time settings, steaming time of 20 min and pressure of 170 kg/cm^2 are shown in Table 1. In general, results indicated that power setting significantly influenced the EE, β-carotene and lycopene ($P < 0.001$). The highest EE was obtained at the microwave power of 630 W. There was a significant loss of β-carotene and lycopene contents as the power increased from 630 to 900 W, 56% and 38%, respectively. At the low power of 495 W, the reduction of carotenoids observed was not statistically significant ($P > 0.05$).

It can be seen in Table 1 that the power of 630 W resulted in higher EE and better retention of nutrients. A possible explanation is that this microwave power provided enough driving force to break down the plant cell matrix but not enough to destroy the nutrients. Under a light microscope, the cell membranes of microwave dried samples were observed to be more ruptured than those of air dried ones (result not shown). It is generally agreed that the extraction yield increases proportionally with increasing microwave power up to a limit after that the increase results in poorer yield (Chan et al., 2011; Mandal and Mandal, 2010). Furthermore, higher microwave power might cause deterioration of nutrients (Chan et al., 2011). Therefore, the content of β-carotene and lycopene in the samples treated at the power of 900 W was much lower than those of the powers of 495 and 630 W.

3.2. Effect of microwave time on EE, β-carotene and lycopene

After choosing the power of 630 W, as most suitable, it was desirable to find out the best microwave processing time to enhance the EE, and β-carotene and lycopene contents during the extraction process. The EE, β-carotene and lycopene were affected by the microwave time as shown in Fig. 2. Generally, a significant increase in the microwave time in the investigated range of 50–65 min resulted in higher EE, β-carotene and lycopene ($P < 0.001$). However, the EE remained unchanged while β-carotene and lycopene contents were slightly and notably decreased respectively, as the microwave time increased from 65 to 70 min. Statis-

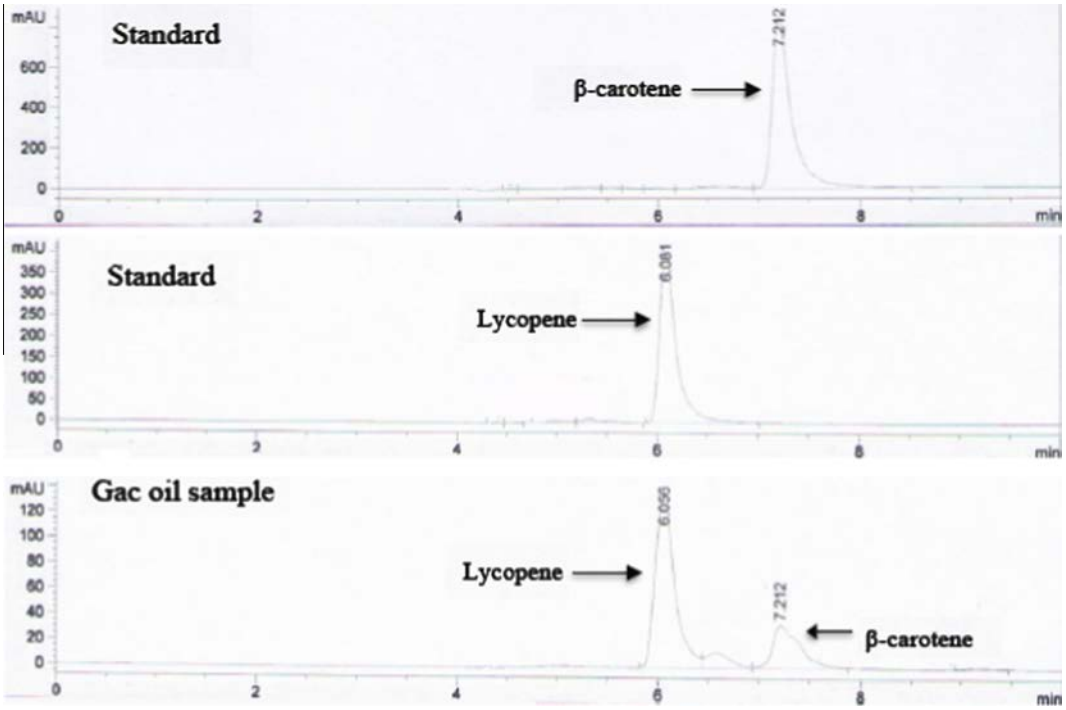

Fig. 1. Typical chromatograms of carotenoid standards and Gac oil sample.

Table 1
Effect of microwave power settings on the EE, and β-carotene and lycopene contents.

Microwave power/time (W/min)	495/95	630/60	900/45
EE (%)	51 ± 2[a]	84 ± 1[b]	65 ± 2[c]
β-Carotene (mg/100 mL)	135 ± 7[a]	134 ± 8[a]	59 ± 8[b]
Lycopene (mg/100 mL)	287 ± 12[a]	293 ± 21[a]	180 ± 17[b]

The values in the same row followed by different superscripts (a–c) were significantly different ($P < 0.05$).

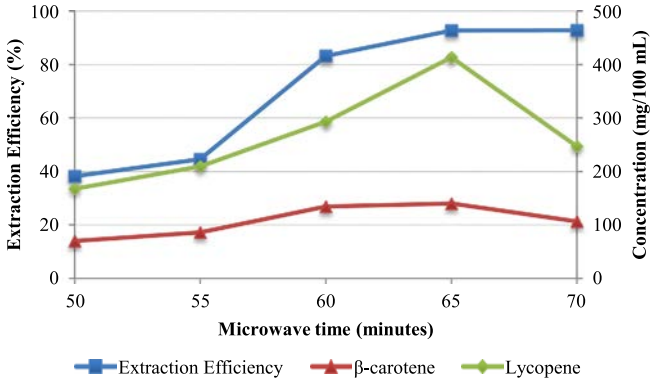

Fig. 2. Effect of microwave time on EE, β-carotene and lycopene.

tical results showed that there was insignificant difference in EE as samples were microwave-treated between the time of 65 and 70 min ($P > 0.05$). However, there were insignificant differences in β-carotene as treated between the time of 50 and 55 min, and between 60 and 65 min ($P > 0.05$).

It is evident from Fig. 2 that the EE improves with increases in microwave treatment time. This is in agreement with several studies reporting that the microwave time had a positive effect on the EE of oil plant materials such as hazelnuts (Uquiche et al., 2008), palm (Cheng et al., 2011) and rapeseeds (Azadmard-Damirchi et al., 2010). Increase in microwave time results in lower moisture content in samples (Fig. 4) which makes them more brittle and renders a greater cell membrane rupture (Uquiche et al., 2008), therefore more oils would be released later during the mechanical pressing. Furthermore, higher EE can be obtained due to modifications in the cellular walls and a greater porosity achieved by microwave treatment (Oberndorfer et al., 2000; Uquiche et al., 2008). As a result, the oil can flow out more readily and more completely during the mechanical pressing (Oberndorfer et al., 2000).

In terms of retention of β-carotene and lycopene in the microwave-treated samples (Fig. 2) prior to steaming and pressing, increase in the retention of nutrients can be obtained by increasing the microwave time from 50 to 65 min. The highest retention can be achieved at the microwave time of 65 min. Damage to the plant cell membrane by microwave treatment may assist increased release of β-carotene and lycopene. However, a significant loss was observed by increasing the time up to 70 min. It is because over exposure to microwave radiation may cause loss of carotenoids due to thermal degradation.

3.3. Effect of steaming time on EE, β-carotene and lycopene

To investigate whether steaming time affects the EE, β-carotene and lycopene, the microwave time of 65 min and the hydraulic pressure of 170 kg/cm^2 were fixed while the steaming time was varied. Results indicated statistically that the EE, β-carotene and lycopene were affected by the steaming time ($P < 0.001$). Fig. 3 shows the effect of steaming time on the EE, β-carotene and lycopene. An increase in the steaming time from 10 to 20 min resulted in higher EE, β-carotene and lycopene. In a steaming period of 20–30 min, however, the EE was slightly decreased whereas the β-carotene and lycopene were significantly reduced in quantity. The loss of lycopene was found to be much more pronounced than that of β-carotene.

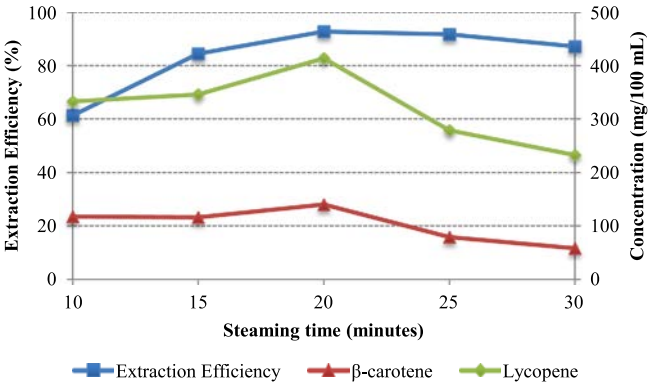

Fig. 3. Effect of steaming time on the EE, β-carotene and lycopene.

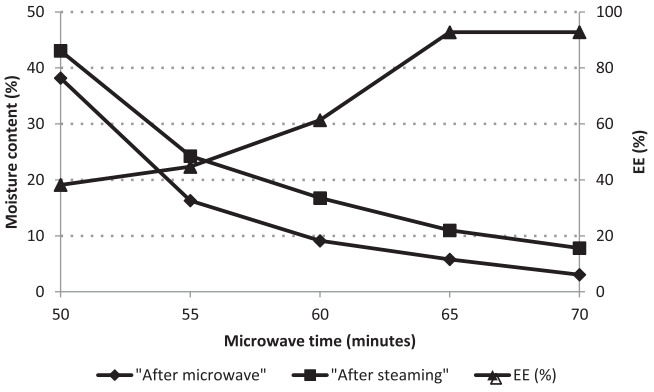

Fig. 4. Effects on EE by moisture content of samples after treatments of microwave time and steaming time before pressing.

In prior preliminary experiments (results not shown), the EE was low when the microwave-dried samples were pressed without steaming. The EE was improved when the samples were steamed prior to pressing. A reason for this is that the microwave-dried tissues would absorb the moisture during the steaming process. The tissues were then expanded, weaken the oil cell walls which broke down, and therefore the oils were easily released as the pressure was applied (Fasina and Ajibola, 1990). Furthermore, the steaming also lowers viscosity of the oil to be pressed and adjusts the moisture content of the sample to an optimal level before pressing (Khan and Hanna, 1983). Therefore, it can be concluded that the steaming prior to the mechanical pressing is a very important step to get more oil from the materials. It can be seen in Fig. 3, steaming time of 20 min would be optimal due to better retention of carotenoids and higher oil yield.

Carotenoid losses during the treatments by microwave (65–70 min) and steaming (20–30 min) were similar. Compared with β-carotene, the lycopene degradation was found to be higher as indicated by the greater slope of the lines in Figs. 2 and 3. This may be due to differences in the chemical structure of carotenoids and their relations to the chemical reactivity (Britton, 1995). For example, lycopene is sensitive to oxidation during extraction processing (Shi et al., 2008) and has a higher singlet oxygen quenching ability and antioxidant capacity (Bohm et al., 2002; Weisburger,

2002; Woodall et al., 1997) due to its unique chemical structure of longer conjugated polyene chain. This result is also consistent with several reports which stated that lycopene was destroyed faster than β-carotene under various conditions (Cao-Hoang et al., 2011; Henry et al., 1998; Nhung et al., 2010).

3.4. Effect of the moisture content on EE

Microwave dried samples were steamed for 20 min to increase their moisture contents then pressed at 170 kg/cm^2. The resulted EE is plotted together with moisture content in Fig. 4.

Fig. 4 shows that the EE was significantly increased as the moisture content of samples was reduced prior to pressing. For example, the EE was very low at about 40% when the moisture content of the sample before pressing was about 43%. However, significant increase in the EE to approximately 93% was achieved as the moisture content of samples (prior to pressing) decreased to between 8% and 11%. This result is in agreement with other studies that reported a maximum oil yield was obtained as moisture content of samples before hydraulic pressing for oil bearing plant materials such as melon seeds (Ajibola et al., 1990), conophor nut (Fasina and Ajibola, 1990) and coconut (Mpagalile and Clarke, 2005). In contrast, to achieve maximum oil yield of pennycress seed oil by screw pressing, the moisture content of sample was much lower, just between 3% and 4%. The difference in the suitable moisture contents is expected to be due to physicochemical characteristics of materials and different pressing equipment used.

3.5. Effect of hydraulic pressure on EE, β-carotene and lycopene

Statistical results showed that hydraulic pressure significantly influenced the EE, β-carotene and lycopene ($P < 0.01$). Generally, increases in EE, β-carotene and lycopene were observed as the pressure increased from 140 to 170 kg/cm^2 (Fig. 5). Interestingly, there were insignificant differences in EE, β-carotene and lycopene as the pressure applied was increased from 170 to 180 kg/cm^2.

It can be seen that the EE increased about 5% or 6% with each 10 kg/cm^2 of pressure increment between 140 and 160 kg/cm^2. However, increase in the EE was twice higher when the pressure rose from 160 to 170 kg/cm^2. At 170 kg/cm^2 and beyond, the EE very slightly decreased. This result is also consistent with several published reports (Bargale et al., 1999; Baryeh, 2001; Mpagalile and Clarke, 2005; Willems et al., 2008). The reason for this phenomenon is that some interfibers or capillary voids of the cell walls may be sealed with increasing pressure; hence the oil flow is prevented (Baryeh, 2001; Ward, 1976). It can therefore be concluded that the mechanical pressure of 170 kg/cm^2 is adequate for maximizing the EE. Any pressure below 170 kg/cm^2 is not recommended.

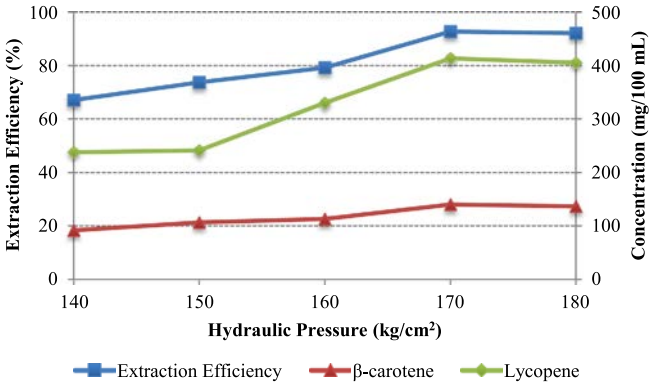

Fig. 5. Effect of hydraulic pressure on EE, β-carotene and lycopene.

Table 2
Effect of microwave and air-drying treatments on the EE, and β-carotene and lycopene contents.

Oil samples	EE (%)	β-Carotene (mg/100 mL)	Lycopene (mg/100 mL)
Microwave-dried	93 ± 1	140 ± 7	414 ± 25
Air-dried	68 ± 3	55 ± 7	240 ± 29
Vuong and King (2003)	na	271	302

na: not available.

Similar to the EE, higher extractability of β-carotene and lycopene in the oil samples could be obtained by increasing pressure. However, further increase in the pressure slightly reduced the amount of carotenoids. As a result the pressure of 170 kg/cm^2 is considered as optimum for the carotenoid extraction.

3.6. Comparison of microwave-dried oil samples and air-dried oil samples

Several authors reported that microwave assisted extraction is one of the most effective methods to obtain higher extraction efficiency and effectively improve the extraction of bioactive compounds (Cravotto et al., 2008; Desai et al., 2010; Tatke and Jaiswal, 2011). It is therefore important for this study to compare microwave-drying and air-drying on the quality of Gac oils in terms of the EE, β-carotene and lycopene. Comparison of these parameters affected by microwave and air-drying treatments prior to pressing is presented in Table 2.

As can be seen in Table 2, microwave-drying treatment prior to pressing greatly enhanced the EE. In this study, the EE could rise up to 25% by using microwave-drying to reduce the moisture in the fresh aril instead of air-drying. This is because the moisture inside the material is heated up due to the effect of microwave, resulting in evaporation and generating high pressure inside the cell wall. As a result, the cell membrane is ruptured (Tatke and Jaiswal, 2011); the oil from the ruptured cells is easily subsequently released and extracted by the hydraulic press. In contrast, the rupturing of cell membranes is not taking place in the air-drying process to the same extent due to the evaporation occurring on the surface of the material.

Compared to air-drying treatment prior to pressing, the β-carotene and lycopene contents in the oil samples using microwave treatment were much higher. In other words, a significant loss of carotenoids was caused by air-drying. The main reason for this degradation is due to heat and oxygen. Shi et al. (1999) reported that tomato tissue was broken down by heat treatment in conventional air-drying and was easily exposed to oxygen, which caused the loss of lycopene. Furthermore, the lycopene content of the microwave-dried oil sample was higher than that of the air-dried sample in this current study and in the published data from Vuong and King (2003). However, the highest β-carotene content of the published oil sample was observed, followed by microwave dried and air-dried samples. According to this study, the fresh aril was also air-dried at 60 °C until reaching the moisture of 12–15%, then smashed and hot steamed for 5 min prior to pressing. The difference in carotenoids of the oil samples between the current and published study may be due to the different varieties, growing conditions, stage of maturity and storage conditions of the fresh Gac fruit, and the varied drying process conditions examined. Furthermore, it is possible to have variability within the fruit.

4. Conclusions

In summary, the EE, β-carotene and lycopene contents of the Gac oil were significantly affected by microwave power, microwave drying time, steaming time and hydraulic pressure. Generally, increase in the EE, and β-carotene and lycopene contents of Gac oil samples was found with increasing these parameters. Microwave and steaming treatments prior to pressing are the most important steps in the Gac oil processing due to significantly increasing the EE, β-carotene and lycopene. Finally, results indicated that the most suitable processing conditions for Gac oil extraction were the microwave power of 630 W, microwave time of 65 min, steaming time of 20 min and hydraulic pressure of 170 kg/cm^2. Under these conditions, the highest EE of 93% was achieved and Gac oil contained highest content of β-carotene and lycopene as 140 and 414 mg/100 mL, respectively. These results could be useful in encouraging Gac oil processors to use microwave and steaming treatments before pressing to improve extraction efficiency and preserve nutrients.

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Paper V

2.1.4 Optimisation of Microwave-assisted Extraction of Gac Oil at Different Hydraulic Pressure, Microwave and Steaming Conditions.

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Original article

Optimisation of microwave-assisted extraction of Gac oil at different hydraulic pressure, microwave and steaming conditions

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Summary The study aimed to optimise Gac (*Momordica cochinchinensis* Spreng) oil extraction conditions, including microwave time, steaming time and hydraulic pressure, for maximising extraction efficiency (EE), and β-carotene and lycopene contents, using response surface methodology. Results indicated that the data were adequately fitted into three second-order polynomial models for EE, β-carotene and lycopene with R^2 values of 0.93, 0.85 and 0.86, respectively. It was predicted that the optimum extraction conditions within the experimental ranges would be the microwaving time of 62 min, steaming time of 22 min and hydraulic pressure of 175 kg cm^{-2}. Under such parameters, the maximum EE of 86%, β-carotene content of 186 mg per 100 mL oil and lycopene content of 518 mg per 100 mL oil were achieved as predicted.

Keywords Carotenoids, Gac oil, microwave, pressing, response surface optimisation, steaming.

Introduction

Several studies reported that Gac fruit aril (*Momordica cochinchinensis* Spreng) is an excellent source of carotenoids, α-tocopherol, flavonoids, medicinal compounds and essential fatty acids (De Shan *et al*., 2001; Aoki *et al*., 2002; Vuong *et al*., 2006; Xiao *et al*., 2007; Kha *et al*., 2010; Nhung *et al*., 2010; Kubola & Siriamornpun, 2011). Importantly, high levels of carotenoids, especially β-carotene and lycopene, were found, in comparison with other fruits and vegetables (Bauernfeind, 1972; Vuong, 2000; Aoki *et al*., 2002). The aril of Gac fruit also contains a significant amount of fatty acids, at 102 mg g^{-1} of edible portion (Vuong *et al*., 2002). About 70% of total fatty acids in the aril are unsaturated, and 50% of these are polyunsaturated (Vuong, 2000). These high content of fatty acids, β-carotene and lycopene are found in Gac aril oil and considered to be beneficial to humans.

To obtain a good quality of plant oil, in general, plant materials such as Gac fruit are extracted using a hydraulic press. The oil yield is often low (Bargale *et al*., 1999; Owolarafe *et al*., 2007); therefore, several treatments such as air drying and hot steaming before pressing were employed to enhance the extraction efficiency (EE) (Fasina & Ajibola, 1990; Mpagalile & Clarke, 2005). However, the oil quality may be degraded due to using heat in the processing steps. Consequently, it is a challenging task to determine the optimum conditions for the extraction process of Gac oil to maximise the oil content while minimising any loss of nutrients.

Recently, conventional extraction using harmful organic solvents has been discarded due to health concerns, environmental problems and quality degradation. It is important to find a suitable alternative extraction method of Gac oil using food grade solvents or eliminating the use of solvents completely. Microwave-assisted extraction (MAE) is a novel alternative method for oil extraction. The principle of this process is to heat up the moisture inside a plant cell using microwave radiation, thus generating evaporation and high pressure within the cell wall. The cell membrane will therefore be ruptured, and the oil from the ruptured cells will be released increasing the oil yield (Uquiche *et al*., 2008; Azadmard-Damirchi *et al*., 2010). In addition, the use of this extraction also offers several benefits such as being environmental friendly, solvent free and allowing reduced processing times and uniform heating (Uquiche *et al*., 2008). The advantages and drawbacks of microwave-assisted extraction from food have been highlighted previously (Kaufmann & Christen, 2002; Desai *et al*., 2010; Tatke & Jaiswal, 2011).

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Response surface methodology (RSM) is the most popular optimisation technique studied in recent years. It is effective to use the RSM method to determine the relationships between the response and the independent variables and optimising the processes or products (Baş & Boyaci, 2007). The technique also allows the evaluation of the effect of multiple parameters and their interactions on the output variables with a reduced number of trials (Lee *et al*., 2000). It is faster and more economical than other approaches required to optimise a process. According to Baş & Boyaci (2007) and Bezerra *et al*. (2008), an optimisation study using RSM includes three main stages: (i) the screening of the independent variables and their levels; (ii) the selection of experimental design and the prediction and verification of the model equation; and (iii) the graphical presentation of the model equation and determination of optimal operating conditions. Furthermore, it is very important to choose the appropriate design. If the data present curvature, experimental design for first-order models cannot be used, hence it should use experimental designs for quadratic response surfaces, including three-level factorial, Box–Behnken, central composite and Doehlert designs (Bezerra *et al*., 2008).

There is a lack of information on optimisation of Gac aril treatments prior to hydraulic pressing using response surface methodology. Therefore, experiments designed for this study aimed to optimise Gac oil extraction conditions including microwave drying and steaming treatments and hydraulic pressure to obtain the maximum extraction efficiency and the highest content of β-carotene and lycopene in Gac oil.

Materials and methods

Chemicals

Solvents and an antioxidant (butylated hydroxytoluene, BHT) were purchased from Merck Pty. Ltd. (Sydney, NSW, Australia). β-apo-8'-carotenal, β-carotene (C4582, type II synthetic, $\geq 95\%$, HPLC grade) and lycopene (L9879, $\geq 90\%$, from tomato) standards were obtained from Sigma-Aldrich Pty. Ltd. (Sydney, NSW, Australia).

Gac fruit

Gac fruit, of a uniform yellow-red skin and size, was purchased from a local market in Ho Chi Minh city, Viet Nam. The fruit was transported in an insulated hard plastic container to avoid light and temperature exposure during transport. The red arils containing seeds were then scooped out and frozen at temperature of −18 °C until use. The freezing may cause cell disruption; however, no evidence of oil release after thawing was found, probably because of the high solid contents.

Table 1 The coded and uncoded levels of independent variables

Coded variable levels	Exposure time X_1, min	Steaming time X_2, min	Hydraulic pressure X_3, kg cm^{-2}
+1.682	66.7	35.1	186.8
+1	65.0	30.0	180.0
0	62.5	22.5	170.0
−1	60.0	15.0	160.0
−1.682	58.3	9.9	153.2

Microwave drying treatment

Frozen Gac aril was thawed at 4 °C prior to microwave drying treatments. The aril, including seeds (about 900 g), was spread onto the turntable plate with a thickness of 5 mm. The samples were then dried at the microwave power of 630 W for different times (Table 1), using a Panasonic Dimension NN-9853 microwave (Panasonic Australia Pty. Ltd., Frenchs Forest, NSW, Australia) with cavity dimensions height × width × depth = 244 × 412 × 426 mm and an operating frequency at 2450 MHz.

Steaming treatment

The microwave-dried samples had the seeds removed by hand and then were powdered using an IKA M20 grinding mill (IKA® Company, Staufen, Germany). The ground samples (with a thickness of 5 mm) were placed inside a stainless steel steam cooker and steamed at ambient conditions for different times as shown in Table 1. Before that, a constant amount of drinking water (2 L) was placed and boiled inside the steam cooker. After steaming, the samples were immediately wrapped inside filtration cloths for pressing. The moisture content of the samples was also determined after cooling to room temperature in a desiccator.

Hydraulic press

The laboratory hydraulic press (length: width: height = 650: 850: 1900 mm) was designed and made by Tinh Anh company, Ho Chi Minh city, Viet Nam. The press was designed for using cages with different dimensions. The stainless steel press cage used in this study was 210 mm high, 112 mm in internal diameter and 3 mm in thickness. The size of the openings located at the cage was 6 mm in diameter. The maximum working hydraulic pressure was up to 250 kg cm^{-2}.

For each test, the samples after steaming were wrapped inside four layers of filtration cloths and pressed at different designed pressures (Table 1). The process was performed in two main stages consisting of a compression stage of 0.2 min, where the direct

target pressure on the material was achieved, and a relaxation period of 5 min, which were repeated three times. The total time for the extraction process was about 15.6 min after which there was no additional oil release.

Experimental design

Response surface methodology (RSM) was employed to optimise the Gac oil extraction conditions on the oil extraction efficiency, β-carotene and lycopene. The levels of the independent parameters including microwave drying time (X_1), steaming time (X_2) and hydraulic pressure (X_3) were based on preliminary experimental results. The coded and uncoded levels of independent variables used in the RSM design are presented in Table 1. The experimental design based on the central composite design (CCD) using a 2^3 factorial and star design with six central points was performed. A second-order polynomial equation was used to express the oil extraction efficiency (Y_1), β-carotene (Y_2) and lycopene (Y_3) as a function of the independent variables as follows:

$$Y_i = a_o + a_1X_1 + a_2X_2 + a_3X_3 + a_{11}X_1^2 + a_{22}X_2^2 + a_{33}X_3^2 + a_{12}X_1X_2 + a_{13}X_1X_3 + a_{23}X_2X_3$$

where Y_i represents the response variables, a_0 is a constant, a_i, a_{ii} and a_{ij} are the linear, quadratic and interactive coefficients, respectively. X_i and X_j are the levels of the independent variables.

Even if several regression coefficients were statistically insignificant, to respect the hierarchical property, the former was still kept in the second-order equation.

Statistical analysis

The experimental data were analysed using JMP software version 9.0. The adequacy of the model was determined by evaluating the lack of fit, coefficient of determination (R^2) and the Fisher's test value (F-value) obtained from the analysis of variance (ANOVA). The test of statistical significance was based on the total error criteria with a confidence level of 95%. The plots of three-dimensional (3D) and two-dimensional (2D) contour of the response were created by varying two variables within the experimental range and holding another constant at the central point.

Different mean values of the measured and predicted responses were analysed by analysis of variance (ANOVA) using SPSS software version 19.0 (IBM Australia Limited, St Leonards, NSW, Australia).

Analytical procedure

All measurements were taken in triplicate.

Moisture content determination

The moisture content of microwave-dried and steamed samples was gravimetrically analysed using an AnD MX-50 moisture analyser (A&D company, Ltd., Tokyo, Japan).

The moisture content of oil samples was determined according to AOCS (1998) Ca 2c-25. Oil samples (about 5 g) were placed into a tared moisture dish and air-dried at 130 ± 1 °C for 30 min. After that, the dish was removed and cooled to room temperature in a desiccator and weighed. The moisture content was calculated when the loss in weight did not exceed 0.05%/30-min drying period.

Soxhlet extraction

The total oil content of microwave-dried Gac sample prior to each pressing experiment was determined using FOSS Soxtec™ system 2045 extraction unit, Hilleroed, Denmark. A method described by Brkić *et al*. (2006) with a slight modification was employed for Gac oil extraction. Briefly, approximately 3 g of the sample was weighed in cellulose thimbles and inserted into the extraction system. Then, 50 mL petroleum ether (boiling point of 60–90 °C) was filled into weighed aluminium vessels and inserted into the extraction unit as well. The temperature was set at 145 °C, and the programme of the oil extraction process was given as follows: 30 min of boiling, 60 min of rinsing, 15 min of evaporation and finally 20 min of drying the aluminium vessels containing the extracted oil. The sample was continually dried in an oven at temperature of 80 °C until constant weight was obtained. The oil content in samples was gravimetrically calculated.

Oil extraction efficiency (EE)

A ratio of mass of extracted oil after subtracting moisture and mass of oil in starting material using Soxtec extraction unit is determined as the extraction efficiency. The oil content (g per g of dry weight) of Gac aril varied from 19 to 33%.

Determination of β-carotene and lycopene

A procedure of β-carotene and lycopene analyses was carried out according to the method of Englberger *et al*. (2006). HPLC analysis was performed with an Agilent 1200 HPLC (Santa Clara, CA, USA) equipped with diode array detector system consisting of a Luna C18 (100 × 4.6 mm i.d; 5 μm) direct-connect guard column coupled to a Jupiter C18 (250 × 4.6 mm i.d; 5 μm) reversed phase column (Phenomenex Australia Pty. Ltd, Lane Cove, NSW, Australia). The mobile phase consisted of acetonitrile, dichloromethane and methanol (ACN/DCMMeOH) 50: 40: 10 v/v/v. The flow rate was 1.0 mL min^{-1}, detection was at 450 nm, and the injection volume was 20 μL. The resultant

Gac oil (10 μL) was dissolved in chloroform (2 mL) and then diluted at a ratio of 1:200 with the HPLC solvent, an internal standard of β-apo-8'-carotenal and an antioxidant (butylated hydroxytoluene [BHT], 0.1% in hexane). All operations were performed under subdued light to minimise oxidation of the carotenoids. The identification of β-carotene and lycopene was based solely on the retention time of a peak compared with the authentic standards. The amounts of β-carotene and lycopene in Gac oils were expressed as mg per 100 mL of crude oil.

Results and discussion

Fitting the models

The EE, and β-carotene and lycopene contents obtained from all the experiments are presented in Table 2. Analysis of variance (ANOVA) indicated that the second-order polynomial model adequately represented the experimental data with the coefficient of multiple determination (R^2) for the responses of EE and content of β-carotene and lycopene being 0.93, 0.85 and 0.86, respectively. The predicted values agreed fairly well with the experimental ones obtained from the RSM design (Table 2). In addition, ANOVA results also showed that the three quadratic regression models were significant ($P < 0.01$), and there was no significance in the lack of fit ($P > 0.05$) in each of the models (Table 3). Therefore, this indicates that the quadratic polynomial models were adequate to describe the influence of the independent variables investigated on the EE, and β-carotene and lycopene contents.

The significance of the coefficients of the quadratic polynomial models was determined using t ratio and *P*-value (significance level) and is listed in Table 3. The larger absolute t ratio and smaller *P*-value would indicate a more significant effect on the corresponding variables (Amin & Anggoro, 2004). It can be seen that the variable with the largest effect on the EE was the interaction term of X_1 and X_2, followed by quadratic of X_3, interaction of X_1 and X_3, linear of X_2, interaction of X_2 and X_3, linear of X_1 and quadratic of X_2. Furthermore, quadratic of steaming time and microwave time and linear of hydraulic pressure were one of the largest effects on β-carotene and lycopene contents. The interaction between steaming time and hydraulic pressure also had a significant effect on β-carotene content.

Response surface analysis

Response surface optimisation of extraction efficiency

To determine the optimal levels of the independent variables for the EE, the 3D surface and 2D contour

Table 2 Experimental (Exp.) and predicted (Pred.) values of EE, and β-carotene and lycopene contents of Gac oil obtained from the CCD design

				EE (%)		β-carotene (mg per 100 mL)		Lycopene (mg per 100 mL)	
Exp. run[a]	X_1 (min)	X_2 (min)	X_3 (kg cm^{-2})	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.
1	60.0	30.0	160	59.27	59.61	90.00	64.02	300.03	251.83
2	62.5	35.1	170	76.16	74.78	51.47	90.37	137.36	181.83
3	62.5	9.9	170	91.49	88.61	111.44	92.22	359.80	289.97
4	65.0	30.0	160	88.43	89.08	61.46	56.27	140.00	167.87
5	62.5	22.5	170	87.80	87.83	149.84	171.16	495.01	496.15
6	60.0	15.0	180	86.54	88.90	139.41	130.69	372.98	363.05
7	58.3	22.5	170	86.33	82.14	92.47	117.12	300.57	310.71
8	65.0	15.0	180	65.89	68.56	105.54	117.61	317.77	383.90
9	60.0	15.0	160	86.69	89.70	86.68	93.46	252.89	309.62
10	65.0	30.0	180	82.19	82.19	165.55	144.85	351.90	313.10
11	62.5	22.5	153	78.63	78.15	102.48	103.78	392.77	368.45
12	65.0	15.0	160	91.82	91.06	111.92	122.84	307.27	326.32
13	62.5	22.5	170	87.68	87.83	185.54	171.16	562.41	496.15
14	62.5	22.5	170	91.33	87.83	194.04	171.16	430.56	496.15
15	60.0	30.0	180	70.66	74.43	219.90	195.07	394.02	392.90
16	62.5	22.5	170	84.11	87.83	166.22	171.16	543.83	496.15
17	62.5	22.5	170	89.44	87.83	170.84	171.16	416.37	496.15
18	66.7	22.5	170	89.88	89.81	104.58	99.60	293.13	257.64
19	62.5	22.5	187	75.46	71.68	191.20	209.58	536.54	535.50
20	62.5	22.5	170	85.90	87.83	163.87	171.16	524.34	496.15

[a]Experiments were run in random order.

Table 3 Regression coefficients of the fitted quadratic equation and standard errors for EE, and β-carotene and lycopene contents

	EE (Y_1)		β-carotene		Lycopene	
Regression coefficient[a]	**Regression coefficients**	***t* ratio**	**Regression coefficients**	***t* ratio**	**Regression coefficients**	***t* ratio**
a_0	87.832		171.163		496.146	
Linear						
a_1	2.280*	2.52	−5.210	−0.77	−15.778	−0.93
a_2	−4.113**	−4.55	−0.548	−0.08	−32.149	−1.89
a_3	−1.923	−2.13	31.453***	4.65	49.664*	2.92
Quadratic						
a_{11}	−0.656	−0.74	−22.203**	−3.37	−74.944**	−4.52
a_{22}	−2.169*	−2.46	−28.238**	−4.29	−92.011***	−5.55
a_{33}	−4.566***	−5.19	−5.120	−0.78	−15.617	−0.94
Interaction						
a_{12}	7.026***	5.95	−9.283	−1.05	−25.165	−1.13
a_{13}	−5.426***	−4.59	−10.614	−1.20	1.040	0.05
a_{23}	3.904**	3.30	23.454*	2.65	21.912	0.98
R^2	0.93		0.85		0.86	
P-value of lack of fit	0.174		0.08		0.43	

*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.
[a]a_0 is a constant, a_i, a_{ii} and a_{ij} are the linear, quadratic and interactive coefficients of the second-order polynomial equation, respectively.

plots were drawn. The response surface plot shows relationship between the independent variables and the responses, while the contour plot helps to visualise the shape of a response surface. Therefore, it is useful to use the plots to evaluate the fits of model (Baş & Boyaci, 2007).

The effects of microwave time, steaming time and hydraulic pressure on the EE are presented in Table 3 by the coefficients of quadratic model. The response surface and contour plots based on these coefficients are shown in Fig. 1a–c. In general, all regression coefficients of the model statistically impacted on the EE, except for the linear term of X_3 and quadratic term of X_1 ($P > 0.05$).

Figure 1a shows the effect of microwave and steaming times on the EE. It can be generally seen that an increase in microwave time resulted in a slight increase in EE at the constant hydraulic pressure of 170 kg cm^{-2}. However, EE gradually decreased with increasing steaming time. Furthermore, at the constant microwave time of 62.5 min (Fig. 1b), a similar trend of EE was also found as the samples were treated in a range of steaming time of 15–30 min. The EE remained unchanged at the constant microwave exposure time of 62.5 min. For Fig. 1c, the EE increased at the constant steaming time of 22.5 min when an increase in microwave time and a decrease in pressure were applied. In addition, results showed that critical values of microwave exposure time, steaming time and hydraulic pressure for Gac oil extraction condition were 64 min, 16 min and 161 kg cm^{-2}, respectively. At this point, the extraction efficiency was about 91.2%.

Response surface optimisation of β-carotene content

The best way to visualise the interaction between the independent variables on the β-carotene content of Gac oil was to draw 3D response surface and 2D contour plots (Fig. 2). Statistical results (Table 3) indicated that linear term of X_3, quadratic terms of X_1 and X_2, and interaction term of X_2 and X_3 significantly influenced the β-carotene content of Gac oil.

Figure 2a showed the effect of treatment times of microwave and steaming prior to the hydraulic pressure of 170 kg cm^{-2}. It indicated that the moderate time of microwave and steaming resulted in the high content of β-carotene in Gac oil extract. The β-carotene concentration was found to be low as shorter or longer time of microwave and steaming treatments was applied. This could be due to β-carotene being lost because of heat as the microwave and steaming times were increasing. However, the low oil level extracted under the shorter times of microwave and steaming may limit dissolving the β-carotene in the oil. Therefore, the lower β-carotene amount that was dissolved in the oil was extracted.

Figure 2b showed the effect of steaming time and the pressure at the constant microwave time of 62.5 min. It indicated that the moderate steaming

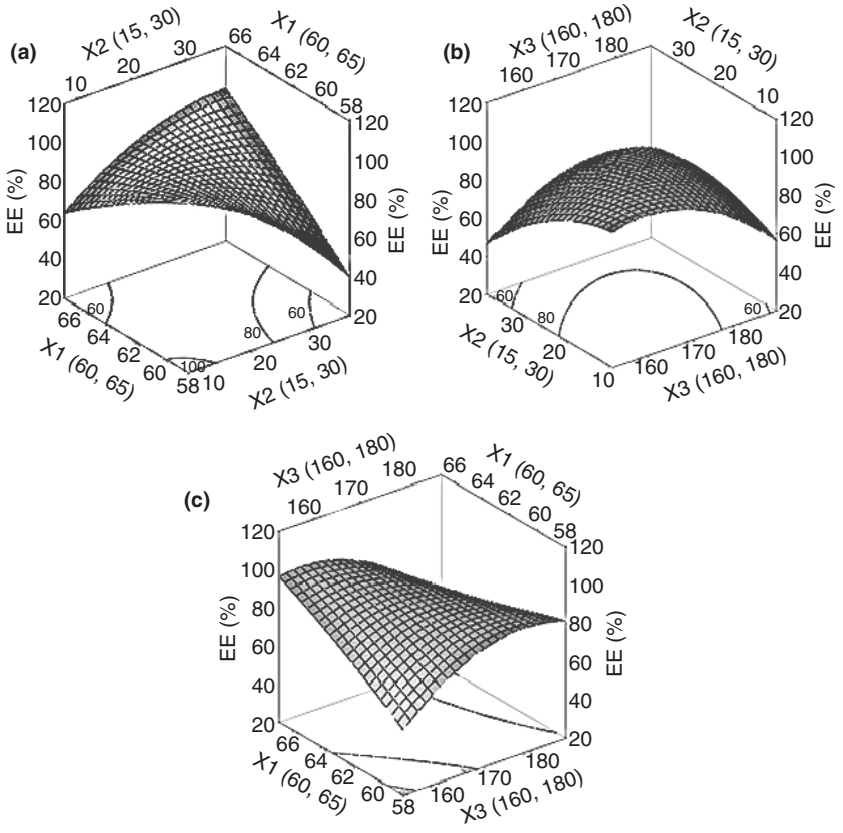

Figure 1 The 3D response surface and 2D contour plots of the EE affected by microwave time (X_1), steaming time (X_2) and working hydraulic pressure (X_3).

time resulted in high content of β-carotene in the oil extract. However, the β-carotene content rose as the pressure increased. A similar result for a trend of the pressure and microwave time (Fig. 2c) was also found when the steaming time of 22.5 min was fixed. Furthermore, it was also found that the high concentration of β-carotene (205 mg per 100 mL) in the oil could be obtained at the optimal conditions of microwave time of 62 min, steaming time of 25 min and pressure of 180 kg cm^{-2}.

Response surface optimisation of lycopene content

Statistical results showed that the lycopene content of Gac oil was significantly affected by linear term of X_3 and quadratic terms of X_1 and X_2 (Table 3). Figure 3a showed the interactions between the microwave and steaming times on the lycopene content of Gac oils. Similar to the trend of β-carotene in Fig. 2a, medium extraction conditions of microwave and steaming time resulted in the highest lycopene content. Moreover, the high content of lycopene was also extracted at the medium of steaming time and the high pressure (Fig. 3b) or at the medium of microwave time and the high pressure (Fig. 3c). In addition, statistical results indicated that the maximum lycopene content (530 mg per 100 mL) could be extracted at the predicted extraction conditions including microwave time, steaming time and the pressure of 62 min, 23 min and 180 kg cm^{-2}, respectively.

A similar trend for the extraction conditions can be explained due to the fact that lycopene and β-carotene are fat-soluble carotenoids. Therefore, they were likely extracted together with Gac oil under the investigated variables. In addition, statistical results indicated that the hydraulic pressure was significantly independent variable affected the β-carotene and lycopene in both models. A combination of microwave drying time and steaming time is also an important factor in the extraction process. A possible explanation is that the optimum microwave drying time resulted in rupturing the cell membrane (Oberndorfer *et al.*, 2000; Chan *et al.*, 2011) and hence assisted the enhanced release of carotenoids. Furthermore, the microwave-dried Gac tissues can be softened and broken down under hot steaming process (Mpagalile & Clarke, 2005). As a result, the oil-dissolving β-carotene and lycopene were easily released by pressing. Furthermore, the presence of other carotenoids such as α-carotene and xanthophylls may be found in the oil in smaller quantities.

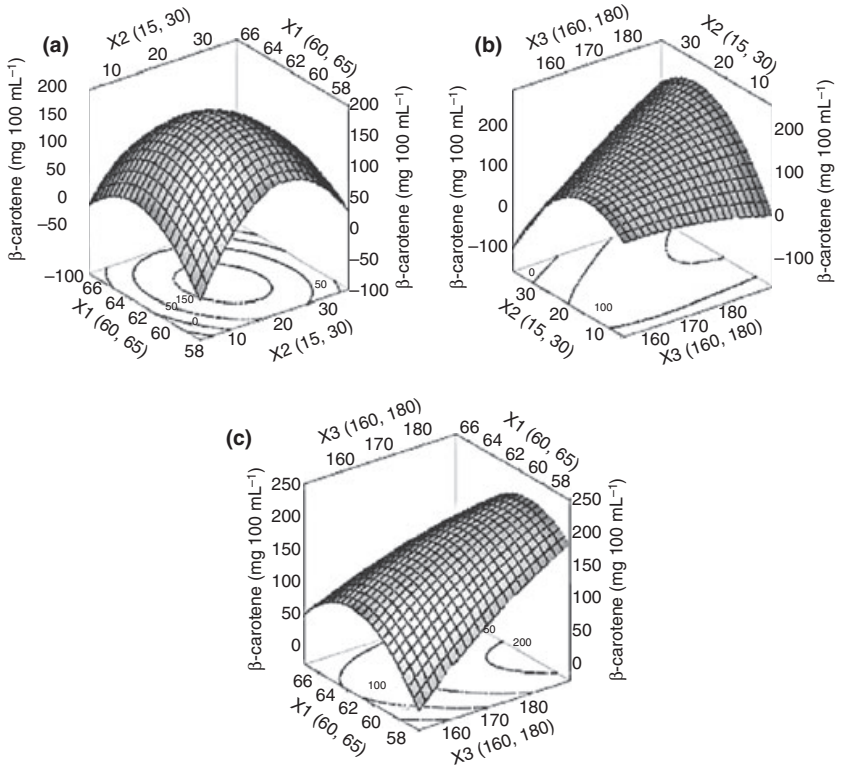

Figure 2 The 3D response surface and 2D contour plots of β-carotene content affected by microwave time (X_1), steaming time (X_2) and working hydraulic pressure (X_3).

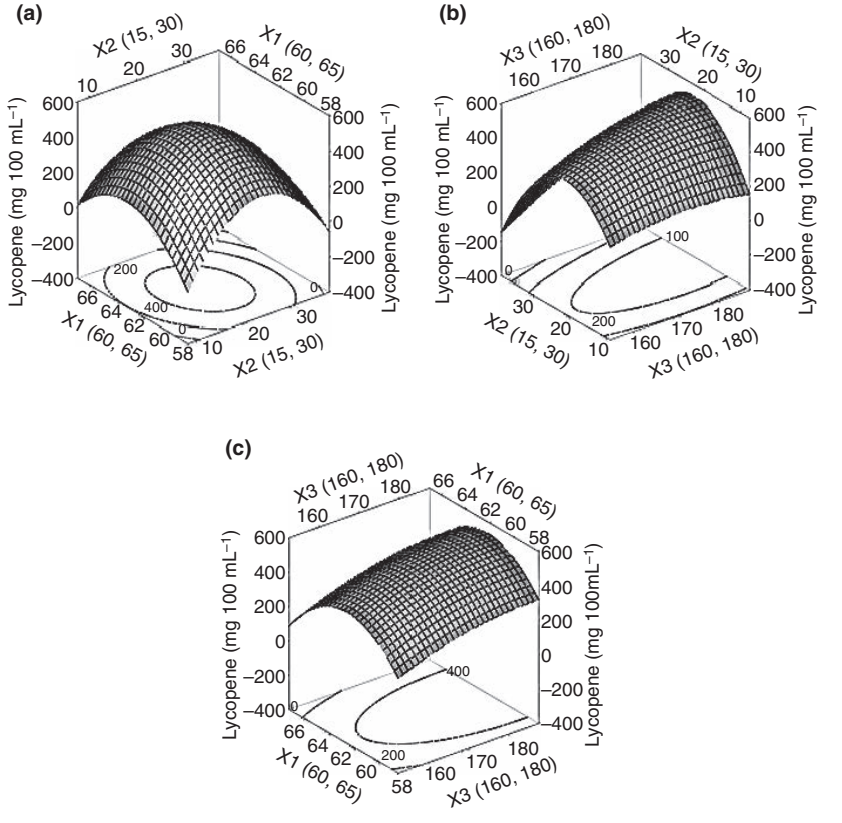

Figure 3 The 3D response surface and 2D contour plots of lycopene content affected by microwave time (X_1), steaming time (X_2) and working hydraulic pressure (X_3).

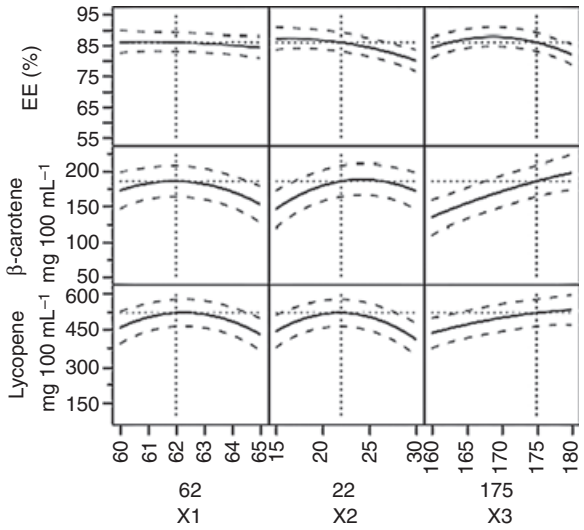

Figure 4 Prediction profilers of EE, β-carotene and lycopene as a function of microwave time, steaming time and hydraulic pressure.

Overall optimal extraction conditions and model validation

This study aimed to determine the optimal aril processing conditions of Gac oil, and a graphical optimisation (Fig. 4) was therefore performed using JMP package. The most important criterion is to obtain the highest extraction efficiency and maximise the β-carotene and lycopene contents. As discussed earlier and can be seen in Fig. 4, an increase in microwave and steaming times resulted in decreasing the EE, and β-carotene and lycopene contents. The EE was also reduced when increasing the hydraulic pressure. In a recently published paper, Kha *et al*. (2012) found that further increase in microwave and steaming times, and the pressure slightly decreased the EE. Some interfibers or capillary voids of the cell walls may be sealed with increasing pressure; therefore, the oil flow is prevented (Baryeh, 2001). However, the β-carotene and lycopene concentrations significantly increased as the pressure rose in the investigated range. Therefore, it is important to determine the optimal conditions so that the highest EE, and β-carotene and lycopene concentrations could be obtained.

Figure 4 shows that the theoretical maximum values of EE, β-carotene and lycopene could be obtained by combining microwave time, steaming time and hydraulic pressure. Based on the prediction profiler, the mathematical model suggested the optimum theoretical values of EE, β-carotene and lycopene under the optimum conditions (microwave time of 62 min, steaming time of 22 min and the pressure of 175 kg cm^{-2}) as shown in Table 4. Moreover, the comparison between predicted and measured response values is a way for establishing how accurately the model describes the studied process. Therefore, an experiment (with three replicates) was performed at the optimum conditions. Statistical results indicated that there was insignificant difference between the predicted and measured responses of EE, β-carotene and lycopene ($P > 0.05$) (Table 4). It is evident that the measured values of the responses were found to be well suited to the predicted ones by the regression model. As a result, the suitability of the quadratic equation for predicting the optimal response values was validated under the optimum conditions. In other words, the response surface optimisation methodology for the aril processing conditions for Gac oil was found to be of practical value.

It is generally agreed that treatments prior to extraction can have effects on the major quality features of the oil. For example, conventional air drying to reduce moisture before oil pressing can take a long time hence can lead to potentially high level of oxidation. Compared to air drying treatment prior to pressing, the extraction efficiency and retention of β-carotene and lycopene in the oil samples using microwave drying treatment were much higher (Kha *et al*., 2012). Therefore, results under this optimisation process for Gac oil could be useful in encouraging Gac oil processers to use microwave and steaming pretreatments prior to pressing to enhance extraction efficiency and preserve carotenoids. Furthermore, a comparison of different treatments and extraction methods on oil yield, fatty acid composition and physicochemical properties will be reported in another paper.

Conclusion

The results indicated that the quadratic polynomial model was sufficient to describe and predict the responses of the EE, β-carotene and lycopene in Gac oil extraction optimisation process. The data could adequately fit three second-order polynomial models

Table 4 Optimum condition of Gac oil extraction

			EE (%)		β-carotene (mg per 100 mL)		Lycopene (mg per 100 mL)	
X_1 min	X_2 min	X_3 kg cm^{-2}	Predicted	Measured	Predicted	Measured	Predicted	Measured
62	22	175	86.02 ± 2.98	88.19 ± 5.08	185.83 ± 22.32	173.87 ± 11.44	517.80 ± 56.17	510.70 ± 37.23

for EE, β-carotene and lycopene with R^2 values of 0.93, 0.85 and 0.86, respectively. The graphical optimisation was adopted and it predicted the optimum extraction parameters within the experimental ranges including microwave time of 62 min, steaming time of 22 min and the pressure of 175 kg cm^{-2}. Under such conditions, the EE, β-carotene and lycopene contents achieved were also predicted and confirmed as 86%, 186 mg per 100 mL and 518 mg per 100 mL, respectively.

Acknowledgments

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Paper VI

2.1.5 Effect of Drying Pre-Treatments on the Yield and Bioactive Content of Oil Extracted from Gac Aril

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Effect of Drying Pre-treatments on the Yield and Bioactive Content of Oil Extracted from Gac Aril

Abstract: Gac fruit (*Momordica cochinchinensis* Spreng) aril contains high levels of bioactive compounds including β-carotene, lycopene and fatty acids. Therefore, it is important to find an extraction method of Gac oil resulting in the highest content of bioactive compounds. The effects of microwave and air-drying pre-treatments of Gac aril prior to hydraulic pressing and Soxhlet extraction on the oil yield, nutrients and chemical properties of the oil were compared. Results showed that the highest oil yield could be obtained when the aril was microwave-dried before Soxhlet extraction. This finding was explained by microstructural changes of the dried arils. Microwave-drying prior to pressing resulted in the highest content of β-carotene (174 mg/100 mL) and lycopene (511 mg/100 mL) in the oil extract. Oleic acid (48%) and linoleic acid (18%) were found to be the dominant fatty acids in the oil extracts. Gac oil with the lowest acid (0.69 mg KOH/g) and peroxide values (1.80 meq O_2/kg) was extracted when microwave-drying was applied prior to pressing. It can be concluded that the microwave drying pre-treatment before pressing was better than the air-drying pre-treatment for extracting Gac oil of high quality.

Keywords: Gac oil, carotenoids, fatty acids, microwave-drying, pressing

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1 Introduction

Gac aril (*Momordica cochinchinensis* Spreng) oil can be utilised in the food industry due to its high nutritional content. Several studies have reported high concentrations of carotenoids such as β-carotene and lycopene in Gac aril [1–3]. It is well-known that carotenoids such as lycopene and β-carotene from plant-based foods play an important role in human health, and are linked to reduced risk of cardiovascular disease and cancers [4–6]. The Gac aril also contains significant amounts of fatty acids, at about 102 mg/g of fresh weight, particularly monounsaturated and polyunsaturated acids [7]. Other bioactive compounds, including vitamin E and flavonoids, found in the aril were also reported by Kha et al. [8]. Furthermore, several studies show that the absorption of carotenoids and other fat-soluble compounds in the body is significantly improved when ingesting with fat [9–11]. Gac oil containing high carotenoids could be used as nutrient supplements and natural food colourants. Therefore, it is important to find a suitable extraction method of Gac oil which results in the highest content of bioactive compounds.

Among extraction methods, mechanical pressing, also referred to as hydraulic pressing, is the common one used in the oil processing industry. The main drawback of this method is a low extraction yield, which is normally limited to about 70% [12]. The two pre-treatments of air-drying and steaming are the key steps in this method [13, 14]. However, it can take a long time to air-dry a sample to reach the desired moisture content. Generally, a low-quality air-dried sample is usually obtained due to high temperature and oxygen exposure. Microwave-drying has recently been of interest as an alternative method of extraction pre-treatment. Several studies have reported that microwave-drying as a pre-treatment prior to mechanical pressing is effective in terms of extraction yield and superior quality [15–18]. As a result, there is a need to evaluate the effects of different drying pre-treatments, prior to pressing, on Gac oil extraction yield and on the quality characteristics of the oil extracts.

Traditionally, the Soxhlet method has been used for fat and oil extraction due to its high extraction efficiency. However, in the Soxhlet method, the use of organic solvents and high temperatures could affect the oil properties and may significantly reduce the nutritional content of the resulting oils. The objective of this study was to compare the Soxhlet and hydraulic pressing extractions in terms of oil yield, carotenoid content, fatty acid

composition and chemical properties of the Gac oil after pre-treatments by microwave- or air-drying. In addition, the microstructure of the dried aril samples was also observed using light and scanning electron microscopes.

2 Materials and methods

2.1 Chemicals

Solvents and an antioxidant (butylated hydroxytoluene, BHT) were purchased from Merck Pty. Ltd. (Kilsyth, Vic, Australia). β-carotene (C4582, type II synthetic, ≥95%, HPLC grade) and lycopene (L9879, ≥90%, from tomato) standards, fatty acid methyl esters (FAME Mix, C8–C24) analytical standards were obtained from Sigma-Aldrich Pty. Ltd. (Castle Hill, NSW, Australia).

2.2 Drying pre-treatments of Gac fruit aril

Fresh Gac fruits, of uniform yellow-red skin and size, were purchased from a local market in Ho Chi Minh city, Vietnam. The red arils containing seeds were scooped out and frozen at −18°C until used. Frozen Gac aril was then thawed at 4°C prior to microwave and air-drying pre-treatments.

For microwave-drying, 900 g of the aril (including seeds), with an initial moisture content of 82%, were spread onto the turntable plate to a thickness of 5 mm. The samples were then dried at a microwave power of 630 W for 62 min, using a Panasonic Dimension NN-9853 microwave oven (Panasonic Australia Pty. Ltd., Macquarie Park, NSW, Australia), with cavity dimensions of height × width × depth = 244 mm × 412 mm × 426 mm and an operating frequency at 2,450 MHz. The microwave conditions were chosen according to the optimisation studies published by Kha et al. [17, 18]. After microwave-drying, the temperature of the arils was immediately measured using an electronic stainless steel thermometer. The temperature after the microwave pre-treatment of the samples was about 80°C.

For air-drying, 900 g of the aril (including seeds) were spread onto a stainless tray (1.2 × 0.8 m) to thickness of 1.5 cm. The samples were air-dried at 60°C for 18 h with an air velocity of 1.1 m/s [19], using an air-drying oven (G.T.C. Australia Trading Pty. Ltd., Alexandria, NSW, Australia). The air-drying temperature of 60°C was chosen because significant losses of carotenoids and fatty acids were found at temperatures higher than 60°C. Furthermore, it took much longer to dry Gac arils at lower temperatures [19].

2.3 Steaming treatment

The microwave-dried and air-dried samples had the seeds removed by hand. The aril was then powdered using an IKA M20 grinding mill (IKA® company, Staufen, Germany). The ground samples (with an average particle size of about 1 mm and stacked to a thickness of 5 mm) were placed inside a stainless steel steam cooker and steamed for 22 min. Before that, a constant amount of potable water (2 L) was placed and boiled inside the steam cooker. After steaming, the samples were immediately wrapped with filtration cloth for pressing [17]. The moisture content of the steamed samples was also determined after cooling to room temperature in a desiccator.

2.4 Hydraulic pressing

For each experiment, the samples after steaming were wrapped inside 4 layers of filtration cloth and pressed at 175 kg/cm^2, using a laboratory hydraulic press (length: width: height = 650: 850: 1,900 mm) (Tinh Anh company, Ho Chi Minh City, Vietnam). The stainless steel press cage used in this study was 210 mm high, 112 mm in internal diameter and 3 mm in thickness. The size of the openings located at the exit from the cage was 6 mm in diameter. The process was performed in two stages, a compression stage for 0.2 min, where the direct target pressure on the material was achieved, and a relaxation period of 5 min, and the process was repeated three times. The total time for the extraction process was about 15.6 min, after which there was no additional oil release.

2.5 Soxhlet extraction

For comparison with the pressing method, the oil of the microwave-dried and air-dried samples was extracted using the FOSS Soxtec™ system 2,045 extraction unit, (Hilleroed, Denmark). A method described by Brkić et al. [20] with a slight modification was employed for Gac oil extraction. Briefly, approximately 3 g of the samples were weighed in cellulose thimbles and inserted into the extraction system. Approximately 50 mL petroleum ether (boiling point of 60–90°C) was then filled into weighed aluminium vessels and inserted into the extraction unit. The temperature was set at 145°C and the program for the oil extraction process was 30 min of boiling, 60 min of rinsing, 15 min of evaporation and finally 20 min of drying the aluminium vessels containing the extracted oil. The sample was then dried in an oven set at a temperature of 80°C until a constant weight was

obtained. The oil content was gravimetrically calculated. Compared with the traditional Soxhlet extraction, the extraction time with the semi-automatic FOSS Soxtec system extraction unit was much shorter, about 1.45 h as compared to more than 4 h.

2.6 Analytical methods

2.6.1 Microstructure of dried Gac arils before grinding

The microstructure of the Gac arils after microwave-drying and air-drying was observed using a light microscope (Novex, Arnhem, Netherlands) equipped with an attached CMEX camera. The samples were cut into thin slices and observed using a 40× objective magnification. The images were processed by ImageFocus® analysis software. In this study, as we just observed the surface structure of Gac aril tissue, the samples needed only simple preparations, not as what was required later for scanning electron microscope (SEM).

The surface morphography of the dried Gac arils was also studied using a JSM-7401F scanning electron microscope (SEM) (Jeol Co. Ltd., Tokyo, Japan). A small amount of the thin slices of the air-dried and microwave-dried arils were fixed onto the surface of a double-coated metallic adhesive tape adhered to a metallic stub. The stub was then coated with a fine layer of gold and argon in a MSP-1S magnetron sputter coater (Vacuum Device, Tokyo, Japan) under vacuum. The morphography was observed at different magnifications operating the SEM at a voltage of 3 kW.

2.6.2 Moisture content determination

The moisture content of microwave-dried, air-dried and steamed samples was gravimetrically analysed using an AnD MX-50 moisture analyser (A&D company, Ltd., Tokyo Japan).

The moisture content of oil samples was determined according to the AOCS Ca 2c-25 method [21]. Oil samples (5 g) were placed into a tared moisture dish and air-dried at 130 ± 1°C for 30 min. After that, the dish was removed and cooled to room temperature in a desiccator and weighed. The moisture content was calculated when the loss in weight did not exceed 0.05%/30 min drying period.

2.6.3 Oil yield

Oil yield was defined as the ratio of the mass of extracted oil, after subtracting its moisture, and the mass of the oil in the starting fresh Gac aril material (dry weight) measured using the Soxhlet extraction as the benchmark method [20] and expressed as a percentage of the oil available in the fresh Gac arils.

2.6.4 Determination of β-carotene and lycopene

The procedure for the β-carotene and lycopene analyses was carried out according to the method of Englberger et al. [22]. The HPLC analysis was performed with an Agilent 1,200 HPLC (Santa Clara, CA, USA) equipped with a diode array detector system and consisting of a Luna C18 (100 × 4.6 mm i.d.: 5 µm) direct-connect guard reverse phase column directly coupled to a Jupiter C18 (250 × 4.6 mm i.d.: 5 µm) reverse phase column (Phenomenex Australia Pty. Ltd., Lane Cove, NSW, Australia). The mobile phase consisted of acetonitrile, dichloromethane and methanol (ACN: DCM: MeOH) 50: 40: 10 v/v/v. The flow rate was 1.0 mL/min, detection was at 450 nm and the injection volume was 20 µL. The resultant Gac oils (10 µL) were dissolved in chloroform (2 mL) and then diluted at a ratio of 1: 200 with the HPLC solvent and an antioxidant (butylated hydroxytoluene [BHT], 0.1% in hexane). All operations were performed under subdued light to minimise oxidation of the carotenoids. The identification of β-carotene and lycopene was based solely on the retention time of the peaks compared with the authentic standards. The amounts of β-carotene and lycopene in Gac oils were expressed as mg/100 mL of crude oil.

2.6.5 Determination of fatty acid composition

The method of Ishida et al. [1] was employed for determining the fatty acid composition of the Gac oils. About 50 mg of sample was weighted into 10 mL glass tubes. The fatty acids were extracted using 2 mL of hexane/2-propanol (8:2, v/v) containing 50 µg/mL BHT. The extraction took place at 55°C for 30 min, with shaking every 10 min. The extracts were then filtered and dried over sodium sulphate and the solvent was evaporated under nitrogen. Toluene (0.5 mL) was added and the fatty acids were methylated for 1 h at 80°C using methanolic HCl 3%. The resulting fatty acid methyl esters (FAMEs) were dissolved in 10 mL cyclohexane (0.01% BHT) for GC analysis.

Quantitative analysis was performed by GC-FID using an Agilent 5890N GC (Santa Clara, CA, USA). The injector and detector temperatures were 250 and 280°C, respectively. The column temperature was 100°C for 1 min, then it was increased by 5°C/min to 250°C and held at 250°C for 1 min. Standard solutions of a mixture of FAMEs at three different concentrations in the range of 5–150 µg/mL were used for generating the standard curves. The

samples (1 μL) were injected into the GC system. The identification of each fatty acid was based solely on the retention times of the peaks compared with the authentic FAME standards.

2.6.6 Chemical properties of oil samples

The chemical properties of the oil samples were analysed according to AOCS methods [21] for determining acid (AOCS Cd 3d-63), peroxide (AOCS Cd 8–53), iodine (AOCS Tg 1–64) and saponification values (AOCS Cd 3–25).

2.7 Statistical analysis

The experiments and subsequent analyses were performed in triplicate and the results were presented as mean values with standard deviations. The one factor experiments were randomly done to investigate the effect of four different treatments, microwave-drying before pressing (MDP), air-drying before pressing (ADP), microwave-drying before Soxhlet extraction (MDS) and air-drying before Soxhlet extraction (ADS), on the oil yield, content of β-carotene and lycopene, fatty acid composition, and chemical properties of the Gac oil samples. The mean values were analysed for significant differences by analysis of variance (ANOVA) and the least significant difference (LSD) post-hoc test using SPSS software version 20.0 (IBM Corp., Armonk, NY, USA).

3 Results and discussion

3.1 Effect of drying pre-treatments and extraction methods on oil yield and content of β-carotene and lycopene in Gac oil

The oil yield and the β-carotene and lycopene content of the Gac oil obtained from the four different extraction methods are presented in Table 1. The statistical results indicated that the oil yield ($P < 0.01$) and the content of β-carotene and lycopene ($P < 0.001$) were significantly affected by the extraction methods.

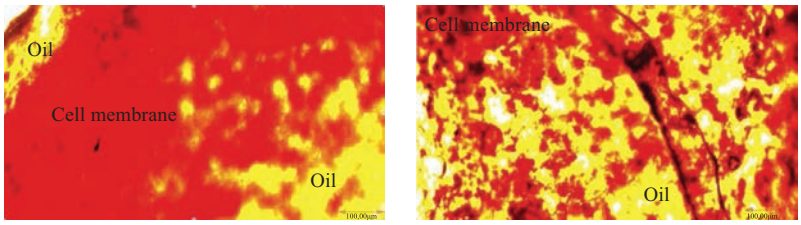
It can be seen in Table 1 that the highest oil content was extracted using the MDS method, followed by ADS, MDP and ADP. However, the values showed statistically insignificant differences among MDS, ADS and MDP ($P > 0.05$). Therefore, microwave-drying could be considered to be the most suitable pre-treatment prior to pressing. This is due to the fact that microwave radiation heats up the moisture inside a plant cell, the moisture is evaporated and then high pressure on the cell wall is generated. As a result, the cell membrane is ruptured, allowing the easy release of oil from the ruptured cells [23]. The effects of different microwave pre-treatment conditions (power and time) for Gac aril drying have been recently reported on [17, 18]. The results indicated that microwave power and time were the most important parameters for Gac oil extraction, increasing oil extraction efficiency and bioactive compounds in comparison with untreated samples. Furthermore, it was previously determined that the microwave-dried Gac aril powder could be used without draining for pressing the oil out because the dried samples effectively retained the moisture they had absorbed during the steaming. In fact, as moisture is absorbed during the steaming process, the tissues can expand and weaken the oil cell walls, which can break down and hence the oils are more easily released when applying the pressure [13]. This is consistent with several reports that the oil yield was enhanced when materials, such as Chilean hazelnut [16] and rapeseed [15], were pre-treated with microwave radiation before pressing. In addition, reduced processing times and uniform heating are also advantages of the use of microwave heating as a drying pre-treatment [16].

Table 1 Effects of drying pre-treatments and extractions on the oil yield and the β-carotene and lycopene in Gac oil

Treatment	MDP	ADP	MDS	ADS
Oil yield (%)	27 ± 2^{a}	20 ± 3^{b}	31 ± 4^{a}	30 ± 2^{a}
β-carotene (mg/100 mL)	174 ± 11^{a}	62 ± 8^{b}	106 ± 6^{c}	30 ± 6^{d}
Lycopene (mg/100 mL)	511 ± 37^{a}	272 ± 32^{b}	322 ± 17^{c}	196 ± 20^{b}

Notes: Values in the same row followed by different superscripts (a–d) were significantly different ($P < 0.05$); MDP: microwave-drying before pressing; ADP: air-drying before pressing; MDS: microwave-drying before Soxhlet extraction; ADS: air-drying before Soxhlet extraction.

The Gac arils after microwave-drying and air-drying were observed under light microscope. Figure 1 shows different microstructural effects of the microwave-drying and air-drying on Gac tissue. It can be seen that the cell wall of the microwave-dried sample (Figure 1B) was of much greater porosity than that of the air-dried sample (Figure 1A), which suggested that the cell wall was easier to break open during pressing or solvent extraction after microwave-drying compared to after air-drying. Therefore, significantly higher oil yield was obtained when the sample was microwave-dried before pressing compared to air-drying as a pre-treatment (Table 1). A similar observation with microwave pre-treated and pressed Chilean hazelnuts was reported by Uquiche et al. [16].

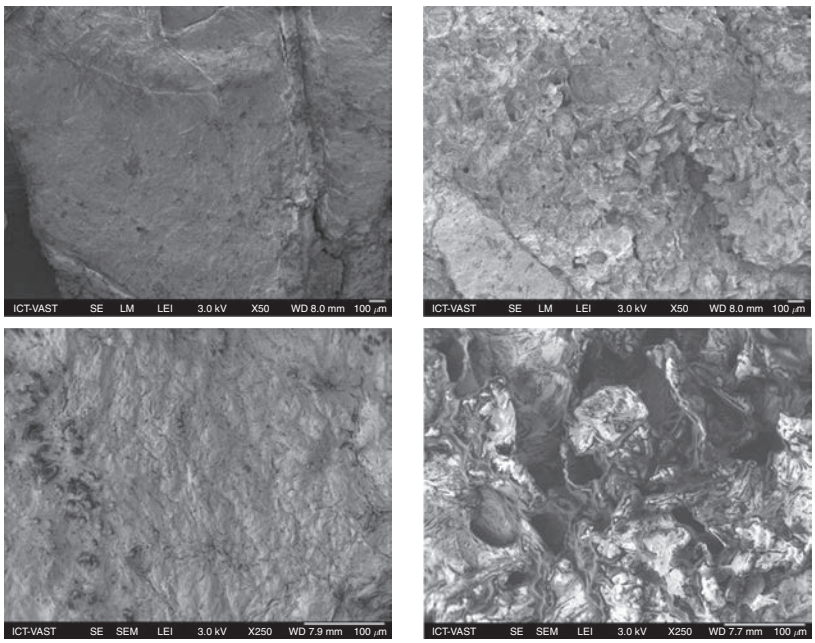


(A) (B)

Figure 1 Light photomicrographs of the dried Gac arils before extraction. (A) Air-dried aril. (B) Microwave-dried aril

In order to facilitate further understanding of the extraction mechanism, SEM was used to observe the microstructural changes in the dried arils under different drying pre-treatments. As can be seen in Figure 2, the cell walls of the microwave-dried arils (Figure 2B) appeared more ruptured than that of the air-dried arils (Figure 2A) at two different magnifications. The cell walls of the microwave-dried sample appeared disintegrated, consistent with several reports indicating disruptive effects of microwave on the microstructure of plant material containing oils and thus increasing the oil yield [24, 25]. In contrast, the cell walls of the air-dried sample (Figure 2A) were closely packed, indicating stronger cells, resulting in less release of oil. This observation clearly showed why a higher oil yield was achieved when the aril was microwave-dried before pressing (Table 1).

The oil yield with hydraulic pressing obtained after air-drying was 34% lower than that obtained by Soxhlet extractuib (Table 1). It is clear that the traditional Soxhlet extraction using organic solvents was effective at dissolving the lipid components in the powdered Gac arils whether they were air-dried or microwave-dried (Table 1). However, this method has been discarded for industrial scale production due to health concerns, environmental problems and quality degradation [26]. For the Soxhlet extraction yield, microwave-drying and air-drying pretreatments were found not to be statistically different. However, microwave-drying pre-treatment prior to the Soxhlet extraction was still preferable due to the



(A) (B)

Figure 2 SEM microstructures of the dried Gac arils before extraction. (A) Air-dried arils and (B) Microwave-dried aril

shorter drying time of about 1 h compared to the air-drying time of 18 h. The percentage of oil left in the pressed cake can be also estimated based on the oil yield of the pressing and the amount of oil available for extraction in the fresh Gac arils as measured using the Soxhlet method (a standard extraction method for oil). Based on the results from Table 1, it was estimated that percentage of oil left in the pressed cake for the air-drying and microwave-drying pre-treatments prior to pressing were 36% and 13%, respectively. Furthermore, it is possible to scale up the microwave pre-treatment for industrial Gac oil pressing. Several parameters including thickness of the material, material load, microwave power and time have now been investigated on a laboratory scale. However, there is still a need to validate optimum parameters for industry-sized microwave ovens.

The HPLC analysis indicated that lycopene and β-carotene in the Gac oil could be detected at 6.056 and 7.212 min, respectively [18]. The content of lycopene and β-carotene in the Gac oils obtained from the four different extraction methods is presented in Table 1. The results showed that the highest retention of both lycopene and β-carotene was achieved using MDP, followed by MDS, ADP and ADS. This is due to the fact that both bioactive compounds belong to the carotenoid group and have similar characteristics.

Compared with the microwave-drying pre-treatment, a significant degradation of carotenoids was found in Gac oil obtained by air-drying prior to either pressing or Soxhlet extraction (Table 1). A possible explanation is that the carotenoids were susceptible to oxidation in conventional air-drying, which caused the high loss of lycopene and β-carotene [19, 27]. In addition, many studies have also reported that microwave-drying was an effective method for preserving the carotenoid content in various plant products [18, 28–32]. This result is consistent with recently published papers by Kha et al. [17, 18], in which the carotenoid content of microwaved-dried Gac arils was higher than in air-dried samples. This is not surprising because, in the present study, the oil was pressed after microwave-drying, using optimised microwave-drying conditions according to their research. Therefore, several studies now strongly support the recommendation that microwave-drying of Gac aril should be performed prior to pressing or Soxhlet extraction for Gac aril oil and carotenoid extraction.

For comparison between pressing and Soxhlet extraction, not taking into account the pre-treatment method, the content of lycopene and β-carotene was much lower after Soxhlet extraction. It is expected that Soxhlet extraction, which is a standard method for oil extraction, would have lower concentrations due to high oil yield. However, Soxhlet extraction was run at high temperatures and underwent several steps for a prolonged time (refer to Section 2.5). As a result, significant degradation of the carotenoids was likely to have resulted owing to heat and air exposure [15].

3.2 Effect of drying pre-treatments and extraction methods on fatty acid composition

Gas chromatography was used to determine the fatty acid composition in Gac oil samples. The chromatograms (Figure 3) show retention times of the individual fatty acids in standards and Gac oils. Generally, the results indicated that the fatty acid composition in the oil extracted was affected by the four different extraction methods (Table 2). The statistical results showed that the treatments significantly affected the C16:0 ($P < 0.001$), C16:1 ($P < 0.05$) and C18:2 ($P < 0.05$) levels. However, C14:0, C18:0, C18:1, C18:3 were not significantly influenced by the treatments ($P > 0.05$).

The most abundant fatty acids found in Gac oil were palmitic acid (C16:0), a saturated fatty acid, oleic acid (C18:1) and linoleic acid (C18:2), unsaturated fatty acids (Figure 3 and Table 2). These finding are generally in agreement with those reported by Ishida et al. [1] and Vuong [33]. Several studies have reported that dietary intake of oleic and linoleic acids has beneficial effects on health [34–37]. Furthermore, Gac oil extracts containing a high concentration of oleic acid (Table 2) can be used as a replacement for oils from other sources such as sunflower, palm and soya [8].

Generally, linoleic and palmitic acids are often used as a reliable indicator of the extent of lipid oxidation during the heating process [38–40]. This is because linoleic acid is more susceptible to oxidation while palmitic acid is more stable towards oxidation [41, 42]. As can be seen in Table 2, this ratio was significantly affected by the different extraction methods ($P < 0.001$). Gac oils obtained by MDP and MDS methods showed a much higher ratio of C18:2/C16:0 compared to ADP and ADS, indicating that unsaturated fatty acids were less degraded by oxidation during the extraction process when microwave-drying was used. In other words, the air-drying pre-treatment prior to either pressing or Soxhlet extraction caused considerable oil oxidation.

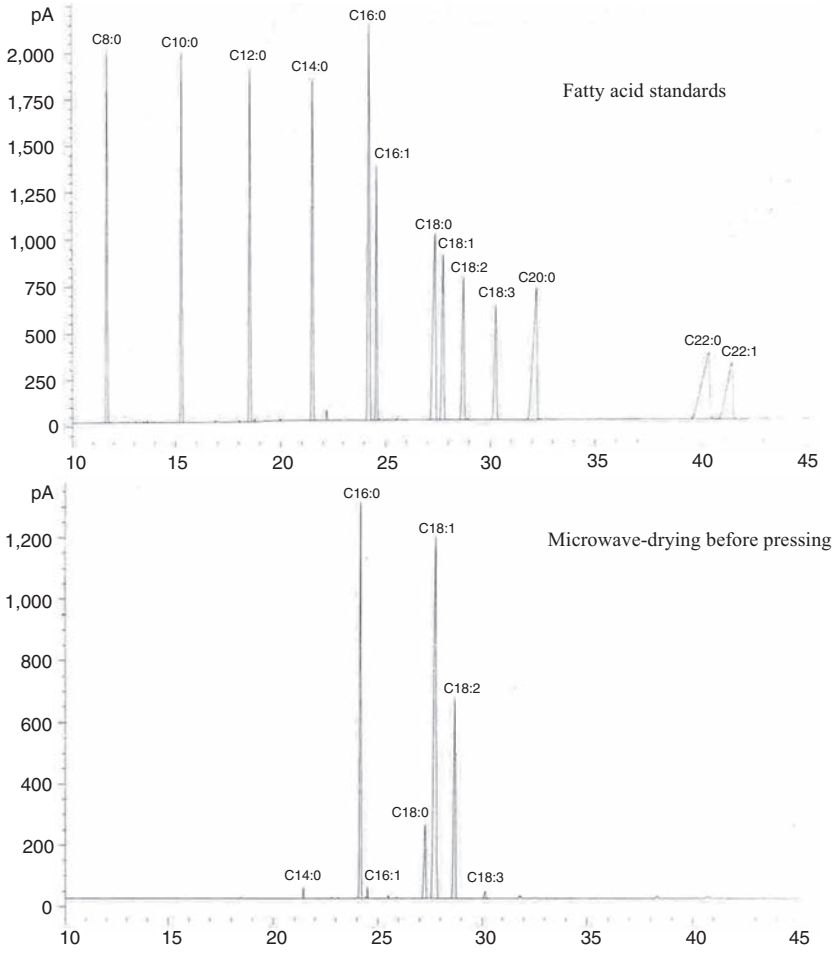


Figure 3 Typical chromatograms of fatty acids standards and a Gac oil sample

Table 2 FAME composition of Gac oil (% total FAMEs) obtained by drying pre-treatments and extraction methods

Fatty acids	Abbreviation	MDP	ADP	MDS	ADS
Myristic	C14:0	0.41 ± 0.04^{a}	1.09 ± 0.58^{a}	0.67 ± 0.10^{a}	0.63 ± 0.19^{a}
Palmitic	C16:0	24.99 ± 2.51^{a}	34.73 ± 1.87^{bc}	22.61 ± 1.29^{a}	34.89 ± 3.19^{c}
Palmitoleic	C16:1 Δ^{9}	0.40 ± 0.05^{a}	0.19 ± 0.08^{b}	0.25 ± 0.06^{b}	0.18 ± 0.07^{b}
Stearic	C18:0	6.85 ± 0.33^{a}	8.45 ± 1.00^{a}	9.14 ± 1.02^{a}	7.78 ± 1.86^{a}
Oleic	C18:1 Δ^{9}	48.25 ± 2.98^{a}	45.04 ± 3.32^{a}	48.92 ± 3.80^{a}	40.58 ± 3.53^{a}
Linoleic	C18:2 $\Delta^{9,12}$	18.28 ± 1.87^{a}	10.14 ± 2.05^{b}	17.88 ± 1.35^{a}	15.60 ± 3.99^{a}
α-Linolenic	C18:3 $\Delta^{9,12,15}$	0.83 ± 0.25^{a}	0.37 ± 0.14^{a}	0.52 ± 0.25^{a}	0.34 ± 0.12^{a}
	Σ MUFA	48.65 ± 3.03^{a}	45.22 ± 3.38^{a}	49.17 ± 3.74^{a}	40.76 ± 3.47^{a}
	Σ PUFA	19.11 ± 1.84^{a}	10.51 ± 2.13^{b}	18.41 ± 1.45^{a}	15.94 ± 4.06^{a}
	Σ SFA	32.24 ± 2.73^{a}	44.27 ± 1.32^{b}	32.42 ± 2.41^{a}	43.30 ± 1.4^{b}
	C18:2/C16:0	0.73 ± 0.11^{a}	0.29 ± 0.04^{b}	0.79 ± 0.03^{a}	0.45 ± 0.13^{b}

Notes: Values in the same row followed by different superscripts (a–b) were significantly different ($P < 0.05$); MDP: microwave-drying before pressing; ADP: air-drying before pressing; MDS: microwave-drying before Soxhlet extraction; ADS: air-drying before Soxhlet extraction.

3.3 Effect of drying pre-treatments and extraction methods on chemical properties of Gac oil

The chemical properties of Gac oil are important parameters to assess the quality of the product. Therefore, how the quality properties, including acid value (AV), peroxide value (PV), iodine value (IV) and saponification value (SV), were affected by the different extraction methods was evaluated. The statistical results (Table 3) indicated that the AV, PV and SV were significantly influenced by the different extraction treatments ($P < 0.001$). However, there was no significant difference among the extraction processes for the IV ($P > 0.05$).

Table 3 shows that the AV of Gac oil extracts obtained from the different extraction processes was in a range of 0.69–2.19 mg KOH/g oil. The Soxhlet extraction method caused higher AVs for the oil extracts compared with the pressing method. The lowest AV was found in the oil extracted by MDP. The increase in AV observed for the other three oils can be attributed to hydrolysis of triacylglycerol to produce free fatty acids [43, 44]. Moreover, the AV can also be increased by the use of solvents, high temperature, air and humidity [45, 46].

The PV, another important parameter used to evaluate the quality of oil, measures the quantity of hydroperoxides in the oil, which are formed by the reaction between oxygen and unsaturated fatty acids. This value is used to evaluate the initial stages of the oxidation process [47]. Together with the low AV, the PV (1.80) of Gac oil extracted by MDP was much lower and significantly different from those obtained by the other three extraction methods. In addition, according to Codex STAN 19-1981 [48], the approved amount of PV is up to 15 meq O_2/kg oil in virgin oils and up to 10 meq O_2/kg oil in cold pressed oil. Therefore, it can be concluded that Gac oil obtained from microwave-dried and pressed samples had a high stability towards oil oxidation.

The IV is a chemical constant and a measure of the unsaturation of fats and oils [49]. This value reflects the ability of an unsaturated carbon-to-carbon bond to absorb halogen atoms [16]. Therefore, if the iodine value is greater, the susceptibility of oil to oxidation is higher. The statistical results indicated that the different extraction processes insignificantly affected the IV in the oil extracts, which ranged from 79.14 to 84.72 g I_2/g oil. This value is within the published range in the literature for vegetable oils, including high oleic sunflower oil (78–88), olive oil (80–88) and peanut oil (84–100) [49].

The SV is a measure of the alkali-reactive groups in oils and can usually be used for predicting the type of glycerides in a sample. The higher the SV the lower is the molecular weight of the oil [16, 50]. Table 3 shows that the microwave-drying pre-treatment before either pressing or Soxhlet extraction caused lower SVs compared to the respective air-dried treatments. This result is consistent with the report by Uquiche et al. [16] who found that the SV was decreased due to microwave pre-treatment prior to pressing Chilean hazelnut oil.

4 Conclusion

High oil yields could be obtained when the aril was microwave-dried before either Soxhlet extraction or hydraulic pressing. These high oil yields could be explained by the observation of microstructural changes in the cell walls of the microwave-dried Gac arils, using light microscope and SEM techniques. The oil yield obtained from Soxhlet extraction was higher than that of pressing for the air-dried Gac arils. However, the

Table 3 Chemical properties of Gac oil obtained from different extraction methods

Treatment	MDP	ADP	MDS	ADS
Acid value (mg KOH/g oil)	0.69 ± 0.12^a	1.80 ± 0.33^b	1.95 ± 0.28^b	2.19 ± 0.24^b
Peroxide value (meq O_2/kg)	1.80 ± 0.35^a	7.70 ± 1.26^b	31.44 ± 2.46^c	33.54 ± 3.21^c
Iodine value (g I_2/g oil)	81.32 ± 1.95^a	83.53 ± 2.53^a	79.14 ± 4.49^a	84.72 ± 2.49^a
Saponification value (mg KOH/g oil)	152.82 ± 1.94^a	173.58 ± 2.48^b	169.58 ± 4.40^b	179.39 ± 3.80^b

Notes: Values in the same row followed by different superscripts (a–c) were significantly different ($P < 0.05$); MDP: microwave-drying before pressing; ADP: air-drying before pressing; MDS: microwave-drying before Soxhlet extraction; ADS: air-drying before Soxhlet extraction.

highest quality of the oil extract was achieved by microwave-drying and pressing, in terms of the highest retention of β-carotene and lycopene, and the lowest values for acidity and peroxides. Furthermore, the desirable oleic acid and linoleic acid were found to be the dominant fatty acids in all Gac oil extracts. Therefore, it can be concluded that the microwave-drying pre-treatment before hydraulic pressing method is more suitable than the air-drying pre-treatment and Soxhlet extraction technique for obtaining Gac oil of high quality.

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Paper VII

2.1.6 Microencapsulation of Gac Oil by Spray Drying: Optimisation of Wall Material Concentration and Oil Load Using Response Surface Methodology

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Microencapsulation of Gac Oil by Spray Drying: Optimization of Wall Material Concentration and Oil Load Using Response Surface Methodology

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Microencapsulation of Gac Oil by Spray Drying: Optimization of Wall Material Concentration and Oil Load Using Response Surface Methodology

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The objective of this study was to optimize the wall material concentration and the oil load on the encapsulation of Gac oil using spray drying by response surface methodology. Results showed that the quadratic polynomial model was sufficient to describe and predict encapsulation efficiencies in terms of oil, β-carotene, lycopene, peroxide value (PV), moisture content (MC), and total color difference (ΔE) with R^2 values of 0.96, 0.95, 0.86, 0.89, 0.88, and 0.87, respectively. Under optimum conditions (wall concentration of 29.5 % and oil load of 0.2), the encapsulation efficiencies for oil, β-carotene, lycopene, PV, MC, and ΔE were predicted and confirmed as 92 %, 80 %, 74 %, 3.91 meq/kg, 4.14 % and 12.38, respectively. The physical properties of the encapsulated oil powders obtained by different formulations were also determined. It was concluded that the protein-polysaccharide matrix as the wall material was effectively used for spray-drying encapsulation of Gac oil.

Keywords Gac oil; Microencapsulation; Response surface methodology; Spray drying; Whey protein

INTRODUCTION

Gac aril (*Momordica cochinchinensis* Spreng) contains extraordinarily high levels of carotenoids, especially β-carotene and lycopene.[1–3] Health benefits associated with these compounds have been extensively demonstrated.[4–7] In addition, significant amounts of unsaturated fatty acids, which are beneficial to human health, are found in the arils.[7] Several studies also showed that fat ingested with carotenoid compounds in plant foods significantly improved their absorption by the body.[8–10]

Recently, microwave-assisted extraction of Gac oil containing a high content of β-carotene and lycopene has been reported.[11,12] Due to the high number of double bonds in the structure of carotenoids and polyunsaturated fatty acids, Gac oil is susceptible to isomerization and oxidation during processing and storage. As a result, it is important to find an effective method to preserve those bioactive compounds. Microencapsulation can be a method to protect, stabilize, and release the compounds while also enabling their solubility in an aqueous medium.[13] Recently, increasing attention has been given to the application of encapsulation of bioactive compounds, particularly unsaturated fatty acids and carotenoids. The encapsulation of fatty acids and carotenoids in plant materials has been reported by numerous studies.[13–15] However, a study of Gac oil encapsulation has not yet been reported.

To effectively encapsulate Gac oil, an appropriate choice of wall materials (encapsulating agent) and ratio of oil to wall material are very important. There are various wall materials effectively used for encapsulating food oils and carotenoids in terms of good protection against heat, light, and oxidation. The agents are classified as carbohydrates, cellulose, gum, lipids, and protein, which have been thoroughly reviewed.[16–19] Generally, different wall materials have different physical and chemical characteristics; therefore, to effectively protect and control the bioactive compounds, combinations of various encapsulating agents are required.[20] A blend of whey protein (WP) and gum arabic (GA) as wall materials may enhance stability of emulsion against significant droplet size increase[21]; an emulsion, which is highly stable over a certain period of time, is a prerequisite for a proper encapsulation. Moreover, among various factors affecting the encapsulation efficiency of encapsulated oil, it is also important to optimize the core concentration.[17] Several reports showed that high stability of encapsulated oil using WP-polysaccharide matrices could be achieved.[22,23]

Among various encapsulation techniques reported,[16,18] spray-drying encapsulation is the most widely used in the food industry.[24,25] This process can potentially offer many benefits, such as economy, flexibility, and good quality of encapsulated products.[26] Thus, it is highly recommended to investigate the effect of formulation of wall material and oil concentration

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as a first step of encapsulation using spray drying. Further optimization of the formulation is also needed using a response surface methodology (RSM). It is well known that RSM is one of the most effective methods to evaluate the relationship between the response and the independent variables and then optimize the process.[27]

Currently, Gac oil encapsulation, using spray drying, has not been optimized by RSM in terms of the encapsulation efficiencies (retention of oil, β-carotene, and lycopene), peroxide value (PV), moisture content (MC), and total color difference between the infeed and reconstituted emulsions (ΔE). Therefore, experiments designed for this study aimed to optimize wall material concentration (WMC) and oil load to obtain high encapsulation efficiencies and to minimize PV, MC, and ΔE. In addition, encapsulation yield, physicochemical properties, color characteristics, and morphology of the encapsulated oil powder were also evaluated.

MATERIALS AND METHODS

Chemicals

Carotenoid standards (HPLC grade), including β-carotene (C4582, type II synthetic, ≥95 %) and lycopene (L9879, ≥90 %, from tomato), were obtained from Sigma-Aldrich Pty. Ltd. (Sydney, NSW, Australia). Solvents and BHT (Butylated hydroxytoluene) were purchased from Merck Pty. Ltd. (Sydney, NSW, Australia).

Wall and Core Materials

Whey protein concentrate (WPC 100) and GA used as wall materials were obtained from Amino Nutrition Co. (Kotara, NSW, Australia). Deionized water was employed in the preparation of all of the formulations. Core material used in this study was Gac oil, which was pressed according to a method described by Kha et al.[11] Briefly, fresh Gac arils (about 900 g) were pre-heated by microwave at 630 W for 62 min and steamed for 22 min prior to hydraulic pressing at 175 kg/cm^2. The fatty acid composition of the pressed Gac oil was 0.41 % C14:0, 24.99 % C:16, 0.40 % C:16:1, 6.85 % C18:0, 48.25 % C18:1, 18.28 % C18:2, and 0.82 % C18:3. Its peroxide value was 1.80 ± 0.35 meq O_2/kg. Gac oil also contained β-carotene (174 mg/100 mL) and lycopene (511 mg/100 mL).[11]

Preparation of the Oil-in-Water Emulsions and Spray-Drying Conditions

Emulsions were prepared using a method from Bellalta et al.[28] with some modifications. Aqueous stock solutions (500 mL) with different concentrations (presented in Table 1) of wall materials (WPC/GA: 7/3, w/w) with 0.1 % sodium benzoate (to prevent the proliferation of microorganisms) were prepared using an Ultra-Turrax T65D homogenizer (Wilmington, DE, USA) at 6000 rpm for 10 min. In the homogenizer, the solution samples were axially drawn into the dispersion head and then forced radially through the slots in the rotor/stator arrangement. The stock solutions were then kept at 4°C for at least 12 h to ensure complete hydration of proteins.

TABLE 1
The coded and uncoded levels of independent variables

Coded variable levels	Wall material concentration (WMC) X_1 (%, w/w)	Oil load X_2 (w/w)
+1.414	32.1	0.36
+1	30.0	0.33
0	25.0	0.27
−1	20.0	0.20
−1.414	17.9	0.17

To create emulsions, Gac oil was added dropwise to the wall material stock solutions while mixing using the homogenizer at 4000 rpm for 10 min to allow full incorporation. Different oil loads (that is, mass ratio of Gac oil to wall powder material, w/w, which is not the concentration of the solution) are also presented in Table 1. The crude emulsions were then re-circulated for 5 min through a twin-stage valve homogenizer (APV-1000, Poland) operated at 500 bars to form stable emulsions.

The stable emulsions (about 500 mL) were spray-dried within a day in a LabPlant SD-06A spray dryer (LabPlant UK Ltd., North Yorkshire, UK). The dryer was equipped with a two-fluid nozzle atomizer (0.5 mm diameter). The operating conditions of the spray drying were inlet air temperature of 150 ± 3°C, outlet air temperature of 95 ± 3°C and pressure of 2 bars, and the feed flow rate was about 400 mL/h. The encapsulated powders were recovered from the collecting chamber. The powders were stored in a desiccator containing silica gel to prevent moisture adsorption, then vacuum-sealed in high-density polyethylene (HDPE) plastic bags and stored at −18°C until analysis (within 24 h). The sample preparation was performed in triplicate.

Experimental Design

RSM was employed to optimize WMC and oil load on the encapsulation efficiencies in terms of retention of oil, β-carotene, lycopene, PV, MC, and ΔE. The levels of independent variables, including WMC (X_1) and oil load (X_2), were based on preliminary trials. The coded and uncoded levels of the independent variables used in the RSM design are shown in Table 1. The experimental design based on the central composite design (CCD) using a 2^2 factorial with three central points was carried out in triplicate. A second-order polynomial equation was used to express the extraction efficiencies in terms of oil (Y_1), β-carotene (Y_2), lycopene (Y_3), PV (Y_4), MC (Y_5), and ΔE (Y_6) as a function of the independent variables as follows: $Y_i = a_o + a_1X_1 + a_2X_2 + a_{11}X_1^2 + a_{22}X_2^2 + a_{12}X_1X_2$. Y_i represents the response variables; a_o is a constant; a_i, a_{ii}, and a_{ij} are the linear, quadratic, and

interaction coefficients, respectively. X_i and X_j are the levels of the independent variables.

To respect the hierarchical property, even if several coefficients were statistically insignificant, the former was still kept in the second-order equation.

Statistical Analysis

The experimental data were analyzed using JMP software version 10.0 (SAS Institute Inc., NC, USA). The adequacy of the models was determined by evaluating the lack of fit, coefficient of determination (R^2), and the Fisher's test value (F-value) obtained from the analysis of variance (ANOVA). The test of statistical significance was based on the total error criteria with a confidence level of 95 %.

Different mean values of the measured and predicted responses, and effects of WMC and oil load on the investigated parameters, were analyzed by ANOVA using SPSS software version 20.0 (IBM Australia Limited, St Leonards, NSW, Australia).

Analytical Methods

All subsequent measurements were carried out in triplicate.

Encapsulation Efficiency (EE, %)

Encapsulation efficiencies (EEs) were determined based on the retention of Gac oil, β-carotene, and lycopene contents in the encapsulated powder as follows:

$$EE_{Oil}(\%) = \frac{\text{total oil content} - \text{surface oil content}}{\text{total oil content}} \times 100$$

$$EE_{\beta-carotene}(\%) = \frac{\beta\text{-carotene content in powder (DW)}}{\beta\text{-carotene content in emulsion (DW)}} \times 100$$

$$EE_{Lycopene}(\%) = \frac{\text{lycopene content in powder (DW)}}{\text{lycopene content in emulsion (DW)}} \times 100$$

The **total oil content** of the encapsulated powder sample (3 g) was extracted with n-hexane (50 mL) using the FOSS Soxtec™ system 2045 extraction unit, Denmark. The total oil content in the powder was gravimetrically calculated.[12]

Surface oil or extractable oil or non-encapsulated oil content was determined according to Jimenez et al.[29] The encapsulated powder sample (5 g) was dissolved in n-hexane for 10 minutes without microcapsule destruction. The solvent was decanted and the residue was dried in a vacuum oven at 70°C until constant weight.

The content of **β-carotene** and **lycopene** in emulsion before spray drying and in the encapsulated powder was determined using HPLC as described by Kha et al.[12] Briefly, about 1 g weighed microcapsule sample was reconstituted in deionized water (2 mL) to form a homogeneous solution. The emulsion samples or the reconstituted samples were dissolved in a 4:3 (v/v) solution of ethanol and hexane (35 mL) and an antioxidant (butylated hydroxytoluene [BHT], 0.1 % in hexane), and the mixture was blended for five minutes at 5,000 rpm. The extract was filtered through Whatman No. 1 filter paper on a Buchner funnel. The residue was re-extracted with another 35 mL of ethanol and hexane (4:3) and then washed twice with ethanol (12.5 mL) and once with hexane (12.5 mL). The combined extracts were washed with deionized water, dried by rotary evaporator, and then diluted with the mobile phase solution. All operations were performed under subdued light to minimize oxidation of the carotenoids.

HPLC analysis of β-carotene and lycopene was performed with an Agilent 1200 HPLC (Santa Clara, CA, USA) equipped with diode array detector system consisting of a Luna C18 (100 × 4.6 mm i.d; 5 μm) direct-connect guard column coupled to a Jupiter C18 (250 × 4.6 mm i.d; 5 μm) reverse phase column (Phenomenex Australia Pty. Ltd., Lane Cove, NSW, Australia). The mobile phase consisted of acetonitrile, dichloromethane, and methanol (ACN: DCM: MeOH) 50: 40: 10 v/v/v. The flow rate was 1.0 mL/min, the detection was at 450 nm, and the injection volume was 20 μL. The identification of β-carotene and lycopene was based solely on the retention time of peaks compared with the authentic standards. The amount of β-carotene and lycopene in the emulsion samples was expressed as μg/g.

Encapsulation Yield (EY, %)

EY was calculated as the ratio of the weight of the resultant powder after spray drying in the collecting bottle and the weight of all solids (including wall and core materials) in the emulsion, expressed as a percentage. Any powders adhering to the walls of the drying chamber or cyclone was not considered, so this yield is only approximate.

Peroxide Value (PV, meq/kg)

The PV value of Gac oil encapsulated powder samples was measured according to AOCS Cd 8-53.[30] Extracted oil (5 g) from the encapsulated powder sample using the FOSS Soxtec™ system 2045 extraction unit was placed in a 250 mL Erlenmeyer flask and dissolved in 30 ml of a mixture containing acetic acid and chloroform (3:2, v/v), and then the mixture was stirred for a few seconds to ensure thorough mixing. Thereafter, 0.5 mL of saturated potassium iodide solution was added. After exactly one minute, deionized water (30 mL) was added and it was titrated with 0.01 N sodium thiosulfate using starch solution as an indicator until the solution became colorless. The mixture was magnetically stirred during the titration procedure. The results were calculated as milliequivalents of active oxygen per kg of sample as follows: $PV\left(\frac{meq}{kg}\right) = (S - B) \times N \times \frac{1000}{\text{Mass of sample (g)}}$, where S and B are the titration amount of 0.01 N sodium thiosulfate for sample and blank (in mL), respectively. N is the normality of the sodium thiosulfate solution.

Moisture Content (MC)

The MC of the samples was determined by drying at a temperature of 105°C in an oven until a constant weight was obtained.

Water Activity (Aw)

An Aqua Lab water activity meter (Aqua Lab Series 4 TEW, Decagon Devices, Inc., WA, USA) was used to measure Aw of the encapsulated powders at 25 ± 0.5°C.

Bulk Density

Bulk density (g/mL) of the powder samples was measured by gently adding 2 g of the powder into an empty 10 mL graduated cylinder and holding the cylinder on a vortex vibrator for 1 min. The bulk density value was determined by the ratio of the mass of the powder and the volume occupied in the cylinder.

pH Determination

The encapsulated powders were reconstituted with deionized water to the same moisture content of the infeed emulsions before measuring the pH. The pH values of the infeed emulsion before spray drying and the reconstituted powder were determined in a beaker maintained at 20°C by a pH meter calibrated with standard buffers at pH 7 and 4.

Water Solubility Index (WSI)

The WSI of the powders was determined using the method described by Anderson et al.[31] The encapsulated powder (2.5 g) and distilled water (30 mL) were vigorously mixed in a 100 mL centrifuge tube, incubated in a 37°C water bath for 30 min, and then centrifuged for 20 min at 11,410 × g in a J2-MC Centrifuge (Beckman, USA). The supernatant was carefully collected in a pre-weighed beaker and oven-dried at a temperature of 103 ± 2°C. The WSI (%) was calculated as the percentage of the dried supernatant weight with respect to the amount of the original 2.5 g Gac fruit powder.

Color Characteristics

The color of the encapsulated powder, the reconstituted powder, and the emulsion was measured using a Chroma meter (CR-400, Konica Minolta Sensing, Inc., Japan) calibrated with a white standard tile. The results were expressed as Hunter color values of L*, a*, and b*, where L* was used to denote lightness, a* redness, and greenness, and b* yellowness and blueness. Before measurement, the samples were packed into a polyethylene pouch and measured.

Chroma (C), indicating color intensity, was calculated by the formula $\sqrt{a^{*2} + b^{*2}}$. The hue angle (H°) was calculated by the formula $H° = \arctan(b^*/a^*)$. The hue angle values vary from 0° (pure red color), 90° (pure yellow color), 180° (pure green color), to 270° (pure blue color). Total color difference between two samples was calculated by the formula $\Delta E = \sqrt{(L_o^* - L^*)^2 + (a_o^* - a^*)^2 + b_o^* - b^*)^2}$, where L_0^*, a_0^*, and b_0^* are the values of the infeed emulsion samples, and L^*, a^*, and b^* are the values of the reconstituted emulsion samples.

Particle Morphology

The external and internal morphography of powder particles were performed using a JSM-7401F scanning electron microscope (SEM) (Jeol Co. Ltd., Tokyo, Japan). A small amount of the encapsulated powder was fixed onto the surface of a double-coated metallic adhesive tape adhered to a metallic stub. The stub was then coated with a fine layer of gold and argon in a MSP-1S magnetron sputter coater (Vacuum Device, Tokyo, Japan) under vacuum condition. The morphography observation was examined at different magnifications using the SEM at a voltage of 5 kV. To evaluate the inner structure of the particles, a small amount of powder (attached to the stub) was fractured by attaching a second piece of adhesive tape on top of the powder and then ripping it off quickly.[32]

RESULTS AND DISCUSSION

Encapsulation Efficiency (EE)

Preliminary trials showed that WMC and oil load were found to be the most significant factors influencing emulsion stability before spray drying, and EEs in terms of retention of oil, β-carotene, and lycopene (data not shown). The most suitable range of the WMC (20 to 30 %, w/w) and the oil load (0.20 to 0.33) was screened and the optimum levels of the two variables were determined using RSM. These results were used for the experimental design (Table 1).

The EEs obtained from all the experiments are shown in Table 2. Analysis of variance (ANOVA) shows in Table 3 that second-order polynomial models adequately represented the experimental data with the coefficient of multiple determination (R^2) for the EEs in terms of retention of oil (0.96), β-carotene (0.95), and lycopene (0.86). In addition, the predicted values agreed well with the experimental ones obtained from the RSM design (Table 2). ANOVA results also indicated that the three models were significant ($P < 0.01$), and there was no significance in the lack of fit ($P > 0.05$) in each of the models (Table 3). Therefore, it can be stated that the three models were adequate to describe the influence of the WMC and the oil load on the EEs. Furthermore, Table 3 shows that the statistical significance of the coefficients of the models was determined using the t ratio and P-value (significance level). According to Amin and Anggoro,[33] a more significant effect on the corresponding variables would be indicated by the larger t ratio and smaller P-value.

The lower surface oil content of the microcapsules, which varied from 0.63 to 7.82 %, resulted in higher EE of Gac oil. The EE of Gac oil varied from 73.59 to 96.67 % and was significantly affected by the linear term of the oil load, and the quadratic terms of the WMC and the oil load (Table 3). As can be seen in Fig. 1, the increase in the EE was obtained when the oil load decreased. Similar trends of the EEs in terms of β-carotene and lycopene were also observed. The lower encapsulation efficiency was caused by the higher loss of β- carotene and lycopene contents in the infeed emulsions during spray drying, which varied from 358 to 738 and from 992 to 2056 μg/g dry weight, respectively. Furthermore, statistical results showed that the linear and interaction terms of the WMC and the oil load significantly

TABLE 2
Experimental (Exp.) and predicted (Pred.) values of the encapsulation efficiencies obtained

			EE_{Oil} (%)		$EE_{\beta-carotene}$ (%)		$EE_{Lycopene}$ (%)		PV (meq/kg)		MC (%)		ΔE	
Pattern	X_1 (%)	X_2	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.
+−	30.0	0.20	96.67	93.67	79.95	80.16	68.83	73.08	3.71	3.94	4.35	4.18	12.30	11.87
0A	25.0	0.36	77.34	76.53	69.84	71.04	70.05	71.83	4.55	4.95	4.16	4.12	14.14	12.02
a0	17.9	0.27	90.91	89.11	87.61	85.43	56.72	55.03	5.39	5.54	4.77	4.76	8.17	7.82
0a	25.0	0.17	92.5	94.27	81.23	79.27	79.88	74.63	4.77	4.50	3.81	3.96	12.34	13.37
00	25.0	0.27	70.73	72.26	76.20	76.40	75.67	75.59	3.90	3.90	4.11	4.07	15.13	15.63
00	25.0	0.27	72.45	72.26	77.89	76.40	76.98	75.59	4.10	3.90	3.95	4.07	16.25	15.63
−+	20.0	0.33	77.00	79.04	86.05	86.59	63.13	62.35	5.15	4.79	4.66	4.72	5.74	7.26
00	25.0	0.27	73.59	72.26	75.11	76.40	74.11	75.59	3.70	3.90	4.15	4.07	15.50	15.63
++	30.0	0.33	85.58	84.40	61.54	59.52	70.85	70.13	5.66	5.42	3.94	3.90	13.10	14.90
A0	32.1	0.27	89.31	92.06	66.68	68.11	69.18	67.41	4.81	4.79	4.03	4.16	13.75	13.01
–	20.0	0.20	94.64	94.86	74.81	77.58	59.17	63.36	5.51	5.63	4.29	4.21	12.90	12.19

X_1: wall material concentration; X_2: oil load.

influenced retention of β-carotene content, whereas retention of lycopene was significantly affected by the linear and quadratic terms of WMC (Table 3).

It is generally agreed that higher core loads result in lower encapsulation efficiency or poorer retention with less core material being encapsulated or higher core material content at the surface of the microcapsules.[34–36] It is due to the amount of wall materials used not being sufficient to encapsulate the core material. Therefore, the core materials, which were not embedded by the wall matrix, were readily destroyed by heat and oxidation, resulting in lower encapsulation efficiency. In other words, a higher concentration of core materials may lead to a greater amount of core materials on the powder surface,[35] thus increasing the surface oil (or carotenoids). In addition, a high concentration of core materials may relate to the droplet size in the emulsion before spray drying, which results in a large core droplet size, thus poor encapsulation efficiency.[37] This behavior could explain why it took a longer time for film formation in large particles, resulting in greater loss of core materials.[35] As a result, it is important to optimize the oil load

TABLE 3
Regression coefficients of the fitted quadratic equation and t ratio for the encapsulation efficiencies and physicochemical properties

	EE_{Oil} (%)		$EE_{\beta-carotene}$ (%)		$EE_{Lycopene}$ (%)		PV (meq/kg)		MC (%)		ΔE	
Regression coefficient[1]	Regression coefficient	t ratio	Regression coefficient	t ratio	Regression coefficient	t ratio	Regression coefficient	t ratio	Regression coefficient	t ratio	Regression coefficient	t ratio
a_o	72.26		76.4		75.59		3.90		4.07		15.63	
Linear												
a_1	1.04	1.14	−6.12***	−7.30	4.38*	3.14	−0.26	−2.17	−0.21**	−4.17	1.83*	3.20
a_2	−6.27***	−6.87	−2.91*	−3.47	−0.99	−0.71	0.16	1.31	0.06	1.11	−0.48	−0.83
Quadratic												
a_{11}	9.17***	8.44	0.18	0.18	−7.18**	−4.34	0.63**	4.36	0.19*	3.20	−2.61*	−3.83
a_{22}	6.57**	6.05	−0.62	−0.62	−1.18	−0.71	0.41*	2.84	−0.01	−0.22	−1.47	−2.15
Interaction												
a_{12}	1.64	1.27	−7.41**	−6.25	−0.49	−0.25	0.58*	3.36	−0.20*	−2.70	1.99	2.46
R^2	0.96		0.95		0.86		0.89		0.88		0.87	
P-value of lack of fit	0.18		0.20		0.08		0.20		0.30		0.07	

*$P < 0.05$; **$P < 0.05$; ***$P < 0.05$.

[1]a_o is a constant; a_i, a_{ii}, a_{ij} are the linear, quadratic, and interactive coefficients of the second-order polynomial equation, respectively.

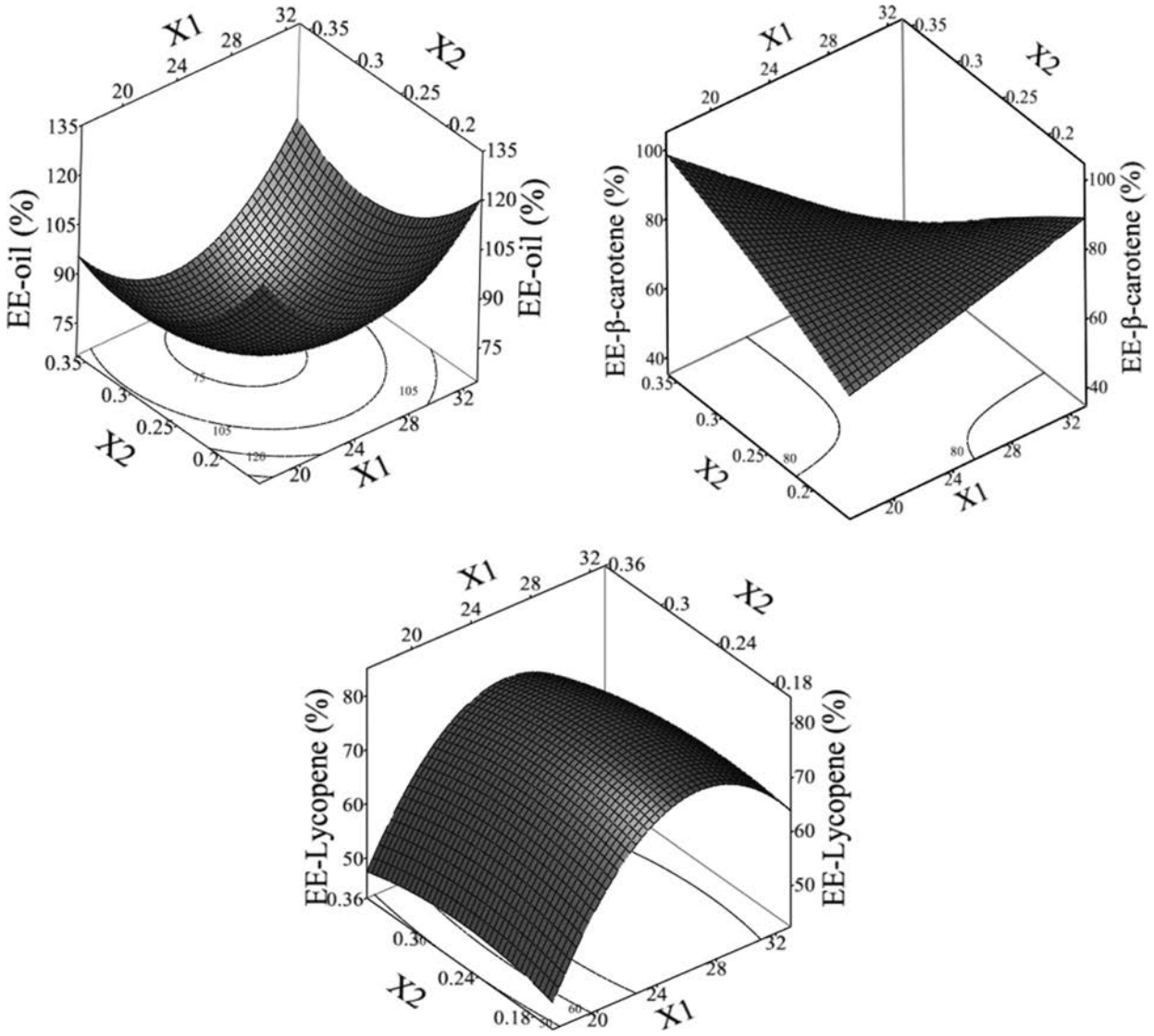

FIG. 1. The 3D response and 2D contour plots of the EEs affected by the wall material concentration (X_1) and the oil load (X_2).

in the emulsion preparation to increase the EE and minimize surface oil content.

The WMC had a positive effect on the EEs in terms of retention of the oil and lycopene; i.e., a higher concentration resulted in a higher encapsulation efficiency (Fig. 1). This phenomenon could be explained by the high concentration providing sufficient content of the continuous phase of the wall materials to properly cover the surfaces of the droplets of the core materials in the infeed emulsion[38] and decrease in circulation movement inside the droplets.[17] As a result, the continuous phase formed a dense, tightly packed matrix surrounding the dispersed oil droplets containing carotenoids, preventing degradation of the oil and carotenoids due to oxidation. Moreover, a high concentration also resulted in a higher emulsion viscosity and lower emulsion droplet size,[17,39] promoting a faster drying and an increase in the film-forming capacity of the wall materials on the surface core, therefore increasing the encapsulation efficiency.[40,41] It can therefore be concluded that the concentration of wall materials and oil loads (as mentioned previously) significantly affected the EE. The two variables are strongly related to the infeed emulsion properties, including viscosity and droplet size.[42]

It is interesting to note that there was an inverse trend of the EE in terms of retention of β-carotene and lycopene (Fig. 1). Higher and lower retentions of lycopene and β-carotene, respectively, were obtained at the higher concentration of wall materials. This behavior is likely due to different supramolecular structures and reactivity of carotenoids, which interacted differently with the matrix of the wall materials.[43,44] Furthermore, the physical and chemical properties of carotenoids can be altered by the interactions between carotenoids and the matrix, such as protein.[44] Therefore, the complex between carotenoids and the mixture

was formed and had an impact on the different degradation rates during spray drying. This result is also partly in agreement with a study by Cao-Hoang et al.,[45] who reported the positive effects of the formation of carotenoids extracted from Gac arils, and polylactic acid as wall material, on oxidation.

Encapsulation Yield (EY)

As well as the EE, it was desirable to obtain higher EY, which is one of the major parameters of the spray-drying operation. The EY of the encapsulated powders formulated by different WMCs and oil load is presented in Table 4. In general, results showed that significantly higher EY was obtained when increasing the WMCs, indicated by a significant value of linear and quadratic terms of the concentration ($P < 0.05$). A similar trend for effect of the oil load on the yield was also found; however, it was not statistically significant ($P > 0.05$). Furthermore, ANOVA results showed that effects of the concentration and the oil load on the yield were inadequately fitted into the quadratic model, indicated by R^2 of 0.83 and $P > 0.05$.

In this study, the yield, which varied from 30.49 to 48.74 %, was significantly affected by the WMC. The yield decreased with decreasing WMC because the wall materials were not sufficient to cover the oil droplets, which were easily stuck to the walls of the drying chamber, leading to a low yield. Therefore, it is important to optimize spray-drying conditions such as inlet and outlet temperature to enhance the EY. For example, Goula and Adamopoulos[35] reported that drying temperatures had a positive effect on the yield.

Peroxide Value (PV)

Table 3 and Fig. 2 show effects of the WMC and the oil load on the PV of the encapsulated powders. According to the ANOVA analysis of the optimization study, the quadratic term of WMC and the oil load had a significant effect on the PV. In addition, interaction between the two variables was found to be significant ($P < 0.05$).

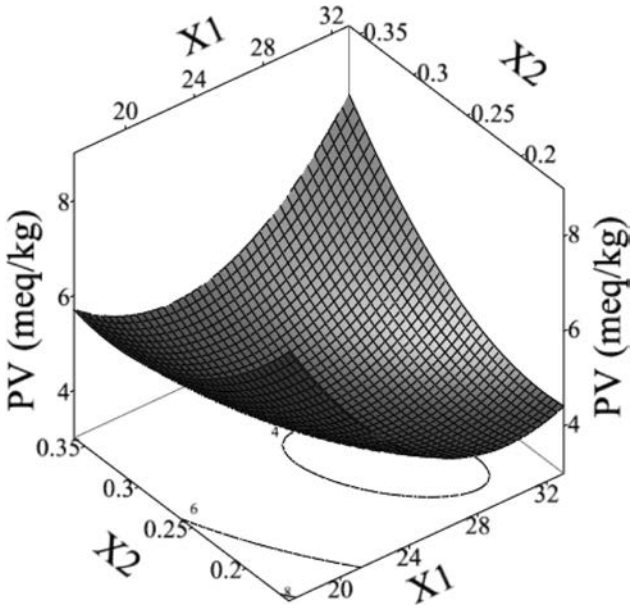

FIG. 2. The 3D response and 2D contour plots of peroxide value (PV) affected by the wall material concentration (X_1) and the oil load (X_2).

Generally, the PV, which measures the quantity of hydroperoxides in the oil formed by the reaction between oxygen and unsaturated fatty acid, is used to evaluate the initial stages of the oxidation process.[46] Results showed that a decrease in PV could be obtained at higher WMC and lower oil load and vice versa. This is due to poorer oil encapsulation efficiency obtained at the lower WMC and the higher oil load, which resulted in the higher content of non-encapsulated oil. As a result, the oil content at the particle surface was easily subjected to oxidation due to the direct contact with oxygen of drying air, leading to an increase in PV, which is in agreement with a study by Aghbashlo et al.[47] on fish oil encapsulation.

TABLE 4
Physical properties and encapsulation yield (EY) of the encapsulated powders obtained by different formulations

WMC (%)	Oil load	Aw	Density (g/mL)	pH	WSI (%)	EY (%)
30.0	0.20	0.35 ± 0.05	0.36 ± 0.05	5.69 ± 0.18	91.15 ± 2.15	48.74 ± 1.62
25.0	0.36	0.36 ± 0.08	0.31 ± 0.03	5.70 ± 0.54	84.76 ± 4.03	37.12 ± 1.98
17.9	0.27	0.44 ± 0.08	0.36 ± 0.05	5.73 ± 0.39	86.16 ± 2.04	30.49 ± 1.84
25.0	0.17	0.38 ± 0.07	0.36 ± 0.08	5.70 ± 0.62	92.28 ± 2.24	42.38 ± 1.90
25.0	0.27	0.40 ± 0.02	0.44 ± 0.04	5.72 ± 0.27	91.12 ± 0.96	42.38 ± 1.80
20.0	0.33	0.38 ± 0.05	0.40 ± 0.05	5.66 ± 0.16	89.73 ± 1.94	38.54 ± 2.08
30.0	0.33	0.41 ± 0.04	0.44 ± 0.07	5.70 ± 0.39	86.25 ± 2.76	38.94 ± 1.09
32.1	0.27	0.27 ± 0.04	0.36 ± 0.04	5.68 ± 0.26	91.71 ± 2.74	38.42 ± 2.17
20.0	0.20	0.39 ± 0.03	0.40 ± 0.08	5.72 ± 0.31	89.79 ± 2.33	37.64 ± 3.34

WPC: whey protein concentration.

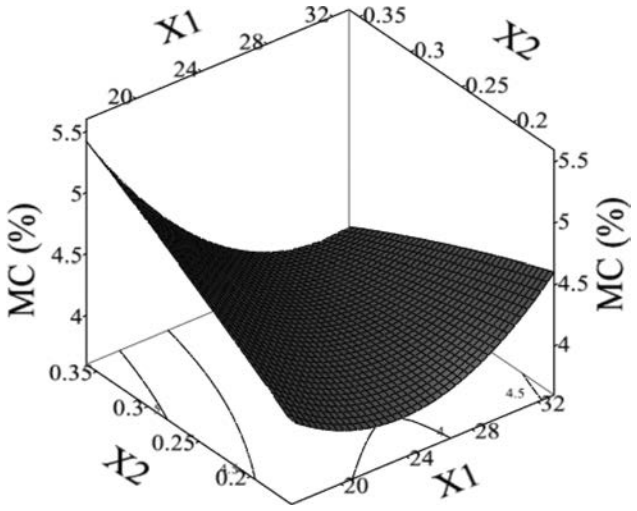

FIG. 3. The 3D response and 2D contour plots of moisture content (MC) affected by the wall material concentration (X_1) and the oil load (X_2).

Moisture Content (MC)

The MC of the resultant powders obtained at different conditions is presented in Table 3 and Fig. 3. Statistical evaluation of results indicated that the MC was significantly affected by linear and quadratic terms of the WMCs. Moreover, there was a significant interaction between the WMC and the oil load on the MC ($P < 0.05$).

Results showed that the WMC had a desirable effect in lowering the MC of the resultant powders. It means that the MC showed a decrease with an increase in the WMC. This observation is due to the effect of WMC on the droplet size. The smaller average particle size in the emulsion can be obtained with increasing WMC, which can be explained by the more complete interaction of WP and GA matrix with the oil droplets.[48] The smaller particle size with a larger surface area results in greater water evaporation rates. Furthermore, an increase in the WMC reduced the total amount of water available to be evaporated during spray drying.[49,50] This result is also in agreement with other previous findings.[22,51,52]

In addition, MC is an important parameter for oil powder because oil oxidation can quickly take place at high MC. The MC of the powders under the investigated conditions was less than 5 % (Table 2), which is in the range of most dried powders used in the food industry.

Water Activity (Aw)

Statistical results showed that the Aw of the resultant powder was insignificantly affected by the WMC and the oil load ($P > 0.05$). It is also indicated that the quadratic model was insignificant ($P > 0.05$). In other words, the model insufficiently described the effect of the WMC and the oil load on the Aw, indicated by R^2 of 0.55. Table 4 presents the Aw of the encapsulated powders obtained at different formulations. In general, the Aw, the mean values of which varied from 0.27 to 0.44, decreased when increasing the WMC. This observation was similar to those for the MC. It is because low water activities are associated with low water contents, which prevent off-flavor caused by the lipid oxidation[53] and are considered to be microbiologically stable.[50,54]

Bulk Density

Analogous to the Aw, the bulk density of the encapsulated powders was insufficiently described by the quadratic model using RSM, indicated by the low R^2 of 0.51 and $P > 0.05$. Table 4 shows that the average bulk density of the powders obtained by different WMCs and oil loads varied from 0.31 to 0.44 g/mL. These values were also similar to those reported by several authors, who showed that the bulk density of oil powder which used WP and/or GA as wall material varied from 0.29 to 0.53 g/mL.[55,56] Generally, low bulk density of the power is not desirable due to the requirement of a greater volume of package.[57] In addition, it is easy to include air within the powders with the lower bulk density, thus oxidation could take place, resulting in shorter storage stability.[58]

pH Value

According to ANOVA results, pH value of the encapsulated powders was not significantly fitted into the quadratic model, indicated by R^2 of 0.66 and $P > 0.05$. Table 4 shows the impacts of different WMCs and oil loads on the pH value, the average values of which varied from 5.66 to 5.73. In general, the pH value slightly decreased with the increasing WMC and the oil load, even though this decrease was found to be statistically insignificant.

In addition, at these pH values, a high stability of the infeed and reconstituted emulsions was observed (data not shown here). This is because those emulsions containing WP have a charge that is enough to prevent the close approach of suspended oil droplets.[59] Although WP is known to be very sensitive to pH with a tendency to aggregation, especially at pH values close to their isoelectric point of 5.2,[59] a continuous phase consisting of WP and GA may make the emulsion less sensitive to pH.[21]

Water Solubility Index (WSI)

In addition to obtaining high EE, WSI was also one of the most important parameters for dried powder. Our previous study showed that the WSI of spray-dried Gac aril powders using maltodextrin as a drying aid agent was very low, 36.91–38.25 %. It was because of a high content of liposoluble substances and insoluble pulp in the aril.[50] As a result, it is important to improve the water solubility for ease of application in the food industry. In this study, the WSI of the encapsulated powders was significantly improved by extracting the oil containing high liposoluble substances from the aril and using a mixture of WP and GA as wall material. This is evident by obtaining the high WSI, which varied from 84.76 to 92.28 % (Table 4). It could be explained by a continuous

phase consisting of high soluble components of WP and GA. Furthermore, the drying conditions for the infeed emulsions may influence the solubility due to denatured WP causing a decrease in dissolution rates.[57,60] Since solubility is one of the key determinants of the overall reconstitution quality,[57] it is highly desirable to optimize the drying conditions to obtain the highest water solubility.

As can be seen in Table 4, the higher water solubility of the oil powder was obtained at higher WMC and lower oil load. It is also evident by calculating from a quadratic model using RSM that the high value of above 90% could be obtained when using the higher WMCs (25 to 32%) and the lower oil loads (0.17 to 0.27). The high solubility could be explained by the positive effects of an interaction between protein and GA on the oil droplets containing carotenoids. At those conditions, GA, having a high molecular mass arabinogalactan polysaccharide, linked to the hydrophobic polypeptide chain that strongly adsorbs at the oil in water interface.[21,61] Since those protein-polysaccharide interactions, which are readily formed, have been shown to possess excellent stabilizing properties at interfaces,[62,63] good solubility in the aqueous continuous phase could be obtained. Similarly, Bouyer et al.[23] reported that high stability of emulsion could be obtained when using these biopolymers under appropriate conditions, due to covalent bonding or electrostatic interactions. However, these data of the water solubility could not be sufficiently predicted using the quadratic model, indicated by R^2 of 0.73 and $P > 0.05$.

Color Characteristics

Table 5 shows the effects of the WMC and the oil load on the color characteristics of the encapsulated powders, including lightness L, chroma C, and hue angle H°. According to ANOVA results, quadratic models insufficiently described the effects of the independent variables on the L, C, and H° ($P > 0.05$). However, the concentration significantly affected L and H° ($P < 0.05$) of the powder, but not C ($P > 0.05$), higher WMCs resulted in a decrease in L and H° of the powders. An inconsistent result was observed in terms of the lightness value of Gac powder spray-dried with maltodextrin addition. A significant increase in L of the products was obtained by increasing maltodextrin content.[50] The difference is due to the different wall materials used; maltodextrin has a white color, whereas the color of WP is light yellow. In addition, since the redness of the encapsulated powders was indicated by the lower values of H°, it can be concluded that it could be effectively preserved at the higher WMC.

For comparison, color characteristics of the infeed and reconstituted emulsions are presented in Table 6. In the experimental range, in general, a significant decrease in the values of L ($P < 0.01$), chroma ($P < 0.001$), and H° ($P < 0.001$) of the infeed emulsions could be obtained when increasing the WMC. A significant reverse effect of the oil load on the color characteristics was observed. Moreover, according to ANOVA results, the color characteristics of the emulsions were adequately fitted into quadratic models. In contrast, quadratic models did not sufficiently describe effects of the WMC and the oil load on the color characteristics of the reconstituted emulsions.

Color is one of the most important appearance attributes of food products as it directly impacts acceptability by consumers. In addition to the high EE and the low PV, it is desirable to obtain high quality of the encapsulated oil powder when the color characteristics of its reconstituted emulsion are close to those of the infeed one. Total color difference (ΔE) between the reconstituted and infeed emulsions is shown in Fig. 4 and Table 2. ANOVA results indicated that linear and quadratic terms of the concentration significantly affected the ΔE ($P < 0.05$). The quadratic model sufficiently described the effect of the independent variables on the ΔE, indicated by R^2 of 0.87 and $P < 0.05$ (Table 3). Generally, a higher ΔE value, which indicates a greater color difference between the infeed

TABLE 5
Color characteristics of the encapsulated powder obtained by different formulations

		Encapsulated powders		
WPC (%)	Oil load	L	C	H°
30.0	0.2	79.66 ± 0.90	46.19 ± 1.38	72.29 ± 0.45
25.0	0.36	81.85 ± 1.67	44.07 ± 0.90	76.26 ± 0.79
17.9	0.27	80.54 ± 1.89	46.65 ± 0.57	74.02 ± 0.74
25.0	0.17	82.05 ± 0.49	46.18 ± 0.48	73.82 ± 0.12
25.0	0.27	78.10 ± 1.43	49.10 ± 0.78	73.24 ± 0.82
20.0	0.33	79.45 ± 0.47	48.01 ± 0.95	73.03 ± 0.59
30.0	0.33	77.92 ± 1.12	46.78 ± 1.13	69.81 ± 0.35
32.1	0.27	76.28 ± 0.63	48.09 ± 1.61	69.24 ± 0.15
20.0	0.2	83.59 ± 0.50	43.94 ± 0.52	75.73 ± 0.46

WPC: whey protein concentration; L: lightness, C: chroma, and H°: hue angle.

TABLE 6
Color characteristics of the infeed and reconstituted emulsions obtained by different formulations

		Infeed emulsions			Reconstituted emulsions		
WPC (%)	Oil load	L	C	H°	L	C	H°
30.0	0.2	52.96 ± 0.85	41.03 ± 0.70	47.79 ± 0.26	61.84 ± 0.44	47.32 ± 0.67	55.21 ± 0.09
25.0	0.36	57.14 ± 0.91	47.63 ± 1.11	52.75 ± 0.09	67.25 ± 1.12	52.63 ± 0.42	62.46 ± 0.82
17.9	0.27	57.09 ± 0.89	45.55 ± 0.61	54.22 ± 0.33	63.10 ± 1.00	49.40 ± 1.15	58.76 ± 0.10
25.0	0.17	55.63 ± 1.07	43.08 ± 1.35	52.46 ± 0.07	62.23 ± 1.20	51.06 ± 1.04	60.46 ± 0.25
25.0	0.27	56.73 ± 1.32	42.27 ± 0.55	50.88 ± 0.06	63.47 ± 0.87	54.48 ± 0.28	59.08 ± 1.21
20.0	0.33	59.59 ± 0.51	48.15 ± 0.75	55.18 ± 0.23	61.54 ± 0.48	53.25 ± 0.56	53.26 ± 0.10
30.0	0.33	53.71 ± 1.32	42.69 ± 0.98	47.59 ± 0.09	60.74 ± 0.26	53.26 ± 0.79	51.39 ± 0.62
32.1	0.27	53.12 ± 0.52	41.07 ± 0.70	46.62 ± 0.13	59.79 ± 0.62	52.26 ± 0.33	51.91 ± 0.66
20.0	0.2	56.54 ± 0.49	43.49 ± 0.66	53.89 ± 0.00	63.28 ± 0.56	51.12 ± 0.50	63.48 ± 0.77

WPC: whey protein concentration; L: lightness, C: chroma and H°: hue angle.

and reconstituted emulsions, was obtained when increasing the WMC (up to about 26%) and the oil load (up to about 0.26). After that, further increases resulted in lower ΔE value, indicating lower color difference.

Particle Morphology

Figure 5 shows size and morphology (inner and outer) of the optimized encapsulated powder (WMC at 29.5% and oil load at 0.2). SEM micrographs confirmed that the powder particles were micro-sized (<30 μm) and had various sizes, which are a typical characteristic of spray-dried powders.[40] Rough and smooth surfaces of the particles were also found. These could be due to the particle shrinkage during spray drying and cooling, resulting in depressions forming.[40] In addition, the microparticles also had spherical shapes and were free of cracks and pores, which are important to effectively prevent the oil and carotenoids from oxidation and the undesired release of the oil droplets to the particle surface.[47]

Furthermore, it is also interesting to observe a broken microcapsule to evaluate its inner porous structure. Small spherical holes were observed in a cross cut of a microcapsule, indicating oil homogeneously distributed in the wall matrix.[53] These observations are also consistent with other reports using different protein types as wall material.[22,55]

Overall Optimization and Model Validation

As discussed above, there are six responses including the EEs in terms of oil, β-carotene, lycopene, PV, MC, and ΔE that were significantly affected by the two independent variables. ANOVA results also indicated that the quadratic models adequately described effects of the variables on those responses. For optimization of formulation conditions, the most important criterion is to obtain the highest EEs of oil, β-carotene, and lycopene, and minimize the PV, MC, and ΔE. A graphical optimization (Fig. 6) was performed using a JMP package for this study.

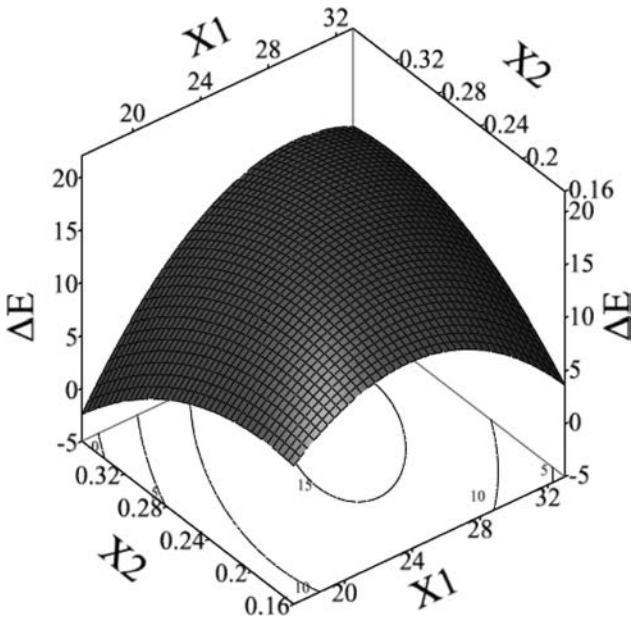

FIG. 4. The 3D response and 2D contour plots of total color difference (ΔE) affected by the wall material concentration (X_1) and the oil load (X_2).

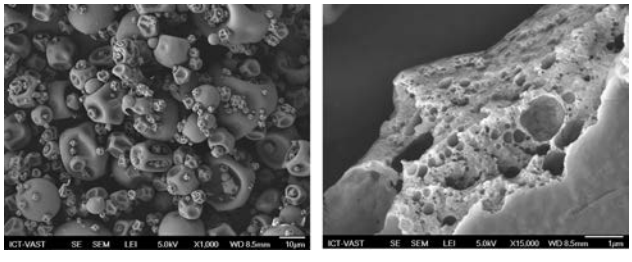

FIG. 5. Outer (left) and inner (right) microstructures of the optimized encapsulated oil powder.

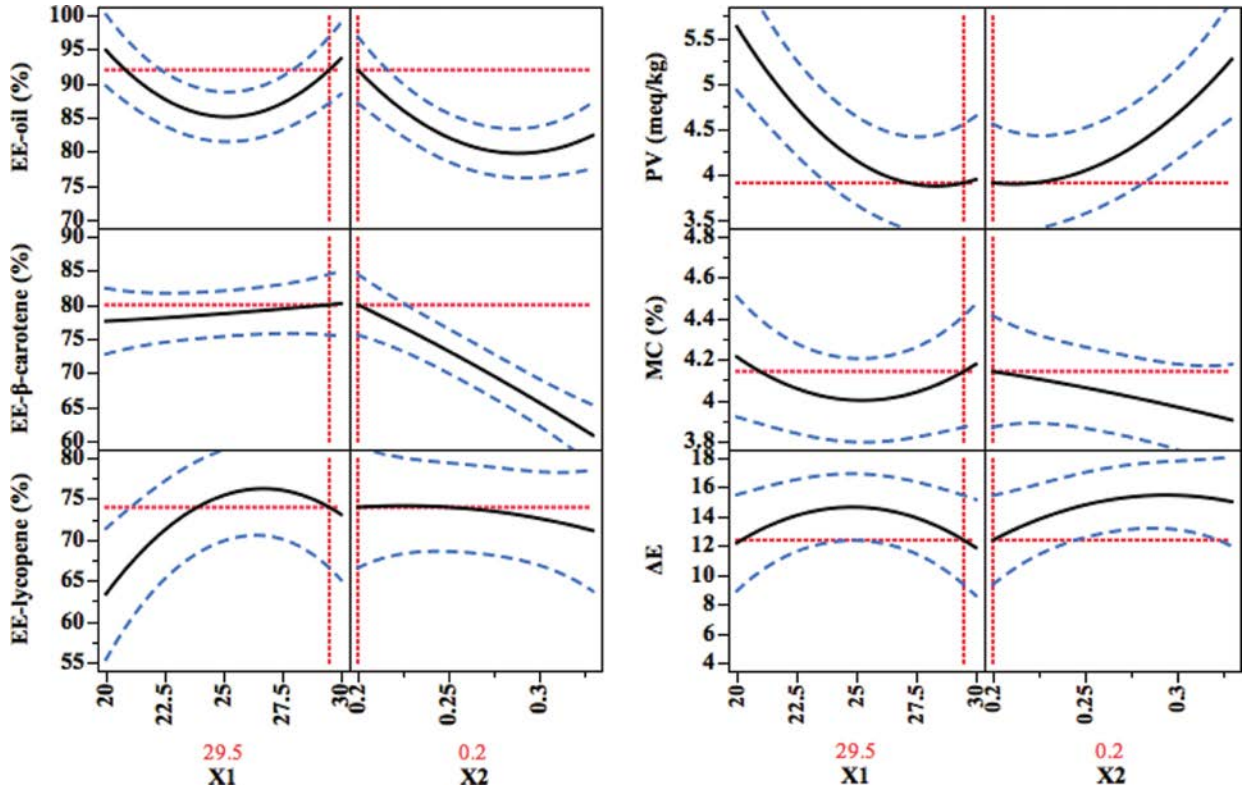

FIG. 6. Prediction profilers of EEs of oil, β-carotene, lycopene, PV, MC, and ΔE as a function of the wall material concentration (X_1) and the oil load (X_2).

As discussed earlier and can be seen in Fig. 6, in general, an increase in the WMC resulted in increasing the EEs of oil, β-carotene, and lycopene, and decreasing PV. An opposite trend of MC and ΔE was observed when the concentration was increased. Moreover, the EEs of oil, β-carotene, lycopene, and MC were decreased with increasing oil load. However, there were great and slight increases found for PV and ΔE, respectively. Therefore, it is desirable to determine the optimum for the WMC and the oil load.

According to Fig. 6, the theoretical maximum values of the EEs of oil, β-carotene, and lycopene, and minimum values of PV, MC, and ΔE, could be obtained by combining the concentration and the oil load. Based on the prediction profiler, the mathematical model suggested the optimum theoretical values of those responses under the optimum conditions (the WMC of 29.5% and the oil load of 0.2), as shown in Table 7. Furthermore, the comparison between the predicted and measured values of the responses is to establish how accurately the model describes the studied process. Hence, an experiment with three replicates was conducted at the optimum conditions. Statistical results showed that the difference between the predicted and measured values of those responses were insignificant ($P > 0.05$) (Table 7). It is evident that the suitability of the quadratic model for predicting the optimum responses was practically validated for the optimum conditions using RSM. Additionally, under the optimum conditions, the properties of the optimum encapsulated Gac oil powder were also evaluated. Results showed that the encapsulation yield (%), Aw, pH, bulk density, and WSI (%) of the optimum powder

TABLE 7
Optimum conditions of the wall material concentration of 29.5 % and the oil load of 0.2

EE-Oil (%)		EE-β-carotene (%)		EE-Lycopene (%)		PV (meq/kg)		MC (%)		ΔE	
Predicted	Measured	Predicted	Measured	Predicted	Measured	Predicted	Measured	Predicted	Measured	Predicted	Measured
91.99 ± 3.86	93.32 ± 1.43	80.00 ± 3.08	77.81 ± 3.18	73.96 ± 3.42	71.73 ± 2.86	3.91 ± 0.12	3.43 ± 0.34	4.14 ± 0.16	4.35 ± 0.42	12.38 ± 0.60	11.29 ± 1.15

were 47.07 ± 2.00, 0.32 ± 0.05, 5.72 ± 0.09, 0.36 ± 0.02, and 92.60 ± 2.19, respectively. Color characteristics of the encapsulated powder (L of 81.30 ± 1.00, C of 48.39 ± 2.66, and H° of 71.60 ± 0.33) were also determined. The L, C, and H° of the reconstituted powders were 61.07 ± 2.00, 48.28 ± 1.70, and 56.94 ± 0.99, respectively.

CONCLUSIONS

The results showed that the quadratic polynomial model was sufficient to describe and predict the investigated responses in the formulation process. The data could adequately fit six second-order equations for the EEs in terms of oil, β-carotene, lycopene, PV, MC, and ΔE with R^2 values of 0.96, 0.95, 0.86, 0.89, 0.88, and 0.87, respectively. The graphical optimization was employed to predict the optimum formulation conditions within the experimental ranges including the WMC of 29.5 % and the oil load of 0.2. Under such conditions, the EEs in terms of oil, β-carotene, lycopene, PV, MC, and ΔE achieved were also predicted and confirmed as 92 %, 80 %, 74 %, 3.91 meq/kg, 4.14 %, and 12.38, respectively.

In addition, the results also indicated that the EY was about 47 %. The color characteristics and the physical properties of the oil powders including Aw, pH, bulk density, and WSI were also determined. The slight difference found when comparing the color characteristics between the infeed and reconstituted emulsions indicated its effectiveness in preserving color. Therefore, it can be concluded that Gac oil containing β-carotene and lycopene was successfully encapsulated in the protein-polysaccharide matrix. The resultant Gac oil powder could then be easily incorporated into various foods for consumers to benefit from the nutrients and enjoy the attractive red-yellow color.

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Paper VIII

2.1.7 Microencapsulation of Gac Oil: Optimisation of Spray Drying Conditions Using Response Surface Methodology

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Microencapsulation of Gac oil: Optimisation of spray drying conditions using response surface methodology

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ABSTRACT

The objective of this study was to optimise the spray drying conditions for the encapsulation of Gac oil using response surface methodology. Results indicated that the corresponding response surface model was sufficient to describe and predict encapsulation efficiencies (EEs) in terms of the oil, β-carotene, lycopene, encapsulation yield (EY), moisture content (MC), water solubility index (WSI) and peroxide value (PV) with R^2 of 0.92, 0.91, 0.89, 0.85, 0.89, 0.98 and 0.97, respectively. Under optimal conditions (inlet and outlet temperatures of 154 and 80 °C), the response variables including the EEs of the oil, β-carotene, lycopene, EY, MC, WSI and PV were predicted and validated as 87.22%, 82.76%, 84.29%, 52.78%, 4.90%, 90.29% and 4.06 meq/kg, respectively. Furthermore, physicochemical, reconstitution and colour properties of the optimally encapsulated powder were also determined. It was concluded that this powder containing high content of unsaturated fatty acids, β-carotene and lycopene, and having the attractive red-yellow colour can be used as nutrient supplement and natural food colourant.

1. Introduction

Many studies showed that red Gac aril (*Momordica conchinchinensis* Spreng) contained very high concentrations of bioactive compounds. For example, high contents of fatty acids (102 mg/g of fresh weight, FW) [1], β-carotene (0.64–0.84 mg/g FW) and lycopene (1.55-3.05 mg/g FW) [2] in the aril were reported. In addition, high level of vitamin E is also found in the aril [3], which is a natural antioxidant, and helps protect Gac oil from oxidation. It is well known that those compounds from plant based foods play a crucial role in human heath, particularly, the absorption of carotenoids in the human body significantly improved in the presence of fat [4,5].

Extracted Gac oil containing high levels of unsaturated fatty acids, β-carotene and lycopene using microwave pre-treatment prior to pressing [6,7] and using supercritical carbon dioxide [8] has been reported. However, the extracted oil is susceptible to oxidation and isomerisation owing to the high number of conjugated double bonds in the chemical structure of fatty acids and carotenoids. Therefore, it is important to preserve these compounds in a convenient oil powder form as nutrient supplement and natural food colourant due to ease of consumption, storage and transportation.

Microencapsulation by spray drying appears to be an effective way for those compounds in the powder form because of its advantages. Excellent properties of the protection, stabilisation, solubility and controlled release of the bioactive compounds can be obtained [9]. The mechanism therein is to enclose the core material by forming an impermeable membrane (wall matrix) against mechanical stress, temperature, light, and oxygen diffusion among others [10,11]. The first step of the microencapsulation process is to select an appropriate wall material, which has excellent functional properties (such as emulsifying, gel and film forming). Whey protein (WP), which has nutritional, physicochemical and functional properties, has been used in foods due to its ability to form thick and flexible films, preventing coalescence; whereas gum Arabic (GA) is a complex blend of natural polysaccharides composed of arabinogalactan, arabinogalactan-protein and glycoprotein. Several studies reported that high stability of encapsulated oil using a blend of WP and GA as the wall material was obtained [12–15].

It is well known that spray drying is commonly used in the food industry. There are many advantages of this process including economy, flexibility and particularly good quality of resultant powder [16,17]. In addition to the properties of the food material, the quality attributes of the powder could be affected by the spray drying conditions such as the feed flow rate, inlet and outlet temperatures, atomiser speed and inlet air flow rate [18]. Among those, provided that infeed emulsion is stable over the processing time, it is important to optimise the inlet and outlet temperatures to obtain higher encapsulation efficiency (EE) and encapsulation yield (EY) [19]. Higher inlet temperature, which is

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directly proportional to the drying rate, may destroy heat sensitive components such as unsaturated fatty acids and carotenoids, resulting in low EE. In contrast, if the inlet temperature is too low, the water will not evaporate fully in short time and the spray-dried powder is still wet. Hence, it is easily stuck on the drying chamber wall, resulting in a low EY. In addition, the outlet temperature, which can be considered as the control indicator of the dryer and is controlled by the inlet temperature, atomisation pressure and feed flow rate, may result in cracking the microcapsules due to over-heating if it is too high [19,20].

Furthermore, encapsulation of carotenoid-rich Gac oil by spray drying is attempted for not only protection from environment, but also more diversified applications of the powder in foods. Therefore, it is desirable to optimise the inlet and outlet temperatures to obtain good quality of the final product in terms of reconstitution and colour characteristics, peroxide value (PV), water activity (Aw), pH and bulk density. Optimisation of the spray drying conditions for encapsulated products has been successfully reported by using response surface methodology (RSM) [21–23]. It involves a group of mathematical and statistical techniques that can be employed to study the relationships between the response and the independent variables [24,25].

Recently, optimisation of wall material concentration and Gac oil load for Gac oil microencapsulation using RSM has been reported [14]. Considering a lack of scientific literature about spray drying microencapsulation of Gac oil, this study aimed to optimise the spray drying conditions using RSM in terms of the EEs (retention of Gac oil, β-carotene and lycopene), EY, MC, water solubility index (WSI), and PV. Furthermore, the physicochemical, reconstitution and colour characteristics of the resultant powder were also evaluated.

2. Materials and methods

2.1. Chemicals and materials

Solvents and BHT (butylated hydroxytoluene) were purchased from Merck Pty. Ltd. (Sydney, NSW, Australia). Carotenoid standards (HPLC grade) including β-carotene (C4582, type II synthetic, ≥95%) and lycopene (L9879, ≥90%, from tomato) were obtained from Sigma-Aldrich Pty. Ltd. (Sydney, NSW, Australia). Wall materials or encapsulating agents including whey protein concentrate (WPC 100) and gum Arabic (GA) were obtained from Amino Nutrition Co. (Kotara, NSW, Australia).

Gac oil used as core material, was pressed according to a method described by Kha et al. [6,7]. Briefly, about 900 g of fresh Gac arils was dried by microwave at 630 W for 62 min and then steamed for 22 min prior to hydraulic pressing at 175 kg/cm^2. The peroxide value of Gac oil was 1.80 ± 0.35 meq O_2/kg (refer to Section 2.6.5). The content of β-carotene and lycopene in Gac oil was 186 and 518 mg/100 mL, respectively [6].

2.2. Preparation of infeed emulsion

The infeed emulsions were prepared according to a method described by Bellalta et al. [26] and Kha et al. [14] with some modifications. An aqueous solution containing WPC/GA: 7/3 (w/w) with the concentration of 29.5% and 0.1% sodium benzoate (to prevent the proliferation of microorganisms) was prepared using an Ultra-Turrax T65D homogeniser (Wilmington, USA) at 6000 rpm for 10 min. The wall material solution was then kept at 4 °C for at least 12 h to ensure complete hydration of proteins.

To create the oil in water emulsion, Gac oil (mass ratio of Gac oil to wall material powder of 0.2, w/w) was added dropwise to the wall material solutions whilst mixing using the Ultra-Turrax T65D homogeniser at 4000 rpm for 10 min, to allow full incorporation. The crude emulsions were then re-circulated for 5 min through a homogeniser (APV-1000, Poland) operated at pressure of 500 bar to form a stable emulsion. The emulsion was then held away from light at room temperature for at least 1 h to ensure homogeneity of infeed prior to spray drying.

2.3. Spray drying conditions for infeed emulsion

The stable emulsion was spray-dried within a day in a LabPlant SD-06A spray dryer (LabPlant UK Ltd., North Yorkshire, UK). The dryer was equipped with a two-fluid nozzle atomiser (0.5 mm diameter). Practically, inlet air temperature has the most important effect on the product. For each inlet temperature, the outlet air temperature is mainly dependent on the feed rate and less so on the aspirator rate, air humidity and spray air flow. The outlet temperature cannot be too high as it affects the product quality or too low as it corresponds to undesirably high residual moisture in the product, hence too high water activity. This study focused on these two temperature parameters to find the optimal conditions within the limitations of the available spray drier. The inlet temperature was controlled by settings of the air heater of the equipment. The outlet temperature was controlled by varying the feed flow rate from about 340 to 970 mL/h at the fixed pressure of 2 bar and air flow speed of 4.3 m/s. The inlet and outlet air temperatures were selected and run according to the generated experimental design from RSM (Table 1). The encapsulated powders were recovered from the collecting chamber. The powders were stored in a desiccator containing silica gel to prevent moisture adsorption, then vacuum sealed in high density polyethylene (HDPE) plastic bags and stored at

Table 1
The central composite design and experimental values obtained for the response variables.

Pattern	X_1 (°C)	X_2 (°C)	Response variable						
			Y_1	Y_2	Y_3	Y_4	Y_5	Y_6	Y_7
			%	%	%	%	%	%	meq/kg
0A	155	87	88.07	78.30	72.83	55.31	4.36	89.73	6.78
00	155	80	85.73	77.40	83.47	53.97	4.59	89.92	4.35
−−	150	75	90.88	85.53	88.42	46.61	5.12	82.38	3.59
++	160	85	79.26	68.68	65.91	57.24	3.29	93.24	7.12
00	155	80	87.22	83.66	82.20	54.61	4.79	91.81	4.10
a0	148	80	84.19	86.45	83.97	44.13	6.46	79.65	3.42
0a	155	73	96.10	80.33	82.77	45.69	5.01	86.58	5.71
−+	150	85	89.10	85.12	87.65	53.89	4.87	84.97	4.11
00	155	80	86.99	83.22	84.73	52.66	4.81	92.27	4.67
+−	160	75	81.88	68.80	89.35	52.94	4.28	92.08	7.85
A0	162	80	77.68	69.83	74.30	65.32	3.31	94.28	7.72

Y_1, Y_2, Y_3, Y_4, Y_5, Y_6, Y_7: response variable of encapsulation efficiencies in terms of oil, β-carotene, lycopene, encapsulation yield, moisture content, water solubility index, and peroxide value, respectively. X_1 and X_2: independent variable of inlet and outlet temperatures, respectively.

−18 °C until analysis (within 24 h). Sample preparation and spray drying conditions were performed in triplicate.

2.4. Experimental design

RSM was employed to optimise inlet and outlet air temperatures on the encapsulation efficiencies in terms of retention of oil, β-carotene, lycopene, as well as EY, MC, WSI, PV, Aw, pH, and bulk density. Also, colour characteristics of the encapsulated powder, reconstituted powder and infeed emulsion, and total colour difference between reconstituted and infeed emulsions (ΔE) were evaluated. The experimental design based on the central composite design (CCD) using a 2^2 factorial with three central points was carried out in triplicate. A total of 33 runs was conducted. The levels of independent variables including inlet air temperature (X_1) and outlet air temperature (X_2) were based on preliminary trials. A second-order polynomial equation was used to express the response variables as a function of the independent variables as follows: $Y_i = a_o + a_1X_1 + a_2X_2 + a_{11}X_1^2 + a_{22}X_2^2 + a_{12}X_1X_2$. Where Y_i represents the response variables, a_o is a constant, a_i, a_{ii}, and a_{ij} are the linear, quadratic and interaction coefficients, respectively. X_i and X_j are the levels of the independent variables. To respect the hierarchical property, even if several coefficients were statistically non-significant, the former was still kept in the second-order equation.

2.5. Statistical analysis

The experimental data were analysed using JMP software version 10.0 (SAS Institute Inc., NC, USA). The adequacy of the models was determined by evaluating the lack of fit, coefficient of determination (R^2) and the Fisher's test value (F-value) obtained from the analysis of variance (ANOVA). The test of statistical significance was based on the total error criteria with a confidence level of 95%.

Different mean values of the measured and predicted responses, and effects of inlet and outlet air temperatures on the investigated parameters were analysed by ANOVA using SPSS software version 21.0 (IBM Australia Limited, St Leonards, NSW, Australia).

2.6. Analytical methods

All analytical measurements were carried out in triplicate.

2.6.1. Encapsulation efficiency (EE, %)

Encapsulation efficiencies (EEs) were determined based on the retention of Gac oil, β-carotene and lycopene contents in the encapsulated powder as follows:

EE_{oil} (%) = [(total oil content − surface oil content) × 100] / total oil content.

$EE_{\beta\text{-carotene}}$ (%) = [β-carotene content in powder (d.b.) × 100] / β-carotene in infeed emulsion (d.b.)

$EE_{lycopene}$ (%) = [lycopene content in powder (d.b.) × 100] / lycopene content in infeed emulsion (d.b.).

The total oil content of the encapsulated powder sample (3 g) was extracted with n-hexane (50 mL) using the FOSS Soxtec™ system 2045 extraction unit, Denmark. The total oil content in the powder was gravimetrically calculated [7,27].

Surface oil or non-encapsulated oil content was determined according to Jimenez et al. [15]. The surface oil content was determined by gently shaking the encapsulated powder sample (5 g) in n-hexane for 10 min without microcapsule destruction. The solvent was decanted and the residue was vacuum-dried at 70 °C until constant weight.

The method described by Kha et al. [7] was employed to analyse the content of β-carotene and lycopene in the infeed emulsion and in the encapsulated powder using HPLC. Briefly, about 1 g weighed microcapsule sample was reconstituted in deionised water (20 mL) to form a homogeneous solution. The emulsion samples or the reconstituted samples were dissolved in a 4:3 (v/v) solution of ethanol and hexane (35 mL) and an antioxidant (butylated hydroxytoluene [BHT], 0.1% in hexane), and the mixture was blended for 5 min at 5000 rpm. The extract was filtered through Whatman No. 1 filter paper on a Buchner funnel. The residue was re-extracted with another 35 mL of ethanol and n-hexane (4:3) and then washed twice with ethanol (12.5 mL) and once with hexane (12.5 mL). The combined extracts were washed with deionised water, dried by rotary evaporator and then diluted with the mobile phase solution. All operations were performed under subdued light to minimise oxidation of the carotenoids.

HPLC analysis of β-carotene and lycopene was performed with an Agilent 1200 HPLC (Santa Clara, CA, USA) equipped with diode array detector system consisting of a Luna C18 (100 × 4.6 mm i.d.; 5 μm) direct-connect guard column coupled to a Jupiter C18 (250 × 4.6 mm i.d.; 5 μm) reserved phase column (Phenomenex Australia Pty. Ltd., Lane Cove, NSW, Australia). The mobile phase consisted of acetonitrile, dichloromethane and methanol (ACN:DCM:MeOH) 50:40:10 v/v/v. The flow rate was 1.0 mL/min, the detection was at 450 nm and the injection volume was 20 μL. The identification of β-carotene and lycopene was based solely on the retention time of a peak compared with the authentic standards. The amount of β-carotene and lycopene in the samples was expressed as μg/g.

2.6.2. Encapsulation yield (EY, %)

EY was calculated as ratio of the weight of the resultant powder after spray drying in collecting bottle and the weight of all solids (including wall and core materials) in the emulsion, expressed as percentage. Any powders adhering to the walls of drying chamber or cyclone were not considered, so this yield will be only approximate.

2.6.3. Moisture content (MC)

The MC of the samples was determined by drying at the temperature of 105 °C in an oven until a constant weight was obtained.

2.6.4. Water solubility index (WSI)

The WSI of the powders was determined using the method described by Anderson et al. [28]. The encapsulated powder (2.5 g) and distilled water (30 mL) were vigorously mixed in a 100 mL centrifuge tube, incubated in a 37 °C water bath for 30 min and then centrifuged for 20 min at 11,410 *g* in a J2-MC Centrifuge (Beckman, USA). The supernatant was carefully collected in a pre-weighed beaker and oven dried at a temperature of 103 ± 2 °C. The WSI (%) was calculated as the percentage of dried supernatant of the original 2.5 g Gac fruit powder.

2.6.5. Peroxide value (PV, meq/kg)

The PV value of Gac oil encapsulated powder samples was measured according to AOCS Cd 8-53 [29]. Extracted oil (5 g) from encapsulated powder and Gac oil samples using the FOSS Soxtec™ system 2045 extraction unit was placed into 250 mL Erlenmeyer flask and dissolved in 30 ml of a mixture containing acetic acid and chloroform (3:2, v/v) and then the mixture was stirred for few seconds to ensure thorough mixing. Thereafter, about 0.5 mL of saturated potassium iodide solution was added. After exactly 1 min, deionised water (30 mL) was added and it was titrated with sodium thiosulfate (0.01 N) using a starch solution as an indicator until the solution becomes colourless. The mixture was magnetically stirred during the titration procedure. The results were calculated as milliequivalents of active oxygen per kg of sample as follows:

PV (meq/kg) = [(S − B) × N × 1000] / mass of sample (g)

where S and B are the titration amounts of 0.01 N sodium thiosulfate for sample and blank (in mL), respectively. N is the normality of sodium thiosulfate solution.

2.6.6. Colour characteristics

The colour of the encapsulated powder, the reconstituted powder and the emulsion was measured using a Chroma meter (CR-400, Konica Minolta Sensing, Inc., Japan) calibrated with a white standard tile. The results were expressed as Hunter colour values of L^*, a^* and b^*, where L^* was used to denote lightness, a^* redness and greenness, and b^* yellowness and blueness. Before measurement, the samples were packed into a polyethylene pouch.

Chroma (C), indicating colour intensity, was calculated by the formula $(a^{*2} + b^{*2})^{0.5}$. The hue angle (H°) was calculated by the formula $H° = \arctan(b^* / a^*)$. The hue angle values vary from 0° (pure red colour), 90° (pure yellow colour), 180° (pure green colour) to 270° (pure blue colour). Total colour difference between two samples was calculated by the formula:

$$\Delta E = \left[(L^*_o - L^*)^2 + (a^*_o - a^*)^2 + (b^*_o - b^*)^2\right]^{0.5}$$

where L^*_o, a^*_o and b^*_o are the values of the infeed emulsion samples, and L^*, a^* and b^* are the values of the reconstituted emulsion samples [30].

2.6.7. Water activity (Aw)

An Aqua Lab water activity meter (Aqua Lab Series 4 TEW, Decagon Devices, Inc., WA, USA) was used to measure Aw of the encapsulated powders at 25 ± 0.5 °C.

2.6.8. pH determination

The encapsulated powders were reconstituted with deionised water to the same moisture content of the infeed emulsions before measuring pH. The pH values of the infeed emulsion and the reconstituted powder were determined in a beaker maintained at 20 °C by a pH meter calibrated with standard buffers pH 7 and 4.

2.6.9. Bulk density

Bulk density (g/mL) of the powder samples was measured by gently adding 2 g of the powder into an empty 10 mL graduated cylinder and holding the cylinder on a vortex vibrator for 1 min. The bulk density value was determined by the ratio of mass of the powder and the volume occupied in the cylinder.

2.6.10. Fatty acid analysis

The method of Ishida et al. [2] was employed for determining the fatty acid composition of Gac oil before and after encapsulation with some modifications. Briefly, about 0.05 g of the oil and the extracted oil from the optimally encapsulated powder sample using the FOSS Soxtec™ extraction unit was weighted into 10 mL glass tube. Toluene (1 mL) was added and the fatty acids were methylated for 1 h at 80 °C using methanolic hydrogen chloride 3%. Resulting fatty acid methyl esters (FAMEs) were dissolved in 10 mL cyclohexane (0.01% BHT) for GC analysis.

Quantitative analysis was performed by GC-FID using an Agilent 5890 N GC (Agilent Technologies Pty. Ltd., CA, USA). The injector and detector temperatures were 250 and 280 °C, respectively. The column temperature was 100 °C for 1 min, then increased by 5 °C/min to 250 °C and held at 250 °C for 1 min. Standard solutions of a mixture of FAMEs at three different concentrations in the range of 5 to 150 μg/mL were used for generating the standard curves. The samples (1 μL) were injected into the GC system. The identification of each fatty acid was based solely on the retention time of a peak compared with the authentic standards.

2.6.11. Particle morphology

The outer and inner structures of powder particles were visualised by a JSM-7401F scanning electron microscope (SEM) (Jeol Co. Ltd., Tokyo, Japan). To observe the inner structure of the particle, a small amount of powder (attached to the stub) was fractured by attaching a second piece of adhesive tape on top of the powder and then ripping it off quickly [31]. The morphography observation was examined at different magnifications using SEM operating at a voltage of 5 kV.

3. Results and discussion

3.1. Fitting the response surface models to independent variables

The encapsulation efficiencies (EEs) in terms of retention of Gac oil, β-carotene, lycopene, encapsulation yield, moisture content, water solubility and peroxide value are shown in Table 1. The linear, quadratic, and interaction effects of inlet air temperature (X_1) and outlet air temperature (X_2) on each response variable (Y_i) of encapsulated Gac oil powder are given in Table 2. The estimated regression coefficients for Y_i with their corresponding R^2, P value of regression and P value of lack of fit are also shown in Table 2. Analysis of variance (ANOVA) indicated that the response surface models were significant (Table 2) and that there was no significance in lack of fit ($P > 0.05$) in each of the models. In addition, the larger absolute t ratio and smaller P value would indicate a more significant effect on the corresponding variables [32]. The significance of the coefficients of the response surface models is listed in Table 2. Therefore, the models with high coefficients of determination (R^2 in a range of 0.85 to 0.98) were adequate to explain the effect of the independent variables investigated on the Y_i. In other words, it indicates that more than 85% of the response variables could be accurately described as function of the inlet and outlet air temperatures investigated.

3.2. Encapsulation efficiency (EE, %)

The effects of inlet air and outlet air temperatures on the EE in terms of the retention of Gac oil, β-carotene and lycopene are shown in Table 2 by the coefficients of quadratic model. For EE of Gac oil, all regression coefficients of the model significantly influenced the EE of oil. However, the interaction term was found to be non-significant ($P > 0.05$), indicating that there was no interaction between the inlet and outlet temperatures on the EE of the oil. For EE of carotenoids, the linear term of inlet temperature significantly affected the EEs of β-carotene and lycopene, whereas only the EE of lycopene was significantly impacted by the linear term of outlet temperature. There was also a significant interaction between the inlet and outlet temperatures on the EE of lycopene. Furthermore, the 3D surface and 2D contour plots (Fig. 1) were drawn to determine the optimal levels of the independent variables. The relationship between the independent and response variables is shown in the response surface plot, whilst the contour plot helps to visualise the shape of a response surface. Therefore, it is important to use these plots to evaluate the fits of model [24].

In this study, high content of total Gac oil, β-carotene and lycopene in the spray-dried Gac oil powder varied from 14.36 to 15.54%, 596.25 to 785.34 μg/g and from 1275.49 to 1591.06 μg/g, respectively. The EE of Gac oil varied from 77.68 to 96.10%, whereas the EE of β-carotene and lycopene varied from 68.68 to 86.45% and 65.91 to 89.35%, respectively (Table 1), and were significantly affected by the inlet temperature (Table 2). As the inlet drying temperature increased, the efficiency of Gac oil encapsulation in terms of the retention of Gac oil, β-carotene and lycopene decreased. According to Gharsallaoui et al. [20], it is important to determine an appropriate drying temperature to avoid damage of the dried food product. There is a directly proportional drying temperature to the evaporation rate and inversely with the final water content of the powder. At low drying temperature, the formation of microcapsules with high density membranes, high water content, poor fluidity and easiness of agglomeration is caused by the low drying rate. In contrast, an excessive evaporation that occurred at high inlet temperatures results in cracks in the membrane inducting subsequent premature release, and therefore a degradation of encapsulated bioactive

Table 2
Regression coefficients, R^2, probability values and lack of fit for the models.

Regression coefficient[a]	EE_{oil} (%)		$EE_{\beta\text{-carotene}}$ (%)		$EE_{lycopene}$ (%)		Encapsulation yield (%)		Moisture content (%)		Water solubility index (%)		Peroxide value (meq/kg)	
	Regression coefficient	t ratio	Regression coefficient	t ratio	Regression coefficient	t ratio	Regression coefficient	t ratio	Regression coefficient	t ratio	Regression coefficient	t ratio	Regression coefficient	t ratio
Constant														
a_o	86.647		81.427		83.467		53.747		4.73		91.333		4.373	
Linear														
a_1	−3.506	−4.73**	−7.084	−6.87**	−4.311	−3.50*	4.956	4.27**	−0.859	−5.82**	4.833	13.91***	1.669	11.61***
a_2	−1.969	−2.66*	−0.425	−0.41	−4.783	−3.89*	3.148	2.71*	−0.270	−1.83	1.026	2.95*	0.163	1.13
Square														
a_{11}	−3.163	−3.59*	−2.067	−1.68	−1.075	−0.73	0.504	0.36	−0.021	−0.12	−2.032	−4.92**	0.538	3.15*
a_{22}	2.412	2.73*	−1.480	−1.21	−1.742	−1.19	−1.609	−1.16	−0.121	−0.69	−1.437	−3.48*	0.876	5.12**
Interaction														
a_{12}	−0.210	−0.20	0.073	0.05	−5.668	−3.26*	−0.745	−0.45	−0.185	−0.89	−0.358	−0.73	−0.313	−1.54
R^2	0.921		0.910		0.888		0.847		0.885		0.979		0.971	
Regression (P value)	0.009		0.012		0.020		0.042		0.021		0.000		0.000	
P value of lack of fit	0.087		0.722		0.078		0.054		0.051		0.786		0.281	

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.
[a] a_o is a constant; a_i, a_j, and a_{ij} are the linear, quadratic and interactive coefficients of the second-order polynomial equation, respectively.

compounds. Moreover, a rapid formation of the semi-permeable membrane on the droplet surface also occurred at a high enough drying temperature, resulting in the high retention of the bioactives. However, drying temperatures above this level are likely to cause heat damage to the powder product [18,33].

EEs in terms of oil and lycopene are also affected by the outlet temperature, which is mainly determined by inlet temperature (Table 2). The EEs were significantly decreased with the increasing outlet temperature, indicated by the "minus" sign of regression coefficient and t ratio in Table 2. This could be explained by the fact that higher inlet temperature has lower encapsulation efficiency due to more loss of volatile oil and more carotenoid degradation by heat and oxidation [33]. According to Bhandari et al. [34], this phenomenon could be explained by the "ballooning" effect, where microcapsules may develop fissures, split and release the encapsulated compounds. Furthermore, the balance between the rate of water evaporation and film formation may be affected by the high outlet temperature [35]. Therefore, it results in low EEs owing to breaking down wall material matrix of particles and then heat damage could easily destroy the bioactive compounds. In contrast, reports indicated that effect of outlet temperature on the EE is also controversial and unclear [18,33]. For example, Danviriyakul et al. [36] reported that the outlet temperature did not influence surface oil content of encapsulated milk fat powders.

3.3. Encapsulation yield (EY, %)

In addition to the EE, the EY is one of the major factors describing the spray-dried powder, it is expected to obtain higher EY. The EY (Y_4) in this study varied from 44.13 to 65.32% (Table 1) and was significantly affected by the inlet and outlet temperatures (Table 2). Higher EY of the process was obtained with increasing drying temperatures, and is shown in Fig. 2a and Table 2. Furthermore, the surface model with a high coefficient of determination R^2 of 0.85 was considered adequately to explain the effect of the independent variables investigated on the EY.

It is generally agreed that if the inlet temperature is not high enough, the water will not vaporise fully, thus the powder is not dry enough and easily sticks on the drying chamber wall. However, the water evaporation occurs easier at higher inlet temperature due to the greater efficiency of heat and mass transfer processes between the chamber walls and the moving fluids [37,38], resulting in higher EY. A similar observation was also reported by several authors [37,39,40]. Similarly, the EY increased with increasing outlet temperature. It can be concluded that the inlet and outlet temperatures had positive effects on the EY. It is therefore desirable to optimise the drying temperatures to obtain higher EY.

There is a consistency of the EY among our results and many other findings [35,37,40], which is less than 65%. In contrast, Gallo et al. [41] reported that higher EY of spray-dried powder could be achieved up to 85% for a laboratory scale spray dryer. The big difference may be due to difference in calculation of the EY. In this study, only the dried powder collected in the collecting bottle, not the powders adhering to the walls of drying chamber or cyclone, was considered for calculating the EY. This could be explained by the fact that retention of powder at the chamber wall or cyclone for a long time is undesirable. According to Goula and Adamopoulos [38], the accumulated powder in the chamber wall received more intense heat and may have different properties such as moisture content, colour characteristics and solubility. Therefore, these powders could not be considered as products. Furthermore, the EE of the resultant powder may be also affected due to more heat exposure. In addition to the quality of the product, the low EY is related to the dimensions of the spray dryer [37] and the design of a dryer system [42]. Theoretically, it may be improved if the drying chamber is sufficiently large or recovery devices may be used in industrial system [42].

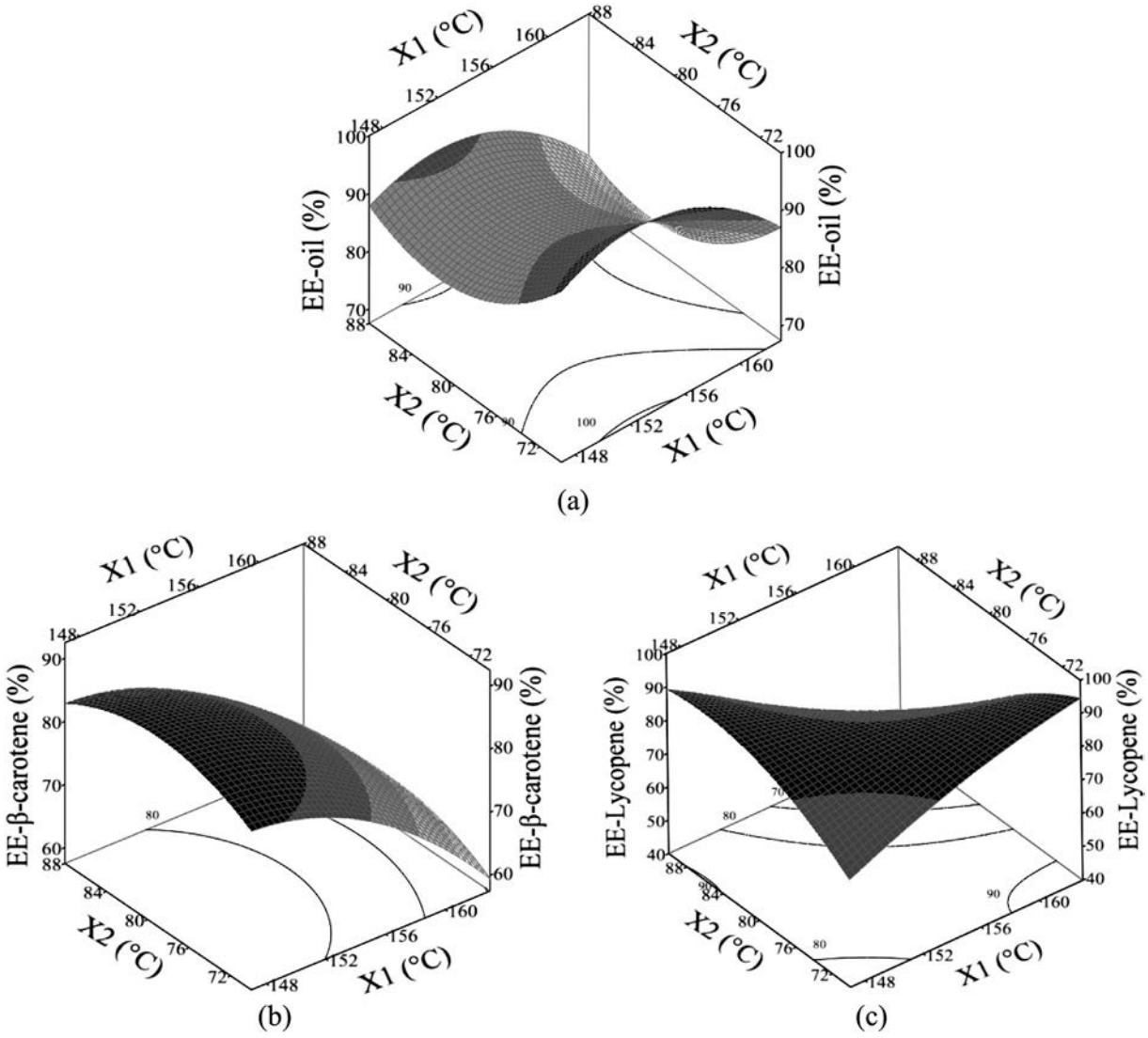

Fig. 1. The 3D response and 2D contour plots of the EEs affected by inlet (X_1) and outlet (X_2) temperatures.

3.4. Moisture content (MC, %)

The moisture content of the encapsulated powder varied from 3.31 to 6.46 (Table 1) and was significantly influenced by the inlet temperature ($P < 0.01$) (Table 2). Although the outlet temperature resulted in the decreasing MC of the resultant powder, statistical results indicated that there was no significant change in the MC ($P > 0.05$). Moreover, Table 2 shows that the model with a high coefficient of determination (R^2) of 0.89 was considered adequately to explain the effect of the independent variables investigated on the MC.

As can be seen in Fig. 2b, an increase in the inlet and outlet temperatures resulted in a decrease in the MC. At higher inlet temperatures, a greater temperature difference between the atomised infeed and the drying air led to a higher rate of heat transfer, providing a greater driving force for water removal. As a result, lower MC of the dried powders was obtained [21,38,43]. This similar observation was also reported by our previous study on spray-dried Gac aril powder [30] and the other powders containing carotenoids [22,43,44]. In addition to the inlet temperature, Bakar et al. [21] reported that the MC of the spray-dried powder also decreased with increasing outlet temperature. As discussed previously, however, a degradation of carotenoids and oil, resulting in lower EEs were also observed at higher inlet and outlet temperatures. Therefore, it is important to optimise these temperatures to possibly obtain higher EE and lower MC, which is discussed in the next section of this paper.

3.5. Water solubility index (WSI, %)

According to the ANOVA analysis of this optimisation study, the WSI of the encapsulated powder was significantly affected by the inlet and outlet temperatures. A second-order polynomial equation with high R^2 of 0.98 was employed to explain the effects of these temperatures on the WSI of the powder (Table 2). The linear and quadratic terms of the inlet and outlet temperatures significantly influenced the WSI. However, interaction between the inlet and outlet temperatures on the WSI of the powder was found to be non-significant ($P > 0.05$).

In this study, the WSI of the powders under different spray drying conditions varied from 79.65 to 94.28%. Fig. 2c shows that the WSI of the powder increased with the increasing inlet and outlet temperatures. The reason for this observation is due to an appropriate reduction of moisture content, which was caused by higher drying temperatures, providing the additional solubility of the powder [21]. Several studies indicated that the MC of the resultant powder is one of the dominant factors affecting its solubility, a lower MC leads to increasing solubility of the dry powder [43,44]. In contrast, a hard surface layer of the powder could be formed at higher inlet temperature, consequently decreasing

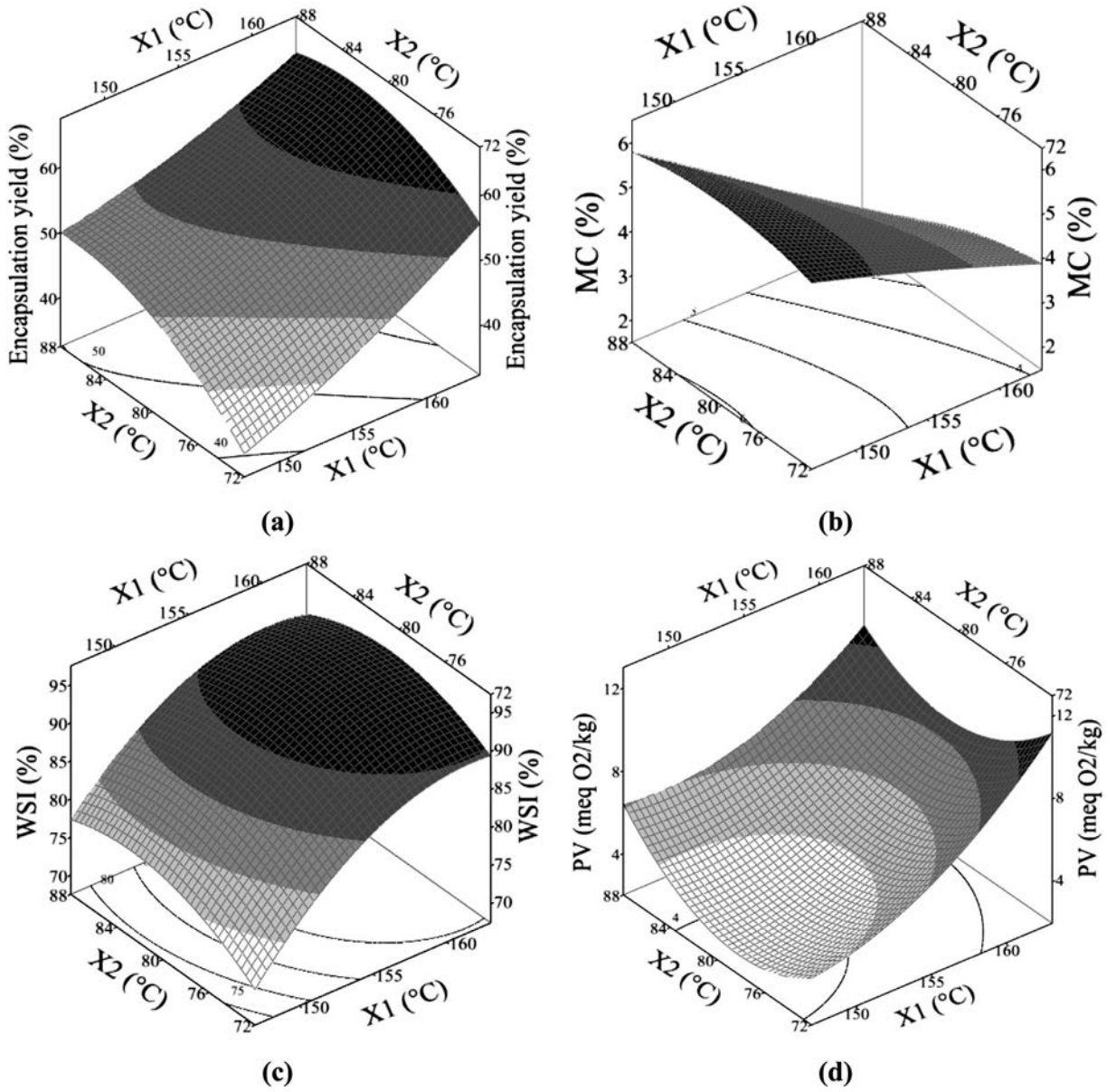

Fig. 2. The 3D response and 2D contour plots of the encapsulation yield (a), moisture content (b), water solubility index (c) and peroxide value (d) affected by inlet (X_1) and outlet (X_2) temperatures.

the solubility of the powder [43,45]. The opposite trend of the solubility among those studies is due to diverse types of encapsulating agents or drying aids used, which may also differently react within the various ranges of inlet temperatures investigated. In this study, it is therefore desirable to optimise the spray drying conditions to achieve the highest water solubility of the encapsulated powder.

Table 3
Colour characteristics of encapsulated and reconstituted powders.

Pattern	X_1 (°C)	X_2 (°C)	Encapsulated powder			Reconstituted powder			
			Lightness	Chroma	Hue angle	Lightness	Chroma	Hue angle	ΔE
0A	155	87	76.18	49.87	68.89	45.65	32.38	36.80	3.42
00	155	80	75.49	49.02	68.09	51.44	40.46	43.55	14.02
−−	150	75	73.58	48.92	66.10	47.33	34.40	38.14	5.97
++	160	85	77.97	48.88	70.36	51.33	39.35	45.63	13.52
00	155	80	74.89	48.80	67.99	49.19	37.54	43.09	10.50
a0	148	80	76.79	48.23	68.83	46.05	33.42	37.37	4.61
0a	155	73	74.39	47.82	66.53	48.68	39.57	43.38	12.09
−+	150	85	74.69	48.93	66.58	48.19	35.16	39.21	7.18
00	155	80	74.73	49.49	68.27	50.54	38.77	43.10	12.11
+−	160	75	76.12	50.39	69.70	52.30	39.45	47.91	14.74
A0	162	80	76.93	49.84	68.16	50.81	38.78	45.39	12.77

X_1 and X_2: independent variable of inlet and outlet temperatures, respectively.

Table 4
Water activity, pH and density of encapsulated Gac oil powder.

Pattern	X_1 (°C)	X_2 (°C)	Water activity	pH	Density (g/mL)
0A	155	87	0.23 ± 0.04	5.87 ± 0.14	0.32 ± 0.04
00	155	80	0.34 ± 0.05	5.72 ± 0.17	0.27 ± 0.04
−−	150	75	0.38 ± 0.06	5.98 ± 0.14	0.29 ± 0.04
++	160	85	0.26 ± 0.06	5.96 ± 0.19	0.30 ± 0.04
00	155	80	0.27 ± 0.07	5.89 ± 0.16	0.30 ± 0.07
a0	148	80	0.30 ± 0.04	6.11 ± 0.21	0.32 ± 0.04
0a	155	73	0.35 ± 0.07	6.01 ± 0.11	0.33 ± 0.06
−+	150	85	0.34 ± 0.07	5.82 ± 0.10	0.27 ± 0.05
00	155	80	0.30 ± 0.03	6.01 ± 0.09	0.25 ± 0.03
+−	160	75	0.43 ± 0.06	5.67 ± 0.17	0.30 ± 0.03
A0	162	80	0.36 ± 0.03	5.78 ± 0.13	0.24 ± 0.02

X_1 and X_2: independent variable of inlet and outlet temperatures, respectively.

3.6. Peroxide value (PV, %)

Generally, the PV of an oil product is used to evaluate the quality of oil, by measuring the quantity of hydroperoxides in the oil, which are formed by the reaction between oxygen and unsaturated fatty acid. This value is used to evaluate the initial stages of the oxidation process [46]. In this study, the PV of the encapsulated Gac oil powder varied from 3.42 to 7.85 meq/kg (Table 1). Statistical results (Table 2) show that the PV of the encapsulated powder was significantly affected by linear term of the inlet temperature, and quadratic term of the inlet and outlet temperatures. In addition, the fit of the response surface model to the PV as a result of the inlet and outlet temperatures was considered to be predictive, indicated by high R^2 of 0.97 and $P < 0.001$ (Table 2).

As can be seen in Fig. 2d, the PV significantly increased with the increasing inlet and outlet temperatures. At higher temperatures, the higher amount of peroxides was generated due to the intensive energy provided for lipid oxidation [47,48]. According to Thomson et al. [49], a strong dependence of lipid oxidation on temperature was found. In this study, for example, the PV of the encapsulated powder spray-dried at the lower inlet/outlet temperatures of 148/80 °C was half lower than that of the dried powder at the higher temperatures of 162/80 °C (Table 1). This observation is also consistent with many reports [48,50]. According to response surface plots from Figs. 1a and 2d, in addition, there is a relationship between the EE of Gac oil and the PV; generally the higher PV relates to the lower EE of the oil and vice versa. This is because the lower the EE, the higher was the surface oil content, which was easily oxidised due to the direct contact with the oxygen of drying air [47,51]. To obtain the higher EE of the oil and the lower PV, therefore, optimisation of the inlet and outlet temperatures is also needed.

3.7. Colour characteristics

Generally the colour of food powder is one of the most important parameters in food industry. In addition to nutrients relating to bioactive compounds presented in plant foods, which are mainly responsible for colour characteristics, the colour of the powders is also an important quality indicator reflecting the sensory attractiveness. In this study, the colour of the encapsulated Gac oil can be described as yellow-red, which is a highly desirable quality attribute. As a result, it is important to investigate the effects of the spray drying conditions on the colour characteristics of the dried and also reconstituted powders. The colour characteristics of the encapsulated Gac oil powders and their reconstitution obtained by the different spray drying conditions are shown in Table 3.

For the encapsulated powder, results showed that the colour characteristics including lightness, chroma and hue angle were non-significantly affected by the inlet and outlet temperatures ($P > 0.05$).

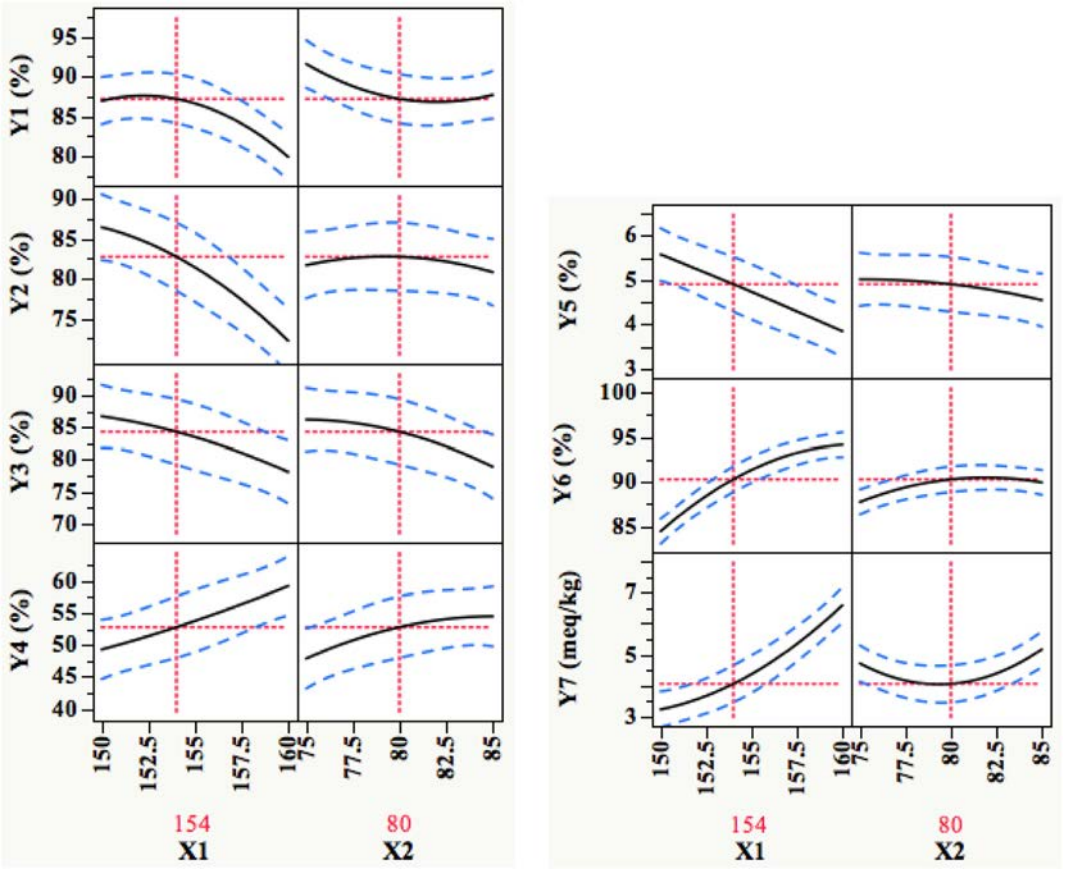

Fig. 3. Prediction profilers of EEs of oil (Y_1), β-carotene (Y_2), lycopene (Y_3), encapsulation yield (Y_4), moisture content (Y_5), water solubility index (Y_6) and peroxide value (Y_7) as a function of inlet (X_1) and outlet (X_2) temperatures.

Moreover, ANOVA results also indicated that the colour characteristics (lightness, chroma and hue angle) of the encapsulated powders affected by the drying temperatures were not explained by the response surface models, indicated by low R^2 of 0.72, 0.46 and 0.46, respectively. Generally, an increase in the drying temperatures resulted in no significant change in the lightness, chroma and hue angle of the powders.

For reconstituted powder, statistical results showed that the colour characteristics of the reconstituted powder affected by inlet temperature were found to be significant ($P < 0.05$). The lightness, chroma and hue angle of the reconstituted powders increased with the increasing inlet temperature. However, ANOVA results indicated that response surface model were not inadequate to explain the effects of the investigated independent variables on lightness and chroma of the reconstituted powders, indicated by low R^2 of 0.75 and 0.73. Furthermore, although the hue angle of the reconstituted powder could be described by the response surface model with $P < 0.05$ and high R^2 of 0.86, statistical results also showed that lack of fit was found to be significant ($P < 0.05$). Similarly, the inlet temperature significantly influenced total colour difference between the reconstituted and infeed emulsions (ΔE) ($P < 0.05$). However, the polynomial model described that effects of the independent variables on the ΔE was found to be non-significant, indicated by $P > 0.05$ and R^2 of 0.78. Therefore, it can be concluded that the polynomial models insufficiently explained the effects of the independent variables on the colour characteristics of the reconstituted powders.

The hue angle of the encapsulated powder varied from 66.10° to 70.36°, whereas the hue angle of reconstituted powder varied from 36.80° to 47.91° (Table 3). As 0° is pure red and 90° is yellow, those values are correspondent to the regions of red to yellow. It is established that a higher hue angle indicates more loss of carotenoid pigment in food products [52,53]. In this study, higher inlet temperatures led to higher hue angle of the spray-dried and reconstituted powders, indicating more colour loss. As discussed in Section 3.2, the EEs in terms of β-carotene and lycopene decreased when the inlet temperatures increased. Carotenoids, which are mainly responsible for the yellow-red colour of Gac oil, were lost by heat; therefore, the colour degradation of the dried and reconstituted powders occurred. This is also consistent with previous studies that the lower colour retention (higher hue angle) of the spray-dried Gac aril powder [30], watermelon powder [43], and watermelon and carrot mixture powder [22] was reported when the inlet temperature increased. Similarly, increasing inlet temperature resulted in increased ΔE, indicating greater colour difference between reconstituted and infeed emulsions.

3.8. Other physicochemical properties

According to ANOVA results, the physicochemical properties, including Aw, pH and density of the encapsulated Gac oil powders were non-significantly affected by the investigated independent variables ($P > 0.05$). Results also indicated that polynomial models were insufficient to explain the effect of the inlet and outlet temperatures on the Aw, pH and density, indicated by low R^2 of 0.77, 0.56 and 0.48, respectively. The Aw, pH and density of the encapsulated Gac oil powder under different spray drying conditions are shown in Table 4.

Generally, Aw, measuring the availability in a food system of free water responsible for microbiological and biochemical reactions, is one of the most important parameters that significantly impact the shelf life of food powders. Therefore, it is undesirable to have a high Aw due to shorter shelf life. According to Tang and Yang [54], the deterioration of dried food powder caused by micro-organisms and biochemical reactions can be prevented at Aw lower than 0.6. However, dried food products containing lipids and with a low Aw of less than 0.2 are particularly sensitive to lipid oxidation due to the action of free radicals. In this study, the Aw of the encapsulated oil powder varied from 0.23 to 0.43 (Table 4) and can be considered to be quite microbiologically and biochemically stable. In addition, the Aw of the

Table 5
Comparison between predicted (Pred.) and experimental (Exp.) values of optimal inlet and outlet temperatures of 154 and 80 °C, respectively.

EE-oil (%)		EE-β-carotene (%)		EE-lycopene (%)		EY (%)		MC (%)		WSI (%)		PV (meq/kg)	
Pred.	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.	Exp.
87.22 ± 3.07	89.51 ± 5.68	82.76 ± 4.28	80.39 ± 4.53	84.29 ± 5.11	83.24 ± 4.71	52.78 ± 4.82	54.69 ± 5.11	4.90 ± 0.61	4.40 ± 0.39	90.29 ± 1.44	88.88 ± 3.75	4.06 ± 0.60	4.10 ± 0.15

encapsulated oil powder was non-significantly affected by the inlet temperature. This observation was also made in other reports to that reported by other researchers [30,40,43].

As can be seen in Table 4, the pH value of the encapsulated powders ranged from 5.67 to 6.11 and was not significantly influenced by the investigated independent variables. Similar results were also reported when investigating effect of spray drying temperature on the pH change of resultant powders from Gac aril [30] and Roselle extracts [55]. Furthermore, Dalgleish [56] stated that emulsion containing whey protein as a continuous phase is sensitive to pH with a tendency to aggregation, especially at pH close to their isoelectric point of 5.2. In this study, no aggregation of the infeed and reconstituted powder emulsions was observed under the pH range. It is because the interaction between protein and polysaccharide in a continuous phase has a charge that is enough to prevent the close approach of suspended oil droplets [56] and makes the emulsions less sensitive to pH [57]. In addition, pH of the food products is one of the most important factors affecting colour of food products containing carotenoids [58,59]. The hue of carotenoids such as β-carotene is often unchangeable over a pH range of 2 to 7 [60]. It can be therefore concluded that the pH range of the encapsulated powder is suitable and should be maintained for application forms of carotenoid food colourant.

In this study, the bulk density of the encapsulated powders obtained by the different drying conditions ranged from 0.24 to 0.33 g/mL. The density of the particles slightly decreased with increased inlet temperatures, however this change was found to be statistically non-significant ($P > 0.05$). Several authors reported that the bulk density of the dried powders decreased with the increasing spray drying temperatures [30,44,47]. This is because evaporation rates were quicker, resulting in the dried products having a more porous or fragmented structure [38] and a higher ratio of surface to volume [39]. In addition to the drying temperature, the difference in the bulk density may also be depending on major food components such as water, carbohydrate, protein, fat, and ash [61]. Moreover, the dried powders with low bulk density have more occluded air within the particles [39]; as a result, oxidative degradation of the unsaturated fatty acids and carotenoids would have occurred, reducing the storage stability. For longer storage, therefore, it is very important to prevent the presence of oxygen or maintain low concentrations of oxygen. There are several ways to modify the gas atmosphere inside food packages; these are creating a vacuum, removing most of the gases present, and flushing the headspace area inside the package with an inert gas such as nitrogen.

3.9. Overall optimisation and model validation

As discussed above, there are seven response variables including EEs (Gac oil, β-carotene and lycopene), EY, MC, WSI and PV, which could be adequately fitted into the corresponding response surface model. A graphical optimisation (Fig. 3) was performed using JMP package for this study to optimise the drying conditions. In general, an increase in the inlet and outlet temperatures resulted in decreasing the EEs and MC. However, an opposite trend of the EY, WSI and PV was observed when the inlet and outlet temperatures were increased. Therefore, it is important to optimise the encapsulated Gac oil powder, which could be considered as an optimal product if the criteria applied for the optimisation process resulted in (1) maximising the EEs, EY, and WSI, and (2) minimising MC and PV.

The prediction profilers (Fig. 3) show that the optimal drying conditions within the investigated range for the encapsulated powder, where the criteria for the response variables were satisfied, can be determined by moving the vertical dot lines on left or right. The corresponding horizontal dot lines indicate the values of the response variables achieved. The conditions were achieved at the inlet and outlet temperatures of 154 and 80 °C, respectively. Under the optimal conditions, the predicted response variable for the drying conditions is shown in Table 5. Moreover, the comparison between the predicted and experimental values of the response variables was to evaluate how accurately the model describes the investigated process. Therefore, an experiment with three replicates was carried out at the optimal drying conditions. Results indicated that differences between those values for all response variables were found to be non-significant ($P > 0.05$). It can be concluded that the adequacy of corresponding response surface model for predicting the EEs (oil, β-carotene and lycopene), EY, MC, WSI and PV as function of the inlet and outlet temperatures was validated.

3.10. Properties of the optimal encapsulated Gac oil powder

In addition to the seven response variables, it is also desirable to evaluate the other properties of the optimal powder including Aw, pH, bulk density, colour of the encapsulated and reconstituted powders, fatty acid composition, and particle morphology. This is because those properties play important roles in application and storage in food industry. The Aw, pH and density of the optimal powder were 0.32 ± 0.03, 5.75 ± 0.07, and 0.33 ± 0.04 g/mL, respectively. As discussed earlier, in general, the optimal powder product could be stable over storage time due to the low Aw. The powder can be also applied in various foods due to colour stability of the powder containing carotenoids, which is often stable at the pH range of 2 to 7 (refer to Section 3.8). However, there is a need to consider for longer storage owing to the low bulk density of the powder. Therefore, it is recommended that a storage study of the optimal powder under different storage conditions should be carried out.

Colour characteristics of food items are one of the most important issues in the food industry, especially natural food colourants. Therefore, it is important to analyse the colour characteristics of the optimally encapsulated and reconstituted powders. The colour characteristics of the optimal powder determined were lightness of 74.79 ± 2.23, chroma of 49.31 ± 1.48 and hue angle of 67.12 ± 1.16°. To evaluate the colour characteristic of the reconstituted emulsion, in addition, the optimally encapsulated powder was reconstituted in deionised water to the same moisture content of the initial emulsion before spray drying. Results indicated that reconstituted emulsion had lightness of 50.80 ± 2.70, chroma of 38.25 ± 2.25 and hue angle of 46.13 ± 0.73. It is interesting to note that the encapsulated powder had the lesser red-yellow

Table 6
Fatty acid composition of Gac oil and encapsulated Gac oil.

Fatty acids	Abbreviation	Gac oil	Encapsulated Gac oil	Significance (P value)
Myristic	C14:0	0.42 ± 0.04	0.45 ± 0.10	0.695
Palmitic	C16:0	24.76 ± 1.50	25.96 ± 1.97	0.450
Palmitoleic	C16:1 Δ^9	0.43 ± 0.03	0.50 ± 0.05	0.072
Stearic	C18:0	6.72 ± 0.38	7.50 ± 1.23	0.351
Oleic	C18:1 Δ^9	49.18 ± 1.93	49.35 ± 2.63	0.931
Linoleic	C18:2 $\Delta^{9,12}$	17.65 ± 0.64	15.51 ± 1.22	0.055
α-Linolenic	C18:3 $\Delta^{9,12,15}$	0.84 ± 0.07	0.73 ± 0.05	0.089
	Σ SFAs	31.90 ± 1.81	33.91 ± 2.93	0.370
	Σ MUFAs	49.61 ± 1.96	49.85 ± 2.62	0.902
	Σ PUFAs	18.49 ± 0.67	16.24 ± 1.27	0.053

Σ SFAs: total saturated fatty acids; Σ MUFAs: total monounsaturated fatty acids; Σ PUFA: total polyunsaturated fatty acids.

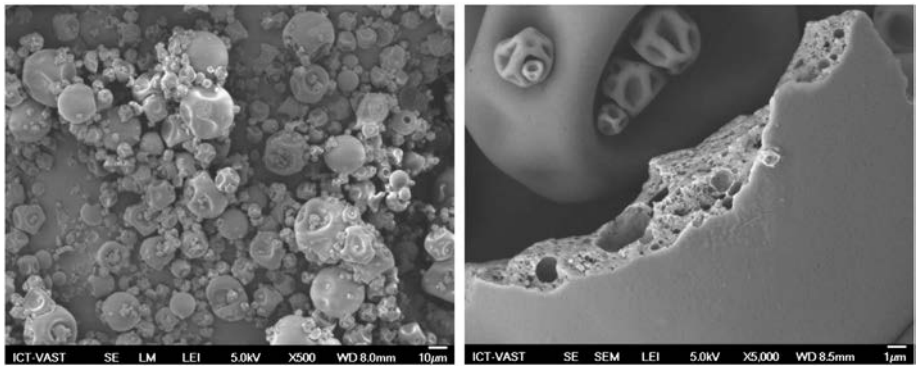

Fig. 4. Outer (left) and inner (right) microstructures of the optimal encapsulated oil powder.

colour compared with the reconstituted equivalent, indicated by higher hue angle [30]. This could be explained by the fact that the carotenoids, which being the main pigment responsible for the red-yellow colour of the product, were encapsulated in the whey protein and gum Arabic matrix. When reconstituting in the water, the carotenoids easily exited from the matrix, therefore, the more attractive colour of the reconstituted emulsion was obtained, an indication of more redness due to lower hue angle. Furthermore, total colour difference between the reconstituted and infeed emulsions (ΔE) was used to compare with the different colours before and after spray drying. Results showed that the average ΔE value was low as 9.95 ± 2.65, indicating that the colour characteristics were slightly changed.

Several authors reported that Gac oil was rich in oleic and linoleic acids [2,62], and dietary intake of those fatty acids has been proven to be beneficial to humans [63,64]. Therefore, it is important to examine whether the fatty acid composition is altered by encapsulation. A comparison between the pressed Gac oil before and after encapsulation is shown in Table 6. Generally, saturated and monosaturated fatty acids slightly increased, whereas a slight decrease in polyunsaturated fatty acids (PUFAs) was detected. However, these observations were found to be statistically non-significant ($P > 0.05$). In other words, it can be concluded that the fatty acid composition in the optimally encapsulated Gac oil powder was preserved. Furthermore, in comparison with PUFAs, monosaturated fatty acids were more stable during the encapsulation process. It is because the chemical structure of PUFAs containing a higher number of double bonds, which could be oxidised easily due to heat and oxygen during spray drying.

In order to evaluate if wall materials can protect the bioactive compounds inside their structure, it is desirable to observe the morphology of particles. As can be seen in Fig. 4, the optimally produced powder had particles with various sizes (less than 20 μm), spherical shape and concave surface, which are the typical characteristics of microcapsules produced by spray drying. Importantly, most of the particles showed no apparent cracks or fissures, ensuring a low permeability for oils and gases, therefore providing better protection and core retention [51]. Furthermore, it is also desirable to observe the inner structure of microcapsules, in which small pores were well distributed. It is indicated that the oil droplet containing carotenoids were homogeneously distributed in the wall matrix of whey protein and gum Arabic. These observations are in agreement with many reports using other types of protein wall materials [10,12,65].

4. Conclusions

Overall the study established that the quality of the encapsulated Gac oil, in terms of the EEs of the oil, β-carotene, lycopene, EY, MC, WSI and PV was adequately fitted and predicted by the corresponding response surface model. The optimal spray drying conditions within the experimental ranges including inlet and outlet air temperatures of 154 and 80 °C were obtained. Under such conditions, the response variables including the EEs of the oil, β-carotene, lycopene, EY, MC, WSI and PV achieved were also predicted and validated as 87.22%, 82.76%, 84.29%, 52.78%, 4.90%, 90.29% and 4.06 meq/kg, respectively.

The colour characteristics of the optimally encapsulated oil powder and its reconstitution were also evaluated. The results indicated that only a slight difference in colour between the infeed and reconstituted emulsions was found, indicating effective preservation of the colour. Furthermore, other physicochemical properties of the powder including Aw, pH, bulk density, fatty acid composition, and particle morphology were also determined. It is expected that the powder could be stored for a long time due to low Aw and good protective structure of particles against light, oxidation, and the unwanted released of the oil droplets and carotenoids. Therefore, it can be concluded that the high quality of the encapsulated Gac oil powder could be then used as nutrient supplement and natural food colourant due to its contents of unsaturated fatty acids, β-carotene and lycopene, and its attractive red-yellow colour.

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Paper IX

2.1.8 A Storage Study of Encapsulated Gac (*Momordica cochinchinensis*) Oil Powder and its Fortification into Foods

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A storage study of encapsulated Gac (*Momordica cochinchinensis*) oil powder and its fortification into foods

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Highlights

- The degradation rate of β-carotene and lycopene in Gac oil powder was best fitted by a first-order reaction
- EMC values at a constant temperature increased with increase in ERH
- The optimal conditions for encapsulated Gac oil powder have been determined.
- Encapsulated Gac oil powder was easily incorporated into dairy and cake mix products
- Encapsulated Gac oil powder could be used as natural colourant and nutrient supplement

Keywords: storage; encapsulation; spray drying; Gac oil; carotenoid; fortification.

Abstract

This study investigated the effects of different storage conditions, temperatures of -20, 10, room temperature (RT), 40 and 63 ^{o}C for up to 12 months in the presence or absence of air and light, on the stability of an encapsulated Gac (*Momordica cochinchinensis*) oil powder. A stability trial of the encapsulated oil powder incorporated into yoghurt, pasteurised milk and cake mix stored at 4±2^{o}C and RT for different storage times was also carried out. The results showed that a progressive degradation of colour, β-carotene and lycopene, and a progressive increase in surface oil content and peroxide value (PV) occurred in the encapsulated powders with increasing storage temperatures and storage times. However, the degradation was much less when the encapsulated powder was stored at low temperature in the absence of air and light. The isotherm curves of the encapsulated Gac oil powder samples had sigmoidal shapes and the equilibrium moisture content at a constant temperature increased with the increase in the equilibrium relative humidity. The results

also showed that the encapsulated Gac oil powder could successfully be incorporated into food products in terms of retention of colour, β-carotene and lycopene, and low PV. In conclusion, the encapsulated Gac oil powder is stable when stored at low temperatures especially in the absence of air and light and can be incorporated into food products.

1. Introduction

It has been reported that Gac oil extracted from Gac fruit (*Momordica cochinchinensis*) arils contains high levels of β-carotene (186 mg/100 mL), lycopene (518 mg/100 mL) and fatty acids (102 mg/g of fresh weight) (Kha et al., 2013a; Kha et al., 2013c, 2014a; Vuong, 2000). These bioactive components play important roles in human health (DeFilippis et al., 2010; Rao and Rao, 2007). The health benefits of the carotenoids, the unsaturated fatty acids and other bioactive compounds in the Gac fruit have been reviewed recently by Kha et al. (2013b). In addition, it is highly desirable to produce Gac oil containing both carotenoids and fatty acids because a significant improvement in the absorption of the carotenoids into the human body occurs when they are digested with fat (Unlu et al., 2005). Having a high antioxidant activity, carotenoids such as β-carotene and lycopene can be used in oils where they can protect unsaturated fatty acids against peroxidation (Pénicaud et al., 2011).

Gac oil is easily susceptible to isomerisation and oxidation during processing and storage because of the presence of a high number of double bonds in the structure of carotenoids and unsaturated fatty acids. It is therefore important to find an effective method to prevent the degradation of the bioactive components in the oil. Microencapsulation by spray-drying is one of the most effective techniques, which can be employed to protect, stabilise, and release the compounds while also enabling their solubility in an aqueous medium (Rocha et al., 2012). Kha et al. (2014b, c) successfully encapsulated Gac oil into a matrix of protein and polysaccharide using spray drying. The authors reported that optimisation of the emulsion formulation and spray-drying conditions is one of the most important prerequisites to achieve successful encapsulation, in terms of high encapsulation efficiencies, high water solubility, low moisture content and low water activity.

In addition to quality loss through processing, the quality of foods may also change during storage and distribution. It is necessary to study the effect of storage conditions on the quality of the encapsulated Gac oil powders, because several deteriorative reactions impacting on the colour and nutrient properties of the powders occur continually during storage. Many factors such as light, temperature and oxygen are considered to influence the quality of powders. The deterioration rate will accelerate in foods containing high unsaturated fatty acids and carotenoids when kept under inappropriate conditions, such as exposure to oxygen or high temperatures. Additionally,

71 degradation reactions can be related to enzymatic action, lipid oxidation and non-enzymic
72 browning; these reactions are often responsible for undesirable changes in colour, flavour and
73 nutritive value (Robertson, 2006). Therefore, an understanding of the causes of deterioration, and
74 the appropriate storage conditions for food products is important. However, the stability of the
75 encapsulated Gac oil powder product during storage over time has not been reported yet and
76 therefore needs to be carried out.
77 The final step of a successful encapsulation process is that the encapsulated powder can be
78 easily incorporated in foods. It is desirable to test the stability of the encapsulated powder in various
79 food products, so that the encapsulated Gac oil powder can be used as a nutrient supplement, food
80 additive and/or natural food colourant. In fact, the demand for carotenoids is still increasing due to
81 their attractive colour and their health benefits (Kong et al., 2010; Ribeiro et al., 2011). It is well
82 known that tomatoes and carrots are good sources of lycopene and β-carotene, respectively, but an
83 encapsulated Gac oil powder containing high levels of both carotenoids would be an excellent
84 alternative source. In addition, as fresh Gac fruit is not available throughout the year, it would be
85 very convenient to use the powder form of Gac in place of the fresh fruit for the fortification of
86 carotenoids into foods such as dairy products.
87 This study aimed to investigate the stability of the encapsulated Gac oil powder under a
88 variety of storage conditions, in terms of colour, surface oil content, peroxide value (PV) and β-
89 carotene and lycopene content. Kinetic parameters and moisture sorption isotherms were examined
90 for predicting the shelf life of the encapsulated powder. In addition, the shelf life of Gac oil-
91 fortified yoghurt, pasteurised milk and cake mix was also evaluated.
92
93 **2. Materials and methods**
94 **2.1 Chemicals**
95 Carotenoid standards (HPLC grade), including β-carotene (C4582, type II synthetic, ≥95%)
96 and lycopene (L9879, ≥90%, from tomato), were obtained from Sigma-Aldrich Pty. Ltd. (Sydney,
97 NSW, Australia). Solvents and butylated hydroxytoluene (BHT) were purchased from Merck Pty.
98 Ltd. (Sydney, NSW, Australia). Whey protein concentrate (WPC 100) and gum Arabic (GA), used
99 as encapsulating wall materials, were purchased from Amino Nutrition Co. (Kotara, NSW,
100 Australia).
101
102 **2.2 Gac oil extraction and encapsulation by spray drying**
103 Fresh Gac fruit was purchased from a local market in Ho Chi Minh City, Viet Nam. A
104 method described by Kha et al. (2013a) was employed to produce Gac oil. Briefly, the fresh Gac
105 arils including seeds (900 g) were scooped out from the fruits and heated by microwave at 630 W

for 62 min. The microwave-dried sample had the seeds removed by hand, was powdered and then steamed for 22 min prior to pressing at 175 kg/cm^2. A batch of fresh Gac fruits (100 kg) was used for production of Gac oil (1.4 kg).

The optimum formulation for the preparation of the Gac oil and wall material emulsion using whey protein concentrate and gum Arabic (7/3, g/g) for spray drying was a 29.5% solution and an oil load of 0.2 g/g (Kha et al., 2014b). For spray drying in a LabPlant SD-06A spray dryer (LabPlant UK Ltd., North Yorkshire, UK), the optimum conditions were an inlet temperature of 154 ^{o}C, outlet temperature of 80 ^{o}C, feed flow rate of 970 mL/h, air flow speed of 4.3 m/s, and pressure of 2 bar (Kha et al., 2014c).

2.3 Storage conditions of encapsulated Gac oil powder

The encapsulated Gac oil powders (10 g) were vacuum-packed and non-vacuum-packed into high barrier vacuum pouches (Caspak Products Pty Ltd, Melbourne, Vic, Australia). The packed samples were then stored at different temperatures of -20 ^{o}C, 10 ^{o}C and room temperature (RT, 25 - 30 ^{o}C) for 360 days, at 40 ^{o}C for 120 days and at 63 ^{o}C for 28 days. For storage at RT, the samples were also packed into laminated and non-laminated vacuum pouches in the presence or absence of air. For practical reasons, due to the time consuming nature of the testing of storage conditions at 40 ^{o}C or lower, the simple accelerated method of using oven storage at 63 ^{o}C (Schaal Oven test) was carried out as is often done to evaluate the stability of food products containing oils. Duplicate samples were periodically withdrawn during the storage in order to measure surface oil content, peroxide value (PV), carotenoid (β-carotene and lycopene) content and colour.

2.4 Water sorption isotherms

The encapsulated Gac oil powders (2 g) were weighed in aluminium containers and then put into a series of hermetic glass desiccators containing saturated salt solutions of sodium hydroxide (NaOH), lithium chloride (LiCl), potassium acetate (CH_3COOK), potassium carbonate (K_2CO_3), magnesium nitrate ($Mg(NO_3)_2$), sodium chloride (NaCl), and potassium chloride (KCl), providing a range of relative humidities from 6 to 87% (Greenspan, 1977). The desiccators were tightly closed and placed at 10, 30 and 40 ^{o}C. The samples were then weighed every five days until they reached equilibrium. The final moisture content of the samples was determined by the air drying method at 103 ^{o}C until a constant weight was reached.

The monolayer moisture content M_o (d.b.) was calculated using the Brunauer Emmett Teller (BET) and Gugenheim Anderson de Boer (GAB) equations as follows (Lee and Lee, 2008):

BET: $$\frac{Aw}{(1-Aw)MC} = \frac{1}{M_oC} + \frac{(C-1)}{M_oC}Aw$$

GAB: $MC = \frac{M_o C_o K_G Aw}{(1-K_G Aw)(1-K_G Aw+C_G K_G Aw)}$

where MC is moisture content of powders expressed in g per 100 g solids; M_o is g of water equivalent to monomolecular layer adsorbed per 100 g dry solids; A_w is water activity at moisture content MC; C is BET constant; C_o, K_G and C_G are GAB equation parameters.

2.5 Fortification of foods with encapsulated Gac oil powder

The encapsulated Gac oil powders were incorporated into three types of foods: yoghurt, pasteurised milk and cake mix, using typically standard formulations. For fortified yoghurt, raw milk (1 L) purchased from the morning milking at the experimental farm of the University of Nong Lam, (Ho Chi Minh City, Viet Nam), was heated and stirred until 90 ^{o}C was reached. The temperature was quickly reduced from 90 ^{o}C to 45 ^{o}C by placing the steel container containing the milk in a cold water bath and the encapsulated Gac oil powder (20 g), sugar (100 g) and a good quality commercial live yoghurt (125 g, Vinamilk Corp., Ho Chi Minh City, Viet Nam) were added into the heated milk and stirred thoroughly. The mixture was then incubated at 42±2 ^{o}C for 4.5 h in an IF55 incubator (Memmert GmbH + Co.KG, Munich, Germany). Afterwards, the mixture, with a pH value of 4.3±2, was poured into sterilised jars and stored under refrigeration at 4±2 ^{o}C. Duplicate samples were periodically withdrawn for testing (β-carotene and lycopene content, PV and colour), after 0 (control), 5, 10, 20 and 30 days.

For fortified pasteurised milk, the raw milk was heated at 63 ^{o}C for 20 seconds. The encapsulated Gac oil powder (20 g) was added into the milk (1 L) and stirred until completely dissolved. The milk was then filtered using a cloth filter and heated to 95 ^{o}C for 30 seconds. After heating, the milk, with a pH of 5.31±0.25, was hot filled into glass bottles (330 mL) with a 1.0 cm headspace. The heating pasteurisation process (90 ^{o}C for 5 min) was performed using an autoclave (Korimat KA 160, Haiger, Germany) with a thermocouple measuring a "cold region" temperature of the bottle. After thermal treatment, the milk bottles were immediately cooled in an ice-water bath. For the storage study, the milk products were kept under refrigeration at 4±2 ^{o}C and then periodically withdrawn for testing (β-carotene and lycopene content, PV and colour), after 0, 5, 10, 20 and 30 days. Samples of the milk before pasteurisation were also measured as a control before pasteurisation.

For a fortified cake mix, the encapsulated Gac oil powder (160 g) was thoroughly mixed with whey flour (200 g), sugar (40 g), raising agents (sodium bicarbonate, 10 g) and salt (4 g). The cake mix (30 g) was packed into high barrier vacuum pouches and stored at RT for 4 months. Duplicate samples were periodically withdrawn during the storage in order to measure total oil content, PV, carotenoid (β-carotene and lycopene) content and colour.

175 **2.6 Analyses**

176 **2.6.1 Surface oil content**

177 The surface oil content of the encapsulated Gac oil powders during storage was determined
178 by gently shaking the encapsulated powder sample (5 g) in n-hexane for 10 min without
179 microcapsule destruction. The solvent was decanted and the residue was vacuum-dried at 70 ^{o}C
180 until constant weight (Jimenez et al., 2006).

181

182 **2.6.2 Peroxide value (PV)**

183 The PV value of Gac oil encapsulated powder and cake mix samples was determined
184 according to AOCS Cd 8-53 (AOCS, 1998). Extracted oil (3 g) from the encapsulated Gac oil
185 powder or the fortified products using n-hexane was placed into 200 mL Erlenmeyer flask and
186 dissolved in 20 ml of a mixture containing acetic acid and chloroform (3:2, v/v) and then the
187 mixture was stirred for a few seconds to ensure thorough mixing. Thereafter, about 0.5 mL of
188 saturated potassium iodide solution was added. After exactly 1 minute, deionised water (20 mL)
189 was added and it was titrated with 0.01 N sodium thiosulfate, using a starch solution as an indicator,
190 until the solution became colourless. The mixture was magnetically stirred during the titration
191 procedure. The results were calculated as milliequivalents of active oxygen per kg (meq/kg) of oil
192 sample as follows:

193 PV (meq/kg) = [(S – B) x N x 1000]/ Mass of sample (g)

194 where S and B are the titration amounts of 0.01 N sodium thiosulfate for the sample and
195 blank (in mL), respectively. N is the normality of sodium thiosulfate solution.

196

197 **2.6.3 Determination of carotenoid content**

198 The content of β-carotene and lycopene of the encapsulated Gac oil powder and the fortified
199 products was analysed using an HPLC method (Kha et al., 2013c). Briefly, the microcapsule
200 samples (1 g) or the fortified samples (5 g) were weighed and reconstituted in deionised water (20
201 mL) to form an homogeneous solution. The reconstituted samples were dissolved in a 4:3 (v/v)
202 solution of ethanol and hexane (35 mL) containing 0.1% BHT, and the mixture was blended for 5
203 minutes at 5,000 rpm. The extract was filtered through a Whatman No. 1 filter paper on a Buchner
204 funnel. The residue was re-extracted with another 35 mL of the ethanol and hexane (4:3) with BHT
205 solution and then washed twice with ethanol (12.5 mL) and once with hexane (12.5 mL). The
206 combined extracts were washed with deionised water, dried by rotary evaporator and then diluted
207 with the mobile phase solution. All operations were performed under subdued light to minimise
208 oxidation of the carotenoids.

HPLC analysis of β-carotene and lycopene was performed with an Agilent 1200 HPLC system (Santa Clara, CA, USA) equipped with a diode array detector. He carotenoids were separated using two reverse phase columns; a Luna C18 column (100 x 4.6 mm i.d; 5μm) was directly connected to a Jupiter C18 column (250 x 4.6 mm i.d; 5μm) (Phenomenex Australia Pty. Ltd., Lane Cove, NSW, Australia). The mobile phase consisted of acetonitrile, dichloromethane and methanol (ACN: DCM: MeOH) 50: 40: 10 v/v/v. The flow rate was 1.0 mL/min, the detection was at 450 nm and the injection volume was 20 μL. The identification of β-carotene and lycopene was based solely on the retention time of the peaks compared with authentic standards. The amount of β-carotene and lycopene in the samples was expressed as μg/g.

2.6.4 Colour determination

The colour of encapsulated Gac oil powder and fortified product samples was determined using a Minolta Chroma Meter calibrated with a white standard tile. The results were expressed as Hunter colour values of L*, a*, and b*, where L* was used to denote lightness, a* redness and greenness, and b* yellowness and blueness. Prior to colour measurement, the encapsulated Gac oil powder samples and cake mix products were packed into a polyethylene pouch and measured. For the yoghurt and pasteurised milk products, the samples were placed in clear Petri dishes and filled to the top before the measurement.

Chroma, representing colour intensity, was calculated by the formula $(a^{*2} + b^{*2})^{1/2}$. The hue angle (H^o) was calculated by the formula $H^o = \arctan(b^*/a^*)$. The hue angle values vary from 0^o (pure red colour), 90^o (pure yellow colour), 180^o (pure green colour) to 270^o (pure blue colour). The desirable hue angle is about 45^o. Total colour difference or change (ΔE) between two samples was calculated by the following formula:

$$\Delta E = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2}$$

where L_o^*, a_o^* and b_o^* are the values of the samples at zero time, and L*, a* and b* the measured values of each sample with time.

2.6.5 Calculation of kinetic parameters

The degradation of β-carotene and lycopene in the encapsulated Gac oil powders was calculated by using the standard equation for a first-order kinetic model as follows:

$$\ln C = \ln C_o - k(t)$$

where C is the concentration at time t; C_o is the concentration at time zero; k, the degradation rate constant (day^{-1}) is obtained from the slope of a plot of the natural log of C/C_o versus time; and t, being the storage time (days).

The half-life was calculated at a specific temperature by the equation: $t_{1/2} = \ln 2/k$

244

245 **2.7 Statistical analysis**

246 The experiments were carried out in duplicate and the subsequent analyses were performed
247 in triplicate. The results were presented as mean values with standard deviations. The three-factor
248 experiments (temperature, air and time in the absence of light; and air, light and time at RT) were
249 randomly done to investigate the effects of the storage conditions on the oil surface content, PV,
250 carotenoid content and colour of the encapsulated powder. For the fortification, the one-factor
251 experiments (storage time) were randomly carried out to investigate the effect of storage time on
252 PV, carotenoid content and colour characteristics of the fortified food products. The mean values
253 were analysed for significant difference by analysis of variance (ANOVA) and the least significant
254 difference (LSD) post-hoc test using SPSS software version 22.0 (IBM Corp., Armonk, NY, USA).

255

256 **3. Results and discussion**

257 **3.1 Effects of storage conditions on the surface oil content and peroxide value**

258 To consider the effectiveness of spray-dried powder for food application, it is necessary to
259 guarantee its stability and functionality during storage (Da Silva et al., 2013). Therefore,
260 investigation of the effects of storage conditions on the quality of the encapsulated Gac oil powders
261 is important. The Gac oil powders were stored at different temperatures of -20, 10, RT, 40 and 63
262 ^{o}C in the presence or absence of air and light, for a period of time. There was no change in the water
263 activity and moisture content of the samples during storage (data not shown). This was most likely
264 due to the very high water barrier packaging material used for storing the samples. However, the
265 statistical results indicated that the investigated factors significantly impacted the surface oil content
266 and the PV ($P < 0.001$). As anticipated, higher storage temperatures and longer times resulted in an
267 increase in the surface oil content (Fig. 1) and the PV (Fig. 2). Similarly, the surface oil content and
268 the PV during storage also increased when the samples were in the presence of air and light ($P <$
269 0.001).

270 During storage at higher temperatures for a longer time, the surface oil content of the
271 encapsulated Gac oil powder significantly increased, especially at 40 and 63 ^{o}C for 120 and 28
272 days, respectively. Therefore, the oil was released to the surface of the powder during storage under
273 these conditions, due to physical and chemical changes in the wall materials and the molecular
274 diffusion of the oil through them (Aghbashlo et al., 2013). For example, proteins can denature at
275 high temperatures (60 ^{o}C) during storage (Lim et al., 2012) and lead to low protection properties of
276 protein wall materials, due to the changes in their structure, and therefore, oil diffusion from inside
277 the matrix to the outside surface can be promoted. The encapsulating structure might also be

weakened when the samples are exposed to increased temperatures for a longer time, causing high release rates of the oil to the surface.

In addition, the increased surface oil content could decrease the amount of Gac oil encapsulated inside the microcapsules, resulting in a poorer oil protection against oxidation. Similar to carotenoids, it is well known that the oil containing high levels of unsaturated fatty acids is much more susceptible to oxidation due to temperature, air and light. In this study, it is evident that the PV of the encapsulated Gac oil powder significantly increased during storage and is related to the increased surface oil content (Figs. 1 and 2). Particularly, the PV of the samples increased up to 212 and 374 meq/kg when the samples stored at the higher temperatures of 40 and 63 ^{o}C in the presence of air, for up to 120 and 28 days, respectively (Fig. 2). As a result, it is recommended that the encapsulated Gac oil should be kept at as low a temperature as possible in the absence of air and light.

3.2 Effects of storage conditions on the β-carotene and lycopene content

Statistical results indicated that the investigated storage conditions significantly affected the content of β-carotene and lycopene in the encapsulated Gac oil powder ($P < 0.001$). Significant degradation of the carotenoids occurred in the powder samples and increased with higher storage temperatures and longer storage times, particularly at 40 and 63 ^{o}C in the presence of air. The results indicated that at those storage conditions, the loss of β-carotene and lycopene was -56% and -93%, and -60% and -97%, respectively. Many studies have reported that the main reasons for this phenomenon are oxidation and isomerisation (Chen and Tang, 1998; Çinar, 2004). According to these studies, the possible mechanism of carotenoid degradation is that heat and available oxygen promote isomerisation of *trans*-carotenoids to the *cis*-forms, which are less stable and more susceptible to degradation during storage. As a result, low molecular mass compounds are formed, resulting in significant degradation of the carotenoids. In addition, compared to the higher temperatures of 40 and 63 ^{o}C, loss of carotenoids in the encapsulated powder samples at the lower temperatures of -20, 10 ^{o}C and RT after storage periods of 360 days, was much lower. This could be explained by the re-isomerisation from *cis*-isomers to all-*tran* forms, which have greater stability, occurring during storage at low temperatures (Xianquan et al., 2005). Alternatively, the degradation of carotenoids during storage at higher temperatures for a longer time could be explained by degradation of the carotenoids into colourless forms at these conditions, rather than isomerisation or re-isomerisation (Goula and Adamopoulos, 2012).

Storage of the dried samples at RT is more convenient. Therefore, the effects of storage of the encapsulated Gac oil powders at RT in laminated packaging (no light) and non-laminated packaging (light) in the presence or the absence of air, were also determined. The results showed

313 that the samples stored at RT exposed to light in the presence or absence of air had significantly
314 decreased retention of β-carotene and lycopene ($P < 0.001$). However, the interaction between the
315 presence or absence of air and the exposure or not to light, on the degradation of carotenoids was
316 found to be statistically insignificant ($P > 0.05$). It has been previously reported that samples
317 containing carotenoids stored in the presence of air and light at a constant temperature had a faster
318 degradation rate than those stored with exposure to air and light (Pénicaud et al., 2011; Sharma and
319 Maguer, 1996). However, Çinar (2004) reported that the carotenoid degradation in freeze-dried
320 plant samples such as orange peel, sweet potato and carrot was not affected by exposure to light
321 when the samples were stored at 25 ^{o}C for 45 days. Similarly, the stability of lycopene in tomato
322 puree was insignificantly changed by exposure to light (Shi et al., 2008). Furthermore, according to
323 Pénicaud et al. (2011), light can also favour the isomerisation of carotenoids such as β-carotene,
324 resulting in the formation of *cis*-isomers, but to a smaller extent compared to the effect high
325 temperatures can have. It can be concluded that, depending on the type of food matrix and the
326 storage conditions used, the extent of the loss of carotenoids during storage may differ.
327 It is also desirable to investigate the kinetic degradation of β-carotene and lycopene in the
328 encapsulated powder in order to predict the shelf life of the product during storage. As can be seen
329 in Figs. 3 and 4, the degradation of β-carotene and lycopene in all samples during storage fitted a
330 first-order reaction. The findings are consistent with other studies (Chen and Tang, 1998; Desobry
331 et al., 1999). Table 1 shows the kinetic parameters of β-carotene and lycopene degradation in the
332 encapsulated Gac oil powder under the different storage conditions. Generally, higher degradation
333 rates and lower half-life values for β-carotene and lycopene were obtained as a result of increasing
334 storage temperatures from -20 ^{o}C to 63 ^{o}C. For example, the apparent degradation rate ($k = 0.0002$
335 day^{-1}) of β-carotene in the powder samples stored at -20 ^{o}C was much lower than for samples stored
336 at higher temperatures in the absence of air and light, i.e. 2 times higher at 10 ^{o}C, 2.5 times at RT,
337 19 times at 40 ^{o}C and 331 times at 63 ^{o}C. Similar trends were also found for the degradation rate for
338 lycopene in the powder samples. However, compared to β-carotene, the degradation rate of
339 lycopene was higher in most samples, except for the samples stored at 10 ^{o}C in the presence of air
340 and under dark conditions, indicating a greater loss of lycopene during storage. This observation is
341 in agreement with several reports that lycopene was degraded faster than β-carotene (Henry et al.,
342 2000; Nhung et al., 2010). Furthermore, the different degradation rates for the carotenoids could be
343 due to the different supramolecular structure and reactivity of the carotenoids, which would cause
344 them to interact differently with the protein-polysaccharide matrix of the encapsulating material
345 during storage. Differing interactions between different carotenoids and their surrounding matrix
346 have been reported, which can lead to changes in the physical and chemical properties of the

carotenoids (Britton, 1995). As a result, different effects on the degradation rate of different carotenoids during storage were found.

According to Nhung et al. (2010), the degradation rates of β-carotene and lycopene in Gac oil stored at different temperatures of 5, RT, 45 and 60 ^{o}C, which followed a first order kinetics model, were much higher than those in the encapsulated Gac oil powder samples under the current storage conditions. Similar results were found in that higher storage temperatures caused a greater loss of carotenoids. However, the lower rates observed in the present study indicate that the carotenoids in Gac oil degraded much faster than those in the encapsulated Gac oil powder. Therefore, it is evident in this study, that prominent protective effects on the carotenoids were achieved by the encapsulation using the protein-polysaccharide matrix. Similarly, Selim et al. (2000) reported that improved resistance of carotenoids to oxidation can be achieved by applying encapsulation. In the present study, high stability, as seen by low surface oil content, PV and carotenoid degradation rates, was achieved when the encapsulated oil powder samples were stored at low temperatures, including RT in the absence of air and light.

3.3 Effects of storage conditions on total colour difference

Fig. 6 shows how the total colour difference (ΔE), between the encapsulated Gac oil samples at time zero and time t, was affected by the different storage conditions (temperature, air and light). The results indicated that a higher storage temperature and a longer storage time led to significantly larger differences in the total colour of the encapsulated powder samples ($P < 0.001$). Higher lightness and lower redness values were obtained for the powder stored at the higher temperatures and for the longer times, resulting in greater total colour differences. Similarly, other studies have also reported that the colour of samples containing carotenoids was significantly affected by high storage temperatures and times (Chen and Tang, 1998; Duangmal et al., 2008). In contrast, only a slight change in the ΔE value was found for the samples stored at low temperatures. As can be seen in Fig. 5, the ΔE values of the samples stored at temperatures of -20 ^{o}C, 10 ^{o}C and RT (^{o}C) and in the absence of air and light for 1 year only changed slightly, as indicated by measurements lower than 10 units. However, the results also indicated that the samples stored in the presence of air and light resulted in significantly greater ΔE values. Therefore, it is important to note that low temperatures and the absence of air and light should be recommended for storage of the encapsulated Gac oil in order to minimise colour change.

In addition to determining the optimal storage conditions for preserving the high quality of the encapsulated powders, it was also deemed necessary to determine whether there were relationships among the dependent variables such as ΔE, carotenoids and surface oil content. The results indicated that there was a high correlations between ΔE and β-carotene content ($R^2 = 0.80$)

and between ΔE and lycopene content (R^2 = 0.82) during storage. According to the Pearson correlation test correlation coefficients for ΔE and β-carotene and for ΔE and lycopene were -0.895 and -0.904, respectively, and the correlations were significant at the 0.01 level (2-tailed). The negative correlations meant that the increases in total colour difference most likely resulted from a decrease in the carotenoid content during storage. Chen and Tang (1998) also reported a similar strong relationship between colour differences and the carotenoid content of carrot pulp waste powder stored under various conditions.

High correlations between the surface oil content and β-carotene (0.89) and between the surface oil and lycopene (0.89), were also found. The Pearson correlation coefficients were -0.945 and -0.941 for the surface oil content and β-carotene and for the surface oil content and lycopene, respectively, and the correlations were significant at the 0.01 level (2-tailed). This indicates that the surface oil content increased as the carotenoid content decreased in the stored samples. There was also a high correlation between the surface oil content and the ΔE (R^2 = 0.81). The positive Pearson correlation coefficient of 0.90 between these values was also significant at the 0.01 level (2-tailed).

The encapsulated Gac oil powder was previously shown to be highly dissolvable in water (Kha et al., 2014b, c) and in the present study to be highly dissolvable in the dairy products tested. There was an insignificant difference in colour when the encapsulated Gac oil powder was reconstituted in the fortified yoghurt and the pasteurised milk products or in water (results not shown). Colour is one of the most important appearance attributes of food products as it directly influences acceptability by consumers. In products such as the encapsulated Gac oil powder, colour can be more easily measured and more quickly evaluated during storage compared to analysing other parameters such as surface oil, β-carotene and lycopene. Therefore, high correlations between colour and the other parameters are useful for predicting the retention of carotenoids and the surface oil content during storage based on the colour.

3.4 Moisture sorption isotherms

The moisture sorption isotherms are extremely useful for predicting the shelf life of a product in terms of its physical, biochemical and microbial stability when determining packaging and storage conditions (Janjai et al., 2006; Yan et al., 2008). The graphical relationship between the equilibrium moisture content (EMC_{db},%) and the equilibrium relative humidity (ERH, %) at a constant temperature is described by moisture sorption isotherms. Fig. 6 shows the sorption isotherm curves constructed for the encapsulated Gac oil powder samples stored at the three different temperatures of 10, 30 and 40 ^{o}C, which all showed sigmoid shapes and similar trends. Generally, the EMC values at a constant temperature increased as the ERH increased. This is in agreement with several authors who stated that the moisture isotherms for dried plant food usually

417 exhibit a sigmoidal curve (Janjai et al., 2006; Yan et al., 2008). In the ERH range of 6 - 43%,
418 depending on the storage temperature, the encapsulated powder samples showed a very slight
419 increase in the EMC. The moisture content and the water activity of the samples in this range were
420 very low, indicating that there was a very small amount of free water within the powders. Thus, the
421 samples could be more stable physically, chemically and biologically. However, it is very difficult
422 and costly to bring foods within this range of ERH with drying processes.
423 In contrast, deterioration of samples occurs easily and rapidly at ERH higher than 75% due
424 to a high moisture uptake, a condition that supports chemical, biological and microbial degradation.
425 In the present study, the greatest increase in water uptake behaviour was observed as the ERH was
426 increased from 43% to 57%, as displayed by the highest gradient slope in this section of the curves.
427 This indicated that the encapsulated powder samples should not be stored within this range of ERH,
428 unless a very good quality packaging material with very high water barrier properties is used. At
429 ERH values from 48 to 75%, the EMC almost remained unchanged, indicating that the water uptake
430 was stable and did not increase further.
431 Fig. 6 also shows that the EMC values decreased as the storage temperature was increased
432 from 10 to 40 ^{o}C, at a constant ERH. This is because the physicochemical changes in the wall
433 material matrix caused by the increasing temperature most likely resulted in a decrease in the total
434 number of active sites for binding water (Goula et al., 2008). Furthermore, the mobility of water
435 molecules is also increased by increasing temperatures; the higher heat energies can activate the
436 water molecules and cause them to separate from their water-binding sites on the food (Goula et al.,
437 2008; Palipane and Driscoll, 1993). As a result, at a constant relative humidity, the amount of
438 adsorbed water decreases with increasing temperature, thus decreasing the EMC.
439 According to the BET equation, the monolayer moisture content, M_o (%, d.b.) obtained for
440 the samples stored at 10, 30 and 40 ^{o}C was 3.53 (R^2 = 0.95), 2.43 (R^2 = 0.91) and 2.25% (R^2 =
441 0.90), respectively. However, the values using the GAB equation were higher at 4.75 (R^2 = 0.84),
442 4.93 (R^2 = 0.86) and 4.78 (R^2 = 0.84), respectively. These results indicated that the BET equation
443 was more accurate for predicting the M_o because the values it generated had higher R^2 values. In
444 general, the M_o of a dried food product is considered to be the safest condition in terms of storage
445 stability. This is because a higher rate of lipid oxidation is promoted at moisture levels below the
446 M_o and at higher moisture levels, deterioration of food occurs due to promotion of Maillard
447 browning and of enzymatic and microbiological activities. However, foods are not usually dried to
448 below M_o values due to the extra heat needed for evaporation. Therefore, the initial moisture
449 content of a product is preferred to be at or slightly above its M_o value in order to achieve a
450 maximal shelf life with minimal spoilage (Fellows, 2009). The encapsulated Gac oil powder sample
451 used in this study had a moisture content of 4.2 ± 0.31%, which was close to the M_o, indicating that

452 the sample was likely to be able to be stored for an extended period of time. As discussed
453 previously, it is evident that the encapsulated Gac oil powder was highly stable at low temperatures
454 in the absence of air and light for up to 1 year.
455
456 **3.5 Fortification with the encapsulated Gac oil powder**
457 Foods containing carotenoids are currently receiving considerable attention from food
458 manufactures and consumers. Generally, carotenoids such as β-carotene and lycopene are widely
459 used within the food industry as colourants and nutrient supplements due to an increased awareness
460 of consumers of the benefits of these natural nutrient components (Zulueta et al., 2007). As
461 mentioned earlier, the final purpose of the encapsulation process is to fortify foods by incorporation
462 of the encapsulated product in foods. In this study, it was important to test whether the encapsulated
463 Gac oil powder could be incorporated into yoghurt, pasteurised milk and cake mix.
464 The stability of the encapsulated Gac oil powder when used for the fortification of yoghurt
465 (Table 2), pasteurised milk (Table 3) and cake mix (Table 4) followed by storage at 4 ^{o}C or RT for
466 4 weeks or 4 months was evaluated. In general, the results showed that the colour characteristics
467 (lightness, chroma, hue angle and ΔE) of the fortified products remained unchanged or only
468 changed slightly during storage. Compared to the fortified yoghurt and pasteurised milk samples at
469 time zero (control), there was only a very slight change in colour for the samples stored under
470 refrigeration at 4 ^{o}C, as indicated by the low ΔE value (less than 4 units). According to Obón et al.
471 (2009), the total colour difference between fresh and processed products can be generally
472 distinguished when the ΔE value is higher than 5 units. Similarly, the colour characteristics of the
473 cake mix fortified with the encapsulated Gac oil powder was stable for up to 4 months. In other
474 words, the colour of the fortified products remained unchanged during storage.
475 There are a number of commercial dairy products that have been fortified with bioactive
476 compounds from fruits and vegetables such as tomato, apricot, carrot, guava and passion fruit.
477 According to Zuleta et al. (2007), these beverages come in a variety of different colours, indicated
478 by a large range of hue angle values from 36 to 116. The fruit and vegetable juice-milk products,
479 among others which were typically observed to have a yellow-red colour, exhibited hue angle
480 values of less than 90. Therefore, from the commercial point of view, the yoghurt, pasteurised milk
481 and cake mix products fortified with the encapsulated Gac oil powder, which have an attractive
482 yellow-red colour, could be competitively marketed.
483 The PV is mainly used to evaluate the initial stages of the oxidation process in food products
484 containing unsaturated fatty acids (Van Hoed et al., 2010). When it was measured in the fortified
485 products during storage, Tables 2 shows that the PV values of the fortified yoghurt product were
486 found to be insignificantly changed ($P > 0.05$). The PV of the pasteurised milk (Table 3) and the

cake mix (Table 4) fortified with the encapsulated Gac oil powder were only slightly increased during storage for up to 4 weeks and 4 months, respectively ($P < 0.05$). According to Codex STAN 19-1981 (2003), the PV of oil products should not exceed 10 meq/kg. Therefore, it can be concluded, due to their low PV, that the fortified products in the current study were highly stable in terms of oil oxidation.

Generally, the results from Tables 2, 3 and 4 show that only a slight decrease in β-carotene and lycopene occurred in some of the fortified products as the storage time increased. The degradation of carotenoid content in the products during storage is mainly due to auto-oxidation, photo-oxidation and photo-isomerisation. These reactions can occur simultaneously and competitively in the presence of catalyst, oxygen and light intensity. The oxidation severity also depends on the carotenoid structure and environmental conditions (Chen et al., 1996).

The apparent high stability of the carotenoids in the fortified products during storage is highly desirable. However, another issue, which will be important to investigate, is the release of the carotenoids from the protein-polysaccharide matrix and the maintenance of their antioxidant activity under gastric and intestinal conditions. The way the encapsulation system behaves under digestive conditions will ultimately determine the bioavailability of the carotenoids and whether the encapsulated Gac oil is likely to be useful for meeting the recommended daily intake levels for these carotenoids.

The recommended daily intake levels for β-carotene and lycopene are 0.2 - 9.7 mg (Müller, 1996) and 5 - 7 mg (Rao and Rao, 2007), respectively. As can be seen from the results in Tables 2, 3 and 4, one serve of 100 g of yoghurt, pasteurised milk and cake mix contained 4 to 14 mg of β-carotene and 8 to 41 mg of lycopene. After refrigeration for up to 4 weeks, despite some losses (refer to Tables 2 and 3), the content of β-carotene and lycopene in the fortified yoghurt and pasteurised milk were still higher than those reported for some other pasteurised fruit and vegetable juices, such as tomato juice, which ranged from 32 to 56 μg/g (Podsędek et al., 2003; Sánchez-Moreno et al., 2006).

As a result of having established in the present study that the levels of the carotenoids remained high during storage in the products fortified with the encapsulated Gac oil powder, these products could be recommended as good sources of β-carotene and lycopene for their value as a vitamin A precursor and for the prevention of prostate cancer, respectively. Indeed, consumption of Gac fruit, particularly rich in β-carotene, has been reported by Vuong et al. (2002) to reduce the incidence of vitamin A deficiency. Studies have also indicated that the consumption of fruits and vegetables rich in lycopene, has been linked with a lower risk of developing prostate cancer (Chan et al., 2009; Guns and Cowell, 2005).

4. Conclusions

It can be concluded that a significant progressive degradation of colour, β-carotene and lycopene, and a significant increase in the surface oil content and PV of the encapsulated Gac oil powder occurred as a result of increasing the storage temperature from -20 to 10, RT, 40 and 63 ^{o}C, and increasing the storage period. The degradation rate of β-carotene and lycopene in the powder was best fitted by a first-order reaction. The loss of colour, β-carotene and lycopene, and the increase in the surface oil content and PV in the encapsulated Gac oil powder were less when the powder was packed in the absence of air and light. There were high correlations among total colour difference, PV, β-carotene and lycopene in the powder samples under the various storage conditions tested. Moreover, the isotherm curves of the encapsulated Gac oil powder samples stored at 10, 30 and 40 ^{o}C had sigmoidal shapes and similar patterns to those which are usually observed in dried food products. At a constant temperature, the EMC values increased with the increase in ERH.

The fortification experiments showed that the encapsulated Gac oil powder was easily incorporated into three food products. In these experiments, the stability of the encapsulated Gac oil powder incorporated into yoghurt, pasteurised milk and cake mix was tested during storage under refrigeration at 4±2 ^{o}C and at RT for different storage times. The stability was found to be satisfactory, in terms of the retention of colour, β-carotene and lycopene and low PV during storage for 4 weeks for the yoghurt and the pasteurised milk, and for 4 months for the cake mix.

In conclusion, the encapsulated Gac oil powder is stable when stored at low temperatures especially in the absence of air and light. It can also be successfully incorporated into food products.

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Figure captions

Fig. 1. Effects of storage conditions on the surface oil content of encapsulated Gac oil powder.

Samples were stored at -20, 10 (A), 40 and 63 ^{o}C (C) in the presence or absence of air (vacuum or non-vacuum) and in the absence of light (dark). B: samples were stored at room temperature (RT) in the presence or absence of air and light (vacuum or non-vacuum and light or dark).

Fig. 2. Effects of storage conditions on the peroxide value (PV) of encapsulated Gac oil powder.

Samples were stored at -20, 10 (A), 40 and 63 ^{o}C (C) in the presence or absence of air (vacuum or non-vacuum) and in the absence of light (dark). B: samples were stored at room temperature (RT) in the presence or absence of air and light (vacuum or non-vacuum and light or dark).

Fig. 3. First-order degradation plots for β-carotene in encapsulated Gac oil powder under different storage conditions.

Samples were stored at -20, 10 (A), 40 and 63 ^{o}C (C) in the presence or absence of air (vacuum or non-vacuum) and in the absence of light (dark). B: samples were stored at room temperature (RT) in the presence or absence of air and light (vacuum or non-vacuum and light or dark).

Fig. 4. First-order degradation plots for lycopene in encapsulated Gac oil powder under different storage conditions.

Samples were stored at -20, 10 (A), 40 and 63 ^{o}C (C) in the presence or absence of air (vacuum or non-vacuum) and in the absence of light (dark). B: samples were stored at room temperature (RT) in the presence or absence of air and light (vacuum or non-vacuum and light or dark).

Fig. 5. Effects of storage conditions on total color difference (ΔE).

Samples were stored at -20, 10 (A), 40 and 63 ^{o}C (C) in the presence or absence of air (vacuum or non-vacuum) and in the absence of light (dark). B: samples were stored at room temperature (RT) in the presence or absence of air and light (vacuum or non-vacuum and light or dark).

Fig. 6. Sorption isotherm curves of the encapsulated Gac oil powder samples at different temperatures of 10, 30 and 40 ^{o}C.

EMC: equilibrium moisture content; ERH: equilibrium relative humidity.

List of Figures

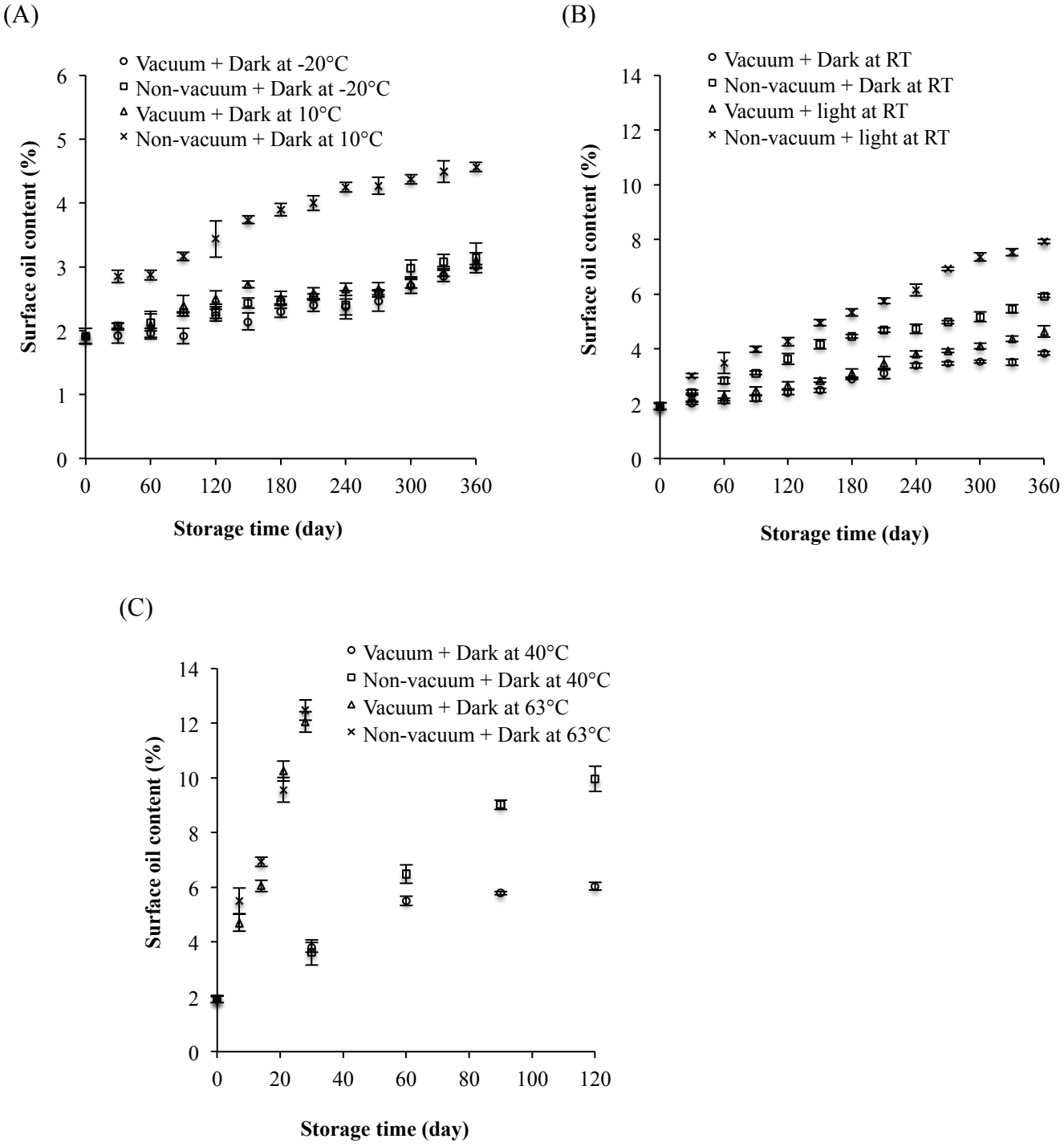


Fig. 1

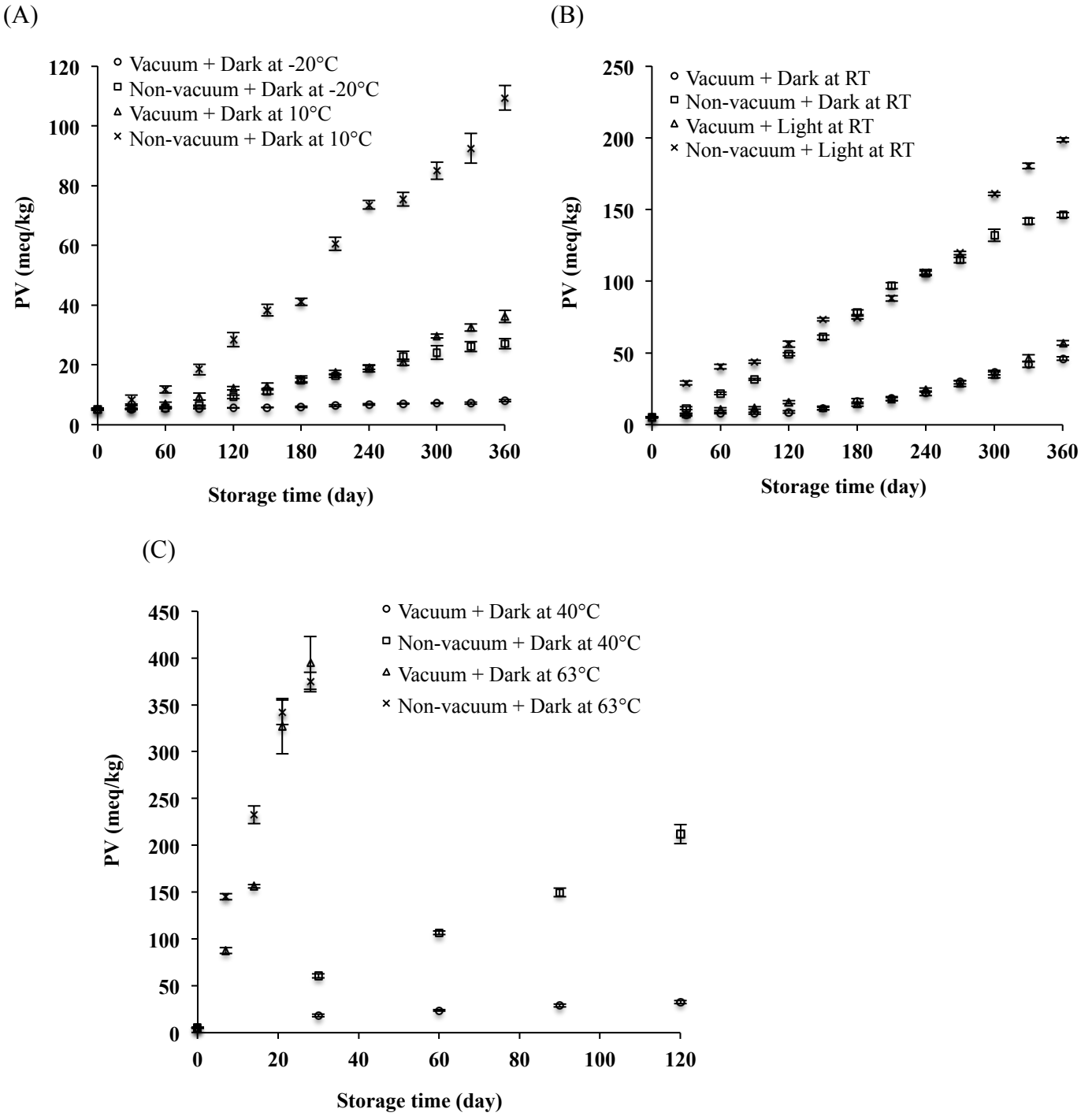
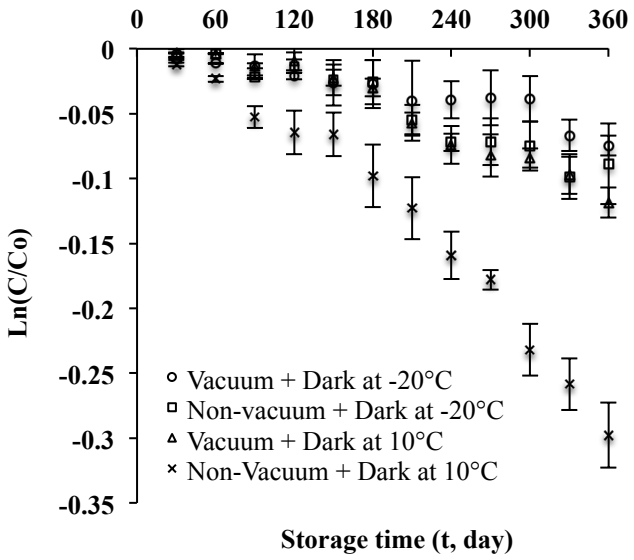
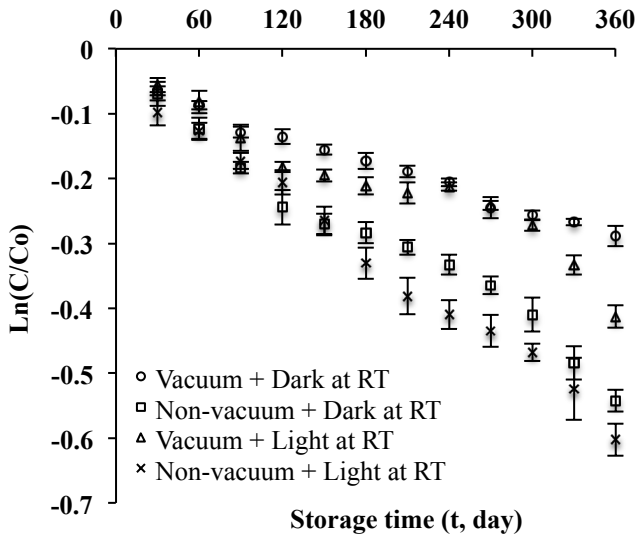

(A)
Vacuum + Dark at -20°C
Non-vacuum + Dark at -20°C
Vacuum + Dark at 10°C
Non-vacuum + Dark at 10°C
PV (meq/kg)
Storage time (day)
(B)
Vacuum + Dark at RT
Non-vacuum + Dark at RT
Vacuum + Light at RT
Non-vacuum + Light at RT
PV (meq/kg)
Storage time (day)
(C)
Vacuum + Dark at 40°C
Non-vacuum + Dark at 40°C
Vacuum + Dark at 63°C
Non-vacuum + Dark at 63°C
PV (meq/kg)
Storage time (day)

Fig. 2

(A)


0
60
120
180
240
300
360
0
-0.05
-0.1
-0.15
-0.2
-0.25
-0.3
-0.35
Ln(C/Co)
Vacuum + Dark at -20°C
Non-vacuum + Dark at -20°C
Vacuum + Dark at 10°C
Non-Vacuum + Dark at 10°C
Storage time (t, day)

(B)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60
120
180
240
300
360
0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
Ln(C/Co)
Vacuum + Dark at RT
Non-vacuum + Dark at RT
Vacuum + Light at RT
Non-vacuum + Light at RT
Storage time (t, day)

(C)

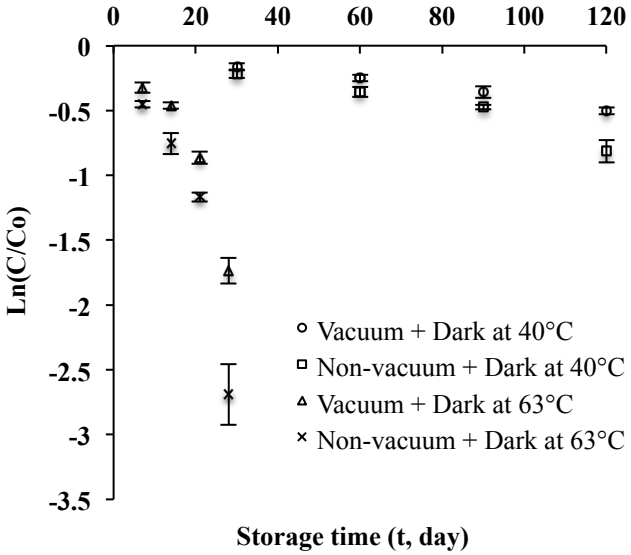
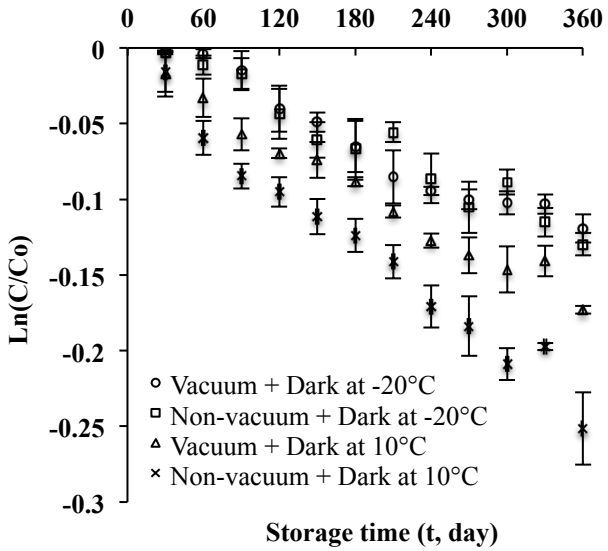
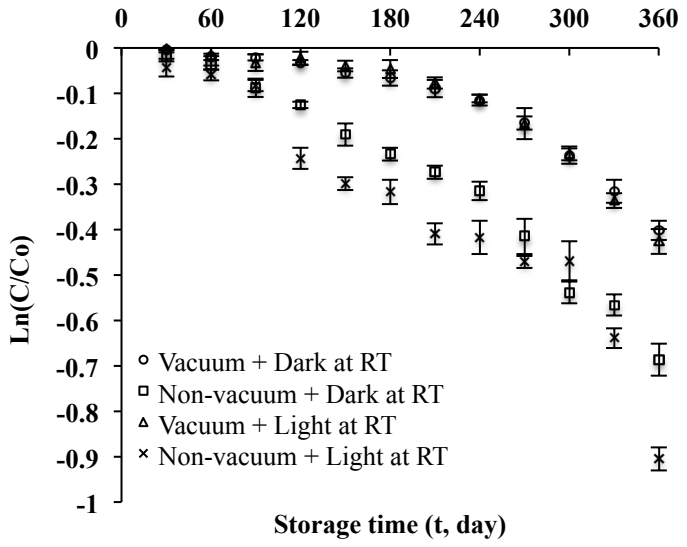

0
20
40
60
80
100
120
0
-0.5
-1
-1.5
-2
-2.5
-3
-3.5
Ln(C/Co)
Vacuum + Dark at 40°C
Non-vacuum + Dark at 40°C
Vacuum + Dark at 63°C
Non-vacuum + Dark at 63°C
Storage time (t, day)

Fig. 3

(A)


0
60
120
180
240
300
360
0
-0.05
-0.1
-0.15
-0.2
-0.25
-0.3
Ln(C/Co)
Vacuum + Dark at -20°C
Non-vacuum + Dark at -20°C
Vacuum + Dark at 10°C
Non-vacuum + Dark at 10°C
Storage time (t, day)

(B)


0
60
120
180
240
300
360
0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
-0.8
-0.9
-1
Ln(C/Co)
Vacuum + Dark at RT
Non-vacuum + Dark at RT
Vacuum + Light at RT
Non-vacuum + Light at RT
Storage time (t, day)

(C)

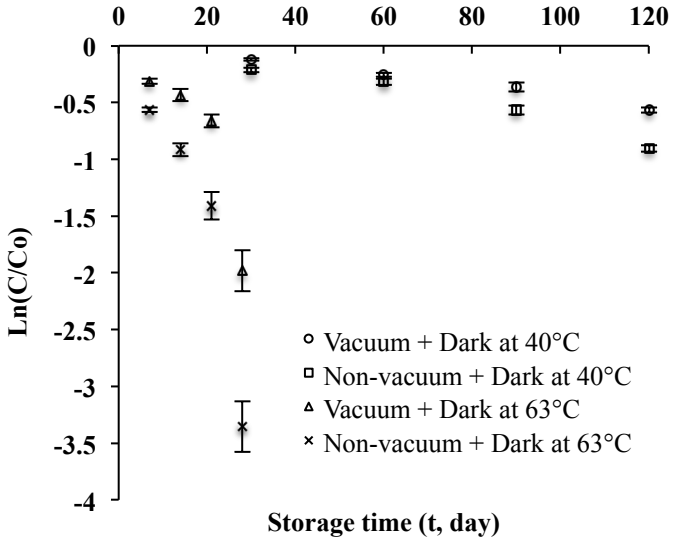
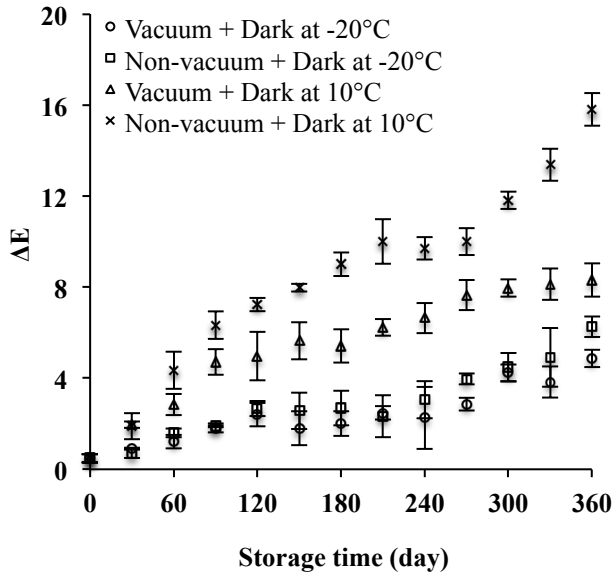
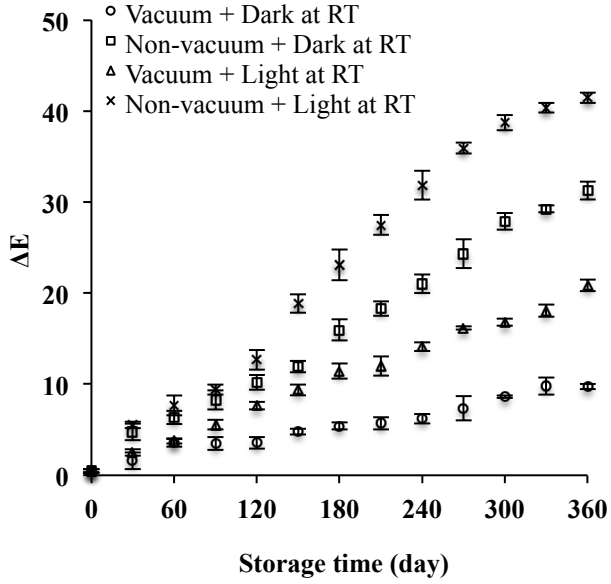

0
20
40
60
80
100
120
0
-0.5
-1
-1.5
-2
-2.5
-3
-3.5
-4
Ln(C/Co)
Vacuum + Dark at 40°C
Non-vacuum + Dark at 40°C
Vacuum + Dark at 63°C
Non-vacuum + Dark at 63°C
Storage time (t, day)

Fig. 4

(A)


Vacuum + Dark at -20°C
Non-vacuum + Dark at -20°C
Vacuum + Dark at 10°C
Non-vacuum + Dark at 10°C
ΔE
Storage time (day)
0
4
8
12
16
20
0
60
120
180
240
300
360

(B)


Vacuum + Dark at RT
Non-vacuum + Dark at RT
Vacuum + Light at RT
Non-vacuum + Light at RT
ΔE
Storage time (day)
0
10
20
30
40
50
0
60
120
180
240
300
360

(C)

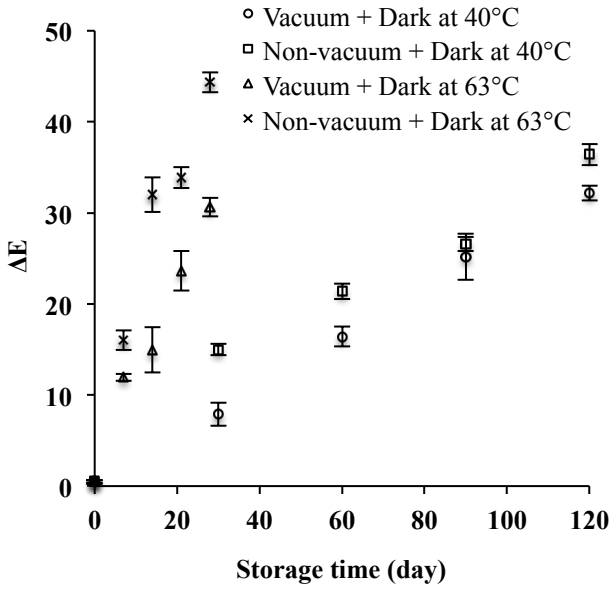

Vacuum + Dark at 40°C
Non-vacuum + Dark at 40°C
Vacuum + Dark at 63°C
Non-vacuum + Dark at 63°C
ΔE
Storage time (day)
0
10
20
30
40
50
0
20
40
60
80
100
120

Fig. 5

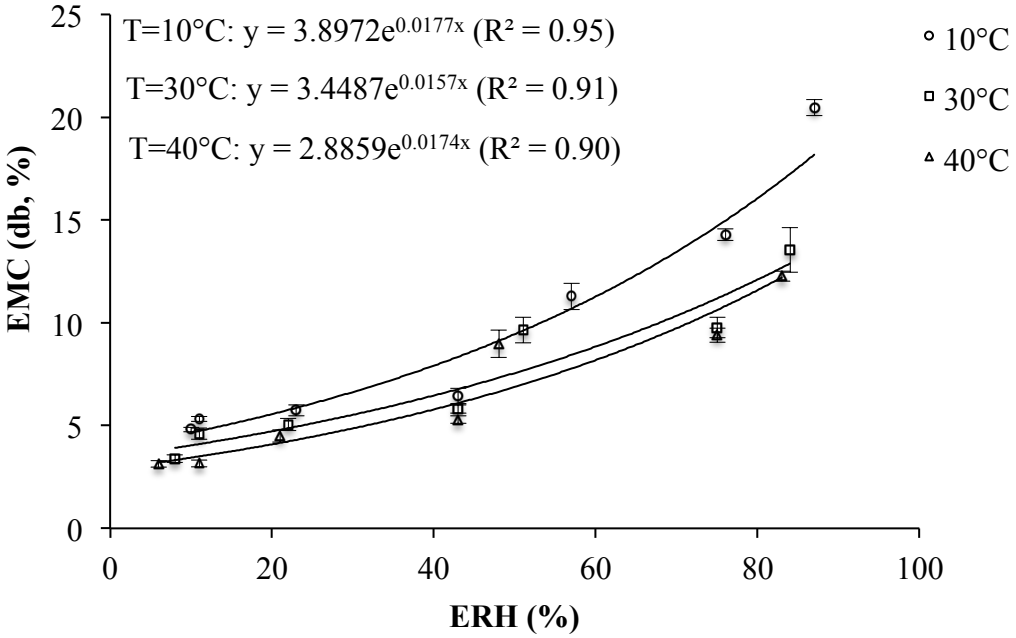

T=10°C: y = 3.8972e^0.0177x (R² = 0.95)
T=30°C: y = 3.4487e^0.0157x (R² = 0.91)
T=40°C: y = 2.8859e^0.0174x (R² = 0.90)
10°C
30°C
40°C
EMC (db, %)
ERH (%)
0
5
10
15
20
25
0
20
40
60
80
100

Fig. 6

Table 1. Degradation kinetics of β-carotene and lycopene in the encapsulated Gac oil powder

Storage conditions	β-Carotene				Lycopene			
	k (day^{-1})	Half life (day)	First-order degradation	r^2	k (day^{-1})	Half life (day)	First-order degradation	r^2
Vacuum + dark at -20°C	0.0002	3466	y = -0.0002t + 0.0029	0.894	0.0004	1733	y = -0.0004t + 0.0097	0.971
Non-vacuum + dark -20°C	0.0003	2310	y = -0.0003t + 0.0124	0.923	0.0004	1733	y = -0.0004t + 0.0075	0.948
Vacuum + dark at 10°C	0.0004	1733	y = -0.0004t + 0.0219	0.952	0.0004	1733	y = -0.0004t - 0.0107	0.977
Non-vacuum + dark at 10°C	0.0009	770	y = -0.0009t + 0.0374	0.964	0.0006	1155	y = -0.0006t - 0.0157	0.973
Vacuum + dark at RT	0.0005	1386	y = -0.0007t - 0.0535	0.989	0.0011	630	y = -0.0011t + 0.087	0.852
Non-vacuum + dark at RT	0.0013	533	y = -0.0013t - 0.0545	0.969	0.002	347	y =-0.002t + 0.0978	0.966
Vacuum + light at RT	0.0009	770	y = -0.0009t - 0.043	0.912	0.0012	578	y =-0.0012t + 0.1001	0.819
Non-vacuum + light at RT	0.0015	462	y= -0.0015t - 0.0437	0.991	0.0022	315	y = 0.0022t + 0.0686	0.912
Vacuum + dark at 40°C	0.0038	182	y = -0.0038t - 0.035	0.989	0.0048	144	y = -0.0048t + 0.0334	0.981
Non-vacuum + dark at 40°C	0.0063	110	y = -0.0063t + 0.0099	0.932	0.0077	90	y = -0.0077t + 0.0808	0.951
Vacuum + dark at 63°C	0.0662	10	y = -0.0662t + 0.3119	0.887	0.0748	9	y = -0.0748t + 0.462	0.771
Non-vacuum + dark at 63°C	0.1018	7	y = -0.1018t + 0.5168	0.858	0.1265	5	y = 0.1265t + 0.6537	0.845

$y = ln(C/C_o)$; C: amount of β-carotene or lycopene at storage time t; C_o: initial amount of β-carotene or lycopene; k: apparent degradation rate constant

Table 2. Colour characteristics, peroxide value (PV) and carotenoid content of the yoghurt incorporated with encapsulated Gac oil powder

Sample	Lightness	Chroma	Hue angle	ΔE	PV (meq/kg)	β-carotene (μg/g)	Lycopene (μg/g)
Week 0	75.1 ± 0.1[a]	47.2 ± 0.7[a]	73.5 ± 0.1[a]		2.0 ± 0.2[a]	50.6 ± 13.9[a]	119.3 ± 26.4[a]
Week 1	75.3 ± 0.6[a]	50.0 ± 1.7[b]	73.4 ± 0.5[a]	2.9 ± 1.7[a]	2.1 ± 0.3[a]	43.1 ± 8.2[a]	112.0 ± 32.9[a]
Week 2	75.4 ± 0.5[a]	50.3 ± 1.1[b]	73.7 ± 0.4[a]	3.2 ± 1.2[a]	2.2 ± 0.1[a]	48.2 ± 11.9[a]	87.2 ± 15.3[a]
Week 3	75.4 ± 0.6[a]	50.8 ± 0.7[b]	73.3 ± 0.6[a]	3.7 ± 0.7[a]	2.3 ± 0.4[a]	44.7 ± 5.3[a]	85.6 ± 12.6[a]
Week 4	75.5 ± 0.3[a]	50.2 ± 0.6[b]	74.7 ± 0.8[a]	3.3 ± 0.4[a]	2.6 ± 0.2[a]	42.7 ± 7.7[a]	81.2 ± 13.1[a]

Values in the same column followed by different superscripts (a - c) were significantly different ($P < 0.05$); ΔE: total color difference.

Table 3. Colour characteristics, peroxide value (PV) and carotenoid content of the pasteurised milk incorporated with encapsulated Gac oil powder

Sample	Lightness	Chroma	Hue angle	ΔE	PV (meq/kg)	β-carotene (μg/g)	Lycopene (μg/g)
Before pasteurisation	77.6 ± 0.2^{a}	48.7 ± 0.7^{a}	74.2 ± 0.1^{a}		2.2 ± 0.3^{a}	61.3 ± 2.7^{a}	162.6 ± 12.6^{a}
Week 0	76.9 ± 0.2^{ab}	48.5 ± 0.4^{a}	74.3 ± 0.4^{a}	1.0 ± 0.2^{a}	3.8 ± 0.2^{b}	57.6 ± 8.5^{a}	148.5 ± 20.0^{ab}
Week 1	76.8 ± 0.5^{b}	48.5 ± 0.5^{a}	74.2 ± 0.2^{a}	0.9 ± 0.6^{a}	4.1 ± 0.2^{bd}	56.6 ± 5.7^{a}	128.3 ± 18.0^{bc}
Week 2	76.1 ± 0.5^{b}	48.5 ± 0.6^{a}	75.2 ± 0.3^{b}	2.0 ± 0.2^{b}	4.2 ± 0.2^{bd}	54.8 ± 3.6^{a}	117.0 ± 11.4^{c}
Week 3	76.0 ± 0.8^{b}	48.1 ± 0.3^{a}	75.3 ± 0.3^{b}	2.0 ± 0.5^{b}	4.2 ± 0.1^{bd}	52.0 ± 5.6^{a}	110.1 ± 10.7^{c}
Week 4	76.8 ± 0.3^{b}	48.7 ± 0.4^{a}	75.8 ± 0.5^{c}	1.7 ± 0.6^{b}	4.3 ± 0.1^{cd}	52.9 ± 6.7^{a}	108.4 ± 12.5^{c}

Values in the same column followed by different superscripts (a - d) were significantly different ($P < 0.05$); ΔE: total color difference.

Table 4. Colour characteristics, peroxide value (PV) and carotenoid content of the cake mix incorporated with encapsulated Gac oil powder

Samples	Lightness	Chroma	Hue angle	ΔE	PV (meq/kg)	β-carotene (μg/g)	Lycopene (μg/g)
Control	80.9 ± 0.8^{a}	40.4 ± 0.4^{a}	73.3 ± 0.2^{a}		3.4 ± 0.2^{a}	140.0 ± 8.6^{a}	410.1 ± 17.3^{a}
Month 1	80.8 ± 1.3^{a}	40.8 ± 1.0^{ab}	74.6 ± 1.1^{b}	1.8 ± 0.7^{a}	4.6 ± 0.1^{b}	139.3 ± 14.1^{a}	406.1 ± 28.9^{a}
Month 2	81.2 ± 1.0^{a}	40.8 ± 0.5^{ab}	74.8 ± 0.7^{b}	1.6 ± 0.6^{a}	6.2 ± 0.2^{c}	134.8 ± 13.5^{a}	381.3 ± 16.8^{a}
Month 3	82.3 ± 0.5^{a}	41.9 ± 0.4^{b}	76.1 ± 0.6^{c}	3.0 ± 0.7^{b}	7.1 ± 0.3^{d}	123.7 ± 7.7^{a}	376.4 ± 12.0^{a}
Month 4	81.9 ± 0.1^{a}	44.3 ± 1.0^{c}	77.4 ± 0.4^{d}	5.2 ± 0.6^{c}	7.7 ± 0.5^{d}	101.3 ± 8.1^{b}	372.3 ± 20.2^{a}

Values in the same column followed by different superscripts (a - d) were significantly different ($P < 0.05$); ΔE: total color difference.

PART 3: GENERAL DISCUSSION AND CONCLUSIONS

3.1 General discussion

The current research work has two main parts: extraction and encapsulation. In the extraction part, several novel extraction methods and the use of water as extracting solvent were employed to effectively extract the oil and carotenoids from the fresh Gac arils. In a study of the Gac oil extraction, it is necessary to investigate the effects of different extraction methods on extraction efficiencies in terms of the oil, β-carotene and lycopene. As a result of such investigations, depending on the desired final product quality and other considerations, a suitable extraction method can be chosen. For the encapsulation, to effectively preserve the bioactive compounds in the resultant Gac oils in the powder form, the encapsulation by spray drying was used. A storage study of the encapsulated Gac oil powder also needed to be carried out to examine whether the encapsulated Gac oil powder product has the potential for long storage at ambient temperature. Finally, an investigation of incorporation into a range of foods was carried out in this study as the use of carotenoid-based foods as natural colourants and nutrient supplements is currently receiving considerable attention from consumers and food industry.

The main results and discussion of this study have been discussed in the eight research papers (section 2.2). Therefore, general discussion and recommendations are presented in the following sections.

3.1.1 Gac oil extraction

It is well known that different extraction techniques have different advantages and drawbacks. In this section, the three different extraction methods of Gac oil from the Gac arils, including supercritical carbon dioxide (SC-CO_2) extraction, ultrasound-assisted aqueous extraction and microwave-drying before pressing are discussed. A comparison of different extraction methods, in terms of extraction efficiency and physicochemical properties, is also presented.

It is expected that the extraction efficiencies in terms of Gac oil, β-carotene and lycopene were affected by conditions applied during the extraction. The main findings of the Gac oil extraction supported the hypothesis that the extraction efficiencies could be improved by applying suitable extraction conditions and optimisation of the independent variables. For example, the three different extraction methods for extracting oil from Gac aril significantly improved the oil extraction efficiency by at least 18% compared to the traditional extraction methods. Furthermore, the bioactive compounds in the Gac oil extracts were also significantly better preserved (refer to Papers II, III, IV, V and VI).

SC-CO_2 extraction

Generally, the SC-CO_2 extraction has been applied for extracting oils from plant materials as an alternative to traditional extraction using hazardous solvents. This is because it offers a number of advantages of having non-solvent residues, shorter extraction times, higher extraction yields and better retention of valuable bioactive compounds (Azmir et al., 2013; Herrero et al., 2006). It is also well known that improvement of the extraction efficiency could be achieved by applying optimal extraction conditions, including pressure, temperature, CO_2 flow rate, and extraction time. However, pre-treatments prior to SC-CO_2 extraction have also been reported as improving the extraction efficiency (Del Valle & Uquiche, 2002). Therefore, this study as reported in **Paper II** was the first to comprehensively investigate the impact of different pre-treatments (enzymatic treatment, air-drying temperature and powder particle size) on the oil yield and β-carotene and lycopene contents of the Gac oil extract using SC-CO_2 extraction.

According to the results of **Paper II**, enzymatic pre-treatment for the fresh Gac arils prior to the SC-CO_2 extraction improved the oil yield compared to the un-treated sample. The cell walls are opened up by a positive effect of enzyme; the complex lipoprotein and lipopolysaccharide molecules are also broken up into simpler molecules (Li et al., 2012; Shankar et al., 1997). However, a significant degradation of β-carotene and lycopene contents in the oil extracts with enzymatic hydrolysis was found. This is because the contacting surface area of the enzymatic-treated arils with oxygen was

larger than the surface of the untreated samples. As a result, more carotenoids were substantially degraded due to oxidation (Tran et al., 2008).

Air-drying and particle size were also important parameters to achieve high oil yield and content of β-carotene and lycopene. Reducing the moisture content of the Gac arils before the SC-CO_2 extraction allows an adequate diffusion of SC-CO_2 through the cell membrane due to the microstructural changes of the dried arils (Gutiérrez, Ratti, & Belkacemi, 2008). The air-drying temperature of 50^{o}C provided enough heat to break down the cell walls and the bonding forces between carotenoids and the tissue matrix (Chang et al., 2006). Moreover, a smaller particle size of 0.45 mm not only increased surface area to volume ratio of materials but also ruptured cell membranes. As a result, a faster rate of the CO_2 diffusion could be obtained (Del Valle & Uquiche, 2002; Panfili et al., 2003); the oil and carotenoids were then easily extracted.

Ultrasound-assisted aqueous extraction

As mentioned earlier, the use of hazardous industrial chemical solvents has been traditionally used for extracting vegetable oil and bioactive compounds from plant materials. The main disadvantages of this process are health concerns and increased environmental regulations due to the toxicity of hazardous solvents. Moreover, the extracted oils can be of low quality in terms of their content of unwanted free fatty acids, waxes and unsaponifiable matter (Do & Sabatini, 2011). Therefore, it is desirable to use "green" solvent such as CO_2, which was reported in Paper II, to extract the oils and bioactive compounds from Gac arils. As expected higher oil yield could be obtained when the SC-CO_2 extraction was used. However, processing costs are also one of the most important considerations when SC-CO_2 equipment is used for Gac oil extraction. To achieve effective Gac oil extraction efficiency compared to SC-CO_2 extraction, use of "green" solvent and cheaper equipment are the main criteria. Water is relatively cheap, environmentally friendly, non-flammable, non-toxic and readily available. Generally, the ultrasound water bath is much cheaper compared to SC-CO_2 equipment. Therefore, in this study ultrasound-assisted aqueous extraction was also employed to extract the oil from microwave-dried Gac arils.

Several important parameters such as ultrasound power, extraction time, particle size and ratio of water to solid have been reported as impacting the extraction efficiency and quality of the oil extract (Azmir et al., 2013; Toma et al., 2001; Vinatoru, 2001). **Paper III** reported the most suitable conditions of ultrasound-assisted aqueous extraction for Gac oil from microwave-dried Gac aril powder. The results in this paper pointed out that high extraction efficiencies in terms of Gac oil, β-carotene and lycopene, and low PV could be obtained using the optimal extraction conditions including ultrasound power of 320 W, extraction time of 20 min, powder particle sizes of 0.3 - 0.5 mm and a ratio of water to powder of 9 g/g. The finding supported the hypothesis, which is presented in section 1.5.

Furthermore, the results from **Paper III** also confirmed that the use of microwave-drying method as a pre-treatment for Gac arils significantly improved the extraction process compared to air-drying method. It is evident that microwave-drying can be used as an alternative drying method to conventional hot air-drying with several advantages such as uniform energy delivery, high thermal conductivity to the interior of the material, better space utilisation, sanitation, energy saving, and fast start-up and shutdown conditions (Izli et al., 2014; Krokida, Maroulis & Saravacos, 2001). Importantly, appropriate microwave-drying conditions could result in better retention of nutrients, colour characteristics and less drying time (Zhao et al., 2013).

In addition to the above-mentioned advantages of the microwave-drying, in the vegetable oil extraction process it is important to break down the cell walls of the material. As a result, the oils and bioactive compounds can be easily extracted. In this study, **Paper III** also showed the surface morphology of the microwave-dried Gac arils appeared more disintegrated compared to the air-dried Gac arils, using SEM. It suggested that microwave-drying disrupted their membrane structures, as seen with other plant materials (Choi et al., 2006). A comparison of the microstructural changes in the dried Gac aril under different drying methods using light microscope and SEM was also presented (see Paper VI).

Interestingly, **Paper III** also showed that major disruption of the Gac aril cells was observed when the combination of microwave-drying the Gac aril followed by the ultrasound-assisted aqueous extraction was used. It allows a better contact between the

components inside the cells and the solvent. Therefore, high extraction efficiencies in terms of Gac oil, β-carotene and lycopene, and low PV were obtained. It can be concluded that microwave-drying followed by ultrasound-assisted aqueous extraction is an effective method for the extraction of Gac oil enriched in β-carotene and lycopene and with a low PV.

Microwave-drying and pressing extraction

In the first two research papers (**Papers II and III**), the extraction methods of the Gac oil including SC-CO_2 extraction and ultrasound-assisted aqueous extraction were carried out under laboratory scale conditions. A small amount of Gac aril material was used and a small quantity of the oil extracts was obtained. As a result, to obtain a larger quantity of Gac oil with high quality, which can be further used for encapsulation study, it is desirable to find another effective extraction method. Hydraulic pressing extraction of vegetable oil has been investigated under pilot scale conditions, which can be further scaled up to commercial operation. Therefore, in this study, microwave-drying and pressing extraction was also investigated to extract Gac oil with larger quantity. The resultant Gac oil will be then used for encapsulation study (refer to section 3.1.2). Furthermore, it is also important to compare those different Gac oil extraction methods in terms of Gac oil extraction efficiency and quality of the oil extract.

The results from **Paper IV** showed that the extraction efficiencies in terms of Gac oil, β-carotene and lycopene were significantly impacted by microwave power, microwave time, steaming time and hydraulic pressure. In practice, mechanical extraction by hydraulic or screw press is one of the most common extraction methods in vegetable oil processing industry. This is because high quality of oil extract could be obtained in terms of better retention of nutrients and chemical free compared to traditional extraction methods using hazardous solvents. However, the main drawback of this mechanical extraction method is that the oil extraction efficiency is low being about 70% (Bargale et al., 1999; Owolarafe, Osunleke & Oyebamiji, 2007). As a result, there is a need to overcome this disadvantage which bioactive compounds are also effectively preserved. In this study, application of microwave-drying and steaming processing steps significantly enhanced the oil extraction efficiency and better retention of β-carotene and lycopene in the Gac oil extract.

In general, it is well known that optimisation using RSM is commonly applied to improve the performances of a system, a process or a product to obtain the maximum benefit from it (Bezerra et al., 2008). Therefore, it is important to use RSM to optimise the Gac oil pressing extraction to obtain the extraction efficiency as high as possible. To achieve this goal, the significant impact and the most suitable range of independent variables in **Paper IV** were used to optimise the microwave-drying and pressing extraction in **Paper V**. The results from **Paper V** confirmed that the maximum Gac oil extraction efficiency and retention of β-carotene and lycopene contents in the oil extract were achieved as predicted using optimisation by RSM.

Therefore, the results from **Paper IV** and **Paper V** confirmed that the Gac oil extraction efficiency could be improved by applying microwave-drying as a pre-treatment prior to the conventional hydraulic pressing. According to those results, the Gac oil extraction efficiency (86%) was much higher, compared to the conventional pressing extraction without microwave-drying pre-treatment (70%). Furthermore, it is possible to apply the optimised parameters for other materials rich in oil and carotenoids, provided that they have similar characteristics.

Comparison of different extraction methods

In addition to achieving the high extraction efficiencies in terms of Gac oil, β-carotene and lycopene, it is also important to compare different extraction methods based on those extraction efficiencies and other physicochemical properties. As a result, depending on the available extraction facility, processing cost and other considerations, a right choice of extraction methods can be considered.

Different drying pre-treatments (air-drying and microwave-drying) and followed by different extraction methods (pressing and Soxhlet extraction) were compared in terms of Gac oil, β-carotene, lycopene, fatty acid composition, chemical properties (acid value, peroxide value, iodine value and saponification value). The microstructural changes in the different pre-treatment and extraction methods were also compared. The results from **Paper VI** indicated that microwave-drying pre-treatment followed by pressing was an effective extraction method to extract the Gac oil with high oil yield,

better retention of β-carotene and lycopene, and the lowest values for acidity and peroxides compared to air-drying pre-treatment followed by pressing or Soxhlet extraction and microwave-drying followed by Soxhlet extraction. Moreover, the desirable oleic acid and linoleic acid were found to be the dominant fatty acids in the Gac oil extracts for all methods.

For the Gac oil extraction efficiency, the highest oil extraction efficiency (95%) was obtained by SC-CO_2 extraction (**Paper II**), followed by ultrasound-assisted aqueous extraction from microwave-dried Gac aril (90%, **Paper III**) and microwave-drying pre-treatment prior to pressing (86%, **Paper V**). A high content of β-carotene and lycopene and a low PV value in all the Gac oil extracts from the different extraction methods was obtained. Furthermore, Vuong and King (2003) reported that the Gac oil extraction efficiency obtained was 68% when air-drying pre-treatment and pressing method were used. In this study, microwave-drying pre-treatment prior to pressing significantly enhanced the extraction efficiency in comparison with a study by Vuong and King (2003). As a result, it can be seen that the extraction of Gac oil employed the different three novel and "green" extraction methods (**Papers II, III, IV and V**), and water as extracting solvent (**Paper III**) significantly enhanced the extraction efficiency.

The results of the surface morphology from **Papers III** and **VI** confirmed that microwave and ultrasound caused major disruption of the Gac aril cells during the treatments. This is consistent with previous studies (Azmir et al., 2013; Knorr et al., 2011; Soria & Villamiel, 2010; Uquiche et al., 2008). The most important extraction principle of oil and bioactive compounds is to break the cell walls of the material so that the solutes can be easily released into the extracting medium. The findings in this study confirmed that the use of microwave and/or ultrasound is suitable for extracting Gac oil with high extraction efficiency and high quality.

In summary, it can be concluded that Gac oil could be effectively extracted using different so called "green" technologies including SC-CO_2 extraction, microwave-dried followed by ultrasound-assisted aqueous extraction and microwave-drying followed by pressing, and "green" solvent. As a result, Gac oil enriched in β-carotene and lycopene can be considered to be chemical free and suitable for use as a natural food colourant, a nutraceutical or an additive in the food industry.

3.1.2 Encapsulation by spray drying

As mentioned above, the Gac oils extracted using microwave-drying followed by hydraulic pressing in the pilot scale conditions were used for encapsulation process. The crude Gac oil extracts are susceptible to isomerisation and oxidation during processing and storage due to the high number of double bonds in the structure of polyunsaturated fatty acids and carotenoids. The health benefits of fatty acids and carotenoids have been highlighted in **Paper I** and section 1.1.2. Hence, it is desirable to effectively preserve those bioactive compounds. Insolubility of Gac oil in water is also one of the main drawbacks when the Gac oil is to be incorporated into a range of foods. It is desirable to investigate the most suitable methods to overcome these issues. Encapsulation by spray drying can be an effective method to protect, stabilise and also to enable the solubility in an aqueous medium (Rocha et al., 2012).

Importantly, although health benefits associated with carotenoids such as β-carotene and lycopene have been reported (Agarwal & Rao, 2000; Strobel, Tinz & Biesalski, 2007), their maximum absorption from natural plant sources is often low due to resistance of carotenoid-protein complexes and the plant cell walls (Rein et al., 2013; Yeum & Russell, 2002). Therefore, it is strongly recommended to encapsulate the extracted Gac oil (refer to section 3.1.1) for delivering as nutrient supplements and/or in fortified foods to enhance bioavailability and to obtain maximum health benefits.

In this section, the encapsulation process is divided into three parts including encapsulation by spray drying, storage study of encapsulated Gac oil powder and its fortification into a range of foods.

Encapsulation

Generally, in the encapsulation process, optimisation of the wall material concentration and the oil load is a prerequisite for a proper encapsulation and is a first step of encapsulation using spray-drying. In this study, the results (**Paper VII**) showed that the concentration of wall material (whey protein and gum Arabic) and the ratio of Gac oil to wall material (oil load) significantly influenced the response variables including the encapsulation efficiencies in terms of oil, β-carotene and lycopene, PV, MC and ΔE.

The optimisation of the wall material concentration and the ratio of Gac oil to wall material were successfully achieved based on the response variables using RSM.

It is well known that the concentration of wall material and the oil load play a crucial role in effective encapsulation of oils and bioactive compounds. In addition, an appropriate selection of wall materials is very important. Generally, to effectively protect and control the bioactive compounds, combinations of various encapsulating agents are required (Sun-Waterhouse et al., 2011). Whey protein (WP), which has nutritional, physicochemical and functional properties, has been used in foods due to its ability to form thick and flexible film, preventing coalescence, whereas Gum Arabic (GA) is a complex blend of natural polysacharides composed of arabinogalactan, arabinogalactan-protein and glycoprotein. Therefore, a blend of whey protein (WP) and GA as the continuous phase may enhance stability of emulsion against significant droplet size increase (Klein et al., 2010). The matrix with the hydrophilic carbohydrates dissolved in the water phase and the hydrophobic polypeptide chain adsorbed onto the oil-water phase can be obtained. As a result, high stability of emulsion obtained before spray drying is desirable to achieve successful encapsulation processs. Our results (Research Paper presented at the FaBE conference proceedings, refer to **Appendices**) confirmed that the emulsions containing Gac oil and the mixture of WP and GA with the ratios of 1:3, 1:4 and 1:5 and the concentration of 30% remained highly stable for at least 24 h at the storage temperatures. Emulsions with excellent stability, low viscosity, and small and uniform particle sizes can be formulated for microencapsulation.

In general, there are many techniques for encapsulation of oil and bioactive compounds. Among them, encapsulation by spray-drying is the most widely used in the food industry due to its advantages as mentioned previously (refer to section 1.1.5). It is important to minimise the loss of Gac oil and bioactive compounds, to maximise the encapsulation efficiency, during the spray-drying process. Therefore, after optimising the concentration of wall material and the oil load (**Paper VII**), there is a need to optimise the spray-drying conditions including the air inlet and outlet temperatures (**Paper VIII**).

The results from **Paper VIII** confirmed that the maximum values for encapsulation efficiencies in terms of Gac oil, β-carotene, lycopene, EY and WSI, and the minimum

values for MC and PV were obtained when the air inlet and outlet temperatures were optimised using RSM. It is reported that many spray-drying conditions affecting the spray-drying process include the feed flow rate, inlet and outlet temperatures, atomiser speed and inlet air flow rate (Gharsallaoui et al., 2007; Jafari et al., 2008; Jena & Das, 2007). However, the air inlet and outlet temperatures are confirmed as the most important variables affecting the oil and bioactive compounds during spray-drying process (Gharsallaoui et al., 2007; Liu et al., 2004).

Finally, the optimised parameters including the concentration of wall material and the oil load (**Paper VII**) and the air inlet and outlet temperatures (**Paper VIII**) can be used for other materials rich in oil and carotenoids.

Storage study

In addition to optimisation of the wall material concentration and the oil load, and the spray-drying conditions, in order to successfully achieve the encapsulation process, the high stability of the encapsulated Gac oil powder at ambient temperature for a long time is required. Therefore, it is of interest to investigate the Gac oil powder stability under a variety of different storage conditions.

It is well known that lipid oxidation, and isomerisation and oxidation of carotenoids are major problem in storage of fat and carotenoid containing foods (Dzondo-Gadet et al., 2005; Pénicaud et al., 2011). Many studies reported that encapsulation is the best way to protect the oil and bioactive compounds from the environment for long preservation (Dzondo-Gadet et al., 2005; Márquez-Ruiz, Velasco & Dobarganes, 2000; Quispe-Condori, Saldaña & Temelli, 2011). The results from **Paper IX** confirmed that the encapsulated Gac oil powder was highly stable at ambient temperature for at least 12 months if it was vacuum-packed and stored at dark condition. This finding supports the hypothesis and the aim of the study presented in section 1.5.

According to the results from Paper VIII, the encapsulation efficiencies in terms of β-carotene and lycopene were predicted and confirmed as 83 and 84%, respectively. The content of β-carotene and lycopene in the encapsulated Gac oil used for a storage study at time 0 was 0.34 and 1.12 mg/g (d.w.). Depending on the storage conditions (at

temperatures of -20, 10 ^{o}C and room temperature in the absence of air (vacuum) and light, a loss of β-carotene and lycopene in the powder after a 12-month storage was from 11 to 33%. Although, there is no standard recommendation for the dietary intake of carotenoids, many published studies reported that the dietary intake of β-carotene and lycopene in the United States should be 2 - 5.4 mg/day (Donhowe & Kong, 2014; Grune et al., 2010; Krinsky & Johnson, 2005) and 5 - 10.5 mg/day (Porrini & Riso, 2005; Story et al., 2010). Furthermore, the dietary intake of carotenoids varies greatly depending upon the population studied. For example, the average dietary intake of lycopene in several countries including the United Kingdom, Spain, Australia, France, Netherlands and Italia was reported as 1.1, 1.6, 3.8, 4.8, 4.9 and 7.4 mg/day, respectively (Lucarini et al., 2006; Porrini & Riso, 2005). Therefore, it can be concluded that the encapsulated Gac oil containing high levels of β-carotene and lycopene is satisfactory to use as a natural food colourant, a nutrient supplement or fortification into foods, which is currently receiving considerable attention from food industry and consumer.

Fortification

The final purpose of encapsulation process is to fortify by incorporation of the encapsulated product into a range of foods. As discussed above, in this study, the encapsulation process addressed the issue of degradation of Gac oil containing carotenoids during the processing and storage due to environmental conditions such as light, heat and oxygen. In general, these deteriorations can affect colour flavour, aroma, and shelf life which limit the use of the encapsulated Gac oil powder for food fortification (Kolanowski et al., 2001; Ye et al., 2009).

As a result, it is of interest to test whether the encapsulated Gac oil powder can be incorporated into foods such as yoghurt, pasteurised milk and cake mix products. The results from **Paper IX** showed that the fortification of the encapsulated enriched in β-carotene and lycopene powder with high water solubility into those foods was achieved. The stability of these fortified products was found to be satisfactory, in terms of retention of colour, β-carotene and lycopene and low PV during their shelf lives. Therefore, it can be concluded that the three fortified products with the encapsulated

Gac oil are excellent representative foods for the delivery of unsaturated fatty acids, β-carotene and lycopene.

3.2 Conclusions

Gac oil enriched in β-carotene and lycopene was effectively extracted using the three extraction methods including air-drying followed by supercritical carbon dioxide (Paper II), microwave-drying followed by ultrasound-assisted aqueous extraction (Paper III) and microwave-drying followed by pressing (Papers IV and V). The extraction method of microwave-drying followed by pressing was then optimised using response surface methodology to maximise the extraction efficiencies in terms of Gac oil, β-carotene and lycopene (Paper V). To minimise isomerisation and oxidation of the bioactive compounds, the resultant Gac oil was subjected to an encapsulation study. The independent variables including wall materials and the oil load, and spray-drying conditions for the encapsulation of Gac oil was optimised based on the encapsulation efficiencies and other physicochemical properties, using response surface methodology. A storage study was also carried out to predict the shelf life of the encapsulated Gac oil product. Finally, an experiment was carried out to examine whether the encapsulated Gac oil powder can be incorporated into a range of foods.

For the extraction, the aim was to compare the extraction of Gac oil from the arils with conventional pressing by using the three different extraction methods. The highest Gac oil extraction efficiency (95%) was obtained using the supercritical carbon dioxide extraction (Paper II). The conditions of this extraction was the air-drying temperature of 50 ^{o}C and particle size of 0.45 mm as pre-treatments before the supercritical carbon dioxide extraction at pressure of 200 bar, extraction temperature of 50 ^{o}C and extraction time of 120 min. Using these extraction conditions, high content of β-carotene (83 mg/100 mL) and lycopene (508 mg/100 mL) was also achieved.

High extraction efficiencies in terms of Gac oil (90%), β-carotene (84%) and lycopene (83%), and a low peroxide value (2.2 meq/kg) were also obtained using microwave-drying followed by ultrasound-assisted aqueous extraction (Paper III). The fresh Gac arils (900 g) microwave-dried at 630 W for 62 min were used for the ultrasound-assisted aqueous extraction. The optimal aqueous extraction conditions were ultrasound

power of 320 W, extraction time of 20 min, powder particle sizes of 0.3-0.5 mm, a ratio of water to powder of 9 (g/g), and a centrifugal force of 6750 × *g*.

In addition to the two extraction methods under laboratory conditions above, the microwave-drying and pressing for the Gac oil extraction was also studied under pilot plant pressing conditions. First, effects of the different independent variables including microwave power, microwave-drying time, steaming time and hydraulic pressure on the Gac oil extraction efficiency, and β-carotene and lycopene contents were investigated (Paper IV). The most suitable range of independent variables including microwave-drying time (60 - 65 min), steaming time (15 - 30 min) and hydraulic pressure (160 - 180 kg/cm^2) was determined. The optimisation for Gac oil extraction (Paper V) was then also investigated based on the results from Paper IV. The results showed that under optimal extraction conditions (microwave-drying time of 62 min, steaming time of 22 min, and hydraulic pressure of 175 kg/cm^2), the maximum Gac oil extraction efficiency (86%), and content of β-carotene (186 mg/100 mL) and lycopene (518 mg/100 mL) were achieved as predicted for the current available equipment.

Furthermore, the high efficiency for Gac oil extraction was confirmed by examining the microstructural changes in the dried Gac arils before and after extraction processes (Papers III and VI).

For encapsulation, the results (Paper VII) showed that the quadratic polynomial model as sufficient to describe and predict the encapsulation efficiencies (Gac oil, β-carotene and lycopene), peroxide value, moisture content and total colour difference with R^2 values of 0.96, 0.95, 0.86, 0.89, 0.88 and 0.87, respectively. The encapsulation efficiencies in terms of Gac oil (92%), β-carotene (80%) and lycopene (74%), peroxide value 3.9 meq/kg), moisture content (4.1%) and total colour difference (12.4) were achieved as predicted under optimal conditions (wall material concentration of 29.5% by weight and oil load of 0.2, g/g).

To optimise the spray-drying conditions for Gac oil encapsulation, the results (Paper VIII) showed that the corresponding response surface model adequately describe and predict encapsulation efficiencies in terms of Gac oil, β-carotene and lycopene, encapsulation yield, moisture content, water solubility index and peroxide value with R^2

values of 0.92, 0.91, 0.89, 0.85, 0.89, 0.98 and 0.97, respectively. Under optimal conditions (inlet and outlet temperatures of 154 and 80^{o}C, respectively), the encapsulation efficiencies in terms of Gac oil, β-carotene, lycopene, encapsulation yield, moisture content, water solubility index and peroxide value were predicted and validated as 87.2%, 82.8%, 84.3%, 52.8%, 4.9%, 90.3% and 4.1 meq/kg. Moreover, the colour characteristics of the optimally encapsulated Gac oil powder and its reconstitution, and physicochemical properties of the powder (including Aw, pH, bulk density, fatty acid composition and particle morphology) were also determined. The results showed that slight difference in colour between the infeed and reconstituted emulsions was found, indicating effective preservation of the colour. It is expected that the encapsulated Gac oil powder could be stored for a long time due to low Aw, and good protective structure of particle against light, oxidation and the unwanted release of the oil droplet and carotenoids.

In the study of the encapsulated Gac oil powder under different storage conditions, a progressive degradation of colour, β-carotene, lycopene and an increase in peroxide value were found as storage temperature increased and over a longer period. The results from Paper IX suggested that preservation of colour, β-carotene and lycopene in the encapsulated oil powder with lower peroxide value was more effective when vacuum-packed into laminated aluminum bag and stored at ambient temperature or lower.

Finally, the encapsulated Gac oil powder was found to be easily incorporated into yoghurt, pasteurised milk and cake mix products. The results (Paper IX) also indicated that during their shelf-lives, the fortified Gac oil products were found to be satisfactory in terms of maintaining an attractive colour and providing a sufficient daily intake of β-carotene and lycopene, while having a low peroxide value.

In conclusion, the hypothesis was supported and the aims and objectives were achieved in this thesis. The extraction of Gac oil and carotenoids was improved using different extraction methods. The Gac oil enriched in β-carotene and lycopene was effectively encapsulated by spray-drying with whey protein and gum Arabic as wall material. In addition, the encapsulated Gac oil powder was found to be easily incorporated into a range of food products. Finally, this encapsulated Gac oil powder, being produced by

“green” technology is considered suitable for use as natural food colourant, a nutraceutical or an additive in the food industry.

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APPENDICES

1. CONFERENCE PROCEEDINGS

Research Paper presented at International Conference on Food and Biosystems Engineering (FaBE). June 2013.

Tuyen C. Kha, Minh H. Nguyen, Paul D. Roach & Costas E. Stathopoulos (2013). Effects of formulations on Gac oil emulsion stability for microencapsulation. *Proceedings of International Conference on Food and Biosystems Engineering FaBE*, Technological Educational Institute of Thessaly, Vol. 2, pp. 180-195, ISBN 978-960-9510-11-0. Full paper and Oral presentation. Refereed. (E1).

EFFECTS OF FORMULATIONS ON GAC OIL EMULSION STABILITY FOR MICROENCAPSULATION

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ABSTRACT

The objective of this study was to determine the most suitable formulations for a high stability Gac oil emulsion and a high retention within the oil of lycopene and β-carotene contents. We investigated the effects (i) of the concentration of the wall materials (WPC/GA, 7/3, w/w) (20, 30 and 40%, w/w), (ii) the ratios of Gac oil to wall material (2:1, 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7 and 1:8, w/w) on the emulsion stability and retention of lycopene and β-carotene contents at different storage temperatures (4, 22 and 37 °C), as well as on viscosity, pH, mean particle size and particle size distribution.

Results showed that emulsions obtained using the ratios of 1:5 up to 2:1 and a concentration of 30% remained highly stable for at least 24 h at the storage temperatures. Among these samples, the retention of carotenoids in the emulsions formulated with the ratios of 1:3, 1:4, and 1:5 was found to be the highest. At those formulations, emulsions had viscosity of < 30 cP, mean particle size of < 10 μm and pH of 5.7 to 5.8. It is concluded that emulsions with excellent stability, low viscosity, and small and uniform particle sizes can be formulated for microencapsulation.

Key words: *Gac oil, carotenoids, emulsion stability, whey protein concentrate, Gum Arabic*

1. INTRODUCTION

Gac (*Momordica cochinchinensis* Spreng) oil containing high amounts of β-carotene and lycopene, and essential fatty acids was discussed in a recent review on Gac fruit by Kha et al. (2013a). These bioactive compounds play crucial roles in human health (Agarwal and Rao, 2000; Gibson et al., 2011). Moreover, fat ingested with carotenoid compounds in plant foods significantly improved the absorption in human body (Brown et al., 2004; Unlu et al., 2005).

For emulsification, an appropriate selection of wall materials and emulsifier is very important. Generally, to effectively protect and control the bioactive compounds, combinations of various encapsulating agents are required (Sun-Waterhouse et al., 2011).Whey protein concentrate (WPC), which has nutritional, physicochemical and functional properties, has been used in foods due to its ability to form thick and flexible film, preventing coalescence, whereas Gum Arabic (GA) is a complex blend of natural polysacharides composed of arabinogalactan, arabinogalactan-protein and glycoprotein. Therefore, a blend of whey protein (WP) and GA as the continuous phase may enhance stability of emulsion against significant droplet size increase (Klein et al., 2010). The matrix with the hydrophilic carbohydrates dissolved in the water phase and the hydrophobic polypeptid chain adsorbed onto the oil-water phase can be obtained.

There are various techniques including mechanical emulsification, high-pressure homogenisation and ultrasound emulsification commonly applied to produce emulsions. Among those methods, emulsification by ultrasound is an effective technique which has been found to produce high stability of W/O emulsions due to very small particle sizes (Abismaïl et al., 1999; Behrend et al., 2000; Djenouhat et al., 2008).

Several studies showed that microemulsions containing carotenoids were successful in providing solubility and bioavailability of carotenoids and fatty acids (Amar et al., 2003; Yuan et al., 2008). However, there has been no work reported on emulsion preparation of Gac oil rich in β-carotene and lycopene using ultrasound. The objectives of this study were to investigate effect of formulation including the wall material concentrations and ratios of Gac oil to wall material on the emulsion stability, viscosity, pH, mean particle size and particle size distribution, and retention of lycopene and β-carotene contents at different storage conditions.

2. MATERIALS AND METHODS

2.1 Materials

Gac oil used for this study was pressed according to a method described by Kha et al. (2013b). Briefly, Gac arils were microwave pre-treated at 630 W for 62 min and steamed for 22 min prior to hydraulic pressing at 175 kg/cm^2. WPC 100% and GA used as encapsulating materials or wall materials were obtained from Amino Nutrition Co. (Kotara, NSW, Australia).

2.2 Emulsion preparation

Emulsions were prepared using a method from Bellalta et al. (2012) and Rodea-González et al.(2012) with some modifications. Aqueous stock solutions (20, 30 and 40%, w/w) of wall material (WPC/GA: 7/3) with 0.1% sodium benzoate (to prevent the proliferation of microorganisms) were prepared using a Silverson L4RT high shear mixer at 6000 rpm for 10 min. The stock solutions were then kept at 4 °C for at least 12 h to ensure complete hydration of proteins.

To create emulsions, Gac oil was added dropwise to the wall material solutions while mixing using the high shear mixer at 4000 rpm for 10 min, to allow full incorporation. Different ratios of oil to wall material (w/w) were 2:1, 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7 and 1:8. The homogenised emulsions were then subjected to sonication using an ultrasonic bath (Extech Equipment Pty. Ltd., Australia). The emulsions were sonicated at frequency of 43-47 kHz and power of 260 W for 30 min.

2.3 Storage study of the emulsion samples

The emulsions with different ratios and wall material concentrations filled into glass and plastic tubes with caps were stored at different temperatures of 4, 22 and 37 °C for up to 30, 20, 12 d, respectively. Emulsion stability, retention of β-carotene and lycopene, pH, viscosity, particle size and particle size distribution, and light microscopic observation were evaluated daily and weekly.

2.4 Stability of emulsions

The stability of the samples (10 mL) was measured at the different formulations and storage temperatures by visual observation of the height of the serum layer formed at the bottom of the graduated test tubes (H_S), expressed as a percentage of the total height of the

emulsions in the tubes (H_E). The emulsion stability (ES, %) was calculated as follows: $$ES(\%) = 100 - \left(\frac{H_S \times 100}{H_E}\right).$$

2.5 pH and viscosity

pH of the emulsion samples after emulsion preparation and during storage time intervals at different storage temperatures was determined with a digital pH-meter.

Immediately after emulsion preparation and during storage time intervals at different storage temperatures, the viscosity of the samples was measured by means of a steady stress Brookfield viscometer, Model DV-II (Brookfield Engineering Laboratories, Inc., Stoughton, MA, USA) equipped with a No. 3 spindle. The measurement of emulsion viscosity was recorded at 60 rpm at ambient conditions.

2.6 Particle size analysis

The particle size and size distribution of the emulsions were determined using a Mastersizer 2000 particle size analyser (Malvern Instruments Ltd, Worcestershire, United Kingdom). The emulsions were diluted with deionised water prior to analysis so that the obscuration range was between 10 and 20%. The refractive index of the emulsion droplets taken was 1.456 and that for the dispersion medium was 1.33. The particle size was expressed as the volume weighted mean diameter which was calculated by the following formula: $d_{4,3} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3}$. Where n_i is the number of droplets in the i^{th} fraction and d_i is the diameter of a droplet in the i^{th} fraction.

2.7 Determination of β-carotene and lycopene contents

A method of Englberger et al. (2006) was employed for analysing β-carotene and lycopene with some modifications. The emulsion samples were dissolved in a 4:3 solution of ethanol and hexane (35 mL), an internal standard of β-apo-8'-carotenal and an antioxidant (butylated hydroxytoluene [BHT], 0.1% in hexane), then blending the mixture for 5 minutes at 5,000 rpm. The extract was filtered through Whatman No. 1 filter paper on a Buchner funnel. The residue was re-extracted with another 35 mL of ethanol and hexane (4:3) and then washed twice with ethanol (12.5 mL) and once with hexane (12.5 mL). The combined extracts were washed with water, dried by rotary evaporator and then diluted with the mobile

phase solution. All operations were performed under subdued light to minimise oxidation of the carotenoids.

HPLC analysis of β-carotene and lycopene was performed with a Shimadzu HPLC system (Shimadzu Australia, Rydalmere, NSW, Australia) consisting of a Luna C18 (100 x 4.6 mm i.d; 5μm) direct-connect guard column coupled to a Jupiter C18 (250 x 4.6 mm i.d; 5μm) reserved phase column (Phenomenex). The mobile phase consisted of acetonitrile, dichloromethane and methanol (ACN: DCM: MeOH) 50: 40: 10 v/v/v. The flow rate was 1.0 mL/min, the detection was at 450 nm and the injection volume was 20 μL. The identification of β-carotene and lycopene were based solely on the retention time of a peak compared with the authentic standards. The amount of β-carotene and lycopene in the emulsion samples was expressed as μg/g. All measurements were carried out in triplicate. Retention of carotenoid content (%) was calculated as percent of the carotenoid content in the samples before and after storage period.

2.8 Statistical analysis

The experiments were randomly designed with three and four factors, including wall material concentration, ratio of Gac oil to wall material, storage temperature and storage time. The experiments were carried out in triplicate, and results were presented as mean values. Different mean values were analysed by analysis of variance (ANOVA) and least significant difference (LSD) using SPSS software version 20.0.

3. RESULTS AND DISCUSSION

3.1 Stability of the emulsion samples

A three-factor experiment was randomly designed to investigate the effects of the concentration, the ratio and storage time on the stability of the emulsion samples at different temperatures of 4, 22 and 37 °C for up to 30, 20 and 12 d, respectively (Table 1). Statistical results showed that the stability of the samples stored at different temperatures was significantly affected by the concentration, the ratio and the storage time ($P < 0.001$). In addition, the interaction among the factors was found to be significant ($P < 0.001$).

As can be seen in Table 1, the higher stability of the emulsion samples is obtained at the higher concentrations. It can be explained that the high concentration was attributable to the sufficient content of the continuous phase of the wall materials to cover the surfaces of the oil droplets properly, which prevent the coalescence or flocculation of the droplets

(McClements, 2005). The movement of the oil droplet was slowed down sufficiently to prevent the phase separation with increasing the amount of the wall materials. Moreover, Table 1 also showed that a decrease in the ratio of Gac oil to the wall materials resulted in increasing instability of the emulsions. This effect could be explained by the fact that the number of the oil particles in the emulsion matrix is augmented by increasing the oil content due to enhancing emulsion viscosity (refer to section 3.3) and improving resistance to flow (Mirhosseini et al., 2009; Soleimanpour et al., 2013). As a result, the gravitational phase separation is limited.

TABLE 1. Effects of wall material concentrations and ratios of Gac oil to wall material on the stability of the emulsion samples stored at different temperatures during storage

Parameters		**Stability (%)**		
		4°C	**22 °C**	**37 °C**
Concentration of wall material	20%	88.3 ± 18.1[a]	92.6 ± 8.2[a]	93.2 ± 9.1[a]
	30%	91.9 ± 15.9[b]	93.5 ± 8.6[b]	93.4 ± 8.0[a]
	40%	95.8 ± 7.3[c]	96.4 ± 6.0[c]	95.5 ± 6.6[b]
Ratio of Gac oil to wall material	2:1	99.6 ± 1.7[a]	100 .0± 0.0[a]	99.9 ± 0.3[a]
	1:1	99.5 ± 1.9[a]	99.5 ± 1.4[a]	99.3 ± 1.5[a]
	1:2	99.4 ± 2.0[a]	97.5 ± 3.7[b]	98.1 ± 2.6[b]
	1:3	98.6 ± 3.3[a]	96.9 ± 4.0[c]	97.0 ± 4.6[bc]
	1:4	97.2 ± 4.8[b]	96.4 ± 4.2[c]	96.6 ± 4.1[c]
	1:5	96.1 ± 5.4[c]	95.1 ± 4.4[d]	95.5 ± 5.2[c]
	1:6	88.7 ± 7.6[d]	90.4 ± 7.0[e]	88.7 ± 7.1[d]
	1:7	78.7 ± 17.1[e]	86.7 ± 9.5[f]	85.9 ± 9.1[e]
	1:8	70.4 ± 25.6[f]	85.2 ± 10.4[g]	85.3 ± 10.4[e]

*Values are mean ± SD after statistical analyses; *** indicate significant at P = 0.001. The values within the same column followed by different superscripts (a-g) were significantly different (P<0.05). Emulsion samples were stored at 4°C, 22°C and 37°C for 30, 20 and 12 d, respectively.*

In addition, results also indicated that the high stability of emulsions formulated by the ratios of 1:5 up to 2:1 was obtained. The positive effects of an interaction between protein and GA may explain for this observation. According to Mirhosseini et al. (2009) and Dickinson (2003), the complex structure of protein and GA is responsible for the emulsifying properties which highly stabilize the food emulsion. GA containing a high molecular mass

arabinogalactan polysaccharide linked to the protein chain that strongly adsorbs at oil in water interface, therefore the emulsion stability is promoted. This is in agreement with several reports that those protein-polysaccharide interactions, which is readily formed, have been shown to possess excellent stabilizing properties at interfaces (Dickinson, 2011; Schmitt and Kolodziejczyk, 2010).

Results also showed that the stability of emulsions significantly decreased over time at the different investigated temperatures. The higher temperatures and the longer times resulted in the greater unstable emulsions the droplet aggregation may have occurred during storage (McClements, 2007). The reason may be due to the size of the particles changes, which is discussed in the next section. Furthermore, unstable emulsions could be due to a number of physicochemical instability mechanisms including gravitational separation, flocculation, coalescence, and particle coalescence (Dickinson, 1992; McClements, 2005).

3.2 Particle size and particle size distribution of the emulsions

A three-factor experiment was randomly designed to investigate the effects of the concentration, the ratio and storage time on particle size and size distribution of the emulsion samples at different temperatures of 4, 22 and 37 °C for up to 30, 20 and 12 d, respectively (Figure 1). Statistical results showed that the particle size was significantly affected by the three independent variables ($P < 0.001$). In addition, interactions among the variables on the particle size were also found to be significant ($P < 0.001$).

In general, emulsion particle size is an important parameter affecting its physical stability. It is generally accepted that smaller particle size and higher viscosity result in more physically stable emulsion than larger particle size and low viscosity. This is evidenced in this study (refer to sections 3.1 and 3.3). In addition, results (Figure 1) also indicated that increase in the wall material concentration and decrease in the oil/wall ratio resulted in smaller particle size of the emulsions. It has been reported that the smaller particle size could be obtained by the more complete interaction of WP-GA matrix with the oil droplets (Kim et al., 1996). Furthermore, the increase of oil phase concentration resulted in increase in average droplet size due to increase in emulsion viscosity (Mirhosseini et al., 2009).

For the storage of the emulsions, greater particle sizes were significantly observed when the emulsions were stored at higher temperatures and longer times ($P < 0.001$). All the initial emulsions had monomodal particle size distribution (Figure 1) and relative small particle diameter ($d_{4,3}$ = 4 to 24 μm). After storage, the mean particle size increased significantly ($d_{4,3}$ = 7 to 37 μm). An increase in particle size of the emulsions containing the ratios of 1:6, 1:7

and 1:8 during storage was larger than that of the other emulsions, indicating more instability to gravitational separation. This can be explained why the emulsions containing these ratios were unstable (refer to section 3.1). Therefore, it indicates that these ratios are not suitable for the preparation of high stability emulsion.

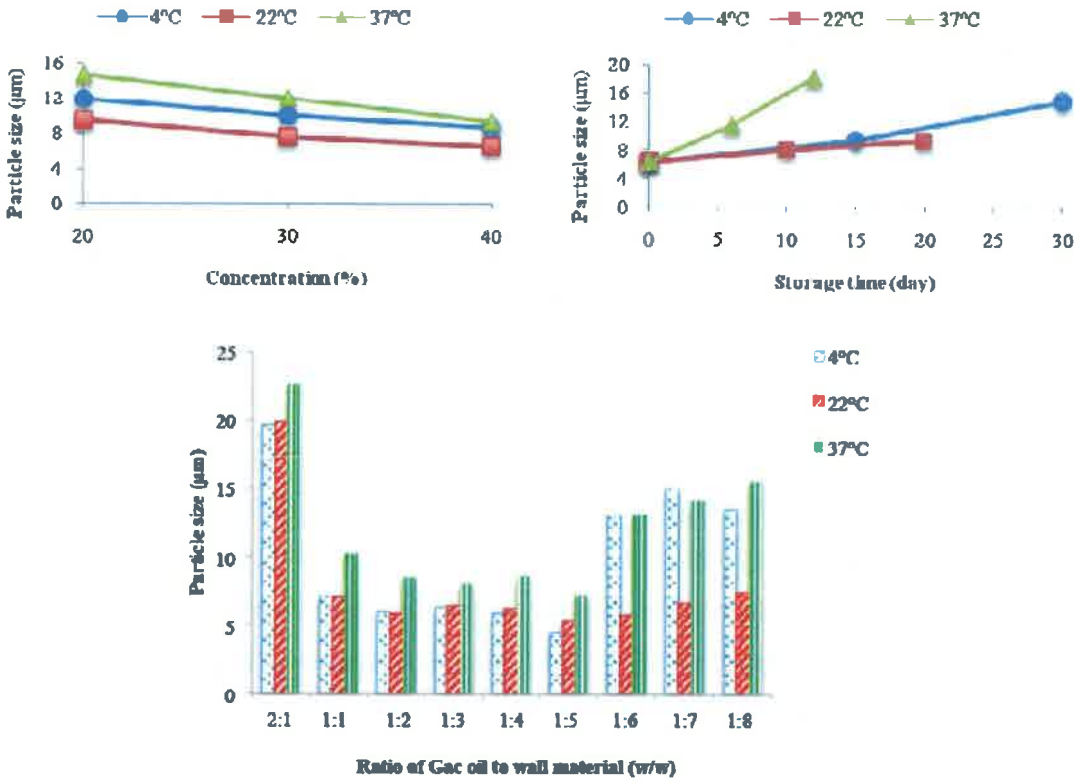
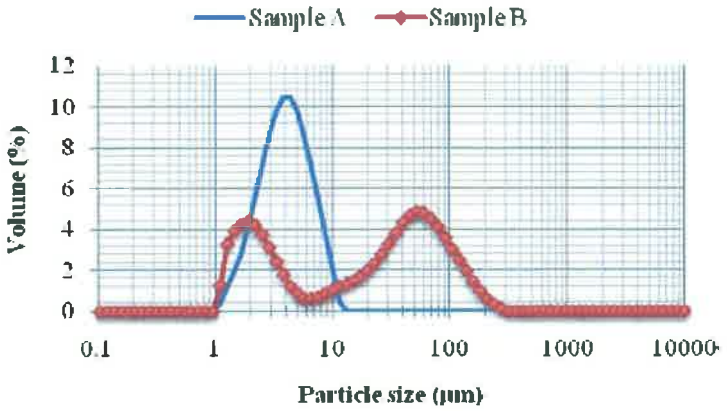


Figure 1. Effects of concentration (a), ratio (b) and storage time (c) on particle size stored at different temperatures

Interestingly, increase in particle size of the emulsions stored at 22 °C was lowest, followed by stored at 4 and 37 °C (Figure 1). This phenomenon could be because the length of storage time was different (refer to section 2.3). In any case, to maintain stability of emulsions, the low storage temperature is highly recommended.



Sample A: prepared with concentration of 30% and the ratio of 1:5 stored at 22°C for 20 d

Sample B: prepared with concentration of 20% and the ratio of 1:8 stored at 37°C for 12 d

Figure 2. A typical profile of particle size distributions of Gac oil emulsions

Moreover, Figure 2 showed that the size distributions were monomodal and bimodal, which is normal distribution of the stable and unstable emulsions, respectively (McClements, 2007). Generally, the oil droplets of the high stable emulsions during storage were small and evenly distributed, especially the emulsions containing higher concentration and ratio stored at lower temperature. The emulsions with two peaks in the particle size distribution (biomodal) indicated less stability. This is evident for explanation of the instability of emulsions in section 3.1. Furthermore, microscopy images (not shown) confirm the stability and instability of the emulsions during storage. Sample B reveals that the oil droplets of emulsion were coalesced whereas sample A shows the oil droplets were small and evenly distributed in emulsion. Results also indicated that the stable emulsions lacked any visual particle aggregation or noticeable presence of non-encapsulated or free oil.

3.3 Changes of pH and viscosity of emulsion

A four-factor experiment was randomly designed to investigate the influence of the concentration, ratio, and storage temperature and time on the viscosity and pH of the emulsions. The mean values of each independent variable (Table 2) were calculated based on calculated mean values of the fixed other variables. Statistical results indicated that the viscosity of the samples was affected by the concentration, the ratio and storage temperature ($P < 0.001$). However, the impact of storage time on the viscosity was found to be insignificant ($P > 0.05$). Interaction among the variables was also found significant except for

interaction among concentration, ratio and time; and among concentration, time and temperature. In addition, the pH value of the samples was not significantly affected by the four investigated factors ($P > 0.05$).

TABLE 2. Effects of wall material concentrations and ratios of Gac oil to wall material on the viscosity and pH of the emulsion samples under storage conditions

Parameters		**Viscosity (cP)**	**pH**
Concentrations of wall material	20%	30.05 ± 16.34^{a}	5.76 ± 0.05
	30%	49.80 ± 48.91^{b}	5.77 ± 0.05
	40%	63.74 ± 56.79^{c}	5.76 ± 0.05
Ratio of Gac oil to wall material	2:1	145.33 ± 64.39^{a}	5.78 ± 0.04
	1:1	86.28 ± 25.73^{b}	5.77 ± 0.05
	1:2	45.17 ± 7.40^{c}	5.77 ± 0.04
	1:3	36.42 ± 8.64^{d}	5.76 ± 0.05
	1:4	29.28 ± 9.27^{e}	5.76 ± 0.05
	1:5	27.17 ± 7.82^{f}	5.77 ± 0.05
	1:6	23.17 ± 5.75^{g}	5.75 ± 0.05
	1:7	20.06 ± 6.09^{h}	5.76 ± 0.05
	1:8	17.89 ± 5.30^{i}	5.75 ± 0.06
Storage temperature	4°C	46.58 ± 45.53^{a}	5.76 ± 0.05
	22°C	49.56 ± 47.08^{b}	5.76 ± 0.05
	37 °C	47.44 ± 46.57^{c}	5.76 ± 0.05
Storage time	Before storage	47.90 ± 46.72^{a}	5.77 ± 0.05
	After storage	47.82 ± 45.96^{a}	5.76 ± 0.05

*Values are mean ± SD after statistical analyses; N.S and *** indicate not significant at P >0.05 and significant at P = 0.001, respectively. The values within the same column followed by different superscripts (a-i) were significantly different (P<0.05). The emulsion samples were stored at 4°C, 22°C and 37°C for 30, 20 and 12 d, respectively.*

Table 2 shows that pH value of the emulsions was not statistically affected by the investigated factors including concentration, ratio, storage temperature and time. This is indicated that at those pH values, the emulsions containing WP have a charge that is enough to prevent the close approach of suspended oil droplets (Dalgleish, 1997). This is proven why the high stable emulsions formulated by different concentrations of 20, 30 and 40%, and ratios of 0.2 up to 2 were obtained (Table 1). Although WP is known to be very sensitive to

pH with a tendency to aggregation, especially at pH values close to their isoelectric point (pI) of 5.2 (Dalgleish, 1997), a mixture of WP and GA may make the emulsions less sensitive to pH (Klein et al., 2010). In contrast, Chuah et al. (2012) reported that high stability of WP-stabilised emulsions was observed at the pH close to the pI even after storing for one week.

In general, viscosity is one of the most important factors affecting the emulsion stability, therefore it is important to measure viscosity of the emulsion before and after storage time periods. According to Stokes' law, destabilization of food emulsions can be retarded by increasing the viscosity of the continuous phase. Table 2 shows that the viscosity of emulsions was significantly increased with increasing wall material concentration and the ratio. These results therefore support the emulsion stability described earlier (Table 1). The increases viscosity at higher concentration may be explained by the presence of high molecular weight of WP and GA, which increases the resistance to the flow. At higher oil contents, furthermore, viscosity increased due to the increase in the packing fraction of oil droplets (Soleimanpour et al., 2013). This result is also in agreement with previous findings (Mirhosseini et al., 2009; Soleimanpour et al., 2013).

In addition, the high stability of initial emulsion before spray drying over a realistic period of time is a prerequisite for a proper encapsulation by spray drying. During the encapsulation process, the emulsion must be stable due to small and relatively uniform droplet sizes, for short-term holding, pumping and drying stages. The stable emulsion with a sufficiently low viscosity to be pumpable and spray-dryable is also one of the most critical parameters (Laine et al., 2011; Rosenberg et al., 1990). Hence, emulsions containing the concentrations of 20 and 30%, and ratios of 1:5 up to 1:3 with the low viscosity and high stability should be considered for encapsulation by spray drying.

3.4 Retention of β-carotene and lycopene in the emulsions

A three-factor experiment was randomly designed to investigate the retention of carotenoids in the emulsion samples under different formulations and storage conditions. Results (Table 3) showed that the concentration of wall material did not significantly affect the retention of β-carotene in the samples stored at 4 °C and 22 °C, and the retention of lycopene stored at 4 °C. However, the retention of carotenoids stored at the other temperatures was significantly affected by the concentration. In addition, the retention of carotenoids in the samples was strongly affected by the ratio ($P < 0.001$). There was no interaction among the concentration and the ratio ($P > 0.05$).

TABLE 3. Effects of wall material concentrations and ratios of Gac oil to wall material on the retention of carotenoids of the emulsion samples stored at different temperatures

Parameters		Retention of β-carotene (%)			Retention of lycopene (%)		
		4 °C	22 °C	37 °C	4 °C	22 °C	37 °C
Concentration of wall material	20%	81.3±11.5	70.7±13.1	65.6±17.2^{a}	81.9±11.1	77.3±11.1^{a}	79.9±14.2^{a}
	30%	81.1±11.5	71.7±12.1	70.5±13.8^{b}	84.4±8.7	78.4±10.1^{a}	73.1±12.9^{b}
	40%	82.3±10.8	71.7±12.1	69.9±13.0^{b}	82.2±8.3	81.5±10.4^{b}	67.9±14.7^{c}
Ratio of Gac oil to wall material	2:1	77.9±9.4^{ac}	70.1±9.1^{a}	64.5±15.5^{ac}	82.1±8.2^{a}	70.9±12.1^{a}	57.2±19.0^{a}
	1:1	77.8±10.1^{ac}	66.4±9.9^{ac}	64.2±15.2^{ac}	80.5±9.0^{ad}	69.8±12.7^{a}	63.2±17.0^{a}
	1:2	75.6±8.9^{ab}	67.4±10.2^{a}	68.2±16.8^{a}	79.5±10.4^{abc}	77.3±9.4^{b}	71.3±12.2^{b}
	1:3	91.7±3.8^{d}	82.7±9.7^{d}	76.9±10.7^{d}	89.3±4.7^{e}	87.3±4.4^{c}	77.7±11.5^{cd}
	1:4	92.1±3.3^{d}	83.2±5.9^{d}	79.2±9.6^{d}	91.4±3.9^{e}	87.0±4.9^{c}	79.1±10.7^{cd}
	1:5	92.7±3.7^{d}	81.7±5.8^{d}	75.9±8.9^{d}	88.9±6.1^{e}	84.6±6.9^{c}	77.9±9.0^{cd}
	1:6	79.5±11.0^{a}	69.1±10.5^{a}	67.5±13.9^{a}	80.6±9.2^{ab}	79.3±9.0^{b}	75.7±11.0^{bcd}
	1:7	74.5±9.8^{cb}	59.6±10.8^{b}	60.2±16.0^{bc}	77.4±8.1^{bcd}	79.1±8.5^{b}	73.7±11.4^{bcd}
	1:8	72.2±9.3^{b}	62.1±9.1^{bc}	61.4±13.6^{bc}	75.7±9.7^{c}	76.3±9.2^{b}	77.8±8.9^{d}

*Values are mean ± SD after statistical analyses; N.S, *, ** and *** indicate not significant and significant at P = 0.05, 0.01 and 0.001. The values within the same column followed by different superscripts (a-d) were significantly different (P<0.05). The emulsion samples were stored at 4°C, 22°C and 37°C for 30, 20 and 12 d, respectively.*

As can be seen in Table 3, generally the wall material concentration has a positive effect on the retention of carotenoids in the emulsions. Concentration of 30% and 40% appear to be suitable for minimising the loss of carotenoids, especially at the lower storage temperatures of 4 and 22°C. This is likely because the carotenoids could be encapsulated in the protein-GA matrix. This is also improved by the greater stability of the emulsions formulated at the higher concentrations (Table 1).

Results also showed that the higher retention of carotenoids was significantly obtained at the ratios of 1:3, 1:4 and 1:5 ratios at different storage temperatures. In contrast, the lower retention of emulsions having the higher or lower carotenoid contents was significantly observed (Table 3). This is likely because there were much free carotenoids on the surface of the matrix, which were not linked in the matrix and easily degraded during the storage conditions due to sensitive to light, oxygen and heat (Che Man and Tan, 2003). The higher amount of free lycopene and β-carotene could be readily oxidised by the available oxygen present in the media (Chen et al., 2009). However, at the lower content, the carotenoids was significantly lost due to instability of the emulsions in which the surface active interface of protein-polysaccharide was not strong enough to incorporate the carotenoids in the matrix.

According to statistical analysis, the retention of β-carotene and lycopene in the samples was strongly affected by storage time ($P < 0.001$). Results indicated that the retention profile of carotenoids as a function of storage time at different storage temperatures. Retentions of carotenoids (β-carotene and lycopene) in emulsions stored at 4 °C/ 30 d, 22 °C/ 20 d and 37 °C/ 12 d were about 70%, 66-73% and 64-58%, respectively. This result is in agreement with several studies which reported negative effects of higher storage temperature and longer time on the carotenoid retention in the emulsion (Tan and Nakajima, 2005; Yuan et al., 2008). In addition, a possible explanation for the degradation of carotenoid is increase in particle size during storage conditions resulting in instability of emulsions, therefore promoting loss of carotenoids due to more contact surface between carotenoids and the aqueous environment (Tan and Nakajima, 2005).

4. CONCLUSION

It can be concluded that the emulsions containing high concentrations of wall materials and high ratios of Gac oil to wall material (1:5 up to 2:1) were found to be significantly more stable than the others. The high retentions of carotenoids in the emulsions prepared with the concentration of 30% and the ratios of 1:3, 1:4, and 1:5 were found to be most significant at the low storage temperatures of 4 and 22 °C for 30 and 20 d, respectively. All those formulations, having excellent stability, low viscosity, small mean particle size and uniform particle size distribution can be considered as most suitable for microencapsulation by spray drying. In addition, influence of pH of emulsions during storage was found to be insignificant.

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2. POSTERS PRESENTD AT CONFERENCES

2.1 **Tuyen C. Kha**, Minh H. Nguyen, Paul D. Roach & Costas E. Stathopoulos (2014). A storage study of encapsulated Gac oil powder. The 47th Annual AIFST convention, 22-25th June, 2014, Melbourne. Poster Presentation. (E3).

A Storage Study of Encapsulated Gac Oil Powder

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Introduction

Gac fruit (*Momordica cochinchinensis* Spreng) (**Fig. 1**) is considered as one of the "super" fruits, containing extraordinarily high levels of β-carotene and lycopene, and a significant amount of polyunsaturated fatty acids. These nutrients have proved to be beneficial to humans(1). Therefore, it is of interest to extract Gac oil rich in these nutrients and process it into a stable and convenient powder form, which could be used as a nutrient supplement and/or as a natural red-orange colourant.

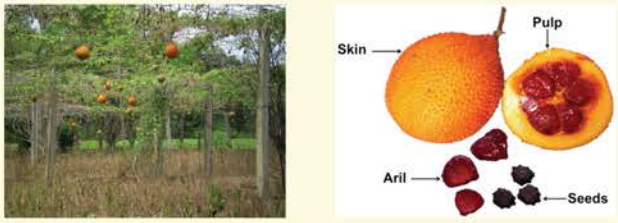

Fig. 1 Gac fruit on vines and its components

Recently, Gac oil has been successfully extracted using microwave drying and hydraulic press(2,3). However the oil is susceptible to isomerisation and oxidation during processing and storage due to the high number of double bonds in the structure of the carotenoids and the polyunsaturated fatty acids. Encapsulation can be a method to protect, stabilise and release the compounds while also enabling their solubility in a aqueous medium. Kha et al.(4,5) successfully encapsulated Gac oil into a matrix of protein and polysaccharide using spray drying. In addition to quality loss through processing, the quality of foods may also change during the periods of storage and distribution. It is desirable to study the effect of storage conditions on the quality of the encapsulated Gac oil powders.

Aims

This study aimed to investigate the stability of the encapsulated Gac oil powder under a variety of storage conditions, in terms of colour, surface oil content, peroxide value (PV), β-carotene and lycopene contents. Kinetic parameters and moisture sorption isotherms were examined for predicting the shelf life of the encapsulated powder.

Materials and methods

A production process of Gac oil and an encapsulated Gac oil powder is presented in Fig. 2.

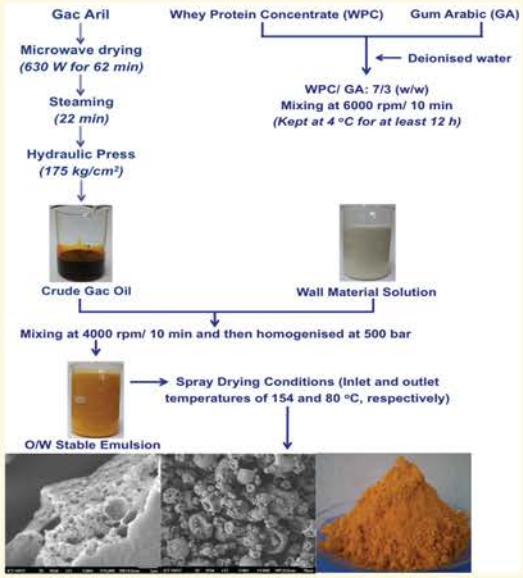

Fig. 2. A production process of Gac oil and encapsulated Gac oil powder

The encapsulated Gac oil powders were packed into high barrier vacuum pouches and stored at different storage conditions:

- ✓ **Temperatures of -20 °C, 10 °C for 360 days in absence of air and light.**
- ✓ **Room temperature (RT, 25 - 30 °C) for 360 days in presence or absence of air and light.**
- ✓ **Temperature of 40 °C for 120 days in absence of air and light.**
- ✓ **Temperature of 63 °C (Schaal Oven test) for 28 days in absence of air and light.**

Duplicate samples were periodically withdrawn during the storage in order to measure colour, surface oil content, peroxide value (PV), and carotenoid (β-carotene and lycopene).

Results

Statistical results indicated that the investigated factors significantly impacted the surface oil content and the PV ($P < 0.001$). As anticipated, higher storage temperature and longer time resulted in an increase in total colour difference (ΔE) (Fig. 3), the surface oil (Fig. 4) and the PV (Fig. 5). Similarly, the surface oil and the PV during storage also increased when the samples were in presence of air and light ($P < 0.001$).

Degradation kinetics of β-carotene and lycopene contents in the encapsulated Gac oil powder under different storage were shown in Table 1. The degradation rate of β-carotene and lycopene in the powders was best fitted by a first-order reaction.

Fig. 6 The isotherm curves of the encapsulated Gac oil powder samples stored at 10, 30 and 40 °C are typically sigmoidal in shape and indicate EMC values at a constant temperature increased with increase in ERH.

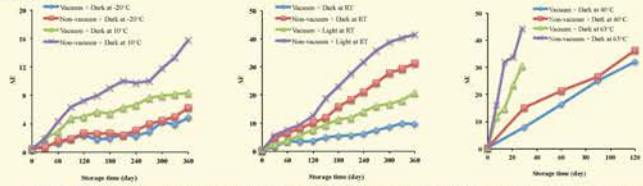

Fig. 3. Effects of storage conditions on total colour difference (ΔE) of encapsulated Gac oil powder

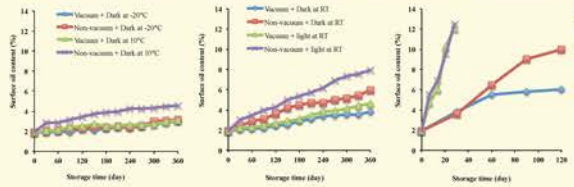

Fig. 4. Effects of storage conditions on the surface oil content of encapsulated Gac oil powder

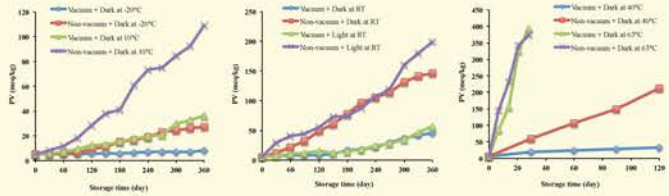

Fig. 5. Effects of storage conditions on PV of encapsulated Gac oil powder

Table 1. Degradation kinetics of β-carotene and lycopene in the encapsulated Gac oil powder

Storage conditions	β-Carotene				Lycopene			
	k (day^{-1})	Half life (day)	First-order degradation	r^2	k (day^{-1})	Half life (day)	First-order degradation	r^2
Vacuum + dark at -20°C	0.0002	3466	y = -0.0002t + 0.0029	0.894	0.0004	1733	y = -0.0004t + 0.0097	0.971
Non-vacuum + dark -20°C	0.0003	2310	y = -0.0003t + 0.0124	0.923	0.0004	1733	y = -0.0004t + 0.0075	0.948
Vacuum + dark at 10°C	0.0004	1733	y = -0.0004t + 0.0219	0.952	0.0004	1733	y = -0.0004t - 0.0107	0.977
Non-vacuum + dark at 10°C	0.0009	770	y = -0.0009t + 0.0374	0.964	0.0006	1155	y = -0.0006t - 0.0157	0.973
Vacuum + dark at RT	0.0005	1386	y = -0.0007t - 0.0535	0.989	0.0011	630	y = -0.0011t + 0.087	0.852
Non-vacuum + dark at RT	0.0013	533	y = -0.0013t - 0.0545	0.969	0.002	347	y =-0.002t + 0.0978	0.966
Vacuum + light at RT	0.0009	770	y = -0.0009t - 0.043	0.912	0.0012	578	y =-0.0012t + 0.1001	0.819
Non-vacuum + light at RT	0.0015	462	y= -0.0015t - 0.0437	0.991	0.0022	315	y = 0.0022t + 0.0686	0.912
Vacuum + dark at 40°C	0.0038	182	y = -0.0038t - 0.035	0.989	0.0048	144	y = -0.0048t + 0.0334	0.981
Non-vacuum + dark at 40°C	0.0063	110	y = -0.0063t + 0.0099	0.932	0.0077	90	y = -0.0077t + 0.0808	0.951
Vacuum + dark at 63°C	0.0662	10	y = -0.0662t + 0.3119	0.887	0.0748	9	y = -0.0748t + 0.462	0.771
Non-vacuum + dark at 63°C	0.1018	7	y = -0.1018t + 0.5168	0.858	0.1265	5	y = 0.1265t + 0.6537	0.845

$y = \ln(C/C_0)$; C: amount of β-carotene or lycopene at storage time t; C_0: initial amount of β-carotene or lycopene; k: apparent degradation rate constant.

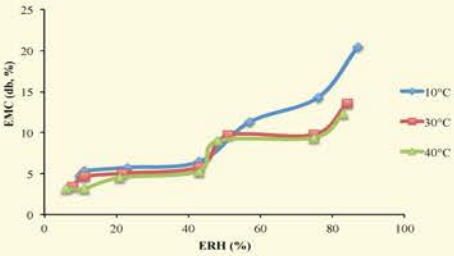

Fig. 5. Sorption isotherm curves of the encapsulated Gac oil powder samples at different temperatures (*EMC: equilibrium moisture content; ERH: equilibrium relative humidity*).

Conclusions

The encapsulated Gac oil powder could be stored at low temperature for at least 1 year in absence of light and air and in high barrier packaging. The oil powder containing high levels of β-carotene and lycopene, and fatty acids could be used for natural colourant, food additive or nutrient supplement.

Acknowledgments

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2.2 **Tuyen C. Kha**, Minh H. Nguyen, Paul D. Roach & Costas E. Stathopoulos (2014). Gac oil extraction and microencapsulation by spray drying. The 2nd International Conference on Food and Applied BioScience, February 6 - 7, 2014, Chiang Mai Province, Thailand. Abstract and Oral presentation. (pp 75). (E3).

GAC OIL EXTRACTION AND MICROENCAPSULATION BY SPRAY DRYING

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Introduction

Gac (*Momordica cochinchinensis* Spreng) (Fig. 1) is considered a 'super fruit' due to its extraordinarily high levels of β-carotene and lycopene and its significant amounts of unsaturated fatty acids and vitamin E[1,2] in its aril - nutrients which are beneficial to human health[3]. Therefore, it is of interest to extract Gac oil rich in these nutrients and process it into a stable and convenient powder form, which could be used as a nutrient supplement and/or as a natural red-orange colourant. Gac oil is susceptible to isomerisation and oxidation during processing and storage due to the high number of double bonds in the structure of the carotenoids and the polyunsaturated fatty acids. Encapsulation can be a method to protect, stabilise and release the compounds while also enabling their solubility in a aqueous medium. However, there is limited information on the extraction and processing of oil from the Gac aril, which is a relatively new area of endeavor.

Aims

The aims of this study were to optimise the conditions in terms of the extraction efficiency (EE) for oil, β-carotene and lycopene from Gac arils and to maximise their retention during the microencapsulation of the oil by spray drying in terms of encapsulation efficiency.

Methods

The Gac Oil Extraction: methodology is presented in Fig. 2. The oil was then microencapsulted by spray drying as illustrated in Fig. 3. The spray-drying conditions were optimised using the Response Surface Methodology (RSM) for the following parameters:

- Gac oil emulsions, wall material concentration (WMC) and oil load[6]
- Spray drying conditions (inlet and outlet temperatures)[7]

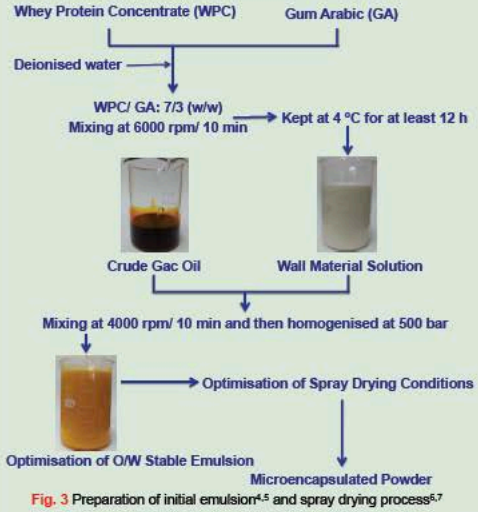

Fig. 3 Preparation of initial emulsion[4,5] and spray drying process[6,7]

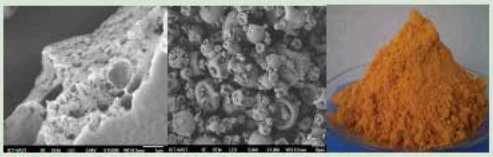

Fig. 4 Gac oil powder (right) microencapsulated using the optimum spray-drying conditions and its outer (middle) and inner (left) microstructures as seen under the Scanning Electron Microscopy.

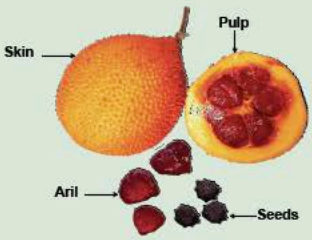

Fig. 1 Gac Fruit and its Components

Fig. 2 Gac Oil Extraction [4,5]

Results

Gac Oil Extraction

The optimum extraction conditions for the oil extraction methodology (Fig. 2) were: microwave time of 62 min, steaming time of 22 min and hydraulic pressure of 175 kg/cm². Under such parameters, the maximum EE for the oil was 85.3% and the β-carotene and lycopene content of the oil was 192 and 527 mg/100mL oil, respectively [4,5].

Microencapsulation by spray drying

The optimum formulation for the optimal preparation of the Gac oil (O) and wall material (WMC) emulsion using whey protein concentrate (WPC) and gum Arabic (GA) for spray drying (Fig. 3) was found to be a WMC of 29.5% and a oil load of 0.2 g/g. For the spray-drying, the optimum conditions were found to be inlet and outlet temperatures of 154 and 80 °C, respectively.

The RSM results indicated that the quadratic polynomial models were sufficient to describe and predict the investigated responses (encapsulation efficiencies, peroxide value, moisture content and water solubility index). The optimal encapsulation efficiencies of the oil, the β-carotene, lycopene and moisture content, the water solubility index and the peroxide value for the Gac oil powder were predicted and validated as 87.22%, 82.76%, 84.29%, 4.90%, 90.29% and 4.06 meq/kg, respectively.

The other physicochemical properties of the powder including water activity (Aw = 0.30), pH (5.87), density (0.27g/ml), fatty acid composition (49.18% oleic and 17.65% linoleic) and the yellow-orange colour (Fig. 4) were stable for at least 12 months.

Under Scanning Electron Microscopy (Fig. 4), the powder particles were micro-sized (< 20μm) and had spherical shapes, which should effectively protect the encapsulated Gac oil.

Conclusions

In conclusion, a microencapsulated Gac oil powder, containing a high content of β-carotene and lycopene, was effectively produced using microwave drying, steaming and the hydraulic press for extraction of the oil from Gac arils and spray drying with whey protein concentrate and gum Arabic for the microencapsulation of the oil.

The resultant Gac oil powder could easily be incorporated into various foods for consumers to benefit from its nutrients or for food manufacturers to use for its attractive yellow-orange/red colour.

Acknowledgments

The authors acknowledge the University of Newcastle, Australia for the financial support through a PhD scholarship for Tuyen Kha.

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For more information: http://gacfruit.weebly.com

2.3 **Tuyen C. Kha**, Minh H. Nguyen, Paul D. Roach & Costas E. Stathopoulos (2013). Optimisation of encapsulation by spray drying Gac oil using whey protein concentrate and gum Arabic. The 46th Annual AIFST convention, 14-16th July, 2013, Brisbane. Oral and Poster Presentations. (pp 43). (E3).

Optimisation of Encapsulation by Spray Drying for Gac Oil using Whey Protein and Gum Arabic

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Introduction

Gac (*Momordica cochinchinensis* Spreng) oil is rich in nutrients including carotenoids (β-carotene and lycopene), unsaturated fatty acids and vitamin E. Several studies showed that fat ingested with carotenoid compounds in plant food significantly improved their absorption by the body.[1] Recently, we have reported on the use of microwave-assisted extraction to improve on the conventional pressing of Gac aril for oil. [2,3]

Gac oil is susceptible to isomerisation and oxidation during processing and storage due to the high number of conjugated double bonds in the structure of carotenoids and fatty acids. Encapsulation can be a method to protect, stabilise and released the compounds while also enabling their solubility in a aqueous medium. Spray drying encapsulation is the most widely used in the food industry due to potentially offering many benefits such as economy, flexibility and good quality of encapsulated products.

Response surface methodology (RSM) is one of the most effective methods to evaluate the relationships between the response and the independent variables and then optimise the process.

The study aimed to optimise wall material concentration (WMC) and oil load to obtain high encapsulation efficiencies and to minimise peroxide value (PV), moisture content (MC) and total colour difference (ΔE). Furthermore, encapsulation yield, physicochemical properties, colour characteristics and morphology of the encapsulated oil powder were also evaluated.

Materials and methods

Preparation of the O/W emulsions and spray drying conditions are shown in Fig. 1.

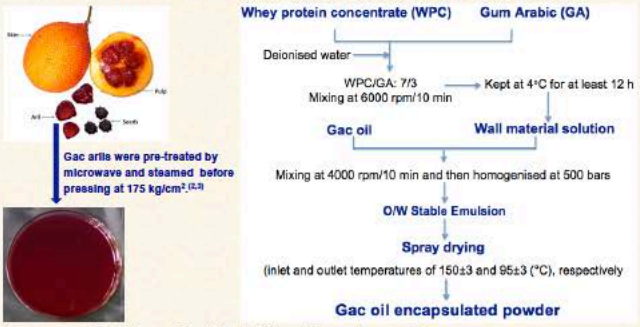

Fig. 1 Preparation of the initial emulsion and spray drying process

- **Encapsulation efficiencies (EEs, %)**: determined based on the retention of Gac oil, β-carotene and lycopene contents in the encapsulated powder.
- **The total oil content of the encapsulated oil powder**: using Soxhlet method.
- **Surface oil content**: the powder was dissolved in n-hexane for 10 min without microcapsule destruction. The solvent was decanted and the residue was dried in a vacuum oven at 70 °C until constant weight.
- **The content of β-carotene and lycopene of samples**: was measured at 450 nm using an Agilent 1200 HPLC.
- **Encapsulation yield (EY, %)**: ratio of the weight of the resultant powder after spray drying and the weight of all solids in the initial emulsion.
- **Peroxide value (PV, meq/kg)**: AOCS Cd 8-53.
- **Moisture content (MC, %)**: air-dried at 105 °C until constant weight.
- **Bulk density (g/mL)**: ratio of mass of the powder and the volume occupied in the cylinder.
- **pH value of samples**: using a pH meter.
- **Water solubility index (WSI, %)**: the percentage of dried supernatant in the solution to the amount of the original powder.
- **Colour characteristics**: used Hunter values of L*a*b* and converted to Chroma and Hue angle.
- **Particle morphology**: using a JSM-7401F scanning electron microscope (SEM).

Results

Results showed that the data were adequately fitted into six second-order polynomial models for EEs (Gac oil, β-carotene and lycopene), PV, MC and ΔE with R^2 values of 0.96, 0.95, 0.86, 0.89, 0.88 and 0.87, respectively (Table 1).

The response surface plot shows relationship between the independent variables and the responses, whilst the contour plot helps to visualise the shape of a response surface (Figs 2 and 3). Therefore, it is useful to use the plots to evaluate the fits of model.

The suitability of the quadratic model for predicting the optimum responses was practically validated under the optimum conditions using RSM (Table 2).

Table 3 and Fig. 4 show the properties of the optimum encapsulated Gac oil powder.

Table 1. Regression coefficients of the fitted quadratic equation and t ratio for the encapsulation efficiencies and physicochemical properties

	EE_{Oil} (%)		$EE_{\beta carotene}$ (%)		$EE_{Lycopene}$ (%)		PV (meq/kg)		MC (%)		ΔE	
Regression coefficient	Regression coefficient	t ratio	Regression coefficient	t ratio	Regression coefficient	t ratio	Regression coefficient	t ratio	Regression coefficient	t ratio	Regression coefficient	t ratio
a_0	72.26		76.4		75.59		3.90		4.07		15.63	
Linear												
a_1	1.04	1.14	-6.12**	-7.30	4.38*	3.14	-0.26	-2.17	-0.21*	-4.17	1.83*	3.20
a_2	-6.27**	-6.87	-2.91*	-3.47	-0.99	-0.71	0.16	1.31	0.06	1.11	-0.48	-0.83
Quadratic												
a_{11}	9.17**	8.44	0.18	0.18	-7.18**	-4.34	0.63**	4.36	0.19*	3.20	-2.61*	-3.83
a_{22}	6.57**	6.05	-0.62	-0.62	-1.18	-0.71	0.41*	2.84	-0.01	-0.22	-1.47	-2.15
Interaction												
a_{12}	1.64	1.27	-7.41*	-6.25	-0.49	-0.25	0.58*	3.36	-0.20*	-2.70	1.99	2.46
R^2	0.96		0.95		0.86		0.89		0.88		0.87	
P-value of lack of fit	0.18		0.20		0.08		0.20		0.30		0.07	

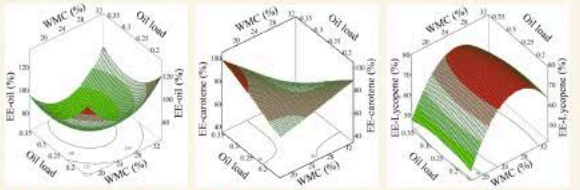

Fig. 2 The 3D response and 2D contour plots of the EEs affected by wall material concentration (WMC) and oil load

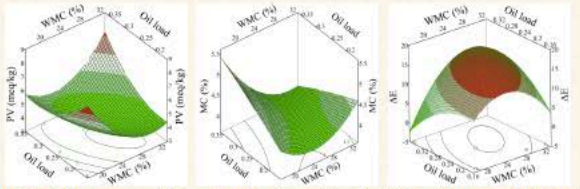

Fig. 3 The 3D response and 2D contour plots of peroxide value (PV), moisture content (MC) and total colour difference (ΔE) affected by wall material concentration (WMC) and oil load

Table 2. Optimum conditions of the wall material concentration of 29.5% and the oil load of 0.2

EE-oil (%)		EE-β-carotene (%)		EE-lycopene (%)		PV (meq/kg)		MC (%)		ΔE	
Predicted	Measured	Predicted	Measured	Predicted	Measured	Predicted	Measured	Predicted	Measured	Predicted	Measured
92.0±3.9	93.3±1.4	80.0±3.1	77.8±3.2	74.0±3.4	71.7±2.9	3.9±0.1	3.4±0.3	4.14±0.2	4.4±0.4	12.4±0.6	11.3±1.2

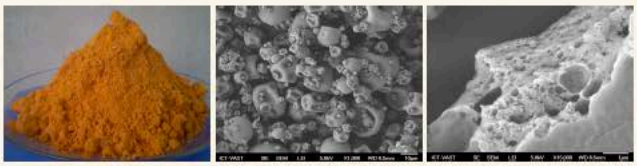

Fig. 4 The optimised encapsulated oil powder (left) and microstructures: outer (middle) and inner (right)

Table 3. Colour characteristics, physical properties and encapsulation yield (EY) of the optimal encapsulated powders under the optimum formulation conditions

Colour characteristics			Physicochemical properties				EY (%)
Lightness	Chroma	Hue angle	Aw	Density	pH	WSI (%)	
81.3±1.0	48.4±2.7	71.6±0.3	0.3±0.1	0.4±0.1	5.7±0.1	92.6±2.2	47.1±2.0

Conclusions

- ✓ The quadratic polynomial model was sufficient to describe and predict the investigated responses in the formulation process by using RSM.
- ✓ The graphical optimisation was employed to predict the optimum formulation conditions within the experimental ranges including the WMC of 29.5% and the oil load of 0.2.
- ✓ It can be concluded that Gac oil containing β-carotene and lycopene was successfully encapsulated in the protein-polysaccharide matrix.
- ✓ The resultant Gac oil powder could then be easily incorporated into various foods for the consumers to benefit from the nutrients and enjoy the attractive red-yellow colour.

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Acknowledgments

The authors acknowledge the University of Newcastle, Australia for the financial support through a PhD scholarship for Tuyen Kha.

2.4 **Tuyen C. Kha**, Minh H. Nguyen (2013). Gac fruit: Bioactive compounds, Processing and Utilizations. International Conference on Food and Biosystems Engineering FaBE 2013 – May 30 to June 02, 2013, Skiathos Island, Greece, Technological Educational Institute of Thessaly, Vol. 2, page 39, ISBN 978-960-9510-11-0. (E3).

GAC FRUIT: BIOACTIVE COMPOUNDS, OPTIONS FOR PROCESSING, AND UTILISATION OF PROCESSED PRODUCTS

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Introduction

Gac fruits (*Momordica cochinchinensis* Spreng) are rich in nutrients including carotenoids, fatty acids, vitamin E, polyphenol compounds and flavonoids. Medicinal compounds are also found in the seeds. The plant has the potential to be a high value crop particularly as parts of the fruit can be processed into nutrient supplements and/or natural red and orange colourants. However, the plant remains underutilised. There is limited information on its requirements in production, and the processing of health products from the fruits is a relatively new area of endeavour. This review will focus on production of Gac fruit, fruit nutrient and phytochemical composition, and the use of Gac products as nutrient supplements and natural food colorants. A potential processing scheme for Gac fruit is proposed to help facilitate greater use of this fruit.

Gac fruit

Gac fruit, *Momordica cochinchinensis* Spreng, is botanically classified as Family *Cucurbitaceae*, Genus *Momordica*, and Species *Cochinchinensis*. It is also known as baby jackfruit, sweet gourd or cochinchin gourd in English. The fruit is one of the traditional fruits in Southeast Asia in general, and in Vietnam in particular. The Gac plant can be cultivated from seeds or root tubers, and grows as dioecious vines that are separate male and female plants (Fig. 1). The Gac fruit is typically round or ovoid in shape, with the exterior skin covered in short spines. Its green skin colour becomes red or dark orange when ripe. Gac fruit (Fig. 2) comprises orange/yellow skin containing spines, yellow pulp and aril (red flesh surrounding the seeds). The fruit components are also presented in Table 1.

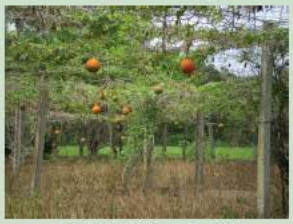

Fig. 1 Gac fruit on vines[10]

Fig. 2 Gac fruit and its components[10]

Table 1 Weight distribution of fresh Gac fruit (10 fruits)[10]

Gac fruit	Fresh weight (g)	Percentage (%)
Whole fruit	7527±475	100
Skin	1332±87	18
Yellow pulp	3700±195	49
Aril	1375±147	18
Seeds	1084±52	14

Bioactive compounds

Gac fruit is considered as one of the "super" fruits, containing extraordinarily high levels of β-carotene and lycopene, and a significant amount of polyunsaturated fatty acids. Moreover, vitamin E concentration is also comparatively high. Vitamin E, as a natural antioxidant, helps protect Gac oil from oxidation. Other bioactive compounds including polyphenol and flavonoids are also present in Gac fruit. Those nutrients (Tables 2 and 3) have proved to be beneficial to humans.

Table 2. Bioactive compounds of fresh Gac fruit (mg/100g)

Bioactive compounds	Skin	Pulp	Aril
β-carotene	38.4 - 141.6[1]	24.0 - 43.2[1] 2.2[2]	160.0[1] 63.6 - 83.6[3]
Lycopene	38.4 - 81.6[1]	14.4 - 49.6[1] 0.1[2]	154.6 - 305.4[3] 140.0[1]
Lutein	189.6 - 1248[1]	16.0 - 144.8[1]	na
Zeaxanthin	na	0.2[2]	0.9[2]
β-cryptoxanthin	na	0.4[2]	0.2[2]
Vitamin E	na	na	7.6[4]
Polyphenolics	45.6 - 67.2[1]	12.2 - 21.0[1]	85.8[1]
Flavonoids	43.0 - 67.2[1]	12.6 - 15.0[1]	41.8[1]

Table 3. Fatty acid composition and total oil content of Gac aril and seeds[3,4]

Fatty acids	Abbreviation	% of total fatty acids	
		Seeds	Aril
Myristic	14:0	na	0.87
Palmitic	16:0	5.6	22.04
Palmitoleic	16: 1 Δ^9	0.1	0.26
Stearic	18:0	60.5	7.06
Oleic	18:1 Δ^9	9.0	34.08
cis-vaccenic	18:1 Δ^{11}	0.5	1.13
Linoleic	18:2 $\Delta^{9,12}$	20.3	31.43
α-linolenic	18:3 $\Delta^{9,12,15}$	0.5	2.14
Arachidic	20:0	1.6	0.39
Gadoleic	20:1 Δ^9	na	0.15
Arachidonic	20:4	1.1	0.10
Behenic	22:0	3.0	0.19
Lignoceric	24:0	na	0.14

Processing of Gac fruit[5]

If the fruit was to be used for all the applications indicated above and more then appropriate processing would be needed. However, little information is available on how the Gac fruit might be processed to make full use of its components and maintain its quality characteristics. It is envisaged that Gac fruit can be processed in several ways (Fig. 3) including drying, extraction of oil, encapsulation and incorporation into foods such as pasteurised juice and milk beverages, glutinous rice, yoghurt, pasta and sauces.

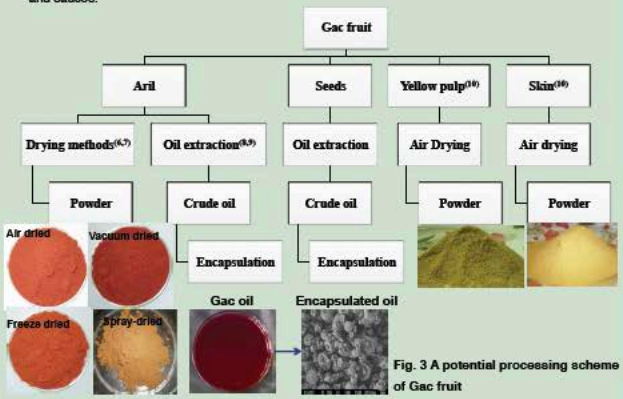

Fig. 3 A potential processing scheme of Gac fruit

Conclusions

- ✓ The processing scheme of all the parts of Gac fruit including drying, oil extraction and oil encapsulation is proposed.
- ✓ The proposed processing scheme should generate products with potential to be utilised in a wide range of foods.
- ✓ The utilisation of air-dried powder from the pulp and skins prevents environmental pollution from waste disposal problem and enhances the overall value of Gac fruit.

Acknowledgments

The authors acknowledge the University of Newcastle, Australia for the financial support through a PhD grant for Tuyen Kha.

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2.5 **Tuyen C. Kha**, Minh H. Nguyen, Constantinos Stathopoulos & Paul D. Roach (2012). Optimisation of pretreatments prior to hydraulic pressing of Gac aril oil using response surface methodology. The 45th Annual AIFST convention, 15-18th July, 2012, Adelaide. (E3).

Optimisation of pretreatments prior to hydraulic pressing of Gac aril oil using response surface methodology

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Introduction

Gac fruit (*Momordica cochinchinensis* Spreng) is considered as one of the "super" fruits, containing extraordinarily high levels of β-carotene and lycopene, and a significant amount of polyunsaturated fatty acids. Moreover, α-tocopherol (vitamin E) concentration is also comparatively high. Vitamin E, as a natural antioxidant, helps protect Gac oil from oxidation. These nutrients have proved to be beneficial to humans.[1-3]

Figure 1. Gac fruit and its components

Recently, traditional extraction of plant oil (using harmful organic solvents) has been discarded due to health concerns, environmental problems and quality degradation. It is important therefore to find a suitable alternative extraction method of Gac oil using food grade solvents or eliminating the use of solvents completely. Microwave-assisted extraction prior to hydraulic pressing, a novel alternative method for oil extraction offers several benefits: it is environmental friendly, solvent free, and allows for reduced processing times and for uniform heating.

Response surface methodology (RSM) is the most popular optimisation technique used in recent years. It is effective in determining the relationships between the response and the independent variables and optimising the processes or products. The technique also allows the evaluation of the effect of multiple parameters and their interactions on the output variables with reduced number of trials.

This study aims to optimise Gac oil extraction conditions, including microwave drying time (X_1), steaming time (X_2) and working hydraulic pressure (X_3), for maximising extraction efficiency (EE), β-carotene and lycopene, using response surface methodology.

Materials and methods

Fresh Gac fruits, of uniform yellow-red skin and size, were purchased from a local market in Ho Chi Minh City, Vietnam. The red arils containing seeds were then scooped out and frozen at temperature of -18°C until use.

Microwave drying treatment: Frozen Gac aril was thawed at 4°C prior to microwave drying treatments. About 900 g of the aril including seeds were spread into the turntable plate with a thickness of 5 mm. The samples were then dried at the microwave power of 630W for different times (Table 1).

Steaming treatment: The microwave dried Gac samples were powdered using a laboratory blender. The ground samples were placed into a stainless-steel tray with a thickness of about 0.5 mm and then placed inside a stainless-steel steam cooker. The samples were steamed under atmosphere pressure for different times (Table 1).

Hydraulic pressing: For each test, the samples after steaming were wrapped inside 4 layers of filtration cloths and pressed at different designed pressures (Table 1).

Analysis: - the EE (%) was determined as ratio of mass of extracted oil after subtracting moisture (AOCS Ca 2c-25, 1998) and mass of oil in starting material using Soxhlet extraction.

- β-carotene and lycopene contents in the oil samples were measure at 450 nm using an Agilent 1200 HPLC.

Table 1. The coded and uncoded levels of independent variables

Coded - variable levels	Exposure time X_1, minutes	Steaming time X_2, minutes	Hydraulic pressure X_3, kg/cm^2
+1.682	66.7	35.1	186.8
+1	65.0	30.0	180.0
0	62.5	22.5	170.0
-1	60.0	15.0	160.0
-1.682	58.3	9.9	153.2

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Results

Results showed that the data were adequately fitted into three second-order polynomial models for EE, β-carotene and lycopene with R^2 values of 0.93, 0.85 and 0.86, respectively (Table 2).

The response surface plot shows relationship between the independent variables and the responses, whilst the contour plot helps to visualise the shape of a response surface (Figures 2, 3 and 4). Therefore, it is useful to use the plots to evaluate the fits of model.

Table 2. Regression coefficients of the fitted quadratic equation and standard errors for EE, β-carotene and lycopene

Regression coefficient*	EE (%)		β-carotene (mg/100 mL)		Lycopene (mg/100 mL)	
	Regression coefficients	t ratio	Regression coefficients	t ratio	Regression coefficients	t ratio
a_0	87.832		171.163		496.146	
Linear						
a_1	2.280*	2.52	-5.210	-0.77	-15.778	-0.93
a_2	-4.113**	-4.55	-0.548	-0.08	-32.149	-1.89
a_3	-1.923	-2.13	31.453***	4.65	49.664*	2.92
Quadratic						
a_{11}	-0.656	-0.74	-22.203**	-3.37	-74.944**	-4.52
a_{22}	-2.169*	-2.46	-28.238**	-4.29	-92.011***	-5.55
a_{33}	-4.566***	-5.19	-5.120	-0.78	-15.617	-0.94
Interaction						
a_{12}	7.026***	5.95	-9.283	-1.05	-25.165	-1.13
a_{13}	-5.426***	-4.59	-10.614	-1.20	1.040	0.05
a_{23}	3.904**	3.30	23.454*	2.65	21.912	0.98
R^2	0.93		0.85		0.86	
P-value of lack of fit	0.174		0.08		0.43	

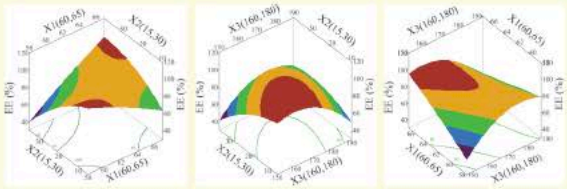


Figure 2. The 3D response surface and 2D contour plots of the EE affected by microwave time (X_1), steaming time (X_2) and working hydraulic pressure (X_3)

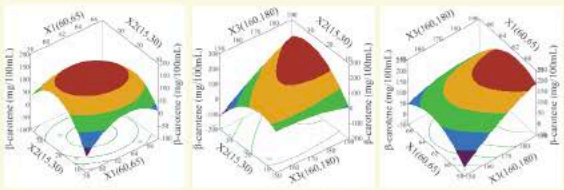


Figure 3. The 3D response surface and 2D contour plots of β-carotene content affected by microwave time (X_1), steaming time (X_2) and working hydraulic pressure (X_3).

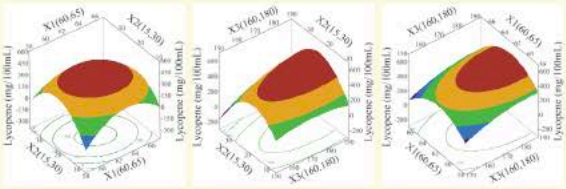


Figure 4. The 3D response surface and 2D contour plots of lycopene content affected by microwave time (X_1), steaming time (X_2) and working hydraulic pressure (X_3).

Conclusions

It was predicted that the optimum extraction conditions within the experimental ranges would be the microwave time of 62 minutes, steaming time of 22 minutes and hydraulic pressure of 175 kg/cm². Under such parameters, the maximum EE of 85.27%, β-carotene content of 191.71mg/100mL oil and lycopene content of 527mg/100mL oil could be achieved.

It can be concluded the response surface optimisation for the aril processing conditions for Gac oil is useful and has been successfully applied.

Acknowledgments

The authors acknowledge the University of Newcastle, Australia for the financial support through a PhD grant for Tuyen Kha. The authors would also like to thank the Faculty of Food Science and Technology, Nong Lam University for their equipment support.

3. GAC FRUIT RESEARCH WEBSITE

Home page of the website about Gac fruit research: http://gacfruit.weebly.com/

Gac Research

University of Newcastle, Australia

HOME | RESEARCH TEAM | RESEARCH ACTIVITY | PUBLICATIONS | CONTACT US

Fig. 1 Gac fruit on vines in rural garden in Viet Nam

Fig. 2 Gac fruit on vines growing in green house with hydroponic technique (IDP, Ourimbah, Australia)

Welcome to our web site **"Gac research"**, the University of Newcastle, Australia.

Ourimbah Gac research is a team of Professional staffs and PhD students in the University of Newcastle, Australia and NSW Department of Primary Industries (DPI), Australia.

Gac fruit (*Momordica cochinchinensis* Spreng.) is now growing of interest. English names for the fruit include baby jackfruit, sweet gourd, and cochinchin gourd. Gac fruit is rich in nutrients including carotenoids (especially beta-carotene and lycopene), polyunsaturated fatty acids, vitamin E, polyphenol compounds, and flavonoids. Furthermore, medicinal compounds are also present in Gac seeds. This fruit has potential to be a high-value crop due to high nutrients. Particularly, all parts of the fruit can be processed into nutrient supplements and/or natural yellow, orange and red colorant.

Since the fruit remains underutilized, this website was created to help facilitate greater use of this fruit. We greatly appreciate if there are any contributions and/or questions relating to our Gac research. Your feedback is important to us in preparing future information in this website.

Gac Research Team

School of Environmental and Life Sciences
University of Newcastle, Australia

4. ACHIEVEMENTS AND AWARDS

4.1 The Best Poster Presentation Award granted by the Australian Institute of Food Science and Technology at the 47th Annual AIFST Convention in Melbourne, 22 - 25th June, 2014.

the australian institute of
food science and technology
incorporated
ABN 24 874 852 254

Mr Tuyen Kha Chan
Student
University of Newcastle
Unit 1, 31 Dianella Street
Ourimbah NSW 2258

1st July 2014

Dear Tuyen,

RE:- AIFST Poster Competition 2014

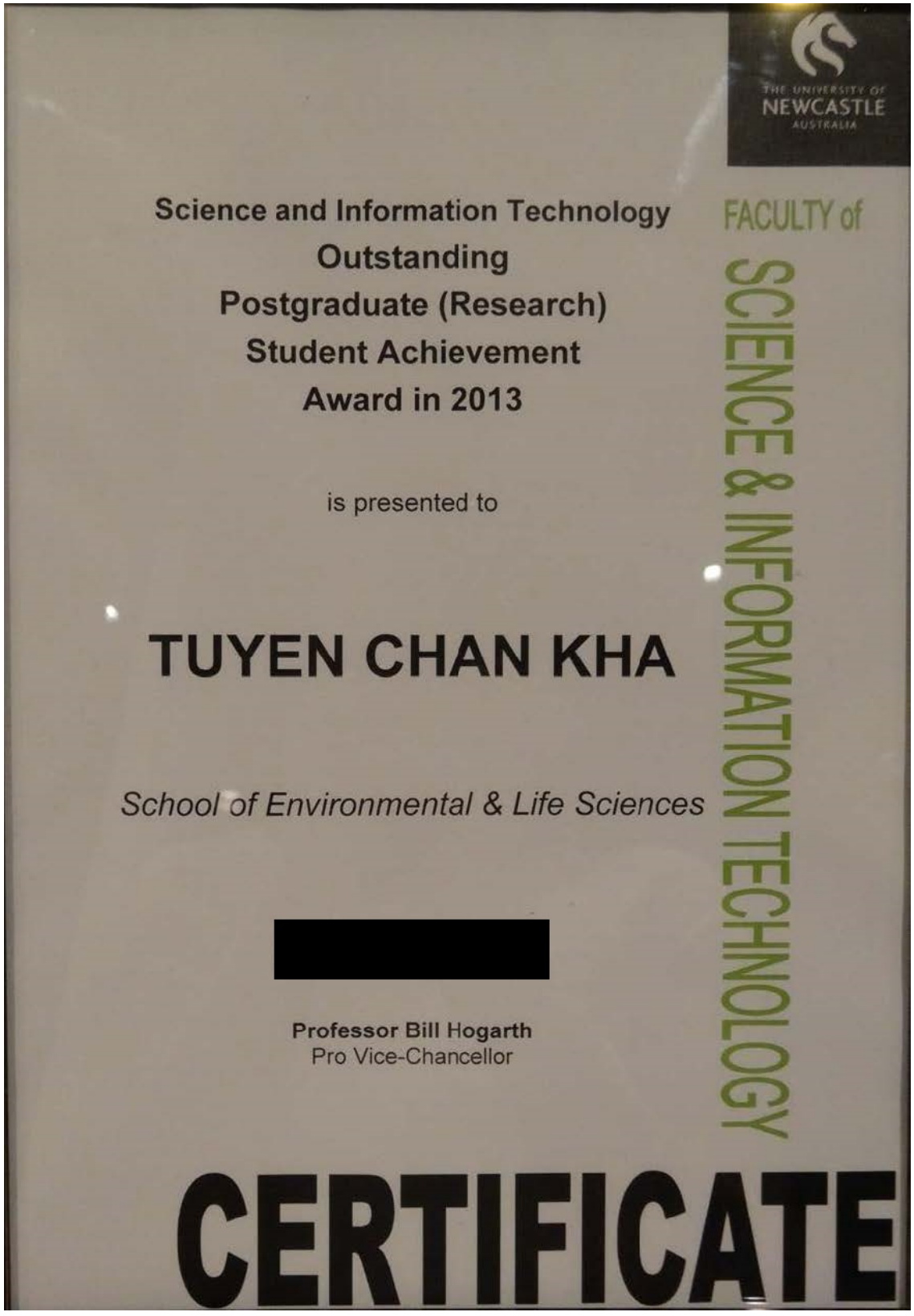
At the 47th Annual AIFST Convention 22-25 June 2014, the Institute conducted a Poster competition. Your poster titled "A Storage Study of Encapsulated Gac Oil Powder" was chosen as the winner.

Congratulations on your success in the competition and we look forward to your participation in future Conventions.

Kind regards,

Mel Malloch
Executive Manager
AIFST Inc

4.2 Outstanding Postgraduate (Research) Student Achievement Award granted by the Faculty of Science and Information Technology, The University of Newcastle, in 2013.


THE UNIVERSITY OF
NEWCASTLE
AUSTRALIA
FACULTY of
SCIENCE & INFORMATION TECHNOLOGY
Science and Information Technology
Outstanding
Postgraduate (Research)
Student Achievement
Award in 2013
is presented to
TUYEN CHAN KHA
School of Environmental & Life Sciences
[redacted]
Professor Bill Hogarth
Pro Vice-Chancellor
CERTIFICATE

4.3 The 1st Best Poster Presentation Award granted by International Conference on Food and Biosystems Engineering, Skiathos, Greece, 30 May - 02 June, 2013.

I.C. FaBE 2013
International Conference on
Food and Biosystems Engineering

International conference on
Food and Biosystems Engineering
FaBE 2013 - May 30 to June 02, 2013
Skiathos island-Greece
www.fabe.gr

FaBE-Lab

1ST BEST POSTER AWARD

Skiathos- Greece, 02/06/2013

ster Award for the participants of FaBE 2013 International Conference, held in Skiathos island-Greece,

the 1ST Best Poster Award to:

Tuyen Chan Kha

:

fruit: bioactive compounds, processing and utilizations.

Tuyen Chan Kha, Minh H. Nguyen.

Dr

4.4 The Best Poster Presentation Award (Nutrition category) granted by the Australian Institute of Food Science and Technology at the 45th Annual AIFST Convention in Adelaide, 15 - 18th July, 2012.

the australian institute of
food science and technology
incorporated
ABN 24 874 852 254

Mr Tuyen Chan Kha
Student
University of Newcastle
Unit 1, 31 Dianella Street
Ourimbah NSW 2258

Dear Yuyen,

RE:- AIFST Poster Competition

At the 45th Annual AIFST Convention 15-17 July 2012 in Adelaide, the Institute conducted a Poster competition. Your poster titled "*Optimisation of pretreatments prior to hydraulic pressing of Gac aril oil using response surface methodology*" was chosen as one of top three posters. Congratulations on your success in the competition and we look forward to your participation in future Conventions.

Kind regards

Mel Malloch
Executive Manager
AIFST Inc