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Optimisation and characterisation of red palm carotene-based microcapsules stabilised by rice protein isolate-flaxseed gum complex using various coating materials and core-to-wall ratios

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Abstract

The present work explores the impact of various wall materials and core-to-wall material ratios on carotene microcapsules' physicochemical properties. Palm carotene-based microcapsules were prepared using spray drying with different wall materials (maltodextrin, starch sodium octenyl succinate (OSS), inulin) and core-to-wall ratios (1:2, 1:3, 1:4), followed by physicochemical characterisation including moisture content, water activity, microencapsulation efficiency, and morphological analysis *via* scanning electron microscope (SEM). Microcapsules created with a 1:4 core-to-wall material ratio, combining maltodextrin and OSS, exhibited optimal traits. These microcapsules possessed the lowest moisture content (2.19%) compared to 1:2 (2.73%), 1:3 (2.65%), and water activity (0.20 a_w), maintaining adequate flowability and intermediate cohesiveness. The same sample demonstrated the highest MEE at 70.50%, compared to 1:2 (24.57%) and 1:3 (35.80%), corresponding to α-carotene (60.85%) and β-carotene (72.79%) content. SEM confirmed their smooth, undented surface, indicating successful encapsulation. Furthermore, microcapsules stabilised with this combination at a 1:4 ratio displayed a superior WSI of 79.59% and a lower WAI of 0.50%, enhancing storage stability and suitability for diverse food systems. The present work thus demonstrated the significance of wall materials and core-to-wall ratios in producing high-quality, functional carotene microcapsules.

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Introduction

Bioactive lipids, such as natural antioxidants, fatty acids, and carotenoids, are commonly used as active components to fortify various food systems. Carotenoids, a family of natural pigments predominantly found in vegetables and fruits, have garnered significant attention for their health benefits. Numerous studies have demonstrated the beneficial effects of carotenoids in preserving human health, including protection against cataracts, muscle degeneration, cardiovascular diseases, and cancers (Liang *et al*., 2018). Among the carotenoids, β-carotene stands out as a crucial member, renowned for its potent antioxidant capabilities and provitamin A activity. As a result, the food industry has taken a keen interest in utilising β -carotene and other carotenoids as valuable additives. However, β-carotene has some limitations, mainly owing to its crystalline structure, making it insoluble in water and poorly soluble in oil at room temperature. This characteristic poses challenges in integrating it into many food formulations, and hinders its bioavailability. Moreover, β-carotene is sensitive to light, heat, and oxygen, further restricting its applicability in the food sector. To overcome these limitations, it is essential to incorporate β- carotene into oil-in-water (O/W) emulsions, and then transform it into dry microcapsules and agglomerates through suitable dehydration methods (Jo and Kwon, 2014).

Microcapsules derived from emulsions represent a unique category of food emulsions, characterised by a high concentration of biopolymers

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serving as wall materials for encapsulation purposes. In the present work, wall materials such as maltodextrin, starch sodium octenyl succinate (OSS), and inulin are employed. Maltodextrin is produced through starch hydrolysis, consisting of D-glucose units connected by (1-4) glycosidic linkages. Its dextrose equivalence (DE), inversely correlated with average molecular weight, serves as a well-known measurement. Maltodextrin finds extensive use in the spray drying process for various food products due to its cost-effectiveness and strong hygroscopicity (Seregelj *et al.*, 2022). On the other hand, OSS is obtained by hydrophobising natural starch. It has proven effective for encapsulation, emulsification, film formation, coating, and gel formation due to its affordability, excellent emulsifying capacity, and film-forming ability. Another wall material used is inulin, composed of fructosyl fructose units linked together by (2-1) connections to form a branching polysaccharide, with glucose units at the end of the chain. Inulin's limited hydrolysis capability makes it widely recognised as a dietary fibre and suitable for manufacturing microcapsules resistant to pH variations in the gastrointestinal system, enabling the controlled release of biocomponents in the colon. Inulin also exhibits prebiotic properties that promote the growth of bifidobacteria in the human gut, thus enhancing human health and improving calcium bioavailability (Dima *et al*., 2015).

Spray drying is a commonly applied microencapsulation methods for various food ingredients and materials, such as edible oils, flavours, spice resins, and essential oils. The technique offers several advantages, including excellent sanitary conditions during processing, cost-effectiveness, and short exposure time. An essential aspect of spray drying process is to minimise the amount of unencapsulated oil on the surface of microcapsules particles to reduce volatile losses, and enhance product shelf life (Shang *et al.*, 2023). However, several variables, including the qualities of wall materials and core materials (bioactive ingredients), emulsion characteristics, and drying parameters can significantly impact the encapsulation effectiveness of bioactive compounds, particularly oils (Jafari *et al*., 2007). Therefore, the present work aimed to investigate the impact of different core-to-wall ratios and the use of various wall materials on the physical and morphology properties of the spray-dried carotene microcapsules.

Materials and methods

Materials

Natural palm-based carotene concentrates with 20% purity in the mixture of α, β, γ, and lycopene, and remaining 80% of refined bleached deodorised palm olein in the mixture of monoglyceride/diglyceride/ triglyceride was provided by ExcelVite Sdn. Bhd. (Chemor, Malaysia). Besides, medium chain triacylglycerol (Radiamuls MCT2107K) oil which comprised of glycerides, mixed decanoyl, and octanoyl was supplied by Oleon Sdn. Bhd. (Klang, Malaysia). The rice protein isolate (RPI) of 85% purity with remaining mixture of starch and fibre in 15% was procured from Anhui Shunxin Shengyuan Biological Food Co. Ltd. (Anhui, China), while flaxseed gum (FG) was purchased from Qingdao Dehui Halobios Science and Technology Co. Ltd. (Shandong, China). Maltodextrin and starch sodium octenyl succinate E1450 (OSS) were obtained from Ingredion Inc. (Westchester, United States), and inulin was obtained from V.I.S. Foodtech Ingredient Supplies Sdn. Bhd. (Kepong, Malaysia). Deionised water and analytical-grade chemicals were used in the present work.

Preparation of palm carotene-based emulsion

The emulsion was prepared by using 8% oil and 92% aqueous phase. The emulsifier, consisting of a 7:1 ratio of RPI mixed with FG, was used at a concentration of 2.5%. The oil phase was prepared by dissolving 10% carotene concentrate in 90% MCT oil at 55°C for 40 min, followed by 20 min of sonication using a bath sonicator (Branson Ultrasonics Corporation, Connecticut, US) at 50°C to ensure complete dissolution. The aqueous phase, consisting of RPI emulsifier, underwent pH treatment at pH 12, and was stirred overnight, followed by centrifugation at 4,300 *g* for 15 min. The FG was also treated similarly but without pH treatment. The RPI and FG mixture was then mixed together, and adjusted to pH 12 to form complexes for 1 h. The wall materials were added in, and stirred for 30 min to form the aqueous phase. Sodium azide (0.1 mg/mL) was added to the aqueous phase to prevent microbial growth. The dispersed and continuous phases were mixed and initially homogenised for 6 min at 7,000 rpm in a high shear homogeniser (Silverson L4R, Silverson Machines Ltd., Chesham, Buckinghamshire) to generate a coarse emulsion. The coarse emulsion was

then subjected to high-pressure homogenisation using high pressure homogeniser (Panda 2K, Niro Soavi, Lubeck, Germany) at 80 MPa for five cycles to produce the fine emulsion.

Preparation of palm carotene microcapsules

The palm carotene emulsions produced from the RPI-FG complex at a ratio of 7:1 was converted into microcapsules using the spray drying microencapsulation methods with three different wall materials: maltodextrin, maltodextrin + OSS, and maltodextrin + inulin, in a 1:1 ratio, and core-to-wall material ratios of 1:2, 1:3, and 1:4 were tested. The palm carotene microcapsules were characterised based on moisture content, water activity, colour, flowability and cohesiveness, Water Solubility Index, Water Absorption Index, microencapsulation efficiency, carotene concentration, and morphological properties.

Spray drying

The palm carotene emulsion-coating materials mixture, with a total volume of 500 mL, was prepared using a high-pressure homogeniser (HPH). The mixture was then subjected to spray drying using a pilot-scale spray dryer (Niro Soavi, Lubeck, Germany) with air inlet and outlet temperatures of 170 and 90°C, respectively, and a fixed flow rate of 10 mL/ min (Chen *et al*., 2017). The microcapsule samples were collected and stored in airtight glass bottles in 25°C incubator for further analyses.

Characterisation of spray-dried palm carotene microcapsules

Moisture content

The moisture content of the palm carotene microcapsules was measured using a moisture analyser at a temperature of 120°C (MX-50, A&D Medical, Tokyo, Japan) (Chang *et al*., 2018). Triplicate samples of the microcapsules weighing 1 g each were dried in the moisture analyser, and the readings were recorded.

Water activity (a_w)

The water activity (a_w) of the palm carotene microcapsules was evaluated using a water activity analyser (Series 3TE, AquaLab, Washington, USA) (Sharifi *et al*., 2015) at room temperature. Measurements were taken in triplicate.

Colour measurement

The colour analysis was performed using a Minolta Chroma Meter CR-300 (Konica Minolta Co., Osaka, Japan) based on the CIE L*a*b* colour space (Quek *et al*., 2007). Colour variations were quantified based on L* (lightness), a*(redness/greenness), and b* (yellowness/blueness) values. The samples were calibrated using a white colour tile (L* = 91.0, a* = +0.3165, and b* = +0.3326), and the mean of three replicate samples was reported.

Flowability and cohesiveness

The bulk and tapped densities of the microcapsules were determined to assess their flowability and cohesiveness. To determine the bulk density (ρbulk), a 10-mL graduated tared cylinder was filled with 2 g of powder without tapping. The bulk density was calculated using the formula: mass (g)/volume (cm^3), where the volume was directly read from the cylinder. To determine the tapped density (ρtapped), the same sample-filled cylinder was tapped until it reached constant volume (ten times) on a smooth, soft surface from a height of 15 cm, and the volume of the sample was recorded. The tapped density was calculated using the relationship: mass (g)/volume (cm^3) (Wang *et al*., 2019).

The Carr Index (CI) (Carr, 1965) and the Hausner Ratio (HR) (Hausner, 1967) were used to assess the microcapsule's flowability and cohesiveness, respectively. The microcapsule's bulk and tapped densities were employed to calculate both CI and HR using Eqs. 1and 2:

$$CI = (\text{Tapped density} - \text{Bulk density}) / (\text{Tapped density}) \times 100 \quad \text{(Eq. 1)}$$

$$HR = (\text{Tapped density}) / (\text{Bulk density}) \quad \text{(Eq. 2)}$$

Water Absorption Index and Water Solubility Index

The Water Absorption Index (WAI) and Water Solubility Index (WSI) of the microcapsules were determined. For WAI, 2.5 g of dry microcapsules were dissolved in 30 mL of distilled water in a 50-mL pre-weighed centrifuge tube, and stirred intermittently for 30 min at 30°C. The mixture was then centrifuged for 10 min at 3,000 *g*, and the supernatant was poured into a tarred Petri plate. The left sediment was weighed, and the WAI was calculated as the ratio of the wet sediment weight to the dry sample weight.

For WSI, the remaining supernatant from the WAI experiment that was placed in the tarred Petri plate was dried overnight at 100°C, and the dried particles were weighed. WSI was calculated as the percentage of dried solids over the initial dry sample weight (Nishad *et al*., 2017).

Microencapsulation efficiency (MEE)

The microencapsulation efficiency (MEE) of the wall materials for the palm carotene microcapsules was evaluated following the approach outlined by Bae and Lee (2008). First, the total oil (TO) was measured by mixing 1 g of microcapsules with 6 mL of boiling water, and vortexing the mixture for 60 s. After allowing the mixture to reach room temperature, 1 mL of 25% ammonia solution and 7.5 mL of methanol were added. The liquid was then vortexed for 2 min before adding 17 mL of petroleum ether and 17 mL of diethyl ether. The resulting mixture was manually agitated, and then centrifuged for 10 min at 6,700 *g*. The top layer of the supernatant was carefully pipetted out into a round bottom flask, and evaporated using a rotary evaporator at 60°C for 5 min (EyeLa, NY, USA). Then the round bottom flask was dried at 105°C for 1 h in a hot air oven (MMM-Group, Semmelweisstra, Germany) until a constant weight was achieved.

To determine surface oil (SO), 1.5 g of microcapsules were mixed with 15 mL of hexane in a glass container wrapped with a cover. The mixture was manually shaken for 2 min at room temperature (around 27°C) to remove any free oil. The solvent mixture was filtered three times through a Whatman filter paper no. 1, and the accumulated microcapsules on the filter paper were rinsed with 20 mL of hexane each time into a round bottom flask. The solvent in the round bottom flask was evaporated using a rotary evaporator at 60°C for 5 min. Then the round bottom flask was dried at 105°C for 1 h in the hot air oven (MMM-Group, Semmelweisstra, Germany) until a constant weight was achieved (Jafari *et al*., 2008).

Based on the mass difference between the empty flask and the flask containing the extracted oil residue, the SO and TO of the non-encapsulated oil present on the surface of the particles was calculated. The initial oil, which comprised the core and surface oils, was considered to be equal to TO. Thus, the MEE was determined using Eq. (3):

$$\text{MEE (\%)} = (\text{TO} - \text{SO}) / (\text{TO}) \times 100 \quad \text{(Eq. 3)}$$

Carotene content

The 2 g of carotene-based microcapsules was reconstituted into a liquid using 5 mL of double distilled water, and transferred to a 50-mL centrifuge tube. Subsequently, 2 mL of isopropanol was added, and the tube was vortexed for 1 min. Next, 10 mL of hexane was added, and the tube was vortexed for an additional 1 min before centrifuging at 6,700 *g* for 5 min. After centrifugation, three distinct layers were observed: a yellowish organic phase at the top, a colourless aqueous phase at the bottom, and a thin protein layer between these two phases. The top solution, containing the carotene was carefully collected in a scintillation vial, and dried using blowdown evaporation technique with nitrogen evaporator (Organomation, Berlin, USA). The nitrogen gas was continuously blown onto the sample's surface to evaporate the excess solvent, and further concentrate the samples. The carotene extract was then combined and diluted with acetone, then the resulting mixture was filtered through a 0.45 μm polytetrafluoroethylene syringe filter, and the filtrate was collected in an auto-sampler HPLC vial for subsequent high-performance liquid chromatography (HPLC) analysis (Chen *et al*., 2017).

HPLC analysis of the carotene content was conducted using a Prominence LC-20AD HPLC system equipped with a UV-Vis detector (Shimadzu, Kyoto, Japan) and a C18 column (250 × 4.6 mm, 5 μm; Thermo Scientific, Waltham, USA). The mobile phase consisted of 7:1.5:1.5:0.01 ratio of methanol/acetonitrile/ethyl acetate/triethanolamine (TEA). The injection volume was 15 μL, and the column temperature was maintained at 25°C. The detector wavelength was set at 450 nm. The carotene entrapment efficiency of the microcapsules was calculated using Eq. (4):

$$\text{Entrapment efficiency of carotene (\%)} = C_m / C_{BO} \times 100 \quad \text{(Eq. 4)}$$

where, C_m = carotene content in the microcapsule, and C_{BO} = carotene content in the bulk oil.

Morphological analysis

The microstructure of the palm carotene microcapsules was observed using a field emission scanning electron microscope (JSM-IT100, JOEL, Tokyo, Japan), operated at 5 kV. Prior to examination, the samples were mounted on metallic

stubs, and coated with a layer of platinum for enhanced imaging (Chang *et al*., 2019). The examination was conducted at magnifications of 500× and 2,000×.

Statistical analysis

The experimental results were analysed using Minitab software (Version 17, Minitab, Pennsylvania, United States). Data were presented as means and standard deviations from triplicate replications. One-way analysis of variance (ANOVA) at a 5% significance level was used to assess the significant differences ($p < 0.05$) between the means, and Tukey's *post hoc* test was used to identify significant differences between pairs.

Results and discussion

Moisture

The moisture content of the spray-dried carotene microcapsules ranged from 2.19 to 2.93%. Figure 1a illustrates that the moisture content decreased with the increased ratio of core-to-wall material used, regardless of the type of wall materials. Specifically, the combination of maltodextrin with OSS at core-to-wall material ratio of 1:4 resulted in the lowest moisture content of 2.19% compared to other ratios with different wall materials. All ratios with the combination of maltodextrin and OSS as the wall material showed lower moisture content of 2.73% in ratio 1:2, 2.65% in ratio 1:3, and 2.19% in ratio 1:4, compared to the other combinations with maltodextrin alone and maltodextrin with inulin at three different ratios.

The decrease in moisture content with increased wall material ratio can be explained by the spray drying process. The incorporation of wall materials into the feed during spray drying increases the total solid content, and decreases the amount of water for evaporation, leading to lower moisture content in the microcapsule (Quek *et al*., 2007). Microcapsules with lower moisture content are desirable as moisture content higher than 14% can adversely affect storage quality due to the excess availability of water, which can support the growth of bacteria, yeasts, and moulds, promote insect infestation and agglomeration, and decrease the shelf life, microbial stability, and physical properties such as flowability and encapsulation efficiency of the microcapsules.

Water activity (a_w)

Water activity (a_w) represents the ratio of the vapour pressure of water in a food system to that of pure water at the same temperature. It is a critical index for spray-dried microcapsules as it significantly impacts the product's shelf life. Unlike moisture content, which reflects the total water composition in a food system, water activity measures the amount of free water available for biological processes (Yusof *et al*., 2022). Higher water activity is associated with shorter shelf life, as it provides more free water accessible for biological reactions. Foods with a water activity below 0.6 a_w are generally considered microbiologically stable, and any deterioration that occurs is more likely due to chemical reactions rather than microbial growth (Quek *et al*., 2007).

Figure 1b illustrates the water activities of the carotene microcapsules, ranging from 0.20 to 0.29 a_w, indicating that the spray-dried microcapsules produced were chemically and microbiologically stable (Tan *et al*., 2022). Moreover, the results showed that water activity decreased with the increased ratio of oil-to-wall materials added, and this was due to higher wall material ratios increasing the total solid content, thus reducing the availability of free water in the microcapsules, and subsequently decreasing water activity (Tan *et al*., 2022). Microcapsules produced using the oil-to-wall material ratio of 1:4 in all combinations of wall materials significantly differed from those produced using the ratio of 1:2.

Colour measurement

The colour assessment for the carotene microcapsules is depicted in Figures 1c - 1e. The L* value increased, the a* value decreased, and the b* value also decreased significantly ($p < 0.05$) when the ratio of oil-to-wall materials used increased from 1:2 to 1:4, regardless of the type of wall materials used. This indicated that the colour of the microcapsules had become lighter with higher ratios of wall materials. One explanation for this result is that as the percentage of wall materials increased from 1:2 to 1:4, the feed became diluted with wall materials, resulting in a decrease in the red-orange colour of the carotene emulsion. Therefore, if less wall material was added into the feed, it would lead to better colour retention of the produced microcapsules. This finding agreed with those reported by Quek *et al*. (2007) on spray-dried watermelon powders, and Cynthia *et al*.

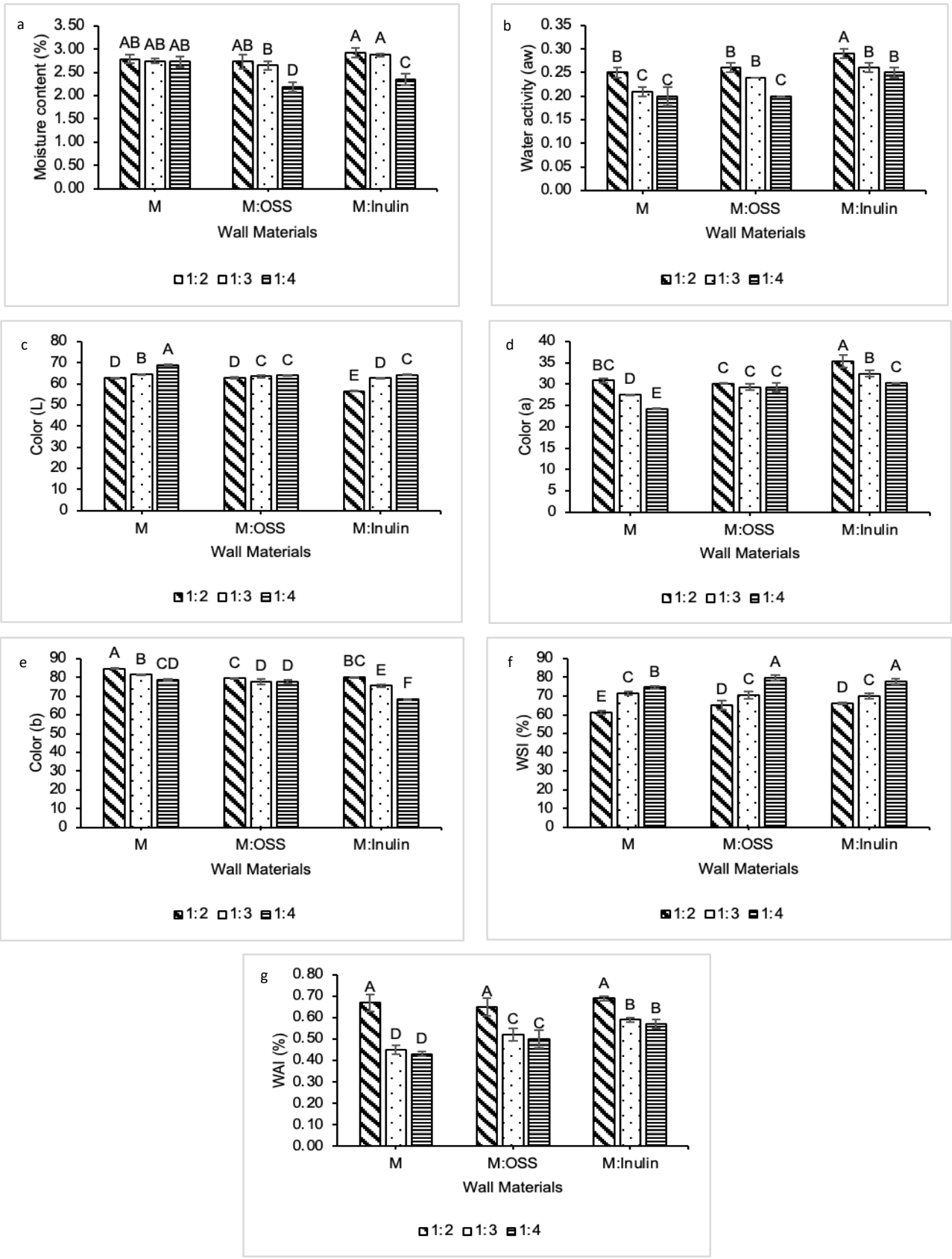

Figure 1. Physical properties of spray-dried carotene microcapsules produced using different types of wall materials and different ratios of core-to-wall material: **(a)** moisture content, **(b)** water activity, **(c)** colour (L*), **(d)** colour (a*), **(e)** colour (b*), **(f)** Water Solubility Index (WSI), and **(g)** Water Absorption Index (WAI). Values are mean ± standard deviation (n = 4). Means with uppercase letters within similar column are significantly different ($p < 0.05$).

(2015) on spray-dried tamarind (*Tamarindus indica* L.) pulp extract powder with encapsulating hydrocolloids.

Flowability and cohesiveness

Flowability and cohesiveness are crucial quality control parameters for dried microcapsules, often measured together as the Carr Index (CI) for flowability, and the Hausner Ratio (HR) for cohesiveness (Nishad *et al*., 2017). A higher CI (> 45%) indicates undesirable flowability, while a lower HR (< 1.2) represents better cohesiveness (Wang *et al*., 2019).

As shown in in Table 1, it was observed that, overall, the spray-dried carotene microcapsules produced using oil-to-wall materials ratio of 1:4 exhibited better flowability and low cohesiveness, regardless of the type of wall materials used. The CI ranged from 12.95 to 30.95%, and the HR ranged from 1.15 to 1.44. There were significant differences ($p < 0.05$) observed among microcapsules produced using different ratios and types of wall materials. Microcapsules produced using oil-to-wall materials ratio of 1:4 showed lower CI and HR compared to those produced using a ratio of 1:2, indicating that they were less sticky, and flowed more freely. Carr Index and Hausner Ratio are important as they act as indicators, helping to predict the behaviour of materials during manufacturing processes, which directly influences product formulation and compliance with regulatory standards (Kaleem *et al*., 2020).

Table 1. Flowability and cohesiveness of carotene microcapsules based on Carr Index and Hausner Ratio values.

Sample	Flowability	Carr Index (%)	Cohesiveness	Hausner Ratio
1:2 M	Fair	30.77 ± 1.98^{A}	Intermediate	1.39 ± 0.08^{A}
1:3 M	Fair	23.00 ± 2.45^{C}	Intermediate	1.30 ± 0.04^{B}
1:4 M	Good	18.60 ± 0.93^{D}	Intermediate	1.24 ± 0.02^{C}
1:2 M:OSS (1:1)	Fair	30.42 ± 3.94^{A}	High	1.44 ± 0.08^{A}
1:3 M:OSS (1:1)	Fair	24.61 ± 1.99^{C}	Intermediate	1.33 ± 0.04^{B}
1:4 M:OSS (1:1)	Very Good	12.95 ± 1.55^{E}	Low	1.15 ± 0.02^{D}
1:2 M:Inulin (1:1)	Fair	30.95 ± 2.75^{A}	High	1.41 ± 0.03^{A}
1:3 M:Inulin (1:1)	Fair	28.83 ± 1.83^{B}	Intermediate	1.39 ± 0.06^{A}
1:4 M:Inulin (1:1)	Fair	21.81 ± 2.37^{CD}	Intermediate	1.28 ± 0.04^{C}

Values are mean ± standard deviation ($n = 4$). Means with different uppercase superscripts within similar column are significantly different ($p < 0.05$). M: maltodextrin; and OSS: starch sodium octenyl succinate.

Several factors, such as moisture content, particle size, and particle morphology can influence the flowability and cohesiveness of microcapsules (Dirim and Caliskan, 2012; Yusof *et al*., 2022). In this case, the fair flowability and high cohesiveness shown by microcapsules produced with oil-to-wall material ratio of 1:2 was likely related to the higher moisture content of these microcapsules. The low amount of wall material used in the ratio of 1:2 may not have been sufficient to coat all of the oil droplets, resulting in higher moisture content in these microcapsules (Jinapong *et al*., 2008). In addition, the differences in the chemical structures of the polysaccharide utilised in the present work might also account for these outcomes. As observed, microcapsules produced using the combination of M:OSS as wall materials in core-to-wall materials ratio of 1:4 produced microcapsules with very good flowability and low cohesiveness, but for same core-to-wall materials ratio of 1:4, microcapsules produced using combination of M:Inulin as wall materials showed fair flowability and intermediate cohesiveness. This can be explained by inulin which is believed to be extremely hygroscopic due to its branched structure, which promotes hydrogen bonding, and consequently, moisture absorption from ambient air occurs easily, and causes the carotene microcapsules to have high moisture content compared to OSS (Lacerda *et al*., 2016).

Water Solubility Index (WSI) and Water Absorption Index (WAI)

The Water Solubility Index (WSI) and Water Absorption Index (WAI) are critical functional characteristics of food products, indicating their ability to hydrate in water. These indices are used to

estimate whether the food products are suitable for use as binders, stabilisers, emulsifiers, or protein sources in further processing (Du *et al*., 2023). WSI represents the product's capacity to dissolve in water, with higher solubility indicated by a high WSI (Nishad *et al*., 2017). Conversely, WAI assesses a microcapsule's ability to reassociate with water under restricted water conditions, making a high WAI undesirable for product storage due to increased susceptibility to gelatinisation and microbial infection.

The WSI of spray-dried carotene microcapsules ranged from 61.25 to 79.59%. Figure 1f demonstrates a significant increase in WSI ($p < 0.05$) as the ratios of oil-to-wall materials increased from 1:2 to 1:4. The combination of maltodextrin with OSS as the wall materials at a ratio of 1:4 yielded the highest WSI of 79.59%, while microcapsules produced using maltodextrin alone at a ratio of 1:2 yielded the lowest WSI of 61.25%. The high solubility of microcapsules produced with the combination of maltodextrin and OSS (ratio of 1:4) can be attributed to the good solubility of OSS and the high ratio of wall materials used (Jindal and Boonyai, 2001). A high WSI is generally preferred in the food sector, as it allows the product to be readily dissolved and incorporated with various other products (Tan *et al*., 2015).

The WAI of spray-dried carotene microcapsules varied from 0.43 to 0.69%. Figure 1g shows significant decrease in WAI ($p < 0.05$) as the ratios of wall materials increased from 1:2 to 1:3, but further increase to a ratio to 1:4 resulted in an approximately stable WAI for all types of wall materials used. The low WAI observed in the microcapsules indicated their limited capacity to absorb water, making them less susceptible to humidity-related effects during storage (Vidovic *et al*., 2014).

However, as the ratio of oil-to-wall materials increased, a decreasing trend in WAI and an increasing trend in WSI were observed. This agreed with the findings of Ahmed *et al.* (2010), who noted that particles leading to lower WAI tended to exhibit improved water solubility and *vice versa*. In conclusion, good-quality microcapsules should have high WSI and low WAI. Desirable microcapsules are expected to moisten quickly and fully, sink rather than float, and disperse or disintegrate without lumping (Phoungchandang and Sertwasana, 2010).

Microencapsulation efficiency (MEE)

Figure 2 illustrates that the microencapsulation efficiency (MEE) of the spray-dried carotene microcapsules was significantly affected by the ratio of core-to-wall material and the type of wall material used. The MEE showed an increasing trend ($p < 0.05$) as the ratio of oil-to-wall material increased from 1:2 to 1:4 for all types of wall materials. The MEE values ranged from 23.28 to 70.50%.

The combination of maltodextrin with OSS as the wall material at oil-to-wall material ratio of 1:4 exhibited the highest MEE of 70.50% compared to other wall material combinations at the same ratio. Similar results were reported by Charved and Reineccius (2009), who found that microencapsulate particles produced using modified starch *via* spray drying displayed higher oil retention compared to particles formed using gum Arabic and whey protein. Jeon *et al*. (2005) also mentioned that chemically altered (succinylated) corn and barley starches showed higher oil retention capability than native starches, which can be attributed to their excellent emulsion stabilisation properties. Moreover, a sufficient amount of wall material used at the ratio of 1:4 could coat all the emulsion, contributing to higher microencapsulation efficiency. According to Barbosa *et al*. (2005), the higher microencapsulation efficiency results in a more stable emulsion with lower non-encapsulated material on particles surface.

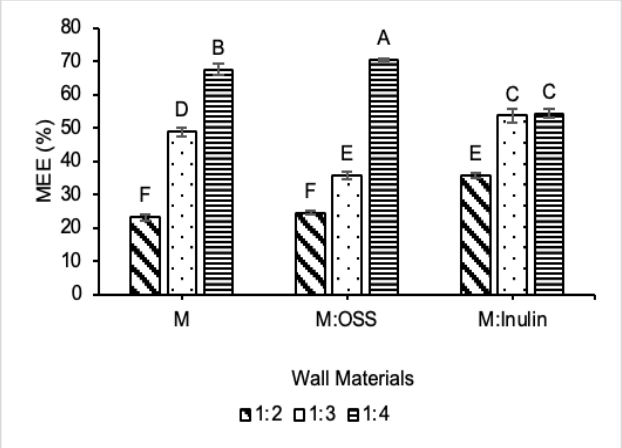

Figure 2. Microencapsulation efficiency (MEE) of spray-dried carotene microcapsules produced using different types of wall materials and different ratios of core-to-wall material. Values are mean ± standard deviation (n = 4). Means with different uppercase letters within similar column are significantly different ($p < 0.05$).

Carotene content

Figures 3a - 3b highlights the substantial impact of the ratio of core-to-wall material and the type of wall material on the carotene contents of the spray-dried carotene microcapsules. The carotene contents showed an increasing pattern ($p < 0.05$) as the ratio of oil-to-wall material increased from 1:2 to 1:4. The entrapment efficiency ranged from 37.47 to 72.79% for β-carotene, and 28.95 to 60.85% for α-carotene, indicating that β-carotene had higher concentration compared to α-carotene in the carotene microcapsule, and this may be because β-carotene is the more common form of carotenoid, and highly distributed in plant sources (palm oil = β-carotene: 60%; α-carotene: 30%), suggesting a higher thermal resistance (Jayesree, 2017). Therefore, the coexistence of α-carotene and its functional values in food applications are often less noticeable, as β-carotene is always more abundant (Jayesree, 2017). The combination of maltodextrin with OSS as the wall material at a core-to-wall material ratio of 1:4 exhibited the highest carotene entrapment efficiency for both α- and β-carotenes. However, in the ratio of 1:4, the entrapment efficiency for the combination of maltodextrin alone and maltodextrin with OSS showed no significant difference for α-carotene.

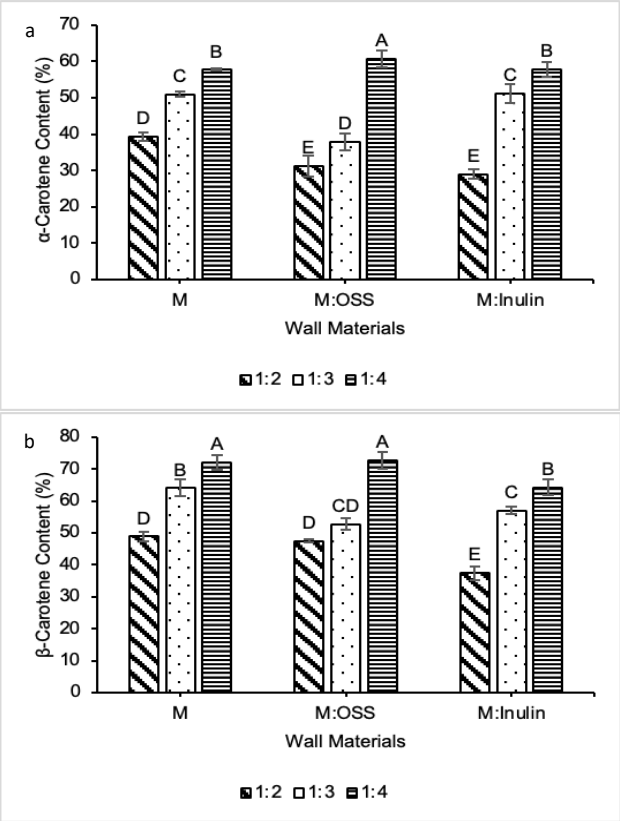

Figure 3. Carotene contents of spray-dried carotene microcapsules produced using different types of wall materials and different ratio of core-to-wall material: **(a)** α-carotene content, and **(b)** β-carotene content. Values are mean ± standard deviation ($n = 4$). Means with different uppercase letters within similar column are significantly different ($p < 0.05$).

A higher ratio of oil-to-wall material (1:4) resulted in the formation of a thicker coating layer on the surface of the core materials, preventing major light penetration and degradation of carotene compared to lower ratios (1:2 and 1:3). Thus, the carotene entrapment efficiency was higher in core-to-wall material ratio of 1:4 compared to other core-to-wall material ratios. In addition, a thicker layer formed by a higher ratio of core-to-wall material can reduce heat destruction by high inlet temperature during spray drying (Ramakrishnan *et al*., 2018). Carotene is highly susceptible to thermal degradation and oxidation due to its highly unsaturated chemical composition (Seregelj *et al.*, 2022).

Morphological analysis

Figure 4 displays the SEM microstructures image of spray-dried carotene microcapsules using a 1:4 ratio of oil-to-wall material, providing insight into the microcapsule morphology. The images reveal that the type of wall material used significantly influenced the microstructures, with the spherical shape and variation in sizes being distinctive traits of spray-dried microcapsules (Carneiro *et al*., 2013). Microcapsules produced using maltodextrin with OSS as the wall material exhibited smoother and less dented surfaces compared with those produced using maltodextrin alone and maltodextrin with inulin. This was due to OSS being a good encapsulation agent which is suitable for the encapsulation of bioactive ingredients as it has high viscosity when solubilised in water, plus it is resistance to oxidation which makes it efficient to form a good coating layer to protect sensitive bioactive ingredients (Montoya *et al*., 2023), such as carotene in the present work. This may also indicate that carotene microcapsules formed using maltodextrin with OSS had lower gas permeability, and were less porous, allowing for better preservation of carotene. This result was also supported by the highest microencapsulation efficiency (70.50%) and highest carotene contents (α: 60.85% and β: 72.79%) observed in this sample.

On the other hand, microcapsules formed using maltodextrin with inulin as the wall material showed rougher and more dented surface. This condition will increase gas permeability, and make the microcapsule

more porous and prone to poor carotene retention, leading to lower microencapsulation efficiency (54 - 57%) and lower carotene contents (α: 57.88% and β: 64.33%). In addition, the appearance of the microcapsules will be affected, becoming stickier and more prone to caking, indirectly decreasing consumer acceptance (Montoya *et al*., 2023). This phenomenon is basically caused by the crust forming more slowly during the spray drying procedure, resulting in the creation of tiny voids in the microcapsules shortly after the crust develops. The uneven surface of the microcapsules is a consequence of the swelling of the crust due to the microcapsule's temperature exceeding the surrounding boiling point, while the internal pressure in the tiny voids surpasses atmospheric pressure (Nijdam and Langrish, 2006). According to Yusof *et al*. (2022), the dents and cracks of the microcapsules can also be caused by the rapid evaporation of the liquids droplets during the spray drying, or due to the capability of the wall material that resists the formation of the crust structure during the drying process.

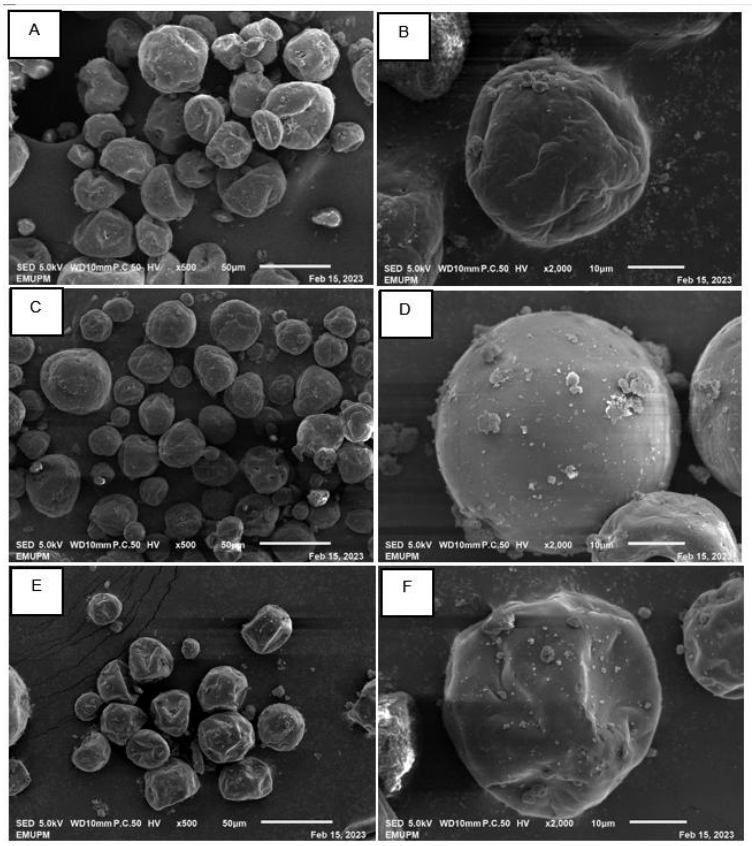

Figure 4. Carotene microcapsules (spray-dried at 170°C, 1:4 ratio of core-to-wall material) formed using maltodextrin [**(A)** and **(B)**], maltodextrin:OSS [**(C)** and **(D)**], and maltodextrin:inulin [**(E)** and **(F)**]. Scale bar represents 50 μm for **(A)**, **(C)**, **(E)**, and 10 μm for **(B)**, **(D)**, **(F)**.

Conclusion

Stable palm-carotene-based microcapsules were successfully produced using a 1:4 ratio of oil-to-wall material with a combination of maltodextrin and OSS as wall materials. The combination of these wall materials formed an effective coating that preserved carotene as the bioactive compound. The findings clearly demonstrated that carotene microcapsules encapsulated using this formulation exhibited the lowest moisture content and water activity, good flow properties, and low cohesiveness. The higher WSI of this microcapsule indicated its ease of dissolution and incorporation with other products. Simultaneously, the lower WAI suggested higher stability, reducing the microcapsules' propensity to absorb water, and making them less susceptible to humidity and microbial activity compared to microcapsules formed using maltodextrin alone and a combination of maltodextrin with inulin. Furthermore, microcapsules formed using a 1:4 ratio of oil-to-wall material with a combination of maltodextrin and OSS also achieved a high microencapsulation efficiency, resulting in a high percentage of carotene content and good morphology compared to those formed using maltodextrin alone and the combination of

maltodextrin with inulin. The positive findings contributed to a deeper understanding of using different types of wall materials for encapsulating functional bioactive compounds to produce stable microcapsules. These stable carotene microcapsules can be widely applied and incorporated into food production, such as in ice cream or mayonnaise products, due to their longer shelf life and scalability. The formulation can be easily scaled up once optimised.

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