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Physicochemical Characteristic of Microencapsulated Fish Oil by Freeze-drying using Different Combinations of Wall Materials

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The objective of this study was to evaluate the influence of different wall material component in the microencapsulation of kilka oil (*clupeonella cultriventris caspia*) by freeze drying. In this study, kilka oil as a core material was microencapsulated with Maltodextrin (MD), Sodium Caseinate (SC), whey protein concentrate (WPC) and modified starch (Hi-Cap 100™) at a ratio of 1:3 (core: wall) in four formula. The emulsions used for particle production were characterized for stability, viscosity and droplet size in emulsion and moisture, microencapsulation efficiency (ME) and bulk density in powder. Emulsion containing MD: WPC: Hi-cap had lower stability compared to others. Results showed emulsion with MD: WPC: Hi-cap: SC had biggest particle size and lowest value in viscosity in emulsion. The best encapsulation efficiency was obtained for MD: Hi-Cap: WPC combination which also showed poorer emulsion stability and lowest value in moisture. The microencapsulated fish oil produced has good physicochemical properties which lead to its more widespread application in food industry as a food additive.

Key words: Fish oil, Microencapsulation, Freeze drying, Microencapsulation efficiency.

There is high tendency to nutritive and healthy foods in the market and this fact increased attention to research on products of this nature in food industry. Marine lipids contain high concentrations of polyunsaturated fatty acids (PUFA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) [1].

Kilka (*clupeonella cultriventris caspia*) is one of the most important industrial and commercial fish in the Caspian Sea. Kilka oil is a rich source of polyunsaturated and omega-3 fatty acids as the most important fish species of the south regions of Caspian Sea) [2]. One of the major problems associated with oils rich in polyunsaturated fatty acids (PUFAs) is due to high susceptibility to oxidative deterioration and development of undesirable flavor [3]. So, there is a necessity to protect these oils in order to increase the storage stability [4,5]. Microencapsulation of fish oils is one of the preserving methods has been extensively used in foods and beverages to control the release of bioactive components, protect ingredients from the environment, lower flavor loss during processing and storage, prolong the flavor mouth feel over a longer period of time. In this way Choosing the best wall materials and encapsulation

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technique are important steps in food encapsulation.

The present microencapsulation is based on spray-drying techniques. One disadvantage of spray-drying technology is the elevated temperature which is necessary for drying. High temperatures lead to an increased oxidation of PUFA so that a drying process at low temperatures (freeze-drying) is expected to be an alternative for the microencapsulation of fish oil. Maltodextrin is a hydrolyzed starch commonly used as a wall material aid because of their relative benefits such as cheapness with no colour, neutral little taste, low viscosity and protection against oxidation used as wall material in microencapsulation of food components [6]. However, the main problem of this wall material is its low emulsifying capacity. So, maltodextrin used in combination with other surface active biopolymers, modified starches (Hi-Cap 100™, Capsul TA) [7, 8] and proteins such as Sodium Caseinate and whey protein concentrate [9, 10] in order to obtain a suitable microencapsulation by freeze drying.

There is available information on microencapsulation of fish oil [11, 12, 13] but none of the published works reported the effect of two different types of carbohydrate and protein wall materials simultaneously on the encapsulation efficiency of kilka oil. The aim of this work was to evaluate the potential of modified starch (Hi-Cap100™) combination with 3 types of wall materials (Sodium Caseinat, whey protein concentrate and Maltodextrin) for fish oil microcapsulation by Freeze drying. The presented study focused to determine Fatty acid profile of kilka oil, the emulsions stability, viscosity and droplet size and encapsulation efficiency, bulk density, moisture content of microcapsules.

MATERIAL AND METHODS

MaterialS

Fresh Kilka fish (*Clupeonella cultriventris caspia*) were caught from Anzali quay located in Guilan province (Iran). And then were transported in isothermal iceboxes to the laboratory 5 h after being caught. The fish were deheaded and gutted and then washed with cold hygienic water. Lipid was extracted by the method of Bligh and Dyer (1959)[14] and was used for lipid quantification and for determination of the fatty acid profile. The wall materials used were: maltodextrin with 18-20 DE (Kirsh pharma, Germany) (MD), whey protein concentrate WPC 80 (Friesland Campina, Netherland) (WPC), sodium caseinate (Friesland Campina, Netherland) (SC), modified starch: Hi-Cap 100™ (derived from waxy maize) (National Starch, Germany).

Methods

Preparation of emulsions

Emulsions were prepared according to previous studies [15, 16]. The wall materials were added to distilled water and mixture was dissolve completely. In this study, all of these combinations prepared with 1 g tween 100 g^{-1} fish oil and 0.02% (w/v) sodium azide in emulsion as antimicrobial agent. The total concentration of dissolved solid (wall material + oil) was 20 % (w/w). The fish oil was added in a 1:3 ratio (w/w) to emulsions, then were formed using an Ultra-Turrax homogenizer operating at 15,000 rpm for 5 min. emulsions were freezed at -70 °C overnight, and dried in a freeze drier for 72 hr. When the freeze-drying process was completed, the encapsulated powder was kept in moisture-impermeable plastic bags and stored at -20 °C for further characterization of its properties.

Fatty acid composition in kilka oil

The fatty acid composition of fish oil samples was determined using a gas chromatography (GC) (Agilent- 6890, USA) equipped with a flame ionization detector and a fused silica capillary (120m×0.25mm ID×0.20 µm). Operating conditions were as follows: temperatures-injection port 260 °C; detector temperature 300°C; oven programmed from 180 to 220 °C at 10 °C min^{-1}. Nitrogen was the carrier gas. The fatty acids were expressed as percentage of the total fatty acid content.

Characterization of the emulsions

Emulsion stability

Each emulsion (25 ml) was moved to 25 ml tube and stored at room temperature for 24 hr. Emulsions were separated to opaque creamy layer on top and distinctive clear serum lower phase. The results were expressed as creaming index (%) of total emulsion height in the tubes. Creaming index= 100× (the height of formed serum layer/total height of the emulsion) [17].

Emulsion droplet size

The droplet size distribution of emulsion droplets was determined using a laser light diffraction (Model Zetasizer nano, Malvern, UK). The emulsion droplet size was expressed as:

$$D_{4,3} = \Sigma z_i d_i^{4} / \Sigma z_i d_i^{3}$$

Distributions the poly dispersity index (PDI) was calculated according to particle size distribution curve by the software.

Apparent Viscosity

The apparent viscosity of emulsions was determined at 25 °C after their preparation using a Brookfield rotational viscometer (RVDV- II^{+}, USA). The Apparent Viscosity was indicated as a function at a shear rate of 40 s^{-1}.

Characterization of the microcapsules

Extraction of total oil

The procedure was described by the Rose–Gottlieb method [18]. 4 g of powder was dispersed in 40 mL of water heated at 65°C. After it was stirred, 8 mL of 25% NH_4OH was added and the solution was heated at 65°C for 20 min in a shaking water bath. Then, the solution was cooled at room temperature and the oil was extracted by using three type of liquid–liquid extractions as follows: first, 20 mL of ethanol, 50 mL of diethyl ether and 50 mL of n-hexane; second, 10 mL of ethanol, 50 mL of diethyl ether and 50 mL of hexane; and, third, 50 mL of diethyl ether and 50 mL of hexane without ethanol. The solvent was filtrated by filter paper containing anhydrous Na_2SO_4 and evaporated in a rotary evaporator. Then oil was collected and dried to constant weight using a stream of nitrogen.

Extraction of free oil

The free oil fraction was extracted according to Ba & Li (2008)[10]. In this method 15 mL of n-hexane was added to 2 g of powder. Then, it was stirred for 2 min at room temperature. After filtration through a filter paper, the solvent was evaporated in a rotary evaporator and the extracted oil was dried to constant weight using a stream of nitrogen.

Encapsulation efficiency

After the free oil is removed from powder and dried to constant weight, the microencapsulated oil fraction was extracted using the same method as that described for the extraction of total oil[15]. Microencapsulation efficiency (ME) was calculated from the quantitative determinations detailed above as follows:

$$ME\ (\%) = \frac{\text{Encapsulated oil (g/100 g powder)}}{\text{Total oil (g/100 g powder)}}$$

Moisture content

Two grams of powder was dried in a oven (Memmert, Germany) at 105 °C [19] until constant weight was reached. Percent loss in weight was reported as water content.

Bulk Density

The bulk density of microcapsule was measured by transferring 2 grams of powder into a 50 ml graduated cylinder. Bulk density of product was then determined based the volume of powder in the cylinder after cylinder was beat 50 times [20].

Statistical analysis

The data were calculated by the analysis of variance (ANOVA) using SPSS (ver.15) software. Differences among mean values were examined by Duncan's test at $p < 0.05$ significance level.

RESULTS AND DISCUSSION

Fatty acids composition

The individual fatty acid composition of starting fish oils expressed in Table 1. the most abundant fatty acids in kilka oil were C18:1 and C16:0, followed by C22:6, C20:5, and C16:1; Fatty acid analysis was also considered according to the composition on saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids, as well as to the ù3/ù6 and polyene (PI) ratios (Table 1). ù3/ù6 ratio has recently attracted a great attention because of its important effects on the development of several health human problems.

Emulsion characterization

Particle size in emulsion

The average particle size ($d_{4,3}$) of these emulsions ranged from 0.58 to 0.89 µm and was independent of the oil load and composition. The use of different wall materials had significant effect on emulsions droplet size (Table 2). The emulsion containing MD: SC: WPC and Hi-cap had the biggest droplets compared to the other materials, while the emulsion containing SC: WPC and Hi-cap showed the smallest ones. This last result can be related to the highest viscosity presented in

SC: WPC and Hi-cap emulsion, which shows in a greater resistance to droplets action, avoiding coalescence and resulting in smaller diameters. Distributions the poly dispersity index (PDI) was used to compare the emulsion properties. A lower PDI indicates limited particle size distribution and vice versa. PDI results varied from 0.288 to 0.575 and showed significant difference ($p < 0.05$) between emulsions.

Creaming index

Creaming index values varied from 0 to 66% (Table 2). The stability study showed that most of the emulsions were kinetically stable, with exception of those prepared without SC. which showed the formation of a separation layer and a foam phase, 24 h after its homogenization. This was unexpected, since whey proteins are well known by their good emulsifying capacity. Creaming index of emulsion without sodium caseinate showed the highest value (66%) as compared to the others. but there was no creaming in emulsions containing MD mixed with SC, Hi-cap and SC mixed with WPC and Hi-cap. Dickinson (2003)[21] identified the most important indication of instability of oil in water emulsion is creaming which can lead to phase separation with a distinctive clear or semi-transparent lower serum phase and cream. The results of our study showed that tween alone is unable to produce a stable emulsion. The emulsion is most stable when mixed with Sodium caseinate. These results are in agreement with Laplante, Turgeon, & Paquin (2005)[22] who have also observed the inability of SC or WPC alone to produce stable emulsions. Our results are according to the generally accepted theory indicating the smaller emulsion droplets are more stable than the larger emulsion droplets.

Table 1. Fatty acid composition of kilka oil (Mean ± Standard deviation)

Fatty acids	Kilka oil (%)
C14: 0	3.83 ± 0.17
C16: 0	19.73 ± 2.26
C16: 1	6.74 ± 0.47
C18: 0	4.27 ± 0.21
C18: 1	26.77 ± 1.73
C18: 2n-6	2.27 ± 0.07
C18: 3n-3	2.1 ± 0.30
C20: 1n-9	2.63 ± 0.17
C20: 5n-3 (EPA)	7.03 ± 0.29
C22: 5n-3 (DPA)	1.29 ± 0.10
C22: 6n-3 (DHA)	16.1 ± 1.34
SFA = 31.59%	
MUFA = 39.31%	
PUFA = 29.08%	
ω3/ω6 = 1.4%	
Polyene Index = 0.92%	

SFA: saturated Fatty Acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty Acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; DPA: docosapentaenoic acid

Viscosity

The viscosity of emulsions prepared with different wall materials combination is shown in table2. The mixture of SC: WPC: Hi-cap has shown higher viscosity in comparison to others. Our results showed smaller droplet mean diameters, indicating that droplets size was not affected only by the emulsion viscosity, but also by the basic emulsifying properties of each type of material. Tonon *et al* (2011)[23] observed that there is an inverse relation between particle size and emulsion viscosity which is similar to our results in current study.

Microcapsule analysis

Microencapsulation efficiency (ME)

The powder prepared from MD: SC: Hi-cap: WPC had a higher ME than the other one (Figure 1). Microcapsules showed significant difference in ME% ($p \geq 0.05$). The

Table 2. Characterization of emulsions prepared with different types of wall materials.

Formulation	$D_{4,3(\mu m)}$	PDI	Viscosity (mPa.s)	Creaming Index (%)
MD: SC: Hi-cap	0.691 ± 0.018 [b]	0.288 ± 0.012 [d]	1443.97 ± 46.37 [b]	0 ± 0 [c]
MD: SC: Hi-cap: WPC	0.896 ± 0.0 27 [a]	0.575 ± 0.0 04 [a]	16.86 ± 1 [c]	4 ± 0.2 [b]
SC: Hi-cap: WPC	0.581 ± 0.016 [c]	0.334 ± 0.002 [c]	1872.4 ± 15.87 [a]	0 ± 0 [c]
MD: Hi-cap: WPC	0.825± 0.1[a]	0.535± 0.03[b]	19.96 ± 0.38 [c]	66 ± 1 [a]

Different letters indicate significant difference between samples at p d" 0.05. (MD: Maltodextrin, SC: Sodium Caseinate, Hi-Cap 100: Modified Starch, WPC: Whey Protein Concentrate).

microencapsulation efficiency (ME) shows the presence of free oil on the surface of the particles within the powder and the degree to which the wall can prevent extraction of internal oil through a leaching process (Hogan *et al* 2001)[24]. In our study, the ME values (70.74–88.55%) (Fig 1). Previous studies have reported ME values from 0% to 95% depending on the type and composition of wall material, the ratio of core material to wall material, the stability and physicochemical properties of the emulsions (Baik *et al* 2004; Hardas *et al* 2000; Heinzelmann *et al* 2000; Hogan *et al* 2001; Lin *et al* 1995; Velasco, Dobarganes & Marquez-Ruiz 2000; Klinkesorn *et al* 2005).[25, 26, 11, 24, 27, 28, 12].

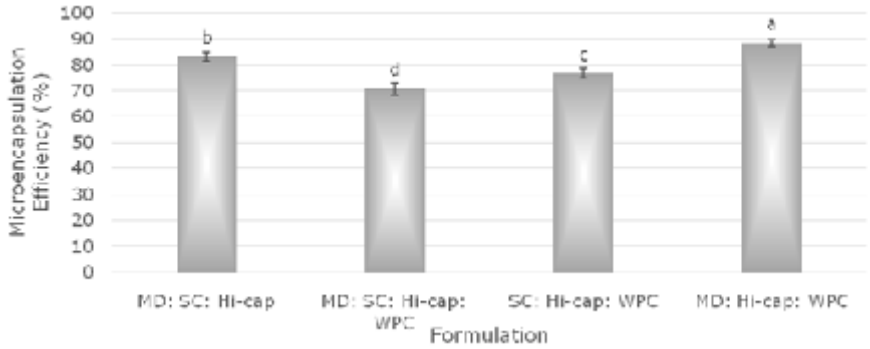

Fig. 1. Microencapsulation efficiency (ME %) of powder

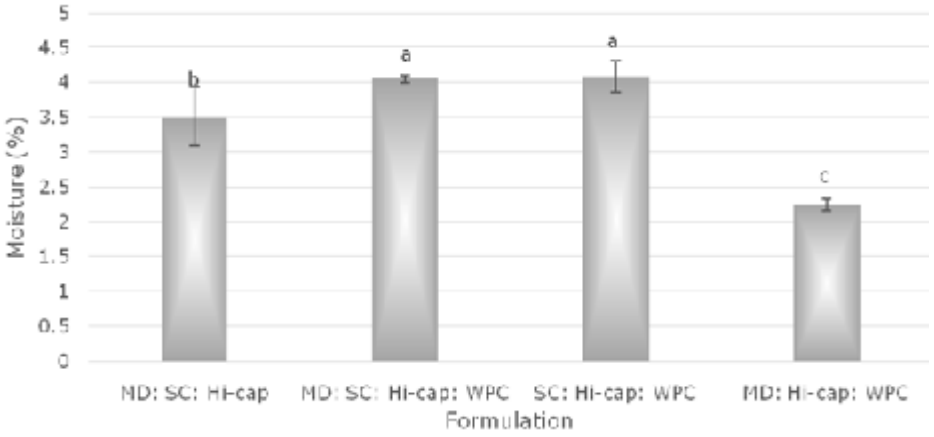

Fig. 2. Moisture percent of powder

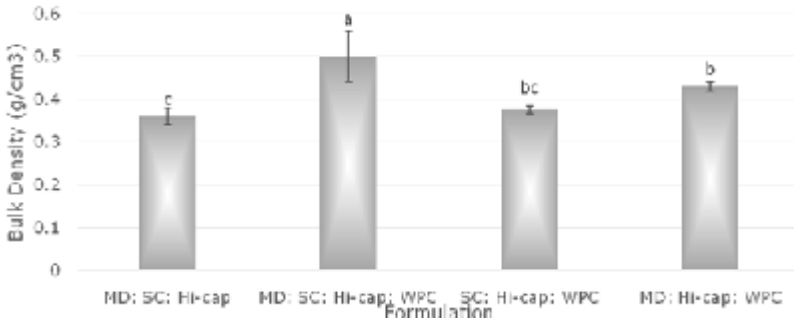

Fig. 3. Bulk density of powder

Moisture

Moisture contents of emulsions varied from 2.25% to 4.09% (Figure 2). This is inconsistent with the results of Hogan *et al* (2001)[24] and Sankarikutty *et al* (1998)[29], who found that moisture content was not affected by type of wall material or core/wall ratio. There was significant difference in the moisture content of microcapsules as affected by composite of wall material except between formula with MD: SC: Hi-cap: WPC and SC: Hi-cap: WPC. Hogan *et al* (2001) reported moisture content values from 1% to 3% in soybean oil microencapsulated by spray-drying that these values were not affected by the type of wall material as well.

Bulk density

Microcapsules showed significant difference in bulk density in formula (Fig 3) according to the type of wall material was used. However, microcapsule containing SC: Hi-cap: WPC didn't have significant difference with two others formula. Formula containing MD: WPC: Hi-cap and SC had highest value (0.5 g cm^{-3}) and showed significant variation with others. Bulk density values ranged from 0.36 (WPC: SC: Hi-cap) to 0.5 g cm^{-3} (MD: WPC: Hi-cap: SC). The advantage of obtaining powders with higher density is that they can be stored in large amounts into smaller containers when compared to products with lower densities. Moreover, higher bulk density may indicate lower amount of air occluded in the spaces between particles, which can help to prevent lipid oxidation. Goula & Adamopoulos (2004)[20] and Tonon *et al* (2011)[23] confirmed that the increasing particle size decreased the bulk density.

CONCLUSION

In this study the efficiency of different wall materials combinations in the fish oil microencapsulation was evaluated. Results obtained during this study indicate that the emulsion properties were significantly affected by wall material composition. Suggestion of the best combinations for microencapsulating of fish oil represents necessary. The MD: Hi-Cap: WPC combination showed the best encapsulation efficiency result. However, it showed poorer emulsion stability and viscosity. This result indicated the powdered fish oil produced has good physicochemical properties which lead to its more widespread application in food industry as a food additive.

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