Aquatic Food Studies 1(1), AFS16
https://doi.org/10.4194/AFS16

RESEARCH PAPER

Microencapsulation of Anchovy Fish Oil (*Engraulis encrasicolus*) with Fish Protein (*Equulites klunzingeri*) Isolate: Nutritional Assessment

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How to cite

Özyurt, G., Yeşilsu, A.F. (2021). Microencapsulation of Anchovy Fish Oil (*Engraulis encrasicolus*) with Fish Protein (*Equulites klunzingeri*) Isolate: Nutritional Assessment. *Aquatic Food Studies*, *1(1), AFS16*. https://doi.org/10.4194/AFS16

Article History
Received 18 June 2021
Accepted 13 July 2021
First Online 14 July 2021

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Keywords
Microencapsulation
Spray drying
Fish protein isolate
Fish oil
Amino acid composition

Abstract

Fish protein isolates extracted from underutilised fish species were used for coating material of anchovy oils and their nutritive value was investigated in this study. For this purpose, Klunzinger's ponyfish (*Equulites klunzingeri*) proteins were extracted by using pH shifting process. Micro particles were prepared with anchovy oil (*Engraulis encrasicolus*) as core material (10%), and as wall materials a ratio of 5% and 10% fish protein isolate (FPI) was used. Maltodextrin (DE: 18:20) was added to both groups in a ratio of 10%. The emulsions were fed immediately into a Buchi Mini Spray Dryer (B-290, Switzerland). The inlet temperatures, feed rate and aspiration rate were maintained at 160 °C, 15 mL/min and 35m^3/h, respectively. The lipid, protein and moisture contents of anchovy oil microcapsules containing 5% FPI and 10% FPI were found as 43.76-43.09%, 4.34- 9.82% and 3.95-3.92%, respectively. The main amino acids in microcapsule samples were lysine, glutamic acid, and leucine which constituted in the range of 349-578 mg/100 g sample for microcapsules containing 5% FPI, and 805-1547 mg/100 g sample for microcapsules containing 10% FPI. In addition to that, essentials and non-essential amino acids (E/NE) ratio for microencapsulated fish oil with 5% FPI and 10% FPI were determined 0.92 and 0.95, respectively. As a result of this study, it can be concluded that the addition of fish protein isolate enhanced the nutritive value of microencapsulated fish oil.

Introduction

Fish oils are rich in long-chain ω3-series fatty acids that have proven health-worthy, but are highly sensitive to lipid oxidation. Traditionally, they are used for healthy diets, and there is a growing interest of consumers for these oils in industrial foods. It is emphasized that the regular consumption of fish oil can provide a remedy to the problem of the micronutrient deficiency and thus significantly reduces the quantity of nutrition-related diseases (cardiovascular, neurodegenerative, oncological and mental diseases, etc.). It has been recommended by the Food and Agriculture Organization (FAO) and World Health Organization (WHO) that people consume 0.25-2 g of EPA+DHA per day for a healthy and balanced diet (FAO-WHO, 2010). On the other hand, The American Heart Association (AHA) recommends higher rates such as 2-4 g EPA+DHA intake per day for hypertriglyceridemic patients (Kris-Etherton et al., 2003). Nowadays, consuming higher ω-6 than ω-3, as is mostly happening in modern westernized diet styles, has been shown to exert an adverse effect on human health (Mariamenatu and Abdu, 2021). Therefore, dietary supplementation of ω3 fatty acids seems to be an alternative way to meet of these fatty acids by many consumers, and there has been an increasing consumer demand for foods enriched with especially EPA and DHA fatty acids.

The smell and taste of fish oils limit their usage in foods. Microencapsulation technology is the most

effective method for the solution of these problems. The microencapsulation technique is a particular method used not only to convert liquids into solid materials, but also to add functionality or enhanced oxidative stability to components. Widespread use of this technique in the industry is spray drying, as it provides the advantage of low-cost commercial processing in large-scale production.

The selection of the appropriate coating material is the first important step in the microencapsulation process. The features sought in the wall material are that it is highly soluble in water, inert to active components, stabilized internal material, inexpensive and easily available. In practice, it may be preferred to mix more than one material because a single coating material does not meet all these features. Proteins are excellent wall materials for encapsulation using spray drying due to their functional properties. Proteins, in particular whey protein and sodium caseinate, have been highly studied as oil encapsulates due to their amphiphilic characteristics, which facilitate emulsion formation, enhance stability and produce desired physicochemical properties. In addition, although there are reports that food proteins such as soy proteins, milk proteins and egg proteins can be used as coating materials in microencapsulation technology, research on fish proteins is quite insufficient.

Process, such as surimi production, hydrolysate production or pH shifting method, can be selected for protein recovery from fish with low economic value. The pH shifting method enables efficient recovery of high quality fish protein, thus they can be successfully integrated into food products on the market. Although there are many studies on the application of the protein isolates from low economic valued fish in different food systems, such as fish ball, fish sausage (Ozyurt et al., 2019; Shaviklo, and Etemadian, 2019; Khan et al., 2020) the studies on fish protein isolates obtained by pH shifting for use as coating material, especially in fish oil microencapsulation, are limited. In this study, fish protein isolates extracted from underutilised fish species were used for coating material of anchovy oils and their nutritive value was investigated.

Materials and Methods

Klunzinger's ponyfish (*Equulites klunzingeri*) which is a discarded fish was caught by the staff of the Fisheries Faculty University of Çukurova in the Northeast Mediterranean Sea, and their proteins were extracted by using pH shifting process according to Hultin and Kelleher (2001) method. Klunzinger's ponyfish (3.65±1.5 g average weight) samples were ground and homogenized for 1 min on a Waring blender (Waring Products, Torrington, Connecticut, USA) with cold distilled water at 1:6 ratios (g:ml). The pH of the solution was adjusted to 11 by adding 2 M NaOH and then homogenates were centrifuged at 13000 x g (Sigma 16 SK, Germany) for 20 min at 4°C. After the centrifugation, the homogenate was separated into three layers as a top (fish oil), middle (fish muscle protein solution) and bottom (bones, skin, scale, etc.). The middle phase was then filtered through a double layer of cheesecloth. The pH of the middle phase was adjusted to 5.5 by adding 2 M HCl for isoelectrically precipitating fish proteins. Then the precipitated protein was centrifuged for 20 min at 13000 x g at 4°C to set the aggregated proteins. After the second centrifuging step, precipitated and de-watered fish protein, which was called as fish protein isolates (FPI), was collected and stored at -18^{o}C until used.

Microparticles were prepared with anchovy oil (*Engraulis encrasicolus*) as core material (10%), and as wall materials a ratio of 5% and 10% fish protein isolate (FPI) was used. Maltodextrin (DE: 18:20) was added to both groups in a ratio of 10%. Before the emulsion, the FPI was dissolved in distilled water and pH was adjusted to 9 by adding 2 M NaOH. The wall solutions were kept in benmari (50^{o}C) for one hour prior to emulsification. After cooling, fish oil was added and homogenized at 14000 rpm for 10 min in ultra-turrax. The emulsions were fed immediately into a Buchi Mini Spray Dryer (B-290, Switzerland). The inlet temperatures, feed rate and aspiration rate were maintained at 160^{o}C, 15 mL/min and 35 m^{3}/h, respectively.

Proximate and Amino Acid Composition Analyses

Moisture and crude ash content of microencapsulated fish oils with 5% FPI and 10% FPI were detected in an oven at 103ºC and 550ºC, respectively until the weight became constant. Lipid content was detected according to procedure of Bligh and Dyer (1959) and crude protein was found by Kjeldahl's method (AOAC 1999). Amino acid compositions of microencapsulated fish oils were detected by the MAM (Food Institute of Marmara Research Centre), TUBITAK (Scientific and Technological Research Council of Turkey). A Shimadzu 20 series ultra-fast liquid chromatography with UV detection was used. The method was adapted from literature and modified by TUBITAK MAM (Dimova, 2003). Carbohydrate content was calculated by difference.

Statistical Analyses

All data were subjected to analysis of variance (one-way ANOVA), at 5% confidence level using t-test.

Results and Discussion

Proximate and amino acid compositions of microencapsulated anchovy oil prepared with different rates of fish protein isolate are given in Table 1 and 2. The moisture, lipid and protein contents of anchovy oil microcapsules containing 5% FPI and 10% FPI were found as 3.95-3.92%, 43.76-43.09%, and 4.34-9.82%, respectively. It is known that moisture content greatly influences physical, chemical, and microbial stability of

Table 1. Proximate composition of microencapsulated fish oils (%)

	5% FPI	10% FPI
Moisture	3.95±0.03^{x}	3.92±0.02^{x}
Ash	0.19±0.03^{x}	0.19±0.02^{x}
Lipid	43.76±3.37^{x}	43.09±5.63^{x}
Protein	4.34±0.02^{x}	9.82±0.2^{y}
Carbohydrate (Maltodextrin)	47.76^{x}	42.98^{y}

The values are expressed as mean and standard deviation, n = 3.
Means followed by different superscript letters are significantly different (P<0.05).
FPI: fish protein isolate

Table 2. Amino acid composition of microencapsulated fish oils (mg/100g)

	5% FPI	10% FPI
Aspartic acid	278±91.92^{x}	797±14.14^{y}
Glutamic acid	431±38.89^{x}	1227±11,31^{y}
Serine	138±2.12^{x}	379±0.00^{y}
Glycine	156±0.71^{x}	347±0.71^{y}
Arginine	166±4.24^{x}	500±4.24^{y}
Alanine	216±16.97^{x}	511±7.78^{y}
Proline	208±29.70^{x}	417±5.66^{y}
Tyrosine	116±7.78^{x}	276±2.12^{y}
Histidine	72±0.71^{x}	173±1.41^{y}
Threonine	138±0.71^{x}	382±14.14^{y}
Valine	70±8.49^{x}	269±4.24^{y}
Methionine	21±0.00^{x}	233±0.71^{y}
Isoleucine	170±4.24^{x}	383±2.12^{y}
Leucine	349±13.44^{x}	805±3.54^{y}
Phenylalanine	180±9.90^{x}	428±2.83^{y}
Lysine	578±22.63^{x}	1547±14.85^{y}
E/NE	0,92	0,95

The values are expressed as mean and standard deviation, n=3. Means followed by different superscript letters are significantly different (P<0.05).
E/NE: Essential Amino Acid/Non Essential Amino Acid Ratio.

foods. Moisture content for microencapsulated fish oil is particularly a significant parameter, as high water activity increases lipid oxidation. In addition to that, at high humidity levels, the wall material transforms from the glassy state to a high molecular mobility amorphous rubbery state, leading to the release of microencapsulated oil during storage (Charles et al., 2021; Velasco et al., 2000). The moisture content of microencapsulated oil has been reported by some researchers in the range of 0.24–4.25% (Ashokkumar et al., 2018; Charles et al., 2021). Generally, the food industry suggests a maximum moisture level for the dry powder as 3–4% (Klinkesorn et al., 2006). The results of this study for both groups were lower than the maximum moisture content limit (set below 4%) for dry powdered food products. Crude ash content was found at 0.19% for both groups. The calculated carbohydrate content of the microencapsulated fish oils was mainly due to the added maltodextrin. Therefore, it was found in range of 47.76% for 5% FPI group and 42.98% for the 10% FPI group.

The lipid content of microencapsulated fish oils was 43.76% for 5% FPI and 43.09% for 10% FPI. It is an expected result since the lipid ratio was formulated to be 1/3 of the dry matter ratio in the emulsion. As for protein content of microencapsulated fish oils, the addition rate of fish protein was reflected in the protein content of the microcapsules. Accordingly, it was determined that the protein content was 4.34% in the 5% FPI added to group, while protein content was 9.82% in the 10% FPI added group.

The amino acid composition of fish protein isolate used in this study was shown in Figure 1. The main amino acids of Klunzinger's ponyfish protein isolate extracted by pH shifting method were lysine, glutamic acid, leucine, threonine and alanine. These findings were in accordance with Özyurt et al (2015) who reported that leucine, threonine and alanine were basic amino acids in Klunzinger's ponyfish isolate produced with pH shifting method. Lysine and methionine, which are highly essential for human, were found to be 4162 and 788 mg/100g, respectively. After spray drying proses, the main amino acids of microencapsulated fish oil were also lysine, glutamic acid, and leucine (Table 2). Following these amino acids, aspartic acid, arginine and alanine were detected in significant amounts, while threonine was not existed in this sequence after microencapsulation. Therefore, it can be concluded that the most affected amino acid by heat treatment was threonine. Kaczmarek et al. (2013) studied the effect of

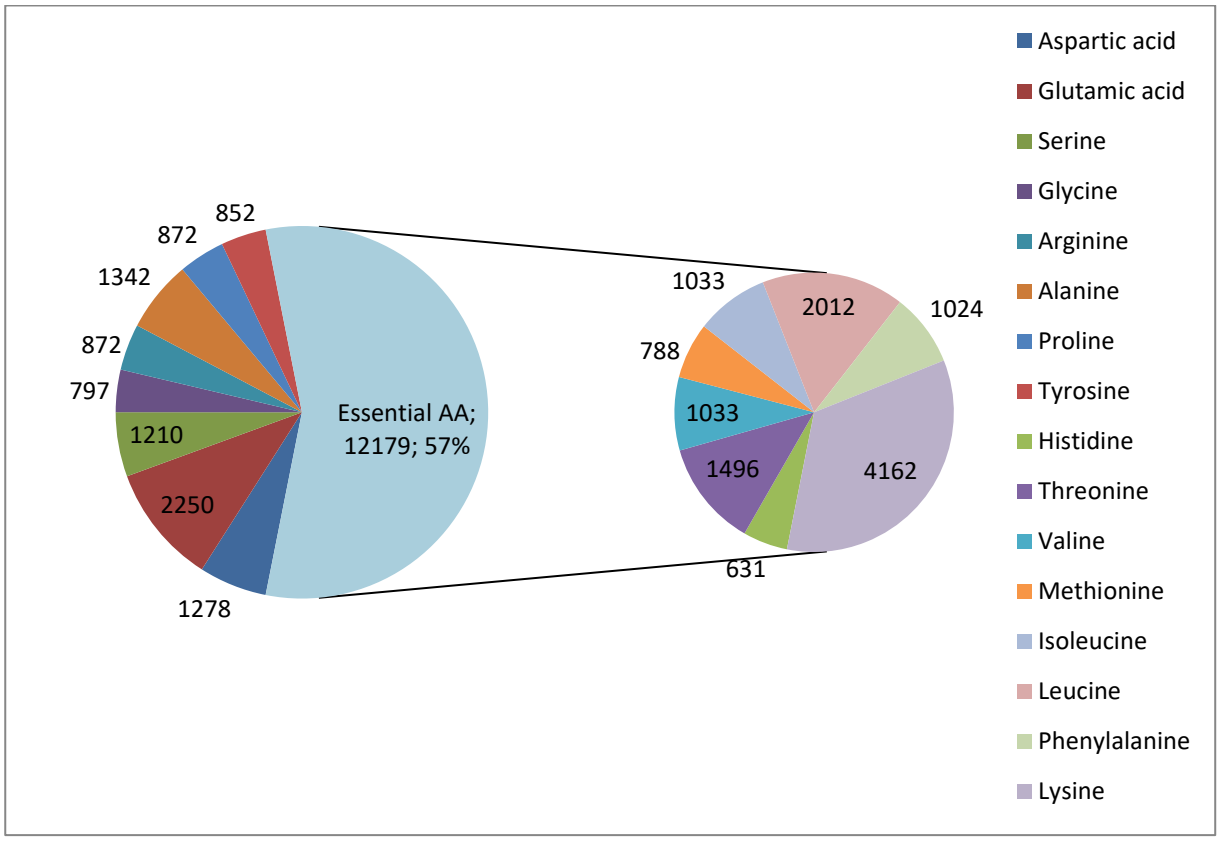

Figure 1. Amino acid composition of fish protein (*Equulites klunzingeri*) isolate added in microencapsulation of fish oil. The values are expressed as mean value (mg/100g), n=3.

oven drying at 60, 100 and 140 °C on the amino acids. It was found that the most affected amino acids by the heat treatment were cysteine, tyrosine, threonine, glycine, isoleucine, serine and aspartic acid. In addition to that, the researchers reported that the mechanisms involved in the denaturation of protein during drying and heat treatments are still unclear. Similarly, in this study, it can be concluded that besides the proportional change in all amino acids, there was also a decrease with the effect of spray drying.

The protein quality of food can be assessed using the ratio between essential and non-essential amino acids (E/NE). The ratio of E/NE was determined to be 1.29 for fish protein isolate extracted pH shifting method in this study. Özyurt et al. (2015) found 0.95 E/NE for the raw Klunzinger's pony. They reported that the ratios of E/NE in freeze dried alkali-aided protein isolate and acid-aided protein isolate of Klunzinger's ponyfish were also found to be 0.90 and 0.81, respectively. In this study, E/NE ratio for microencapsulated fish oil with 5%FPI and 10% FPI were determined as 0.92 and 0.95, respectively. These ratios were considerably higher than those of many unprocessed fish that previously reported by some researchers (Iwasaki and Harada, 1985; Özyurt and Polat, 2006; Mol et al., 2008; Gómez-Limia et al., 2021). These results indicated that ponyfish isolate has well balanced protein thanks to favourable E/NE ratio, and may be considered as a nutrient-rich coating material for the microencapsulation process. In our previous study (Ozyurt et al., 2020), it was found that the addition of fish protein isolate in the wall material did not adversely affect the lipid stability of fish oil microcapsules. Based on these data, it can be concluded that fish protein isolates can be used as a wall material for microencapsulation of fish oil and have a high potential for application as a nutraceutical agent in food systems.

Ethical Statement

This article does not contain any studies involving animals performed by any of the authors.

Funding Information

There is no any funding institution for this study.

Author Contribution

GÖ: Conceptualization, Analysis, Methodology, Writing -review and editing; **AFY:** Analysis, Methodology, Statisctical Analysis

Conflict of Interest

There is no conflict of interest.

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