

Evaluation of n-3 polyunsaturated fatty acid content in various foods: health impact assessment

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Abstract: The objectives of this study were to verify the on-label claims of foods labelled as rich in n-3 polyunsaturated fatty acids (FA) and to assess their potential effects on human health in relation to European legislation. All the foods tested, i.e., chicken meat, anchovy fish oil, linseed oil, shellfish, capsule oil concentrate, egg, cold-smoked mackerel, frozen seafood, squid, hake, salmon and sardine, were evaluated for their contribution to the amount of n-3 polyunsaturated FA (n-3 PUFA) and the ratio of n-6/n-3 PUFA in relation to European dietary regulations. Lipids were extracted from the samples and then detected using capillary gas chromatography with flame ionization. An intake of 250 mg eicosapentaenoic acid plus docosahexaenoic acid (EPA+DHA) per day, which is sufficient for primary prevention of chronic diseases in healthy volunteers, was found for 100 g of the edible part of shellfish, frozen seafood, squid, salmon, anchovy fish oil, capsule oil concentrate, cold-smoked mackerel and sardine. The European regulation defines high n-3 PUFA food as food with a content of at least 0.6 g 100 g⁻¹ α -linolenic acid or at least 80 mg 100 g⁻¹ EPA+DHA. This means that linseed oil and anchovy fish oil were the foods best suited to fulfil the first recommendation (>0.6 g 100 g⁻¹ α -linolenic acid). The edible part of shellfish, frozen seafood, squid, hake, salmon, sardine, cold-smoked mackerel, capsule oil concentrate and anchovy fish oil met the second recommendation (>80 mg 100 g⁻¹ EPA+DHA). With regard to the nutrition recommendations, the least favourable foods in terms of EPA+DHA content were eggs and chicken meat. An n-6/n-3 PUFA ratio closer to 4:1 is necessary for the prevention and treatment of chronic diseases. The results obtained in this study should be relevant for the establishment of Serbian tables of nutritional values of products.

Key words: total fat content, n-3 PUFA, n-6 PUFA, ratio n-6/n-3 PUFA.

Introduction

Fat in food consists mainly of fatty acids (FA), which are chemically coupled to glycerol. FA can be saturated (SFA), monounsaturated (MUFA) or polyunsaturated (PUFA) (Simopoulos, 2008; Gibson *et al.*, 2013). Whereas PUFA have historically contained about one n-3 FA (omega in popular literature) for every four n-6 FA (1:4), modern diets can contain up to fifty to a hundred times more n-6 FA than n-3 FA (50:1) (Simopoulos *et al.*, 2013). The evidence that this imbalance contributes to disease is now strong, and governments should formulate agricultural and food policies to influence costs (Simopoulos *et al.*, 2013, Simopoulos, 2008). The n-6/n-3 PUFA ratio could again approach that to which we are genetically adapted, i.e. four to one (4:1) (Simopoulos, 2004; Simopoulos and Cleland, 2003). A high n-6/n-3 PUFA ratio is typical of Western and, increasingly, global diets and is associated with an increased risk of cardiovascular disease, obesity,

type 2 diabetes and breast and prostate cancer, especially in people with genetic predispositions. Of concern, animal studies show that low intake of docosahexaenoic acid (DHA, C22:6n-3), an n-3 PUFA, combined with high intake of fructose leads to a metabolic syndrome in the brain (Agrawal and Gomez-Pinilla, 2012).

n-3 PUFA have numerous functions in the human body. They play an important role in the structure and function of biological membranes. Any increase in n-3 PUFA could cause changes in membrane fluids that can affect enzymatic activity, receptor-ligand interaction, cell interaction and nutrient transport through the membranes (Horrobin, 1995; von Schacky *et al.*, 1985). Studies have shown that n-3 PUFA are essential for infant growth and development and for the prevention of various clinical conditions such as arthritis, diabetes, cancer and skin diseases.

Most diets, although with regional differences, are deficient in n-3 PUFA and too high in n-6 PUFA. A concerted effort is needed to narrow the n-6/n-3

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PUFA ratio in the diet. Consumers should be encouraged, through education and, if necessary, through government intervention to switch from oils with high n-6 PUFA content such as corn, safflower, and sunflower oil, to those with high n-3 PUFA content such as rapeseed and linseed oils and oils with high MUFA content such as olive oil or hazelnut oil in combination with rapeseed oil. The increased consumption of fish should also be emphasized. Scientists should work with the fishing industry to achieve this goal. Aquatic organisms and fish from aquaculture are the main source of the essential FA (Arts *et al.*, 2001; Hunter and Roberts, 2000). The nutritional and health benefits of consuming fish and fish products are the reason for increased consumer demand for fish (Hunter and Roberts, 2000). Specifically, a 4:1 ratio of n-6/n-3 PUFA in the diet should be the goal (Simopoulos, 2008, Simopoulos and Cleland, 2003). The aims of the present study were to verify the on-label claims of foods declared to be rich in n-3 PUFA and to assess their potential effects on human health.

Materials and Methods

Food samples

All foods tested were labelled as rich in n-3 PUFA: three chicken meat samples with skin, three anchovy fish oil samples, three linseed oil samples, eighteen edible shellfish samples, two capsule oil concentrate samples present on the Serbian market, six whole egg samples, three cold-smoked mackerel samples, three frozen seafood samples, three frozen squid samples, three frozen hake samples, three frozen salmon samples and eighteen edible part of sardine samples.

FA analysis by capillary gas chromatography

Total lipids for FA determination were extracted from products by accelerated solvent extraction (ASE 200, Dionex, Sunnyvale, CA) using a 33 ml stainless steel cell according to the method of Spiric *et al.* (2010). Fatty acid methyl esters (FAMES) in the extracted lipids were transesterified using 0.25 M trimethylsulfonium hydroxide (TMSH) in methanol (EN ISO 5509:2000). FAMES were determined by gas-liquid chromatography (GLC, Shimadzu 2010, Japan) equipped with flame ionization detector and capillary HP-88 column (length 100 m, i.d. 0.25 mm, film thickness 0.20 μm). Injector and detector temperature were set at 250°C and 280°C, respectively. Nitrogen was used as the carrier gas at

flow rate of 1.33 mL min⁻¹. The injector split ratio was set at 1:50 and programmed column oven temperature started at 125°C and ended at 230°C. Total analysis time was 50.5 min. The chromatographic peaks in the samples were identified by comparing relative retention times of FAME peaks with peaks in Supelco 37 Component FAME mix standard (Supelco, Bellefonte, USA).

Results and Discussion

The average total fat, the total n-3 PUFA, α -linolenic acid (ALA), eicosapentaenoic acid plus docosahexaenoic acid (EPA+DHA) and n-6/n-3 ratio of the 69 samples are presented in Table 1.

This study included twelve food types and provided total n-3 PUFA in g 100 g⁻¹ of samples, along with ALA, EPA and DHA contents. An intake of 250 mg per day of EPA+DHA is sufficient for primary prevention in healthy volunteers (EFSA, 2010). This recommendation would be fulfilled when at least 100 g of shellfish, frozen seafood, squid, salmon, anchovy fish oil, capsule oil concentrate, cold-smoked mackerel or sardine are consumed (Table 1). The American Heart Association (AHA) recommends at least two portions of fish per week for general health; cardiovascular patients are advised to consume 1 g EPA+DHA per day and patients with hypertriglyceridaemia, 2 to 4 g EPA+DHA per day (Lichtenstein *et al.*, 2006). As shown in Table 1, foods, if consumed in 100 g amounts, that fulfil the minimal AHA recommendation for EPA+DHA intake (1 g per day) were anchovy fish oil, sardine, capsule oil concentrate and cold-smoked mackerel.

ALA cannot be synthesized by the body, but it is necessary to maintain “metabolic integrity” and is, therefore, considered an essential FA. However, there is not enough scientific data to derive an average requirement or a population reference intake (EFSA, 2010). The foods that were relatively high in ALA were linseed oil, anchovy fish oil, frozen salmon, chicken meat and cold-smoked mackerel. However, the Annex of Regulation EC No 1924/2006 defines a high n-3 PUFA food as a foodstuff containing at least 0.6 g 100 g⁻¹ ALA or at least 80 mg 100 g⁻¹ EPA+DHA. For fulfilling the first recommendation (0.6 g 100 g⁻¹ ALA), linseed oil and anchovy fish oil were the most suitable foods. Shellfish, frozen seafood, squid, hake, salmon, sardine, cold-smoked mackerel, capsule oil concentrate and anchovy fish oil complied with the second recommendation (80 mg 100 g⁻¹ EPA+DHA). Samples of eggs and chicken meat were the most unfavourable

Table 1. The content of total fat, n-3 PUFA, ALA, EPA+DHA and the n-6/n-3 FA ratio in foods

Food sample	Total fat (g 100 g ⁻¹)	n-3 FA (g 100 g ⁻¹ of sample)	ALA (g 100 g ⁻¹ of sample)	EPA+DHA, (g 100 g ⁻¹ of sample)	n-6/n-3 FA ratio
Eggs whole (n = 6)	9.85	0.07	0.04	0.03	12.01
Chicken meat with skin (n = 3)	8.34	0.46	0.45	0.007	5.62
Anchovy fish oil (n = 3)	100	10.48	5.80	4.68	0.87
Linseed oil (n = 3)	100	58.14	58.14	0	0.19
Shellfish (n = 18)	1.72	0.34	0.05	0.29	0.26
Capsule oil concentrate (n = 2)	3.33	1.27	0.04	1.23	0.73
Mackerel cold-smoked (n = 3)	20.12	0.50	0.24	2.96	0.15
Seafood frozen (n = 3)	3.92	0.84	0.15	0.69	0.48
Squid frozen (n = 3)	2.48	0.48	0.03	0.45	0.11
Hake frozen (n = 3)	1.66	0.20	0.01	0.19	0.10
Salmon frozen (n = 3)	11.69	1.10	0.54	0.56	0.53
Sardine (n = 18)	10.0	2.53	0.11	2.42	0.26

Legend: n – Number of samples examined

foods examined in terms of EPA+DHA content and n-6/n-3 ratio. The n-6/n-3 PUFA ratios in eggs were above the recommended levels of 4:1 (*Simopoulos, 2002*) and averaged 12.01 (egg samples examined), which was consistent with the previously published data for eggs from Hy-line hens housed in a cage system (*Pavlovski et al., 2011*). The n-6/n-3 PUFA ratios were higher in our study than in similar studies with Hy-line free range and Naked neck free range eggs (*Pavlovski et al., 2011*). The n-6/n-3 PUFA ratio of 5.62 in our chicken samples was lower than in the studies of *Živković et al. (2017)* and *Miličević et al. (2014)*. With dietary manipulation, chicken meat enriched with n-3 PUFA with n-6/n-3 <5 can be produced (*Penko et al., 2015*).

The n-6/n-3 PUFA ratio in frozen fish ranged from 0.10 (frozen hake) to 0.48 (frozen seafood), which were similar ratios to those of freshwater fish such as silver carp, Wells catfish and zander, namely from 0.33 to 0.93 (*Čirković et al., 2011*). The n-6/n-3 PUFA ratio of cold-smoked mackerel was 0.15, similar to that of smoked salmon (*Djordjević et al., 2016*). Fish generally has high EPA+DHA ratios with low n-6/n-3 PUFA ratios, as was shown for rainbow trout with an n-6/n-3 PUFA ratio of 0.62–0.72 (*Trbović et al., 2012; Lušnic Polak et al., 2017*) and carp reared with extruded or pelleted feed, with an n-6/n-3 PUFA ratio of 3.79 (*Čirković et al., 2011*). Certainly, even more favourable n-6/n-3 ratios in fish can be achieved by animal dietary measures.

Conclusion

Whereas the PUFA content of food declared as rich in n-3 PUFA have historically contained an n-6/n-3 PUFA ratio of about 1:4, modern diets can contain as much as 50:1. A concerted effort is needed to decrease the ratio of n-6/n-3 PUFA in modern human diets. The aim of the present study was to verify food samples labelled as rich in n-3 PUFA. The foods tested were chicken meat, fish and linseed oil, shellfish, capsule oil concentrate, egg, cold-smoked mackerel, frozen seafood, squid, hake, salmon and sardine. Sufficient intake for primary prevention in healthy subjects is 250 mg EPA+DHA per day. Consumption of 100 g of shellfish, frozen seafood, squid, salmon, anchovy fish oil, capsule oil concentrate, cold-smoked mackerel or sardine meets this recommendation. The AHA recommends for general health at least two portions of fish per week, while cardiovascular patients are advised to consume 1 g of EPA+DHA per day and patients with hypertriglyceridaemia to take 2 to 4 g of EPA+DHA per day. Foods meeting the AHA recommendation for EPA+DHA content were anchovy fish oil, sardine, capsule oil concentrate and cold-smoked mackerel. Eggs and chicken meat contained the least favourable EPA+DHA ratios. The results obtained in this study should be relevant for the establishment of Serbian food composition tables in the field of meat and meat products.

Procena sadržaja n-3 polinezasićenih masnih kiselina u različitim namirnicama: procena uticaja na zdravlje

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A p s t r a k t: Cilj ove studije su verifikacija uzoraka hrane označeni kao bogata n-3 polinezasićenim masnim kiselinama (PUFA) i procena njihovog uticaja na zdravlje ljudi u odnosu na evropsko zakonodavstvo. Svi ispitivani uzorci, poput pilećeg mesa, ribljeg i lanenog ulja, školjki, koncentrata ulja u kapsuli, jaja, hladno dimljene skuše, smrznute morske hrane, lignji, oslića i lososa, kao i sardine, ocenjeni su zbog njihovog doprinosa količini n-3 PUFA i odnos n-6 / n-3 PUFA u odnosu na evropske propise. Izvršena je ekstrakcija lipida iz uzoraka i ispitano je kapilarnom gasnom hromatografijom sa detekcijom plamenske jonizacije. Unos 250 mg eikosapentaenske kiseline plus dokozaheksanske kiseline (EPA + DHA) dnevno, koji je dovoljan za primarnu prevenciju kod zdravog subjekta, za 100 g jestivog dela školjki, smrznutih morskih plodova, lignji i lososa, ribljeg ulja, koncentrata kapsula ulja, hladno dimljena skuša i sardina. Evropska uredba definiše visoki nivo n-3 PUFA kao hranu sa sadržajem od najmanje 0,6 g / 100 g α -linolenske kiseline ili najmanje 80 mg / 100 g EPA + DHA. To znači da su laneno i riblje ulje najprikkladnije za prvu preporuku. Hrana, poput jestivog dijela školjkaša, smrznute morske hrane, lignje, oslić i losos, sardina, hladno dimljeni skuša, koncentrat ulja u kapsuli i riblje ulje ispunjava drugu preporuku. U pogledu ishrane, najmanje povoljni uzorci su jaja i pileće meso. Bliži odnos PUFA n-6 / n-3 neophodan je za prevenciju i lečenje hroničnih bolesti. Rezultati dobijeni ovom studijom trebalo bi da budu relevantni za formiranje tabela hranjivih vrednosti proizvoda.

Ključne reči: određivanje ukupne masti, n-3 PUFA, n-6 PUFA, odnos n-6/n-3 PUFA

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