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.

Tuyen Chan Kha
Date: 26/09/2014

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Finally, I wish to acknowledge my wife and children for their support and encouragement during my PhD research program.

LIST OF PUBLICATIONS INCLUDED AS PART OF THE THESIS

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. (C1).

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).

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).

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).

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).

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).

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).

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).

STATEMENTS OF CONTRIBUTION BY OTHERS

THE UNIVERSITY OF
NEWCASTLE
AUSTRALIA

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,

Associate Professor
Minh H. Nguyen

Doctor
Paul D. Roach

Doctor
Costas E. Stathopoulos

Doctor
Sophie E. Parks

Doctor
Dong T. Phan

Doctor
Huan Phan-Tai

Endorsed By

Associate sor Jenny Cameron
Assistant Dean (Research Training)

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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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LIST OF ADDITIONAL PUBLICATIONS, ACHIEVEMENTS AND AWARDS

CONFERENCE PROCEEDINGS

1. 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, 180-195, ISBN 978-960-9510-11-0. Full paper and Oral presentation. Refereed. (E1).

2. Tuyen C. Kha, Dong T. Phan, Minh H. Nguyen (2012). Traditional and industrial products from Gac fruit. *International Conference on Food Science and Nutrition Proceeding*, 2-4 April, 2012, Kota Kinabalu, Sabah, Malaysia, p.p 484-498. Full paper and oral presentation. Non-refereed. (E2).

CONFERENCE PRESENTATIONS (In Abstract Form)

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. (pp. 47). (E3).

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).

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).

4. **Tuyen C. Kha**, Minh H. Nguyen (2013). Gac fruit: Bioactive compounds, Processing and Utilisations. 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, pp. 39, ISBN 978-960-9510-11-0. (E3).

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. Poster Presentation. (E3).

BOOK CHAPTER

Tuyen C. Kha & Minh H. Nguyen. *Extraction and Isolation of Plant Bioactives*. In C. Scarlett & Q.V. Vuong (Ed.), Plant Bioactives and Pancreatic Cancer Prevention and Treatment. Nova Science Publishers, Inc. In Press. (A1).

ACHIEVEMENTS AND AWARDS

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.

3. Outstanding Postgraduate (Research) Student Achievement Award granted by the Faculty of Science and Information Technology, The University of Newcastle, in 2013.

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).

TABLE OF CONTENTS

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.