UNIVERSITI PUTRA MALAYSIA

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DOCTOR OF PHILOSOPHY
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# 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

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

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**Chairman : Assoc. Prof. Tan Chin Ping, PhD**

**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 (16-66ºC) 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**

**Oleh**

**NAVIDEH ANARJAN KOUCHEHBAGH**

**Januari 2012**

**Pengerusi : Prof. Madya Tan Chin Ping, PhD**

**Fakulti : Sains dan Teknologi Makanan**

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 (16-66ºC) ke atas purata saiz partikel, indeks kepoliserakan (PDI); dan ke atas kepekatan astaxanthin di dalam sistem serakan nano astaxanthin yang distabilkan oleh kedua-dua polisorbat 20 (PS20) dan natrium kaseinat (SC). Kaedah pengoptima-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 mengandungitiga 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 pengoptima-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 dioptimakan dengan menggunakan kaedah tindakbalas permukaan dan pengoptima 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 antioksida, dan mikrostruktur yang dikehendaki berbanding dengan serakan nano astaxanthin yang tidak dioptimakan.

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.

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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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