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Research Article

Studies on the extraction and characterization of fish oil from wastes of seafood processing industry

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Abstract

This paper presents a study on the extraction of fish oil from fish leaching waste obtained as a by-product from fish processing plant. Three different extracting methods i.e wet reduction, acetone and Bligh and Dyer methods were evaluated. Different ratio of mixtures of chloroform, methanol and water used as the extracting solvent were also evaluated in order to obtain highest yield of oil. The extracted fish oil was further characterized for its peroxide value, free fatty acid, colour, saponification value and acid value. The yield of fish oil obtained (54.6 ± 1.6%) was found to be highest with chloroform/methanol/water (2:4:1). This proves the potential for oil recovery from such wastes. Furthermore, the peroxide value of the extracted oil was 9.9 ± 0.1 meq/kg, which was still within the acceptable quality limit. Fish oil powder was also produced via spray drying technique and it was found that microencapsulation of 50% oil loading with dextrose equivalent (DE) of 25 and 10% sodium caseinate blend gave sufficient spray dried fish oil powder with microencapsulation efficiency of 84.0 ± 0.2%.

Keywords: fish wastes, fish oil, peroxide value, saponification value, microencapsulation, Malaysia

Introduction

Marine capture fisheries contribute over 50% of total world fish production and more than 70 % of this production has been utilized for processing [1]. As a result, every year a considerable amount of total catch is discarded as processing leftovers and that include trimmings, fins, frames, head, skin and viscera. Some of the by-products are utilized, but the main bulk is

dumped to waste, creating both disposal and pollution problems. Annual discard from the world fisheries is estimated to be about 25 % of the total production of marine capture fisheries [1]. Recent studies have identified a number of bioactive compounds such as fish muscle proteins, collagen and gelatine, fish oil, fish bone, internal organs and shellfish and crustacean shells from marine by-products [2] There is a great potential in marine bioprocess industry to convert and utilize these by-products into value-added food ingredients [3]. Fish oils are excellent dietary sources of the important fatty acids, contain especially polyunsaturated fatty acid (PUFA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [3]. These n-3 polyunsaturated fatty acids have been shown to be important for the prevention of a range of human disease and disorder [4,5]. They occur as triglycerides in fish oils at levels between 10 and 25 % [6]. Recognition of the rules played by these fatty acids in human health and nutrition and the resulting growth in new markets have stimulated much interest in methods of extracting and concentrating them from natural sources. Incorporation of fish oil into the normal food components offers an opportunity to elevate the intake of EPA and DHA in line with recent recommendation. However, due to high degree of unsaturation, these fatty acids are prone to oxidation. The autoxidation of fish oils is the most significant cause of deterioration in quality which leads to the development of volatile secondary oxidation products [7]. This means that fish oil that is incorporated into food has to be protected against oxidation. Unpleasant flavours and odours develop even at very low peroxide values at early stage of oxidation; even during the induction period [8]. A technique normally use for the fish oil protection is microencapsulation. Microencapsulation allows converting fish oil into a stable and more convenient dry powder product. The wall materials of the microcapsules protect the core substance against influence of environment conditions such as oxygen, light, humidity. Thus, the stability of fish oil is improved. Microencapsulation of fish oil can be achieved via spray drying technique. The process is flexible, efficient, and a good quality powder could be produced [9].

This paper describes the study performed on the extraction of fish oils from fish waste materials discarded from a local surimi processing industry. During surimi production, a lot of waste is generated which is usually discarded. It was estimated that 7-8 tones of fish waste produced daily from this company (personal communication). The objective of this work was to investigate the most suitable solvent mixtures that would give optimum yield of fish oil based on extraction technique described by Bligh & Dyer [10]. The extracted fish oil was further characterized for its physico-chemical properties to evaluate its quality and stability. Microencapsulation study on the fish oil extracted was also conducted using spray drying technique to produce microencapsulated fish oil powder.

Materials and Methods

Fish waste sample

Fish waste known as leaching fish waste (LFW) was supplied by a local seafood industry in Perak, Malaysia. This LFW was obtained as a by-product during the leaching process of surimi processing. The waste material was kept frozen in big plastic bags, transported in an insulated icebox to the laboratory. The waste material was then kept in plastic bags in portions of 300 g each and stored at -18°C until further use. Before extraction, these waste were thawed again overnight in the chiller at 5^0C.

Chemicals

Maltodextrin of dextrose equivalent (DE) of 15 and 25, kindly donated by Grains Processing Corporation (USA) and sodium caseinate (Sigma Chemical, USA) were used as the

microencapsulating agent. All reagents used for the extraction and chemical analyses were of analytical grade, unless specified otherwise.

Extraction and optimization process for fish oil

In preliminary experiments, three different extraction methods were used to extract fish oil from sample leaching fish waste (LFW). These were Bligh & Dyer method [11], acetone extraction method [12] and wet reduction method [13]. From these preliminary experiments, it was found that the Bligh and Dyer method gave the highest yield of fish oil that could be recovered from the fish wastes compared to the other methods. Thus the extraction procedures described by Tanamati et al. [11] which was a modification from Bligh and Dyer method, was adopted in this optimization experiments to evaluate the suitable solvent mixtures that would give high yields of fish oil extract. Four different ratios of chloroform, methanol and water mixtures were used as given in Table 1. In this extraction procedure, 50 g portions of LFW sample was taken and thawed. The sample was mixed thoroughly with the mixture of solvents for 15 minutes with an electronic shaker (IKA-Labortechnik, Germany). The homogenized mixture was then filtered by using a Whatman filter paper no.2 and the filtrate was collected. The tissue residue was re-extracted with the same amount of chloroform in order to get high recovery of oil. The combined filtrate solutions were poured into a separatory funnel to allow for separation. The lower organic layer containing the lipids was collected. The solvent was evaporated off in a rotary vacuum evaporator at 25ºC until all chloroform and methanol were removed and lipids remaining were determined gravimetrically. The resultant oil was collected and kept in a screw capped bottle, flushed with nitrogen gas and stored at -18 ˚C for further analysis. The extraction methods were carried out in triplicates.

Table 1. Solvent mixture and ratio used in the extraction method.

Solvents	Ratio based on volume			
	Ratio 1	Ratio 2	Ratio 3	Ratio 4
Chloroform	4	2	2	2
Methanol	2	4	2	1
Water	1	1	1	2

Note: Weight of LFW used for each extraction ratio is 50 g

Physico-chemical analyses on extracted fish oil

The Peroxide values (PV) of fish oil were determined according to AOAC method [14]. Oil sample (5 g) was weighed into a 200 ml conical flask and mixed with 300 ml of glacial acetic acid and chloroform (3:1) and mixed thoroughly by swirling the flask. Saturated potassium iodide (0.5 ml) was then added and the mixture was left in the dark for 1 min with occasional swirling, followed with further addition of 30 ml distilled water. The mixture was titrated with 0.1 N sodium thiosulphate solution with 1 ml of 1.0 % soluble starch as indicator until the blue colour disappeared. A blank sample titration was also carried out in the same manner but with no oil added.

The saponification value (SV) of the fish oil was determined following procedures described in AOCS method [15]. Oil sample (1 g) was dissolved in 12.5 ml of 0.5 N ethanolic potassium hydroxide. The mixture was refluxed for 30 min until oil droplets disappeared and was left to cool to room temperature. Phenolphthalein indicator was then added and the hot soap solution was titrated with 0.5 N HCl until the pink colour disappeared. A blank titration was also carried out in the same manner except no oil was added.

Free fatty acids (FFA) value was determined according to the method describe in AOCS method [15]. An amount of 5 g oil sample was mixed with 75 ml of 95 % neutral ethyl alcohol and swirled. Phenolphthalein was added as indicator. The solution was titrated with 0.1 N sodium hydroxide until pinkish colour was observed at end point. FFA values were determined from equation given in the method. From the FFA value, acid value (AV) was also calculated.

Preparation of microencapsulated fish oil powder

Preparation of oil-in-water emulsion

Fish oil powders were prepared by microencapsulation via spray drying technique. Prior this step, oil-in-water emulsions were prepared by combining fish oil, sodium caseinate, maltodextrin and water according to formulation given in Table 2. The aqueous solutions were prepared by dispersing sodium caseinate and maltodexrin in water to 50°C with stirring. Fish oil was then added in the mixture. The emulsion was then homogenized using a homogenizer (IKA-Labortechnik, Germany) operated at 10000 rpm for 5 min. Two types of maltodextrin were used, DE 15 and DE 25 as the carrier agent to evaluate their efficiency. Fish oil was added in 3 different percentages of 50%, 75% and 85% based on weight of oil and maltodextrin. Thus 3 emulsion formulations using each type of maltodextrin were prepared and labeled as D15O50, D15O75, and D15O85 for maltodextrin DE15, while D25O50, D25O75 and D25O85 for maltodextrin DE25. In addition, to evaluate the emulsification stability of the sodium caseinate as an emulsifier, 5% and 10% based on total soluble solid were also used and labeled as D25O85-5 and D25O85 respectively.

Spray drying of encapsulated fish oil

The emulsions were spray dried in the concurrent mode using B290 type spray dryer (Büchi Mini Spray Dryer, B 290, Switzerland). The water evaporation rate of the dryer was at 3 kg/h and an inner chamber diameter was 800 mm. The emulsions were atomized by a centrifugal atomizer operated at 30000 rpm. The inlet air at 170°C and the outlet air at 90 to 100°C were used. The spray dried fish oil powders obtained were kept in air-tight bottles and stored in the desiccators before conducting SEM analysis.

Determination of emulsion stability index

Emulsion stability was determined by procedures described by Cho et al. [16]. A sample of liquid emulsion was transferred to a 10 ml mass cylinder, which was then capped and stored for 24 h. The volume of oil separated from emulsion in the mass cylinder was measured. Results are reported as an emulsion stability index (ESI) with a range of possible results from 0 to 1.

ESI = 1 (total volume of separated oil/ total volume of oil in emulsion)

Determination of microencapsulation efficiency

The amounts of surface and total oil were determined to calculate the microencapsulation efficiency. Surface oil or non-encapsulated oil was determined by a modified method described by Varavinit et al. [17]. Hexane (50 ml) was added to an accurately weighed amount (5 g) of powder followed by stirring for 10 min. The suspension was then filtered and the residue rinsed three times by passing 20 ml of hexane. The residual powder was then air dried for 30 min and weighed. The amount of surface oil was calculated by the difference in weights of the microcapsule, before and after washing. The total oil which includes both the encapsulated oil and surface oil was estimated by Soxhlet extraction [14]. Encapsulated oil was calculated by deducted the total oil with surface oil. Then microencapsulation efficiency was calculated by formula:

Microencapsulation efficiency = (Encapsulated oil/Total oil) x 100%

Table 2. Emulsion composition.

Constituents	Emulsion samples						
	DE15 O50	DE15 O75	DE15 O85	DE25 O50	DE25 O75	DE25 O85	DE25 O85-5
Ratio of Oil: Maltodextrin	50:50	75:25	85:15	50:50	75:25	85:15	85:15
Oil loading (%)	50	75	85	50	75	85	85
Sodium caseinate (%)*	10	10	10	10	10	10	5
Water (%)**	66.7	66.7	66.7	66.7	66.7	66.7	66.7
Total weight of soluble solid (g)***	83.25	83.25	83.25	83.25	83.25	83.25	83.25
Total weight of emulsion (g)	250	250	250	250	250	250	250

* Percentages of sodium caseinate are based on total soluble solid.
** Percentages of water are based on total weight of emulsion.
*** Total soluble solid = oil + maltodextrin + sodium caseinate.
Note:
DE15 O50 = Maltodextrin DE 15 with oil loading 50%
DE15 O75 = Maltodextrin DE 15 with oil loading 75%
DE15 O85 = Maltodextrin DE 15 with oil loading 85%
DE25 O50 = Maltodextrin DE 25 with oil loading 50%
DE25 O75 = Maltodextrin DE 25 with oil loading 75%
DE25 O85 = Maltodextrin DE 25 with oil loading 85%
DE25 O85 = Maltodextrin DE 25 with oil loading 85% and 5% sodium caseinate

Scanning electron microscopy (SEM)

The overall morphology and the outer topography of powders were observed by scanning electron microscope (SEM). Samples were mounted on metal stubs using a double-sided tape and then coated with a layer of gold (40–50 nm) under vacuum (sputtering), allowing surface and cross section visualization. Micrographs of the samples were obtained with scanning electron microscope (LEO SUPRA 55VP, Ultra High resolution analytical FESEM, Germany).

Statistical analysis

Anova was performed to determine differences in the emulsion stability, surface oil and microencapsulation efficiency attributable to different formulation. Duncan test was performed for *post hoc* multiple comparison at $\alpha = 0.05$. All statistical analysis was conducted by using SPSS 16.0 for Windows (SPSS Inc, Chicago, Illinois).

Results and Discussion

Yield of recovered fish oil from wastes

Figure 1 shows the results for oil yield obtained using different ratios of solvent mixtures. The mixture ratios of chloroform/methanol/water (4:2:1 and 2:4:1) both gave higher yields compared to the other two ratios. Bligh and Dyer method has been recognized as the most reliable method currently available for total lipid extraction [18]. Bligh & Dyer method uses polar solvent, chloroform and methanol mixture to extract the oil from fish by-products. The polar solvents can penetrate into the cells and extract the lipid from the cell membrane and muscle fibres including the phospholipids materials [10]. Many researchers who have employed this particular extraction method have also reported higher yields [11,19-21]. It was

propounded by Smedes & Thomasen [22] that the extraction of polar material to chloroform is promoted by the presence of methanol and that the solubility of free fatty acids was actually higher in a mixture of methanol and chloroform than in either of the separate solvents. This statement, together with the fact that non-lipid contaminants were additionally extracted into the methanol/water layer, was significant to the higher oil yield observed during the extraction of fish oil from our samples, as opposed to the acetone extraction. According to Smedes & Askland [8] the higher lipid content obtained by the Bligh and Dyer extraction method is not related to the different solvent compositions but mainly due to higher recovery of the organic phase containing the lipids. Smedes & Askland [8] also pointed out that methanol also gave a positive effect on the extraction yield. This was either due to less organic phase sticking to the tissue or better extraction kinetics, due to higher solubility in the mono-phasic situation and in the aqueous phase. Thus, the order of adding the solvent seemed to have a significant effect on extraction kinetic. The results show that ratios chloroform/methanol/water (4:2:1 and 2:4:1) had more chloroform and methanol content compared to ratio chloroform/methanol/water (2:2:1 and 2:1:2). Factors such as dietary, gender, season, water temperature and maturity may affect the makeup distribution of fatty acid content. Fish oil extracted with chloroform/methanol/water (2:4:1) was then used in latter experiment.

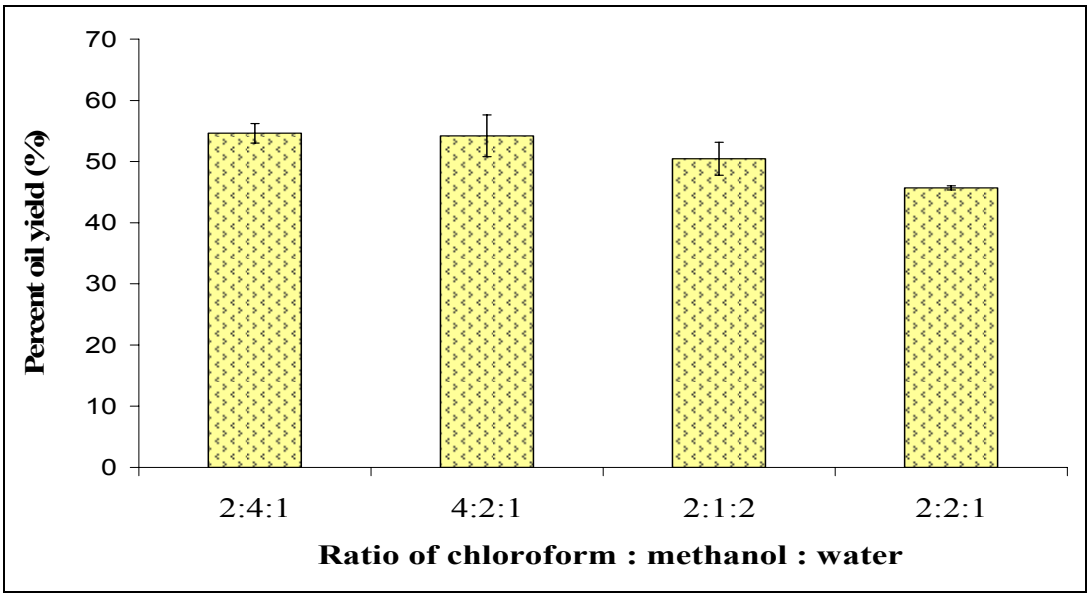

Figure 1. Percentage of oil yield obtained from different solvent ratios.

Physico-chemical properties of fish oil extracts

In order to determine the stability and quality of fish oil extracts, some quality assessment was conducted. These results are shown in Table 3. Undeland et al [21] indicated that unsaturated character of the lipids and the strong pro-oxidative systems naturally present in fish tissue could cause susceptibility of lipids to oxidize during processing and storing especially for species Herring. Young [23] has reported that peroxide value (PV) of crude fish oil was between 3 and 20 meq/kg. In this study, the PV was found to be 9.9 ± 0.1 meq/kg, which is well below acceptable limit of 20 meq O_2/kg oil. This indicated that the fish oil extracted had low lipid oxidation rate [24]. The values of acid value (AV) and free fatty acids (FFA) in extracted fish oil were found to be 23.7 ± 2.5 mg KOH/g and 11.9 ± 2.0 %, respectively. Increase in AV is generally associated with the lipase activity originating from microorganism or biological

tissue [25]. The acceptable limit for AV was reported to be 7-8 mg KOH/g [26]. According to De Koning [27] hydrolysis of fish was greatly reduced upon sterilization. Since we did not sterilize the samples nor studied them under aseptic conditions, it is possible that some enzyme or microorganism contamination might have occurred during sample removal. Chantachum [28] had reported that high heating temperature during oil extraction deactivated the enzyme and the release of free fatty acids by the lipase activity thus lowered the FFA value. Due to low temperature used during the extraction of oil in this study, enzyme lipase present may not have been deactivated and thus more free fatty acids could be release by lipase activity. Thus caused high fatty acid value in this study which was also probably due to enhanced oxygen transfer which led to increased lipid oxidation, as propounded by Daukšas et al. [29]. Saponification is the process of breaking down a neutral fat into glycerol and fatty acids by alkali treatment. The SV of fish oil obtained in this study was higher (295.4 ± 3.0 mg KOH/g) than standard value for fish oil (180-200 mg KOH/g), given by AOCS [15]. Bimbo & Crowther [26] reported that crude oil contains minor amount of non-triglyceride substances. Thus, it is possible that high SV was due to impurities present in crude fish oil. Additionally, higher saponification value may be contributed by the unsaponifiable matter present in the leaching wastes materials such as sterols, glyceryl ethers, hydrocarbons, fatty alcohols and some minor quantities of pigments and vitamins.

Table 3. Physico-chemical analysis of extracted fish oil.

Analysis	Fish oil
Peroxide value (meq/kg)	9.9 ± 0.1
Saponification value (mg KOH/g	295.4 ± 3.0
Free fatty acid (%)	11.9 ± 2.0 %
Acid value (mg KOH/g)	23.7 ± 2.5

Emulsion stability index

Stability of the emulsion is necessary for successful for microencapsulation process. Emulsifying agents can be used to form an emulsion by lowering the surface tension of droplets and form a barrier to help prevent droplet coalescence [30]. Protein which generally has both hydrophilic and hydrophobic groups can function as emulsifying agent [31]. During homogenization, these proteins rapidly absorbed to the surface of the newly formed oil droplets, where they lowered the interfacial tension (thereby facilitating further droplet disruption) and formed a protective layer around the droplets (thereby retarding droplet coalescence). The stability of oil-in-water emulsions was improved by the adsorbed protein layer because it is resistant to rupture and can generates repulsive interactions between droplets that prevents them from coming into close proximity. In this work, sodium caseinate (one of protein-type emulsifier) at 5% and 10% concentration level was used as an emulsifying agent for maintaining oil-in-water (O/W) emulsion stability. The amount of 5% of sodium caseinate was used for sample D25O85-5 while 10% of sodium caseinate was used for sample D15O50, D15O75, D15O85, D25O50, D25O75 and D25O85. It was found that the usage of 10% sodium caseinate had a more stable O/W emulsion than 5% sodium caseinate as shown in Table 4. The stability of the emulsion was increased with an increasing sodium caseinate concentration. The result obtained shows the same finding with Hwang et al. [32] as they reported that the increased protein concentration favours the stability of the emulsion. Possibly, emulsion sample with 10% sodium caseinate showed good emulsion stability index, which were in the range of 0.65 to 0.70 by having no significant difference between these samples (Table 4). This suggested that there was minimal phase separation and that emulsifier concentration of 10%

sodium caseinate based on total soluble solid was sufficient to stabilize the emulsion. In contrast phase-separation was observed in sample D25O85-5 containing 5% of sodium caseinate. It was probably due to the insufficient amount of emulsifier added (5% of total soluble solid) in the formulation to form films around the oil droplets. This insufficient amount of emulsifier might also be due to the higher oil loading content in this formulation. Because of that, the emulsion was not stable and then coalescence of the emulsion droplets occurred in the reservoir of the emulsion.

Table 4. Emulsion stability index of oil-in-water emulsion before spray-drying.

Sample	Emulsion stability index (ESI)
D15 O50	0.70 ± 0.01^{a}
D15 O75	0.67 ± 0.03^{a}
D15 O85	0.65 ± 0.01^{a}
D25 O50	0.70 ± 0.02^{a}
D25 O75	0.67 ± 0.03^{a}
D25 O80	0.66 ± 0.01^{a}
D25 O80-5	0.42 ± 0.03^{b}

Means within a column are significantly different when not followed by the same letter ($p<0.05$)

Scanning electron microscopy (SEM)

Scanning electron micrographs (SEM) show that the fish oil encapsulated powders prepared from the sample DE15O50, DE15O75, DE15085, DE25O50 and DE25O75 were generally spherical with surface indentations and no apparent surface cracks (Figure 2). This was the typical appearance of spray-dried products. The surface indentations were probably due to rapid particle shrinkage during the early stage of the drying process as similiarly reported by Kim and Morr [33]. These microcapsules were likely to offer good protection of the encapsulated oil as they had a continuous surface without cracks [34]. In contrast, although microcapsules prepared from D25O85 were also spherical in shape, it also exhibited some surface pores (Figure 3). These pores were probably due to the footprints of core droplets that were originally present at the surface and were lost during the microencapsulation process [35]. In addition, it was found that microcapsules containing maltodextrin DE15 had less surface dents and shrinkage than their maltodextrin DE25 counterparts (Figure 2). Incorporating maltodextrin DE15 into emulsion composition had a profound influence on the structure and surface morphology of microcapsules, resulting in particles with smooth surfaces and less indentations. This suggested a slower rate of wall matrix solidification with maltodextrin DE15 samples compared to maltodextrin DE25, and possibly a higher elasticity of these wall systems.

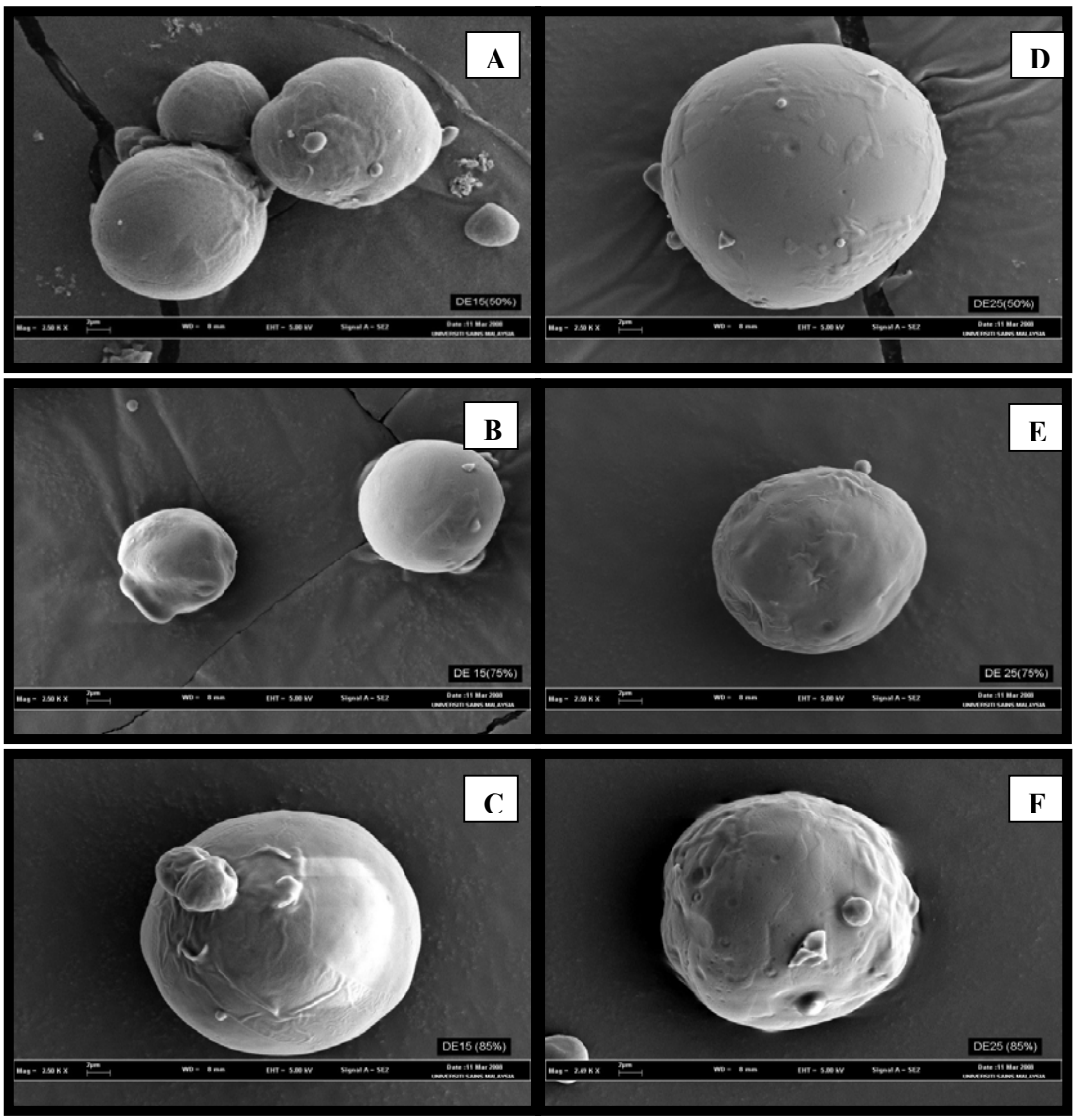

Figure 2. Scanning electron micrographs of fish oil encapsulated powders at 2.5k magnification: (A) D15 O50, maltodextrin DE15 with 50% oil; (B) D15 O75, maltodextrin DE15 with 75% oil; (C) D15 O85, maltodextrin DE15 with 85% oil; (D) D25 O50, maltodextrin DE25 with 50% oil; (E) D25 O75, maltodextrin DE25 with 75% oil; (F) D25 O85, maltodextrin DE25 wiith 85% oil.

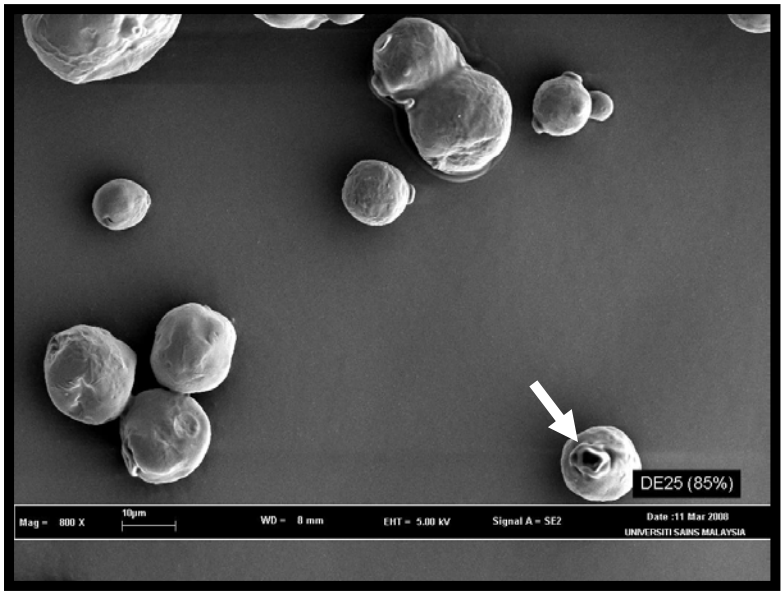

Figure 3. White arrow shows one of the pored microcapsules identified from D25O85, maltodextrin DE25 with oil 85% scanning electron micrograph (800X magnifications).

In general, it was observed that microcapsules for D15O50 and D25O50 were the smallest, compared to microcapsules prepared with D15O75, D25O75, D15O85 and D25O85 (Figure 2). This could possibly be due to the lower oil content; allowing more extensive microcapsules shrinkage during the early stage of the drying process [36, 37]. At a higher oil loading, the oil occupied a larger volume of the microcapsule. It also contributed to the mechanical strength of the microsphere matrix and may reduce microcapsule shrinkage during drying. Thus, the more oil loading, lesser shrinkage could be observed and the bigger size of microcapsules being produced. Oil loading of 75% and 85% gave quite similar shape in term of the size of microcapsules and it was observed that with high oil loading, the yield of spray dried fish oil powders was seems lower than that with of 50% oil loading. The low yield of microcapsules produced was due to microcapsules adhering to the inner walls of the spray dryer as a result of condition being not optimized. The results indicate that with higher oil loading in sample D15O85 and D25O85, wet microcapsules were obtained. On the other hand, high oil loading also caused the amount of maltodextrin was insufficient to encapsulate the oil, causing part of the oil to be unencapsulated and remained on the surface of the microcapsules. The shrinking could also force oil in the core to leach out as microcapsules shrunk during drying, producing wet microcapsules. In addition, the leached out oil also resulted in sticky spray dried powder being produced and agglomerated.

Microencapsulation efficiency

Microencapsulation efficiency is one of the important parameters in microencapsulation. Microencapsulation efficiency obtained with the various formulations are shown in Tables 5. The result revealed that there is a significant difference ($P < 0.05$) between maltodextrin DE15 and DE25 for each level of oil loading. It was found that the maltodextrin DE25 mixtures performed better as wall material than corresponding formulations containing maltodextrin DE15. This was evident by the higher microencapsulation efficiency of microcapsules containing maltodextrin DE25 compared with those containing maltodextrin DE15 where there

was an equivalent oil loading in microcapsules (50, 75, and 80% oil loading). This indicated that the properties of the coating material surrounding the oil droplets may affect the microencapsulation efficiency. With microcapsules containing maltodextrin DE25, there was a fast crust formation (fast rate of wall matrix solidification) that could contribute to the low levels of surface oil and high of microencapsulation efficiency values as there is less opportunity for the fish oil droplets to come onto the surface of microcapsules. While in maltodextrin DE15, crust formation is slower (slow rate of wall matrix solidification) inferring that more oil droplets could come onto the surface resulting high level of surface oil and low in microencapsulation efficiency. This lower microencapsulation efficiency might also be due to the thicker layer of surface oil on microcapsules containing maltodextrin DE15 compared with thinner layer on maltodextrin DE25. In other words, the surface area occupied by fish oil on maltodextrin DE15 was smaller (but thicker) than maltodextrin DE25.

Table 5. Microencapsulation efficiency of six formulation of fish oil powder.

Sample	Microencapsulation efficiency (%)
D15 O50	80.20 ± 1.84^{b}
D15 O75	54.34 ± 0.76^{d}
D15 O85	47.30 ± 1.13^{e}
D25 O50	83.97 ± 0.19^{a}
D25 O75	61.67 ± 1.38^{c}
D25 O85	55.35 ± 0.46^{d}

Means within a column are significantly different when not followed by the same letter ($p < 0.05$).

In addition, the percentage of oil loading could also affect the microencapsulation efficiency of microcapsules produced. It was found that an increase in oil loading resulted in lower microencapsulation efficiency value, with less oil being encapsulated. Table V shows that sample D25O50 had highest percentage of microencapsulation efficiency while sample D25O85 was the lowest. Same finding could also be seen for sample with maltodextrin DE15 where sample D15O50 was highest in microencapsulation efficiency and sample D15O85 was the lowest. This lower percentage of microencapsulation efficiency obtained most probably due to lower amount of wall material used to encapsulate the oil as the oil load was increased [46]. On the other hand, pores formed on the surface of the microcapsule could also reduce the microencapsulation efficiency. Observation with SEM shows that sample D25O85 exhibited some surface pores (Figure IV). The pores might cause the internal oil to leach out to the surface of microcapsule, thus resulting in high surface oil and low microencapsulation efficiency value. Nevertheless, microencapsulation efficiency reflects not only the encapsulated oil present on the microcapsule surface but also the proportion of microencapsulated oil extracted from near the surface of the capsule.

Conclusions

Bligh and Dyer method give more oil yield than acetone extraction and wet reduction method. Optimization of this method is necessary to get the highest oil yield. Both ratio mixtures of chloroform/methanol/water (4:2:1 and 2:4:1) resulted in higher yields of fish oils. Although peroxide value is seems to be good. But, fish oil prone to oxidation due to the oil is crude. It was found that the stability of the emulsion was increased with an increasing of sodium caseinate concentration thus; emulsion which used 10% sodium caseinate exhibited the higher emulsion stability than emulsion with 5% sodium caseinate. For microencapsulation process, it

was found maltodextrin with DE 15 and 25 can be used as encapsulating agents. However, the higher dextrose equivalent of maltodextrin was found to be the higher in microencapsulating efficiency of fish oil than the lower dextrose equivalent. The microencapsulation efficiency of the spray-dried fish powder was also affected by the oil loading. Thus, the opposite effect was observed with microencapsulation efficiency of fish oil powders as the oil loading increase.

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