# INFLUENCE OF PARTIAL REPLACEMENT OF PORK FAT BY FISH OIL ON THE FATTY ACIDS PROFILE IN LIVER PÂTÉ

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**Abstract – Liver pâté was reformulated to produce better lipids composition (with healthier fatty acid profile). The pork back fat was replacement by fish oil at 50 and 75% of total fat. As expected, the results indicate that the fat replacement highly affect the fatty acids profile. The amount of total n-3, and in particular the amounts of EPA, DPA and DHA increased as increase the proportion of fish oil in the pâté. The major increase was obtained for DHA content, which increased from 0.13 g/100 g of fat in control batch to 8.00 and 11.92 g/100 g of fat in 50 and 75% batches, respectively. In contrast, the incorporation of oil caused a decrease in the content of SFAs (due to the decrease of C16:0 and C18:0), MUFAs (due to the decrease of C18:1n-9c) and n-6 PUFAs (due to the decrease of C18:2n-6). The n-6/n-3 ratio also decreased from 12.81 in control batch to 0.92 and 0.45 in 50 and 75% batches, respectively. To sum up, replacing fat for fish oil in liver pâté can be a good alternative to balance the intake of PUFAs and reduce the intake of SFA in the diet, and thus improve the health of the consumer.**

**Key Words –fat sustitute, Pâté, Fish oil, Healthy meat product**

## I. INTRODUCTION

Healthier lipid formulation based on processing strategies is one of the most important current approaches to the development of potential meat-based functional foods [1]. According to Jiménez-Colmenero [1], there is growing evidence that dietary fat may play a role in the prevention and therapy for a number of chronic disorders, particularly coronary heart disease.

According to dietary recommendations [2] for the intake of specific fatty acids as a proportion of total diet energy, no more than 10% of calorie intake should be from saturated fatty acids (SFAs), 6–10% should be from polyunsaturated fatty acids (PUFAs) (n-6, 5–8%; n-3, 1–2%), around 10–15% should be from monounsaturated fatty acids (MUFAs), and less than 1% should be from *trans* fatty acids. In addition, there is abundant evidence to suggest that regular consumption and/or dietary supplementation with long chain n-3 PUFAs (eicosapentaenoic – EPA C20:5n-3, docosapentaenoic – DPA C22:5n-3 and docosahexaenoic – DHA C22:6n-3 acids) confers a number of health benefits [3,4]. So, the aim of this work was to study the effect of the pork fat replacement by fish oil (with high amounts of long n-3 PUFAs) on fatty acid profile and nutritional indices.

## II. MATERIALS AND METHODS

### II.1 Manufacture of the liver Pâté

The pâtés were prepared in the pilot plant of the Meat Technology Center of Galicia according the methodology described by Lorenzo & Pateiro [5]. The recipe used for the preparation of liver pâtés is presented in the Table 1. The day before the preparation, liver and meat were ground through 10 mm diameter mincing plate in a cooled chopped (La Minerva, Bologna, Italy) at 4 °C and mixed with the nitrificant ingredients (sodium chloride, sodium nitrite and sodium ascorbate). This blend was kept in darkness and refrigerated until the following day. On the day of manufacture, the fat was chopped using the same conditions used for the meat and liver, and heated in water to

65 °C. Then, the remaining ingredients were added, sodium caseinate to the heated fat, and water, milk powder and potassium phosphates to the meat mixture. Finally, both mixtures were blended to obtain a homogeneous raw paste. The liver pâtés were packed in glass containers prior to thermal treatment (80 °C/30′). The samples were cooled in a blast chiller (-21 °C/30′) and then analyzed.

Table 1 Recipe used for the preparation of liver pâtés with different fish oil contents

| | Fat replacement | | |
|---|---|---|---|
| **Ingredients (%)** | **0%** | **50%** | **75%** |
| Pork meat | 20 | 20 | 20 |
| Pork liver | 33 | 33 | 33 |
| Pork back fat | 30 | 15 | 7.5 |
| Fish oil | 0 | 15 | 22.5 |
| Water | 11.5 | 11.5 | 11.5 |
| Sodium chloride | 2 | 2 | 2 |
| Milk powder | 2 | 2 | 2 |
| Sodium caseinate | 1 | 1 | 1 |
| Potassium phosphate | 0.5 | 0.5 | 0.5 |
| Sodium nitrite | 0.05 | 0.05 | 0.05 |
| Sodium ascorbate | 0.025 | 0.025 | 0.025 |

II.2 Fatty acid analysis

Total lipids were extracted from 15 g of each sample, according to Folch *et al.* [6] procedure. Fifty milligrams of fat was used to determine fatty acid profile. For the fatty acids transesterification 4 mL of a sodium methoxide (2%) solution were added to the fat, vortexed every 5 min during 15 min at room temperature, then 4 mL of a $H_2SO_4$ solution (in methanol at 33%), vortexed a few seconds and vortexed again before adding 2 mL of distilled water. Organic phase (containing fatty acids methyl esters) was extracted with 2.5 mL of hexane.
Separation and quantification of FAME's was carried out using a gas chromatograph, GC-Agilent 6890N (Agilent Technologies Spain, S.L., Madrid, Spain) equipped with a flame ionization detector and an automatic sample injector HP 7683, and using a Supelco SPTM-2560 fused silica capillary column (100 m, 0.25 mm i.d., 0.2 μm film thickness, Supelco Inc, Bellafonte, PA, USA). Chromatographic conditions were the same as described by Lorenzo *et al.* [7]. Individual FAMEs were identified by comparing their retention times with those of authenticated standards (Supelco 37 component FAME Mix) and the results were expressed as g/100 g of fat.

II.3 Statistical analysis

The effect of the pork fat replacement by fish oil on fatty acid profile and nutritional indices was examined using a mixed-model ANOVA, where the fatty acid profile and nutritional indices was set as dependent variables, the pork fat replacement by fish oil as fixed effect, and replicate as random effect. The pairwise differences between least-square means were evaluated by Duncan's method. Differences were considered significant if $P<0.05$. The values were given in terms of mean values and standard error (SEM). All analyses were conducted using the IBM SPSS Statistics 19.0 program (IBM Corporation, Somers, NY, USA) software package.

III. RESULTS AND DISCUSSION

The influence of the partial replacement of pork fat by fish oil on fatty acid profile is shown in Table 1. As expected, statistical analysis indicated that fatty acid profile was highly modified by the inclusion of fish oil.
In the pâtés without fat replacement and with fat replacement at 50% MUFAs were the major fatty acids (39.07 and 32.42 g/100 g of fat for 0 and 50% batches, respectively) followed by SFAs (30.14 and 24.27 g/100 g of fat for 0 and 50% batches, respectively) and finally PUFAs (19.15 and 22.64 g/100 g of fat for 0 and 50% batches, respectively). In contrast, in the pâtés with 75% of replacement MUFAs were the major group (29.58 g/100 g of fat) followed by PUFAs (22.16 g/100 g of fat) and SFAs (22.16 g/100 g of fat).
The most affected fatty acids were n-3 PUFAs. In this study the content of total n-3 increased from 1.38 g/100 g of fat in control batch to 16.86 g/100 g of fat for batch with 75% of replacement. This increase was directly related to the contents of C20:5n-3 (0.04 *vs.* 1.48 *vs.* 2.73 g/100 g of fat for 0, 50 and 75% batches, respectively), C22:5n-3 (0.17 *vs.* 1.04 *vs.* 1.46 g/100 g of fat for 0, 50 and 75% batches, respectively), and mainly with the

amounts of C22:6n-3 (0.13 *vs.* 8.00 *vs.* 12.92 g/100 g of fat for 0, 50 and 75% batches, respectively). These results agree with those reported by Jiménez-Colmenero *et al.* [8], who also found an increase of these fatty acids with the addition of fish oil on dry fermented sausages.

In contrast, the content of n-6 PUFAs decreased from 17.59 to 7.58 g/100 g of fat, mainly due to the decrease of C18:2n-6 amount (15.94 *vs.* 8.40 *vs.* 4.87 g/100 g of fat for 0, 50 and 75% batches, respectively) and, to a lesser extent due to the content of C20:2n-6. On the contrary, the amount of C20:4n-6 increased ($P$<0.001) as increase the proportion of fish oil in liver pâté.

Regarding to SFA content, as commented above, the increase of fish oil decreased ($P$<0.001) these fatty acids. The major differences were observed in the amounts of C16:0 (from 18.59 to 13.63 g/100 g of fat) and C18:0 (from 9.61 to 5.25 g/100 g of fat). To this regards, Jiménez-Colmenero *et al.* [8] also found a decrease of these fatty acids as increase the fish oil replacement in dry-fermented sausages. Finally, MUFAs content also decreased with the incorporation of fish oil in pâté. This decrease was only related with the amount of C18:1n-9c, which decreased from 33.51 to 18.40 g/100 g of fat. In fact, the content of the others MUFAs (C16:1n-7, C18:1n-7c, C20:1n-9 and C22:1n-9) increased as increase the level of fat replacement.

In this study the highest amounts of n-3 PUFAs and some MUFAs were described in pâté with fat replacement. This fact is related, as commented above, with the content of EPA (3.71 *vs.* 0.01 g/100 g of fat), DPA (1.87 *vs.* 0.10 g/100 g of fat) and DHA (16.29 *vs.* 0.04 g/100 g of fat) was higher in fish oil than in back fat pork. These results agree with Jiménez-Colmenero [1] who found that the long chain n-3 PUFAs (EPA, DPA and DHA) are found largely in seafood. Moreover, the increase of other fatty acids such as C20:4n-6, C16:1n-7, C18:1n-7c, C20:1n-9 and C22:1n-9 as increase fat replacement also is related with the higher amounts of these fatty acids in fish oil (data not shown).

Table 2 Fatty acid profile (g/100g of fat) of the different pâtés

| | *Fat replacement* | | | | |
|---|---|---|---|---|---|
| Fatty acids | 0% | 50% | 75% | SEM | Sig |
| C10:0 | $0.05^c$ | $0.02^b$ | $0.01^a$ | 0.002 | *** |
| C12:0 | $0.06^c$ | $0.04^b$ | $0.03^a$ | 0.002 | *** |
| C14:0 | $1.08^a$ | $1.54^b$ | $1.76^c$ | 0.048 | *** |
| C14:1n-5 | $0.03^a$ | $0.07^b$ | $0.09^c$ | 0.004 | *** |
| C15:0 | $0.09^a$ | $0.28^b$ | $0.37^c$ | 0.020 | *** |
| C16:0 | $18.59^c$ | $14.99^b$ | $13.63^a$ | 0.357 | *** |
| C16:1n-7 | $1.93^a$ | $2.64^b$ | $2.99^c$ | 0.075 | *** |
| C17:0 | $0.53^b$ | $0.50^a$ | $0.51^a$ | 0.003 | *** |
| C17:1n-7 | 0.37 | 0.37 | 0.38 | 0.001 | n.s. |
| C18:0 | $9.61^c$ | $6.46^b$ | $5.25^a$ | 0.312 | *** |
| C18:1n-9t | $0.29^b$ | $0.34^c$ | $0.22^a$ | 0.009 | *** |
| C18:1n-7t | $0.22^b$ | $0.19^a$ | $0.29^c$ | 0.007 | *** |
| C18:1n-9c | $33.51^c$ | $23.06^b$ | $18.40^a$ | 1.072 | *** |
| C18:1n-7c | $2.05^a$ | $2.09^b$ | $2.13^c$ | 0.008 | *** |
| C18:2n-6c | $15.94^c$ | $8.40^b$ | $4.87^a$ | 0.782 | *** |
| C20:0 | $0.12^a$ | $0.31^b$ | $0.41^c$ | 0.020 | *** |
| C20:1n-9 | $0.65^a$ | $2.72^b$ | $3.71^c$ | 0.216 | *** |
| C18:3n-3 | $0.92^c$ | $0.68^b$ | $0.56^a$ | 0.026 | *** |
| C18:2n-7 | $0.18^c$ | $0.14^b$ | $0.11^a$ | 0.005 | *** |
| C20:2n-6 | $0.59^c$ | $0.53^b$ | $0.50^a$ | 0.006 | *** |
| C22:0 | $0.00^a$ | $0.04^b$ | $0.05^c$ | 0.003 | *** |
| C20:3n-6 | $0.14^a$ | $0.15^b$ | $0.16^c$ | 0.002 | *** |
| C22:1n-9 | $0.01^a$ | $0.51^b$ | $0.76^c$ | 0.053 | *** |
| C20:3n-3 | $0.11^a$ | $0.17^b$ | $0.19^c$ | 0.006 | *** |
| C20:4n-6 | $0.86^a$ | $1.62^b$ | $1.95^c$ | 0.078 | *** |
| C23:0 | $0.01^a$ | $0.08^b$ | $0.13^c$ | 0.011 | *** |
| C22:2n-6 | $0.01^a$ | $0.04^b$ | $0.05^c$ | 0.003 | *** |
| C20:5n-3 | $0.04^a$ | $1.84^b$ | $2.73^c$ | 0.189 | *** |
| C24:1n-9 | $0.01^a$ | $0.42^b$ | $0.62^c$ | 0.043 | *** |
| C22:5n-3 | $0.17^a$ | $1.04^b$ | $1.46^c$ | 0.091 | *** |
| C22:6n-3 | $0.13^a$ | $8.00^b$ | $11.92^c$ | 0.829 | *** |
| SFA | $30.14^c$ | $24.27^b$ | $22.16^a$ | 0.577 | *** |
| MUFA | $39.07^c$ | $32.42^b$ | $29.58^a$ | 0.681 | *** |
| PUFA | $19.15^a$ | $22.64^b$ | $24.56^c$ | 0.386 | *** |
| n-6 | $17.59^c$ | $10.77^b$ | $7.58^a$ | 0.708 | *** |
| n-3 | $1.38^a$ | $11.73^b$ | $16.86^c$ | 1.090 | *** |
| n-6/n-3 | $12.81^c$ | $0.92^b$ | $0.45^a$ | 0.969 | *** |

[a-c] Mean values in the same row (corresponding to the same parameter) not followed by a common letter differ significantly ($P$<0.05)
Sig: significance: *** ($P$<0.001), n.s. (not significant)

Western diets are deficient in n-3 PUFAs (especially long chain) and contain excessive amounts of n-6 PUFAs, with an n-6/n-3 PUFA ratio of 15–20 as opposed to the recommended range of 1–4 [4]. Therefore, in order to improve the health status of the population, health agencies and professional organizations have issued recommendations to increase the consumption of food rich in n-3 PUFAs, as a means of promoting a reduction in the n-6/n-3 PUFA ratio [3,4]. In our study, as expected, the n-6/n-3 ratio decrease as increase the fat replacement. In fact, the values of this ratio decreased from 12.81 in control batch to 0.92 and 0.45 in 50 and 75% batches, respectively.
According to Kolanowski *et al.* [9] a reasonable estimate of optimal intake would be 0.8–1.4 g (or even more) for EPA and DHA, or 3–5.5 g for total n-3 PUFAs per day. Enrichment of food products with long chain n-3 PUFA by fish oil addition may significantly improve level and profile of PUFA in the diet.
According with the conclusions of Kolanowski & Laufenberg [10] it is expected that frequent consumption of foods enriched with n-3 PUFA of marine origin increase the amount of long chain n-3 PUFA in the diet, thus significantly improving its nutritional quality.

## IV. CONCLUSION

In view of the results and international recommendations, replacing pork fat for fish oil in liver pâté can be a good alternative to balance the intake of n-6 and n-3 fatty acids and reduce the intake of SFA in the diet, and thus improve the health of the consumer.

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