Attila Tanai, Zsuzsanna Lelovics[1], Anett Kovács[2], Hajnalka Hingyi[2], Éva Csavajda[4], Péter Kovács[3], Nándor Kovács[3], Gyöngyi Kanyó Princes[3], György Grosz[5], Tamás Tóth[1]

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Analysis of meat products supplemented with omega-3 fatty acid sources

1. Summary

Our research goal was to increase the n-3 fatty acid content of three meat products (letscho sausage, Kaiserroulade, duck liver pâté), so that the added value can be declared on the product labels. Increasing the amount of n-3 fatty acids was performed by the addition of flax seed sprout oil rich in alpha-linolenic acid (min. 300 mg of alpha-linolenic acid/100 g or 100 kcal product) or fish oil rich in EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) (min. 40 mg EPA + DHA/100 g or 100 kcal product).

We found that the n-3 fatty acid proportion of the products tested were increased significantly ($p < 0.05$) by the oil supplements used. Of the products supplemented by bio flax seed sprout oil, letscho sausage performed the best, with an alpha-linolenic acid content of 644 mg/100 kcal, sufficient to be able to claim „rich in omega-3 fatty acids" on the label. The value was 575 for the Kaiserroulade and 504 mg for the duck liver pâté. These values allow the legitimate use of the statement „omega-3 fatty acid source". The measured EPA + DHA content of the Kaiserroulade supplemented with fish oil was 99 mg/100 kcal, sufficient to be able to claim „rich in omega-3 fatty acids" on the label. The EPA + DHA contents of letscho sausage and duck liver pâté failed to reach the level required to be able to claim the above statement on the label by 1 and 4 mg/100 kcal, respectively.

The more important microbiological parameters of the finished products were not affected negatively by the oil supplements used, and the shelf lives of the products did not decrease either. The appearance of the products was not influenced by the supplements, however, foreign flavors and odors were identified by testers in several instances, due to the fish oil and bio flax seed sprout oil supplements.

2. Introduction

n-3 and n-6 polyunsaturated fatty acids play an important role in a healthy diet. The most significant representative of the n-6 group linoleic acid (C18:2), while the member of the n-3 group most often encountered is α-linolenic acid (C18:3) **[1] [2]**. Several studies have shown that the individual fatty acids affect health in different ways, due to their various physiological roles **[3]**. The risk of developing coronary heart disease, hypertension, diabetes and several other inflammatory and autoimmune diseases can be reduced by consuming appropriate amounts of n-3 fatty acids **[4]**. n-3 fatty acids exert their anti-inflammatory effect through the synthesis of eicosanoids **[5]**. α-Linolenic acid (ALA), EPA and DHA prevent blood clots and reduce serum triglyceride levels **[6]**, **[7]**, **[8]**. Their consumption also decreases the risk of thrombosis, because they increase bleeding time by their incorporation into the membranes of the

[1] Kaposvár University, Faculty of Agricultural and Environmental Sciences, Institute of Nutrition and Product Development Sciences, Department of Nutritional Science and Production Technology;
[2] Adexgo Kft.;
[3] Funkció Kft.;
[4] University of West Hungary, Faculty of Agricultural and Food Sciences;
[5] PharmAgora Quality of Life Cluster

platelets, thus inhibiting the aggregation of the latter **[9]**. Some studies also showed that proper n-3 fatty acid intake during pregnancy contributes to decreasing the incidence of gestational hypertension and premature births **[10], [11]**. For optimal brain and retina development, in addition to arachidonic acid (ARA, C20:4, n-6), an appropriate supply of DHA is also important for the fetus. As a result of insufficient supply, visual disturbances and reduced visual acuity might develop **[12]**. There are also favorable research results regarding the effects of n-3 fatty acids on different tumor cells **[13]**.

In addition to the appropriate amount of fatty acid intake, another important aspect is the relative ratio of the individual fatty acids. While the optimal n-6/n-3 fatty acid ratio is 3-5:1 **[14], [15], [16]**, from the data of the National Diet and Nutritional Status Survey we know that this ration in Hungary was 28-30:1 at the beginning of the last decade **[17]**. According to the latest results, this ratio by gender is 29:1 for men and 26:1 for women **[18]**. The unfavorable dominance of n-6 fatty acids is a combined result of a culinary culture based on margarine and sunflower oil and the low intake of n-3 fatty acids.

There are several possibilities for improving the fatty acid ratio ingested by the population with the foods consumed. Foods that are natural sources of long-chain, polyunsaturated n-3 fatty acids include saltwater fish (e.g., hake, mackerel, salmon, tuna, cod, sardines) and vegetable oils (soy, rapeseed or linseed oil, etc.). Another possibility for improving the intake ratio is the consumption of dietary supplements. For this purpose, a wide range of notified goods is available to consumers **[19]**.

Consumption of health protecting, so-called functional foods is also an effective solution to increase the intake of polyunsaturated fatty acids. Due to their special nutrient content, functional foods can prevent or slow down the development of certain diseases. Foods containing elevated levels of n-3 fatty acids can be produced in two ways. Special oils are either introduced into the products during food production, or the n-3 fatty acid content of products of animal origin (milk, meat, eggs) is increased through special feeding of the animals (feeds supplemented with vegetable oils, feeding of „full fat" oilseeds) **[20]**.

Several studies have already demonstrated that the fatty acid composition of meats or meat products can be modified successfully through feeding. Fattening pigs were fed a mixture supplemented with tuna oil by the research group lead by Ratchaneewan Khiaosa-ard in the different phases of fattening **[21]**. When analyzing the products made from the meat of the animals (two kinds of sausages, bacon), a clear increase in the n-3 fatty acid content was observed in all cases. The breast meat of Japanese quail whose diet was supplemented with n-3 polyunsaturated fatty acids (PUFA) also showed a clear increase in PUFA content **[22]**. Taulescu and his coworkers found that the PUFA content of the muscle tissue of broiler chickens increased significantly due to mixing linseed into their feed **[23]**. According to the research of Schneiderová and her colleagues **[24]**, fattening feed supplemented with linseed oil changed the fatty acid profile of the lipids of poultry meat, by increasing significantly the amount of ALA in the meat samples analyzed.

The goal of the present study is to determine how the fatty acid composition and the energy content of some commercially available meat products (letscho sausage, Kaiserroulade, duck liver pâté) change as the result of omega-3 fatty acid supplements (bio flax seed sprout oil, fish oil). It was also evaluated which statements („omega-3 fatty acid source" or „rich in omega-3 fatty acids") can be applied on the product packaging as a result of this (1924/2006/EK **[25]**; 116/2010/EU **[26]**). In addition, the microbiological and sensory characteristics of the products supplemented with omega-3 fatty acid sources were also examined.

3. Materials and methods

Plant experiments aimed at increasing the omega-3 fatty acid content of meat products were performed in the meat plant of Funkció Kft. (Darnózseli), within the framework of the Economic Development Operational Program titled *„Development of a tradition-based, health-protecting, premium quality meat product family"*.

The following meat products, produced by Funkció Kft., were included in the studies:

Letscho sausage K
(control product with no supplement)

Letscho sausage H
(+ fish oil supplement, min. 40 mg EPA + DHA/100 g or 100 kcal product) /manufacturer: Biosearch S.A., Granada, Spain/

Letscho sausage L
(+ bio flax seed sprout oil supplement, min. 300 mg ALA /100 g or 100 kcal product) /manufacturer: Omega Bázis Kft., Lábatlan, Hungary/

Kaiserroulade K
(control product with no supplement)

Kaiserroulade H
(+ fish oil supplement, min. 40 mg EPA + DHA/100 g or 100 kcal product) /manufacturer: Biosearch S.A., Granada, Spain/

Kaiserroulade L
(+ bio flax seed sprout oil supplement, min. 300 mg ALA /100 g or 100 kcal product) /manufacturer: Omega Bázis Kft., Lábatlan, Hungary/

Szigetköz duck liver pâté K
(control product wih no supplement)

Szigetköz duck liver pâté H
(+ fish oil supplement, min. 40 mg EPA + DHA/100 g or 100 kcal product) /manufacturer: Biosearch S.A., Granada, Spain/

Szigetköz duck liver pâté L
(+ bio flax seed sprout oil supplement, min. 300 mg ALA/100 g or 100 kcal product) /manufacturer: Omega Bázis Kft., Lábatlan, Hungary/

The chemical compositions and fatty acid profiles of the raw materials and the finished products were analyzed, and the energy contents of the finished products were calculated. Based on the results, the necessary oil supplements were determined, and the recipes were developed. 100 g of product contained more than 418 kJ (100 kcal) energy, therefore, according to the relevant law (116/2010/EU), this value was taken into consideration when determining the amount of oil supplement. When implementing the changes, oil supplements were used instead of the high fat content ingredients (e.g., bacon) originally listed in the recipe. This way, we tried to avoid creating large differences between the energy contents of the control and experimental products.

The amount of n-3 fatty acids was increased using flax seed sprout oil rich in alpha-linolenic acid (manufacturer: Omega Bázis Kft., Lábatlan, Hungary), and fish oil rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (manufacturer: Biosearch S.A., Granada, Spain). Fatty acid compositions of the oil sources used as supplements are summarized in **Table 1**.

Product chemical composition (dry matter, protein, fat, ash) analytical methods:

– Dry matter: MSZ ISO 6496:2001

– Raw protein: MSZ 6830 - 4:1981

– Raw fat: FVM decree 44/2003. (IV. 26.)

– Raw ash: MSZ ISO 5984:1992

The fatty acid compositions of the oil sources used as supplements (bio flax seed sprout oil, fish oil), and also of the finished products were determined, following extraction **[27]**, as prescribed in the Hungarian Standard (MSZ ISO 5508:1992). The fatty acid compositions of the samples analyzed were determined using an HP Agilent Technologies 6890N (Agilent Technologies Inc., USA) model gas chromatograph. Particulars of the column: Supelco SP™ 2560 Fused Silica Column (Supelco, Bellefonte, PA, USA) 100 m × 0.25 mm × 0.2 μm film thickness; carrier gas: H.

Chemical and fatty acid composition analyses were performed at the laboratories of the Institute of Animal Sciences and/or the Institute of Food Sciences of the Faculty of Agricultural and Food Sciences of the University of West Hungary.

Finished products from the test productions were analyzed from a microbiological point of view as well, with the help of GalenBio Kft. (Mosonmagyaróvár), comparing them to control samples with known shelf lives (letscho sausage: 5 days; Kaiserroulade: 21 days; Szigetköz duck liver pâté: 30 days). Accordingly, letscho sausages were analyzed on the 5th day, Kaiserroulades on the 21st day and Szigetköz duck liver pâtés on the 30th day. Analytical methods regarding the microbiological state are summarized in **Table 2**.

With the help of the employees of Campden BRI Kft. (Budapest), sensory (organoleptic) testing was also performed. Quantitative descriptive and organoleptic profile tests were carried out according to Section 5.4.3 of standard MSZ ISO 6685:2007.

Statistical evaluation *(Kolmogorov-Smirnov test; t-test, Kruskal-Wallis test, Mann-Whitney test, one-factor variance analysis, Newman–Keuls method)* of the analytical results was performed using the program SPSS 15.0. for Windows (*SPSS Inc.*, Chicago, USA). The selected significance level was min. $p \leq 0.05$.

4. Results and evaluation

4.1. Chemical composition

Chemical compositions and energy contents of the products are summarized in **Table 3**. Examining the dry matter contents of the products, there is a significant difference between the sample treated with flax seed sprout supplement and the control sample in the case of the letscho sausage. The 43.73% dry matter content of the control letscho sausage samples increased to 46.50% as a result of the flax seed sprout oil supplement, while there was no observable difference in the case of the fish oil supplements for any of the products (letscho sausage, Kaiserroulade, duck liver pâté).

The protein contents of the products were not influenced significantly by the oil supplements ($p > 0.05$). And, looking at the fat content, statistically verifiable difference could only be found between the results of experimental letscho sausages ($p < 0.05$). In the case of the product supplemented with fish oil, the measured fat content was 23.71%, while for the bio flax seed sprout oil product it was 26.23%.

When examining the ash contents of the products, Kaiserroulade products supplemented with fish oil and flax seed sprout oil showed a statistically verifiable difference ($p < 0.05$) compared to the control. The 3.90% ash content of the control samples increased to 5.45% as a result of the fish oil supplement, while to 5.10% as a result of the bio flax seed sprout supplement.

The calculated energy contents of the products did not change to a great extent as a result of the different treatments.

In summary, it can be stated that the chemical compositions of the products were not influenced significantly by the oil supplements. The resulting differences are not considered significant from a nutritional physiology point of view.

The same conclusion was reached by Severini et al. when examining the effect of replacing the fat content of different salamis by extra virgin olive oil **[28]**. According to the study of Makala, published in 2007 **[29]**, differences between the chemical compositions of a sausage product (mortadella) supplemented with linseed oil and fish oil compared to the control sample were only due to the biological diversity of the raw materials used.

4.2. Fatty acid profile

The fatty acid compositions of the products examined are shown in **Table 4**. Based on the results we determined that the proportion of saturated fatty acids (SFA) was not affected by the fish oil supplement, compared to the control products (letscho sausage, Kaiserroulade, duck liver pâté). At the same time, a significant decrease was observed for myristic acid (C14:0) and palmitic acid (C16:0) in the case of all products as an effect of flax seed sprout oil, while the proportion of stearic acid (C18:0) decreased to a statistically verifiable extent ($p < 0.05$) only in the case of the Kaiserroulade. The SFA ratio of the products was not affected by the fish oil supplement. Addition of flax seed sprout oil resulted in a significant decrease in the SFA ratio ($p < 0.05$) of letscho sausage, Kaiserroulade and duck liver pâté as well, meaning 10, 16 and 7.5% decreases, respectively. This result can be explained by the low, 10.56% saturated fatty acid (SFA) ratio of the flax seed sprout oil used as a supplement (see the data in **Table 2**).

The palmitoleic acid (C16:1) proportion was not influenced significantly by the fish oil supplement, however, it reduced the amount of oleic acid (C18:1) in the case of the Kaiserroulade significantly, from 41.18% to 38.82%. Statistically verifiable reduction in the proportion of palmitoleic acid (C16:1) was the result of the flax seed sprout oil supplement in the case of the letscho sausage and the duck liver pâté, while the amount of oleic acid (C18:1) was reduced significantly in the Kaiserroulade and duck liver pâté products. Nevertheless, neither the fish oil, nor the flax seed sprout oil supplement had a significant effect ($p > 0.05$) on the proportion of monounsaturated fatty acids (MUFA).

Examining the proportion of polyunsaturated fatty acids (PUFA) in the products, it can be stated that the proportion of PUFA fatty acids increased significantly ($p < 0.05$) for all three product groups as a result of the oil supplements. As a result of the fish oil supplement, the PUFA ratio increased by 4.8, 14.2 and 8.5 percent in the case of the letscho sausage, Kaiserroulade and duck liver pâté, respectively. This result is mainly due to the significant increase in the amounts of EPA and DHA. The proportion of EPA increased from 0.03% to 0.48%, while that of DHA from 0.03% to 0.78%. The change in PUFA ratio as a result of the flax seed sprout oil supplement was also statistically verifiable when compared to the PUFA ratio of the fish oil supplement. Compared to the control products, the measured PUFA ratio was significantly higher ($p < 0.05$) for both the letscho sausage (33.2%), the Kaiserroulade (65.4%) and the duck liver pâté (30%). The change can be explained mainly by the significant increase ($p < 0.05$) in the alpha-linolenic acid ratio.

Compared to the control products, the alpha-linolenic acid ratio of the letscho sausage increased from 1.19% to 7.01%, while the increase was from 0.88% to 7.89% in the case of the Kaiserroulade and from 1.40% to 5.65% for the duck liver pâté products.

As expected, the n-3 fatty acid proportion of the products was increased by the oil supplements. Accordingly, there was a favorable change in the ratio of n-6 to n-3 fatty acids in the products. In the case of all three product groups, best results (n-6/n-3) were achieved by the flax seed sprout oil supplement (1.4–2.5:1).

Changes in the amount of n-3 fatty acids were also evaluated from a product labeling point of view. For all three product groups, the energy content of 100 g of product exceeded the 100 kcal value greatly, therefore, to support the claim regarding the n-3 fatty acid content, both the alpha-linolenic acid content and the EPA + DHA content were compared to the energy content of the product (**Figures 1 and 2**). n-3 fatty acid levels necessary for the claims „omega-3 fatty acid source" and „rich in omega-3 fatty acids" are indicated by the dotted and continuous lines, respectively.

In accordance with the fatty acid composition – per 100 kcal energy content – the highest alpha-linolenic acid level was achieved by the products with flax seed sprout oil supplements. In this regard, letscho sausage was the best with a value of 644 mg/100 kcal, which is sufficient for using the claim „rich in omega-3 fatty acids". The value for the Kaiserroulade was 575 mg, and it was 504 mg for the duck liver pâté, both of which allow the use of the claim „omega-3 fatty acid source".

Looking at the EPA + DHA amount of the products, it can be stated that best results were achieved by the fish oil supplement. For all fish oil supplemented products, indication of the claim "omega-3 fatty acid source" on the label became available. However, the claim "rich in omega-3 fatty acids" can only be indicated on the label of the Kaiserroulade. The measured EPA + DHA content of the product was 99 mg/100 kcal. EPA + DHA contents of the letscho sausage and the duck liver pâté were below the level that allows for the use of this claim by 1 and 4 mg/100 kcal, respectively.

4.3. Microbiological testing

Microbiological test results of the shelf life experiments are summarized in **Table 5**. The data clearly show that the more important microbiological parameters of the finished products were not affected negatively by the oil supplements used, shelf lives did not decrease. Based on Commission Regulation (EC) No 2073/2005, all of the products met the microbiological criteria for foodstuffs **[30]**.

4.4. Organoleptic testing

Results of the organoleptic testing of letscho sausage are shown in **Table 6**. In terms of external appearance, there was no difference between the products: both the control products and the ones that received oil supplements were orange-brown in color, with a pinkish cut surface and uniform mosaicness.

In terms of both smell and taste, best results were achieved by the control products. Foreign taste was observed by the testers in the case of flax seed sprout oil supplement for the raw products and in the case of fish oil supplement for the cooked products. Highlighting the properties of harmonious taste and intensity of meat flavor, control products were given the highest scores, 7.1 and 5.7, respectively. Products that had received the fish oil supplement were deemed to be the worst by testers. For harmonious taste the score was 5.1 and for intensity of meat flavor it was 3.9, which were different from the control product and the product with the flax seed sprout oil to a statistically verifiable extent. Product flavor was less negatively influenced by flax seed sprout oil, when compared to fish oil, however, a foreign taste was identified by testers in the case of both oil supplements.

There were observable differences in the consistency of the samples analyzed. It was statistically verifiable ($p < 0.05$) that products treated with oil supplements were softer. And, based on the oily feeling in the mouth, both experimental products were considered by testers to be too greasy.

The external appearance of the Kaiserroulade samples (**Table 7**) did not differ from each other significantly ($p > 0.05$). Cut surfaces of the samples were of bright pink color, showing less evenly distributed larger whitish and dark pink mosaic elements.

In terms of smell, samples were harmonious, there were only small differences reported by the testers. Nevertheless, significantly lower scores ($p < 0.05$) were awarded to products that had received the bio flax seed sprout oil supplement, in terms of harmonious smell, compared to control and fish oil supplemented products. In the case of meat odor intensity, both oil supplements resulted in significantly lower scores, while the smoke odor intensity score increased significantly in the case of the product supplemented with fish oil. No foreign smell was detected for any of the products.

The taste of the control and the fish oil supplemented Kaiserroulades was judged by testers to be more harmonious than that of one with linseed oil, to a statistically verifiable extent. Smoky flavor improved as a result of the oil supplements, however, in the case of the bio flax seed sprout oil supplement, a foreign taste was detected by the testers.

The consistency of the samples was sufficiently soft, easy to chew. The consistency of the control sample was slightly more compact than that of the products that had received oil supplements, which was judged by the testers to be better to a statistically verifiable extent. The product with bio flax seed sprout oil proved to be significantly ($p < 0.05$) more greasy than both the control product and the one with the fish oil.

Duck liver pâté samples that had received oil supplements (**Table 8**) differed significantly ($p < 0.05$) from control samples in terms of external appearance, but these differences were negligible, based on the written evaluations of the testers.

In terms of smell, all the samples were harmonious, with a recognizable liver smell and a slightly smoky, spicy character. Control samples scored significantly higher than the products with fish oil or flax seed sprout oil in terms of harmonious smell and liver smell, and higher than the products with flax seed sprout oil in terms of smoky smell. No foreign smell was detected for any of the products.

The taste of the samples was harmonious, slightly smoky and spicy, recognizably livery and free of foreign taste. In terms of liver taste and spiciness, the highest score was awarded to the control sample, and this was significantly higher than those of the samples with fish oil. The taste of the duck liver pâté was not affected in a statistically verifiable way by the bio flax seed sprout oil supplement.

There was no observable difference between the consistencies of the samples tested. The products were suitably greasy, sufficiently soft and easy to spread.

5. Conclusions

Based on our results, the following conclusions and recommendations can be formulated:

- Oil supplements (bio flax seed sprout, fish oil) did not alter significantly the chemical compositions of the products. The resulting differences cannot be considered significant from a nutritional physiology point of view.

- The alpha-linolenic acid content of 644 mg/100 kcal of the letscho sausage receiving the flax seed sprout oil supplement satisfies the requirement for using the claim “rich in omega-3 fatty acids”.

- The values for the Kaiserroulade and the duck liver pâté supplemented with flax seed sprout oil were 575 and 504 mg, respectively, allowing the use of the claim “omega-3 fatty acid source”.

- The measured EPA + DHA content of the Kaiserroulade supplemented with fish oil was 99 mg/100 kcal, enough to claim “rich in omega-3 fatty acids” on the label.

- The 79 and 76 mg/100 kcal EPA + DHA contents of the letscho sausage and the duck liver pâté supplemented with fish oil, respectively, allow for the use of the claim “omega-3 fatty acid source”.

- More important microbiological characteristics of the finished products were not affected negatively by the oil supplements used, shelf lives were not diminished.

- Supplements did not affect the appearance of the products negatively, however, foreign tastes and smells were detected by the testers in several instances, in the case of samples receiving fish oil or bio flax seed sprout oil supplements.

- For carrying out further experiments, the recommendation can be formulated for the manufacturer to use only enough flax seed sprout oil or fish oil in its recipe that is sufficient to reach ALA, EPA and DHA levels necessary for the use of the claim “omega-3 fatty acid source”. This way, negative effects of the oil supplements could be minimized.

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