Encapsulation Efficiency, Microstructur and Oxidation Stability of Fish Oil Encapsulated Powder Made by Using whey Protein Concentrate

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ABSTRACT

Fish oil encapsulated powder was prepared with four ratios (0.6:1, 0.8:1, 1:1 and 1.2:1) of fish oil:whey protein concentrate with addition of 100 ppm in dry matter antioxidant (α-tochopherol) and 25 ppm in dry matter nisin as an antimicrobial agent. The emulsion was homogenized, pasteurized and spray or freeze dried. The produced powder was freshly analyzed for moisture content, encapsulation efficiency and microstructure while degree of oxidation was determined every 15 days in the powder kept at 4°C for 90 days.

Spray dried powders have lower moisture content than freeze dried one. Moisture in both powders tended to decrease with the increasing of oil ratio.

Encapsulation efficiency showed the highest values (70.2 % for spray and 63.18% for freeze dried powder) at the ratio of (0.6:1), while it was decreased with the increasing of fish oil ratio up to (1.2:1). Encapsulation efficiency was dropped by about 13% and 10% from the ratio of 1:1 to the ratio of 1.2:1 in spray and freeze dried powder respectively. Spray dried powder had higher encapsulation efficiency than freeze dried. In addition, the ratio of (1:1) can be considered as the most suitable ratio could be applied as it allows reasonable concentration of omeg-3 in the same time, the encapsulation efficiency is acceptable. Many authors and foundations suggested 500 mg of ω-3 fatty acids as daily requirement, this amount can be covered by 4.38g of powder of 1:1 fish oil:WPC.

Oxidation stability was determined for fish oil as a control and for the encapsulated fish oil powder (EFOP) along 90 days storage at 4°C.

Degree of oxidation (TBAR values) was gradually increased in both fish oil (control) and EFOP with the advance of storage time. TBAR values of fish oil were dramatically increased throughout storage time (10 at zero time, and it become 325 nmolMAD/kg oil at day 90). On the other hand the increment of TBAR values in EFOP was not so remarkable (18 as minimum at zero time, and it become 53 as maximum nmol MDA/kg oil at day 90). Encapsulation of fish oil strongly delayed the oxidation development in the powder beside the influence of antioxidant (α-tochopherol) which was added. The third factor which considerably impaired oxidation is the low storage temperature (4°C).

Scanning electron microscope (SEM) showed that spray drying resulted in spherical particles of different sizes with collapse or shriveling, visible wrinkles or dimples on the surface, but no apparent pores. On the other hand, freeze dried powder showed a completely different morphology of particles. Relatively low magnification (x1500) showed the powder in plates-like layers without pores on the surface, while magnification of (x3500) was not clear enough to describe the image content. At the same magnification of (x3500), spray dried powder showed clear individual spherical particles with different sizes. Images of spray dried powder with magnifications of (x10000 and x15000) were very sharp and distinctive. The particles were mostly about 2um diameter with smooth surface and visible wrinkles. Magnification more than (x3500) for freeze dried powder was not successful to show any useful details.

Key words: Spray drying, freeze drying, fish oil powder, encapsulation, oxidation, microstructure, whey protein concentrate.

INTRODUCTION

Omega-3 fatty acids are long chain polyunsaturated fatty acids (LC PUFAs). These acids are essential nutrients for normal metabolism, enhancing life quality, contributing to the prevention of coronary heart disease, hypertension, type 2 diabetes, rheumatoid arthritis, support the normal physical development of the brain and eyes.

Fish oil is a predominant dietary source of omega-3 fatty acids since it contains 15-40% omega-3 fatty acids.

Omega-3 fatty acids (eicosapentaenoic acid (EPA 20:5), and docosahexaenoic acid (DHA 22:6) are very susceptible to oxidation due to numerous double bonds existing in the fatty acid molecule. Attempts to incorporate fish oil into food formulations have had limited success mainly because of 'fishy' flavors coming through in the consumer products and the great susceptibility to oxidation.

Oxidation has negative effect on both nutritional and organoleptic properties, consequences; changes in nutritional value of products such as the destruction of essential fatty acids and the lipid-soluble vitamins A, D, E, and K; decrease in caloric content; rancidity which produces off-flavors and pronounced odors; color changes such as darkening of fats and oils and lightening of pigments, as well as flavor loss. Many attempts have been made to prevent the oxidative deterioration by using natural antioxidants (Frankel, 1998). Some components in natural products such as vitamin E, carotenoids, flavonoids, anthocyanins, and

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phenolic compounds are known to function as scavengers in both 1 and 2 oxidation processes (Ahn et al., 2008).

The encapsulation of sensitive or active components such as fish oil has become a very attractive process in the last decades. Encapsulation is defined as a process in which tiny particles or droplets are surrounded by a coating, or embedded in a homogeneous or heterogeneous matrix, to give small capsules with many useful properties. Encapsulation can provide a physical barrier between the core compound and the outside environment through the encapsulating agent which protects the core against deterioration.

Therefore, the aim of this study is producing a dried food ingredient in which the fish oil was protected from oxidation and the fish flavor was relatively masked by means of encapsulation using the whey protein concentrate as a capsulation material. Encapsulation and drying aimed to concentrate the omega-3 PUFAs content of the prepared ingredient and extend the shelf-life of oil in the powder thus increasing its versatility as a nutritional ingredient may incorporate into a number of food products, at levels to satisfy the recommended daily intake of omega-3 fatty acids.

MATERIALS AND METHODS

Whey protein concentrate (WPC minimum 80% protein), a product of Agri Mark, Inc (USA), with the following chemical composition: Moisture 5.00%, fat 4.60%, protein 83.70 %, lactose 5.50% and minerals 1.20%.

Fish oil as a Source of omega 3-fatty acids, a product of china, Jiangsu Weisikang Food Science and Technology Development Co., Ltd.

Antioxidant

α-tocopherol, a product of Sigma Aldrich, Co., St. Louis, MO.

Preservative (nisin).

Nisin, a product of China, Tianjin Ecobio Biotech Co. LTD.

Analysis of fish oil:

1. Refractive index measurement: Abbey refractometer was used in determining the refractive index of the oil.

2. Determination of Free Fatty Acids (Acid value):

Free fatty acids (FFA) value was determined according to the method describe in AOCS (1992). 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 color was observed. FFA concentration in oil is calculated as percentage oleic acid (grams oleic acid per 100 gram oil) according to the following equation:

$$\text{FFA \% as oleic acid} = \frac{\text{ml NaOH x NaOH normality x 28.2}}{\text{Weight of sample (g)}}$$

3. Determination of Peroxide value (PV):

The Peroxide values (PV) of fish oil was determined according to the method of (AOCS, 1992). 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 (0.5ml) was then added and the mixture was left in the dark for 1 minute 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 disappears. A blank sample titration was also carried out in the same manner but with no oil added.

$$\text{Peroxide value} = \frac{\text{a - b x 10}}{\text{Weight (g) of the sample}}$$

Where; a =Volume (ml) of 0.1 mol/l sodium thiosulfate consumed in the blank test,

b = Volume (ml) of 0.1 mol/l sodium thiosulfate consumed in the test.

4. Determination of lipid oxidation

Thiobarbituric acid reactive substances

The oxidative level of fish oil and microcapsules was determined by the thiobarbituric acid reactive substance (TBAR) method, as described by Hu and Zhong (2010).

To completely dissolve the microcapsule matrix and fish oil, a ternary solvent mixture composed of 1-butanol/isopropanol/HCl 0.5 M (2:2:1, v/v/v) was used. TBA stock solution was obtained by mixing 15 g of trichloroacetic acid, 0.75 g of TBA, and a solution of 0.8 g of BHT dissolved in 100 mL of the ternary solvent mixture. Forty milligrams of samples was placed into a centrifuge tube and, subsequently, 10 mL of TBA stock solution was added and mixed using a vortex. Then, tubes were introduced in a water bath at 95°C for 2 h and, after that, immediately cooled at room temperature. Concurrently, a calibration curve with solutions of 1,1,3,3-tetramethoxypropane (TMP) was prepared ranging from 0.2 to 20.0 μM, which were processed as the samples. Finally, absorbance at 532 nm was measured in a spectrophotometer Jenway 7305 (Roissy, France) using ternary solvent mixture as a blank.

Malondialdehyde (MDA) equivalence was calculated from the calibration curvel. Thiobarbituric acid reactive substances TBARS level was expressed as nmol Malondialdehyde MDA /kg oil.

5. Determination of fatty acids composition of fish oil by gas chromatograph

- Preparation of fatty acid methyl ester:

Preparation of fatty acids methyl ester (FAME) was carried out as described by DeMan (1964) and modified by Jumat et al.(2006). 1ml of hexane was put into .01 ml oil to be analyzed and 1 ml sodium methoxide (1.55 g of NaOH in 50 ml of methanol) is sealed into a glass bulb. The sealed bulb is placed in an oven at 60 C and methylation is complete in 1 hr, as is evident from the change of a two-phase to a one-phase system. After opening the bulb the esterification mixture is used without further treatment for injection into the GLC column. The identification of the peaks was carried out by retention times.

- Gas chromatography Conditions:

Device Model: HP (Hewlett Packard) 6890 GC

Detector: FID (Flame Ionization Detector)

Detector temperature: 280°c

Injector temperature: 220°c, injector volume 3 µl, split ratio 50:1

Column: HP-5 (5% diphenyl, 95% dimethyl polysiloxane), 30 m, 0.32 mm ID, 0.25 µm film thickness

Carrier gas: Nitrogen, gas flow: 1 ml/min

Oven program:

Initial temp. 150°c for 2 min

Ramps	Rate ° C/min	Final temp	Hold time
1	10	200	-
2	5	250	9 min

Preparation of encapsulated fish oil powder (EFOP).

Fish oil was added in four concentrations in relation to WPC (fish oil: WPC) (0.6:1, 0.8:1, 1:1 and 1.2:1) in order to obtain the favorable ratio for the favorable encapsulation efficiency with considering the amount of omega -3 fatty acids must be supplemented/day . Antioxidant was added to be in a constant concentration of 100 ppm of dry matter and nisin was added to be in a constant concentration of 25ppm in dry matter as shown in table (1)

Encapsulation was carried out as described by Helena et al., (2013). WPC, antioxidant and nisin were added into distilled water, heated to 50°C and stirred continuously with Electric-Hand-Mixer (Philips-HR1459-300W) until complete dissolving, then fish oil was added. The blends were mixed thoroughly until it becomes homogenous emulsion. The four blends were homogenized at pressure of 10MPa and temperature of 55°C and then pasteurized at 65°C / 30 min. The homogenized pasteurized emulsions were cooled down to 4ºC. Part of each emulsion was spray dried by (BüCHI 190 Mini Spray Dryer, Germany) and another part was freeze dried by (vir Tis sp scientific, USA). The resultant powder was filled in dark brown glass jar and kept in refrigerator at 4ºC until use. The following flow diagram represents the steps of preparation of encapsulated fish oil powder (EFOP).

Analysis of encapsulated fish oil powder (EFOP).

1. Moisture content of fish oil powder (EFOP)

Moisture content was determined gravimetrically in EFOP by drying in an oven at 70°C until constant weight (AOAC, 2006).

2. Encapsulation efficiency of fish oil powder (EFOP)

Encapsulation efficiency (EE) was determined for EFOP according to the method described by Bae and Lee (2008). Fifteen milliliters of hexane were added to 1.5 g of EFOP in a glass jar with a lid, which was shaken by hand for the extraction of free oil, during 2 min, at room temperature. The solvent mixture was filtered through a Whatman filter paper no. 1 and the powder collected on the filter was rinsed three times with 20 mL of hexane. Then, the solvent was left to evaporate at room temperature and after drying heated at 60□C, until constant weight. The non-encapsulated oil (surface oil) was determined by mass difference between the initial clean flask and that containing the extracted oil residue (Jafari et al., 2008). Total oil was assumed to be equal to the initial oil, since preliminary tests revealed that all the initial oil was retained (unvolatile).

Encapsulation efficiency (EE) was calculated from the following equation.

$$EE = (TO - SO / TO) \times 100$$

where TO is the total oil content and SO is the surface oil content.

3. Degree of oxidation of encapsulated fish oil powder (EFOP)

Degree of oxidation of (EFOP) was determined according to the method of Hu and Zhong (2010) described above. Along 90 days of storage at 4ºC, degree of oxidation was determined in EFOP every 15 days interval and expressed as nmol Malondialdehyde MDA /kg oil.

4. Microstructure of encapsulated fish oil powder (EFOP)

Scanning electron microscope JEOL JSM-5300-Japan was applied on selected two samples of encapsulated fish oil powder (EFOP), one sample from spray-dried and the other sample from freeze-dried

Table 1. Composition of the microencapsulation emulsion

Blends	WPC (80%) (kg)	Fish oil (kg)	Antioxidant α-Tochopherol (ppm)	Nisin (E234) (ppm)	Distilled Water (kg)	Total (kg)
1	1.0	0.6	100	25	8.4	10
2	1.0	0.8	100	25	8.2	10
3	1.0	1.0	100	25	8.0	10
4	1.0	1.2	100	25	7.8	10

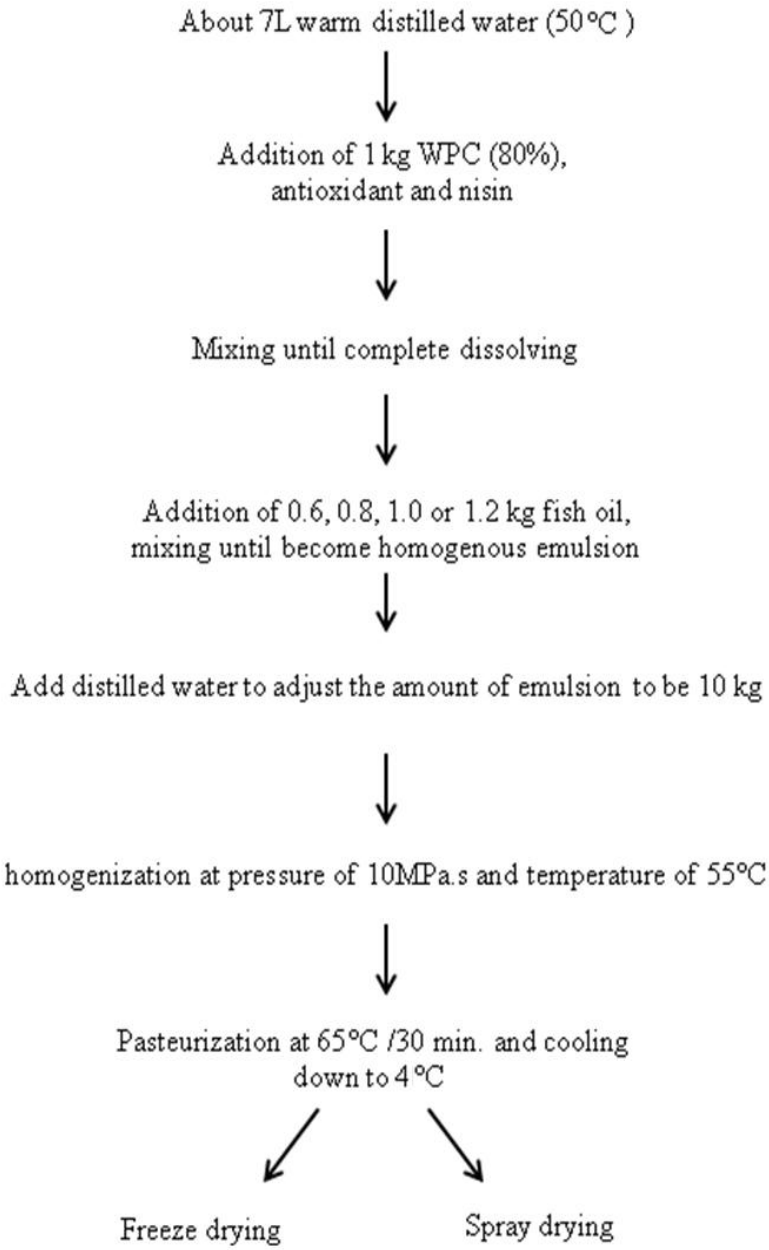


Figure 1. Flow diagram represents the steps of preparation of encapsulated fish oil

EFOP in order to examine its microstructure. The two selected samples contained 1:1 Fish oil:WPC which contains reasonable concentration of n-3 fatty acids and gave good results from the encapsulation efficiency test. All chemicals used in this work are analytical grade.

RESULTS AND DISCUSSION

1. Analysis of fish oil

Fish oil was analyzed for qualitative physical and chemical properties in order to assure its freshness and usability as food ingredient (table 2). The measured refractive index of fish oil (1.4740) is in the normal range obtained by Young (1986) for Herring oil (1.4730-1.4750 with average value of 1.4735) and Horse Mackerel oil (1.4741-1.4758 with average value of 1.4750). Acid value, peroxide value and TBA value are in the normal range of unoxidized fresh fish oil and consistent with those obtained by Bako et al (2014) and Jiménez-Martín et al.(2015). The peroxide value (PV) is a usual indicator for the determination of primary oxidation products (Chávez-Servín, et al., 2008). According to Gracey et al. (1999), oil with a PV below

5 milliequivalents of peroxide (meq)/kg oil can be considered fresh oil.

Data of gas chromatography analysis of fish oil has been shown in figure 2 and table 3. Medium chain fatty acids (C_{12}-C_{16}) are 3.5045%, saturated fatty acids are 15.1275%, monounsaturated fatty acids are 7.9318% and polyunsaturated fatty acids are 76.8775%. The ratio of omega-3: omega-6 fatty acids is 1.7:1 and the ratio of polyunsaturated fatty acids: saturated fatty acids is 5.08:1. These results are agreed with the data published in the Fish Oil Bulletin number 18 appendix 1-5 by Young (1986). Fatty acid composition of analyzed fish oil showed high percentage of polyunsaturated fatty acids that make fish oil very susceptible to oxidation. Oxidation in oils is of paramount importance because it results in loss of nutritional value and development of undesirable flavors.

Table 2. Physical and chemical properties of fish oil

Physical and chemical properties	Specification
	Pale yellow liquid
Refractive index	1.4740
Acid value (as oleic acid %)	0.28
Peroxide value*	0.66
TBA value**	10.0

* meq/kg oil, ** nmol malondialdehyde (MDA)/kg oil

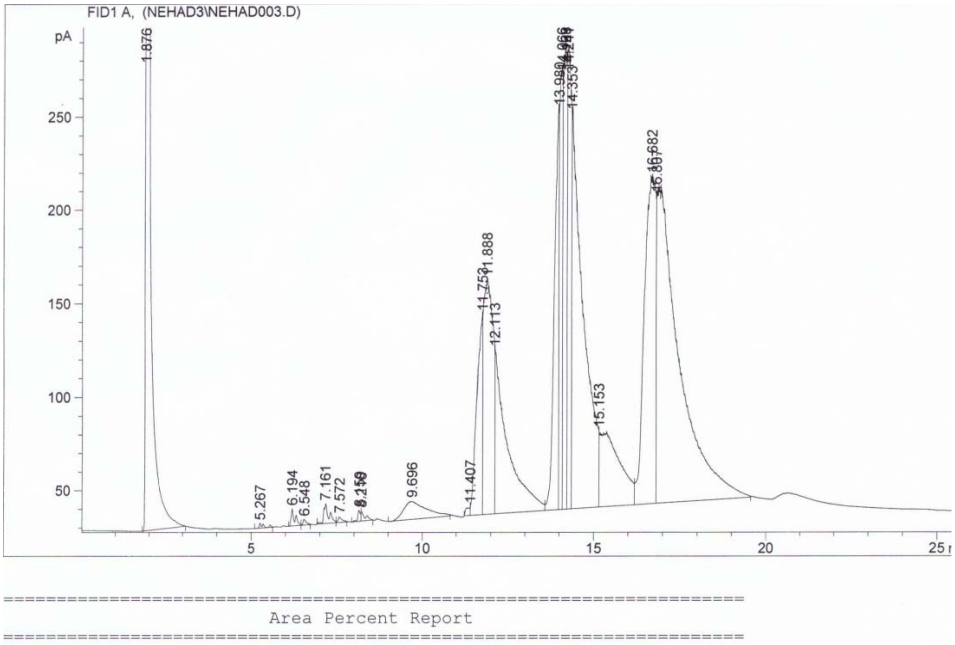


Fig 2. Fatty acids pattern of fish oil (as fatty acids methyl esters)

Table 3. Fatty acids composition of fish oil (as fatty acids methyl esters)

Fatty acids composition of fish oil as methyl esters	Content (%)
C12:0 lauric acid	0.0668
C13:0 Tridecylic acid	0.2085
NI*	0.0631
C14:1 Myristoleic acid	0.3340
C14:0 Myristic acid	0.0767
C15:1 cis-10-Pentadecenoic acid	0.1002
C15:0 pentadecanoic acid	1.2714
C16:0 Palmitic acid)	1.3838
C18:3 ω3 α-Linolenic acid (ALA)	*0.1074

C18:2 ω6 Linoleic acid (LA)	**3.6125
C18:1 ω9 Oleic acid	7.4977
C18:0 Stearic acid	6.9809
C20:5 ω3 Eicosapentaenoic acid (EPA)	*5.6012
C20:4 ω6 Arachidonic acid (AA)	**4.4976
C20:3 ω3 Eicosatrienoic acid (ETA)	*5.9063
C20:3 ω6 Dihomo-γ-linolenic acid	**5.7622
C20:2 cis-11,14-Eicosadienoic acid	15.4086
C20:0 Arachidic acid	5.1395
C22:6 ω3 Docosahexaenoic acid (DHA)	*11.9799
C22:2 cis-13,16-Docosadienoic acid	24.0020
Other	0.06314

*Omega-3 fatty acids, **Omega-6 fatty acids, NI = not identified

Therefore, α-tochopherol was added as antioxidant in the preparation of encapsulated fish oil. Fatty acid composition of fish oil is significantly differs according to type of fish, geographic area and time of year. Fatty acids pattern resulted from gas chromatography analysis, indicated that polyunsaturated fatty acids (PUFAs) are predominant with total omega-3 fatty acids content of 23.5948% and total omega-6 fatty acids content of 13.8723%. The ratio of omega-3 fatty acids is high enough to allow incorporation of fish oil as a supplement in some food with small quantities that does not allow the fishy flavor to be appeared in the food product.

2. Analysis of encapsulated fish oil powder (EFOP)

Microencapsulation is a process by which particles of sensitive or bioactive materials are packed into thin films of a coating material (Shahidi and Han, 1993). Benefits of applying microencapsulation in food industry are summarized as follows: 1) to reduce the core reactivity with environmental factors; 2) to decrease the transfer rate of the core material to the outside environment; 3) to promote easier handling; 4) to control the release of the core material; 5) to mask the core taste; and finally 6) to dilute the core material when it should be used in only very small amounts (Gharsallaoui et al., 2007).

2.1. Moisture content of EFOP

In the present study, fish oil was encapsulated with using whey protein concentrate (80% protein) as wall material (coating or capsulating material) by making emulsion of oil and WPC in water, homogenized and spray dried or freeze dried. The resultant powder was analyzed for moisture content, encapsulation efficiency, degree of oxidation over 90 days of storage at 4ºC and microstructure by using scanning electron microscope.

Data in table (4) revealed that there are slight differences in moisture content among oil:WPC ratios in both spray and freeze dried powders. Spray dried powders have lower moisture content than freeze dried powders. Moisture content in both spray and freeze dried powders tended to decrease with the increasing of oil ratio in the powder. Values obtained for moisture content of powder are in agreement with the results obtained in most dried powders used for food purposes (Gallardo et al. 2013). It is logic that the moisture content is associated with the relative WPC content, therefore as the fish oil ratio increased, the relative WPC content decreased and moisture content is also decrease. Gharsallaoui et al. (2012) stated that, in spray drying, the final moisture in the microcapsules has been related to the composition of the feed emulsions that subsequently constitutes the matrix material of the microcapsules. Partanen et al., (2008) and Fuchs et al., (2006) found that spray drying results in powders with good quality and low water activity. In addition, Sliwinski et al., (2003) studied the effect of spray-drying on the physico-chemical properties of oil-in-water emulsions stabilized by milk proteins. It was shown that spray-drying resulted in a denaturation and aggregation of β-lactoglobulin. It can be concluded that spray draying technique is more suitable than freeze drying technique in preparation of encapsulated fish oil powder.

2.2. Encapsulation efficiency of EFOP

Encapsulation efficiency of oil powder is a measure for the completion of coating material in covering the oil droplets. As the coating material covered more oil droplets as the encapsulation efficiency is higher. Free oil (surface oil) must be minimal because it will rapidly deteriorated due to exposure to oxygen, light and the other environmental factors.

Kaushik et al., (2014) stated that a successful microencapsulation system is evaluated on the basis of encapsulation efficiency and storage stability of omega-3 oil microcapsules.

Table (5) showed the encapsulation efficiency of

fish oil powder (EFOP). The highest efficiency (70.2 % for spray and 63.18% for freeze dried powder) was obtained when the fish oil to WPC ratio was the minimum (0.6:1), while the efficiency was gradually decreased with the increasing of fish oil ratio up to (1.2:1). Worth noting that the decrease in the efficiency between the ratio of 0.6:1and 1:1 was only 3%, while the efficiency was dropped by about 10% from the ratio of 1:1 to the ratio of 1.2:1 in spray dried and about 7% in freeze dried powders. This result is consistent with the findings of Hogan et al., (2001) who stated that oil/protein ratio

should not exceed 1 or the formed emulsion could be destabilized during spray drying. He studied the microencapsulation of soy oil in sodium caseinate as wall material. Our results are also showed that spray drying technique led to higher encapsulation efficiency than freeze drying.

Partanen et al., (2008) and Fuchs et al., (2006) stated that spray drying is widely used for microencapsulation of oils and flavors results in powder with good quality, easier handling and storage and also protects the active material against undesirable reactions. Furthermore, (Gharsallaoui et al., 2007) reported that spray drying is the most common and cheapest technique to produce microencapsulated food materials. Equipment is readily available and production costs are lower than most other methods. Compared to freeze-drying, the cost of spray-drying method is 30–50 times cheaper. Spray drying has been considered as a solution for conventional drying problems because the process has usually proved not only efficient but also economic. From the observed data, it can be concluded that spray drying technique led to more encapsulation efficiency than freeze drying technique for the encapsulated fish oil powder.

2.3. Degree of oxidation of encapsulated fish oil powder (EFOP)

Results for TBAR values of fish oil as a control and encapsulated fish oil powder (EFOP) along 90 days storage at 4°C are shown in Table 6.

Degree of oxidation (TBAR values) was gradually increased in both fish oil (control) and EFOP with the advance of storage time. TBAR values of fish oil were dramatically increased throughout storage time (10 at zero time, and it become 325 nmolMAD/kg oil at day 90).

Table 4. Moisture content of encapsulated fish oil powder EFOP

Item	Moisture%	
	Spray dried	Freeze dried
Oil:WPC		
0.6:1	3.33	4.38
0.8:1	3.32	4.33
1:1	3.23	4.26
1.2:1	3.20	4.15

Table 5. Encapsulation efficiency of fish oil powder (EFOP)

Item	Encapsulation efficiency%	
	Spray dried	Freeze dried
Oil:WPC		
0.6:1	70.20	63.18
0.8:1	66.33	61.32
1:1	67.21	60.25
1.2:1	57.59	53.21

Table 6. Degree of oxidation of fish oil and encapsulated fish oil powder (EFOP) along storage period of 90 days at 4°C

Degree of oxidation of fish oil (control) along storage period of 90 days at 4°C (nmol MDA /kg oil)							
(days)	Fresh	15	30	45	60	75	90
Oxidation	10	38	77	122	178	242	325

Degree of oxidation of (EFOP) along storage period of 90 days at 4°C (nmol MDA /kg oil)			
Oil: WPC	Storage period (days)	Encapsulated fish oil powder (EFOP)	
		Spray dried	Freeze dried

		Oxidation (nmol MDA /kg oil)	
0.6:1	Fresh	18	20
	15	21	22
	30	21	22
	45	26	30
	60	30	35
	75	34	38
	90	40	40
0.8:1	Fresh	18	18
	15	20	21
	30	22	22
	45	28	30
	60	32	35
	75	37	39
	90	41	43
1:1	Fresh	20	22
	15	23	26
	30	27	29
	45	31	33
	60	38	40
	75	40	44
	90	45	48
1.2:1	Fresh	24	29
	15	26	32
	30	32	39
	45	40	42
	60	42	47
	75	45	50
	90	50	53

On the other hand the increment of TBAR values in EFOP was not so remarkable (18 as minimum at zero time, and it become 53 as maximum nmol MDA/kg oil at day 90). It is clear that encapsulation of fish oil strongly delayed the oxidation development in the powder beside the influence of antioxidant (α-tochopherol) which was added to the emulsion before spray or freeze drying and producing the EFOP. The third factor which considerably impaired oxidation is the low storage temperature (4°C).

McClements and Decker, (2000) stated that PUFAs in fish oil are readily oxidized to produce off flavor volatiles when exposed to light, oxygen, pro-oxidants, and high temperatures.

Refering to the influence of storage temperature, our results are consistent with the findings of Klinkesorn et al. (2005) who found that the remarkable differences in lipid oxidation between fish oil, emulsions, and microcapsules were found at 60°C of storage temperature whereas the differences at 4 and 30°C were not significant. They also found that at 60°C, lower TBAR values were observed in the microcapsules in comparison to the fish oil and the emulsion indicating the protective effect of microencapsulation against ω-3 fatty acid oxidation. They concluded that temperature is one of the most important factors determining the stability of dried powders.

The ratio of oil:WPC had a slight effect on oxidative stability. As the oil ratio increased, the degree of oxidation increased. It seems that it is direct quantitative relationship between the amount of oil in the environment and the amount of TBAR materials produced. On the other hand, the drop in the encapsulation efficiency in the oil: WPC ratio of 1.2:1 comparing with the other ratios has no effect on the oxidation stability. Klinkesorn et al. (2005) reported that it seems that, in agreement with other authors, lipid oxidation in spray dried powders was not influenced by the amount of free fat (surface fat).

Our data also showed that oxidative stability of produced powder either by spray or freeze drying followed the same trend, but Freeze dried powder always had oxidation values slightly higher than spray dried one. Encapsulating material which has been used in our study is concentrated whey proteins. It is well known that whey proteins have the advantage of

antioxidative effect due to its high content of reducing agent 'glutathion'. This coating material may shared in the protective effect on the encapsulated fish oil powder against oxidation.

Kaushik et al., (2014) reported that microencapsulation of omega-3 oils minimizes oxidative deterioration and allows their use in stable and easy-to-handle form. The key parameter in this process is the selection of coating material. For spray dried emulsions and complex coacervates protein or polysaccharides are primarily used as coating material.

Depending on the obtained results it can be suggested that encapsulated fish oil powder may be successfully produced with extended shelf life up to 90 days if kept at 4°C. In addition, these results must be taken into account in transportation of fish oil or fish oil powder and direct addition of encapsulated ω-3 fatty acids to enriched food products.

Microstructure of encapsulated fish oil powder (EFOP) by scanning electron microscope

Figure 3 shows the scanning electron microscope (SEM) images of the microcapsules. Spray drying resulted in microcapsules appeared as spherical particles of different sizes, which is expected in the powders produced by this method (Carneiro et al. 2013), and they were constituted by skin-forming layer with no apparent pores. Skin-forming layer composed of a continuous non-liquid phase which is primarily denatured whey protein particles. The absence of pores in the skin of the microcapsules has been highlighted as an advantage, since it ensures better protection and retention of the encapsulated fish oil, as the presence of pores can lead to an increase in the permeability of the wall material, decreasing the protective effect of the core (Carneiro et al. 2013).

Some visual differences between microcapsules can be observed. Spherical defined particles of smoother surface, is a sign of better stability of the microcapsules. Some common microstructural features are present, such as particle collapse or shriveling. Other common morphological features for these capsules were visible wrinkles or dimples on the surface that have been attributed to rapid evaporation of the drops during atomization in the drying process (Rosenberg et al. 1985).

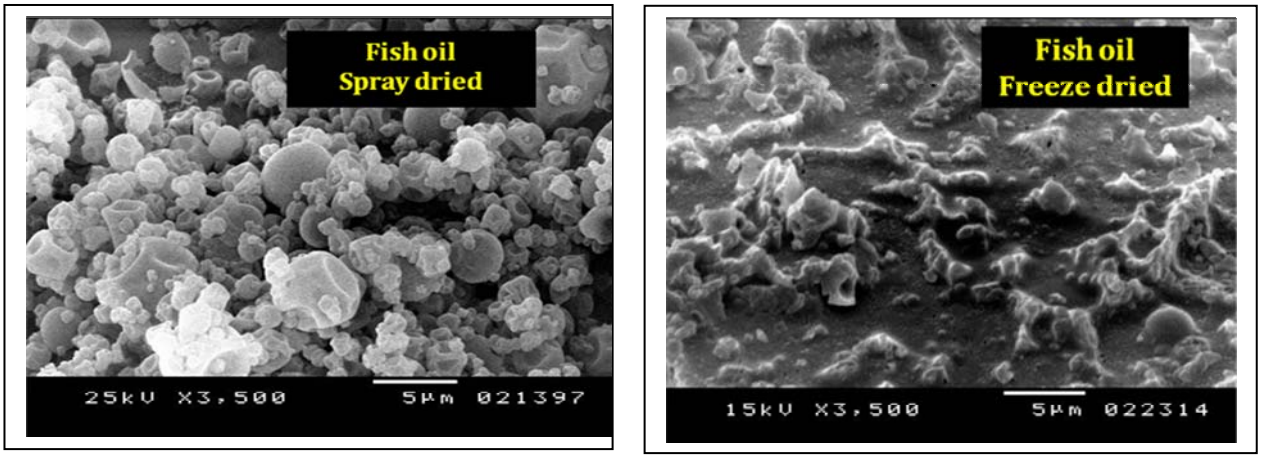
On the other hand, freeze dried powder showed a completely different morphology of particles. Relatively low magnification (x1500) showed the powder in plates-like layers without pores on the surface, while magnification of (x3500) was not clear enough to describe the image content. At the same magnification of (x3500), spray dried powder showed clear individual spherical particles with different sizes. Images of spray dried powder with magnifications of (x10000 and x15000) were very sharp and distinctive. The particles were mostly about 2um diameter with smooth surface without pores but with visible wrinkles. Magnification more than (x3500) for freeze dried powder was not successful to show any usefull details.

Anandharamakrishnan and Karthik (2013) studied the powder morphology of spray dried and freeze dried microencapsulated DHA by scanning electron microscope (SEM). He found spherical shape with smooth surface for spray-dried microencapsulated powder as compared with nonspherical freezedried microencapsulated particles. The freeze dried powder exhibits the cakelike structure with uneven surfaces (figure 4)

Earlier reports also showed the spherical shape of microencapsulated spray dried powder (Lee and Rosenberg 2000; Anandharamakrishnan et al. 2007; Dolly et al. 2011). Moreover, whey and skimmed milk products exhibits smooth skin forming behaviour (Sheu and Rosenberg 1998; Anandharamakrishnan et al. 2007).

Through morphology analysis, it is clear that spray dried technique gave better characteristics for the dried powder which leeds to extended shelf life with good tolerance against oxidation

Figure 3. Microstructure of encapsulated fish oil powder (EFOP)



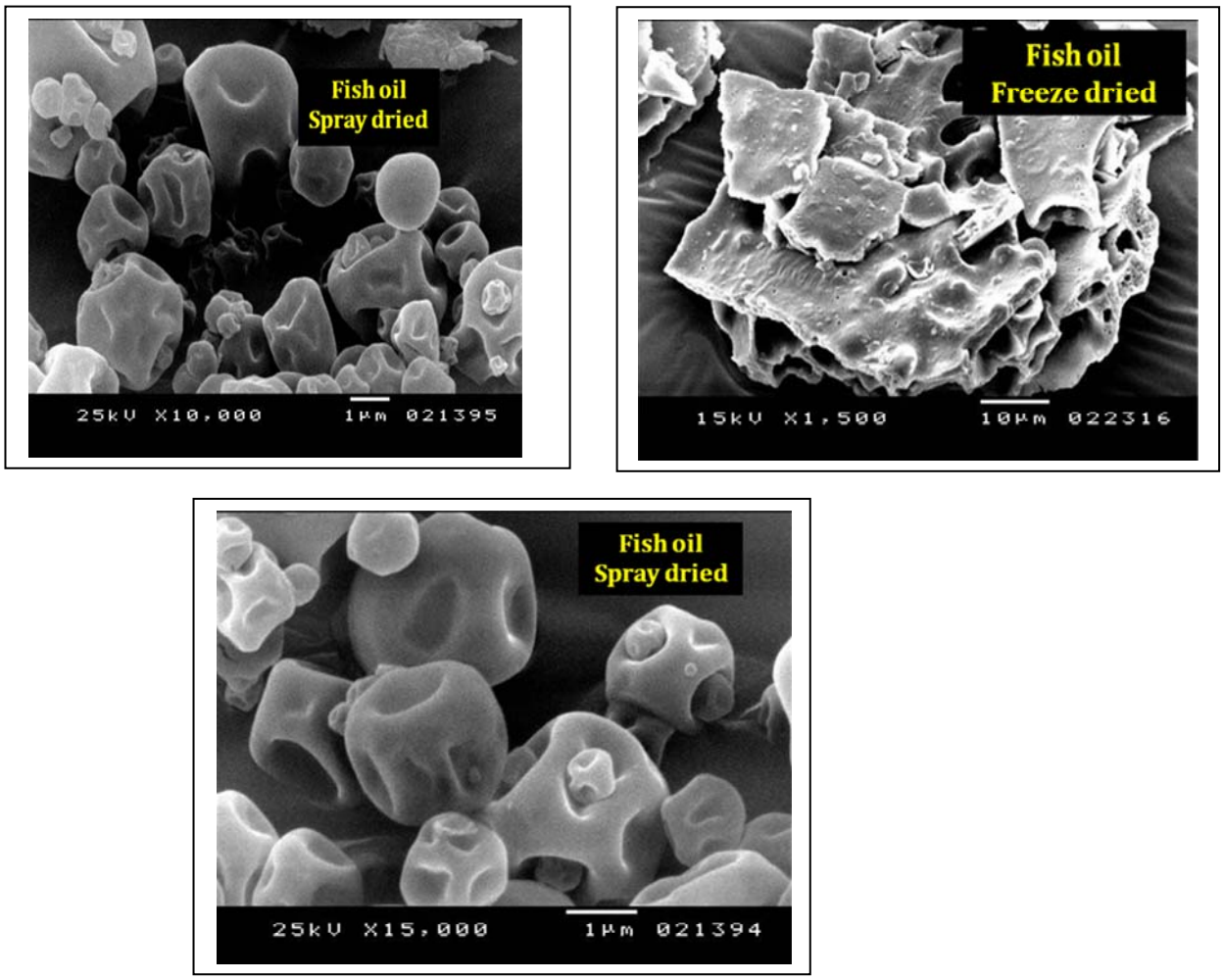


Figure 3. Images of encapsulated fish oil powder (EFOP) taken by scanning electron microscope

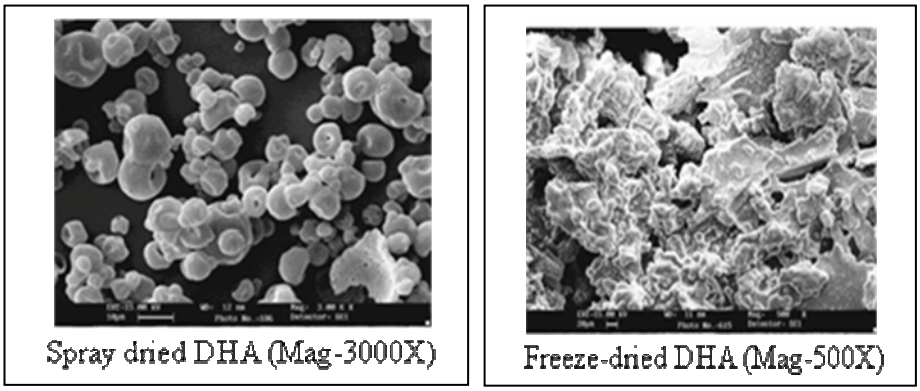


Figure 4. Spray and freeze dried of Docosahexaenoic Acid

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الملخص العربي

القدرة على الكبسلة والتركيب الدقيق والثبات ضد الأكسدة لمسحوق زيت السمك المحضر بواسطة الكبسلة باستخدام مركز بروتينات الشرش

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إستهدفت الدراسة إنتاج بودرة لزيت السمك المحمى بطبقة من بروتينات الشرش لاستخدامها كمكمل غذائى غنى بالأوميجا-٣ يضاف لمنتجات الألبان وذلك بتطبيق تكنولوجيا الكبسلة. تم تحضير بودرة زيت السمك بأربعة تركيزات من زيت السمك إلى تركيز ثابت من مركز بروتينات الشرش (١:٠,٦، ١:٠,٨، ١:١، ١:١,٢) كما أضيف ألفا-توكوفيرول كمضاد للأكسدة بتركيز ١٠٠ جزء/المليون فى المادة الجافة، وكذلك أضيف النيسين كمضاد للميكروبات بنسبة ٢٥ جزء/المليون فى المادة الجافة. تم خلط المستحلب وبسترته وتجنيسه، ثم تجفيفه بالرذاذ أو بالتجفيد وتم الحصول على البودرة بالطرقتين. تم تحليل البودرة الناتجة بتقدير نسبة الرطوبة وكفاءة الكبسلة والتركيب الدقيق لحبيبات البودرة باستخدام الميكروسكوب الإلكترونى كما تم تحليلها لتقدير الأكسدة عند التحضير وكل ١٥ يوما من التخزين على حرارة ٤ °م ولمدة ٩٠ يوم.

أظهرت النتائج إحتواء البودرة الناتجة من التجفيف بالرذاذ على نسبة رطوبة أقل من تلك المحضرة بالتجفيد. كذلك لوحظ أنخفاض نسب الرطوبة فى نوعى البودرة كلما ارتفعت نسبة زيت السمك فى البودرة.

أظهرت نتائج كفاءة الكبسلة أن أعلا القيم وهى ٧٠,٢% للبودرة المحضرة بالرذاذ، ٦٣,١٨% لتلك المحضرة بالتجفيد وكانت لأقل نسبة لزيت السمك(١:٠,٦) وتناقصت كفاءة الكبسلة مع ارتفاع نسبة الزيت حتى نسبة (١:١,٢). إنخفضت كفاءة الكبسلة بشدة بزيادة نسبة الزيت من (١:١) إلى (١:١,٢) حيث وصلت نسبة الإنخفاض إلى ١٣%، ١٠% لكل من البودرة المحضرة بالرذاذ والمحضرة بالتجفيد على التوالى. البودرة المحضرة بالرذاذ أظهرت كفاءة أعلا على الكبسلة مقارنة بنظيرتها المحضرة بالتجفيد. من النتائج يمكن القول بأن نسبة(١:١) هى أفضل النسب بإعتبارها تحتوى على كمية مقبولة من الأوميجا-٣ فى البودرة بالإضافة لكفاءة كبسلة مقبولة. العديد من

الباحثين والمؤسسات المعنية حددت الحتياجات اليومية من أحماض الأوميجا–٣ بـ ٥٠٠ ملليجرام/ يوم، هذه الكمية توجد فى ٤,٣٨ جم بودرة طبقا للتحليل الكيماوى لزيت السمك والبودرة المحضرة بطريقة الرذاذ ذات النسبة (١:١).

تم تقدير الأكسدة فى الزيت(كنترول) وفى بودرة الزيت على مدى ٩٠ يوم من التخزين على درجة ٤°م. إرتفعت درجة التأكسد تدريجيا مع التقدم فى زمن التخزين فى كل من الزيت والبودرة. إزدادت درجة التأكسد بشدة فى الزيت حيث كانت ١٠ فى بداية التخزين ووصلت فى نهاية التخزين إلى ٣٢٥ nmol MAD/kg oil. من جهة أخرى وجد أن الزيادة فى التأكسد فى البودرة لم يكن مؤثراً حيث كان ١٨ عند بداية التخزين ووصل إلى ٥٣ nmol MAD/kg oil فى نهاية مدة التخزين. إتضح من النتائج أن عملية الكبسلة قد أعاقت بشدة حدوث التأكسد فى البودرة. هذا إلى جانب تأثير مضاد الأكسدة(ألفا-توكوفيرول) الذى أضيف أثناء تحضير البودرة. كما أن هناك عامل ثالث أعاق عملية التأكسد وهو حرارة التخزين المنخفضة (٤°م).

أظهرت صور الميكروسكوب الإلكترونى عند درجة تكبير(x3500) أن البودرة الناتجة بطريقة الرذاذ عبارة عن حبيبات كروية الشكل ذات سطح ناعم خالى من الثقوب مع ظهور منخفضات كبيرة على السطح. من جهة أخرى ظهرت البودرة الناتجة بالتجفيد بشكل مختلف تماماً حيث عند درجة تكبير(x1500) ظهرت كما لوكانت رقائق وبدون ثقوب على السطح بينما لم تظهر تفاصيل واضحة عند تكبير (x3500).

عند تكبير (x10000, x15000) ظهرت حبيبات البودرة الناتجة بالرذاذ أكثر وضوحاً كروية الشكل ذات سطح أملس وأحجام متفاوتة ولكن معظمها بقطر حوالى ٢ ميكرون خالية تماماً من الثقوب ووجود المنخفضات بوضوح.