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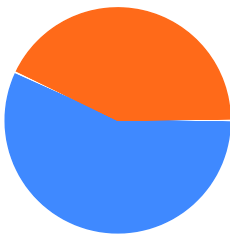

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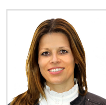

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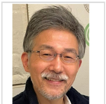

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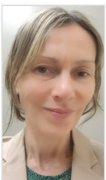

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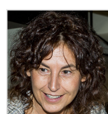

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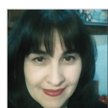

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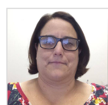

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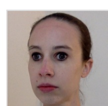

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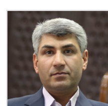

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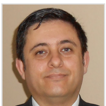

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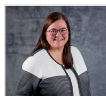

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ISSN: 0268-005X

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Food Hydrocolloids 112 (2021) 106281

Contents lists available at ScienceDirect

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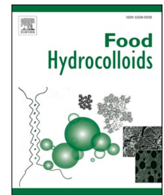

Characterization of freeze-dried microencapsulation tuna fish oil with arrowroot starch and maltodextrin

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ARTICLE INFO

Keywords:
Tuna fish oil
Arrowroot starch
Freeze drying
Microencapsulation
Maltodextrin
Oxidative stability

ABSTRACT

Arrowroot starch (AS), extracted from a tropical rhizome (*Maranta arundinacea*), was investigated and compared to maltodextrin (MD) in whey protein (WP) combinations for its potential as a novel wall material to microencapsulate and limit oxidative rancidity of tuna fish oil. Tuna fish oil was mixed into six emulsions composed of 5:1, MD:WP (P1); 6.5:1, MD:WP (P2); 5:1, AS:WP (P3); 6.5:1, AS:WP (P4); 3.344:1.668:1, MD:AS:WP (P5); and 3.25:3.25:1, MD:AS:WP (P6) to produce microcapsules by freeze drying. The microcapsules were studied for encapsulation efficiency (EE), moisture content, water activity, peroxide value (PV), acid value (AV), microstructure, and the degree of primary oxidation was evaluated every 15 days for 90 days at ±25 °C. The fish oil microcapsules demonstrated high EE (80.5–86.4%), low moisture content (0.24–3.47%), and low water activity (0.05–0.23). The microcapsules exhibited PV (4.80–9.20 mEq/kg oil) and AV (1.46–2.24 %FFA) values that remained below industry-set maximum limits of PV (10 mEq O_2/kg oil) and AV (<3) for refined fish oil. Particle size of the freeze-dried microcapsules ranged 26.72–103.14 μm with irregular to smooth surfaces. Microcapsules of MD:AS:WP (P6) combination exhibited the highest oxidation stability during the 90-day storage test. The results demonstrated that arrowroot starch: maltodextrin combinations successfully encapsulated tuna fish oil, improved shelf life, and demonstrated oxidative stability. Moreover, arrowroot starch behaved as a cryoprotectant during freeze-drying, and as wall material in tuna fish oil microencapsulation indicated its microencapsulation carrier and application potential in food fortification.

1. Introduction

Modern food research has shifted from qualitative and quantitative nutrition studies to investigating the roles of functional foods, whereby a food product and its components can impart additional health benefits and reduce the risk of chronic diseases (Grajek et al., 2005). Consumers are increasingly aware of the need to maintain their health by adopting healthier dietary choices. Fish oils, a popular dietary ingredient, are a rich source of omega-3 polyunsaturated fatty acids (PUFAs), and is composed of mainly docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (Di Giorgio, Salgado, & Mauri, 2019; Jeyakumari et al., 2015; Kurek et al., 2018). However, fish oils have several undesirable characteristics such as low water solubility, high susceptibility to oxidation, pungent odor, and unpleasant taste (Encina et al., 2016; Klaypradit & Huang, 2008), which limit their use in the diet despite their acceptance as rich sources of the bioactive omega-3 fatty acids. Hence, the challenge to food processors is to prevent or reduce oxidative deterioration of fish oils and improve their incorporation into foods.

Microencapsulation involves entrapping, packaging and sealing a solid, liquid, or gaseous (core) material inside a secondary, more stable (wall) material. This technique is useful in the pharmaceutical and food industries as it enables the protection of bioactive compounds and their release at controlled rates under specific conditions (Klaypradit & Huang, 2008; VILSTRUP, 2004). Microencapsulation methods

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https://doi.org/10.1016/j.foodhyd.2020.106281
Received 22 May 2020; Received in revised form 12 July 2020; Accepted 25 August 2020
Available online 14 September 2020
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commonly used in foods and beverages are spray drying and freeze drying, although other methods such as spray granulation and liposome entrapment are also used to protect natural or inherent bioactive ingredients and prevent the loss of flavor during storage, and extend product shelf life (Berry, 2004; Shefer & Shefer, 2003). Factors such as total solids concentration, oil content, viscosity, droplet size, and emulsification influence the stability of the prepared emulsions. Moreover, the stability of these emulsions plays a significant role in maximizing the encapsulation efficiency and stability of the microencapsulation of bioactive ingredients (Cao et al., 2018; Di Giorgio, Salgado, & Mauri, 2019).

Unfortunately, one disadvantage of using spray drying is exposing fish oil to high temperatures that lead to oxidation (Anwar & Kunz, 2011). This makes freeze drying or lyophilization an attractive alternative as the process uses low temperature and an absence of air minimizing the risk of oxidation (Fang & Bhandari, 2012, pp. 73–109). However, as the process can generate stress on the wall material, the wall material has to also act as a cryoprotectant to help stabilize the wall material (of sensitive products) such as probiotics (Kailasapathy & Sureeta, 2004). Anwar and Kunz (2011) also reported that the morphology of microencapsulated fish oil using freeze drying was structurally irregular, highly porous, and had large surface area, which indicated that the wall material used in freeze drying methods must showed cryoprotection ability.

Among polysaccharides, starches are a low-cost hydrocolloid that provide an effective oxygen barrier, high capacity to form a matrix and have low viscosity at high solids concentration in an emulsion (Jafari et al., 2008; Sartori & Menegalli, 2016). The inclusion of starches in the wall material can also serve as a substrate for probiotic cores increasing the viability, while simultaneously supplying prebiotics (Fang & Bhandari, 2012, pp. 73–109) for gut microflora. Prebiotics are non-digestible or low-digestible food ingredients that stimulate the growth of one or a few bacteria classified as probiotics such as bifidobacteria, thereby providing the host with health benefits. Prebiotics undergo little to no digestion in the small intestines, and their fermentation in the gut, in addition to delivering probiotics, exhibit synergistic effects in the host digestive system leading these combinations to be referred to as synbiotics (Grajek et al., 2005).

The most practical microcapsule consists of a core substance surrounded by a wall or barrier. There are many substances that can be used as wall material in the encapsulation process of tuna fish oil. Among the various wall materials, polysaccharides are the most widely used material for microencapsulation. Starches used in microencapsulation are usually modified starches (Fang & Bhandari, 2012, pp. 73–109). However, research on the use of traditional sources of or native starch in fish oil microencapsulation using lyophilization are either rare or non-existent. Arrowroot (*Maranta arundinacea*) is a plant of South American origin that has been distributed along the tropics, and its rhizomes produce an easily digested starch (Charles et al., 2016). Arrowroot starch possesses low protein, lipids, ashes, and rich in amylose content, which promises many applications in food product development (Nogueira et al., 2018). However, arrowroot starch as wall material for fish oil microencapsulation products is a novelty that remains unreported in the literature. Polysaccharide derivatives such as maltodextrin are commonly used as wall materials (Wandrey et al., 2009), in combinations with whey protein, which is widely used in the food industry because of its excellent surface activity and its ability to develop and stabilize oil in water (o/w) emulsions (Encina et al., 2016).

Research on microencapsulation of tuna fish oil has focused on finding the best wall material as the material used will affect the final properties of the microcapsules (Anwar & Kunz, 2011). Therefore, the purpose of this study was to microencapsulate tuna fish oil by freeze-drying and investigate the wall material potential of arrowroot starch compared with maltodextrin, and to compare their effects on the physicochemical characteristics, oxidation, and oxidative stability of tuna fish oil microencapsules.

2. Materials and methods

2.1. Sample description

Omega oil 0525 TG Gold (kindly provided by BASF, Taichung, Taiwan and referred to as tuna fish oil in this study), was used as the core material. The wall materials selected for the study were maltodextrin (MD) (Lansida CV, Yogyakarta, Indonesia); and native industrial arrowroot starch (AS), which was a gift from the St. Vincent Arrowroot Association. Whey protein (WP) isolate (AMPC Inc., Ames, Iowa) was used as the emulsifier. All sample materials were stored in a digital humidity controller (Temperature: ±25 °C; relative humidity: 50%). All other chemicals were of analytical grade. Deionized water was obtained from Milli-Q (Millipore) water purification system (Billerica, MA, USA).

2.2. Preparation of emulsion and freeze drying

The selection of wall material ratio combinations to prepare emulsions for freeze drying followed Anwar and Kunz (2011) with slight modification. The emulsions were prepared with 37.5% (w/v) total solid wall material, 12.5% 9 (v/v) tuna fish oil, and 50% (v/v) water. Tuna fish oil (core) and wall material were used in various ratio combinations (P1, P2, P3, P4, P5, and P6) to prepare the tuna fish oil microcapsules (Table 1). An aqueous phase was prepared at predetermined ratios (Table 1) by dissolving the wall materials, AS or MD, with WP in deionized water in centrifuge tubes (immersed in an ice bath) using a laboratory homogenizer (OMNI TH, Omni Inc, Kennesaw GA, US) at 10, 000 rpm for 5 min. After complete dissolution of the wall materials, tuna fish oil (core material) (Table 1) was added slowly to the wall material solutions. The mixtures were emulsified using a homogenizer at 10,000 rpm for 6 min. The emulsions (Table 1) were transferred to freeze dryer flasks, which were kept at −20 °C for 24 h to initiate the freeze drying method. The samples were freeze dried in a Labconco Freezone 6 Plus model freeze dryer (Kansas, USA) at a condenser temperature set lower than −50 °C for ± 48 h. When the freeze-drying process was completed, the microencapsulated powders were gently ground and filtered to obtain a uniform granule size fine flowing powder. All samples were kept in air tight amber-colored sample bottles, and stored in a digital controller humidity (Temperature: ±25 °C; relative humidity:50%) for further analysis.

2.3. Encapsulation efficiency (EE)

The encapsulation efficiency (EE) of tuna oil was determined according to the method of Bejrapha et al. (2010). Hexane (15 mL) was added to 2.5 g of tuna fish oil microencapsulates and vortexed for 2 min. Then, the mixtures were centrifuged at 3000 rpm for 30 min. The supernatant was collected and evaporated in the fume hood. The amount

Table 1
Emulsion ratios to microencapsulate tuna fish oil.

Formulation Code	Wall materials		Whey Protein (g)	Tuna oil (g)	Water (mL)
	Maltodextrin (g)	Arrowroot Starch (g)			
P1[a]	12.5	–	2.5	5	20
P2[b]	13	–	2	5	20
P3[c]	–	12.5	2.5	5	20
P4[d]	–	13	2	5	20
P5[e]	8.33	4.17	2.5	5	20
P6[f]	6.5	6.5	2	5	20

[a] Maltodextrin: Whey Protein (5.0: 1.0) (Control).
[b] Maltodextrin: Whey Protein (6.5: 1.0) (Control).
[c] Arrowroot Starch: Whey Protein (5.0: 1.0).
[d] Arrowroot Starch: Whey Protein (6.5: 1.0).
[e] Maltodextrin: Arrowroot Starch: Whey Protein (3.344:1.668:1.0).
[f] Maltodextrin: Arrowroot Starch: Whey Protein (3.25:3.25: 1.0).

of free tuna fish oil was determined gravimetrically. Encapsulation efficiency was calculated quantitatively using equation (1):

$$EE\ (\%) = \left[\left(\frac{fish\ oil\ in\ the\ powder(g) - surface\ fish\ oil\ in\ the\ powder\ (g)}{fish\ oil\ in\ the\ powder\ (g)}\right) \times 100\%\right] \quad (1)$$

2.4. Measurement of moisture content

The moisture content was measured using MX-50 moisture analyzer (A&D Company, Tokyo, Japan) according to a method described in Charles and Alamsjah (2019). A specific program was created to analyze sensitive powder containing tuna oil. Briefly, 5.0 g microencapsulate powder was accurately placed into an aluminum pan and heated at 105 °C. The moisture content (%) was recorded for each sample after a stable weight was obtained.

2.5. Water activity (a_w)

Water activity of tuna fish oil microencapsulates was determined by the Dew Point method using AquaLab CX-2 water activity meter (Decagon Devices, Inc., Pulman, WA, US) at ±25 °C and followed the method by Klaypradit and Huang (2008). One gram of microcapsules was inserted into the tube provided, and placed in the instrument. The analysis took approximately 2 min for each sample and the results were recorded upon reaching equilibrium.

2.6. Peroxide value analysis (PV)

The PV of tuna fish oil microcapsules was determined by iodometric titration as described by Firestone (2009). This method is based on the titration of iodine released from potassium iodide by peroxide using a standardized thiosulfate solution as the titrant and a starch solution (0.5 mL, 1% w/v) as the indicator. Moreover, this method detects all substances that oxidize potassium iodide under acidic conditions. Peroxide value (mEq peroxide/kg sample) was determined using the following equation (2):

$$PV = \left[\frac{(S - B) \times N \times 1000}{W}\right] \quad (2)$$

Where B is the titration of blank (mL), S is the titration of sample (mL), N is the normality of sodium thiosulfate solution, and W is the weight of sample (g).

2.7. Acid value analysis (AV)

The AV of tuna fish oil microcapsules was determined by Gooch (2011). Samples were weighed (5.0 g), transferred to Erlenmeyer flasks, and mixed with 100 mL hot neutral alcohol. The contents were swirled to dissolve the sample, and phenolphthalein (1 mL, 1% w/v) was added as an indicator. The solutions were titrated with 0.5 N sodium hydroxide (NaOH). Acid values (mg KOH per g of fatty acids) were determined using the following equation (3):

$$AV = \left[\frac{V \times M \times 56.10}{W}\right] \quad (3)$$

Where V is volume of titrant (mL), M is molarity of accurately standardized sodium hydroxide solution, and W is the weight of sample (g).

2.8. Degree of oxidation

Degree of oxidation in the microcapsules was determined by the thiobarbituric active reactive substance (TBARS) method of Hu and Zhong (2010) at 15-day intervals for 90 days, at ± 25 °C. The data collected was expressed in mmol malondialdehyde (MDA) equivalent/kg oil. TBARS value was determined using the following equation (4):

$$TBARS = \left[\left(\frac{A_{532}/(\varepsilon b)}{M \times wt.\%}\right) \times 10^7\right] \quad (4)$$

Where A_{532} is the absolute absorbance at 532 nm, ϵ is the molar absorptivity ($M^{-1}cm^{-1}$), b is the light path length (1 cm), M is sample weight (mg), and wt.% is the weight oil percentage in microencapsulated sample.

2.9. Surface observation by scanning electron microscopy (SEM)

A Hitachi S–3000 N scanning electron microscope (Tokyo, Japan) was used to investigate the structural properties and to determine particle size of the tuna oil microcapsules. The samples were placed on double-sided adhesive carbon tabs, and were subsequently coated with gold. The coated samples were analyzed with the SEM operating at an accelerating voltage of 10.0 kV, according to the procedure by Charles et al. (2016).

2.10. Statistical analysis

One-way analysis of variance (ANOVA) using the SAS statistical program 9.1 (SAS Institute, Cary, NC, US) was conducted to evaluate the differences in the mean values (moisture content, water activity, PV, AV, EE, and degree of oxidation) with statistical significance determined at $p < 0.05$. Each treatment had five replicate determinations and comparison of the means was conducted by Duncan's multiple range test to determine significant differences between the tested wall materials used for the microencapsulation of tuna fish oil.

3. Results and discussion

3.1. Encapsulation efficiency (EE)

Encapsulation efficiency (EE) is a key factor used to evaluate the potential of different wall materials for encapsulating core materials. EE also reflects the quantity of tuna fish oil encapsulated in the wall material matrix of the freeze-dried microcapsule, which is vital for stable and long storage. In this study, efficient of microencapsulation tuna fish oil using freeze drying were analyzed and the influence of arrowroot starch on the quality of tuna fish oil microcapsules was determined. According to the results obtained, the amount of arrowroot starch, the ratio of individual wall materials (MD:WP and AS:WP), wall materials combinations (MD:AS:WP), and fish oil content had significant influence on encapsulation efficiency ($p < 0.05$). EE values of the tuna fish oil microcapsule ranged from a minimum of 80.52% (P3) to a maximum of 86.4% (P2). A similar study by Klaypradit and Huang (2008) on tuna fish oil encapsulated with chitosan, maltodextrin, and whey protein produced microcapsules that exhibited 79.3–85.54% EE, whereas a 60–80% EE was reported by Bejrapha et al. (2010) using freeze drying

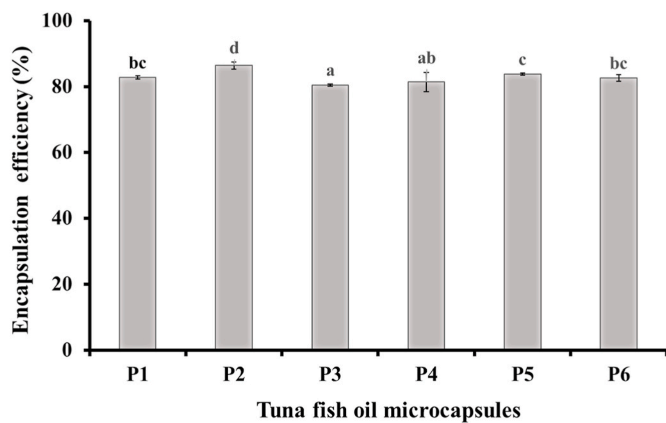

Fig. 1. Encapsulation efficiency of microencapsulated tuna fish oil from (P1) Maltodextrin: Whey Protein (5.0:1.0) as control; (P2) Maltodextrin: Whey Protein (6.5:1.0) as control; (P3) Arrowroot starch: Whey Protein (5.0:1.0); (P4) Arrowroot starch: Whey Protein (6.5:1.0); (P5) Maltodextrin: Arrowroot Starch: Whey Protein (3.344:1.668:1.0); and (P6) Maltodextrin: Arrowroot Starch: Whey Protein (3.25:3.25: 1.0). Different letters show significant differences between treatments ($p < 0.05$).

method. The individual wall material MD:AS (P2) exhibited the highest value of EE (86.4%). However, microcapsules composed of arrowroot starch and maltodextrin combinations (P5 and P6) (Fig. 1) demonstrated significantly similar levels of EE compared to (MD:WP) P1 and P2 microcapsules samples made from maltodextrin. Moreover, microcapsules (P4) made of arrowroot starch as the major wall material component showed similar EE with P1 microcapsules, which were made of maltodextrin as the major wall material. The high EE value range of this study suggested that arrowroot starch behaved to a certain degree as a cryoprotectant, which helped stabilize and protect against spontaneous oxidation and increased the shelf life of the air-sensitive tuna fish oil stored in the microcapsules.

3.2. Moisture content and water activity (a_w)

Moisture content of microencapsulated fish oil is an important parameter, as high water activity enhances lipid oxidation. Moreover, at high moisture levels, the wall material changes from the glassy state to amorphous rubbery state with high molecular mobility, leading to the release of microencapsulated oil during storage (Velasco et al., 2000). Reportedly, moisture content greatly influences physical, chemical, and microbial stability of foods, whereas water activity is usefull to determine non-enzymatic and enzymatic activities, lipid oxidation, and

Table 2
Biochemical properties of tuna fish oil microcapsules[a,b].

Sample Code	Peroxide Value (PV) (mEq/kg oil)	Acid Value (AV)	Moisture Content (%)	Water Activity (a_w)
P1	4.80 ± 1.09[a]	1.56 ± 0.25[a]	1.45 ± 0.16[b]	0.12 ± 0.008[b]
P2	5.20 ± 1.09[a]	1.90 ± 0.31[ab]	1.36 ± 0.30[b]	0.13 ± 0.021[b]
P3	8.00 ± 1.41[b]	2.35 ± 0.47[b]	0.45 ± 0.24[a]	0.06 ± 0.013[a]
P4	9.15 ± 2.28[b]	2.24 ± 0.39[b]	0.24 ± 0.08[a]	0.05 ± 0.005[a]
P5	7.60 ± 0.89[b]	1.46 ± 0.50[a]	3.47 ± 0.39[c]	0.21 ± 0.021[c]
P6	9.20 ± 1.79[b]	1.46 ± 0.30[a]	3.35 ± 0.29[c]	0.23 ± 0.035[c]

[a] Results are means ± SD, n = 5.
[b] Values within a column with different superscript letters are significantly ($P < 0.05$) different.

microbial growth in foods (Velasco et al., 2000). Based on this theory, the moisture contents and water activities of the sample treatments were in agreement; for example, microcapsules with only arrowroot starch as wall material exhibited significantly lower moisture contents and water activities (Table 2) compared to those samples with wall material composed of arrowroot starch and maltodextrin, phenomena attributed to the wall material composition. The results of this study were consistent with Klaypradit and Huang (2008), which reported less than 0.3 a_w of microencapsulated fish oil with combinations of chitosan and maltodextrin as wall materials. In addition, moisture content of all microencapsulated fish oil treatments ranged 0.24–3.47%, which was lower than the maximum moisture content limit (set below 4%) for dry powdered food products (Klinkesorn et al., 2006). The freeze drying process works by reducing the moisture content and water activity of dried material (Gharsallaoui et al., 2007). Moreover, morphology studies indicated that freeze-dried powders are highly porous, which facilitate oxygen diffusion (Anwar & Kunz, 2011). Based on the literature, the rate of diffusivity, which is influenced by different contents of moisture content and water activity, depends on matrix (wall coating) porosity, and adsorbed water that forms a protective layer against oxidation. Based on this theory, microcapsules (P5 and P6) with higher moisture contents (3.47 and 3.35%) and a_w (0.21 and 0.23) should demonstrate (and our study demonstrated significantly) better acid values (AV) than the other samples (Table 2). Our findings proved that higher moisture content (1.45,1.36, 3.47, and 3.35%) and a_w (0.12, 0.13, 0.21, and 0.23) in the microcapsule samples (P1, P2, P3 and P4), respectively correlated and indicated better stability against oxidation, which disagreed with the conclusions of Anwar and Kunz (2011) on the effects of moisture content and a_w on oxidative stability of freeze-fried powders.

3.3. Peroxide value (PV)

The PV is a standard assay used to evaluate fish oil oxidation after microencapsulation and during storage (Kolanowski et al., 2007). The PV is attributed to increasing formation of hydroperoxides as the primary oxidation products that reflect oxidative deterioration of oils (Gordon, 2004; Kong & Singh, 2011). Table 2 summarizes PV values for 6 tuna fish oil microcapsule samples stored at ±25 °C for one week. The statistical analysis indicated that PV values were significantly ($p < 0.05$) affected by different wall material combinations. The samples with maltodextrin (MD) wall material exhibited significantly lower PV values; treatments P1 and P2 indicated the highest oxidative stability (4.80 mEq/kg oil and 5.2 mEq/kg oil) followed by P3, P4, P5, and P6 (8.00, 9.15, 7.60, and 9.20 mEq/kg oil), respectively. However, all samples exhibited PV values within the limits accepted for microencapsulated fish oil that is set at PV ≤ 10 mEq/kg of oil (Bannenberg et al., 2017), and by the British Pharmacopeia Fish Oil Type I, EU Pharmacopeia Fish Oil Type I (De Boer et al., 2018), and Australian government guidelines. Moreover, a similar study using freeze-dried microencapsulated fish oil recorded PV < 10 mEq/kg oil (Anwar & Kunz, 2011). In general, newly refined oil must have PV < 1 mEq/kg oil and stored oil with PV values of up to 10 mEq/kg of oil before the production of undesirable rancid odors (Hogan et al., 2003). The PV results showed that all 6 preparation treatments of microencapsulated tuna fish oil exhibited acceptable PV values.

PV values within each group wall material combinations (P1 and P2, P3–P6) (Table 2) exhibited similar oxidation behavior, which demonstrated that different whey protein content failed to influence oxidation. Therefore, differences in oxidation of the microcapsules were attributed to polysaccharide components of the wall material. The low PV values of the microcapsules suggested that tuna fish oil was well protected in the matrix, which was attributed to the development of a better physical shield or barrier reinforced by whey protein. The formed protein network might have helped to delay release of the core material during the freeze-drying process, and during storage; moreover, the strength of

the physical barrier was further improved from cross-linking protein and arrowroot starch combinations in P3 and P4 microcapsules. The combination between protein, arrowroot starch and maltodextrin in P5 and P6 showed similar protection from oxidation with P3 and P4, which confirmed arrowroot starch could potentially supplement maltodextrin as wall material in tuna oil microencapsulation.

3.4. Acid value (AV)

The AV is reported as one indicator of hydrolytic rancidity of many fish oil products in the market (De Boer et al., 2018). Hydrolytic rancidity increases when triglycerides are hydrolyzed and FFA are produced due to slow hydrolysis of oils by heat, microbial, and enzymatic activities, and serve as the main substrates of lipid oxidation (Gibson & Newsham, 2018). Rancidity occurs in food products during storage and affects odor, taste, and lowers nutritive values (Riaz & Rokey, 2012). A low AV value indicates better oil quality, and based on Codex Standard for Fish Oils Rep 15/FO Appendix III (CAC, 2017) and United States Pharmacopeia (De Boer et al., 2018), the acid value of fats/oils intended for human consumption should be ≤ 3 mg KOH/g. In our study, all AV of tuna fish oil microcapsule treatments were below the acceptable limit, which indicated the wall material combinations either significantly inhibited or delayed hydrolytic rancidity of tuna fish oil. The statistical analysis demonstrated that AV were significantly affected ($p < 0.05$) by combination of wall material treatment. The lowest AV were recorded for P1, P5, and P6 (1.56, 1.46, and 1.46, respectively), which were slightly significantly lower than P2 (1.90), and the highest AV were determined for P3 and P4 (2.35 and 2.24), respectively. Similar AV values between maltodextrin-whey protein (P1) and maltodextrin, arrowroot, and whey protein (P5 and P6) combinations indicated that arrowroot starch could improve the ability of the wall material to protect the core material from hydrolytic rancidity. The lower AV values of both treatment groups suggested that increases in whey protein and arrowroot starch component likely delayed the development of hydrolytic rancidity. The low AV (and corresponding PV values, Table 2) were attributed to the effect of freeze-drying, which is an alternative method, although costly, for creating dried foods that are highly soluble with extended shelf life (low water activity). Freeze drying conducted at temperatures lower than ambient temperature, and in the absence of air, prevents product damage caused by oxidation or chemical modification. Rancidity process starts during oil processing and continues under refrigerated or frozen temperature at a slower rate (Gunstone, 2001). In this study, the synergy between arrowroot starch and maltodextrin to produce a cryoprotectant role could be further investigated in freeze drying process and during refrigeration. Arrowroot starch demonstrated a similar cryoprotectant effect by minimizing freeze-thaw damage and improving thermal stability in cassava and sweet potato gel pastes (Charles et al., 2016).

3.5. Degree of oxidation

Thiobarbituric active reactive substances (TBARS) is one of the secondary oxidation products produced by the breakdown of oxidized PUFA, which is a widely used assay to measure lipid peroxidation end product malondialdehyde (Kurek et al., 2018). The nutritional content, shelf life, and consumer appeal of dietary oil can be compromised by oxidative rancidity, which involves an undesirable series of chemical reactions that involves degradation of oil quality due to exposure to oxygen. In this study, TBARS values were significantly ($p < 0.05$) affected by the various wall material combination treatments. Generally, microencapsulated tuna fish oil showed higher oxidation stability than the unmicroencapsulated tuna fish oil, which demonstrated rapid increase in oxidation breakdown from 13.63 (15 days) to 33.67 (90 days) mMol MDA/kg oil stored at ± 25 °C (Fig. 2). Oxidation values of microencapsulated tuna fish oil were significantly lower than the unmicroencapsulated fish oil (untreated fish oil) at the end of the 90-day storage test. The maltodextrin: whey protein microcapsules (P1 and P2) exhibited lower TBARS value (1.7 and 2.1 mMol MDA/kg oil, respectively) at 0-day microcapsule production, but failed to inhibited oxidation after 15 days (10.8 and 9.8 mMol MDA/kg oil), which then steadily increased (17.9 and 17.4 mMol MDA/kg oil) at the end of the 90-day storage test. Similar oxidation patterns of P1 and P2 microcapsules were demonstrated by arrowroot starch-whey protein microcapsules (P3 and P4), which exhibited oxidation instability of 3.5 and 3.9 mMol MDA/kg oil, respectively, at day 0; 10.3 and 8.9 mMol/kg oil, respectively, after 15 days, and 18.3 and 18.0 mMol MDA/kg oil, respectively, at day 90. However, this study demonstrated (Fig. 2) that microcapsules made from arrowroot starch-maltodextrin combinations (P5 and P6) exhibited significantly higher oxidation inhibition of microencapsulated tuna fish oil than microcapsules made of only maltodextrin or arrowroot starch from 15 to 90 days storage at ± 25 °C. At the start of the experiment, arrowroot starch-maltodextrin combinations (P5 and P6) showed the highest oxidation values (7.3 and 6.23 mMol MDA/kg oil, respectively), then by day 15, P5 and P6 had exhibited delayed oxidation (7.24 and 7.19 mMol MDA/kg oil, respectively) and after 90 days, oxidation was significantly lower than the other treatments at 10.7 and 11.1 mMol/kg oil, respectively. These results were attributed to the influence of arrowroot starch and indicated the potential of arrowroot starch as an

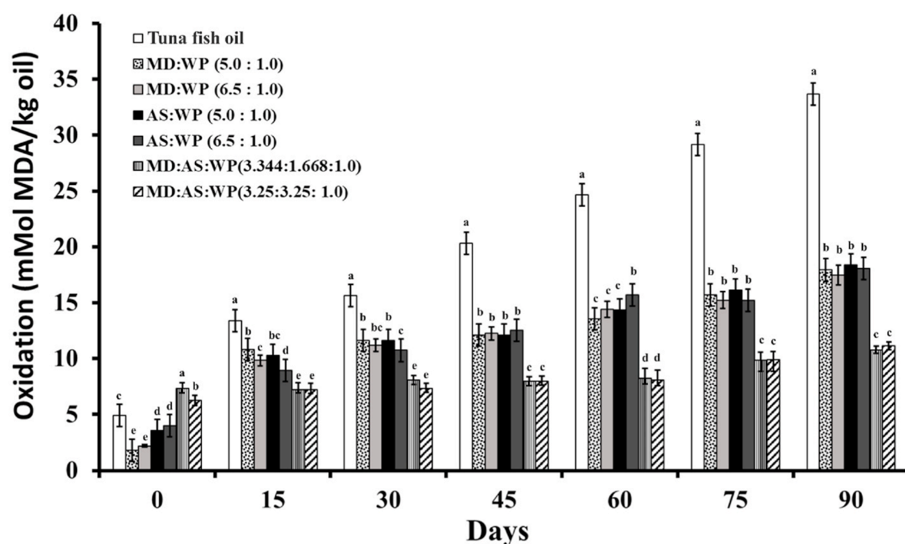

Fig. 2. Oxidative stability of microencapsulated tuna fish oil from (P1) Maltodextrin: Whey Protein (5.0:1.0) as control; (P2) Maltodextrin: Whey Protein (6.5:1.0) as control; (P3) Arrowroot starch: Whey Protein (5.0:1.0); (P4) Arrowroot starch: Whey Protein (6.5:1.0); (P5) Maltodextrin: Arrowroot Starch: Whey Protein (3.344:1.668:1.0); and (P6) Maltodextrin: Arrowroot Starch: Whey Protein (3.25:3.25: 1.0). Different letters show significant differences between treatments within 90 days observation ($p < 0.05$).

alternative or supplement to maltodextrin in reducing or delaying the synthesis of primary or secondary oxidation products to below regulatory thresholds, which was also demonstrated by peroxide and acid values of the microcapsules. The mechanism involved in delaying or inhibiting oxidation of the microencapsulated fish oil demonstrated by P5 and P6 microcapsules was attributed to their wall matrix composed of maltodextrin arrowroot starch crosslinked with whey protein and reinforced by higher moisture contents (Table 2) in the form of adsorbed water that formed a protective layer against oil leakage that resulted in the lowest acid values (Table 2) and the highest oxidation stability (Fig. 2) among the microcapsules.

These results were in agreement with other reports that combinations of chitosan, fish gelatine, and maltodextrin as microencapsulation wall material in fish oil delayed lipid oxidation storage (Pourashouri et al., 2014). Moreover, fish oil microencapsulated with fish gelatine and maltodextrin as wall material exhibited better oxidative stability of polyunsaturated fatty acids stored at refrigerated conditions than unmicroencapsulated fish oil (Jeyakumari et al., 2015). Interestingly, microcapsule samples produced from either maltodextrin and or arrowroot starch combinations as wall material showed similar delayed oxidation behaviours, which reinforced the conclusion that arrowroot starch could supplement or potentially replace maltodextrin in the microencapsulation process of tuna fish oil. Moreover, a lighter color (by observation) was exhibited by microencapsulated tuna fish oil (P5 and P6) than other treatment (P1, P2, P3, and P4) at day 90 (Fig. 3). Color visualization of microencapsulated tuna fish oil powder confirmed maltodextrin and arrowroot starch combined with whey protein demonstrated better oxidation stability.

3.6. Scanning electron microscopy (SEM) micrograph

SEM micrographs (Fig. 4) showed that P1 and P2 microcapsules (composed of maltodextrin wall material) were irregular in structure, lacked normal spherical shape, highly porous, with particle size ranging 73.04–103.14 μm. However, surface morphology of P3 and P4 microcapsules prepared using arrowroot starch appeared round and smooth; furthermore, their particle sizes were smaller (26.72–52.97 μm) compared to P1 and P2. The morphology of P5 and P6 microcapsules using maltodextrin combined with arrowroot starch showed less structural irregularity than maltodextrin without combination (P1&P2), which showed size dimensions from 39.61 to 95.31 μm. Theoretically, smaller particle sizes indicated a large surface area, which could have led to a higher volume of unmicroencapsulated oil and corresponding higher TBARS values on the surface of P5 and P6 microcapsules compared with P1, P2, P3, and P4 oxidative stability results (Fig. 2). However, the significantly lower TBARS demonstrated by P5 and P6 confirmed the enhanced stability of the microcapsules composed of arrowroot starch and maltodextrin combinations compared to the other wall material ratios of P1–P4 microcapsules, and the potential wall material of arrowroot starch in microencapsulation technology.

Based on their microstructures, P1 and P2 microcapsules could have facilitated diffusion of oxygen from the air to the particle surface, which was attributed to the particle state of the matrix. Moreover, oxygen could easily decompose the core material (tuna fish oil) inside the matrix and on the surface of the particles (Anwar & Kunz, 2011). In addition, images of P3, P4, P5, and P6 revealed that the smaller-sized microcapsules were embedded in the arrowroot-whey protein matrix forming aggregates. Charles et al. (2016) reported that arrowroot starch minimizes freeze-thaw damage, which explained the smoother surface and uniformly round smaller sizes of P3, P4, P5, and P6 microcapsules. Moreover, in Nogueira et al. (2018), SEM images confirmed combinations of arrowroot starch with glycerol created a homogenous (smooth) microstructure on edible film surface. These findings led to the conclusions that arrowroot starch, behaved as a microencapsulation carrier and potential wall material agent, and created a similar wall material as maltodextrin to protect the core material during the freeze-drying process. Although oxidative stability thresholds of PV and AV were within industrial standards for all the microcapsules, P5 and P6 microcapsules with arrowroot starch and maltodextrin demonstrated relatively high oxidative stability and encapsulation efficiency compared with P1 and P2 microcapsules. Nevertheless, long storage (90 days) at ±25 °C conditions indicated that both maltodextrin and arrowroot similarly

Fig. 3. Microencapsulated tuna fish oil powder from (P1) Maltodextrin: Whey Protein (5.0:1.0) as control; (P2) Maltodextrin: Whey Protein (6.5:1.0) as control; (P3) Arrowroot starch: Whey Protein (5.0:1.0); (P4) Arrowroot starch: Whey Protein (6.5:1.0); (P5) Maltodextrin: Arrowroot Starch: Whey Protein (3.344:1.668:1.0); and (P6) Maltodextrin: Arrowroot Starch: Whey Protein (3.25:3.25: 1.0).

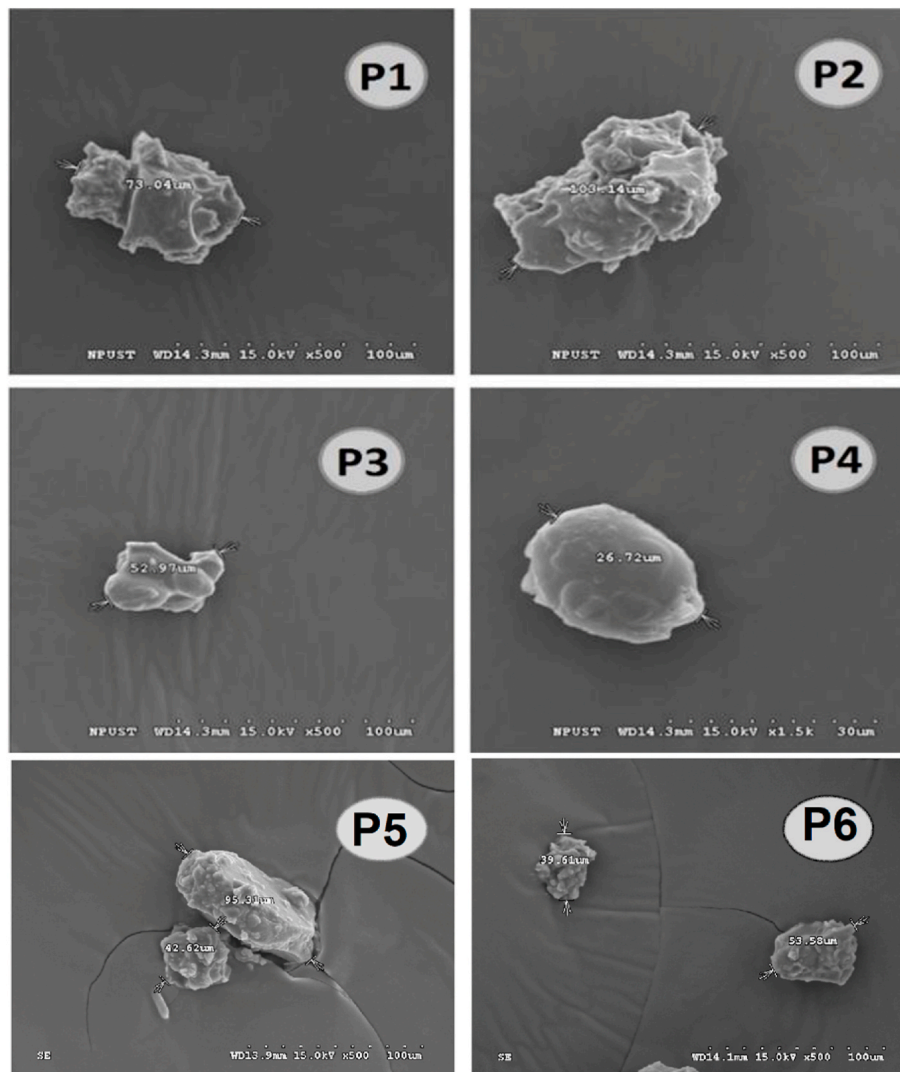

Fig. 4. SEM micrographs of microencapsulated tuna fish oil from (P1) Maltodextrin: Whey Protein (5.0:1.0) as control; (P2) Maltodextrin: Whey Protein (6.5:1.0) as control; (P3) Arrowroot starch: Whey Protein (5.0:1.0); (P4) Arrowroot starch: Whey Protein (6.5:1.0); (P5) Maltodextrin: Arrowroot Starch: Whey Protein (3.344:1.668:1.0); and (P6) Maltodextrin: Arrowroot Starch: Whey Protein (3.25:3.25: 1.0).

delayed oxidation of the core material.

4. Conclusions

Microencapsulated tuna fish oil developed using arrowroot starch and maltodextrin combination as wall material demonstrated suitable physicochemical properties and inhibited or delayed oxidation of tuna fish oil below industrial values suggested by various food regulators. In addition, SEM showed that freeze-dried microcapsules containing arrowroot starch demonstrated better morphology properties, which was attributed to lower water activity and acid value, relatively high encapsulation efficiency, improved oxidative stability, and increased shelf life in a simulated 0-to-90-day storage test. The use of arrowroot starch as a wall material component to produce microcapsules was reported for the first time, and this study demonstrated the novelty of arrowroot starch as a microencapsulation carrier, supplementing maltodextrin with a cheaper polysaccharide, arrowroot starch, to produce microencapsulated tuna fish oil to meet industrial demands for food fortification and consumer needs of the omega-3-rich tuna fish oil in a more palatable form. Therefore, composite matrices involving arrowroot starch and maltodextrin combinations will be further studied to optimize their properties as wall materials or carrier of bioactive compounds such as vegetable oils, enzymes, secondary metabolites, and probiotics in microencapsulation technology.

CRediT authorship contribution statement

Albert Linton Charles: Conceptualization, Methodology, Writing - original draft, Supervision. **Annur Ahadi Abdillah:** Data curation, Writing - original draft. **Yuniar Rizky Saraswati:** Investigation, Resources. **Kandi Sridhar:** Investigation, Software. **Christian Balderamos:** Investigation. **Endang Dewi Masithah:** Supervision. **Mochammad Amin Alamsjah:** Supervision.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to express their gratitude to the Directorate of Education Universitas Airlangga, Faculty of Fisheries and Marine

Universitas Airlangga, and National Science Council (MOST) of Taiwan. (NSC100-2313-B-020-004-) for financial support throughout this research.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodhyd.2020.106281.

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