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Utilization of Different Wall Materials to Microencapsulate Fish Oil Evaluation of its Behavior in Bread Products

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Abstract: The aim of this study was to assess the possibility of adding fish oil microcapsules to bread products in order to evaluate four different types of wall material: methyl cellulose (M), soybean protein isolates (S), calcium-gelatin casein (CG) and whey protein concentrate (W). The M, W and CG microcapsules were made by the spray drying method, whereas S microcapsules were made by polymerization with transglutaminase. Both, the microencapsulation efficiency and the microcapsule morphology were determined. The microcapsules were added to bread and this was evaluated by a triangle sensory test and a rehological test. In conclusion, it is possible to add fish oil to bread products with no significant modification of their sensory characteristics using the M and S treatments, being the spray drying method the one with greater potential due to more efficiently encapsulated fish oil and better performance.

Key words: Microencapsulation, fish oil, omega-3 fatty acid, bread, spray dried, soybean protein, transglutaminase

INTRODUCTION

New lifestyles have caused changes in man's eating habits which have led many people to ignore an appropriately balanced diet. Due to the fact that bread is a highly consumed product at low cost, it is of great importance to improve its nutritional quality.

Functional foods help to compensate imbalanced diets and make up for food disorders as they exert a preventive role reducing the risk of diseases (Shi *et al*., 2002).

Among the functional foods that provide major health benefits, we can find fish oil rich in omega-3 fatty acids, which reduce cardiovascular disease risk, autoimmune disorders, diabetes, arrhythmia and certain inflammatory diseases, among others (Nettleton, 1995). Most of the benefits can be attributed to its high concentration of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), vital in the structure formation and the functionality of the nervous system (Fomuso *et al*., 2002).

Due to the fact that fish oil is easily damaged by oxygen and that it has a strong taste and smell, it represents a great challenge for scientists. Microencapsulation provides an individual package for sensitive ingredients; it is an oxygen, light and temperature barrier which retards the damage and prevents unpleasant taste (Kagami *et al*., 2003).

A critical point in the microencapsulation is the adequate selection of the wall material depending on the active ingredient, the system in which it is going to be applied and the release mechanism (Pedroza-Islas *et al*., 1999).

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Several wall materials and encapsulation methods have been tested for fish oil microencapsulation. Díaz-Rojas *et al.* (2004) used pectin, sodium alginate and chitosan as wall material, other wall materials studied were modified cellulose (Kolanowski *et al.*, 2004), gelatin and sodium caseinate (Lin *et al.*, 1995; Hogan *et al.*, 2003) and soybean protein isolate (Cho *et al.*, 2003). Concentrated and isolated whey proteins have also been used for microencapsulation (Sheu and Rosenberg, 1995; Lee and Rosenberg, 2000) and they showed an antioxidant activity when used in salmon oil emulsions (Tong *et al.*, 2000; Hu *et al.*, 2003).

There are also some studies about the addition of fish oil to different food products. Kolanowski and Weiâbrodt (2007) added fish oil to dairy products. Fish oil microcapsules have also been added to instant food products with acceptable results at limited levels of addition. Regarding the addition of fish oil to bread, Yep *et al.* (2002) enriched bread with tuna fish oil microcapsules and studied the nutritional influence of its consumption. There is also a study in which Serna-Saldivar *et al.* (2006) added bulk fish oil to bread and concluded that flavour and overall acceptability were lost during the last stages of storage.

Based on the studies mentioned above the idea of creating an omega-3 fatty acids enriched bread that is similar to regular bread could be an attractive idea for a highly consumed functional product. One possibility to create this bread could be employing fish oil microcapsules.

The aim of this research was to study the oxidative stability and the encapsulation efficiency of fish oil in different types of encapsulation matrixes and to evaluate the sensory and technological quality of bread products added with fish oil microcapsules.

MATERIALS AND METHODS

Materials for Microencapsulation

The following materials with their sources in parentheses were used: Methyl cellulose (Methocel A15 Premium LV, Colorcon, Mexico), maltodextrine 10 DE (Maltadex, Mexico), soy lecithin (food grade, Droguería Cosmopolita, Mexico), cod liver oil (Farmacia Paris, Mexico), whey protein concentrate (WPC-80, Ingredientes Funcionales de México, S.A.), calcium caseinate (Droguería Cosmopolita, Mexico), gelatin 200 bloom USP (Coloidales Duche, Mexico), sodium carboxymethyl cellulose (Avicel RC-591, FMC, Mexico), transglutaminase in powder MTGase (Nutrer, México), soybean protein isolate (Droguería Cosmopolita, Mexico), Span 80 (Droguería Cosmopolita, México), corn oil (100% puro Mazola, Alimentos Capullo, Mexico), ethanol (ethyl alcohol, Fermont, Mexico). All the experiments were conducted in laboratories of Chemical Engineering Sciences Department (Mexico Iberoamerican University).

Materials for Bread Manufacture

The following materials were used: wheat flour (La Selecta, Mexico), yeast (Instant dry yeast, Magidely, Mexico), refined salt (La fina, Mexico), sugar (refined sugar, Great Value, Mexico), whole pasteurized milk (Lala, Grupo Lala, Mexico) and unsalted butter (Unifoods, Mexico).

Experimental Design

Fish oil was encapsulated following the experimental design shown in Table 1 where wall materials and encapsulation methods used are described.

Formation of the Spray Dried Microcapsules

Following the method of Kolanowski *et al.* (2004), the wall materials were dissolved in water with a Silverson L4R mixer (Silverson Machines Ltd., United Kingdom) at 4000 rpm and rested for a night at 5°C. For the CG treatment (calcium-gelatin casein) the solution was made at 60°C.

Table 1: Experimental design

	Treatments			
Constituents	M*	CG*	W*	S**
Methyl cellulose (g)	40	-	-	-
Gelatin (g)	-	20	-	-
Calcium caseinate (g)	-	20	-	-
Whey protein (g)	-	-	54	-
Soybean protein isolate (g)	-	-	-	50
Transgultaminase (mg)	-	-	-	125
Maltodextrine (g)	20	20	6	-
Soy lecithin (g)	6.5	5.5	6.5	-
Carboximethylcellulose (g)	-	1	-	-
Deionized water (mL)	1000	1000	1000	500
Corn oil (g)	-	-	-	2000

In all the treatments the proportion of coating material: fish oil was 2:1, *Spray drying encapsulation, **Encapsulation by enzyme cross-linking,

On the other hand, the lecithin was dissolved in the fish oil and this solution was added in drops to the wall solution, mixing constantly. This emulsion was homogenized in a Silverson L4R mixer at a rate of 10,000 rpm for 5 min. It was left to settle to reduce the foam formed. For the CG treatment, the temperature was above 35°C.

The emulsions were dried in a Niro Atomizer (Niro Copenhagen-Denmark) at 20.0 mL min^{-1} flow, 0.4 bar pressure, inlet air temperature was about 160±5°C and outlet air temperature of about 78±5°C. The microencapsulated products were recollected in a glass flask and were stored at -5°C.

Formation of Microcapsules by Enzyme Cross-Linking

Following the method of Cho *et al.* (2003), the soybean protein isolate was dissolved in distilled water and the transglutaminase was added. The primary emulsion oil/water was prepared by mixing the fish oil for 10 min at 9500 rpm in a Silverson L4R homogenizer. The corn oil was preheated at 50°C to make it easier for the emulsifier (Span 80) to dissolve. The primary emulsion was slowly added to the corn oil and to the emulsifier mixture and it was maintained at 37°C for 4 h. The microcapsules were separated from the oil using a N^0 4 filter paper; they were washed with pure ethanol three times and were vacuum dried at 0.04 bar and at room temperature for 24 h.

Oil Encapsulation Efficiency in Microcapsules

Surface Oil

To quantify the surface oil, the microcapsules were drip washed with ethyl ether for 15 min at room temperature.

Total Oil

Acid hydrolysis was made in treatments that have protein in their coating to obtain the total extraction of oil using the Soxtec System 1047 Hydrolyzing Unit (Foss Tecator, Sweden). Next, the total oil was determined by Soxhlet extraction method using a Soxtec 2055 (Foss Tecator, Sweden). The encapsulation efficiency (EE) was determined by Eq. 1

$$EE = ((\text{Total oil} - \text{Surface oil})/\text{Total oil}) \times 100 \quad (1)$$

Oxidation Evolution of Oil Microcapsules

The oxidation evolution was measured by determination of peroxide values (AOAC Official Method 965.33, 1999). The samples were stored in a controlled environment in glass flasks with constant exposure to light at 60°C for 24 days. A sample of oil without encapsulation was used as the control system (C).

The delta of color change for the microcapsules was measured during 24 days with a colorimeter Accuprobe hh06 (Accuracy Microsensors, Inc., USA) and using Eq. 2.

$$\Delta \text{ color change} = \sqrt{(L_1-L_2)^2 + (a_1-a_2)^2 + (b_1-b_2)^2} \quad (2)$$

where, L, a and b values (Wyszecki and Stiles, 1982) denote lightness (white-black), red-green and yellow-blue, respectively (first color parameter of each axis is positive direction, second is negative).

Morphology and Microstructure of the Microcapsules

For this study microcapsules were placed on SEM specimen holders using a two-sided adhesive tape and coated with a thin layer of gold. Micrographs were taken using a scanning electron microscope JEOL JSM-5600 LV Scanning Microscope (Akishima, Japan) of low vacuum and using a retrodispersed electrons technique with a 20 kV at 10 Pa voltage acceleration. Micrographs were made at 1000x and 1400x.

Evaluation of Dough Extensibility

For uniaxial extension 20 g of dough was used. Samples were formulated with 1% of microcapsules and a control (C) without microcapsules. The quantity of water added was 1:0.65 solid:water. The ingredients were mixed in a kneader (Oster 4450-08A, Oster Inc., China) for 2.5 min at velocity 1. Kieffer dough extensibility tests were made with a texturometer TAXT Plus software Texture Exponen 32 version 2.0 (Exponen Stable Micro System, USA). The parameters measured were maximum resistance to extension (R_m), maximum extensibility (E) and area under the curve (A).

Bread Manufacture

The bread was made with the following formulation: white flour 1000 g, salt 20 g, sugar 80 g, unsalted butter 100 g, yeast 12 g, milk 550 mL. Microcapsules were added to make up for 600 mg of fish oil, for every 100 g of dough that equals two pieces of bread. The amount of microcapsules added was based on the daily requirement of EPA and DHA (Artemis *et al*., 1999). Control bread (C) was made without microcapsules.

Milk, salt and sugar were mixed in a kneader (Kitchenaid K55S Kitchenaid Inc, USA) for 3 min at velocity 6. The flour, yeast and butter were added together, adjusting the velocity of the kneader to 1, kneading for 3 more min. Then, the velocity was adjusted to 8 and kneaded for 15 min. The dough formed was rested for 10 min. Then, 50 g pieces were placed for 1 h in a fermentation chamber (Italform, Sime Alpha, Mexico) with humidity of 100% at 37°C. The bread was baked in an oven (Ruvamex, 260 Type A6 Serie B, Mexico) for 30 min at 180°C.

Evaluation of Bread Texture

Bread was cut in cubes of 4 cm^2 the first day of manufacture. The TPA (Texture Perfil Analyses) test was made with a texturometer TAXT Plus software Texture Exponen 32 version 2.0 (Exponen Stable Micro System, USA) using a cylinder of 75 mm with relaxation period of 5 sec, the deformation obtained was of 40%. The parameters of texture measured were hardness, elasticity, cohesiveness and chewiness.

Sensory Evaluation of Bread with Fish Oil Microcapsules

A total of twelve triangular tests performed by trained judges were used to compare the bread with and without microcapsules (Stone and Sidel, 1993).

Statistical Analysis

To evaluate the differences between microcapsules, a one way analysis of variance was used with a significance of $p<0.05$ with the Minitab 14 program for Windows (Minitab Inc., USA). The measurements were made at least in duplicate.

For the results of sensory evaluation, a table with the least number of correct answers was used to establish a significant difference to different levels of probability for triangular tests (Stone and Sidel, 1993).

RESULTS AND DISCUSSION

Encapsulation Efficiency

The EE results of the treatments evaluated are shown in Table 2. There was significant differences ($p<0.05$) between the spray dried microcapsules and the ones made by enzyme cross-linking which showed the smallest value. This could be due to losses in the double emulsion formation during the cross-linking of the wall or during the washing (Cho *et al*., 2003).

Buma (1971) showed that free fat was strongly related ($r = 0.94$) to particle porosity, this could explain the decrease of EE in spray dried microcapsules.

The surface oil values were greater than 5% (data not shown), which could be diminished if the proportion of the encapsulating agent was increased (Keogh and O'Kennedy, 1999). Diaz-Rojas *et al*. (2004) used a similar spray dry conditions and almost the same load of fish oil (30%) comparing our work it can be concluded that our surface oil amount is higher. This difference may be due to the different conditions of the emulsion formation, considering that the oil droplet size in the emulsions influences directly the amount of surface oil (Soottitantawat *et al*., 2003) on the other hand different wall materials were used in both works.

Morphology and Microstructure of the Microcapsules

In general the microcapsules had spherical form, even though this was not very clear for the ones obtained in the S treatment. Figure 1A-C show the presence of some defects, specifically the pores formed, which could explain the relatively high values of surface oil found in the microcapsules CG, W and M, though M microcapsules presented the smaller pore number. The microcapsules obtained in the S treatment (Fig. 1D) showed a less smooth and not well defined structure with respect to those spray dried. Probably the roughness presented comes from the traces left from the drops of wall that originally were present in the surface and were lost during the encapsulation process (Cho *et al*., 2003).

In the photographs obtained by SEM (Fig. 1). It was observed that the microcapsules made by spray drying showed a great variety of sizes, which indicates that during this process a very different family of drops were formed. The largest sizes obtained for the microcapsules of CG, W and M were approximately 59, 61 and 46 μm, respectively, while the smallest sizes were of 1μm in all the cases. The S microcapsules were presented in great aggregates which did not allow establishing the precise size of the microcapsules, possibly due to a drying deficiency after washing, which caused a poor separation of particles.

Table 2: Encapsulation efficiency of several wall materials (EE)

Treatments	EE (%)
CG	82.2±3.7^{b}
M	84.1±4.3^{b}
S	65.0±5.0^{a}
W	87.2±6.2^{b}

Values are presented as mean±SD, ^{ab}Values with different superscript within a column are significantly different ($p<0.05$). EE (%), Percent of encapsulation efficiency; CG: Calcium-gelatin Casein; M: Methyl cellulose; S: Soybean protein isolate; W: Whey protein concentrate

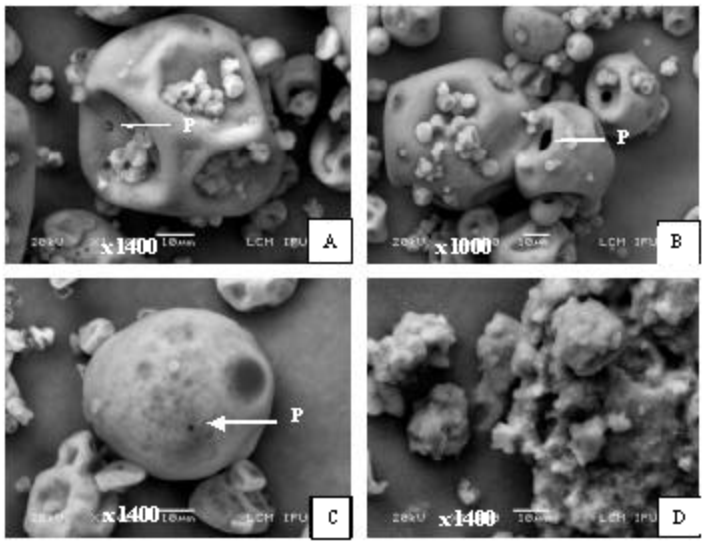

Fig. 1: Scanning electron microscopy (SEM) of the four types of microcapsules. References: A: Calcium-gelatin casein; B: Whey protein concentrate; C: Methyl cellulose and D: Soybean protein isolate, P: Pores

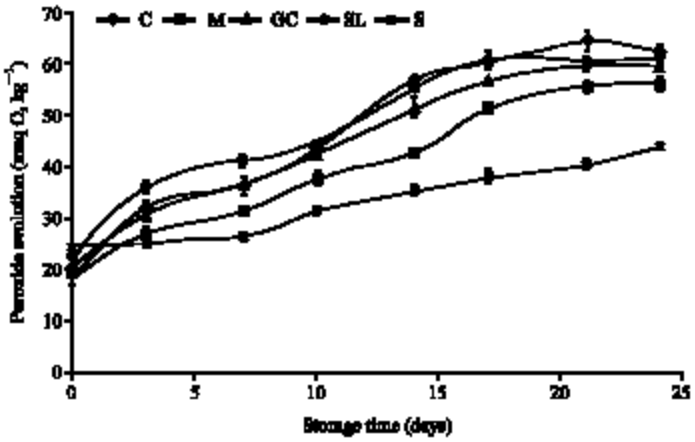

Fig. 2: Evolution of the oxidation of the four treatments and control sample stored at 60°C with a relative humidity of 45% and a permanent exposure to light. References: C: Control sample (unencapsulated fish oil); M: Methyl cellulose; CG : Calcium-gelatin casein; W: Whey protein concentrate; S: Soybean protein isolate. The tests were conducted during 24 days and the dates made in duplicated

Oxidative Stability

Figure 2 shows the oxidation evolution during the storage of fish oil with and without microencapsulation (M, CG, W, S and the control C respectively) for 24 days, the values of oxidation showed a constant increment throughout the time.

Table 3: Average of delta values for the change of color during the 24 days of storage

Treatments	Delta for the change of color
CG	4.02±2.09
M	3.69±2.10
S	2.21±1.12
W	3.12±1.76

Values are presented as mean±SD, CG: Calcium-gelatin casein, M: Methyl cellulose; S: Soybean protein isolate; W: Whey protein concentrate

Table 4: Effect of the microcapsules addition on dough extensibility parameters

Treatments	R_m (g)	E (mm)	A (gs)
C	16.21±1.64^{c}	71.84±2.80^{b}	195.89±15.77^{c}
M	12.45±1.01^{b}	77.80±4.15^{b}	189.93±15.08^{c}
CG	11.98±0.92^{b}	61.74±8.18^{a}	140.55±14.64^{b}
S	12.79±1.80^{b}	55.63±3.32^{a}	117.92±6.03^{a}
W	8.48±0.58^{a}	77.64±9.05^{b}	133.29±15.08^{a,b}

Values are presented as mean±SD, $^{a-c}$Values with different superscript within a column are significantly different ($p<0.05$). R_m: maximum resistance to extension; E: Maximum extensibility and A: Area under the curve. C: Control; M: Methyl cellulose; CG: Calcium-Gelatin casein; S: Soybean protein isolate; W: Whey protein concentrate

Table 5: Effect of microcapsules addition on bread texture parameters

Treatments	Hardness (g)	Cohesiveness	Elasticity	Chewiness
C	124.92±8.94^{a}	0.86±0.01^{a}	0.97±0.03^{a}	105.06±9.77^{a}
CG	157.84±6.27^{a,b}	0.83±0.03^{a}	0.96±0.00^{a}	131.49±15.30^{a,b}
M	180.05±21.39^{b}	0.79± 0.02^{a}	0.99±0.03^{a}	147.14±2.12^{b}
S	162.02±10.02^{a,b}	0.84±0.01^{a}	1.00±0.04^{a}	130.84±0.74^{a,b}
W	267.25±6.73^{c}	0.82±0.03^{a}	0.97±0.01^{a}	207.89±3.14^{c}

Values are informed ± standard deviation, $^{a-c}$Values with different superscript within a column are significantly different ($p>0.05$). The parameters of cohesiveness, elasticity and chewiness do not have units. C: Control; M: Methyl cellulose; CG: Calcium-Gelatin casein; S: Soybean protein isolate; W: Whey protein concentrate

The S treatment showed the lower oxidative rate respect to the other treatments, while the W and CG present the highest values, being W the most similar to the control C suggesting that these microcapsules did not improve stability. This was probably due to the presence of pores in the microcapsules which could favor the oxygen transference and promote the oxidation reactions (Kagami *et al*., 2003) or due to the interstitial air or air spaces between particles entrapment during processing and drying (Keogh and O'Kennedy, 1999).

On the other hand, considering the delta of color change in the microcapsules (Table 3), there was no significant difference ($p>0.05$). The color change could be associated with the migration of the lipidic phase to the surface of the microcapsules.

Uniaxial Extension Analysis

Microcapsules addition to the dough modified significantly the rheological parameters of extensibility with $p<0.05$ (Table 4). Uniaxial extension analysis showed a decrease in R_m produced by the incorporation of microcapsules in the dough, it was the lowest value with W. The parameter E decreased with the incorporation of CG and S. The area A showed a general decrease when the microcapsules were present in the mixture, even though M did not show significant difference in respect to the control C. These results proved the strong modification of the rheological properties of the dough produced by the addition of microcapsules. In all treatments the microcapsules produced less resistance to extension dough. CG and S produced dough with less extensibility. The reduction of A confirmed the dough weakness produced by the incorporation of the microcapsules CG, S and W. Therefore, it can be concluded that M is the one that less modified the parameters of extensibility and produced the most similar dough to C.

Texture Analysis

Table 5 shows the TPA parameters values analyzed in control sample (C) and samples containing microcapsules (CG, M, S and W). Elasticity and cohesiveness did not show significant differences

Table 6: Types of sensory attributes that marked the differences in relation to the control bread

	Attribute (No. of time this attribute was mentioned by a judge as the reason for the difference)							
Treatments	More sweet	More firm	More acid	More compact	Light taste of fish	More sticky	More dry	More soft
M	3	0	1	2	0	1	0	1
SL	3	0	0	1	2	1	0	0
GC	2	1	1	2	2	0	1	0
S	0	0	1	0	0	1	0	1

CG: Calcium-Gelatin casein; M: Methyl cellulose; S: Soybean protein isolate; W: Whey protein concentrate

($p<0.05$) among the samples, for which it can be concluded that the chewiness was mainly affected by the hardness. In bread products, low values of chewiness are wanted. W showed the highest value and M an intermediate value for this parameter while CG and S did not show significant difference with C.

Sensory Evaluation of Bread

For the triangular sensory evaluation of bread the minimum of correct answers to assure that there is a significant difference between the samples to the 5% of significance is 8 for 12 tests (Stone and Sidel, 1993). Only bread samples containing S microcapsules showed less than 8 correct answers concluding that they have similar sensory properties to the control bread. A corn oil was used as liquid phase in the S treatment; consequently a less water-soluble microcapsule was expected. On the other hand the microcapsule obtained by the spray-dried technique could be partially dissolved during the dough preparation explaining the differences with the control samples (more than 8 correct answers). Although M microcapsules presented significant difference with respect to the control, this difference was not due to the presence of fish taste (Table 6), being the main difference a sweeter taste, which could be the result from the amylases action that comes from yeasts over the maltodextrines, creating mono and disaccharides. This hypothesis could be proved because in each case that maltodextrine was part of the microcapsule composition the bread was sweeter than the control. Breads added with CG and W microcapsules, presented a slight fish taste for which it could be concluded that probably these microcapsules are more soluble in water and less thermoresistant than the other treatments. As was mentioned previously, it is probable that the aroma and taste of fish present in these breads were due to the presence of pores in CG and W microcapsules.

Another difference mentioned between the breads with and without microcapsules was that in general the first ones were more compact, specially the breads containing CG and W.

CONCLUSIONS

It is possible to add fish oil to bread product without a significant modification of their sensory and technological characteristics increasing their nutritional value. The wall materials that less changed the characteristics of bread were the S treatment, which resulted with the least oxidation rate and did not present a taste of fish. Another treatment was M microcapsules which also did not present fish taste and proved to be the wall material that less affected the rheological characteristics of dough and bread.

Although, cross-linking is a very cheap method, S microcapsules presented some difficult in it separation of corn oil. The spray drying technology is the most commonly technique used in the food industry due to its low cost and the availability of equipment.

Therefore, we recommend the spray drying method and methyl cellulose as encapsulation matrix to enrich the bread with fish oil omega-3, because it is easier and faster to elaborate these microcapsules using this technique than using the enzyme cross-linking treatment.

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