



UNIVERSITI PUTRA MALAYSIA

**PREPARATION, CHARACTERIZATION AND STABILITY
EVALUATION OF ASTAXANTHIN NANODISPERSIONS**

NAVIDEH ANARJAN KOUCHEHBAGH

FSTM 2012 2

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NANODISPERSIONS**

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**DOCTOR OF PHILOSOPHY
UNIVERSITI PUTRA MALAYSIA**

2012

**PREPARATION, CHARACTERIZATION AND STABILITY EVALUATION
OF ASTAXANTHIN NANODISPERSIONS**



By

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**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

January 2012

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

PREPARATION, CHARACTERIZATION AND STABILITY EVALUATION OF ASTAXANTHIN NANODISPERSIONS

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January 2012

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Faculty : Food Science and Technology

Incorporating functional lipid nutraceuticals, such as carotenoids, which suffer from poor water solubility and low bioavailability, into nano-sized delivery systems, such as nanodispersions, can strongly improve the lipid nutraceuticals' solubility, stability, and bioavailability.

In this study, stable astaxanthin nanodispersions were prepared and characterised. Response-surface methodology was employed to investigate the effects of applied pressure (20-90 MPa), the number of cycles (0-4) in the homogeniser, and the evaporation temperature (16-66°C) on the mean particle size, polydispersity index (PDI) and astaxanthin concentration of polysorbate 20 (PS20)- and sodium caseinate (SC)- stabilised astaxanthin nanodispersion systems. On the basis of this multiple-optimisation procedure, the optimum processing conditions were predicted to be 50 MPa, 2 cycles, and 47°C for a PS20-stabilised nanodispersion system and 30 MPa, 3 cycles, and 25°C for a SC-stabilised nanodispersion system.

To evaluate the formulation parameters, PS20 and gum Arabic (GA) were selected through screening evaluations, and SC was selected based on the literature. A

simplex centroid mixture design was used to develop a three-component stabilising system to produce nanodispersions with minimal particle size, PDI, and astaxanthin loss and maximal physical and chemical stabilities. The multiple-response optimisation results predicted that a stabiliser system composed of 29% w/w PS20, 6% w/w GA and 65% w/w SC would produce astaxanthin nanodispersions with the most desirable physicochemical and stability characteristics. Another simplex centroid mixture design was employed to study the effects of the organic phase in the formation and characteristics of astaxanthin nanodispersions. Accordingly, dichloromethane (DCM) and acetone (ACT) were selected as organic phase components. The results predicted that an organic phase with 38% w/w DCM and 62% w/w ACT would be most appropriate for the production of astaxanthin nanodispersions. Subsequently, the astaxanthin concentrations (0.02–0.38% w/w), stabiliser concentrations (0.2–3.8% w/w) and organic phase (dichloromethane) concentrations (2–38% w/w) were optimised using response surface methodology and a response optimiser. Overall, optimum conditions for obtaining stable astaxanthin nanodispersions were obtained by combining 0.08% w/w astaxanthin, 2.5% w/w stabiliser and 11.5% w/w organic phase. Such optimally formulated astaxanthin nanodispersions also showed the most desirable characteristics in terms of flow behaviour, cellular uptake, colour, antioxidant activity and microstructure compared with nonoptimised astaxanthin nanodispersions.

Stability studies were performed on these optimally produced astaxanthin nanodispersions while varying the heat treatments, pH and concentrations of ions, and these studies confirmed that these astaxanthin nanodispersions were more stable compared with nonoptimised nanodispersions. In addition, although the astaxanthin

contents of our prepared nanodispersions were decreased significantly ($p < 0.05$) during 8 weeks of storage at different temperatures, luminances and atmospheres, the optimally formulated nanodispersions showed less rapid astaxanthin loss and higher chemical stability compared with other nanodispersions.

As a result of these optimisation methods, the high chemical stability of astaxanthin nanodispersions in real food systems (orange juice and skimmed milk) has proven their suitability as functional ingredients in a wide range of food, feed (via the aquaculture industry), pharmaceutical, cosmetic and personal-care product formulations.

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

**PENYEDIAAN, PENCIRIAN, DAN ANALISA KESTABILAN SERAKAN
NANO ASTAXANTHIN**

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Penyediaan sistem lemak fungsian nutraseutikal seperti karotenoid yang mempunyai keterlarutan air dan ketersediaan bio yang rendah, dalam bentuk sistem penghantaran bersaiz nano seperti serakan nano telah terbukti dapat meningkatkan keterlarutan, kestabilan, dan ketersediaan bio sistem tersebut. Dalam kajian ini, serakan nano astaxanthin yang stabil telah disediakan dan ciri-cirinya telah diselidik. Kaedah tindakbalas permukaan telah digunakan untuk menganalisa kesan tekanan yang dikenakan (20-90 MPa) dan bilangan kitaran (0-4) di dalam penghomogenasi, serta kesan suhu penyejatan (16-66°C) ke atas purata saiz partikel, indeks kepoliserakan (PDI); dan ke atas kepekatan astaxanthin di dalam sistem serakan nano astaxanthin yang distabilkan oleh kedua-dua polisorbate 20 (PS20) dan natrium kaseinat (SC). Kaedah pengoptimuman pelbagai meramalkan 50 MPa, 2 kitaran, dan 47°C sebagai keadaan optima untuk sistem serakan nano yang distabilkan oleh PS20, manakala 30 MPa, 3 kitaran, dan 25°C adalah keadaan pemprosesan optima yang diramalkan untuk sistem serakan nano yang distabilkan oleh SC.

Dalam kajian ini juga, PS20 dan gam arab (GA) telah dipilih melalui kaedah penskrinan, manakala SC telah dipilih berdasarkan kajian-kajian relevan terdahulu.

Tujuan pemilihan ini dilakukan adalah untuk membentuk sistem penstabil yang mengandung tiga komponen dalam menghasilkan serakan nano dengan purata saiz partikel (PDI) dan kehilangan astaxanthin terkecil, di samping kestabilan fizikal dan kimia tertinggi, dengan menggunakan rekabentuk percampuran sentroid simpleks. Kaedah pengoptimuman pelbagai meramalkan bahawa sistem penstabil yang mengandungi 29% berat/berat PS20, 6% berat/berat GA, dan 65% berat/berat SC menghasilkan serakan nano astaxanthin dengan ciri-ciri fizikokimia dan kestabilan yang dikehendaki. Rekabentuk percampuran sentroid simpleks juga telah digunakan sekali lagi untuk mengkaji kesan fasa organik ke atas pembentukan dan ciri-ciri serakan nano astaxanthin. Diklorometan (DCM) dan aseton (ACT) telah dipilih sebagai komponen-komponen fasa organik di dalam kajian ini. Hasil kajian telah mendapati bahawa fasa organik yang mengandungi 38% berat/berat DCM dan 62% berat/berat ACT adalah fasa organik yang paling sesuai untuk penghasilan serakan nano astaxanthin. Seterusnya, kepekatan astaxanthin (0.02–0.38% berat/berat), kepekatan penstabil (0.2–3.8% berat/berat), dan kepekatan fasa organik (DCM) (2–38% berat/berat) telah dioptimumkan dengan menggunakan kaedah tindakbalas permukaan dan pengoptimuman tindakbalas. Keseluruhan rantau optima yang menunjukkan serakan nano astaxanthin yang dikehendaki telah didapati menerusi penggabungan 0.08% berat/berat astaxanthin, 2.5% berat/berat penstabil, dan 11.5% berat/berat fasa organik. Formulasi serakan nano astaxanthin optima itu juga menunjukkan ciri-ciri aliran, serapan ke dalam sel-sel, warna, aktiviti antioksidan, dan mikrostruktur yang dikehendaki berbanding dengan serakan nano astaxanthin yang tidak dioptimumkan.

Kajian penstabilan telah dijalankan ke atas formula serakan nano astaxanthin optima di bawah suhu, pH, dan kepekatan ion yang berbeza-beza. Hasil kajian telah membuktikan bahawa sistem ini memiliki kestabilan yang tinggi berbanding dengan serakan nano yang tidak dioptimakan. Walaupun kandungan astaxanthin di dalam serakan nano yang disediakan menurun secara ketara ($p < 0.05$) sepanjang 8 minggu penyimpanan pada suhu, luminans, dan keadaan atmosfera yang berbeza-beza, namun formula serakan nano optima itu telah menunjukkan kadar kehilangan astaxanthin yang rendah dan kestabilan kimia yang lebih tinggi berbanding dengan serakan-serakan nano yang lain.

Akhirnya, serakan nano optima yang stabil di dalam sesetengah sistem makanan (jus oren dan susu tepung tanpa lemak) telah menunjukkan kebolehannya sebagai bahan tambah fungsian di dalam pelbagai jenis makanan, ubat-ubatan, kosmetik, dan produk-produk penjagaan diri.

ACKNOWLEDGEMENT

First of all, I thank God for allowing me to complete my PhD thesis. I am honored and grateful to have the full support from a number of persons in my life for their commitment during my PhD's degree.

I would like to express my sincere appreciation and gratitude to Associate Professor Dr. Tan chin Ping for his kindness, support and guidance in all throughout my PhD program. I am extremely thankful that he genuinely cared not only about my academic and professional success, but also about my well-being as a person.

Special thanks are expressed to Dr. Seyed Hamed Mirhosseini and Associate Professor Badlishah Sham baharin , the members of my supervisory committee, and also Associate Professor Dr Yoke Kqueen Cheah, for their helpful suggestions throughout my research.

I would also like to thank the faculties and staff of food science and technology, medicine and health science and also the stuff of institute of advanced technology, in particular, Mr Mohd Kadri Masaud the science officer of advanced material and nanotechnology laboratory.

My warmest thanks go also to my parents and husband, Hoda Jafarizadeh Malmiri. I cannot express how much I would like to say thank you to them for their love and supporting me from day one. They have always been there for me no matter what the situation and I am forever in their debt.

Also, many thanks to Masni Mat yusoff , Cheong kok Whye, Thoo Yin Yin and all my lab members. I couldn't have completed this research without their support, friendship and encouragement.



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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TABLE OF CONTENTS

	Page
ABSTRACT	ii
ABSTRAK	v
ACKNOWLEDGEMENTS	viii
APPROVAL	x
DECLARATION	xii
LIST OF TABLES	xvii
LIST OF FIGURES	xx
LIST OF ABBRIVIATIONS	xxvi
CHAPTERS	
1 INTRODUCTION	1
2 LITERATURE REVIEW	5
2.1 Functional foods	5
2.1.1 Carotenoids as functional foods	6
2.1.2 Bioavailability and Solubility of carotenoids	7
2.1.3 Carotenoid market	9
2.1.4 Astaxanthin	11
2.1.5 Increasing the water solubility of functional lipid compounds	20
2.2 Emulsions	24
2.2.1 Theory and Background of Emulsions	24
2.2.2 Emulsion stability	26
2.2.3 Surface active molecules	29
2.2.4 Emulsion classification (based on droplet size)	42
2.3 Nanodispersions	46
2.3.1 Stabilizing mechanisms of nanodispersions	47
2.3.2 Preparation method of nanodispersions	50
2.3.3 Characterization of nanodispersions	57
2.3.4 Applications of nanodispersions	61
	Physical Stability of nanodispersions 65
	Chemical stability of nanodispersions 66
3 EFFECT OF PROCESSING CONDITION ON PHYSICO-CHEMICAL PROPERTIES OF ASTAXANTHIN NANODISPERSIONS	
3.1 Introduction	67
3.2 Materials	69
3.3 Preparation of astaxanthin nanodispersions	70
3.3.1 PS20-stabilized astaxanthin nanodispersions	70
3.3.2 Sodium caseinate -stabilized astaxanthin nanodispersions	71
3.4 Analytical methods	72
3.4.1 Mean droplet size and polydispersity index (PDI)	72
3.4.2 Determination of astaxanthin content	73
3.5 Statistical analysis	74

	3.5.1	Experimental design	74
	3.5.2	Optimization and validation procedures	76
3.6		Results and discussion	77
	3.6.1	PS20-stabilized astaxanthin nanodispersions	77
	3.6.2	SC-stabilized astaxanthin nanodispersions	88
3.7		Optimization and validation procedures for the optimum processing condition	101
3.8		Micro structure analysis using transmission electron (TEM)	105
3.9		Summary	106
4		EFFECT OF FORMULATION PARAMETERS ON PHYSICO-CHEMICAL PROPERTIES OF ASTAXANTHIN NANODISPERSIONS	
	4.1	Introduction	108
	4.2	Materials	113
	4.3	Preparation of astaxanthin nanodispersions	114
	4.3.1	Screening of small molecular emulsifier	114
	4.3.2	Screening the polysaccharides	115
	4.3.3	Optimization the solvent proportions in organic phase mixture	116
	4.3.4	Optimization the selected components' proportions in stabilizing mixture	116
	4.3.5	Optimization the concentrations of astaxanthin, stabilizing mixture and organic phase	117
	4.3.6	Characterization of optimum astaxanthin nanodispersions	119
	4.4	Analytical methods	119
	4.4.1	Average droplet size and polydispersity index (PDI)	119
	4.4.2	Zeta-potential, mobility and conductivity measurements	119
	4.4.3	Determination of astaxanthin content	120
	4.4.4	Viscosity measurement	121
	4.4.5	<i>In vitro</i> tests of carotenoid uptake	121
	4.4.6	Solvent residual measurements using gas chromatography	122
	4.4.7	Color measurement	123
	4.4.8	DPPH assay	124
	4.4.9	ABTS assay	124
	4.4.10	X-ray diffraction (XRD)	125
	4.4.11	TEM	125
	4.5	Statistical analysis	125
	4.5.1	Screening of small molecular emulsifier and hydrocolloids	125
	4.5.2	Optimization the solvent proportions in organic phase mixture and stabilizer mixture component proportions in preparation the astaxanthin nanodispersions	126
	4.5.3	Optimization the concentrations of astaxanthin, stabilizing mixture and organic phase	127

4.6	Results and discussion	128
4.6.1	Performance of selected polysorbates and sucrose esters as emulsifier in preparation of astaxanthin nanodispersions	128
4.6.2	Performance of selected polysaccharides as stabilizer in preparation of astaxanthin nanodispersions	135
4.6.3	The effect of organic phase solvents on physicochemical properties of astaxanthin nanodispersions	144
4.6.4	Developing a three component emulsifier and stabilizer system for producing astaxanthin nanodispersions	156
4.6.5	The Influence of astaxanthin, emulsifier and organic phase concentration on physicochemical properties of astaxanthin nanodispersions	181
4.6.6	Characterization of optimum (OPT) astaxanthin nanodispersions	198
4.7	Summary	217
5	STABILITY OF ASTAXANTHIN NANODISPERSIONS	
5.1	Introduction	220
5.2	Materials	221
5.3	Preparation of astaxanthin nanodispersions	222
5.4	Preparation of simulated gastric fluid (SGF) and simulated intestinal fluid (SIF)	222
5.5	Characterization of astaxanthin nanodispersions	223
5.5.1	Particle size, PDI and zeta-potential measurements	223
5.5.2	Determination of astaxanthin content	223
5.6	Statistical analysis	224
5.7	Results and discussion	224
5.7.1	Effect of pH	224
5.7.2	Effect of Na ⁺ and Ca ²⁺	227
5.7.3	Effect of heat treatment	230
5.7.4	Freeze-thaw stability	233
5.7.5	Stability in SGF and SIF	237
5.7.6	Effect of storage temperature and atmosphere	240
5.7.7	Effect of Light	245
5.8	Summary	247
6	CHEMICAL STABILITY OF OPTIMUM ASTAXANTHIN NANODISPERSIONS IN FOOD SYSTEMS	
6.1	Introduction	249
6.2	Materials	250
6.3	Preparation of astaxanthin nanodispersions	251
6.4	Determination of astaxanthin content	251
6.5	Statistical analysis	251
6.6	Results and Discussion	253

	6.6.1	Effect of Food system	253
	6.6.2	Effect of stabilizer	256
	6.6.3	Effect of dilution factor (astaxanthin concentration)	258
	6.6.4	Effect of additional antioxidant compounds	260
	6.7	Summary	266
7	SUMMARY, CONCLUSION AND RECOMMENDATIONS		269
	REFERENCES		274
	BIODATA OF STUDENT		397
	LIST OF PUBLICATIONS		298
	AWARDS		299
	LIST OF PAPERS PRESENTED AT TECHNICAL MEETINGS		300

