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DEVELOPMENT OF A WATER-DISPERSIBLE PHYTOSTEROL NANODISPERSION SYSTEM AND ITS APPLICATION IN SOY MILK STORAGE

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By

LEONG WAI FUN

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DEVELOPMENT OF A WATER-DISPERSIBLE PHYTOSTEROL NANODISPERSION SYSTEM AND ITS APPLICATION IN SOY MILK STORAGE

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Chairman: Associate Professor Tan Chin Ping, PhD

Faculty: Food Science and Technology

This work was aimed to develop a stable water-dispersible phytosterol nanodispersion system. In the first part of this work, the formation and characterization of phytosterol nanodispersions prepared using Tween 20 was investigated. The experiment demonstrated the feasibility of phytosterol nanodispersion production using hexane as organic phase through an emulsification-evaporation technique. The mean particle diameter of phytosterol nanoparticles produced was 50 nm in diameter and had a spherical shape. The dispersed phase ratio, conventional homogenization parameters and the homogenization pressure showed significant ($p < 0.05$) effects on the final phytosterol particles size and their distribution profiles. High-pressure homogenization caused significant phytosterol loss ($p < 0.05$).

Two response surface methodology (RSM) processes were applied to optimize the processing and formulation parameters for preparing phytosterol nanodispersions. The

optimized processing parameters were 15.25 min of mixing time, 7000 rpm of mixing speed and a homogenization pressure of 42.4 MPa. The corresponding responses for the optimized preparation conditions were a mean particle size (PS) of 52 nm and a phytosterol concentration (Phyto) of 336 mg/l. The optimized formulation parameters determined were a phase ratio (PR) of 3.54 and a mixture ratio (MR) of 0.19, and the corresponding optimized responses were a PS of 55.4 nm and 87.6% phytosterol concentration. The PS showed no significant ($p > 0.05$) change over a period of 8 weeks of storage at 4 ºC.

The Tween 20 was replaced by four different types of sucrose fatty acid esters (SEs), namely sucrose palmitate (P-1570), sucrose laureate (L-1695), sucrose steareate (S-1570) and sucrose oleate (OWA-1570). The physicochemical properties of SE-stabilized water-dispersible phytosterol nanodispersions were examined. The PS and the %Phyto of the prepared phytosterol nanodispersions ranged from 2.8 to 259.9 nm And from 230.4 to 504.6 mg/l. All of the prepared phytosterol nanodispersions exhibited pseudoplastic flow behavior, with a low yield stress ranging from 0.630 to 9.183 mPas and a low consistency coefficient of 0.608 to 88.710 mPas. Less than 1.5 ppm of hexane residues in the prepared nanodispersions was detected. Sucrose esters P-1570, L-1695 and S-1570 were found to be appropriate for use in preparing phytosterol nanoparticles with small PS at a monomodal distribution, with high clarity. The high phytosterol-loaded nanodispersions prepared with co-solvents ethanol and L-1695 had small spherical PS of approximately 5 nm, with low viscosity and high clarity. The solvent residue levels in the final prepared nanodispersions were acceptable.

L-1695 was selected for further optimization of the production of L-1695-stabilized water-dispersible phytosterol nanodispersions through RSM. The optimized parameters were 5.5% of Ph (phytosterol concentration), 1.0% of L (L-1695 concentration), 3 C (homogenization cycle), and P(homogenization pressure) of 37 MPa. The corresponding responses for the optimized condition were a PS of 3 nm and a %Ph of 90.4%. The optimized phytosterol nanodispersions had a polydispersity index of 0.550 at a monomodal distribution. The pH value and hexane and ethanol residues concentration were 6.45, 48.2 µl/l and 930.3 µl/l, respectively. The optimized nanodispersions were stable to heat treatment up to 121 °C, chilling at 4 and 10 °C and freezing with a cryoprotectant at – 4 and – 20 °C.

The stability of the optimized phytosterol nanodispersions and phytosterol-fortified soy milk (SMP) over a 12-week period was investigated. The storage resulted in increases in PS and reduced the total phytosterol concentration of the autoclaved phytosterol nanodispersions. Adding phytosterol nanodispersions increased the mean particle size of the soy milk. The fortified phytosterol nanoparticles became entrapped in the fat droplets of the soy milk. The stability of the SMP depended on the stability of the soy milk. The fortification of phytosterol nanodispersions in soy milk was feasible.

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

PEMBANGUNAN NANOSERAKAN FITOSTEROL TERSERAK DALAM AIR DAN APLIKASINYA DALAM PENYIMPANAN SUSU SOYA

Oleh

LEONG WAI FUN

November 2010

Pengerusi: Profesor Madya Tan Chin Ping , PhD

Fakulti: Sains dan Teknologi Makanan

Kajian ini bertujuan untuk membangunkan nanoserakan fitosterol terserak dalam air untuk penggunaan makanan. Bahagian pertama kajian ini melibatkan kajian permulaan untuk menyiasat pembentukan dan perwatakan nanoserakan fitosterol yang disediakan dengan mengunakan Tween 20 sebagai agen pengemulsi. Eksperimen menunjukan kemungkinan penghasilan nanoserakan fitosterol dengan mengunakan pelarut organik heksana melalui kaedah pengemulsian-penyejatan. Diameter purata partikel nanoserakan yang dihasilkan berukuran 50 nm dan berbentuk sfera. Nisbah fasa serakan, parameter penghomogenan biasa dan tekanan penghomogenan menunjukkan kesan ketara ($p < 0.05$) ke atas saiz dan pengagihan partikel fitosterol. Selain itu, penghomogenan bertekanan-tinggi menyebabkan kehilangan fitosterol yang ketara ($p < 0.05$).

Due kaedah permukaan gerak balas (RSM) telah digunakan untuk pengoptimuman parameter pemprosesan dan formulasi bagi penyediaan nanoserakan fitosterol.

Parameter pemprosesan yang optimum ialah 15.25 min masa pengaulan, 7000 rpm halaju pengacauran and tekanan penghomogenan ialah 42.4 MPa. Parameter-parameter pemprosean pada keadaan optimum tersebut menghasilan saiz purata partikel (PS) bernilai 52 nm and kepekatan fitosterol (Phyto) bernilai 336 mg/l. Parameter formulasi yang optimum ialah nisbah serakan (PR) bernilai 3.54 and nisbah campuran (MR) bernilai 0.19. Formulasi optimum ini telah menghasilkan PS bernilai 55.4 nm dan 87.6% kepekatan fitosterol. Tiada perbezaan ketara ($p < 0.05$) pada PS selama 8 minggu penyimpanan pada suhu 4ºC.

Seterusnya, Tween 20 telah digantikan dengan ester sukrosa (SEs) iaitu palmitat sukrosa (P-1570), lauriat sukrosa (L-1695), stearat sukrosa (S-1570) dan oliat sukrosa (OWA-1570). Kajian ke atas sifat fisikokimia nanoserakan fitosterol yang disediakan dengan SEs telah dijalankan. PS dan %fitosterol dalam nanoserakan fitosterol yang dihasilkan adalah berukuran 2.8 hingga 259.9 nm dan 230.4 hingga 504.6 mg/l masing-masing. Semua nanoserakan fitosterol yang disediakan menunjukan sifat pengaliran pseudoplastik dengan hasil stress yang rendah bernilai 0.630 hingga 9.183 mPa and nilai ketekalan yang rendah di antara 0.608 hingga 88.710 mPas. Kurang daripada 1.5 ppm sisa heksana telah dikesan dalam nanoserakan yang telah disediakan. Ester sukrosa P-1570, L-1695 dan S-1570 didapati bersesuaian untuk digunakan dalam penyediaan nanoserakan fitosterol dengan PS yang kecil, corak serakan monomodel dan kejernihan yang tinggi. Nanoserakan fitosterol dengan kepekatan fitosterol yang tinggi telah disediakan dengan sepelarut etanol and L-1695 dan menghasilkan PS yang kecil dan sfera pada ukuran kira-kira 5 nm, kekentalan yang rendah dan kejernihan yang tinggi.

Kepekatan sisa pelarut pada nanoserakan fitosterol akhir adalah pada tahap yang munasabah.

L-1695 telah dipilih untuk kajian pengoptimuman bagi menghasilkan nanoserakan fitosterol terserak dalam air melalui RSM. Parameter optimum ialah 5.5% Ph (kepekatan fitosterol), 1.0% L(kepekatan L-1695), 3C (pusingan penghomogenan) dan 37 MPa P (tekanan penghomogenan). Parameter optimum ini telah menghasilkan PS berukuran 3 nm and %Ph bernilai 90.4%, Nanoserakan fitosterol yang dioptimumkan mengandungi indek poly-serakan berukuran 0.550 pada corak serakan monomodel. Nilai pH, kepekatan sisa heksana dan etanol, ialah 6.45, 48.2 µl/l dan 930.3 µl/l masing-masing. Nanoserakan fitosterol yang dioptimumkan adalah stabil terhadap rawatan pemanasan sehingga suhu 121°C, penyejukan pada 4 dan 10°C, dan pembekuan dengan cryoprotectant pada – 4 dan – 20°C.

Kestabilan nanoserakan fitosterol yang telah dioptimumkan dan kestabilan susu soya yang telah ditambah dengan nanoserakan fitosterol (SMP) telah dikaji selama 12 minggu. Penyimpanan telah meningkatkan PS dan mengurangkan kepekatan fitosterols pada nanoserakan yang telah diautoklaf. Penambahan nanoserakan fitosterol dalam susu soya meningkatkan PS susu soya. Nanopartikel fitosterol yang ditambahkan dalam susu soya telah terperangkap dalam titisan lemak susu soya. Sehubugan dengan ini, kestabilan SMP bergantung pada kestabilan susu soya. Penambahan nanoserakan fitosterol dalam susu soya adalah boleh diterima.

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I certify that a Thesis Examination Committee has met on 23 November 2010 to conduct the final examination of Leong Wai Fun on her thesis entitle "Development of water-dispersible phytosterol nanodispersion system and its application in soy milk " is accordance with Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Examination Committee were as follows:

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

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DECLARATION

I declare that the thesis is my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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LEONG WAI FUN

Date: 23 November 2010

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