

Research Paper / Araştırma Makalesi

Use of Gelatin, Pullulan, Lactose and Sucrose as Coating Material for Microencapsulation of Fish Oil by Freeze Drying

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ABSTRACT

Encapsulating properties of gelatin, lactose, pullulan, sucrose and starch for the coating of the fish oil by freeze-drying technique were evaluated. Two groups of solutions containing 10% fish oil, 4.5% lactose, 4.5% sucrose, 1% gelatin and 80% water (L) and 10% fish oil, 4.5% pullulan, 4.5% sucrose, 1% gelatin and 80% water (P) on weight basis were prepared. As a control sample, the mixture containing 10% fish oil, 10% starch and 80% water (S) was used. All microencapsulated samples were stored in dark for 45 days at 25°C to determine changes in physicochemical properties. In order to determine the microencapsulation efficiency, the non-microencapsulated or/ free fish oil content and changes in color (ΔE) were also studied. Use of lactose as a coating material increased both the moisture content and water activity of encapsulated products. In terms of oxidation stability, the highest oxidation level was found in the control sample. The scanning electron microscope images revealed that surface structures of the sample P was mostly fibrous whereas the sample L had smooth surface.

Key Words: Microencapsulation, Fish oil, Freeze drying, Oxidation degree, Coating materials

Jelatin, Pullulan, Laktoz ve Sakkarozun Balık Yağının Dondurarak Kurutma Tekniği ile Mikroenkapsülasyonunda Kaplama Materyali Olarak Kullanımı

ÖZET

Jelatin, laktoz, pullulan, sakkaroz ve nişasta kullanılarak balık yağının dondurarak kurutulma tekniği ile kaplanması işleminde enkapsülasyon özellikleri incelenmiştir. Yüzde 10 balık yağı, % 4.5 laktoz, % 4.5 sakkaroz, % 1 jelatin ve % 80 su (L) ile % 10 balık yağı, % 4.5 pullulan, % 4.5 sakkaroz, % 1 jelatin ve % 80 su (P) içeren iki farklı formülasyon hazırlanmıştır. Kontrol örneği olarak % 10 balık yağı, % 10 nişasta ve % 80 su (S) içeren karışım kullanılmıştır. Mikroenkapsüle edilmiş örneklerin fizikokimyasal değişimlerini gözlemek için bütün örnekler karanlık ortamda 25°C'de 45 gün boyunca depolanmıştır. Bunların yanı sıra örneklerin mikroenkapsülasyon verimini belirlemek için kaplanmamış balık yağı içeriği ve renk değişimleri (ΔE) belirlenmiştir. Laktozun kaplama materyali olarak kullanılması nem ve su aktivitesi değerlerinde daha fazla artışa sebep olmuştur. Oksidasyon stabilitesi açısından, kontrol örneğinde (S) en yüksek oksidasyon derecesi bulunmuştur. Taramalı elektron mikroskobu ile çekilen resimlere göre L örneği düzgün bir yüzeye sahipken P örneğinde ise daha lifli bir yapı gözlenmektedir.

Anahtar Kelimeler: Mikroenkapsülasyon, Balık yağı, Dondurarak kurutma, Oksidasyon derecesi, Kaplama materyalleri

INTRODUCTION

Fish oil is known as a source of *n*-3-polyunsaturated fatty acids (PUFA). The main PUFA of fish oil are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Epidemiological studies have shown that PUFAs have protective effect against coronary heart disease. Furthermore, many of researches indicated that dietary supplementation with fish oil is beneficial for brain and nervous system functions. On the other hand, fish oil is

very susceptible to oxidation because of its high PUFA content. Today, protection of these *n*-3 PUFAs against oxidation reactions is quite important for food processing. Some researchers reported that microencapsulation of fish oil slows down the oxidation [1-7].

Microencapsulation technology is defined as covering an active substance (core material) with one or more covering material (wall material) to obtain microcapsules

in the range of micrometer to millimeter [8]. The most preferred wall material during microencapsulation of food ingredients are gelatin, lactose, sucrose, maltodextrin, pullulan, whey protein and gum arabic. Microencapsulation of food ingredients with coating materials can be achieved by several methods. The selection of the microencapsulation process is governed by the properties (physical and chemical) of core and coating materials and the intended application of food ingredients. The most important microencapsulation processes used to encapsulate food ingredients are spray drying, freeze drying, extrusion and fluidized bed-coating [9]. Although spray drying is the most frequently used microencapsulation method in the food industry, high drying temperatures lead to an increase in the oxidation of food ingredients. On the other hand, the drying processes at low temperature such as freeze drying are potentially suitable for sensitive oils and hence could be an alternative for the microencapsulation of fish oil [1].

Microencapsulation allows transforming liquid fish oil into more convenient to use and stable dry powder. The wall materials of microcapsules protect the core substance against the effects of oxygen, light and humidity [4]. Shelf life of fish oil can reach 12-24 months if stored in a cool place under nitrogen and in the absence of light [1, 6, 7]. The aim of this study was to investigate the effect of microencapsulation process on oxidative stability of fish oil. In addition, the effects of different wall materials on the microencapsulated fish oil were also determined.

MATERIALS and METHODS

Materials

The fish oil used in this study was anchovy (*Engraulis encrasicolu*) oil, which was obtained from Denizden T.A.S. (Turkey, Sinop). Gelatin (Çağdaş Kimya, Turkey), lactose (Merck, Germany), pullulan (Hayashibara International Inc., UK) sucrose and starch were used as encapsulating agents.

Sample Preparation

Mainly two different emulsion formulations containing lactose (L) and pullulan (P) were prepared. The formulation of the emulsions was given in Table 1. Protein contents, gelatin and another carbohydrate based wall material and sucrose were kept constant in these emulsions. The emulsion containing starch (S) was also prepared as the control sample especially to determine the degree of oxidation. During the preparation of emulsions, gelatin was initially dissolved in distilled water at 40°C. Then, sucrose and either lactose or pullulan were added to mixture, and the mixture was dissolved. The sample containing only

starch as a wall material was prepared in a similar way. The fish oil was added to the mixture as the last component prior to homogenization. The ratio of wall material to core material (fish oil) was fixed at 1:1 on weight basis. The mixture was homogenized to form oil in water emulsion by using a homogenizer (Edmund Bühler H04, Germany) operating at 30,000 rpm for 2 min to produce fine droplets. After homogenization, the emulsions were poured on petri-dishes as 3 mm thick layer and frozen at -10°C, in an air blast freezer (Frigoscandia, Helsinborg, Sweden). The frozen samples were dried under vacuum for 10h in a freeze-dryer set at -50°C condenser and 10°C plate temperatures (Armfield Limited-FT33 Vacuum Freeze Drier, England).

Table 1. Formulation for fish oil microcapsules

Ingredient (% w/w)	Samples		
	L	P	S
Gelatin	1.0	1.0	-
Sucrose	4.5	4.5	-
Lactose	4.5	-	-
Pullulan	-	4.5	-
Starch	-	-	10.0
Fish oil	10.0	10.0	10.0
Deionised water	80.0	80.0	80.0

L: Sample containing lactose, P: Sample containing pullulan, S: Control sample

Physico-chemical Analyses

The microencapsulated fish oil powders (20g) were filled in glass jars, and then stored in dark at 25°C for 45 days. The samples were analyzed every 15 days for quality parameters such as moisture content, water activity and peroxide value during the storage. Moisture content was determined gravimetrically in a vacuum oven (Shel-Lab, Sheldon Manufacturing, Inc., USA) at 70°C and 20 in Hg pressure [10]. The water activity (a_w) values of the powders were determined with a water activity measurement device (Testo AG 400, Germany) with ± 0.001 sensitivity. Peroxide value analysis was used as an indicator of the oxidation of microencapsulated fish oil. Peroxide value was determined by the iodometric assay according to IUPAC standard method 2.501 [11]. The morphological properties of the samples (the appearance and the particle shape) were investigated by taking images via a scanning electron microscope (SEM, JSM-6060 JEOL, Japan). The free or non-encapsulated oil content was determined according to the method by Sankarikutty et al. [12]. Microencapsulated fish oil (8g) was added to 200 mL light petroleum (bp: 60-80°C) and stirred for 15 min at 25°C with a magnetic stirrer. After filtration through anhydrous Na_2SO_4 , the solvent was evaporated and the extract was dried to constant weight under nitrogen. The microencapsulation efficiency (ME) was calculated as follows:

$$ME(\% \text{ encapsulated oil in total oil}) = \frac{(\text{total oil} - \text{free oil})}{\text{total oil}} \times 100 \quad (1)$$

The color of microencapsulated fish oil samples (Hunter L, a and b-value) were measured with a colorimeter (Colorflex, CFLX 45-2 Model Colorimeter, HunterLab,

USA). The total color change (ΔE) of 45 days stored samples with respect to initial color values was calculated by Eq. (2).

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$$

$$\Delta L = L_{0.day} - L_{45.day}; \Delta a = a_{0.day} - a_{45.day}; \Delta b = b_{0.day} - b_{45.day} \quad (2)$$

RESULTS and DISCUSSION

Changes in the moisture content and water activity values of L and P samples during storage are shown in Figure 1(a) and (b), respectively. First 15 days of storage, the moisture content of the samples did not change. Heinzelmann et al. [1] also reported that the moisture content of the dried microencapsulated fish oil was below 1% (w/w) before the storage. At the 45th days

of storage, the moisture content of L and P samples increased to 2.87 and 2.35%, respectively. The water activity values of L sample slightly increased from 0.23 to 0.35 during storage. However, the water activity of the sample containing pullulan (P) was stable at the 30th day storage. These results may be explained by the granular structure and wide surface area of the samples containing lactose.

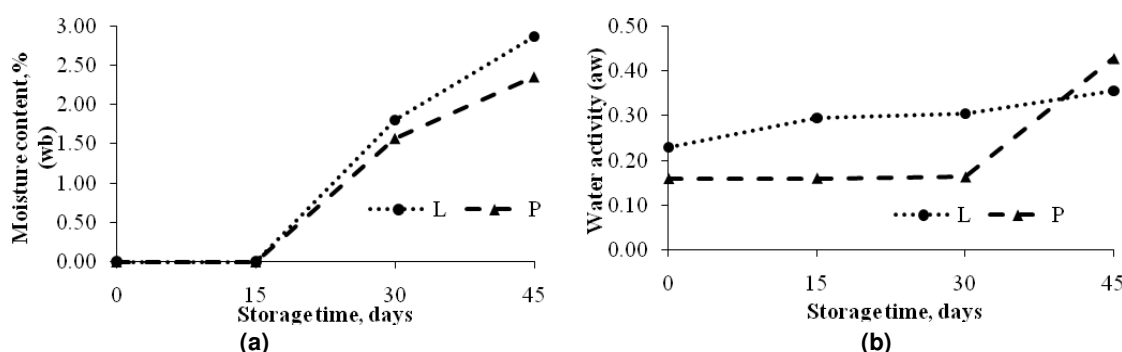


Figure 1. The change in the moisture content (a) and water activity (b) of L and P samples during storage

The results of peroxide analyses indicated that the sample containing lactose as a coating material was exposed to oxidation more than the one with pullulan. The changes in peroxide values of samples during storage are shown in Figure 2. Wider surface area of the L sample than the other explains its high oxidation

degree. Furthermore, pullulan has the ability to form strong films, which are impermeable to oxygen [13, 14]. The sample containing starch alone (S) had the maximum peroxide value among the dried microencapsulated fish oil samples.

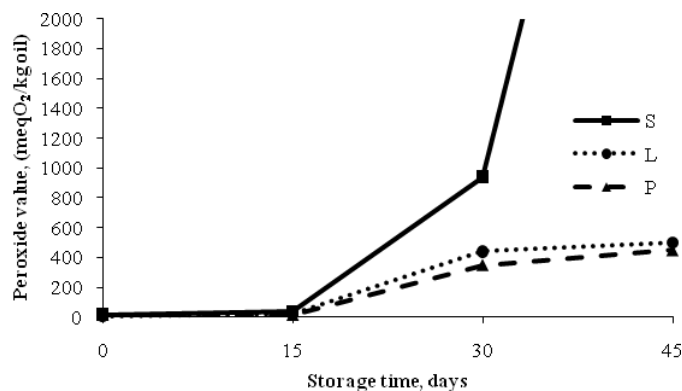


Figure 2. Peroxide values of S, L and P samples during storage

The scanning electron microscope images of powders microencapsulated with different wall materials are shown in Figure 3. Powder particles of sample L were

observed to have a smooth surface. However, the powder particles of sample P were mostly observed to have fibrous structures.

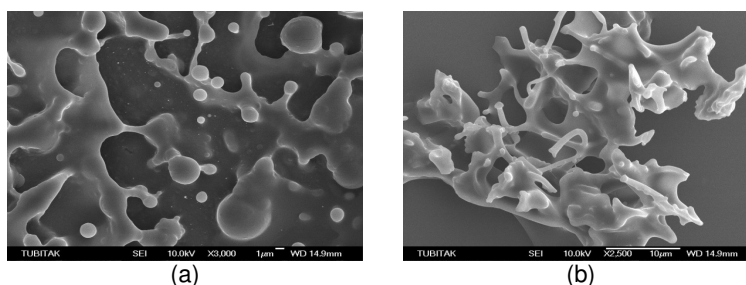


Figure 3. The scanning electron microscope images of microencapsulated fish oil powders containing lactose (a) and pullulan (b).

Initial microencapsulation efficiencies of the samples containing lactose and pullulan were 43.1% and 33.9%, respectively. At the 45th day of storage, this value reduced to 28.5 for L and 20.9% for P because of the breaking of the microcapsules during storage. Final value of ME and the reduction ratio for the sample L were higher than those for the sample P. This condition could be resulted from the increase in peroxide value of sample containing lactose more than that of containing pullulan. Furthermore, color change value (ΔE) of sample containing lactose (6.66) was determined higher than sample containing pullulan (1.95). This situation was partly related to breaking of microcapsules during storage. The higher the breaking ratio of microcapsules, the higher the oozing out the oil content, which means lower microencapsulation efficiency.

CONCLUSION

This study indicated that the use of gelatin, sucrose and either pullulan or lactose as wall materials for preparation of freeze-dried fish oil microcapsules is suitable to obtain more stable fish oil powder against oxidation. Although microencapsulation efficiency of L was higher than P, the peroxide value of P was lower than L during storage. This condition was probably caused by wider surface area, more granular form and higher breaking of microcapsules coated with lactose.

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