Extraction and Encapsulation of Bioactive Compounds from Bitter Melon

By

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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 persons, except where due reference has been made in the text.

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DECLARATION OF AUTHORSHIP

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

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2. **Paper II: Tan, S.P.,** Parks, S.E., Stathopoulos, C.E., Roach, P.D (2014). Extraction of Flavonoids from Bitter Melon. *Food and Nutrition Sciences*, 5(5), 458-465. (C1)
3. **Paper III: Tan, S.P.,** Parks, S.E., Stathopoulos, C.E., Roach, P.D (2014). An optimised aqueous extract of phenolic compounds from bitter melon with high antioxidant capacity. *Antioxidants,* 3 (4), 814-829. (C1)
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STATEMENT OF CONTRIBUTION BY OTHERS

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To whom it may concern,

This statement outlines Sing Pei Tan's contribution to the series of papers that are submitted as a part of her PhD. All papers that are contributing to her thesis are listed below, with a statement of her contribution for each.

Regards,

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   This project was led by Sing P. Tan. She conducted all data collection and all analyses, and was primarily responsible for manuscript preparation. Numerically, the contribution from the authors were: Sing Tan, 80%; Sophie Parks, 10%; Costas Stathopoulos, 5% & Paul Roach, 5% each.

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OTHER RELATED ACTIVITIES

1. Student Membership of Australian Institute of Food Science and Technology (AIFST).
2. Student Membership of Institute *of Food Technologists (IFT).*
3. Student Membership of Australian Society of Horticultural Science (AuSHS).

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LIST OF ABBREVIATIONS AND UNITS OF MEASUREMENT

Abbreviations

AAPH	2, 2'- azobis (2 - amidinopropane) dihydrochloride
AC	Antioxidant capacity
ABTS	2,2'-azinobis-(3-thylbenzothiozoline-6-sulfonic acid
AE	Aecsin equivalents
Aw	Water activity
BET	Brunauer Emmett Teller
BM	Bitter melon
C	Chroma
CCD	Central composite design
CE	Catechin equivalents
CH_3COOK	Potassium acetate
DI	Deionised water
DPPH	2,2'-diphenyl-1-picrylhydrazyl
DW	Dry weight
D (3,4)	Particle size distribution
EA	Encapsulating agent
EC	Electrical conductivity
EE	Encapsulation efficiency
EY	Encapsulation yield
FC	Folin-Ciocalteau
FRAP	Ferric reducing antioxidant power
GA	Gum Arabic
GAB	Guggenheim, Anderson, and DeBoer
GAE	Gallic acid equivalents
H°	Hue angle
HCl	Hydrochloric acid
KCI	Potassium chloride
K_2CO_3	Potassium carbonate
L	Lightness
LiCl	Lithium chloride
MC	Moisture content
MD	Maltodextrin
$MgCl_2$	Magnesium chloride
$Mg(NO_3)_2$	Magnesium nitrate
MeLo	Oxidation of linoleic acid methyl ester
M_0	Monolayer moisture content
NaCl	Sodium chloride
NaOH	Sodium hydroxide
ORAC	Oxygen radical absorbance capacity
R^2	Coefficient of determination
RSM	Response surface methodology
RE	Rutin equivalents
RT	Room temperature
SEM	Scanning electron microscopy
SPSS	Statistical package for social science
TAA	Total antioxidant activity

TE	Trolox equivalents
TFC	Total flavonoid content/compounds
TPC	Total phenolic content/compounds
TPTZ	2,4,6-tripyridyl-s-triazine
TSC	Total saponin content/compounds
v/v	Volume per volume
WAI	Water absorption index
w/v	Weight per volume
w/w	Weight per weight
WSI	Water solubility index
WAI	Water absorption index
ΔE	Total colour difference

Units of measurement

%	Percentage
°C	degree Celsius
× *g*	g force
bar	Bar
Cp	Centipoises
dS/m	Siemens per meter
g	Gram
g/g	gram per gram
g/L	gram per litre
g/mL	gram per millilitre
h	Hour
kPa	kilopascal
kV	Kilovott
L/h	litre per hour
m^3/h	cubic meter per hour
mbar	Millibar
mg	Milligram
mg/g	milligram per gram
mg/kg	milligram per kilogram
mL	Millilitre
mL/g	millilitre per gram
mL/h	millilitre per hour
mL/L	millilitre per litre
mm	Millimetre
mM	Millimolar
mol/L	mole per litre
mmol/L	micromole per litre
min	Minute
nm	Nanometre
nmol/L	nanomole per litre
rpm	revolution per minute
µmol/g	micromole per gram
µmol/L	micromole per litre
µL	Microliter

SYNOPSIS

In the realms of therapeutic ethnobotanical practices and Asian traditional remedies, bitter melon (*Momordica charantia* L.), a common tropical fruit, is claimed to have several therapeutic effects. These health benefits have been associated with its content of saponins (TSC), phenolics (TPC) and flavonoids (TFC) and its antioxidant capacity (TAA). Many different varieties of bitter melon are cultivated worldwide. However, it remains to be demonstrated whether varieties grown under controlled conditions, such as in a greenhouse, differ in yield and bioactive characteristics.

In ethnobotanical practices and Asian traditional medicine, plant materials are usually extracted for their active ingredients. Although organic solvents are often used to extract bioactive compounds from plant materials, water is a more desirable extractant because it is non-toxic, environmentally friendly and inexpensive compared to organic solvents. However, if water is to be the solvent of choice, the optimal conditions for the aqueous extraction of flavonoids, phenolics and saponins from bitter melon need to be determined, as there is a lack of knowledge on the extraction of these bioactives from bitter melon with water.

Once active components are extracted from their original source, it is often desirable to remove the solvent in order to prepare dry powders, which are easier to handle and often more stable than solvent extracts. This is especially true for aqueous extracts, which are susceptible to oxidative degradation. Spray-drying, with or without encapsulating agents, is often used to dry aqueous extracts because of its wide availability and low cost. However, little has been published on the optimal conditions for spray-drying bitter melon aqueous extracts.

Therefore, the experimental aims of this research were to determine 1) the best variety of bitter melon for greenhouse production in terms of yield, bioactive content and antioxidant activity, 2) the optimal conditions for the extraction of the bioactive compounds and antioxidant activity from bitter melon using water, 3) the optimal infeed emulsion formulation and the optimal drying temperatures for the encapsulation by spray-drying of the optimised bitter melon aqueous extract in terms of yield, bioactive content and antioxidant activity and 4) whether a stable high-quality powdered form of the bitter melon aqueous extract can be produced.

In the initial studies (**Papers I and II**), the production and quality characteristics of six different bitter melon varieties (Big Top Medium, Hanuman, Jade, White, Indra and Niddhi), grown under the same greenhouse conditions were investigated in order to determine the best variety of bitter melon for greenhouse production in terms of yield, bioactive content and antioxidant activity.

The variety 'Big Top Medium' was the best of the six varieties grown under controlled greenhouse conditions; it was high yielding and had a high bioactive content (**Paper I**). However, the greenhouse grown bitter melon varieties generally had a lower bioactive content than the field-grown variety 'Moonlight', which was purchased from a local market (**Paper II**). As a result, the 'Moonlight' variety was chosen for the subsequent extraction and encapsulation studies (**Papers III to VI**). This finding suggests that the greenhouse conditions may have limited the production of bioactive compounds.

Next, the conditions for the extraction of flavonoids (**Paper II**), phenolics (**Paper III**) and saponins (**Paper IV**) from bitter melon using water were optimised. The parameters studied for the aqueous extraction conditions were extraction temperature, time, ratio of water to bitter melon (mL/g) and number of times the same material was extracted.

The conditions for the aqueous extraction of flavonoids from bitter melon were optimised to be a double extraction of the same material at 40°C for 15 min at a water-to-powder ratio of 100:1 mL/g (**Paper II)**. However, after optimisation, the content of flavonoids in the aqueous extract was still much less than that obtained with the best organic solvent extract (acetone). Therefore, as for other plant materials, acetone is the best solvent for extracting flavonoids from bitter melon. For phenolic compounds, the optimal conditions for the aqueous extraction of bitter melon were a single extraction at 80°C for 5 min at a water-to-powder ratio of 40:1 mL/g and a powder particle size of 1 mm (**Paper III)**. These aqueous extraction conditions resulted in the same or higher TPC and TAA compared to the 80% ethanol extract. Furthermore, less solvent and less time was required with water than with 80% ethanol. For the aqueous extraction of saponins from bitter melon, the optimal conditions were chosen to be 40°C for 15 min at a water-to-powder ratio of 20:1 mL/g (**Paper IV**).

The conditions for the drying of the bitter melon aqueous extract into a powder were then investigated. In **Paper IV,** the bitter melon aqueous extract, optimised for saponins, was dried by spray-drying without an encapsulating agent. The results showed that, compared to the extract before spray-drying, the retention of saponins in the powder after drying was only 18%. The spray-dried powder also had a significantly lower retention of saponins than a powder prepared from the aqueous extract by freeze-drying.

Therefore, encapsulation of the bitter melon aqueous extract was tried in an attempt to increase the retention of the saponins and the other bioactives compounds after spray-drying. In the encapsulation studies, the formulation of the spray-drying infeed emulsion (**Papers V)** and the spray-drying temperatures (**Paper VI**) were optimised. The optimal formulation of the infeed emulsion for spray-drying was a ratio of 1.5:1 g/g of the aqueous extract to the encapsulating agent solution (35% (w/v) of 1:1 (w/w) MD and GA) (**Paper V**)**.** The optimal inlet and outlet temperatures were 140°C and 80°C, respectively (**Paper VI**). The retention of the saponins was increased from <20% in the absence to >60% in the presence of encapsulating agents. Similarly the retention for the phenolic and flavonoid compounds and the antioxidant activity was ~70%, >60% and >65%, respectively.

The quality of the encapsulated bitter melon powder prepared using the optimal formulation and the optimal inlet and outlet spray-drying temperatures was high due to its small spherical particle sizes, low moisture content, low water activity, high bulk density, high water solubility index and low water absorption index (**Paper VI**).

The stability of the optimised encapsulated bitter melon aqueous extract powder, in terms of the morphology of the powder particles and loss of the bioactive compounds and TAA, was significantly affected by the relative humidity levels (8.2-84.3%) due to water absorption by the powder (**Paper VI**). The safest range for the relative humidity for preserving the powder at 25°C was determined to be from 22.5 to 33.8%, which gave an equilibrium moisture content from 4.41 to 4.75, which was below the M_o = 5.71% predicted by the BET method.

However, the optimised encapsulated bitter melon aqueous extract powder was very stable under storage for 5 months with the greatest observed loss being <15% for the saponins. However, the powder was slightly more stable at -20 and 10°C (<6% loss) than at 30°C (<15% loss), over the 5 months.

In conclusion, six bitter melon varieties were successfully grown under controlled greenhouse conditions and the 'Big Top Medium' variety was the best of the six varieties in terms of fruit yield and bioactive content. However, the field-grown variety 'Moonlight', which was purchased from a local market, had a higher bioactive content than the greenhouse-grown varieties. Water was found to be an effective solvent for the extraction of the bitter melon bioactive compounds, phenolics and saponins but not flavonoids, and the yield was improved by optimising the aqueous conditions for their extraction from the fruit, Finally, optimisation of the spray-drying encapsulation process with MD and GA as encapsulating agents, resulted in a very stable encapsulated powder of the bitter melon aqueous extract.