FORMULATION AND QUALITY EVALUATION OF MARGARINE ENRICHED WITH ANTHOCYANINS EXTRACT FROM ROSELLE

NUR SHAKIRA BINTI SAHAT

A thesis submitted in fulfilment of

requirements for the award of the degree of

Master of Engineering (Bioprocess)

Faculty of Chemical and Energy Engineering

Universiti Teknologi Malaysia

JULY 2017

Specially dedicated to my beloved parents, Sahat bin Daud and S.Rosidah binti Jumat, my siblings and friends for their continuous support, prayers, encouragement and also understanding during my master programme.

ACKNOWLEDGEMENT

In the name of Allah SWT, Most Gracious and Most Merciful. First and foremost, all praise to Allah SWT for his guidance, blessing and grace that give me health and strength to be able to complete this research work and finally come out with this thesis.

Secondly, I would like to take this opportunity to express my deepest gratitude and appreciation to my supervisors; Dr. Dayang Norulfairuz Abang Zaidel and Prof. Dr. Ida Idayu Muhamad for their advice, encouragement and dedicated guidance. Without their advice and consult, this thesis project would not be produced on time.

Furthermore, I would like to express my endless thanks to Bioprocess Engineering Department Laboratory staff and Sime Darby R&D Centre, Pulau Carey officers for providing technical help and guidelines throughout the whole research. They are Mr. Yaakob Sabudin, Mr. Ahmadilfitri Md. Noor, Ms Hidayu Othman, Mr. Mahathir Ismail, Mrs Normala, Mrs Zurainah, Mr. Saiful Azmi and Ms Anis .

To all my friends; Nurul Syafinaz Mohd Daud, Siti Nurazwa Zainol, Norsuhada Abdul Karim, Nor Ayzati, Siti Noorwanie Abdul Hamid, and Nor Azlina, thank you for being with me and for the great memories that we shared together. To all them, I would like to express my sincere gratitude.

Last but not least, I would like to express my earnest appreciation to my beloved parents, family and all people who involved directly and indirectly for their support, understanding and love throughout this meaningful journey.

ABSTRAK

Penghasilan produk menggunakan mikronutrien yang baik dan pewarna semulajadi berbanding pewarna tiruan telah menjadi tarikan baharu dalam industri makanan dan minuman. Objektif kajian ini dijalankan adalah untuk menghasilkan formula marjerin yang mengandungi bahan bioaktif (antosianin) daripada bunga roselle (*Hibiscus sabdariffa L.*) dan mengkaji ciri-ciri kimia, tekstur dan kestabilan marjerin yang dihasilkan. Marjerin telah diformulasikan dengan tiga nisbah pepejal/cecair yang berbeza (88:12. 86:14 dan 84:16). Antosianin yang dimasukkan ke dalam marjerin adalah dalam bentuk kapsul (serbuk) dan tidak berkapsul (cecair). Antosianin dikapsulkan bersama maltodekstrin dengan kaedah gelombang mikro. Antosianin diekstrak menggunakan kaedah pengekstrakan air suling. Jumlah kepekatan antosianin yang diperoleh adalah 135.33±2.0 mg/L. Antosianin berkapsul daripada roselle mengandungi kelembapan yang rendah dan cepat larut di dalam air. Pada suhu 20 °C, semua marjerin menunjukkan peratus lemak pejal (SFC) melebihi 10% dan melebihi 20% pada suhu 25 °C. Pada suhu 30 °C, peratus SFC didapati lebih rendah daripada 5%. Semua marjerin yang diformulasikan mempunyai takat lebur melebihi 28 °C. R12% (adunan lemak 88%: roselle tidak berkapsul 12%) dan R14% (adunan lemak 86%: roselle tidak berkapsul 14%) menunjukkan titik lebur yang stabil sepanjang 12 minggu masa penyimpanan. R12% dan R16 % (adunan lemak 84%: roselle tidak berkapsul 16%) menunjukkan nilai tekstur yang stabil sepanjang 12 minggu penyimpanan pada kedua-dua suhu penyimpanan. Ini menegaskan bahawa R12% dan R16% mempunyai proses pasca pengerasan yang lambat dengan penambahan antosianin. Daripada semua analisis yang dijalankan, marjerin yang terbaik dipilih adalah marjerin tidak berkapsul dengan nisbah pepejal/cecair 88:12 (R12%) manakala marjerin yang diformulasi bersama antosianin berkapsul (RE12%, RE14% dan RE16%) menunjukkan kestabilan yang lebih rendah.

ABSTRACT

There have been increased attractions in the development of food that contains good micronutrients and natural colourants rather than synthetic colourants. The objectives of this study were to formulate a margarine that contains bioactive compound (anthocyanin) from roselle (*Hibiscus sabdariffa L.*) and to study the chemical, texture and stability characteristics of the margarine. The margarine was formulated with three different solid/liquid ratios (88:12, 86:14 and 84:16). The anthocyanins added in the margarine were in the form of encapsulated (powder) and non-encapsulated (liquid). The anthocyanins were encapsulated with maltodextrin by using microwave. Extraction of anthocyanins was performed using distilled water extraction method. Total concentration of anthocyanin obtained for roselle was 135.33±2.0 mg/L. Encapsulated anthocyanins from roselle had low moisture content and soluble fast in water. At 20 ^{0}C, all margarine had more than 10 % solid fat content (SFC) percentages and showed more than 20 % SFC at 25 ^{0}C. At 30^{0}C the SFC percentages was found to be lower than 5 %. The melting points of the margarine formulated were above than 28 ^{0}C. R12 % (fat blend 88 %: roselle non-encapsulated 12 %) and R14 % (fat blend 86 %: roselle non-encapsulated 14 %) showed a stable slip melting point value within 12 weeks storage time. R12 % and R16 % (fat blend 84 %: roselle non-encapsulated 16 %) showed a stable texture values throughout the 12 weeks storage period at both storage temperature. It emphasized that R12 % and R16% had a slow post-hardening process with the addition of anthocyanins. From all the analysis done, the best margarine chosen was non-encapsulated margarine with solid/liquid ratio 88:12 (R12 %) whereas the margarine formulated with encapsulated anthocyanins (RE12 %, RE14 % and RE18 %) showed a slightly lower stability.

TABLE OF CONTENT

CHAPTER	SUBJECT	PAGE
	Title Page	i
	Declaration	ii
	Dedication	iv
	Acknowledgement	v
	Abstrak	vi
	Abstract	vii
	Table Of Content	viii
	List of Tables	xi
	List of Figures	xii
	List of Symbols	xiii
1	**INTRODUCTION**	
	1.1 Background	1
	1.2 Problem Statement	3
	1.3 Objective of Study	4
	1.4 Scope of Study	4
	1.5 Significance of the Study	5
2	**LITERATURE REVIEW**	
	2.1 Introduction	6
	2.2 Margarine	6
	2.2.1.Characteristics of Margarine	9
	2.2.2 Formulation of Margarine	11
	2.2.3 Method of Manufacturing of Margarine	14

2.2.4 Storage and Stability Characteristics of Margarines 16

2.3 Food Colourants 18

2.3.1 Anthocyanin 19

2.3.2 Stability of Anthocyanin 21

2.3.3 Source of Anthocyanins 23

2.3.4 Extraction of Anthocyanins 24

2.4 Background of Roselles 25

2.5 Technologies of encapsulation 30

2.6.1 Microwave-assisted Encapsulation 31

2.6.2 Maltodextrin as Wall Material 33

2.6 Colour Parameter Analysis 35

2.7 Summary of Chapter 2 37

3 METHODOLOGY

3.1 Introduction 38

3.2 Materials and Chemicals 38

3.3 Sample Preparation 40

3.4 Anthocyanins Extraction 40

3.5 Encapsulation of Anthocyanins using Microwave-Assisted Encapsulation Technique 41

3.6 Blend Preparation 41

3.7 Margarine Formulation 41

3.8 Analysis for Extracted Anthocyanins 43

3.8.1 Measurement of Antioxidant Activity 43

3.8.2 Measurement of Total Anthocyanins Content 44

3.8.3 Colour Parameter Analysis 45

3.9 Analysis for Encapsulated Anthocyanins 46

3.9.1 Moisture Content Analysis 46

3.9.2 Dissolution Test 46

3.10 Chemical Analysis for Margarine Formulated 47

3.10.1 Determination of Fatty Acid (FA) Profile by Gas Chromatography (GC) 47

3.10.2 Determination of Triacylglycerol (TAG) Profile by High Performance Liquid Chromatography (HPLC) 48

3.11 Physical Analysis for Margarine Formulated 48

3.11.1 Determination of Solid/Liquid Ratio of Fat or Solid Fat Content (SFC) by pNMR 48

3.12 Texture Analysis for Margarine Formulated (Penetration Analysis) 48

3.13 Statistical Analysis 50

3.14 Summary of Chapter 3 51

4 RESULT AND DISCUSSION

4.1 Introduction 51

4.2 Extraction of Anthocyanins from Roselle 51

4.3 Encapsulation by Using Microwave-assisted Approach 53

4.4 Formulation of Margarine Containing Anthocyanins 54

4.4.1 Preliminary Data of Fat Used 55

4.4.1.1 Fatty Acid Composition Analysis 55

4.4.1.2 Solid Fat Content Analysis 56

4.4.1.3 Slip Melting Point Analysis 58

4.4.2 Binary Blend 59

4.4.2.1 Fatty Acid Composition Analysis 59

4.4.1.2 Solid Fat Content Analysis 61

4.4.1.3 Slip Melting Point Analysis 62

4.4.1.4 Crystallisation Behaviour 63

4.4.3 Tertiary Blend 64

4.4.3.1 Fatty Acid Composition Analysis 64

	4.4.3.2 Solid Fat Content Analysis	66
	4.4.3.3 Slip Melting Point Analysis	67
	4.4.3.4 Crystallisation Behaviour	68
	4.4.4 Summary of Formulation	69
	4.5 Storage and Stability Characteristics of Margarine containing Anthocyanins	70
	4.5.1 Chemical Properties	70
	4.5.1.1 Fatty Acid Composition Analysis	70
	4.5.1.2 Triglycerides (GC) Analysis	72
	4.5.1.3 Free Fatty Acid Analysis	74
	4.5.1.4 Peroxide Value Analysis	76
	4.5.2 Physical Properties	78
	4.5.2.1 Solid Fat Content Analysis	78
	4.5.2.2 Slip Melting Point Analysis	82
	4.5.3 Texture Analysis (Penetration Analysis)	85
	4.6 Summary of Chapter 4	88
5	**CONCLUSION AND RECOMMENDATION**	89
	5.1 Conclusions	89
	5.2 Recommendation	91
	REFERENCES	92

LIST OF TABLES

TABLE NO.	TITLE	PAGE
2.1	Characteristics of hard and soft margarines	10
2.2	Standard Fatty Acid Composition	12
2.3	Fatty Acid Composition of Palm Oil	12
2.4	Fatty Acid Composition of Palm Kernel Oil	13
2.5	Means of Total Antioxidant Activity of Selected Samples and Standard	30
2.6	Moisture Content of Encapsulated Anthocyanins Powder in Different Wall Materials	35
3.1	Formulation Table of Margarine	43
4.1	Physical Properties for Extracted Anthocyanins from Roselle	52
4.2	Physical Properties for Encapsulated Anthocyanins from Roselle using Maltodextrin as Wall Material	53
4.3	Fatty Acid Compositions of Fats Used	55
4.4	Ratio of Binary Blend	59
4.5	Fatty Acid Composition of Binary Blend	60
4.6	Fats Proportion of Tertiary Blends	64
4.7	Fatty Acid Composition of Tertiary Blend	65

LIST OF FIGURES

FIGURE NO.	TITLE	PAGE
2.1	Structural formulas for the cis-fatty acid – oleic acid, and its trans isomer – elaidic acid	7
2.2	Different types of single emulsions	8
2.3	Simplified flow diagram for continuous margarine solidification.	16
2.4	General Anthocyanins Structure	21
2.5	Roselle Plant	27
2.6	Flower of Roselle Plant	28
2.7	FRAP activity in various beverages	29
2.8	Illustration of Microwave Drying System	33
2.9	Framework of CIELAB colour	36
2.10	CIELAB uniform diagram showing lightness L*, chroma C* and hue angle	36
3.1	Flowchart of the Study	39
3.2	Overall margarine making process	42
4.1	Solid Fat Content of Fats Used	57
4.2	Slip Melting Point of Fats Used	58
4.3	Fatty Acid Composition of Binary Blend	60
4.4	Solid Fat Content of Binary Blend	61
4.5	Slip Melting Point Analysis of Binary Blend	62
4.6	Crystallization Behaviour of Binary Blend	63
4.7	Fatty Acid Compositions of Tertiary Blends	66
4.8	Solid Fat Content Analysis of Tertiary Blend	67
4.9	Slip Melting Point of Tertiary Blend	68

4.10	Crystallization Behaviour of Tertiary Blends	69
4.11	Fatty Acid Content (%) margarine formulated for (a) liquid/solid ratio 12% (b) liquid/solid ratio 14% and (c) liquid/solid ratio 16%	71
4.12	Triglycerides Content (%) for margarine formulated (a) liquid/solid ratio 12% (b) liquid/solid ratio 14% and (c) liquid/solid ratio 16%	73
4.13	FFA content of margarines stored for 12 week for three different liquid-to-solid ratio (a) 12%; (b) 14% and (c) 16%.	75
4.14	Peroxide value of margarines stored for 12 week for three different liquid-to-solid ratio (a) 12%; (b) 14% and (c) 16%.	77
4.15	Solid Fat Content (SFC) for Roselle 12% (12%)	79
4.16	Solid fat content (%) at 20^0C for three different liquid/solid ratio (a) 12% (b) 14% and (c) 16% for margarine at 25^0C storage temperature.	80
4.17	Solid fat content (%) at 20^0C for three different liquid/solid ratio (a) 12% (b) 14% and (c) 16% for margarine at 30^0C storage temperature.	81
4.18	Slip Melting Points of margarine formulated with storage temperature at 25 °C (a) liquid/solid ratio 12% (b) liquid/solid ratio 14% (c) liquid/solid ratio 16%.	83
4.19	Slip Melting Points of margarine formulated with storage temperature at 30 °C (a) liquid/solid ratio 12% (b) liquid/solid ratio 14% (c) liquid/solid ratio 16%.	84
4.20	Penetration Analysis of margarine formulated with storage temperature at 25°C (a) solid/liquid ratio 12% (b) solid/liquid ratio 14% and (c) solid/liquid ratio 16%	86
4.21	Penetration Analysis of margarine formulated with storage temperature at 30°C (a) solid/liquid ratio 12% (b) solid/liquid ratio 14% and (c) solid/liquid ratio 16%	87

LIST OF SYMBOLS

β	-	beta
β’	-	Beta prime
α	-	alpha

CHAPTER 1

INTRODUCTION

1.1 Research Background

Margarine has become one of the choices for table spread, bakery, and pastry for many people for reasons of either health or economics (Vaisey-Genser, 2003; Saadi *et al*., 2012). Margarine is a water-in-oil emulsion, in which the oil phase consists of both liquid oil; crystalline fat at room temperature which consists of one liquid being dispersed into another liquid (Ramisetty and Shyamsunder, 2011). The stability of margarine depends on many factors such as the liquid to oil phase ratio, temperature of crystallization, size of water droplets, type of emulsifier, storage temperature, presence of salt or preservative and method of manufacturing.

The appreciation of high and good quality food is a part of life's pleasures. Flavor identification and taste thresholds, influencing food preferences, food acceptability and ultimate food choice can be modulated by colour (Briddle and Timberblake, 1996). Thus, colour acts an ultimate role in enjoyment and gratification of food.

There have been increased attractions in the development of food colourants from natural or natural-derived alternatives as the synthetic pigments are increasingly rejected by the consumer (Stintzing and Carle, 2004). The concern is increasing as the synthetic colourants or synthetic antioxidants have been reported to provide an implication on cardiovascular disease (Nalsen *et al*., 2006). Anthocyanin is one of the example colourant widely used as natural colorant in food industry. The anthocyanins can be widely found as it comprise the largest group of water soluble pigments in the plant kingdom and are especially characteristic of the angiosperms or flowering plants such as cherry, blueberry, apple, acai berry, avocado, guava, kiwifruit, roselle, mango and dragon fruit (Duangmal *et al*., 2004;Ersus and Yurdagel, 2006; Looi, 2008). Anthocyanin from different sources gives different processing and storage stability such as storage temperature, light, pH, concentration, ascorbic acid, sugar and oxygen (Markakis, 1982; Tsai and Huang, 2004; Rein, 2005).

Recently, the biological activities of anthocyanin, such as antioxidant activity, protection from atherosclerosis and anticarcinigenic activity, aphrodisiac properties (Duke and Ducellier, 1993) have been investigated, and shown that anthocyanins can provide some beneficial effects in the treatment of disease such as neuronaland cardiovascular illnesses, cancer and diabetes (Tsai *et al.*, 2002; Lule and Xia, 2005; Nichenametla *et al*., 2006), lower blood pressure and improve the digestive system in humans (Muhammad and Shakib, 1995). As the consequences, anthocyanin as food colourant is becoming increasingly important not only do they contribute to the aesthetic value but also they tend to yield potential positive health effects. Several authors have confimed that *Hibiscus Sabdariffa L.*is a good source of dietary antioxidants, with its calyces containing amounts of anthocyanins as high as $2.5g^{-1}$ DW (Aurelio *et al*., 2008; Juliani *et al*., 2009). So, addition of roselle in margarine formulated is the research serves as added value in the food properties.

1.2 Problem Statement

Margarine sometimes may not stable at room temperature. There are many factors that may affect the stability of margarine such as the liquid to oil phase ratio, addition of ingredients in formulation or method of manufacturing. Addition of anthocyanin will provide a healthy product and attractive preference in term of additional colour to the margarine. However, anthocyanins as natural food colorants are not stable in food products (Duangmal *et al*., 2004). The stability of anthocyanins is affected by several factors such as pH, storage temperature, chemical structure, concentration, light, oxygen, solvents, copigmentation and thin film effects, the presence of enzymes, flavonoids, proteins and metallic ions (Rein, 2005; Markakis, 1982). The formulation of margarine containing anthocyanin is challenging as the addition of anthocyanin may alter the stability of the margarine because it may affect the particle size of emulsion. The stability and storage characteristics of margarine formulated can be achieved by a good formulation of margarine. The margarine that will be formulated is a healthy margarine because the oil blend used is natural vegetable oil. In a nutshell, this product has a high potential to prevent cardiovascular, act as anti-aging and can provide goodness for eye, skin, hair and nails. Currently, there are no products in market similar to margarine formulated with anthocyanin specifically with roselle. The product will provide more choices of healthy food with lower cost to the consumer.

1.3 Objective of the Study

The objectives of this study are

1. To formulate a stable margarine containing anthocyanins from Roselle (*Hibiscus sabdariffa L.*).
2. To study the texture, storage and stability characteristics of the margarine produced.

1.4 Scopes of the Study

The scopes of this study encompass:

1. Extraction of anthocyanins from Roselle calyces using hot water extraction method.
2. Encapsulation of anthocyanins from Roselle using microwave-assisted technique.
3. Formulation of margarine containing non-encapsulated anthocyanins and encapsulated anthocyanins from Roselle calyces with three different oil-to-aqueous ratio starting at 16% with increments of 2%.
4. Investigation of texture, storage and stability characteristics of the margarine containing anthocyanins by using its chemical and physical composition.

1.5 Significance of Study

This study investigates the formulation of stable margarine containing anthocyanin from Roselle (*Hibiscus sabdariffa L.*). As the addition of anthocyanin may alter the stability of margarine, the optimum amounts of anthocyanin that can be formulated with the margarine are determined. It is believed that the commercial value of the margarine will keep on increasing due to the content of anthocyanin as natural colourants as well as its antioxidant activity.

REFERENCES

Abbasi, S. and Rahimi. S. (2008). Microwave-assisted Encapsulation of Citric Acid using Hydrocolloids. *International Journal of Food Science and Technology*. 43, 1226-1232.

Aini, I. N., and Miskandar, M. S. (2007). Utilization of Palm Oil and Palm Products in Shortenings and Margarines. *Journal of Lipid Science Technology*. 109: 422-432.

Akhtar, M., Stenzel J., Murray, B. S. and Dickinson, E. (2005). Factors Affecting the Perception of Creaminess of Oil-in-Water Emulsions. *Food Hydrocolloids*. 19, 521-526.

Andreasan, M. F., Christensen, L. P., Meyer, A. S., and Hansen, A. (2000). Content of Phenolic Acids and Ferulic Acid Dehydrodimers in 17 Rye (*Secale Cereale L*.) Varieties. *Journal of Agriculture and Food Chemistry*. 48: 2837-2842.

Astorg, P., (2005). Dietary fatty acids and colorectal and prostate cancer:epidemiological studies. Bull.*Cancer* 92,670–684.

Balami, A. (1998). The effect of processing conditions, packaging and storage on selected quality attributes of Mungza Ntusa (M.Sc. thesis). Nigeria: University of Ibadan.

Beltran-Orozco, M. C., Oliva-Coba, T. G., Gallardo-Velazquez, T. and Osorio-Revilla, G. (2009). Ascorbic Acid, Phenolic Content, and Antioxidant Capacity of Red, Cherry, Yellow and White Types of Pitaya Cactus Fruit (*Stenocereus stellatus* Riccobono). *Agrociencia*. 43, 153-162.

Binks, B. P. (2002). Particles as Surfactants- Similarities and Differences. *Current Opinion in Colloid & Interface Science 7*. 7: 21-41.

Bridle, P., and Timberlake, C. F. (1996). Anthocyanins as Natural Food Colours-Selected Aspects. *Food Chemistry*. 58: 103-109.

Brouillard, R., and Dangles, O. 1994. Anthocyanin molecular interactions - the first step in the formation of new pigments during wine aging. *Food Chemistry* 51: 365-371.

Bongers, P., and Almeida-Rivera, C. (2011). Dynamic modelling of the margarine production process. Computer Aidded Chemical Engineering, 29, 1301-1305.

Calliste, C. A., Trouillas, P., Allais, D.-P., Simon, A., & Duroux, J.-L. (2001). Free radical-scavenging activities measured by electron spin resonance spectroscopy and b16 cell antiproliferative behaviors of seven plants. *Journal of Agricultural and Food Chemistry,* 49, 3321–3327

Carr R. A. and Vaisey-Genser M. (1993) Methods of Manufacture. *Encyclopaedia of Food Science,Food Technology and Nurtition,,* Academic Press, 3709-3714.

Charcosset, C. (2009). Review. Preparation of Emulsions and Particles by Membrane Emulsification for the Food Processing Industry. *Journal of Food Engineering*. 92, 241-249.

Cheong, L.ZI, Tan, Chin-Ping, Long, K., Yusoff, M.S., Lai, O.M. (2009). Physicochemical, Textural and Viscoelastic Properties of Palm Diacylglycerol Bakery Margarine during Storage. Journal Am Oil Chemical Society, 86:723-731.

Chi, H., Xu, K., Xue, DH. Song, CL., Zhang, WD., Wang, PX. (2007). Synthesis of Dodecenyl Succinic Anhydride (DDSA) Corn Starch. *Food Research International,* 40 (2), 232-238.

Chiaramonti, D., Bonini, M., Fratini, E., Tondi, G., Gartner, K., Bridgwater, A. V., Grimm, H. P., Soldaini, I., Webster, A., and Baglioni, P. (2003). Development of Emulsions from Biomass Pyrolysis Liquid and Diesel and Their Uses in Engines- Part 1: Emulsion Production. *Biomass & Bioenergy*. 25: 85-99.

Chu, Y., Sun, J., Wu, X., & Liu, R. H. (2002). Antioxidant and antiproliferative activities of common vegetables. *Journal of Agricultural and Food Chemistry,* 50, 6910–6916.

Cisse, M., Vaillant, F., Pallet D. and Dornier, M. (2011). Selecting Utrfiltration and Nanofiltration Membranes to Concentrate Anthocyanins from Roselle Extract (*Hibiscus Sabdariffa* L.). *Food Research International*. 44, 2607-2614.

Cissouma, A. I., Tounkara, F., Nikoo, M., Yang, Na. and Xu, X. (2013). Physico-chemical Properties and Antioxidant Activity of Roselle Seed Extracts. *Advance Journal of Food Science and Technology*. 5 (11), 1483-1489.

Constantino, L.; Albasino, A.; Rastelli, G.; Benvenuti, S. Activity of polyphenolic crude extracts as scavengers of superoxide radicals and inhibitors of xanthine oxidase. *Planta Med.* 1992, 58, 342-344.

Dangles, O., Elhabiri, M., and Brouillard, R. 1994. Kinetic and thermodynamic investigation of the aluminium-anthocyanin complexation in aqueous solution. *Journal of American Chemical Society* - Perkin Transactions II 12: 2587-2596.

Dangles, O., Saito, N., and Brouillard, R. 1993. Anthocyanin intramolecular copigment effect. Phytochemistry 34: 119-124.Dembitsky, V.M., Poovarodom, S., Leontowicz, H., Leontowicz, M., Vearasilp, S., Trakhtenberg, S. and Gorinstein, S. (2011). The Multiple Nutrition Properties of Some Exotic Fruit: Biological Activity and Active Metabolites. *Food Research International*. 44, 1671-1701.

Dollimore, D. (1996). Thermal Analysis. *Analytical Chemistry*. 68, 63-71.

Duangmal, K., B. Saicheuaa and S. Sueeprasan. (2004). Roselle Anthocyanins as a Natural Food Colourants and Improvement of its Colour Stability. *AIC 2004 Colour and Paints, Interim Meeting of the International Colour Associations*. pp.155-158.

Duke, J.A. and Ducellier, J.L. (1993).*Handbook of Alternative Cash Crops.CRC Press*. pp. 433-434.

Duttaroy, A. K., & Jorgensen, A. (2004): Effects of kiwi fruit consumption on platelet aggregation and plasma lipids in healthy human volunteers. Platelets, 15(5): 287–292.

Eckel,R.H., Borra,S., Lichtenstein,A.H., and Yin-Piazza,S.Y. (2007). Understanding the complexity of trans fatty acid reduction in the American diet. *Circulation* 115, 2231–2246.

Ersus, S. and Yurdagel, U. (2006). Microencapsulation of Anthocyanin Pigments of Black Carrot (Daucuscarota L.) by Spray Drier. *Journal of Food Engineering.* 80, 805-812.

Figueiredo, P., Elhabiri, M., Toki, K., Saito, N., & Brouillard, R. (1996b). Anthocyanin intramolecular interactions. A new mathematical approach to account for the remarkable colorant properties of the pigments extracted from Matthiola incana. *Journal of the American Chemical Society*, 118, 4788–4793.

Fomuso LB, Akoh CC (2001) Enzymatic modification of high-laurate canola to produce margarine fat. *J Agric Food Chem* 49, 4482-4487.

Frasch-Melnik, S., Norton, I. T. and Spyropolous, F. (2010). Fat-Crystal Stabilized W/O Emulsions for Controlled Salt Release. *Journal of Food Engineering*. 98, 437-442.

Ghosh, S. and Rousseau, D. (2011). Fat Crystals and Water-In-Oil Emulsion Stability. *Current Opinion in Colloid & Interface Science*. 16, 421-431.

Giusti, M. M., & Wrolstad, R. E. (2003). Acylated anthocyanins from edible sources and their applications in food systems. *Biochemical Engineering Journal*, 14, 217–225.

Gunes, G., Liu, R. H., & Watkins, C. B. (2002). Controlledatmosphere effects on postharvest quality and antioxidant activity of cranberry fruits. *Journal of Agricultural and Food Chemistry*, 50, 5932–5938.

Haji-Faraji M., and Haji-Tarkhani A. H. (1999). The effect of sour tea (Hibiscus sabdariffa) on essential hypertension. *Journal of Ethnopharmacology* 65 (1999) 231–236.

Hamm, W. (1995). Trends in Edible Oil Fractionation. *Trends in Food Science & Technology*. 6 : 121-126.

Heinonen, I. M., Meyer, A. S., and Frankel, E. N. (1998). Antioxidant Activity of Berry Phenolics on Human Low-Density Lipoprotein and Liposome Oxidation. *Journal of Agriculture and Food Chemistry*. 46: 4107-4112.

Herrera-Arellano, A., Miranda-Sanchez, J., Avila-Castro, P., Herrera-Alvarez, S., Jimenez-Ferrer, J. E., Zamilpa, A., et al. (2007). Clinical effects produced by a standardized herbal medicinal product of Hibiscus sabdariffa on patients with hypertension. A randomized, double-blind, lisinopril-controlled clinical trial. *Planta Medica*, 73(1), 6–12

Hirunpanich, V., Utaipat, A., Morales, N. P., Bunyapraphatsara, N., Sato, H., Herunsale, A., et al. (2006). Hypocholesterolemic and antioxidant effects of aqueous extracts from the dried calyx of Hibiscus sabdariffa L. in hypercholesterolemic rats. *Journal of Ethnopharmacology*, 103(2), 252–260.

Horozov T. S. and Binks B. P. (2006) Stability of suspensions, emulsions, and foams studied by a novel automated analyzer. *Langmuir*, 20, 9007

Hoshino, T., & Tamura, H. (1999). Anthocyanidin glycosides: color variation and color stability. In R. Ikan (Ed.), *Naturally occurring glycosides* (pp. 43–82). New York/Weinheim: John Wiley & Sons.

Hui, K.H. (2009). *Microwave-assisted Encapsulation of Anthocyanins from Roselle (Hibicus Sabdarifa L.) and its Thermal Stability Analysis*. MSc Thesis. Universiti Teknologi Malaysia, Skudai.

Kahkonen MP, Hopia AI, Heinonen M. (2001). Berry phenolics and their antioxidant activity. *J Agric Food Chem* 49: 4076- 4082.

Karleskind, A. (1992). Manual fats (vol. 1). Edition: Technology and Document (1st ed.). 65-112,318-617.

Kirdpon, S., Nakorn, S. N., & Kirdpon, W. (1994). Changes in urinary chemical composition in healthy volunteers after consuming roselle (Hibiscus sabdariffa Linn.) juice. *Journal of the Medical Association of Thailand*, 77(6), 314–321.

Knoth A., I. Scherze and G. Muschiolik. (2005). Stability of Water-in-Oil-Emulsions Containing Phosphatidylcholine-Depleted Lecithin. *Food Hydrocolloids.*19, 635-640.

Kong, J., Chia, L., Goh, N., Chia, T., and Brouillard R. (2003). Analysis and Biological Activities of Anthocyanins. *Phytochemistry*. 64: 923-933.

Konczak I. and Zhang W. (2004) Anthocyanins-more than nature´s colours. Journal of Biomedicine and Biotechnology, 5, pp. 239–240.

Lai, O. M., Ghazali, H. M., Cho, F., and Chong, C. L. (2000). Physical Properties of Lipase-catalyzed Transesterified Blends of Palm Stearin and Anhydrous Milk Fat. *Food Chemistry.* 70: 215-219.

Lim, Y. Y., Lim, T. T., and Tee, J. J. (2007). Antioxidant Properties of Several Tropical Fruits: A Comparative Study. *Food Chemistry*. 103: 1003-1008.

Liu, R. H. (2004). Potential Synergy of Phytochemicals in Cancer Prevention: Mechanism of Action. *International Research Conference on Food, Nutrition, and Cancer*. 15-16 July. Washington, DC. 3479S-3485S.

Liu, M., Li, X. Q., Weber, C., Lee, C. Y., Brown, J., & Liu, R. H. (2002). Antioxidant and antiproliferative activities of raspberries. *Journal of Agricultural and Food Chemistry*, 50, 2926–2930.

Looi, M.L. (2008). *Nano Encapsulation of Anthocyanins from Brassica Oleracea*. MSc Thesis. Universiti Teknologi Malaysia, Skudai.

Lule, S. U., and Xia, W. (2005). Food Phenolics, Pros and Cons: A Review. *Food Review International*. 21: 367-388.

Markakis, P., G. E. Livingstone, and G. R. Fillers. (1957). Quantitative Aspects of Strawberry Pigments Degradation. *Food Research*, 22, 117-130.

McClements, D. J., Decker, E. A., and Weiss, J. (2007). Emulsion-Based Delivery Systems for Lipophilic Bioactive Components. *Journal of Food Science.* 72 (8): 109-124.

Merken, H. M., & Beecher, G. R. (2000). Measurement of food flavonoids by high performance liquid chromatography: A review. *Journal of Agricultural and Food Chemistry,* 48(3), 577–599.

Meyers, K. J., Watkins, C. B., Pritis, M. P., and Lin, R. H. (2003). Antioxidant and Antiproliferative Activities of Strawberries. *Journal of Agricultural and Food Chemistry.* 51: 6887-6892.

Ming, L. O., Ghazali, H. M. and Let, C. C. (1999). Use of Enzymatic Transesterified Palm Stearin-Sunflower Oil Blends in the Preparation of Table Margarine Formulation. *Food Chemistry*. 64, 83-88.

Mohamed, R., Fernandez, J., Pineda, M. and Aguilar, M. (2007). Roselle (*Hibiscus Sabdariffa*) Seed Oil is Rich Source of γ-tocopherol. *Journal of Food Science*. 72(3), 207-211.

Mohd-Esa, N., Hern, F. S., Ismail, A. and Yee, C. L. (2010). Antioxidant Activity in Different Parts of Roselle (*Hibiscus Sabdariffa* L.) Extracts and Potential Exploitation of the Seeds. *Food Chemistry*. 122, 1055-1060.

Morton, J.F., 1987. Roselle. In: Morton, J.F. (Ed.), *Fruits of Warm Climates*. Miami, FL.

Mozaffarian, D., Clarke, R. (2009). Quantitative effects on cardiovascular risk factors and coronary heart disease risk of replacing partially hydrogenated vegetable oils with other fats and oils. *Eur. J. Clin. Nutr*. 63, S22–S33.

Murkovic M (2003) Phenolic compounds. In: Caballero B, Trugo C, Finglas PM (eds) *Encyclopedia of food sciences and nutrition*, 2nd edn. Academic, Amsterdam, pp 4507–4514

Murthy, K. N. C., Singh, R. P., and Jayaprakasha, G. K. (2002). Antioxidants Activity of Grape (Vitis Vinifera) Pomace Extracts. *Journal of Agricultural and Food Chemistry*. 50: 5909-5914.

Nichenamentla, S. N., Taruscio, T. G., Barney, D. L., and Exon, J. H. (2006). A Review of the Effects and Mechanisms of Polyphenolics in Cancer. *Critical Reviews in Food Science and Nutrition*. 46: 161-183.

Nilsson, L., and Bergenstahl, B. (2007). Emulsification and Adsorption Properties of Hydrophobically Modified Potato and Barley Starch. Journal of Agricultural and *Food Chemistry.* 55: 1469-1474.

Pande, G. and Akoh, C. C. (2013). Enzymatic Synthesis of *Trans*-Free Structured Margarine Fat Analogs with High Stearate Soybean Oil and Palm Stearin and Their Characterization. *LWT-Food Science and Technology*. 50, 232-239.

Pande, G., Akoh, C. C. and Shewfelt, R. L. (2012). Utilization of Enzymatically Interesterified Cottonseed Oil and Palm Stearin-Based Structured Lipid in the Production of Trans-Free Margarine. *Biocatalyst and Agriculture Biotechnology*. 1-9.

Pantzaris (1987) liquid fraction of palm oil

Pazmino-Duran, E. A., Giusti, M. M., Wrolstad, R. E., and Gloria, M. B. A. (2001). Anthocyanins from Banana Bracts (*Musa X Paradisiaca*) as Potential Food Colourants. *Food Chemistry*. 73: 327-332.

Prasongwatana, V., Woottisin, S., Sriboonlue, P. and Kukongviriyapan, V. (2008). Uricosuric Effect of Roselle (*Hibiscus Sabdariffa*) in Normal and Renal-Stone Former Subjects. *Journal of EthnoPharmacology*. 117, 491-495.

Pokorny, J. (2007). Are Natural Antioxidants Better- and Safer- than Synthetic Antioxidants?. *Journal of Lipid Science Technology*. 109: 629-642.

Qi, Y., Chin, K. L., Malekian, F., Berhane, M. and Gager, J. (2005). Biological Characteristics, Nutritional and Medicinal Value of Roselle, *Hibiscus Sabdariffa.Agricultural Research and Extension Centre.* Cicular UFNR, No. 604.

Ramisetty, K.A. and Shyamsunder, R. (2011). Effect of Ultrasonication on Stability of Oil in Water Emulsions. *International Journal of Drug Delivery*. 3, 133-142

Rao, R., K.U. Sankar, K. Sambaiah, andB.R. Lokesh. (2001). Differential Scanning Calorimetric Studies on Structured Lipids from Coconut Oil Triglyceride Containing Stearic Acid. *European Food Research Technology*. 212, 334-343.

Redus, M., Baker, D. C., & Dougall, D. K. (1999). Rate and equilibrium constants for the dehydration and deprotonation reactions of some monoacylated and glycosylated cyanidin derivatives. *Journal of Agricultural and Food Chemistry*, 47, 3449– 3459.

Rein, M. (2005). *Copigmentation Reactions and Color Stability of Berry Anthocyanins.* Ph. D. Thesis. University of Helsinki.

Rodriguez Montealegre, R., Romero Peces, R., Chacon Vozmediano, J. L., Martinez

Gascuena, J., & Garcia Romero, E. (2006). Phenolic compounds in skins and seeds of ten grape Vitis vinifera varieties grown in a warm climate. *Journal of Food Composition and Analysis*, 19, 687± 693.

Rousseau, D. and Hodge, S. M. (2005). Stabilization of Water-in-Oil Emulsions with Continuous Phase Crystals. *Colloids and Surfaces*. 260, 229-237.

Saadi, S., AriffinA. A.,Ghazali, H. M., Abdulkarim, M. S., Huey C. B. and Miskandar, M. S. (2012). Crystallisation Regime of W/O Emulsion [E.g. Multipurpose Margarine] Models During Storage. *Food Chemistry*, 133, 1485-1493**.**

Shui, G., and Leong, L. P. (2006). Residue from Star Fruit as Valuable Source for Functional Food Ingredients and Antioxidant Nutraceuticals. *Food Chemistry*. 97: 277-284.

Stinzting, F. C., Schieber, A. and Carle, R. (2002). Betacyanins in Fruit from Red-Purple Pitaya, *Hylocereus Polyrhizus* (Weber) Britton & Rose. *Food Chemistry*. 77, 101-106.

Stinzting, F. C. and Carle, R. (2004). Fuctional Properties of Anthocyanins and Betalains In Plants, Food, and In Human Nutrition. *Trends in Food Science and Technology*. 15, 19-38.

Su, F., Bray, C. L., Carter, B. O., Overend, G., Cropper, C., Iggo, J. A., Khimyak, Y. Z., Fogg, A. M., Cooper, A. I. (2009). Reversible Hydrogen Storage in Hydrogel Clathrate Hydrates. *Advance Material*. 21: 2382-2386.

Surh, J., Vladisavljevic G. T., Mun, S., and McClements, D. J. (2007). Preparation and Characterization of Water/Oil and Water/Oil/Water Emulsions Conatining Biopolymer-Gelled Water-Droplets. *Journal of Agricultural and Food Chemistry*. 55: 175-184.

Tan, Y., Xu, K., Niu, C., Liu, C., Li, Y. and Wang, P. (2014). Triglyceride-Water Emulsions Stabilized by Starch-Based Nanoparticles. *Food Hydrocolloids*. 36, 70-75.

Thanasukarn, P., Pongsawatmanit, R., and McClements D. J. (2004). Impact of Fat and Water Crystallization on The Stability of Hydrogenated Palm Oil-in-Water Emulsions Stabilized by Whey Protein Isolates. *Colloids and Surfaces*. 246, 49-59.

Tsai, P. and Huang, H. (2004). Effect of Polymerization on The Antioxidant Capacity of Anthocyanins in Roselle. *Food Research International*. 37, 313-318.

Tsai, P., McIntosh, J., Pearce, P., Camden, B. and Jordan, B. R. (2002). Anthocyanins and Antioxidant Capacity in Roselle (*Hibiscus Sabdariffa* L.) Extract. *Food International Research*. 35, 351-356.

Tseng, T., Kao, T., Chu, C., Chou, F., Lin, W. and Wang, C. (2000). Induction of Apoptosis by *Hibiscus* Protocatechuic Acid in Human Leukemia Cells via Reduction of Retinoblastoma (RB) Phosphorylation and Bcl-2 Expression. *Biochemical Pharmacology*. 60, 307-315.

Vaisey-Genser,M. (2003). *Composition and Analysis of Margarine*. The University of Manitoba: Elsevier Science Ltd., pp. 3714-3719.

Vaisey-Genser,M. and Carr R.A.(2003). *Methods of Manufacture Margarine*. The University of Manitoba: Elsevier Science Ltd. pp. 3709-3714.

Vauzour, D., Vafeiadou, K., Rodriguez-Mateos, A., Rendeiro, C., and Spencer, J. P. E. (2008). The Neuroprotective Potential of Flavonoids: a Multiplicity of Effects. *Genes Nutrition*. 3: 115-126.

Vladisavljevic, G. T. and Williams, R. A. (2005). Recent Developments in Manufacturing Emulsions and Particulate Products Using Membranes. *Advances in Colloid and Interface Sciences*. 113, 1-20.

Walkowiak-Tomczak, J.C. 2007. Colour changes of a preparation from red cabbage during storage in a model system. *Food Chemistry* 104: 709-714.

Wang W.L., Morris B., Tonnis B., Davis J., and Pederson G. A. (2012). Assessment of oil content and fatty acid composition variability in two economically important *Hibiscus* species. *Journal of Agricultural and Food Chemistry*, 60 (26) (2012), pp. 6620–6626

Weiss, J., Decker, E. A., McClements, D. J., Kristbergsson, K., Helgason, T., and Awad, T. (2008). Solid Lipid Nanoparticles as Delivery Systems for Bioactive Food Components. *Food Biophysics*. 3: 146-154.

Wiederman, L. H. (1978). Margarine and Margarine Oil, Formulation and Control. *Journal of the American Oil Chemists' Society*. 55: 823-828.

Wolfe, K., Wu, X., & Liu, R. H. (2003). Antioxidant Activity of Apple Peels. *Journal of Agricultural and Food Chemistry*, 51, 609–614

Wu, L., Hsu, H., Chen, Y., Chiu, C., Lin, Y. and Ho, J. A. (2006). Antioxidant and Antiproliferative Activities of Red Pitaya. *Food Chemistry*. 95, 319-327.

Wybraniec, S. and Mizrahi, Y. (2002). Fruit Flesh Pigments in *Hylocereus Cacti*. *Journal of Agriculture and Food Chemistry*. 50, 6086-6089.

Yang, J., Guo, J., and Yuan, J. (2008). In Vitro Antioxidant Properties of Rutin. *Food Science and Technology*. 41: 1060-1066.

Young, D. K. and C.V. Morr. (1996). Microencapsulation Properties of Gum Arabic and Several Food Proteins: Spray-Dried Orange Oil Emulsion Particles. *Journal of Agriculture and Food Chemistry*. 44, 1314-1320.

Zadernowski, R., Czaplicki, S. and Nacz, M. 2009. Phenolic acid profiles of mangosteen fruits (*Garcinia mangostana*). *Food Chemistry* 112: 685-689

Żbikowska A., Wirkowska M., and Kaźmierczyk M. (2007). Quality of margarines marketed in Warsaw. *Tłuszcze Jadalne*, 42 (in Polish;English abstract).

Zhang, H., Jacobsen, C. and Adler-Nissen, J. (2005). Storage Stability Study of Margarine From Enzymatically Interesterified Fats Compared to Margarines Produced by Conventional Methods: I. Physical Properties. *Journal of Lipid Science Technology*. 107, 530-539.