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MICROENCAPSULATION OF BETACYANIN EXTRACT FROM AMARANTH

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Microencapsulation process is used to develop natural pigments by using a spray drying process. Betacyanins are red-violet natural pigment can be found in plant sources. In this project, the betacyanins were obtained from red amaranth. The optimization of spray drying process and types of encapsulating agents used are important to obtain desired pigment values. The objectives of the research are to investigate the effect of solvents on the yield and the colour properties of *Amaranthus gangeticus* and *Amaranthus blitum*, to examine the physicochemical properties and optimise spray drying conditions of maltodextrin-microencapsulated betacyanins as influenced by the inlet temperature and the maltodextrin concentration, to investigate the quality of β-cyclodextrin-encapsulated betacyanin pigment powder as influenced by spray drying conditions: inlet temperature, feed concentration and feed flow rate, and to investigate the drying parameters of spray-dried betacyanin pigment powder encapsulated by maltodextrin and β-cyclodextrin as influenced by spray drying conditions. Amaranth betacyanins are extracted from *A. gangeticus* and *A. blitum* by using water, methanol and ethanol extraction methods. Betacyanin content, total soluble solids and colour parameters are analyzed to assess extraction efficiency. A Principal Component Analysis is used to establish the relationships between the different analytical variables and to detect the most important factors of variability. The extracts are then microencapsulated by using a spray drying process. A Response Surface Methodology (RSM) was employed as a statistical analysis tool throughout the research. *A. gangeticus* extract (152.5 mg/100 g fresh weight) contains about two and half time more betacyanin with half of total soluble solids compared to *A. blitum* (62.1 mg/ 100 g of fresh weight). Water is the best as solvent for extracting betacyanin from *A. gangeticus* and ethanol in case of *A. blitum.* Three principal components are found

among the seven analytical variables accounting 88% of total variability. The first principal component mostly reflects the redness, whereas the second principal component reflects the betacyanin content, total soluble solids and lightness. It is suggested that *A. gangeticus* is selected as raw material and extracted by water extraction method. The results for second objective show that betacyanin retention is affected by inlet temperature whereas antioxidant activity is influenced by maltodextrin concentration. Only the effect maltodextrin concentration is found to have a significant effect on colour value. Particle densities and sizes are slightly affected by the process conditions studied. For β-cyclodextrin was used as the encapsulating agent, the results reveal that all the studied physicochemical properties of betacyanin pigment powder are affected by the three spray drying operating conditions. The glass transition temperature and storage studies are carried out to ensure the powder stability. Results of drying parameters explain that the droplet size is influenced by inlet temperature for both encapsulating agents and β-cyclodextrin concentration remarkably affects the droplet size for β-cyclodextrin-encapsulated pigment. As a conclusion, using of β-cyclodextrin as encapsulating agent exhibits preferable results compared to maltodextrin. The research suggests that using of β-cyclodextrin as encapsulating agent for spray drying of betacyanins with proposed optimized formulation by RSM (Inlet temperature 190 ℃, β-cyclodextrin concentration 13.89% and fee flow rate 9 ml/min) has potential to be utilized in food industries. The studied β-cyclodextrin-encapsulated betacyanin pigment can be applied to food system for future work.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PEMIKROKAPSULAN EKSTRAK *BETACYANIN* DARI *AMARANTH*

Oleh

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Proses pengkapsulan-mikro digunakan untuk membangunkan pigmen semula jadi dengan menggunakan proses pengeringan semburan. Betacyanin yang berwarna merah-ungu pigmen boleh didapati dalam sumber-sumber tumbuhan. Dalam projek ini, betacyanin diperolehi daripada amaranth merah. Pengoptimuman proses semburan pengeringan dan jenis pengkapsulan agen digunakan adalah penting untuk mendapatkan hasil yang diingini. Objektif kajian ini adalah untuk mengkaji kesan bahan pelarut terhadap hasil dan ciri-ciri warna kepada ekstrak *Amaranthus gangeticus* dan *Amaranthus blitum*, mengkaji ciri-ciri fizikal-kimia dan mengoptimumkan keadaan semburan pengeringan terhadap pengkapsulan-mikro betacyanin oleh maltodekstrin yang telah dipengaruhi oleh faktor suhu dalaman dan kepekatan maltodekstrin, mengkaji kualiti serbuk pigmen betacyanin yang telah melaui proses pengmikroenkapulan β-cyclodextrin dengan keadaan semburan pengeringan seperti suhu kemasukan, kepekatan kemasukan dan kadar aliran kemasukan, dan mengkaji parameter pengeringan terhadap serbuk pigmen yang telah melalui proses pengakpsulan maltodekstrin dan β-cyclodextrin oleh semburan pengeringan. Amaranth betacyanin telah diekstrak daripada *A. gangeticus* dan *A. blitum* dengan menggunakan tiga prosedur pengekstrakan yang berlainan iaitu; pengekstrakan dengan menggunakan air, pengekstrakan dengan menggunakan methanol dan pengekstrakan dengan etanol. Kandungan betacyanin, jumlah pepejal terlarut, parameter warna telah dianalisis bagi mengkaji keberkesanan pengekstrakan. Analisis komponen yang utama digunakan untuk mewujudkan dan menilai hubungan antara faktor pembolehubah yang berbeza-beza serta mengesan faktor yang paling penting dalam pembolehubah. Ketiga-tiga proses tersebut dijalankan oleh proses pengkapsulan-mikro oleh semburan pengeringan. Response Surface Methodology (RSM), telah digunakan untuk mnganalisis data kajian.

Ekstak *A. gangeticus* (152.5 mg/100 g berat sayur) mempunyai separuh jumlah pepejal terlarut dan mengandungi dua kali ganda setengah kandungan betacyanin berbanding dengan *A. Blitum* (62.1 mg/100 g berat sayur). Air merupakan pelarut terbaik bagi mengekstrak betacyanin daripada *A. gangeticus* manakala etanol adalah pelarut terbaik bagi mengekstrak betacyanin daripada *A. blitum*. Tiga daripada tujuh pembolehubah analitikal mempunyai 88% daripada jumlah kepelbagaian. Pembolehubah yang pertama kebanyakanya mempengaruhi factor kemerahan, manakala pembolehubah yang kedua mempengaruhi faktor-faktor berikut; kandungan betacyanin, jumlah pepejal terlarut dan kecerahan. Adalah dicadangkan bahawa, *A. gangeticus* dipilih sebagai bahan mentah dan diekstrak dengan menggunakan air. Kajian yang objektif kedua telah menunjukkan, pengekalan betacyanin telah dipengaruhi oleh suhu dalaman dan kepekatan maltodekstrin. Aktiviti antioksida juga telah dipengaruhi oleh kepekatan maltodekstrin. Terdapat kesan ketara pada nilai perubahan warna hanya apabila kepekatan maltodekstrin diubah. Di samping itu, ketumpatan dan saiz zarah sedikit dipengaruhi oleh keadaan proses yang dikaji Keputusan menunjukkan semua ciri-ciri fizikal-kimia serbuk dipengaruhi oleh tiga keadaan semburan pengeringan yang menggunakan β-cyclodextrin untuk pengkapsulan-mikro. Peralihan kaca suhu dan kajian pengyimpanan dijalankan untuk memastikan kestabilan serbuk. Keputusan parameter pengeringan menerangkan saiz titisan dipengaruhi oleh suhu kemasukan untuk kedua-dua ejen pengmikroenkapsulan dan kepekatan β-cyclodextrin memberi kesan besar terhadap saiz titisan untuk pigmen pengmikroenkapsulan β-cyclodextrin. Konklusinya, penggunaan β-cyclodextrin sebagai ejen pengmikroenkapsulan berbanding maltodekstrin menghasilkan keputusan yang lebih baik. Kajian ini mencadangkan bahawa menggunakan daripada β-cyclodextrin sebagai pengkapsulan agen untuk semburan pengeringan betacyanins dengan formulasi dioptimumkan oleh RSM (suhu dalaman 190 ℃, kepekatan β-cyclodextrin 13.89% dan kadar aliran kemasukan 9 ml/min) mempunyai potensi untuk digunakan dalam industry makanan. Betacyanin pigmen boleh digunakan dalam sistem makanan untuk kerja-kerja masa depan.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

*a**	Redness
A_{538}	Absorbance at 538 nm
Ac	Absorbance for control reading
A. blitum	*Amaranthus blitum*
A. gangeticus	*Amaranthus gangeticus*
ANOVA	Analysis of Variance
As	Absorbance for sample reading
a_w	Water activity
*b**	Yellowness
BC	β-cyclodextrin Concentration
BD	Bulk Density
BR	Betacyanin Retention
C	Carrier concentration (regression equation)
*C**	Chroma
CCRD	Central Composite Rotatable Design
D	Nozzle diameter
d_0	Initial diameter of drop
d_p	Droplet size
d_t	Diameter of evaporating drop at time *t*
DE	Dextrose Equivalent
DF	Dilution Factor
DSC	Differential Scanning Calorimetry
EE	Ethanol Extraction

F	Feed flow rate (regression equation)
FFR	Feed Flow Rate
g	Gravitational acceleration
$H°$	Hue angle
HMC	Hygroscopic Moisture Content
IC_{50}	Median Inhibition Concentration
IT	Inlet Temperature
k_f	Thermal conductivity
L	Path length
$L*$	Lightness
m	Mass of the falling droplet
MC	Moisture Content
MD	Maltodextrin concentration
ME	Methanol Extraction
MW	Molecular Weight
PCA	Principal Component Analysis
PC1	First Principal Components
PC2	Second Principal Components
PC3	Third Principal Components
PS	Particle Size
r	Radius of dripping tip
RSM	Response Surface Methodology
SEM	Scanning Electron Microscopy
t	Drying time
T	Inlet temperature (regression equation)
T_g	Glass transition temperature

V	Volume extract
v_{rel}	Gas-liquid relative velocity
W	Fresh weight of extracting material
WE	Water Extraction
X_f	Feed moisture
X_p	Powder moisture
y	Response
λ	Latent heat of vapourization
θ	Coefficient value
σ	Air-water surface tension
ρ_l	Density of liquid
ρ_g	Density of gas
η	Gas-liquid mass ratio
μ	Viscosity of liquid
ΔT	Temperature difference between heated air temperature and wet bulb temperature for heated air

CHAPTER 1

INTRODUCTION

1.1 Food colourants

The appearance of food is one of the crucial factors for acceptability by consumers. One of the attributes that contribute to the appearance of food is colour. For this reason, food industries focus on the physical qualities of food. However, food processing will affect the physical appearance of food by generally losing the colour compound. So food colourants become important either as an additive to food for which the colour has been lost during processing or to intensify the appearance of food.

Food colourants are simply classified into synthetic colourants and natural colourants. Natural colour is defined as the colour extracted from plant or animal sources without changing the pigments chemically. Nature-identical colour is defined as a colour synthesized from chemical feedstocks such that it is structurally identical to the natural pigments (Timberlake and Henry, 1986). Nowadays consumers prefer natural colourants instead of synthetic colourants due to quality issues as well as health concerns. Some certain synthetic colourants provoke intolerance and allergenic reactions which are not acceptable to consumers (Wissgott and Bortlik, 1996). Natural pigments from plant sources such as lycopene and anthocyanins exhibit antioxidant activity that may protect against chronic diseases (Socaciu, 2008).

There are a wide range of plant pigments available in nature, such as green colours from leafy vegetables which are contributed by a combination of chlorophyll and carotenoids, and reddish-purple colours from fruits and vegetables produced by anthocyanins and betacyanins. Other natural sources of colourants from plant pigments include yellowish to reddish carotenoids from eggs, fish, fruits and vegetables, lycopene from tomatoes, watermelons and pink grapefruit, and caramel colour obtained by the caramelisation of sugar (Wissgott and Bortlik, 1996). The chemical properties of natural pigments can alter the stability as well as produce physical changes. Carotenoid is lost due to oxidation with exposure to light, heat, enzymes and peroxides (Socaciu, 2008). The colour of anthocyanins changes due to the change of pH. Red colour is characterised by anthocyanins when the pH is between 2.5 and 3.8. With a pH higher than 6, the anthocyanins will present as a blue colour (Timberlake and Henry, 1986).

Although natural colourants from plants are preferred, the stability of plant pigments becomes a challenge. In general, they are sensitive to pH, heat, or light. The processing of natural colourants such as spray drying can reduce the colour degradation by transforming it into powder and thus prolong the shelf life.

1.2 Betacyanins

Betalains are one type of pigments found in 10 families of the order *Caryophyllales* as well as family *Amaranthaceae*. As a water-soluble nitrogenous pigment it can be structurally divided into two groups: yellow betaxanthins and red-violet betacyanins. The basic structure of betacyanins and betaxanthins and their building block betalamic acid are shown in Figure 1.1. Based on the chemical structures, betacyanins can be further classified into four types which are betanin, amaranthin, bougainvillein and gomphrenin (Cai *et al.,* 2005). Some 50 betacyanins and 20 betaxanthins have been identified to occur in nature (Francis, 1999).

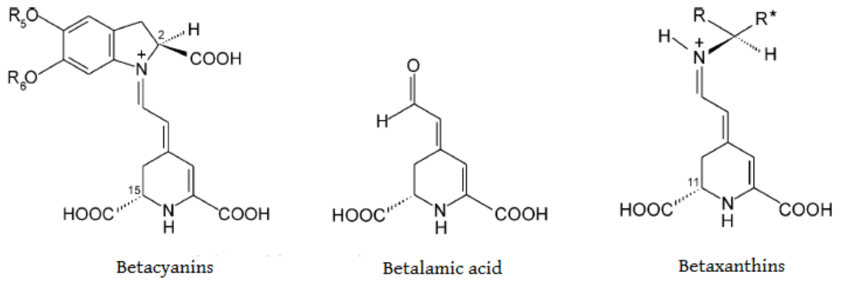

Figure 1.1. Basic structure of betacyanins, betalamic acid and betaxanthins (adopted from Stintzing and Carle, 2004).

Betacyanins can be quantified using a spectrophotometry. Two absorption ranges are displayed that include a UV-range from 270 nm to 280 nm as a result of the cyclo-Dopa structure, and a visible range from 535nm to 538 nm (Stintzing and Carle, 2004).

Betacyanins have been widely used as food colourants in food industries. Previous studies have shown that betacyanins exhibit antioxidant activity. Cai *et al.* (2005) identified 19 different types of betalains from the family *Amaranthaceae* which possesses high antioxidant activity. The glycosylation is decreased in betacyanins while acylation increases the antioxidant potential (Stintzing and Carle, 2004). This is an additional advantage for human health as antioxidants can reduce the risk of cancer, cardiovascular diseases as well as other diseases that are associated with aging (Cai *et al.*, 2003). Red beet is also a source of betalains, but it is it restricted by its earthy smell as well as considerable nitrate level (Herbach *et al.*, 2007). Thus betalains from red amaranth have an advantage in this area.

One of the issues for natural plant pigments is the stability as such plant pigments are easily degraded by exposure to light, oxygen, high temperature causing decomposition, water activity and enzymatic activities. However betalains are suitable to be applied in a food system compared to anthocyanins. The hydrophilic betalains are stable between pH 3 and 7 and this characteristic is applicable from low acid to neutral foods (Stintzing and Carle, 2007). A pH below 2 or above 9 will cause the degradation of betacyanins (Jackman and Smith, 1996).

1.3 Amaranthaceae

As mentioned earlier, betalains are plant pigments found in the family *Amaranthaceae*. Amaranth has been cultivated since 5000 to 7000 years ago and it has been reported as the main food of the ancient Aztecs (Lehmann, 1994). Now it can be found in certain areas of Africa and Asia, Central and South America. The highest production of amaranth is predominantly in China where it is utilised as feed for animals and food (Cai *et al.*, 2005). Over the recent 20 years, Amaranth has been widely cultivated due to its high nutritional value as well as the ability to withstand critical conditions such as drought or poor soil.

In Malaysia, five species of *Amaranthus* species have been identified which include *Amaranthus gangeticus (bayam merah), Amaranthus blitum (bayam itik), Amaranthus paniculatus (bayam putih), Amaranthus viridis (bayam panjang)* and *Amaranthus spinosus (bayam duri)* (Amin *et al.*, 2006). The species *Amaranthus gangeticus* is shown in Figure 1.2. Nowadays the main source of betacyanins is obtained from red beets (Cai *et al.*, 2005). *Amaranthus* has become a topic of research interest as an alternative source of betacyanins since it can be produced in high biomass levels and can be cultivated in Malaysia. In addition, *Amaranthus* pigments are high in crude extracts and protein content, which contribute considerable nutritional value to food (Cai *et al.*, 1998a).

Figure 1.2. *Amaranthus gangeticus*

Amaranthus betacyanins have been identified as isoamranthine and amaranthine (Piattelli *et al.*, 1964; Cai *et al.*, 1998b). *Amaranthus* betacyanins have been applied in food systems such as bread and beverages in south-western United States, Mexico and Argentina (Lehmann, 1990). Further, the *Amaranthus* pigments can be applied to low-temperature food such as yoghurt, frozen fruit desserts and ice cream (Freund *et al.*, 1988). Cai and Corke (1999) used *Amaranthus* betayanins in beverage, ice cream and jelly to compare with commercial colourants. The model foods using betacyanins have a brighter colour than red radish anthocyanins.

1.4 Microencapsulation by spray drying

Microencapsulation in the context is defined as the process in which particles or liquid droplets are coated with a thin film of an edible coating material (Fellows, 2009). In food industries, spray drying can be normally used for microencapsulation. It involves the combination of the ingredient that will be encapsulated and a coating material such as dextrin, modified starch, celluloses, proteins or gums. In general there are a few types of microencapsulated products which include flavourings, fats and oils, bioactives and herbs, vitamins and minerals, and other food ingredients (Barobosa-Canovas *et al*., 2005; Reineccius, 2005).

The microencapsulation process is done by evaporating the moisture from an aqueous surface and the polymeric material will form a coating around the ingredient. For water-soluble material such as aspartame this is encapsulated by either fluidised bed coating or spray coating. In this case the particles are suspended in a heated airstream which is in an upward moving direction. At this time atomisation of the coating material occurs which dries on the particles to coat them properly (Fellows, 2009). Spray drying is widely used as encapsulating tool as it produces a solid spherical powder directly from the liquid phase and has a short drying time over other drying technologies (Devahastin, 2011).

The criteria for coating materials are that they should be insoluble in the entrapped ingredient to avoid reaction, should resist the temperature used in the encapsulation process and must be soluble in food systems. Some general purposes of using encapsulation are to mask the odour or taste of ingredients such as fish oils, to stabilise volatile or heat sensitive ingredients, or ingredients which are susceptible to oxidation, such as colours and flavourings, and to transform from a liquid phase to solid phase for easy handling or storage stability (Fellows, 2009).

1.5 Problem statement and objectives

Artificial colourants applied in food industries contribute side effects which affect certain people by allergic reaction (Wissgott and Bortlik, 1996). It is suggested that use of natural colourants as food additives is much preferred over artificial sources. Nowadays beet roots are the major source of betalains and have been commercialised in food industries. The source of betalains from red amaranth has become of interest over the few years. In addition, two types of *Amaranthus* species: *A. gangeticus* and *A. blitum* have gained much attention due to the amount of betacyanin pigment they contain. The potential sources of betacyanin and methods of extraction become key elements as they can directly affect the yield. The retention of the betacyanin extracted from red amaranth should also be optimised to minimise the loss and thus to save cost. In addition, the choice of encapsulating agents can be another crucial factor influencing the retention. Hence the stability of betacyanin pigment compound is the main concern if it is to be used as a natural food colourant (Stintzing and Carle, 2007). The understanding of the drying behaviour of encapsulated betacyanin pigment from red amaranth during spray drying should be studied. Research is needed in order to overcome these problems stated above. Hence the objectives of this study are:

1. To evaluate the types of betacyanin extraction methods on the yield and the colour properties of *Amaranthus gangeticus* and *Amaranthus blitum*.
2. To determine the physico-chemical properties and optimise spray drying conditions of maltodextrin-microencapsulated *Amaranthus gangeticus* powder.
3. To measure the physic-chemical properties and optimise spray drying conditions of β-cyclodextrin-microencapsulated *Amaranthus gangeticus* extract and its stability.
4. To evaluate the drying parameters of microencapsulated betacyanin powder by spray drying conditions.

1.6 Outline of the thesis

Chapter 1 introduces a basic understanding of the research area related to the study, which includes a brief overview of food colourants, the microencapsulation process by spray drying and types of encapsulating agents used. In addition, the raw material of the research, red amaranth, is briefly presented. The natural pigment betacyanins which form the main body of the study are introduced here. The problem statement and objectives are clearly described at the end of the chapter along with an outline of the thesis.

Chapter 2 provides a literature review of the research data that is related to the study. It informs of the further improvement that the research work can make. The spray drying technology and encapsulating agents are explained. The related research area, including microencapsulation of anthocyanin, *Amaranthus* betacyanin, and the antioxidant activity of the *Amaranthus* species are elaborated here.

Chapter 3 delivers a preliminary study on the selection of the *Amaranthus* species from *Amaranthus blitum* and *Amaranthus gangeticus*. The extraction methods are evaluated and chosen. Principal component analysis is used as a statistical tool in this section. The next stage of the research is explained in the following chapter after the raw material and extraction method are specified. The details of the raw materials, chemicals used, analyses and methodology are described here.

Chapter 4 explains the microencapsulation process for the betacyanin pigment and the spray drying process is modelled and optimised by response surface methodology as a statistical method. The physicochemical properties of betacyanin powder are analysed. Maltodextrin is used as an encapsulating agent in this section. There are two major factors being studied: spray drying inlet temperature and maltodextrin concentration, which involves physicochemical analyses after spray drying: betacyanin retention, redness of powder, antioxidant activity (expressed as IC_{50}), moisture content, water activity, densities, particle size, colour properties and morphology. Optimisation and model validation is conducted based on three important responses: betacyanin retention, antioxidant activity and redness of powder, and finally the formulation is proposed.

Chapter 5 continues the extension work of Chapter 4. The main study is to model the spray drying process of betacyanin powder using different encapsulating agent: β-cyclodextrin with three factors studied: inlet temperature, β-cyclodextrin concentration and feed flow rate. Analyses include betacyanin retention, antioxidant activity, redness of powder, moisture content, hygroscopic moisture content, water activity, bulk density, particle size and particle morphology. The storage studies are conducted for betacyanin powder under different storage conditions. Optimisation and model validation are carried out which emphasise on betacyanin retention, antioxidant activity and redness of powder. The optimised formulation is proposed and the glass transition temperature is determined for optimised betacyanin powder.

Chapter 6 validates the drying parameters of microencapsulated betacyanin powder using established empirical models as affected by the factors studied: inlet temperature, carrier concentration and feed flow rate with using two different encapsulating agents: maltodextrin and β-cyclodextrin, based on the proposed formulations from Chapter 4 and Chapter 5 in Table 4.2 and Table 5.2, respectively. A few equations are proposed to measure the drying rate, droplet size and drying time. The parameters are experimentally determined, including feed moisture, surface tension, viscosity and density of liquid.

Chapter 7 summarises the entire outcome of the research with general conclusions. The optimised formulations of maltodextrin and β-cyclodextrin-encapsulated betacyanin pigment powder are recommended. Future work for the study is suggested in this chapter.

REFERENCES

Abadio, F. D. B., Domingues, A. M., Borges, S. V., Oliveira, V. M. (2004). Physical properties of powdered pineapple (*Ananas Comosus*) juice: effect of maltodextrin concentration an atomization speed. *Journal of Food Engineering*, 64: 285-287.

Ahmed, J., Tiwari, B. K., Imam, S. H., Rao, M. A. (2012). Starch-based Polymeric Materials and Nanocomposites (pp. 96). Boca Raton: Taylor & Francis Group.

Amaro, M. I., Tajber, L., Corrigan, O. I., Healy, A. M. (2011). Optimisation of spray drying process conditions for sugar nanoporous microparticles (NPMPs) intended for inhalation. *International Journal of Pharmaceutics*, 421: 99-109.

Amin, I., Norazaidah, Y., Hainida, K. I. E. (2006). Antioxidant activity and phenolic content of raw and branched *Amaranthus* species. *Food Chemistry*, 94: 47-52.

Azeredo, H. M. C. (2009). Betalains: properties, sources, applications, and stability – a review. *International Journal of Food Science & Technology*, 44: 2365-2070

Barbosa-Canovas, G. V., Ortega-Rivas, E., Juliano, P., Yan, H. (2005). Food powders: Physical Properties, Processing, and Functionality. New York: Springer-Verlag.

Brennan, J. G. (2006). Food Processing Handbook (pp. 84-86, 105-110). Germany: Wiley-VCH.

Bridle, P., Timberlake, F. (1997). Anthocyanins as natural food colours-selected aspects. *Food Chemistry*, 58: 103-109.

Burrows, A. J. D. (2009). Palette of Our Palates: A Brief History of Food Coloring and Its Regulation. *Review in Food Science and Food Safety*, 8: 394-407.

Cai, Y. Z., Sun, M., Corke, H. (1998a). Colorant properties and stability of Amaranthus betacyanin pigments. *Journal of Agricultural and Food Chemistry*, 46: 4491–4495.

Cai, Y. Z., Sun, M., Wu, H. X., Huang, R. H., Corke, H. (1998b). Characterization and quantification of betacyanin pigments from diverse *Amaranthus* species. *Journal of Agriculture Food Chemistry*, 46: 2063-2070.

Cai, Y. Z., Corke, H. (1999). *Amaranthus* Betacyanin Pigments Applied in Model Food Systems. *Journal of Food Science*, 64: 869-873.

Cai, Y. Z., Corke, H. (2000). Production and Properties of Spray-dried Amaranthus Betacyanin Pigments. *Journal of Food Science*, 65: 1248-1252.

Cai, Y. Z., Corke, H. (2001). Effect of Postharvest Treatments on *Amaranthus* Betacyanin Degradation Evaluated by Visible/Near-Infrared Spectroscopy. *Journal of Food Science*, 66: No. 8.

Cai, Y. Z., Sun, M., Corke, H. (2003). Antioxidant Activity of Betalains from Plants of the Amaranthaceae. *Journal of Agricultural and Food Chemistry*, 51: 2288-2294.

Cai, Y. Z., Sun, M., Corke, H. (2005). Characterization and application of betalain pigments from plants of the Amaranthaceae. *Trends in Food Science & Technology*, 16: 370-376.

Carnat, A. P., Carnat, A., Fraisse, D., Lamaison, J. L. (1998). The aromatic and polyphenolic composition of lemon balm (Melissa officinalis L. subsp. officinalis) tea. *Pharmaceutics Acta Helvetiae*, 72: 301-305.

Carmen, S., Tapia, S., Chavez, J., Robert, P. (2009). Microencapsulation by spray drying of bioactive compounds from cactus pear (*Opuntia ficus-indica*). *Food Chemistry*, 114: 616-622.

Castellar, R., Obon, J. M., Fernandez-Lopez, J. A. (2006). The isolation and properties of a concentrated red-purple betacyanin food colourant from Opuntia stricta fruits. *Journal of the Science of Food and Agriculture*, 86: 122-128.

Cevallos, P. A. P., Buera, M. P., Elizalde, B. E. (2010). Encapsulation of cinnamon and thyme essential oils components (cinnamaldehyde and thymol) in β-cyclodextrin: Effect of interactions with water on complex stability. *Journal of Food Engineering*, 99: 70-75.

Chen, X. D., Mujumdar, M. S. (2008). Drying Technologies in Food Processing (pp. 113-125). UK: Blackwell Publishing Ltd.

Chong, P. H., Aziz, M. G., Yusof, Y. A., Nazli, N. M., Chin, N. L., Syed Muhammad, S. K. (2013). Modelling and Determination of Physico-Chemical and Morphological Properties of Microencapsulated Red Amaranth Powder. *The Institute of Engineers, Malaysia*, 74: No 2.

Coisson, J. D., Travaglia, F., Piana, G., Capasso, M., Arlorio, M. (2005). *Euterpe oleracea* juice as a functional pigment for yogurt. *Food Research International*, 38 (8-9): 893-897.

Coulson, J. M., Richardson, J. F., Backhurst, J. R., Harker, J. H. (1978). Chemical Engineering (pp. 735-745). England: Pergamon Press.

Couto, R. O., Conceicao, E. C., Chaul, L. T., Oliveira, E. M. S., Martins, F. S., Bara, M. T. F., Rezende, K. R., Alves, S. F., Paula, J. R. (2012). Spray-dried rosemary extracts: Physicochemical and antioxidant properties. *Food Chemistry*, 131: 99-105.

Cui, S. W. (2005). Food Carbohydrates: Chemistry, Physical Properties and Applications. Boca Raton: Taylor & Francis Group.

Dastmalchia, K., Damien Dormana, H. J., Darwisd, Y., Laakso, I., Hiltunena, R. (2008). Chemical composition and in vitro antioxidative activity of a lemon balm (Melissa officinalis L.) extract. *LWT-Food Science & Technology*, 41: 391-400.

De Castro, A. D., Silva Jr, N. P., Cury, B. S. F., Evangelista, R. C., Freitas, L. A. P., Gremiao, M. P. D. (2010). A new approach to the granulation of β-cyclodextrin inclusion complexes. *Chemical Engineering Journal*, 164: 316-321.

Desai, K. G. H., Park, H. J. (2005). Recent development in microencapsulation of food ingredients. *Drying Technology*, 23: 1361-1394.

Desobry S. A., Netto F. M., Labuza T. P. (1997). Comparison of spray-drying, drum-drying and freeze-drying for b-carotene encapsulation and preservation. *Journal of Food Science*, 62: 1158-1162.

Devahastin, S. (2011). Physicochemical Aspects of Food Engineering and Processing (pp. 6, 24-25). Boca Raton: Taylor and Francis Group.

Dixon, W. J. (1992). BMDP statistical software. University of California Press, Los Anegeles, USA.

Dodziuk, H. (2006). Cyclodextrins and Their Complexes, Chemistry, Analytical Methods, Application (pp. 396). Germany: WILEY-VCH Verlag GmbH & Co.

DPPH Scavenging Activity (Spectrophotometry) (2011). IBS, UPM, Malaysia.

Erdogdu, F. (2008). Optimization in Food Engineering (pp. 115-140). US: CRC Press.

Ersus, S., Yurdagel, U. (2007). Microencapsulation of anthocyanin pigments of black carrot (*Daucuscarota* L.) by spray drier. *Journal of Food Engineering*, 80: 805-812.

Escribano, J., Pedreno, M. A., Garcia-Carmona, F., Munoz, R. (1998). Characterization of the antiradical activity of betalains from Beta vulgaris L. roots. *Phytochem. Anal*, 9: 124–127.

Espinola, F., Moya, M., Fernandez, D. G., Castro, E. (2011). Modelling of virgin olive oil extraction using response surface methodology. *International Journal of Food Science & Technology*, 46: 2576-2583.

Fang, Z. X., Bhandari, B. (2010). Encapsulation of polyphenols – a review. *Trends in Food Science & Technology*, 21: 510-523.

Fazaeli, M., Djomeh, Z. E., Ashtari, A. K., Omid, M. (2012). Effect of spray drying conditions and feed composition on the physical properties of black mulberry juice powder. *Food and Bioproducts Processing*, 90: 667-675.

Fellows, P. (2000). Food Processing Technology. 2nd (Ed.) (pp. 329-330). New York: CRC Press.

Fellows, P. J. (2009). Food processing technology: Principles and practice. 3nd (Ed.) (pp. 709-710). New York: CRC Press.

Francis, F. J. (1999). Anthocyanins and betalains. In F.J. Francis (Ed.), Colorants. St Paul, MN: Eagan Press.

Frau, M., Sinal, S., Femenia, A., Sanjuan, E., Rossello, C. (1999). Use of principal component analysis to evaluate the physical properties of Mahon cheese. *European Food Research and Technology*, 210: 73-76.

Freund, P. R., Washam, C. J., Maggion, M. (1988). Natural color for use in foods. *Cereal Foods World*, 33: 553-559.

Fu, W. Y., Etzel, M. R. (1995). Spray drying of *Lactococcus lactis* ssp. *Lactis* C2 and cellular injury. *Journal of Food Science*, 60: 195-200.

Gallo, L., Llabot, J. M., Allemandi, D., Bucala, V., Pina, J. (2011). Influence of spray-drying operating conditions on *Rhamnus purshiana* (Cascara sagrada) extract powder physical properties. *Powder Technology*, 208: 205-214.

Gharsallaoui, A., Roudaut, G., Chambin, O., Voilley, A., Saurel, R. (2007). Applications of spray-drying in microencapsulation of food ingredients: An overview. *Food Research International*, 40: 1107-1121.

Ghosh, D., Chattopadhayay, P. (2012). Application of principal component analysis (PCA) as a sensory assessment tool for fermented food products. *Journal of Food Science and Technology*, 49 (3): 328-334.

Gibbs, B. F., Kermasha, S., Alli, I., Mulligan, C. N. (1999). Encapsulation in the food industry: A review. *International Journal of Food Sciences and Nutrition*, 50: 213-224.

Harkins, W. D., Humphery, E. C. (1916). The drop weight method for the determination of surface tension (Surface tension 1). *Journal of the American Chemical Society*, 38: 242-246.

Heldman, D. R., Lund, D. B. (2007). Handbook of Food Engineering, 2nd (Ed.)(pp. 631-666). US: Taylor and Francis Group, LLC.

Herbach, K. M., Maier, C., Stintzing, F. C., Carle, R. (2006). Betalain Stability and Degradation – Structural and Chromatic Aspects. *Journal of Food Science*, 71: R41-R50.

Herbach, K. M., Maier, C., Stintzing, F. C., Carle, R. (2007). Effects of processing and storage on juice colour and betacyanin stability of purple pitaya (*Hylocereus polyrhizus*) juice. *Section Plant Foodstuff Technology*, 224: 649-658.

Herodez, S. S., Hadolinb, M., Skergeta, M., Kneza, Z. (2003). Solvent extraction study of antioxidants from Balm (Melissa officinalis L.) leaves. *Food Chemistry*, 80: 275-282.

Huo G. H., Guo, C. Z. (1994). Exploiting study on the red pigment in *Amaranthus* tricolor. *Acta Agricultural University Jiangxiensis* (in Chinese), 16: 106-111.

Ismail, A., Marjan, Z. M., Foong, C. W. (2004). Total antioxidant activity and phenolic content in selected vegetables. *Food Chemistry,* 87: 581-586.

Jackman, R. L., Smith, J. L. (1996). Anthocyanins and betalains. In G.F. Hendry and J.D. Houghton (Eds.), Natural Food colorants. London: Blackie Academic & Professional.

Kalogeropoulos, N., Yannakopoulou, K., Gioxari, A., Chiou, A., Makris, D. P. (2010). Polyphenol characterization and encapsulation in β-cyclodextrin of a flavonoid-rich *Hypericum perforatum* (St John's wort) extract. *LWT – Food Science and Technology*, 43: 882-889.

Kanner, J., Harel, S., Granit, R. (2001). Betalains—a new class of dietary cationized antioxidants. *Journal of Agricultural and Food Chemistry*, 49: 5178– 5185.

Kargozari, M., Moini, S., Emam-Djomeh, Z. (2010). Prediction of some Physical Properties of Osmodehydrated Carrot Cubes using Response Surface Methodology. *Journal of Food Processing and Preservation*, 34: 1041-1063.

Karovicova, J., Kohajdova, Z. (2002). The use of PCA, FA, CA for the evaluation of vegetable juices processed by lactic acid fermentation. *Czech Journal of Food Science*, 20: 135-143.

Kennedya, D. O., Scholeya, A. B., Tildesleya, N. T. J., Perryb, E. K., Wesnes, K. A. (2002). Modulation of mood and cognitive performance following acute administration of Melissa officinalis (lemon balm). *Pharmacology, Biochemistry and Behavior*, 72: 953-964.

Kharidah, M., Hossein, A., Jamilah, B. (2009). Natural colorant and method thereof. Patent WO2010/090508A1.

Khattree, R., Naik, D. N. (2000). Multivariate Data Reduction and Discrimination with SAS Software (pp. 25-26). USA: SAS Institute Inc.

King, A. E. (1985). Studies on the production of banana juice powder using spray drying. *Journal of the Chinese Agricultural Chemical Society* (in Chinese), 23: 62-72.

Lehmann, J. W. (1990). Pigments of grain and feral amaranths. *Legacy,* 3(1): 3-4.

Lehmann, J. W. (1994). Amaranth: Commercialization and industrialization. In O. Paredes-Lopez (Eds.), Amaranth biology, chemistry, and technology. Boca Raton: CRC Press.

Lim, S. D., Yusof, Y. A., Chin, N. Y., Talib, T. A., Endan, J., Aziz, M. G. (2011). Effect of extraction parameters on the yield of betacyanins from pitaya fruit

(*Hylocereus Polyrhyzus*) pulps. *Journal of Food Agriculture and Environment*, 9: 158-162.

Lin, S. Y., Kao, Y. H., (1989). Solid particulates of drug-β-cyclodextrin inclusion complexes directly prepared by a spray-drying technique. *International Journal of Pharmaceutics*, 56: 249-259.

Main, J. H., Clydesdale, F. M., Francis, F. J. (1978). Spray drying anthocyanin concentrates for use as food colorants. *Journal of Food Science*, 43:1693-1694, 1697.

Marshall, W. R. (1954). Atomization and spray drying. *Chemical Engineering Progress Monographs*, 50: Series No 2.

Marshall, W. R. (1955). Heat and mass transfer in spray drying. *Transactions of the American Society of Engineers*, 77.

Masters, K. (1985). Spray Drying Handbook, 4th (Ed.). England: George Godwin.

Masters, K. (1991). Spray Drying Handbook, 5th (Ed.). London: Longman Scientific and Technical.

Maury, M., Murphy, K., Sandeep, K., Shi, L., Lee, G. (2005). Effects of process variables on the powder yield of spray-dried trehalose on a laboratory spray-dryer. *European Journal of Pharmaceutics and Biopharmaceutics*, 59: 565-573.

Montgomery, D. C. (2009). Design and Analysis of Experiments, (7th Ed.). US: John Wiley & Sons (Asia) Pte Ltd.

Mourtzinos, I., Papadakis, S. E., Igoumenidis, P., Karathanos, V. T. (2011). Encapsulation of *Melissa Officinalis* leaf's active compounds in β-cyclodextrin and modified starch. *Procedia Food Science*, 1: 1679-1685.

Mukhopadhyay, P. (2009). Multivariate Statistical Analysis (pp. 3-8). USA: World Scientific Publishing Co. Pte. Ltd.

Nadeem, H. S., Dincer, C., Torun, M., Topuz, A., Ozdemir, F. (2013). Influence of inlet air temperature and carrier material on the production of instant soluble sage (*Salvia fruticosa* Miller) by spray drying. *LWT – Food Science and Technology*, 52: 31-38.

Ng, L. T., Chong, P. H., Yusof, Y. A., Chin, N. L., Talib, R. A., Taip Farah, S., Aziz, M. G. (2012). Physicochemical and Nutritional Properties of Spray-dried Pitaya Fruit Powder as Natural Colorant. *Food Science Biotechnology*, 21(3): 675-682.

Nwabueze, T. U. (2010). Basic steps in adapting response surface methodology as mathematical modelling for bioprocess optimization in the food systems. *International Journal of Food Science & Technology*, 45: 1768-1776.

Oliveira, W. P., Souza, C. R. F., Kurozawa, L. E., Park, K. L. (2010). Spray drying of food and herbal products. *Spray drying technology*, 1: 113-156.

O'Neil, B. T. (1998). Dry Scrubbing Technologies for Fluw Gas. USA: Kluwer Academic Publishers.

Patel, R. P., Patel, M. P., Suthar, A. M. (2009). Spray drying technology: an overview. *Indian Journal of Sciences and Technology*, 2: No. 10.

Peng, Z., Li, J., Guan, Y., Zhao, G. (2013). Effect of carriers on physicochemical properties, antioxidant activities and biological components of spray-dried purple sweet potato flours. *LWT – Food Science and Technology*, 51: 348-355.

Pedreno, M. A., Escribano, J. (2000). Studying the oxidation and the antiradical activity of betalain from beetroot. *Journal of Biological Education*, 35: 49–51.

Phebe, D., Chew, M. K., Suraini, A. A., Lai, O. M., Janna, O. A. (2009). Red-fleshed pitaya (Hylocereus polyrhizus) fruit colour and betacyanin content depend on maturity. *International Journal of Food Research*, 16: 233-242.

Piattelli, M., Minale, L., Prota, G. (1964). Isolation and structure of amaranthine and isoamaranthine. *Annali di Chimica*, 54: 963-968.

Porrarud S., Pranee, A. (2010). Microencapsulation of Zn-Chlorophyll pigment from from Pandan leaf by spray drying and its characteristics. *International Food Research Journal*, 17: 1031-1042.

Quek S. Y., Chok, N. K., Swedlund, P. (2007). The physiochemical properties of spray-dried watermelon powders. *Chemical Engineering and Processing*, 46: 386-392.

Reineccius, G. A. (2001). Multiple-core encapsulation – the spray drying of food ingredients. In: Vilstrup, P. (Ed.) (pp. 151-185), Microencapsulation of Food Ingredients. England: Leatherhead Publishing Surrey.

Reineccius, G. (2005). Flavor Chemistry and Technology, (2nd Edn)(pp. 351-384). Boca Raton: CRC Press.

Ribeiro, M. H. L., Afonso, C., Real, H. J. V., Alfaia, A. J., Ferreira, L. (2010). Contribution of response surface methodology to the modelling of naringin hydrolysis by naringinase Ca-alginate beads under high pressure. *Food Science and Technology*, 43: 482-487.

Richter, T. (2004). Atomization of liquids (pp. 105-109). Germany: Expert Verlag, Renningen.

Rodriguez-Hernandez, G. R., Gonzalez-Garcia, R., Grajales-Lagunes, A., Ruiz-Cabrera, M. A. (2005). Spray-drying of cactus pear juice (*Opuntia streptacantha*): effect on the physicochemical properties of powder and reconstituted product. *Drying Technology*, 23 (4): 955-973.

Sablani, S. S., Kasapis, S., Rahman, M. S. (2007). Evaluating water activity and glass transition concepts for food stability. *Journal of Food Engineering*, 78: 266-271.

Saenz, C., Tapia, S., Chavez, J., Robert, P. (2009). Microencapsulation by spray drying of bioactive compounds from cactus pear (*Opuntia ficus-indica*). *Food Chemistry*, 114: 616-622.

Schauss, A. G., Wu, X., Prior, R. L., Ou, B., Huang, D., Owens, J., Agarwal, A., Jenson, G. S., Hart, A. N., Shanborm, E. (2006). Antioxidant capacity and other bioactivities of the freeze-dried amazoniam palm berry, *Euterpe oleracea* Mart (Acai). *Journal of Agricultural and Food Chemistry*, 54 (22): 8604-8610.

Shahidi, F., Han, X. Q. (1993). Encapsulation of food ingredients. *Critical Review in Food Science and Nutrition*, 33: 501-547.

Sharma, S. K., Mulvaney, S. J., Rizvi, S. H. (2000). Food Processing Engineering, Theory and Laboratory Experiments. Canada: John Wiley & Sons Inc.

Socaciu, C. (2008). Food Colorants Chemical and Functional Properties (pp. 57-58, 87-92, 127). Boca Raton: CRC Press.

Spears, K. (1988). Developments in food colourings: the natural alternatives. *Trends in Biotechnology*, 6: 283-288.

Stintzing, F. C., Carle, R., (2004). Functional properties of anthocyanins and betalains in plants, food, and in human nutrition. *Trends in Food Science & Technology*, 15: 19-38.

Stintzing, F. C., Carle, R. (2007). Betalains – emerging prospects for food scientists. *Trends in Food Science & Technology*, 18: 514-525.

Stintzing, F. C., Schieber, A., Carle, R. (2002). Betacyanins in fruits from red-purple pitaya, *Hylocereus polyrhizus* (Weber) Britton & Rose. *Food Chemistry*, 77: 101-106.

Strack, D., Vogt, T., Schliemann, W. (2003). Review. Research advances in betalain research. *Phytochemistry*, 62: 247-269.

Sudheer, K. P., Indira, V. (2007). Post Harvest Technology of Horticultural Crops. India: New India Publishing.

Szejtli, J. (1988). Cyclodextrin Technology. Dordrecht, the Netherlands: Kluwer Academic Publishers.

Timberlake, C. F., Henry, B. S. (1986). Plant pigments as natural food colours. *Endeavour*, New Series, 10: 31-36.

Tonon, R. V., Brabet, C., Hubinger, M. D. (2008). Influence of process conditions on the physicochemical properties of acai (*Euterpe oleraceae* Mart.) powder produced by spray drying. *Journal of Food Engineering*, 88: 411-418.

Toledo, R. T. (2007). Fundamentals of Food Process Engineering (pp.431). USA: Springer Science+Business Media, LLC.

Truong, V., Bhandari, B. R., Howes, T. (2005). Optimization of co-current spray drying process of sugar-rich foods. Part I – Moisture and glass transition temperature profile during drying. *Journal of Food Engineering*, 71: 55-65.

Van't Land, C. M. (2012). Drying in the Process Industry (pp. 133-149). Hoboken, New Jersey: John Wiley & Sons, Inc..

Vos, P. D., Faas, M. M., Spasojevic, M., Sikkema, J. (2010). Encapsulation for preservation of functionality and targeted delivery of bioactive food components. *International Dairy journal*, 20: 292-302.

Walstra, P. (2003). Physical Chemistry of Foods (pp. 261-262). New York: Marcel Dekker, Inc.

Wang, D. S., Torng, C. C., Lin, I. P., Cheng, B. W., Liu, H. R., Chou, C. Y. (2006). Optimization of Nattokinase Production Conduction using response surface methodology. *Journal of Food Process Engineering*, 29: 22-35.

Wang, F. J., Wang, C. H. (2002). Effects of fabrication conditions on the characteristics of etanidazol spray-dried microspheres. *Journal of Microencapsulation*, 4: 495-510.

Wissgott, U., Bortlik, K. (1996). Prospects for new natural food colorants. *Trends in Food Science & Technology*, 7: 298-302.