PREPARATION, CHARACTERIZATION, AND APPLICATION OF PALM CAROTENE MICROCAPSULE COATED BY RICE PROTEIN ISOLATE FLAXSEED GUM COMPLEX

By

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Chair : Professor Tan Chin Ping, PhD
Faculty : Food Science and Technology

Natural proteins, including plant-based proteins and their complexes with polysaccharides, have received limited attention in terms of their functional properties for food applications. In contrast, carotene has garnered significant interest from consumers and the food industry due to its health-promoting effects. However, the poor solubility of carotene poses substantial challenges for its inclusion in food formulations, particularly in aqueous-based systems. This study aimed to develop a stable palm carotene-based emulsion using a natural-based rice protein isolate-flaxseed gum (RPI-FG) complex, followed by microencapsulation and subsequent incorporation into ice cream. In the first phase, various ratios of RPI-FG (ranging from 3:1 to 11:1) were employed to form complexes, which were then incorporated into the emulsion. The emulsion created using a 7:1 ratio of RPI-FG complex exhibited superior functionality. It featured the smallest droplet size (3.37 μm), a span index of 1.74, a viscosity of 8.48 mPa·s, and a creaming index (CI) of 26.67%. These values fell within an intermediate range compared to the other ratios on the 7th day of storage. Moving to the second phase, the formation of carotene microcapsules was accomplished using the emulsion produced with a 7:1 ratio of RPI-FG complex via spray drying. Various combinations of wall materials and core-to-wall material ratios were explored to create the microcapsules. Microcapsules produced with a combination of maltodextrin and starch sodium octenyl succinate (OSS) as the wall material, maintaining a 1:4 core-to-wall material ratio, displayed enhanced stability. They exhibited the highest microencapsulation efficiency (MEE) at 70.50%, significant carotene content (α-60.85% and β-72.79%), impressive water solubility index (79.60%), and a smooth surface as observed through scanning electron microscopy. Additionally, these microcapsules had the lowest moisture content (2.19%), a low water activity of 0.20, minimal water adsorption index of 0.50%, fair flow properties, and intermediate cohesiveness. In the third phase, the study delved into the storage stability, release characteristics, and in-vitro digestibility of the microencapsulated carotene emulsion, stabilized with a combination of maltodextrin and OSS as the wall material, maintaining a 1:4 core-to-wall material ratio. Over a rigorous

13-week storage period, minimal changes were observed in terms of MEE, carotene degradation, and peroxide value. In contrast, color changes increased, and chroma values decreased throughout the storage. Remarkably, these microcapsules displayed stability against varying ionic strengths. Most significantly, their bioaccessibility and the percentage of carotene release during in-vitro digestion were significantly higher compared to bulk oil. Finally, in the fourth phase, ice cream was fortified with these carotene microcapsules, and assessments of storage stability and sensory evaluations were conducted. Although there were slight differences in overrun and melting rate between ice cream variants with and without carotene microcapsules, the former exhibited a more vibrant color profile. However, carotene degradation occurred over a 4-week storage period, accompanied by an increase in firmness throughout storage. Sensory analysis results indicated moderate satisfaction among panelists, with a preference for ice cream containing carotene microcapsules. This confirmed the feasibility of incorporating carotene into ice cream to enhance its color, flavor, and nutritional value. In conclusion, this study underscores the potential of using plant-based proteins complexed with polysaccharides as a viable alternative to animal-derived emulsifiers. Furthermore, it highlights carotene's versatility in various food products, facilitated by the meticulous microencapsulation process using maltodextrin and OSS at a 1:4 core-to-wall ratio, resulting in impressive stability and microencapsulation efficiency exceeding 70%. This study highlights the promising potential of natural-based protein-polysaccharide complexes and microencapsulation techniques to enhance the incorporation of carotene into a range of food products, offering both functional benefits and improved consumer appeal.

Keywords: Carotene, microcapsule, rice protein isolate, flaxseed gum.

SDG: GOAL 3: Good Health and Well Being.

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

PENYEDIAAN, CIRI-CIRI DAN APLIKASI EMULSI BERASASKAN KAROTENA KELAPA SAWIT MENGGUNAKAN KOMPLEKS GUM PROTEIN BERAS ISOLATE-FLAXSEED GAM

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Protein semulajadi, termasuk protein berasaskan tumbuhan dan kompleksnya dengan polisakarida, telah mendapat perhatian yang terhad dari segi sifat fungsinya untuk aplikasi makanan. Di samping itu, karotena telah mendapat minat yang ketara daripada pengguna dan industri makanan kerana kesannya yang menggalakkan kesihatan. Walau bagaimanapun, keterlarutan karotena yang lemah menimbulkan cabaran besar untuk dimasukkan ke dalam formulasi makanan, terutamanya dalam sistem berasaskan akueus. Kajian ini bertujuan untuk membangunkan emulsi berasaskan karotena sawit yang stabil menggunakan kompleks berasaskan protein beras isolate-gam flaxseed (RPI-FG), diikuti dengan kaedah mikroenkapsulasikan emulsi karotene sawit menjadi mikrokapsul karotene dan penggabungan mikrokapsul karotene ke dalam ais krim. Dalam fasa pertama, pelbagai nisbah RPI-FG (antara 3:1 hingga 11:1) digunakan untuk membentuk kompleks, yang kemudiannya dimasukkan ke dalam emulsi. Emulsi yang dicipta menggunakan nisbah 7:1 kompleks RPI-FG mempamerkan fungsi unggul. Ia menampilkan saiz titisan terkecil (3.37 µm), indeks rentang 1.74, kelikatan 8.48 mPa·s, dan indeks gabungan (CI) sebanyak 26.67%. Nilai ini jatuh dalam julat pertengahan berbanding nisbah lain pada hari ke-7 penyimpanan. Bergerak ke fasa kedua, pembentukan mikrokapsul karotena telah dicapai menggunakan emulsi yang dihasilkan dengan nisbah 7:1 kompleks RPI-FG melalui pengeringan semburan. Pelbagai kombinasi bahan dinding dan nisbah bahan teras ke dinding telah diterokai untuk mencipta mikrokapsul. Mikrokapsul yang dihasilkan dengan gabungan maltodekstrin dan kanji sodium octenyl succinate (OSS) sebagai bahan dinding, mengekalkan nisbah bahan teras-ke-dinding 1:4, menunjukkan kestabilan yang dipertingkatkan. Mikrokapsul karotene mempamerkan kecekapan mikroenkapsulasi (MEE) tertinggi pada 70.50%, kandungan karotena yang ketara (α-60.85% dan β-72.79%), indeks keterlarutan air yang mengagumkan (79.60%), dan permukaan licin seperti yang diperhatikan melalui pengimbasan mikroskop elektron. Selain itu, mikrokapsul ini mempunyai kandungan lembapan paling rendah (2.19%), aktiviti air rendah 0.20, indeks penyerapan air minimum 0.50%, sifat aliran saksama, dan kepaduan pertengahan. Dalam fasa ketiga, kajian menyelidiki kestabilan penyimpanan, ciri pelepasan, dan kebolehcernaan in-vitro emulsi karotena terkapsul mikro, distabilkan dengan gabungan maltodekstrin dan OSS sebagai bahan dinding, mengekalkan teras ke dinding 1:4. nisbah bahan. Sepanjang tempoh penyimpanan 13 minggu yang ketat, perubahan minimum telah diperhatikan dari segi MEE, degradasi karotena dan nilai peroksida. Sebaliknya, perubahan warna meningkat dan nilai kroma menurun sepanjang storan. Mikrokapsul ini menunjukkan kestabilan terhadap pelbagai kekuatan ionik. Paling ketara, bioakses mikrokapsul karotene dan peratusan pelepasan karotena semasa pencernaan in-vitro adalah lebih tinggi berbanding minyak pukal. Akhirnya, dalam fasa keempat, ais krim diperkayakan dengan mikrokapsul karotena ini, dan penilaian kestabilan penyimpanan dan penilaian deria telah dijalankan. Walaupun terdapat sedikit perbezaan dalam kadar kelimpahan dan takat lebur antara varian ais krim dengan dan tanpa mikrokapsul karotena, yang pertama mempamerkan profil warna yang lebih menawan. Walau bagaimanapun, degradasi karotena berlaku dalam tempoh penyimpanan 4 minggu, disertai dengan peningkatan ketegasan sepanjang penyimpanan. Keputusan analisis deria menunjukkan kepuasan sederhana di kalangan ahli panel, dengan keutamaan untuk ais krim yang mengandungi mikrokapsul karotena. Ini mengesahkan kemungkinan menggabungkan karotena ke dalam ais krim untuk meningkatkan warna, rasa dan nilai pemakanannya. Kesimpulannya, kajian ini menggariskan potensi penggunaan protein berasaskan tumbuhan yang dikomplekskan dengan polisakarida sebagai alternatif yang berdaya maju kepada pengemulsi yang berasal dari haiwan. Tambahan pula, ia menyerlahkan kepelbagaian karotena dalam pelbagai produk makanan, difasilitasi oleh proses mikroenkapsulasi yang teliti menggunakan maltodekstrin dan OSS pada nisbah teras-ke-dinding 1:4, menghasilkan kestabilan yang mengagumkan dan kecekapan mikroenkapsulasi melebihi 70%. Kajian ini menyerlahkan potensi yang menjanjikan kompleks protein-polisakarida berasaskan semula jadi dan teknik mikroenkapsulasi untuk meningkatkan penggabungan karotena ke dalam rangkaian produk makanan, menawarkan kedua-dua faedah berfungsi dan daya tarikan pengguna yang lebih baik.

Kata kunci: Karotene, mikrokapsul, protein beras isolate, gam flaxseed.

SDG: MATLAMAT 3: Kesihatan yang Baik dan Kesejahteraan.

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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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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
CI	Creaming index
EAI	Emulsifying activity index
ESI	Emulsifying stability index
FG	Flaxseed gum
kDa	Kilodalton
MCT	Medium-chain triacylglycerol
MEE	Microencapsulation efficiency
MPa	MegaPascal
mPa.s	Millipascal second
mEq/kg	Milliequivalents per kilogram
O/W	Oil-in-water
OSS	Starch sodium octenyl succinate
PV	Peroxide value
RPI	Rice protein isolate
SEM	Scanning electron microscope
SGF	Simulated gastric fluid
SIF	Simulated intestinal fluid
SSF	Simulated saliva fluid
µm	Micrometre (Micron)
WAI	Water absorption index
WSI	Water solubility index

CHAPTER 1

INTRODUCTION

Carotenoids, which constitute a significant portion of plant-based compounds, account for 70% of vitamin A found in the average human diet. Among these compounds, carotenoid pigments serve as key functional lipids used in various food products (Yuan et al., 2008). The composition of carotenoids in palm includes phytoene, phytofluene, cis-β–carotene, β–carotene, α–carotenes, cis-α-carotene, ζ–carotene, δ–carotene, γ–carotene, neurosporene, β–zeacarotene, α–zeacarotene, and lycopene (Siti et al., 2021). Both consumers and the food industry become increasingly interested in the health benefits of carotene. Carotene consumption is associated with the prevention or protection against significant diseases such as coronary artery disease, cancer, macular degeneration, aging-related degeneration, and cataracts (Linnewiel-Hermoni et al., 2015; Liang et al., 2018).

Furthermore, carotene serves as a valuable food additive due to its bioactivity and its coloring properties, which contribute to its economic value. It is commonly used as an orange-red pigment in various foods, including non-alcoholic tropical fruit-flavored beverages, edible fats, cheese, pastries, and ice cream (Radomska & Harasym, 2018). Given these advantages and consumer demand, integrating carotenoids into the food industry in various forms is desirable. However, a significant challenge lies in the poor water solubility, water dispersion, chemical stability, and bioavailability of functional lipids like carotene (Chen & Zhong, 2015; Tan & Nakajima, 2005). For instance, β-carotene's limited solubility in oil at room temperature and its low water solubility due to its hydrophobic structure and high melting point make it challenging to incorporate into aqueous-based meals, resulting in inconsistent oral bioavailability (Luo et al., 2017). Additionally, carotene's susceptibility to oxygen, heat, and light further limits its use in food, nutraceuticals, and pharmaceuticals (Yuan et al., 2008).

The primary concern for food industries when using functional lipids in formulations is solubility. Functional lipids with low water solubility, such as carotene, often have reduced bioavailability. To address these challenges, carotene's solubility and bioavailability are enhanced by incorporating it into colloidal systems, particularly oil-in-water (O/W) emulsions (Mehmood et al., 2018). Emulsions enable the transport, encapsulation, solubilization, and delivery of lipophilic carotenoids to epithelial cells (McClements, 2010; McClements & Xiao, 2012). The critical stage in ensuring carotene absorption is the fat digestion phase (Xu et al., 2021). However, encapsulation reduces the droplet size, enhancing bioavailability (Wang et al., 2012; Salvia-Trujillo et al., 2015).

In recent years, nanotechnology has emerged as a promising area of study for improving the solubility and bioavailability of various components, including carotene (Yuan et al., 2008). However, emulsion systems are thermodynamically unstable and prone to destabilization, leading to dispersed droplets coalescing into separate organic and aqueous phases (Tan et al., 2018). To overcome this challenge, variables such as emulsifier type, concentration, homogenizing conditions, and the water-to-oil phase ratio need careful consideration to achieve stable emulsions.

Most studies employ synthetic emulsifiers like Tween 80, whereas the use of natural emulsifiers, particularly plant proteins like rice protein in combination with polysaccharides such as flaxseed gum, remains relatively unexplored in food applications (Phongthai, Homthawornchoo, & Rawdkuen, 2017). This study aims to utilize natural emulsifiers—specifically, rice protein in combination with flaxseed gum—because rice protein's potential as an emulsifier in food applications has been underexplored due to it low solubility in water but if the solubility of the rice protein is improved, there is a big potential in rice protein to become a versatile plant-based emulsifier. This is due to, rice protein has a minimal record of allergies, have bland taste and widely available thus it can potentially serve as an organic substitute for other plant-based protein emulsifiers such as soybean protein, chickpea protein and pea protein (Amine et al., 2014).

The combination of proteins and polysaccharides in emulsions and foams compensates for their individual limitations, resulting in stable systems (Virangkumar & Murthy 2012). In this study, blended flaxseed gum with rice protein was used to assess its emulsification properties since flaxseed gum is a versatile natural hydrocolloid (Liu et al., 2018). For the oil phase in the emulsion, medium-chain triacylglycerol (MCT) oil was chosen for its ability to dissolve lipid-soluble carotene concentrate at room temperature (Tan & Nakajima, 2005). MCT oil offers various health benefits, including weight management and preventing yeast and bacterial growth due to its fatty acid composition (Sharon, 2018).

To commercialize the product, the liquid emulsion was transformed into microcapsules, reducing storage and transportation costs while improving handling, utilization, and shelf life. Spray-drying was selected as the most widely used method to convert liquid emulsions into microcapsules, particularly in the food and pharmaceutical industries, due to its quick drying properties, which minimize the risk of damaging heat-sensitive components (Yeimy et al., 2017). Incorporating wall materials, such as maltodextrin, before dehydration is crucial for enhancing microcapsule quality. This addition raises the glass transition temperature of the solid matrix around the oil droplets, increasing chemical stability and improving flow properties and water dispersibility (Chen et al., 2017).

This study focuses on incorporating carotene into functional foods, with a particular interest in ice cream due to public consumption trend on the healthy ice cream are showing increasing trend (Nik Fakrulhazri et al., 2021). Palm carotene microcapsules, produced using a rice protein-flaxseed gum complex as an emulsifier, are a valuable addition to ice cream due to its appealing color and nutritional benefits. While there are no palm carotene microcapsule ice cream products available in the market currently, previous research has produced curcumin emulsions for ice cream research purposes (Kumar et al., 2016). Sensory evaluations using a 9-point hedonic scale were conducted to assess the color, appearance, body, texture, melting quality, and flavor of the resulting ice cream.

In summary, this study's objectives were as follows:

i. To develop a stable palm carotene-based emulsion using rice protein isolate-flaxseed gum complex,
ii. To optimize and characterize the prepared palm carotene-based microcapsules using various coating materials and core-to-wall ratios based on the optimized emulsion formulation,
iii. To determine the effect of using a mixture of maltodextrin with starch sodium octenyl succinate as the coating material on the storage stability, release characteristics, and in-vitro digestion of the spray-dried palm carotene-based microcapsules, and
iv. To evaluate the physicochemical stability and sensory acceptability of palm carotene-based microcapsules in vegan ice cream.

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