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DEVELOPMENT AND APPLICATION OF NOVEL LACTOGLOBULIN NANOFIBRILS COACERVATED WITH ALGINATE FOR ENCAPSULATION OF CAROTENOIDS ENRICHED PALM OLEIN

NG SHY KAI

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By

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DEVELOPMENT AND APPLICATION OF NOVEL β–LACTOGLOBULIN NANOFIBRILS COACERVATED WITH ALGINATE FOR ENCAPSULATION OF CAROTENOIDS ENRICHED PALM OLEIN

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β-Lactoglobulin (β-lg) has the ability to form fibrils, and palm carotenoids have important biological activities affecting human health. However, carotenoids are unstable, and their insolubility in water makes them difficult to incorporate into many food matrixes. Therefore, this study addressed the development and application of β-lg nanofibril to produce palm carotenoid emulsions to improve the stability and bioavailability of these carotenoids.

First, the impact of different stirring speeds on the characteristics of β-lg fibrils was investigated. The results showed that stirring significantly ($p<0.05$) enhanced the formation and stability of β-lg fibril. Second, a β-lg nanofibril solution was produced by a homogenization process. The results showed that increasing the homogenization pressure and cycles led to significantly decrease the fibril concentration, particle size, viscosity and turbidity. The emulsifying properties of the β-lgs were generally improved by the homogenization process. Third, different concentrations of sodium alginate (0.2-1.0% w/w) were added to a fixed concentration of β-lg nanofibrils (1.0% w/w) to form a complex. The results showed that the zeta potential decreased from a positive charge to a more negative charge (+13.7 ± 1.4 to -41.7 ± 1.2 mV). The addition of alginate resulted in an increase in the emulsifying properties of the β-lg nanofibrils.

Fourth, the complex was used to produce a palm olein oil-in-water (o/w) emulsion. The results showed that increasing the oil concentration caused an increase in viscosity and particle size and reduced emulsion stability. The homogenization process was shown to produce an emulsion that with smaller particle size (29.92 ± 5.8 to 0.82 ± 0.05 μm), more negative of zeta potential (-59.5 ± 2.0 to -67.5. ± 1.2 mV), less viscous (26.0 ± 0.7 to 11.8 ± 0.1 mPa-s) and more stable of emulsions (75.0 ± 3.5 to 91.3 ± 1.9%). Finally, the palm carotenoids were incorporated into the palm olein oil to produce β-lg nanofibril-alginate complex-stabilized palm carotenoids. The results showed minimal

changes in the carotenoid content of the emulsion upon exposure to environmental stresses and storage, indicating the high stability of the emulsion. In addition, the cellular uptake of the emulsion (931.30 ± 125.50 fmol/ cell) was double compared to bulk oil (509.30 ± 37.30 fmol/ cell).

The findings of this study suggest the suitability of the β-lg nanofibril-alginate complex to produce a palm carotenoid emulsion that exhibits high stability and better bioavailability.

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

PEMBANGUNAN DAN APLIKASI NANOFIBRIL β–LAKTOGLOBULIN NOVEL GABUNGAN DENGAN ALGINAT UNTUK PENGKAPSULAN OLEIN SAWIT YANG DIPERKAYA DENGAN KAROTENOID

Oleh

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β-laktoglobulin (β-lg) mempunyai keupayaan untuk membentuk fibril, manakala karotenoid sawit mempunyai aktiviti biologi yang penting untuk kesihatan manusia. Walau bagaimanapun, karotenoid yang tidak stabil dan ketidaklarutan dalam air menjadikannya sukar disebatikan dalam banyak matriks makanan. Oleh itu, kajian ini bertujuan membangunkan dan mengaplikasikan nanofibril β-lg untuk menghasilkan emulsi karotenoid sawit untuk meningkatkan kestabilan dan bioavailabilitinya.

Pertamanya, kesan kelajuan pengacauan yang berbeza kepada ciri-ciri fibril β-lg telah disiasat. Hasil kajian menunjukkan bahawa pengacauan menghasilkan kesan yang ketara ($p<0.05$) dalam mempertingkatkan pembentukan fibril yang stabil. Keduanya, β-lg nanofibril telah dihasilkan oleh proses penghomogenan. Hasil kajian menunjukkan bahawa tekanan dan kitaran penghomogenan yang semakin meningkat memberi kesan yang ketara dalam menurunkan kepekatan fibril, saiz fibril, kelikatan dan kekeruhan. Sifat-sifat pengemulsi daripada β-lg secara amnya bertambah baik dengan proses penghomogenan. Ketiganya, kepekatan natrium alginat yang berbeza (0.2-1.0% w/w) telah ditambah ke dalam kepekatan β-lg nanofibrils yang tetap (1.0% w/w) untuk membentuk kompleks. Hasil kajian menunjukkan bahawa keupayaan zeta itu menurun daripada cas positif kepada keupayaan zeta caj yang lebih negative (+13.7 ± 1.4 kepada -41.7 ± 1.2 mV). Penambahan alginat menyebabkan peningkatan dalam sifat pengemulsi β-lg nanofibril.

Keempatnya, kompleks itu digunakan untuk menghasilkan emulsi minyak sawit olein-dalam-air. Hasil kajian menunjukkan bahawa peningkatan kepekatan minyak menyebabkan peningkatan kelikatan, saiz zarah emulsi dan mengurangkan kestabilan emulsi. Proses penghomogenan telah menghasilkan emulsi yang lebih kecil (29.92 ± 5.8 kepada 0.82 ± 0.05 μm), keupayaan zeta yang lebih negatif (-59.5 ± 2.0 kepada -67.5. ± 1.2 mV), kurang pekat (26.0 ±

0.7 kepada 11.8 ± 0.1 mPa-s) dan kestabilan emulsi yang lebih baik (75.0 ± 3.5 kepada 91.3 ± 1.9%). Akhir sekali, karotenoid sawit telah disebatikan ke dalam minyak olein sawit untuk menghasilkan kompleks β-lg nanofibril-alginat yang stabil untuk mengkasulkan karotenoid sawit. Hasil kajian menunjukkan bahawa perubahan yang minimum dalam kandungan emulsi karotenoid kepada tekanan alam sekitar dan penyimpanan dan ini menunjukkan kestabilan emulsi yang tinggi. Selain itu, pengambilan selular emulsi (931.30 ± 125.50 fmol/ sel) meningkat dua kali ganda berbanding dengan minyak pukal (509.30 ± 37.30 fmol/ sel).

Hasil kajian ini menunjukkan kesesuaian β-lg nanofibrils-alginat kompleks untuk menghasilkan emulsi karotenoid sawit yang sangat stabil dan bioavailabiliti yang lebih baik.

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I certify that a Thesis Examination Committee has met on 28th March 2016 to conduct the final examination of Ng Shy Kai on his thesis entitled "Development and Application of Novel β-lactoglobulin Nanofibrils Coacervated with Alginate for Encapsulation of Carotenoids Enriched Palm Olein" in accordance with the 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.

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LIST OF ABBREVIATIONS

A.u	Arbitrary units
AFM	Atomic force microscopy
ANOVA	Analysis of variance
BLG-ALG	β-lg nanofibrils-alginate
CCD	Charge-coupled device
CD	Circular dichroism
CR	Congo Red
DLS	Dynamic light scattering
EAI	Emulsifying activity index
ES	Emulsion stability
ESI	Emulsifying stability index
FBS	Fetal bovine serum
FTIR	Fourier transform infrared
HDL	High-density lipoprotein
LDL	Low-density lipoprotein
MPOB	Malaysian Palm Oil Board
NMR	Nuclear magnetic resonance
O/W	Oil-in-water
PBS	Phosphate buffered saline
PDI	Polydispersity index
SDS	Sodium dodecyl sulfate
TEM	Transmission electron microscopy
ThS	Thioflavin S

ThT	Thioflavin T
WPI	Whey protein isolate
β-lg	β-lactoglobulin

CHAPTER 1

INTRODUCTION

Most proteins and peptides are generally unstable and susceptible to denaturation to form various types of aggregates, which accumulate in biological environments and result in pathological conditions (Dobson, 2001). These aggregates are known as amyloid fibrils were first discovered in tissue of patients with Alzheimer's disease (Virchow, 1853) and some other diseases including Type II diabetes and Creutzfeldt-Jakob disease (Tan & Pepys 1994; Kelly, 2002). β-Lactoglobulin (β-lg) represents 0.2-0.4% (w/v) of whey protein, which exists in a dimeric structure at pH 7 but separated into monomers during acidic condition (Uhrinova et al., 2000). The folding of β-lg occurs cause gelation when the β-lg solution is under high temperatures (Relkin et al., 1998), high hydrostatic pressure (Dumay et al., 1998), or in the presence of chemical denaturants (Renard et al., 1999).

Carotenoids contribute various color (orange, yellow or red) to many foods and also play a role as natural antioxidants in the stabilization of foods. Carotenoids have health-promoting effects and also aid in reducing the risk of degenerative diseases such as cancer, cardiovascular diseases, cataracts and macular degeneration (Tapiero et al., 2004; Krinsky & Johnson, 2005; Voutilainen et al., 2006). However, carotenoids are susceptible to oxidation in the existence of heat, light and oxygen (Bradley & Min, 1992). In addition, their insolubility in the water makes carotenoids difficult to incorporate into many food matrixes. Nevertheless, encapsulation techniques are often used to overcome these problems. The active ingredients (core) of the carotenoids are protected by a wall of materials, which helps to protect these active ingredients against oxidation, light and chemical reactions.

Several milk proteins have significant functional properties, i.e., bind with hydrophobic molecules, react with biopolymers, involve in stabilizing emulsions, gelation, and retard oxidation process, which make them suitable for encapsulating the bioactive compounds (Zimet & Livney, 2009). In fact, the formation of a complex between protein and polysaccharides may provide extra protection for hydrophobic molecules (Fioramonti et al., 2014). Sodium alginate is capable to form complexes with β-lg (Harnsilawat et al., 2006), whey proteins (Perez et al., 2009), and lactoferrin (Tokle et al., 2010). Sodium alginate has been used as a stabilizer, thickener and gelling product in the food industry (Whistler & BeMiller, 1999). Encapsulation of α-tocopherol using a β-lg-alginate complex has been studied (Somchue et al., 2009). However, the encapsulation of carotenoids using a β-lg nanofibril-alginate complex has not been studied.

Ionic strength, temperature, pH, and concentration of protein have a strong implication on β-lg fibrillation process (Pearce et al., 2007). Hill et al. (2006) and Dunstan et al. (2009) reported that nucleation time can be shortened by shearing during heating. In fact, rapid stirring can abolish the lag phase of the nucleation process, which promotes the secondary nucleation (Dave et al., 2014). This finding can be explained in that stirring generates shear stresses in

the solution that increase the solubility of the protein molecules. However, no studies have been done to study the impact of different stirring speeds on the characteristics of the β-lg fibrils formed after the fibrillation process has taken place.

Thus, in this study, the impacts of different stirring speeds on the characteristics of β-lg fibril formation were first evaluated to produce the highest yield and the most stable form of β-lg fibrils. Next, the production of β-lg nanofibrils using high-pressure homogenization was conducted, and the homogenizing parameters were chosen according to the good emulsifying properties and stability of the nanofibrils. Subsequently, various concentrations of sodium alginate were added to the β-lg nanofibrils to form protein-polysaccharide complexes by electrostatic interaction. The resulting complex solutions were characterized in terms of their physicochemical, morphological and emulsifying properties. The resulting complex solution with the highest emulsifying capacity and stability was used to produce a palm olein oil-in-water (o/w) emulsion with palm olein oil. The effects of the oil load and homogenization process on the emulsion characterization were also evaluated. The incorporation of concentrated palm carotenoids into the palm olein oil was then stabilized by the β-lg nanofibril-alginate complex to produce a palm carotenoid emulsion. The resulting emulsion was then evaluated in terms of its stability under environmental conditions and eight weeks of storage. The bioavailability of the palm carotenoid emulsion was examined according to its cellular uptake properties.

The objectives of this study were as follows:

1. To investigate the impact of different stirring speeds on the characteristics of the β-lg fibril formation.
2. To examine the effects of the homogenization process on the formation of β-lg nanofibrils to produce and characterize β-lg nanofibrils with specific lengths and desirable emulsifying properties.
3. To evaluate the microstructural changes in β-lg nanofibrils upon the addition of alginate via electrostatic complex formation.
4. To develop a palm olein oil-in-water (o/w) emulsion stabilized by a β-lg nanofibrils-alginate complex and to investigate the effects of the oil load and homogenization process on the emulsion characterization.
5. To develop a palm carotenoid emulsion stabilized by a β-lg nanofibril-alginate complex and also investigate its stability and bioavailability.

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