Sensory and technological properties of developed functional bread enriched by microencapsulated fish oil

Shirin Hasani[1], *Seyed Mahdi Ojagh*[1], *Maryam Hasani*[2], *Mohammad Ghorbani*[3]

[1]Department of Fisheries, Faculty of Fisheries and the Environment, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran, E-mail: Mahdi_ojagh@yahoo.com; [2]Department of Food science and Technology, Shahrood Branch, Islamic Azad University, Shahrood, Iran; [3]Department of Food Chemistry, Faculty of Food Science and Technology, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

Summary. Summary. The object of this study was to develop functional bread enriched with omega-3 fatty acids. Therefore, to produce enriched bread, fish oil containing long-chain omega-3 polyunsaturated fatty acids was microencapsulated with different wall material component [chitosan (CS) and modified starch (Hi-cap 100)] by freeze drying. Then, the size and stability of the emulsion droplet, viscosity and the properties of the microencapsulated powders after freeze drying were determined. The combination of CS: Hi-cap 100 (1%: 9%) as best formulation of wall component in fish oil microcapsules was then incorporated in to bread to develop functional properties. Results indicated that enriched bread with 5% microencapsulated fish oil, had the highest value in firmness, crust and color of bread. In addition, Sensory results showed that there was no significant difference between enriched with 1% fish oil microcapsules and control on texture, appearance and crumb. So, Using of microencapsulated fish oil as an effective additive for improvement of nutritional value of semi volume bread and its application is suggested for production of other bakery products.

Key words: fish oil, freeze drying, semi volume bread, firmness, chitosan

Introduction

Fish oil is a rich source of polyunsaturated fatty acids (PUFAs), especially EPA (eicosapentaenoic acid, C20:5) and DHA (docosahexaenoic acid, C22:6) that cause fish oils are known as functional foods (1). Fish oil has been shown to be important and beneficial for health and prevention of many diseases including Cardiovascular and nervous system disease, blood pressure and inflammatory (2). Kilka oil (*clupeonella delicatula caspia*) contains significant values of PUFAs and may be a suitable food supplement. However, one of the main problems associated with long chain PUFAs is their high sensitivity to oxidative deterioration. Several studies were done to find technologies to prevent deterioration (2- 5- 6). Encapsulation can be as a key technology is used in foods to delay or inhibit oxidation of sensitive food ingredients like omega-3 fatty acids, control the release of valuable compounds, lower flavor loss during storage and raise the stability, component bioavailability and effects. The wall materials of microcapsules protect the core contents against adverse environmental conditions like oxygen, light, and humidity. The use of mixed wall material increases the microencapsulation efficiency (ME), stability and shelf life of core (6- 8). Chitosan (CS) applied as a wall material for encapsulation of sensitive core component such as lipophilic drugs, vitamin D, fish oil and olive oil extract (9). Natural starch and its derivatives (modified starches (Hi-Cap 100™), maltodextrin and β-cyclodextrin) widely are used in the food industry in order to protect the productive taste and odor compounds (10). Bread is one of the main and inexpensive nutritional resources of the human diet in most countries of the world. Bread contains high levels of protein and carbohydrates, but it is poor in materials such as dietary fibers, fatty acids, and phenols.

Recently, consumers trend to functional food which provides additional health benefits that can improve positive health effects. Therefore, in order to support low-income families, it is necessary to pay attention to improving the quality, variety of bread and savings in household income. Borneo et al. (11) used encapsulated omega-3 fatty acids in cream-filled sandwich cookies. They reported that it was possible to make shelf-stable enriched foods with omega-3 fatty acids without any negative effect on sensory and technological properties.

The aim of this work was to evaluate the potential of modified starch (Hi- Cap100™) combination with chitosan as wall materials for fish oil microencapsulation by Freeze drying and evaluate the effects of the addition of nano-encapsulated omega-3 on the technological and the sensory quality of semi volume bread.

Materials and methods

Materials

Kilka oil (*clupeonella delicatula caspia*) was purchased from Fisheries office. (Bandar-Anzali, Iran). The fish oil was transported to the laboratory in a dark sealed container and stored at −18 ˚C until proceeded and experimented. The coating materials were CS with Medium molecular weight (75–85% degree of deacetylation,) (Sigma–Aldrich) and modified starch: Hi-Cap 100™ (derived from waxy maize) (National Starch, Germany). Sodium tripolyphosphate (TPP) and Tween 80 were obtained from Sigma–Aldrich (St. Louis, MO, USA). Distilled and deionized water was used for the preparation of all solutions. Wheat flour with an extraction rate of 87% was purchased from Alborz Flour Company in Karaj, Iran. All general chemicals used in this study were of analytical grade.

Fatty acid composition in fish oil

Fish oil methyl ester was prepared according to AOAC Official Method (12). The fatty acid composition of fish oil was then analyzed 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 reported as a percentage of the total fatty acid content.

Emulsion preparation

Emulsions were prepared as slightly modification according to previous studies (13, 14). CS (0.5% w/v) and Hi-Cap 100 (9.5% w/v) dispersed in glacial acetic acid solution (1% v/v), then fish oil was added to solution and referred to as F1sample code. In addition, CS and Hi-Cap 100 at a ratio (1%: 9%) referred to as F2 sample code and (1.5%: 8.5%) referred to as F3 sample code. In this study, all of these combinations prepared with 1 g tween 80 100 g^{-1} fish oil and TPP (1mg/ml) in the mixture. The total concentration of dissolved solid (wall material + oil) was 20 % (w/w). The fish oil was added in a 1:4 ratio (w/w) to all solution. This mixture agitated using a magnetite stirrer at 200 rpm for 40 min at room temperature. The mixture was then emulsified using a homogenizer operating at 5000 rpm for 30 min. emulsions were frozen 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.

Emulsion particle size measurements

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: $D_{3,2} = \Sigma z_i d_i^{\,4} / \Sigma z_i d_i^3$

Apparent Viscosity

The apparent viscosity of emulsions was examined 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} (15).

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) (15).

Characteristics of microencapsulated fish oil
Microencapsulation efficiency

Microencapsulation efficiency (ME) was determined by measuring the free oil and total oil of the microcapsules. Extraction of total oil was done according to the Rose–Gottlieb method. The free oil fraction was extracted according to Ba & Li (16). 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.

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 (8). 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 an oven (Memmert, Germany) at 105 °C until constant weight was reached. Percent loss in weight was reported as water content (17).

Powder particle size

The particle size of the encapsulated powder was determined by using a light scattering instrument (Mastersizer Model MSS, Malvern Instruments Ltd.).

Scanning electron microscopy

Scanning electron microscopy was used to study the surface structures of the spray-dried powders. Powder particles were fixed directly on door-metallic specimens (stubs) of 12 mm diameter and then subjected to metallization (sputtering) with a layer of gold/palladium in a Sputter Coater SC7620 polaron (VG Microtech, England). The SEM pictures were taken at excitation voltage of 15kV and 100 X magnification.

Preparation of functional bread

The ingredients used for the bread preparation consisted of wheat flour (100 g), water (67 g/100 g), salt (2 g/100 g), sugar (6 g/100 g), instant yeast (2 g/100 g), fat (1 g/100 g). Wheat flour and other material were mixed until bread dough was prepared. Then microencapsulated fish oil was added to the dough at levels of 0.0 (control), 1, 2.5 and 5% (w/w) and mixed with another component thoroughly. The production of bread was performed at the local bakery by using a standard method described by Maleki et al. (18).

Proximate analysis of the wheat flour

Chemical analysis of wheat flour including the moisture content, protein, ash, wet gluten, pH and acidity were carried out according to (12). All the measurements were in triplicates.

Technological characterization of the bread
Texture analysis

Texture analyses were done by using the penetration test in some pieces of different treatment (3×3 cm). A texture analyzer (TA-XT-PLUS Texture Analyzer, UK) was used to measure the force required for penetration of a probe (6 mm) at a rate of 1 mm/s (19). Texture measurements were performed three times for each sample and mean values were reported.

Color analysis

For each treatment, three samples (crust and crumb) were performed using a digital image. For studying on color components of bread, the RGB color space images were converted to L*a*b* space (19).

Sensory assessment

Sensory evaluation of the bread was performed within 1 h of baking. The samples were tested by 30 semi-trained panelists on a 9-point hedonic scale of 9 (like extremely) to 1 (dislike extremely) were involved in the evaluation for crust and crumb color, aroma, taste, texture and overall acceptability.

Statistical analysis

One-way analysis of variance (ANOVA) was performed using SPSS (ver.15) software. Differences among mean values were examined by Duncan›s test

($p \leq 0.05$) significance level. Kruskal-Wallis test was used to sensory evaluation. The Mann-Whitney U-test is used to Paired comparisons test.

Results and Discussion

Fatty acids composition

Fatty acid profile of starting fish oils (*C. delicatula*) is shown in Table 1. The most abundant fatty acid identified was oleic acid (C18:1) at 26.77% (g/100g fatty acids), followed by palmitic acid (C16:0) at 19.73% (g/100g fatty acids), docosahexaenoic acid (C22:6) at 16.1 (g/100g fatty acids), eicosapentaenoic acid (C20:5) at 7.03 (g/100g fatty acids), 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 ω3/ω6 and polyene (PI) ratios (Table 1). The ω3/ω6 ratio has recently attracted a great attention because of its important effects on the development of several health human problems.

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

Emulsion particle size measurements

Average particle diameter [$D_{(3,2)}$] of the oil/water emulsions ranged from 0.85 to 2.05 µm and is listed in Table 2. This results showed that the use of the different ratio of wall materials had a significant influence on emulsions droplet size (Table 2). The sample F2 had the smallest droplets when compared to the other samples. This last result can be related to the highest viscosity presented in F2 sample emulsion, which shows a greater resistance to droplets action, avoiding coalescence and resulting in smaller diameters.

Apparent Viscosity

The viscosity of emulsions prepared with a different ratio of wall materials combination is shown in table 2. The F2 sample CS: Hi-Cap 100 (1%: 9%) has shown higher viscosity in comparison to others. Table 2 showed there was a significant difference between apparent values of formulations. Our results confirmed that emulsion with smaller droplet mean diameters were more viscous compared with others. Tonon et al. (4) observed that there is an inverse relation between particle size and emulsion viscosity which is similar to our results in the current study. The observed decreases in viscosity reflect the decrease in average molecular size and improved solubility of higher DE carbohydrates. The comparatively low viscosity values for emulsions with higher content of modified starch (Hi-cap 100) are most probably due to the sedimentation of starch granules which occurred during testing.

Creaming index

The stability examine noticed that emulsions prepared with CS: Hi-Cap 100 (1%: 9%) and (1.5%: 8.5%) were kinetically stable, with exception of those

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

Formulation	Viscosity (mPa.s)	D_{32} (µm)
F1	137.4 ± 58.20 ^{c}	2.05 ± 0.13 ^{a}
F2	3171.07 ± 133.87 ^{a}	0.65 ± 0.06 ^{c}
F3	1583.07 ± 503.21 ^{b}	0.85 ± 0.08 ^{b}

Reported means (± standard deviations) derived from 3 replications with 3 samples per replication.
Means with in column followed by different superscripts are significantly different at $P \leq 0.05$.

prepared with CS: Hi-Cap 100 (0.5%: 9.5%) which showed the formation of a small separation layer and a foam phase, 24 h after its homogenization. This was unexpected since modified starch (Hi-Cap 100) are well known for their good emulsifying capacity.

Emulsion samples F1 showed the highest creaming index values. However, there was no creaming in emulsion samples F2. On the other hand, a small but not significantly higher ($P \leq 0.05$) creaming rate was observed in emulsion samples F3. Dickinson (20) presented the most usual initial appearance of the instability of oil in water emulsion is creaming which can cause to phase separation with a defined clear or semitransparent lower serum phase and cream. This may then lead to droplets coalescence within the cream and oiling off at the top of the sample. The results of our study clearly indicated that F2 sample was the best formula to produce the stable emulsions. The emulsion is most stable when mixed with CS and Hi-cap 100 in proper ratios. These results are compatible with Laplante et al. (21) who have also reported the inability of CS or whey protein isolated alone to prepare stable emulsions, by opposition to mixed components showing a lot much better stability. In addition, CS is a unique polysaccharide of a cationic nature with hydrophilic part rich in glucosamine and hydrophobic part rich in N-acetyl-glucosamine which enable it to adsorb at the oil/water interfaces. So, Interaction between CS and Hi-cap 100 can co-adsorb on the oil/water interface, causing higher emulsion stability due to increased interfacial electrostatic stability (21). A stable emulsion containing small oil droplets is critical for successful microencapsulation. It is therefore important to select an appropriate emulsifying system, as well as the conditions required to obtain a stable emulsion before the drying process.

Characteristics of microencapsulated fish oil
Microencapsulation efficiency (ME)

The ME results of the treatments evaluated are shown in figure 2. There were significant differences ($p<0.05$) between F2 sample and other samples which showed the smallest value (79.37%) in F1 sample. The highest microencapsulation efficiency (ME) was obtained in F2 samples CS: Hi-cap100 (1%:9%) while the lowest ME was found for the sample using CS: Hi-cap100 (1.5%:8.5%) wall materials.

This could be explained by properties of CS and Hi-cap 100 complex. It has increased emulsifying properties and forms a better membrane at the oil-water interface, why it can cover fish oil with a better microencapsulation. Many research has 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 (22- 27). Also, the investigation results showed that concentration of wall material had very significant influence ($p<0.01$) on the surface oil content of fish oil encapsulated powders. This trend could be explained by the droplet size, similar to the results of Jafari et al. (10). This researcher found that reduces the emulsion droplet size significantly decreased the surface oil content (by using CS and Hi-cap as the wall material because small oil droplets incorporated more efficiently within the wall matrix of microcapsules. Emulsion droplet size plays a key role in the surface oil content of microencapsulated powders. According to Thijssen et al. (28), when water

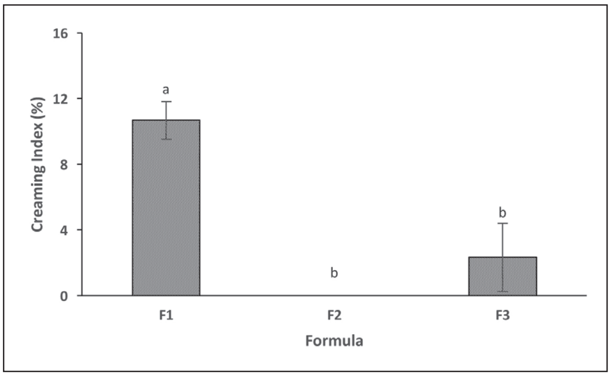

Figure 1. Creaming index (%) of emulsions

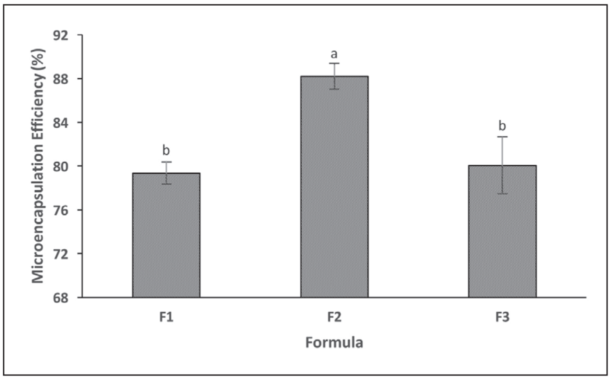

Figure 2. Microencapsulation efficiency (ME %) of powder

content reduces, diffusion coefficient of the oil phase components decreased several times more than water, so while drying the emulsion, water is continuously removed from the shell with a certain speed, but the oil phase negligible velocity because they are trapped inside the mass of material (wall material) being dried. This solid coating, as a semi-permeable membrane, allows water molecules to exit while reducing or even stopping the outflow of oil components through microcapsules. The results obtained in this work are consistent with this theory.

Moisture content of powder

Moisture of microcapsules is shown in the table 3 which ranged from 2.05 to 2.91 %. Results showed that ratio of wall material had very significant effects ($p \leq 0.05$) on the moisture powder. Table 3 showed there was a significant difference between F3 (2.91%) and other samples.

It seems modified starch (Hi-cap 100) compared to CS has a lower water binding capacity. Because its base in the number of linking groups with water in CS and Hi-cap 100 molecules are different. Also, our results showed that concentration and ratio of wall materials had significant influence ($p \leq 0.05$) on the moisture content of the fish oil encapsulated powders. This result are inconsistent with the observations of Sankarikutty et al. (29) and Dian et al. (30), who found that moisture content was not affected by the type of wall material or core/wall ratio.

The moisture content of tree formula encapsulated powder F1, F2 and was under the maximum moisture specification for most dried powder in the food industry which is between 3 and 4 g/100 g (28).

Microcapsules particle size measurements

Table 3. Characterization of fish oil microcapsules with different ratio of wall materials.

Formulation	Moisture (%)	D_{43} (μm)
F1	2.05 ± 0.05 [b]	4.73 ± 0.47 [a]
F2	2.23 ± 0.22 [b]	2.30 ± 0.20 [b]
F3	2.91 ± 0.09 [a]	4.17 ± 0.65 [a]

Reported means (± standard deviations) derived from 3 replications with 3 samples per replication.
Means with in column followed by different superscripts are significantly different at $P \leq 0.05$

The average particle size ($d_{4,3}$) of fish oil microcapsules ranged from 2.30 to 4.73 μm and was independent of the oil load and composition. The use of different wall materials had a significant effect on emulsions particle size (Table 3). The selection of wall material combinations and their concentration affects both the emulsion properties and the particles' characteristics after drying. It is well described that emulsion characteristics such as stability, viscosity, droplets size, as well as powder properties such as surface oil, particle size, morphology and oxidative stability, are influenced by the type of encapsulating agent used (10).

Scanning electron microscopy of microencapsulated powders (SEM)

Figure 3 shows the scanning electron microscope (SEM) images of the microcapsules. Morphological studies on dried particles provide valuable perspective relation to the drying particles, chemical and physical factors affecting the particle structure. Freeze dried microcapsule particles, as expected, were irregular shaped. The edges of the capsules are considerably sharper. This images could be confirmed In Fig 3 (A), (B) and (C), we can see the slab like the structure of irregularly shaped microcapsule, which is expected in the powders produced by this method (8). Anandharamakrishnan & Karthik (31) studied the powder morphology of spray dried and freeze dried microencapsulated DHA by scanning electron microscope (SEM). They found a spherical shape with a smooth surface for spray-dried microencapsulated powder as compared with nonspherical freeze dried microencapsulated particles. The freeze dried powder exhibits the cakelike structure with uneven surfaces (figure 3).

The powder particles appeared to be largely free of cracks and pore or free oil in surface because of higher microencapsulation efficiency (ME %) in fig 3 (b) but the presence of some pores was observed in fig 3 (a, c). The "free oil" measured using the solvent extraction procedure mentioned above may, therefore, have been due to the presence of these pores in the powder particles. A considerable part of the free oil consists of surface fat or fat globules from the interior of the microcapsules (32).

Chemical composition of wheat flour

The proximate composition of the wheat flour was shown in table 4. Physical and chemical results of wheat

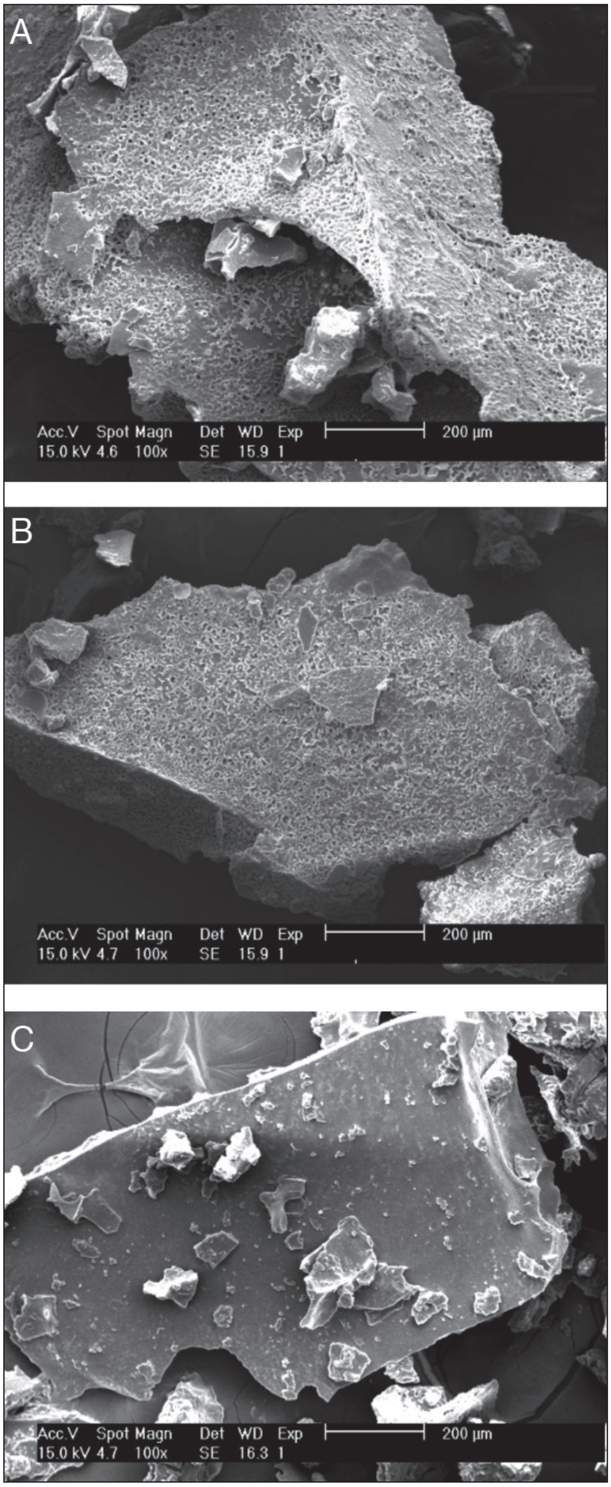

Figure 3. External micro structures of powders produced from different ratio of wall material combinations: (a) F1 sample: CS (0.5% w/v) and Hi-Cap 100 (9.5% w/v); (b) F2 sample: CS (1 % w/v) and Hi-Cap 100 (9 % w/v); (c) F3 sample: CS (1.5 % w/v) and Hi-Cap 100 (8.5 % w/v)

raw material used in this research Reflect the quality of wheat and its usage, the baking quality of wheat and final product. Moisture is one of the effective factors in wheat quality during storage. The moisture content of Wheat flour used in this study is close to the flour used in other studies (33, 34). The values obtained moisture (10.25± 0.06) are showed that this sample is suitable for baking bread according to standard (up to 14.2 %).

Protein content in wheat depends on varieties, wheat types and environmental conditions during the growing season (35). The data presented in Table 4 indicate that this flour is rich in protein and wet gluten as it is desirable for baking semi-volume bread.

The amount of ash is closely related to bran content in wheat and flour extraction rates. So that mineral value in flour will be increased with flour extraction rate increasing and flour color will be darker. According to the results shown in Table 4, the raw wheat flour ash tested was in standard range (0.7- 0.85) for baking Iranian semi-volume bread. Primarily changes in flour pH are low due to buffered flour compounds such as proteins in alkaline solutions and weak acid as they have the ability to balance pH content.

Technological characterization of the bread

Texture analysis

The results of texture analysis of control and enriched with microencapsulated fish oil bread are given in table 5. According to the results, it can be concluded firmness immediately after baking in bread enriched with fish oil microcapsules was significantly ($p \leq 0.05$) higher than the control. The firmness values varied from 3.27 ± 0.12 to 4.80 ± 0.10 N. Adding fish oil microcapsules to bread significantly increases the firmness in the samples were fortified compared to control bread whereas, the control and bread with 1% microcapsules showed the same firmness of the bread. It seems to add the particles of microencapsulated fish oil in amounts exceeding 2.5% decreased the rate of drying during baking of bread. So, this adversely impacts the crumb porosity making the bread harder. Hardening effect may be due to the use of wall components of fish oil is used in microcapsules. Costa de Conto et al. (36) reported that adding omega-3 microcapsules powder and rosemary extract increased specific volume and firmness of white bread that this result was similar to our finding.

Table 4. Physiochemical characteristics of wheat flour (± standard deviations)

Acidity	pH	Wet Gluten	Moisture	Ash	Protein
0.87 ± 0.06	6.04 ± 0.04	34.67 ± 0.31	10.25 ± 0.06	0.72 ± 0.03	12.27 ± 0.03

Table 5. Effect of concentration of microencapsulated fish oil on the technological characteristics of semi volume bread (Mean ± SD)

	Bread Samples			
	A	B	C	D
Firmness (N)	3.27 ± 0.12 c	3.47 ± 0.25 c	4.13 ± 0.12 b	4.80 ± 0.10 a
L^* (Crust)	62.83 ± 0.06 a	57.57 ± 0.49 b	55.03 ± 0.06 c	51.40 ± 0.61 d
a^* (Crust)	-1.23 ± 0.21 d	1.07 ± 0.12 c	1.40 ± 0.20 b	1.87 ± 0.06 a
b^* (Crust)	32.43 ± 0.25 c	34.67 ± 0.06 b	35.10 ± 0.10 a	35.17 ± 0.29 a
L^* (Crumb)	56.47 ± 0.06 a	56.07 ± 0.12 b	52.17 ± 0.15 c	50.03 ± 0.06 d
a^* (Crumb)	-4.63 ± 0.06 d	-3.03 ± 0.06 c	1.03 ± 0.06 b	2.07 ± 0.12 a
b^* (Crumb)	23.40 ± 0.17 a	23.10 ± 0.10 a	24.03 ± 0.15 a	21.77 ± 1.08 b

A: control (without microencapsulated fish oil), B: with 1% microencapsulated fish oil, C: with 2.5% microencapsulated fish oil, D: with 5% microencapsulated fish oil.

Color analysis

The results for crust and crumb color obtained after processing are presented in Table 5. L^* values of crust were found as 62.83 ± 0.06, 57.57 ± 0.49, 55.03 ± 0.06 and 51.40 ± 0.61 for control, containing 1%, 2.5% and 5% of fish oil microcapsule particles added to breads, respectively. The difference in the crust colors of bread was clear for bread incorporated with microencapsulated powder. In terms of crumb color, control bread (without particles) had lighter crumb color while other bread having 1%, 2.5%, and 5% of particles showed light browning in their crumb. a^* value of crust and crumb color had significant differences ($p \leq 0.05$) in all treatments (table 5). Resulting in b^* values in crust and crumb color of bread containing 0%, 1%, 2.5% and 5% microcapsules showing that low concentrations of nanocapsules did not affect the color characteristics of bread. It seems that adding powdered fish oil microcapsule increases Maillard browning reaction rate followed by light decreasing in fortified bread. Previous studies have determined that the instrumental measurement of the color of baked products is necessary for quality assessment of the products, evaluation of effects of different material or formulations, processing conditions, as well as the storage quality of baked products (37).

Sensory evaluation

The sensory analysis of the semi volume bread samples is shown in Table 6. Results indicated that bread incorporated with microencapsulated fish oil (1%, 2.5%, and 5%) had significant values compared with the control sample in preference to taste, aroma

Table 6. Effect of concentration of microencapsulated fish oil on the sensory characteristics of semi volume bread (Mean ± SD)

	Bread Samples			
	A	B	C	D
Appearance	8.4 ± 0.55 a	8 ± 0 a	6 ± 0.71 b	5.6 ± 0.55 b
Taste	8.6 ± 0.55 a	7 ± 0.71 b	5.8 ± 0.45 c	4.2 ± 0.45 d
Texture	8.2 ± 0.45 a	7.8 ± 0.45 a	6.6 ± 0.55 b	5.4 ± 0.55 c
Aroma	9 ± 0 a	7.8 ± 0.45 b	5.4 ± 0.55 c	3 ± 0.71 d
Crumb	8.4 ± 0.55 a	8.2 ± 0.45 a	6.6 ± 0.55 b	5.2 ± 0.45 c
Overall Accessibility	8.4 ± 0.55 a	7.4 ± 0.55 b	5 ± 1 c	4.6 ± 0.55 c

A: control (without microencapsulated fish oil), B: with 1% microencapsulated fish oil, C: with 2.5% microencapsulated fish oil, D: with 5% microencapsulated fish oil.

and overall acceptability. There were no significant differences between the control samples and containing 1% microcapsules for appearance, texture, and crumb. Mahmoud et al (2017) have reported non-encapsulated lemon and orange peel extract decreased acceptability of cake color, odor, taste, texture and overall acceptability unlike encapsulated samples (38). In the present study sensory analysis was in agreements with their results. Thus, it is well found that microencapsulation of fish oil can balance flavor and odor and makes it suitable for use in products.

Conclusion

These research clearly showed that concentration ratio of wall material influences the properties of the micro-emulsion and microencapsulated powders. CS and Hi-cap 100 are the main factor in properties of fish oil encapsulated powder. It has shown potential for encapsulation of fish oil due to their emulsifying properties. In addition, our finding in this study showed that the addition of microencapsulated fish oil caused effects on technological and sensory properties of semi volume bread as bread had good sensory acceptance, even at the maximum dosage of omega-3 microcapsules. So, According to the results obtained in this study microcapsules containing fish oil can be used for enriching bread without causing undesirable odor and taste.

References

1. Klinkesorn U, Sophanodora P, Chinachoti P, Decker E A, McClements D J. Characterization of spray-dried tuna oil emulsified in two-layered interfacial membranes prepared using electrostatic layer-by-layer deposition. Food Research International 2006; 39: 449–457.
2. Chen Q, McGillivray D, Wenb J, Zhong F, Young Quek S. Co-encapsulation of fish oil with phytosterol esters and limonene by milk proteins. Journal of Food Engineering 2013; 117: 505–512.
3. Velasco J, Dobarganes M C, Márquez-Ruiz G. Oxidation of free and encapsulated oil fractions in dried microencapsulated fish oils. Grasas y Aceites 2000; 51: 439–446.
4. Tonon R.V, Grosso C R F, Hubinger M D. Influence of emulsion composition and inlet air temperature on the microencapsulation of flaxseed oil by spray drying. Food Research International 2011; 44 (1): 282–289.
5. Motalebi A A, Seyfzadeh M. Effects of whey protein edible coating on bacterial, chemical and sensory characteristics of frozen common Kilka (Clupeonellia delitula). Iranian Journal of Fisheries Sciences 2015; 11 (1): 132–144.
6. Kaushik V, Roos Y. Limonene encapsulation in freeze-drying of gum Arabic–sucrose–gelatin systems. LWT 2007; 40: 1381–1391.
7. Velasco J, Dobarganes C, Holgado F, Márquez-Ruiz G. A follow-up oxidation study in dried microencapsulated oils under the accelerated conditions of the Rancimat test. Food Research International 2009; 42: 56–62.
8. Carneiro H C F, Tonon RV, Grosso C R F, Hubinger M D. Encapsulation efficiency and oxidative stability of flaxseed oil microencapsulated by spray drying using different combinations of wall materials. Journal of Food Engineering 2013; 115: 443–451.
9. Klaypradit W, Huang Y W. Fish oil encapsulation with chitosan using ultrasonic atomizer. LWT- Food Science and Technology 2008; 41 (6): 1133–1139.
10. Jafari S M, Assadpoor E, Bhandari B, He Y. Nano-particle encapsulation of fish oil by spray drying. Food Research International 2008; 41: 172–183.
11. Borneo R, Kocer D, Ghai G, Tepper B J, Karwe M V Stability and consumer acceptance of long-chain omega-3 fatty acids (eicosapentaenoic acid, 25: 5, n-3) in cream-fi lled sandwich cookies. Journal of Food Science 2007; 72: S50–S54.
12. AOAC. 2000. Office methods of analysis of AOAC international. Method 991, 39: 26–28.
13. Hosseini M H, Razavi S H, Mousavi M A. Antimicrobial, physical and mechanical properties of chitosan-based films incorporated with thyme, clove and cinnamon essential oils. J. Food. Process and Preserv 2009; 33: 727–43.
14. Saloko S, Darmadji P, Setiagi B, Pranoto Y. Antioxidative and antimicrobial activities of liquid smoke nanocapsules using chitosan and maltodextrin and its application on tuna fish preservation, Food bioscience 2014; 7: 71–79.
15. Aghbashlo M, Mobli H, Madadlou A, Rafiee S H. The correlation of wall material composition with flow characteristics and encapsulation behavior of fish oil emulsion. Food Research International 2012; 49: 379–388.
16. Bae E.K, LEE S J. Microencapsulation of avocado oil by spray drying using whey protein and maltodextrin. Journal of Microencapsulation 2008; 25 (8): 549–560.
17. AOAC. 2006. Official Methods of Analysis (18th Ed.). Association of Official Analytical Chemists, Gaithersburg, Maryland.
18. Maleki M, Vetter J L, Hover W J. The effect of emulsifiers, sugar, shortening and soya flour on the staling of barbari flat bread. Journal of Science Food Agriculture 1981; 32: 1209-1211.
19. Razavizadegan Jahromi S H, Karimi M, Tabatabaee Yazdi F, Mortazavi S A. Responce surface optimization of Barbari bread-making process variables: interrelationship of texture,image and organoleptic characteristics; using image analysis for quality and shelflife prediction. Journal of Food

Processing and Preservation 2014; 38: 1608–1621.
20. Dickinson E. Hydrocolloids at interfaces and the influence on the properties of dispersed system. Food Hydrocolloids 2003; 17: 25–39.
21. Laplante S, Turgeon S L, Paquin P. Effect of pH, ionic strength, and composition on emulsion stabilising properties of chitosan in a model system containing whey protein isolate. Food Hydrocolloids 2005; 19: 721–729.
22. Lin C C, Lin S Y, Hwang L S. Microencapsulation of squid oil with hydrophilic macromolecules for oxidative and thermal stabilization. Journal of Food Science 1995; 60 (1): 36–39.
23. Heinzelmann K, Franke K, Jensen B, Haahr A M. Protection of fish oil from oxidation by microencapsulation using freeze drying techniques. European Journal of Lipid Science and Technology 2000; 102 (2): 114–121.
24. Hardas N, Danviriyakul S, Foley J L, Nawar W W, Chinachoti P. Accelerated stability studies of microencapsulated anhydrous milk fat. Lebensmittel- Wissenschaft & Technologie 2000; 33: 506-513.
25. Hogan S A, McNamee B F, D O'Riordan E, O'Sullivan M. Emulsification and microencapsulation properties of sodium caseinate/carbohydrate blends. International Dairy Journal 2001; 11: 137–144.
26. Baik M Y, Suhendro E L, Nawar W W, McClements D J, Decker E A, Chinachoti P. Effects of antioxidants and humidity on the oxidative stability of microencapsulated fish oil. Journal of the American Oil Chemists Society 2004; 81 (4): 355–360.
27. Klinkesorn U, Sophanodora P, Chinachoti P, McClements D J, Decker E A. Stability of spray-dried tuna oil emulsion encapsulated with two-layered interfacial membranes. Journal of Agriculture and Food Chemistry 2005; 53: 8365–8371.
28. Thijssen H A C, Rulkens W H. Retention of aroma in drying food liquids. DeIngenieur 1968; 47: 45–56.
29. Sankarikutty B, Sreekumar M M, Narayanan C S, Mathew A G. Studies on microencapsulation of cardamon oil by spray-drying technique. Journal of Food Science and Technology 1988; 25: 352–356.
30. Dian N L H M, Sudin N, Yusoff M S A. Characteristics of microencapsulated palm-based oil as affected by type of wall material. Journal of the Science of Food and Agriculture 1996; 70: 422–426.
31. Anandharamakrishnan C, Karthik P. Microencapsulation of docosahexaenoic acid by sprayfreeze- drying method and comparison of its stability with spray-drying and freeze-drying methods. Food Bioprocess Technol 2013; 6: 2780–2790.
32. Buma T J. Free fat in spray-dried whole milk. 8. The relation between free-fat content and particle porosity of spray-dried whole milk. Netherlands Milk and Dairy Journal 1971; 25: 123–140.
33. Karimi M, Azizi M H, Abbasi S. Effect of Different Levels of Sodium Estearyl Laktylat on Dough Rheological Properties and Tafton Bread Quality. Iranian Journal of Food Science and Technology 2007; 4 (3): 31-38.
34. Fazeli M R, Hajimohammadali M, Moshkani A, Samadi N, Jamalifar H, Khoshayand M. R. Aflatoxin B1 binding capacity of autochthonous strains of lactic acid bacteria. Journal of Food Protection 2009; 72: 189–192.
35. Gray J A, Bemiller J N. Bread Staling: Molecular Basis and Control. Compr. Rev. Food Sci. Food Safety 2003; 2, 1-20.
36. Costa de Conto L, Porto Oliveira R S, Martin, L, Chang Steel Y C. Effects of the addition of microencapsulated omega-3 and rosemary extract on the technological and sensory quality of white pan bread. LWT - Food Science and Technology 2012; 45: 103-109.
37. Erkan H, Celik S, Bilgi B, Koskel H. A new approach for the utilization of barley in food products: barley tarhana. Food Chemistry 2006; 97: 12-18.
38. Mahmoud KH, Ibrahim M, Mervat E, Shaaban H, Kamil M, Hegazy N. Nano-encapsulation efficiency of lemon and orange peels extracts on cake shelf life. Am J Food Tech 2017; 11: 63-75.

Correspondence:
Seyed Mahdi Ojagh
Department of Fisheries, Faculty of Fisheries and the Environment, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran
Email: Mahdi_ojagh@yahoo.com