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Possibilities of Fisherman's Friend Type Lozenges Fortification with Omega-3 LC PUFA by Addition of Microencapsulated Fish Oil

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Abstract The aim of this study was to evaluate the influence of fish oil powder addition on sensory quality and oxidative stability of Fisherman's Friend type lozenges. Microencapsulated fish oil (MFO) was used for lozenge formulation. Sensory quality of the moderate and strong mint flavored lozenges was not significantly affected by fish oil powder addition up to 60 and 80 g kg^{-1}, respectively. Higher MFO addition resulted in significant reduction in the sensory quality and increases in fishy off-flavor intensity. During 4 months of storage in air permeable packages, a gradual decrease in sensory quality and an increase in fishy off-flavor were detected in lozenges containing MFO, which were more significant in moderately flavored than in strongly flavored ones. The peroxide value increased significantly during air storage. However, the peroxide value of vacuum stored samples increased slightly whereas sensory scores remained stable. A recommendation to pack and store the MFO lozenges under limited oxygen is suggested due to the low stability of the MFO product. A dose of 5 lozenges fortified with MFO might provide 40 mg of omega-3 LC PUFA elevating its average level in the diet.

Keywords Fish oil · Food fortification · Lozenges · Omega-3 PUFA · Sensory quality

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Introduction

Long chain omega-3 fatty acids (LC PUFA) appear to decrease the risk of cardiovascular diseases, many types of cancer and autoimmune disorders. Additionally, omega-3 LC PUFA positively impact the proper development and function of the brain and retina [1,2]. The main omega-3 LC PUFA are eicosapentaenoic and docosahexaenoic acids (EPA C20:5 and DHA C22:6). The richest dietary source of omega-3 LC PUFA is sea fish, especially fish oils [3]. Due to desirable health effects, an increased omega-3 LC PUFA intake is currently strongly advised. At least two servings of fatty fish per person per week, which corresponds to 1.5 g of omega-3 LC PUFA per week and 0.2 g per day, is recommended [4]. Some nutritional authorities recommend higher intakes. The International Society for the Study of Fatty Acids and Lipids (ISSFAL) proposed the minimal intake of omega-3 LC PUFA to be 0.44 g per person day and even higher for pregnant and lactating women—0.6 g [5]. Currently the average level of omega-3 LC PUFA in the Western diet is approximately 0.15 g per person per day and the ratio of omega-6 to omega-3 in the diet is approximately 10–1 in spite of a recommended 5–1 ratio [6,7]. The high omega-6 to omega-3 ratio is the result of elevated consumption of vegetable fats and oils rich in omega-6 PUFA and low fish consumption [7].

The elevation of omega-3 LC PUFA intake may be achieved by regular consumption of fish. However, many people do not like or cannot eat fish. For those individuals the intake of fish oil supplements or foods fortified with fish oil in liquid or powder (microencapsulated) form can be the way to achieve higher omega-3 LC PUFA [8,9]. Nevertheless, due to very high unsaturation level of omega-3 LC PUFA fish oil is extremely susceptible towards oxidation. Antioxidants, especially of natural origin like

flavonoids or carotenoids, are commonly used to stabilize fish oil [4,10]. Oxidative deterioration of fish oil, provoking subsequent development of unacceptable fishy off-flavor, is a major concern with adding fish oil to foods [11].

Microencapsulation is a method that coats the fish oil, resulting in a powder, which might be easy to use in production of dry foods. Moreover, it is believed that microencapsulation may protect fish oil against oxidation by limiting undesirable influences of environment, like oxygen, light, humidity, etc. [12,13]. However, some studies indicated that stability of microencapsulated fish oil might be lower than bulk oil due to increased surface area and insufficient density of capsules structure [14]. Nevertheless, a number of companies have created microencapsulated fish oil powders for use in dry foods as a source of omega-3 LC PUFA [15,16]. In the industrial practice to diminish fishy off-flavor intensity it is proposed to use small amounts of fish oil and an extra addition of flavorings or seasonings to fish oil fortified foods. This may effectively mask unacceptable off-flavor formed during gradual oxidation of fish oil in enriched foods, when processed and stored in air permeable conditions. This makes fish oil-fortified foods palatable longer [8]. The sensory quality of foods is the most important aspect for the consumer and flavor is a crucial sensory discriminator, also for fish oil-containing foods [17]. The results of sensory evaluations are strongly related to the level of fish oil fortification and the amount of fishy flavor masking ingredients. When the fortification level is very low or fishy flavor masking ingredients high, it is almost impossible to detect fish oil or off-flavors formed by oxidation of fish oil in foods [11,18].

Nevertheless, the assortment of fish oil fortified foods available on the international market increases every year [4,8,9]. Fish oil fortified food is considered a functional food. Bread, dairy products, mayonnaise or even ice cream are fortified with fish oil in oily or powder (microencapsulated) form. In this study it was proposed that mint flavored lozenges might be fortified with fish oil powder at levels higher than in the case of other foods due to strong mint flavor. One of the most well known around the World mint flavored lozenges are Fisherman's Friend produced by Lofthouse since 1865. Hence the aim of this study was to evaluate the influence of fish oil powder addition on sensory quality and oxidative stability of Fisherman's Friend type lozenges.

Experimental Procedures

Materials

Glucose, sorbitol, lecithin, magnesium stearate, citric acid, natural peppermint oil powder and chlorophyllin were used for lozenges formulation. Formulated lozenges were similar to typical mint flavored Fisherman's Friend. Fish oil powder was used to fortify the lozenges with omega-3 LC PUFA. Microencapsulated fish oil powder, Ropufa'10'n-3 Food Powder, was given as a grant from DSM Nutritional Products (Switzerland). It is a pale brownish powder containing well purified fish oil dispersed in a matrix of fish gelatin, corn starch and sucrose. Fish oil was stabilized with ascorbyl palmitate and rosemary extract. Fish oil powder was packed under nitrogen and was in the middle of its shelf life. According to the producer's declaration, the average omega-3 LC PUFA content in the fish oil powder was approximately 10% (100 g kg^{-1}).

Determination of Oil Content

Soxhlet extraction was conducted to determinate the oil content in fish oil powder used in the study. The extraction was completed using petroleum ether for 4 h. The petroleum ether was then removed under vacuum at 50 °C. The flask containing oil was stored under a hood for 15 min before determining oil content gravimetrically. Oil content was assessed in triplicate.

Fatty Acid Analysis

Omega-3 LC PUFA content in fish oil powder was determined by gas chromatography (GC). To prepare samples for GC analysis, the oil was extracted from samples according to the Folch method. Folch extraction avoids high temperature and the long time typical of Soxhlet extraction. One gram of sample was homogenized in a cold mixture of chloroform–methanol (2:1). After filtering, the solid residue was washed with a chloroform–methanol mixture and filtered again. The combined filtrates were mixed with water (15–25%, respectively). After phase separation, the lower chloroform phase was removed, dried over anhydrous Na_2SO_4, then roto-evaporated under vacuum at 40 °C. The lipid fraction obtained was covered by nitrogen, weighed and washed out from the evaporation flask using hexane, additionally dried by passing through anhydrous Na_2SO_4, then closed in small vials under nitrogen.

Fatty acid methyl esters (FAME) were prepared using a slight modification to the AOCS method Ce1b-89. After evaporating hexane under nitrogen, the dried lipid fraction was saponified by 0.5 N solution NaOH with methanol, covered with nitrogen, mixed and heated in a water-bath at 100 °C for 40 min. The saponified sample was transmethylated with 14% BF_3 in methanol reagent, covered with nitrogen, at boiling point for 3 min. After that, the mixture

was cooled and 3 ml hexane was added, covered with nitrogen and shaken vigorously for 30 s while still warm. Then 40 ml of saturated water solution of NaCl was added and shaken vigorously. After separation, the hexane layer was transferred by syringe to the thin glass tube and dried over anhydrous Na_2SO_4 and decanted to a clean vial, covered with nitrogen and capped. One µl of prepared FAME was injected into the chromatograph under appropriate conditions. Tricosanoic acid (C23:0) methyl ester was used as an internal standard (Sigma–Aldrich, Steinheim, Germany). FAME were prepared according to a slightly modified AOCS method Ce1b-89 [19].

The GC analysis of FAME was performed using Agilent 6890N GC (Agilent, Böblingen, Germany) equipped with Rtx 2330 silica capillary column of 100 m length, 0.25 mm ID, d_f 0.1 µm (Restek Corp, Bellefonte, USA). Hydrogen was used as a carrier gas at flow rate 0.9 ml s^{-1}. Split-splitless (50:1) injector at 235 °C and flame-ionization detector (FID) at 250 °C were used. Column temp. was programmed as follows: initial 155 °C, time 55 min., next rate 1.5 °C min^{-1}, final temp. 210 °C. Each sample was analyzed in triplicate. Results were collected in the Chemstation and transformed using software HP-Chem (Hewlett Packard, Palo Alto, USA). Peaks were identified by comparison with known standards: menhaden reference oil (Supelco, Bellefonte, USA) and Supelco 37 component FAME Mix (Supelco, Bellefonte, USA). Results were reported as peak area percentages and recalculated with respect to an internal standard according to AOCS method Ce1b-89. The EPA-IS-DHA factors of 0.99-1.00-0.97 were used for these omega-3 LC PUFA [19,20].

Lozenges Preparation

Lozenges were formulated in the laboratory. All ingredients were carefully mixed together and carefully compressed, at ambient temperature, into flat cylinder shaped lozenges of approximately 1.5 g weight each using hand operated equipment. The general composition of the lozenges is shown at Table 1. Samples were divided in two types: moderate and strong mint flavored. Fish oil powder was added to the mixture of components at several levels: 5, 10, 20, 40, 60, 80 and 100 g kg^{-1}. Sensory evaluation was completed shortly after sample preparation.

For the storage test, lozenges were fortified with fish oil powder at upper levels established in the preliminary sensory evaluations, which did not significantly influence sensory quality. Samples were packed in air permeable packages, which is typical for this kind of product. Control samples were packed under vacuum. Samples stored in air were packed in paper sachets closed with the use of an office stapler. Vacuum stored samples were closed using a vacuum packer VAC-STAR 2000 (Vac Star AG, Switzerland). All samples were stored at ambient temperature in darkness for 4 months, simulating typical storage conditions for industrially produced lozenges.

Table 1 Composition of Fisherman's Friend type lozenges evaluated in the study, g kg^{-1}

Ingredient	Moderately flavored sample	Strongly flavored sample
Peppermint oil powder	3.5	6.0
Magnesium stearate	10.0	10.0
Citric acid	15.0	15.0
Lecithin	15.0	15.0
Chlorophyllin	15.0	15.0
Fish oil powder	5.0–100.0	5.0–100.0
Sorbitol	500.0	500.0
Glucose	ad 1,000.0	ad 1,000.0

Sensory Assessment

Acceptability of the stored samples was evaluated using a structured 9-points hedonic scale, anchored "dislike extremely" to "like extremely" [17]. Intensity of fishy off-flavor was measured on a linear scale, anchored "none" to "very strong"; the results were converted to numerical values (0–10 units) [17]. During the first session, the fishy off-flavor attribute was discussed to ensure equal understanding by all panelists and described as typical old fish attribute with rancid, chemical and sour characteristics.

Sensory evaluations of acceptability were made by a group of 30 consumers, mainly students. Evaluations of intensity of fishy flavour were made by a sensory panel consisting of ten assessors trained in sensory analysis. Panelists were formally trained [21] and well experienced. All evaluations were conducted in laboratory conditions according to instructions of the evaluation procedure [17]. For each evaluation a set of four coded samples was given to each assessor on Petri plates in random order at ambient temperature. Assessors were asked to take longer breaks after the evaluation of two samples. All evaluations were made in two replications; 20 individual results were used for statistical data analysis. The sessions were conducted in the morning hours and than after a 4 h break—two sessions per day. Assessors' notes were marked on individual score sheets. The test room for sensory evaluation was air-conditioned and free of disturbing factors (odors, noise, etc.).

The preliminary evaluation was conducted to establish the upper level of fish oil powder addition that did not significantly influence the sensory quality of fortified lozenges. Samples contained from 10 to 100 g kg^{-1} of fish oil

powder and were strong and moderately flavored. Lozenges without fish oil addition were used as the control. Samples were evaluated using sensory tests described above.

Samples fortified with fish oil powder at the upper levels established in the preliminary evaluations were selected for the storage test. Lozenges of moderate and strong mint flavor without fish oil powder addition were used as controls 1 and 2, respectively. Samples stored in vacuum and air permeable conditions were evaluated every 2 weeks during the 4 months using sensory tests described above.

Oxidative Stability

The oil was extracted from lozenges according to the Folch method (described above). For each measurement ten lozenges were homogenized in a cold mixture of chloroform–methanol to achieve oil samples sufficient for measurement. The peroxide value (PV) was measured to determinate the oxidation of fish oil in fortified lozenges during storage [22]. The applied iodometric method was carried out with chloroform and glacial acetic acid as solvents. Results were calculated as milliequivalents of active oxygen per kg of fat sample (mequiv O kg^{-1}). Each measurement was done in triplicate.

Data Analysis

The results of the sensory tests were analyzed using a one-way analysis of variance (ANOVA) to check the significance of differences in acceptability and intensity of fishy off-flavor among samples containing fish oil powder and controls [23]. The statistical analysis of PV data was completed using the same method. Results were analyzed using Statgraphics Plus ver. 4.1 software, at a significance level $P < 0.05$.

Results and discussion

Soxhlet extraction showed 311 mg kg^{-1} of fish oil in fish oil powder used in the study. In the analyzed fish oil over 40 different fatty acids were found. However, significant levels were shown for 19 (Fig. 1). The predominant fatty acid was palmitic acid (C16:0), oleic acid (C18:1), DHA (C22:6) and EPA (C20:5), which were: 20.3, 17.5, 17.4 and 10.7% of total fatty acids, respectively. The total content of omega-3 LC PUFA, including docosapentaenoic acid (C22:5; DPA), calculated according to the results of GC analysis was 291.2 mg kg^{-1} in the oil fraction of the evaluated fish oil powder. Thus the fish oil

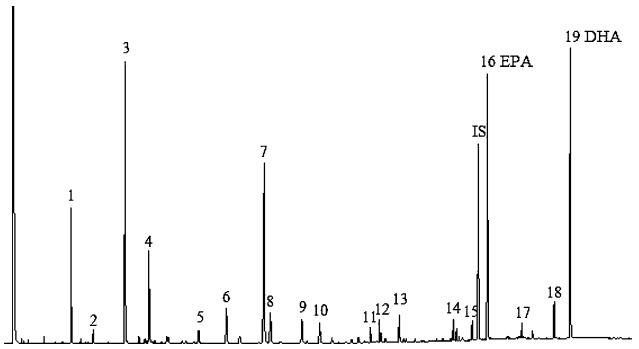

Fig. 1 Chromatogram of FAME analysis of fish oil used in the study: *1* C14:0, *2* C15:0, *3* C16:0, *4* C16:1n-9, *5* C17:1c, *6* C18:0, *7* C18:1n-9, *8* C18:1n-7, *9* C18:2t, *10* C18:2n-6, *11* C18:3n-3, *12* C20:1n-9, *13* C18:4n-3, *14* C20:3n-6, *15* C20:4n-6, *16* C20:5n-3, *17* C24:1c, *18* C22:5n-3, *19* C22:6n-3

powder used in the study contained 90.5 g kg^{-1} of omega-3 LC PUFA.

Preliminary sensory evaluations of samples containing fish oil powder showed that depending on the flavoring agent used, lozenges might be fortified at different levels. Upper levels of fish oil addition, which did not change overall sensory quality of lozenges, were: 60 g kg^{-1} for moderately flavored samples and 80 g kg^{-1} for strongly flavored ones. Higher addition levels resulted in a statistically significant decrease in sensory quality and an increase in fishy off-flavor intensity for both strong and moderately flavored samples. Results of the preliminary evaluation are presented at Fig. 2.

During storage, both strong and moderate mint flavored samples containing 60 g kg^{-1} fish oil powder were evaluated. Gradual decrease of sensory quality and an increase of fishy off-flavor intensity were detected for fish oil containing lozenges stored in air in comparison to control 1 (Fig. 3). Decrease of acceptability was statistically significant for moderately flavored fish oil fortified lozenges. For strongly flavored products, changes were not significant until the second month of storage, after which the

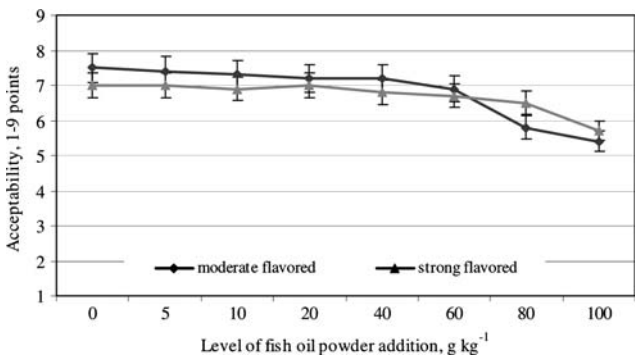

Fig. 2 Preliminary evaluation of overall sensory quality of mint flavored lozenges fortified with fish oil at different levels—hedonic test, with 1 indicating "dislike extremely" and 9—"like extremely"

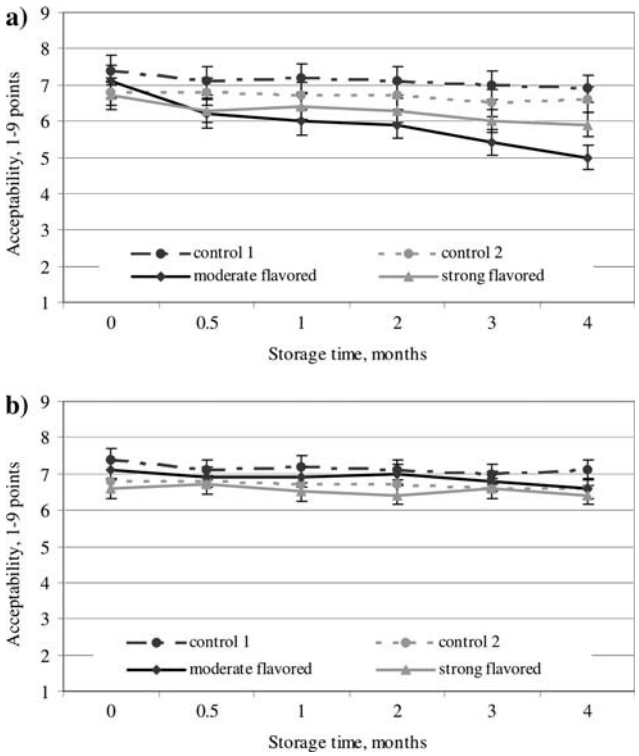

Fig. 3 Overall sensory quality of mint flavored lozenges fortified with fish oil at the level 60 g kg^{-1} evaluated during storage: **a** air permeable conditions, **b** vacuum storage—hedonic testing, with 1 indicating "dislike extremely" and 9—"like extremely"

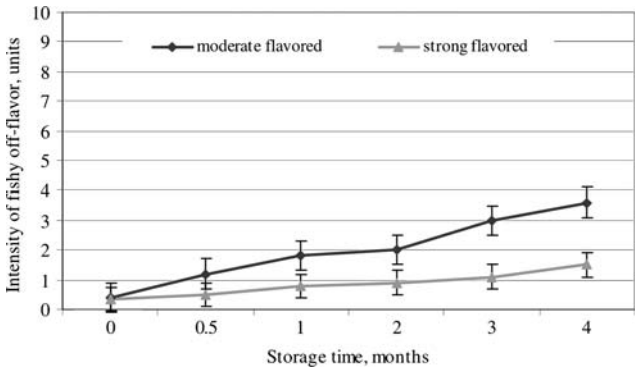

Fig. 4 Fishy off-flavor intensity of mint flavored lozenges fortified in fish oil at the level 60 g kg^{-1} evaluated during storage in air permeable conditions—scaling method, with 0 indicating the lowest intensity (none) and 10—very strong one

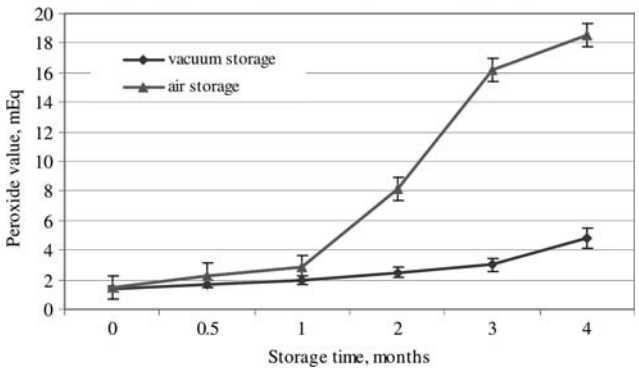

Fig. 5 Oxidative stability of mint flavored lozenges fortified with fish oil at the level 60 g kg^{-1} evaluated during air and vacuum storage

difference compared to the control 2 and to the first evaluation became statistically significant. Sensory qualities of samples stored under vacuum remained stable during the entire storage (Fig. 3). Intensity of fishy off-flavor increased significantly for both moderately and strongly flavored samples stored in air permeable conditions. For moderately flavored samples, a significant increase was detected in the first evaluation (after 0.5 months). In the case of strongly flavored ones this situation took place after 2 months of storage. For moderately flavored samples, the increase during storage was much greater than for strongly flavored lozenges (Fig. 4). The increase of fishy off-flavor was positively correlated with a decrease in overall sensory quality of the samples. However, in vacuum stored samples the increase was not detected.

Peroxide value measurements during storage showed similar results to sensory evaluations. Permanent increase of PV was found in air stored samples, from 1.5 up to 18.6 mequiv O kg^{-1} (Fig. 5). The most intensive PV increase was shown after 1 month of storage. PV increase was positively correlated with increase of fishy off-flavor and negatively correlated with overall sensory quality of the samples. In vacuum stored samples the increase was much lower, from 1.4 up to 4.4 mequiv O kg^{-1} and started to be significant after 3 months of storage. There was no correlation between PV and mint flavoring intensity of evaluated lozenges.

During this study it was shown that microencapsulated fish oil can be potentially used for manufacturing of lozenges containing high amounts of omega-3 LC PUFA. Nevertheless, air permeability during storage significantly reduced the quality of fish oil fortified lozenges. Therefore, such products should be packed and stored in conditions that eliminate air access. Consumption of lozenges fortified with fish oil at levels established in the study might increase omega-3 LC PUFA content in the average Western-style diet (Fig. 6), especially when combined with other fish oil fortified foods [16,24,25]. Consumption of fish oil containing lozenges may raise the average level of omega-3 LC PUFA intake. It can be expected that five strong mint flavored lozenges containing 60 g kg^{-1} of fish oil might provide 40 mg of EPA and DHA, which is approximately 20% of the omega-3 LC PUFA recommended minimal daily intake [6,7].

In previous studies it was established that the level of fish oil fortification of foods depends on food form (liquid or solid), fat content and flavoring ingredients presence, as

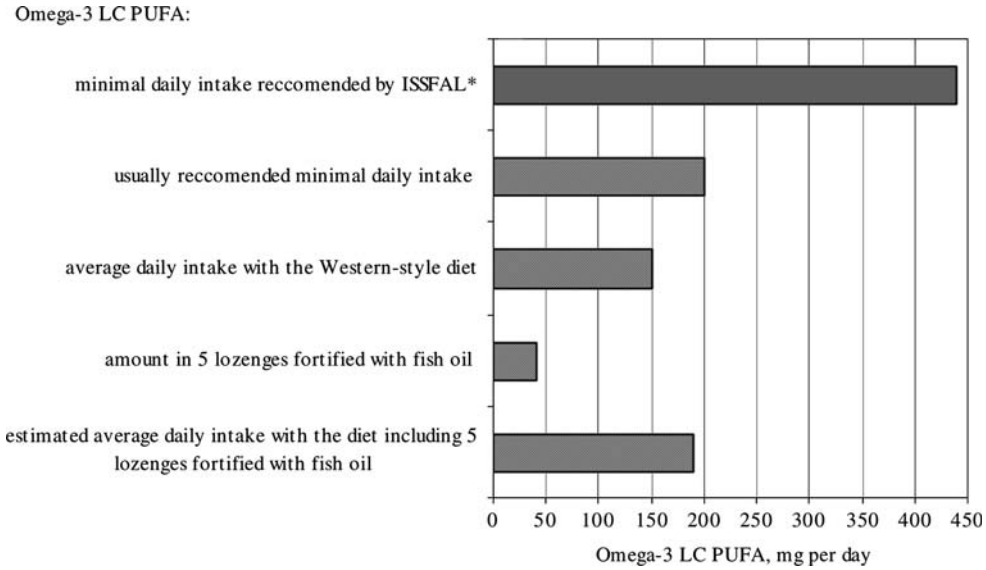

Fig. 6 Levels of omega-3 LC PUFA in the average diet and in five Fisherman's Friend type lozenges fortified with fish oil powder at the level 60 g kg^{-1}. *ISSFAL—the International Society for the Study on Fatty Acids and Lipids

well as on the form of added fish oil [26,27]. Results obtained in the present study showed, that strong and moderately flavored lozenges were suitable for fortification with microencapsulated fish oil at limited levels. However, levels of fortification obtained were higher than for bread, dairy products, instant foods, and lower than for spreadable fats, mayonnaise or salad dressings [27–32]. This was due to the mint flavor used in the formulation.

Flavoring agents present in food might mask unacceptable off-flavor of fish oil added to the food [31–33]. In this study, it was shown that the presence of a strong mint flavor allowed a high level addition of fish oil to the lozenges and significantly masked fishy off-flavor intensity which increased during storage in air permeable conditions. Flavorings mask undesirable sensory changes of fish oil added to the food at low level. Increasing fishy off-flavor is one of the indicators of its oxidation, which can be harmful for the consumer. However, if the off-flavor is masked by flavorings it does not mean that the oxidation has not occurred, which was shown by an increase in PV during air storage of strong mint flavored lozenges fortified with fish oil. For this reason, foods with added fish oil should be strongly protected against oxidation during processing, packaging, storage and distribution to eliminate factors that promote oxidation, such as oxygen, high temperature, UV light, copper and iron ions [8–11]. To prevent oxidation of fortified foods it could be recommended to use vacuum or inert gas packaging in one-portion units and to consume the product shortly after opening the package. Nevertheless, safety, bioavailability and sensory quality of omega-3 LC PUFA fortified food products still remain to be evaluated.

Regular consumption of Fisherman's Friend type lozenges fortified with fish oil, packed and stored in vacuum or inert gas conditions might provide a significant level of EPA and DHA to the diet. Nevertheless, fish oil fortified lozenges cannot be considered an important dietary source of these fatty acids. However, they might contribute to an increased average level of omega-3 LC PUFA in the Western-style diet, especially when combined with fish meal or other fish oil fortified foods.

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