Effect of omega-3 microcapsules addition on the profile of volatile compounds in enriched meat products

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TITLE

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ABSTRACT

This work evaluated the influence of omega-3 enrichment by different types of fish oil microcapsules (monolayered (Mo) and multilayered (Mu)) on the profile of volatile compounds, with special interest on lipid oxidation markers, of different meat products (cooked (C-SAU) and dry-cured sausages (D-SAU)). For that, the volatile compounds of microcapsules and meat products were extracted by Solid-Phase Microextraction (SPME) and analyzed by Gas Chromatography-Mass Spectrometry (GC-MS). Significant differences have been found in the profile of volatile compounds between Mo and Mu, which has been reflected in the meat samples. Thus, in general, volatile compounds from lipid oxidation have shown higher abundance in Mo and C-SAU and D-SAU enriched with this type of microcapsules, indicating that the wall of Mu (chitosan-maltodextrine) might protect the encapsulated bioactive compounds more effectively than that of Mo (maltodextrine). However, this finding is not reflected in the results of previous studies evaluating the sensory perception and oxidation stability of C-SAU and D-SAU batches, but it should be considered since unhealthy oxidation products can be formed in the enriched meat products with Mo. Thus, the addition of Mu as omega-3 vehicle for enriching meat products may be indicated.

KEY WORDS

Volatile compounds; fish oil microcapsule; dry-cured sausage; cooked sausage

1 INTRODUCTION

Numerous epidemiological studies suggest that a diet rich in omega-3 polyunsaturated fatty acids (ω-3 PUFAs), mainly eicosapentaenoic acid (EPA; C20:5 ω-3) and docosahexaenoic acid (DHA; C22:6 ω-3), has an important role in prevention and therapy of a series of chronic disorders [1], particularly coronary heart disease [2–5]. However, western diets are deficient in long chain ω-3 PUFAs and the consumption trends of sources rich in these fatty acids (mainly fish and seafood products) are currently static or declining [6]. Therefore, to improve the welfare state of the population, different professional organizations and health agencies have established a recommended intake of EPA plus DHA, around 0.25 g per person and day [7–10] and the European Union legislation have established the minimum level required of the sum of EPA and DHA to label a food as "source of ω-3 fatty acids" and "high in ω-3 fatty acids": 40 and 80 mg per 100 g and per 100 kcal, respectively [11].

Nowadays, there is a high consumption of meat products, 3-4 times per week [12]. This is in part due to the growing intake of "ready-to-eat" products, related to the current lifestyle. Meat and meat products contain a high amount of proteins of high biological value, but their lipid profile is sometimes questioned due to their high to moderate quantity of saturated fatty acids (SFA) and the higher content of ω-6 PUFA than ω-3 PUFA [13]. Hence, meat industries are interested in producing healthier meat products [14] and some studies have investigated the possibility of incorporating fish oils, as bulk or emulsified, in different foods [15–17]. However, the presence of numerous double bonds in ω-3 PUFAs cause a rapid oxidation in the presence of prooxidants such as iron, light, oxygen and high temperatures [18,19] that accelerate the formation of primary oxidation products, such as hydroperoxides, which easily isomerize and degrade to volatile compounds. Some of them impart undesirable off-fishy and rancid odors and flavors such as, 4-heptenal, 3,5-octadiene or 2-ethylfuran [20–22].

In this context, several studies have investigated the possibility of producing stable foods enriched with ω-3 PUFA microcapsules [15,23–25]. The microencapsulation technique is based on creating a physical barrier between active compounds and the environment, reducing the perception of off-flavors [26] and the contact with oxidant promoters [18,19]. Moreover, this technique is of easy application in the food industry, economic and scalable [27]. Recent studies have pointed out the possibility of adding fish oil microcapsules, to enrich different meat products [28,29].

Most studies on enrichment of meat products with ω-3 PUFAs microcapsules have focused on the evaluation of the proximal composition, oxidative stability, fatty acid profile and sensory attributes of the enriched foods [24,25,30,31], without taking into account the influence of the different processing conditions and microcapsules addition on the volatile compounds profile of these meat derivatives, except for a previous study in chicken nuggets [32].

The main objective of the present study was to evaluate the profile of volatile compounds of cooked and dry cured meat products enriched with monolayered and multilayered fish oil microcapsules, taking special attention on those from oxidation processes. The profile of volatile compounds of the fish oil microcapsules was also aimed.

2 MATERIAL AND METHODS

2.1 Experimental Design

Two different meat products were elaborated, cooked (C-SAU) and dry-cured sausages (D-SAU), which were added with monolayered (Mo) (C-SAU-Mo and D-SAU-Mo) and multilayered (Mu) microcapsules (C-SAU-Mu and D-SAU-Mu), modifying the formulation of the batter by the addition of 2.75 % (w/w) of Mo and 5.26% (w/w) of Mu. A control batch (without enriching) of each meat product was also prepared (C-SAU-Co, D-SAU-Co). In both products, the quantity of Mo and Mu added was 3 and 5 g per 100 g of dough, respectively. These figures were calculated to excess the required quantity of EPA + DHA to label a food as "source of ω-3 fatty acids": at least 40 mg of the sum of EPA and DHA per 100 g and per 100 Kcal [11].

The profile of volatile compounds was analysed in the three batches of C-SAU and D-SAU, and also in Mo and Mu.

2.2 Preparation of omega-3 sources

Fish oil from cod liver with initial peroxide value < 10 meq kg^{-1} and a percentage of 5.96% EPA and 25.83% DHA, kindly provided by Biomega Nutrition (Galicia, Spain), was used as source of ω-3

PUFAs to prepare Mo and Mu microcapsules, according with the methodology of (Jiménez-Martín *et al.*, 2014) with slight modifications. The procedure started with the production of fish oil Mo and Mu emulsions. Fish oil (20 g) and soybean lecithin (6 g), provided by Across Organics (Madrid, Spain), were mixed with a magnetic stirrer overnight. Then, water was added until a total weight of 200 g and homogenized (20000 rpm, 10 min) using an Ultraturrax T-18 basic (IKA, Germany). In this way, the primary emulsion was obtained and then homogenized at high-pressure (SPX, model APV-200a, Silkeborg, Denmark) under the conditions previously optimized, 1200 Ba and 3 passes for Mo and 1100 and 2 passes for Mu [29]. The primary emulsion was blended with 200 g of water, in the case of Mo, and with 200 g of 1 % of chitosan (w/w) with 95 % of deacetylation (Chitoclear FG 95, kindly provided by Trades, Murcia, Spain) in acetic acid 1 %, in the case of Mu, by slowly agitation with a magnetic stirrer for 15 min. In both types of emulsions, the final step consists on adding 400 g of maltodextrin solution (120 g maltodextrin + 280 g water) with a dextrose equivalent of 12 % (Glucidex 12, kindly provided Roquette, Lestrem, France), to obtain the feed emulsion. The Mo and Mu emulsions obtained (800 g) were dried in a laboratory-scale spray drier equipped with a 0.5-mm nozzle atomizer (Mini spray-dryer B-290, Buchi, Switzerland). The emulsions, maintained at room temperature, were constantly and gently agitated in a magnetic stirrer during the spray drying process. The aspirator rate was adjusted at 80 %, feed rate was 1 L/h, inlet temperature was 180 °C, and outlet temperature ranged 85–90 °C. The collected dried powders were stored in containers at 4 °C until being added to the meat products.

2.3 Elaboration of meat products

C-SAU were elaborated with meat mechanically separated from chicken, water, pork fat, salt, pork plasma, stabilizer (E-450), aromas, vegetable fiber, spices, spice extracts, smoke flavor, antioxidant (E-316) and preservative (E-250) and the corresponding microcapsules in the case of the enriched batches, added in the knead phase. All C-SAU batches were stuffed in 18 mm diameter cellulose casings, heated in a water bath at 85 °C during 30 min and allowed to cool at 7 °C during 1 hour. After that, frankfurters were vacuum packed and stored at refrigeration temperatures (0-5 °C) for 7-9 days until been analyzed. Formulation and manufacture of these products were made in a meat industry (remain anonymous). All batches were analyzed after heating at 90 °C during 3 min.

D-SAU was elaborated with Iberian pork meat and fat, which were ground through a 6 mm diameter mincing plate. The rest of ingredients: salt, dextrose, soy protein, spices, aromas, stabilizers (E-451 and E-450), antioxidant (E-301), preservatives (E-252 and E-250), enhancer flavor (E-621), coloring (E-120) and the corresponding microcapsules in the case of the enriched batches were added and mixed for 3 minutes and kept at 4 °C until stuffed. No starter culture was added. The obtained dough was stuffed into collagen casings with a length of 40 cm and a diameter of 60 mm. The sausages followed a dry-cured process under controlled conditions of 4 °C and 82% of relative humidity for 3 days. After this period, the samples were 21 days in the drying-curing chamber at 8 °C and 80% of relative humidity and 14 days in a cellar at 5 °C and 85% humidity, until reaching a percentage of loss of 38-40%. The dry-cured sausages were stored at ambient temperature (18-20 ºC for 7-9 days) until been analyzed. Formulation and manufacture of these products were also made in a meat industry (remain anonymous).

2.4 Analysis of volatile compounds

One g of microcapsules and minced meat products was weighed into a 10 mL glass flask (Hewlett-Packard, Palo Alto, Calif., U.S.A.) sealed with an aluminum cap and PTFE butyl septum (Perkin-Elmer, Foster City, Calif., U.S.A.). Volatile compounds were extracted by solid-phase microextraction (SPME) following the method described by [34] with some modifications. SPME was carried out by using a cross-linked carboxen/polydimethylsiloxane with a 10 mm long and 100 µm thick (Supelco, Bellefonte, Pa., U.S.A.), conditioned at 220 °C for 50 min prior to use by heating in the gas chromatograph (GC) injection port. Absorption was done in a water bath at 40 °C, introducing the fiber in a sealed vial for 30 minutes. The SPME fiber was desorbed and maintained in the injection port for 30 min. Analyses were performed using a Hewlett–Packard 6890 series II GC coupled to a mass selective detector (HP 5973) (Hewlett-Packard, Wilmington, DE, USA). Volatiles were separated using a 5% phenyl–95% polydimethylsiloxane column (30 m × 0.32 mm ID, 1.05 µm film thickness, Hewlett-Packard), operating at 6 psi of column head pressure, resulting in a flow of 1.3 mL min^{-1} at 40 °C. The injection port was in a splitless mode. The temperature program was isothermal for 15 min at 35 °C, increased to 150 °C at 4 °C min−1, and then to 250 °C at 20 °C min−1. The transfer line to the mass spectrometer was maintained at 280 °C. The mass spectra was obtained using a mass selective detector by electronic impact at 70 eV, a multiplier voltage of

1756 V and collecting data at a rate of one scan over the m/z range of 30–550 u.m.a. N-alkanes (Sigma R-8769) were analyzed under the same conditions to calculate the linear retention indexes (LRIs) for the volatile compounds. Compounds were identified by comparison of mass spectrum with database (NIST and Wiley libraries) and by comparison of their LRI with those available in the literature [20,21,43–52,35,53–58,36–42]. Results from volatile analyses are provided in area units (AU).

2.5 Sampling replication and statistical analysis

Replicate experimental samples (n = 3) of Mo and Mu microcapsules and of the three batches (Co, Mo and Mu) of meat products (C-SAU and D-SAU) were analyzed in triplicate. In the meat products, the effects of the addition of different types of fish oil microcapsules and the differences between microcapsules were evaluated by one-way analyses of variance (ANOVA). When a significant effect ($p<0.05$) was detected, paired comparisons between means were conducted using the Tukey's test. A data reduction procedure (Factor analysis) was used to create two-dimensional principal component analysis score plots (PCA) for all volatile compounds that showed significant differences in the ANOVA analysis. The original data were normalized and orthogonal and linear combinations of the original variables were classified depending on the level of information they produced in the first two components: PC1 (the axis, containing the largest possible amount of information of the variance of the data) and PC2 (perpendicular to PC1). The loading of each selected volatile compound on the two first principal components were plotted and the average scores of the three batches (Co, Mo and Mu) in both meat products (C-SAU and D-SAU) on the two first principal components were plotted. The statistics were run using the program IBM SPSS Statistics v.22.

3 RESULTS AND DISCUSSION

3.1 Volatile compounds in fish oil microcapsules

A total of 40 volatile compounds have been identified in the Mo and Mu fish oil microcapsules, which have been grouped in the following chemical families: aliphatic hydrocarbons, alcohols, aldehydes, ketones, furans and acids. Figure 1 shows the area percentage of each chemical family in

Mo and Mu. In both types of fish oil microcapsules, aliphatic hydrocarbon was the major chemical family (around 88 %), followed in decreasing order by aldehydes (around 6.7 %), ketones (around 3 %), alcohols (around 1.5 %), furans (around 0.5 %) and acids (around 0.2 %). This profile of volatile compounds is quite according to a previous study with double and multilayered fish oil microcapsules [43]. The percentage of aliphatic hydrocarbons has been used as an indicator of quality and stability of different commercial fish oils, from salmon, tuna, sardines and shrimp. Considering the relationship between the decrease in the percentage of this family of volatile compounds with an increase of lipid oxidation [58], the high percentage of aliphatic hydrocarbons in Mo and Mu may support the protective effect of the wall materials of these types of microcapsules, minimizing the contact and reactivity of fish oil with oxidizing promoters. Significant differences have been detected in the percentage of the most chemical families of volatile compounds between the Mo and Mu of this study, showing Mo higher percentages of aldehydes (8.43 % vs 4.78 %), ketones (3.82 % vs 2.65 %), alcohols (1.97 % vs 1.10 %) and lower of aliphatic hydrocarbon (87.89 vs 90.43 %) and acids (n.d. vs 0.32 %) than Mu. Accordingly, [59] also found significant differences in the percentage of chemical families of volatile compounds between different types of fish oil microcapsules.

Table 1 listed the individual volatile compounds of Mo and Mu, being expressed as AU x 10^6. Hexane was the major volatile compound in Mo and Mu (around 87.4 AU x 10^6), followed by pentane (around 19.7 AU x 10^6), hexanal (around 2.4 AU x 10^6) and 3-hydroxy-2-butanone (around 2.13 AU x 10^6), and the rest of individual volatile compounds showing less than 1 AU x 10^6. This agrees with results described for double and multilayered fish oil microcapsules [43]. From the 40 individual volatile compounds identified in the fish oil microcapsules of the present study, 9 of them were found in Mu but not in Mo (tridecane, 1,2,4-butanetriol, phenylethyl alcohol, 2-heptenal, 2-octenal, 2-nonanone, 2-butyltetrahydrofuran, heptanoic and sorbic acid) and 7 were only detected in Mo (1-heptene, heptane, decane, 2-propanol, 4-hexen-1-ol, 1-heptanol and 2-hexenal).

Significant differences were found in most individual volatile compounds between Mo and Mu (Table 1). Regarding the aldehydes, which have been described as the most important indicators of fish oil oxidation [58], Mo showed significant higher levels of propanal, pentanal, hexanal, 2-hexenal, heptanal, octanal and nonanal compared to Mu. It has been described that propanal comes from the lipid hydroperoxides derived from ω-3 PUFA while hydroperoxides derived from ω-6 PUFA mainly generates hexanal, as consequence of the breakdown of the first double bond of the n position of the ω-3 and ω-6 fatty acids, respectively [60]. Besides, hexanal has been used in previous studies as a marker to measure the quality and oxidative stability of fish oil microcapsules [20]. On the other hand, Mu had significant higher AU of 2-pentenal and 2-octenal than Mo, but these two volatile compounds have not been related to the oxidation of ω-3 PUFAs. In addition, other relevant indicators of fish oil oxidation, such as 2,4-heptadienal and 2,4-decadienal, which have been associated to the perception of rancid flavor [20,55,60], or other aldehyde volatile compounds related to rancid odors, such as decanal or 2-nonenal [20,55,61], have not been identified in Mo or Mu.

Most individual alcohols and ketones have shown significant differences between Mo and Mu, with higher AU in Mo in comparison to Mu in most cases. However, as our knowledge, either of the ketones detected in Mo and Mu are associated with lipid oxidation process. Most important ketones from lipid autoxidation reactions are 3,5-octadien-2-one and 1-octen-3-one [56]. In fact, they have been detected in mayonnaise and milk enriched with fish oil, being strongly correlated with the strength of the oxidation process [55,57]. However, these ketones have not detected in Mo or Mu. Regarding the alcohols, 1-penten-3-ol and 2-penten-1-ol have been described as one of the most characteristics oxidation markers for PUFA [55,56]. 1-penten-3-ol has been detected in both types of microcapsules, with higher AU in Mo than in Mu, while 2-penten-1-ol was not found in these fish oil microcapsules. Other common oxidation product of ω-3 PUFA is 2-ethylfuran, which can be generated from the 12-hydroperoxide of EPA and 16-hydroperoxide of DHA [43,62]. This volatile compound has been identified in both types of microcapsules, having Mo significantly higher AU than Mu. Two acids volatiles compounds, heptanoic and sorbic acid, have been found in Mu, but not in Mo. Heptanoic acid at high concentrations impart unpleasant rancid odor, but AU of this compound in Mu are very low.

The higher AU in some individual volatile compounds related to lipid oxidation found in Mo in comparison to Mu could be explained by the different wall material of these fish oil microcapsules, being of maltodextrine and of chitosan plus maltodextrine, respectively. In fact, it has been described that chitosan increases the electrostatic force and viscosity of the layers [63], avoids the oxidative damage and could act as a free scavenger [64]. Thus, the Mu coating may be more effective than Mo to protect fish oil from oxidative damage. This aspect can be marked in the case of volatile compounds as hexanal, 1-penten-3-ol and 2-ethylfuran, with low odor thresholds (4.5, 1 and 2.2 μg Kg-1 oil, respectively) and associated with sensory defects [65,66]

Nevertheless, in comparison to the profile of volatile compounds in bulk fish oil, Mo and Mu have not shown polyunsaturated lipid oxidation products with rancid taste perceptions, such as 2,4-heptadienal, 2,4-decadienal, 2-nonenal, 3,5-octadien-2-one and 1-octen-3-one [58,67], which points out the effectiveness of the Mo and Mu microcapsules of the present study to minimize the contact and reactivity of fish oil encapsulated with oxidizing promoters.

3.2 Volatile compounds in dry-cured and cooked sausages enriched with fish oil microcapsules

A total of 53 and 60 volatile compounds have been identified in D-SAU and C-SAU of the present study, respectively, which have been grouped in the following chemical families: aliphatic hydrocarbons, alcohols, aldehydes, furans, ketones, terpenes, acids, esters, aromatics, cyclic hydrocarbons and pyrazines. Figures 2.a and 2.b show the percentages of these chemical families of volatile compounds in D-SAU and C-SAU as affected by type of fish oil microcapsule addition, respectively. The most abundant families in all batches of D-SAU were acids (around 65.1 %) and aldehydes (around 14.5 %), followed far behind by terpenes (around 7.2 %) and esters (around 6.9 %). Minor percentages were found for aliphatic hydrocarbons (around 2.7 %), aromatics (around 1.6%), ketones (around 1 %), cyclic hydrocarbons and alcohols (around 0.9 %) and furans (around 0.8 %). This profile is quite in concordance with previous studies in fermented sausages [37,42]. Moreover, significant differences have been detected in the chemical families of volatile compounds between the D-SAU batches of this study, with Mo showing higher percentages of aldehydes and terpenes (16,03 and 8.2) than Co and Mu batches, and Mu having higher percentage of acids (69,63) and lower of esters (5,58) than Co and Mo.

In C-SAU, the major family of volatile compounds was aldehydes (around 24.7 %) followed by aliphatic hydrocarbons (around 15.1 %), cyclic hydrocarbons (around 14.8 %) and alcohols (around 12.4 %), while minor abundance was detected for terpenes (around 7.6 %), acids (around 6.9 %), esters (around 6.5 %), aromatics (around 5.2 %), ketones (around 4.7 %), furans (around

1.4 %) and pyrazines (around 0.6 %). This agrees with the profile of volatile compounds previously reported in other studies in cooked sausages [50,53]. Besides, significant differences have been found in the chemical families of volatile compounds between the C-SAU batches of this study, showing C-SAU-Mo higher percentages of aldehydes and alcohols than C-SAU-Co and C-SAU-Mu; C-SAU-Mu obtained higher percentage of acids and esters and lower of aliphatic hydrocarbons than C-SAU-Co and C-SAU-Mo; and the percentage of cyclic hydrocarbons was higher in C-SAU-Co than in the enriched batches. Thus, at first, considering the results on the percentage of volatile compounds, the differences between microcapsules are reflected in the bathes of meat products. Giving a step forward, the individual volatile compounds of the control and enriched batches of the meat products of the present study are following analyzed.

Table 2 listed the individual volatile compounds of D-SAU as affected by the type of fish oil microcapsule added. Acetic acid was the major volatile compound in all batches (around 470.3 AU x 10^6), followed by hexanal (around 162.7 AU x 10^6), methyl hexanoate (around 62.4 AU x 106), β-myrcene (around 39.1 AU x 106), pentanoic acid (around 35.9 AU x 10^6), butanoic acid (around 26.6 AU x 10^6) and heptanal (around 10.5 AU x 10^6), showing the rest of the individual volatile compounds less than 10 AU x 10^6. This profile is quite in concordance with previous studies in dry fermented sausages [37,39]. The high content of acetic acid would be related to microbial fermentation of carbohydrates [39,68]. Others compounds also typical of carbohydrate fermentation, such as 3-hydroxy-2-butanone, has also been detected in D-SAU batches [41]. It is also noted the high AU of β-myrcene in these samples, which may be ascribed to the addition of species [69].

The enrichment effect with Mo and Mu fish oil microcapsules significantly influence on the volatile compounds of most chemical groups (Table 2), excluding esters, aromatics and cyclic hydrocarbons. Only 13 in 53 volatile compounds identified in D-SAU showed significant differences among batches: C-SAU-Mo showed higher AU in 6 volatile compounds (1-propanone, 1-penten-3-ol, 1-octen-3-ol, pentanal, 3,5-octadien-2-one and acetic acid) and lower in 1 (butanal) in comparison to C-SAU-Co and C-SAU-Mu; C-SAU-Mu obtained higher abundance in 2 volatile compounds (heptane and β-mycene) and lower in 1 (3-hydroxy-2-butanone) than in C-SAU-Co and C-SAU-Mo, and C-SAU-Co showed higher AU in 1 volatile compound (2-penthyl-furan) and lower in 2 (1-

propanol and 2-ethylfuran) in comparison to the enriched batches. Figure 3.a represents the score plots of PCA of volatile compounds data from the D-SAU samples. The first principal component (PC1) comprised 55.48% of the total variance, and the second principal component (PC2) accounted for 29.47%. The score plot indicates a clear differentiation of samples as affected by the addition of fish oil microcapsules: those with high positive PC1 scores (D-SAU-Mo), those with high positive PC2 scores (D-SAU-Mu) and those with high negative PC2 scores (D-SAU-Co). Several volatile compounds (3,5-octadien-2-one, 1-propanol, pentanal, 1-octen-3-ol, 1-penten-3-ol, acetic acid, 2-ethylfuran, 1-propene, allyl sulphide) are located in the right quadrants (upper and lower), which corresponds to high positive charges in PC1, associated with D-SAU-Mo batch. On the other hand, there were a few volatile compounds allocated in the PC2: heptane, β-myrcene, and butanal in the left upper quadrant, and 2-pentyl-furan and 3-hydroxy-2-butanone in the left lower quadrant, related to D-SAU-Mu and D-SAU-Co, respectively. Thus, in comparison to C-SAU-Co and C-SAU-Mu, C-SAU-Mo are more related to typical volatiles compounds of fatty acid oxidation, such as 1-propanol, 1-octen-3-ol and pentanal [35], and to characteristic oxidation markers for PUFA oxidation, such as 1-penten-3-ol, 2-ethyl-furan and 3,5-octadien-2-one, which have been previously observed in mayonnaise and chicken nuggets enriched with fish oil [23,55], and are correlated with the strength of the oxidation process [55,57]. The low odor thresholds some of these volatile compounds, such as 1-octen-3-ol and 3,5-octadien-2-one (1 and 0.45 μg kg-1 of oil, respectively) [70,71], would lead to the perception of anomalous odor and/or flavor, may having a negative impact in the products enriched with Mo. Nevertheless, in a previous study carried out with the D-SAU samples of the present work [28], no significant differences in acceptability have been found among Co, Mo and Mu samples. So, the influence of the fish oil microcapsules addition on the profile of volatile compounds does not seem to be reflected in the consumer´s perception of D-SAU.

Table 3 showed the individual volatile compounds of C-SAU as affected by the type of fish oil microcapsule added. α-thujene was the major volatile compound in all batches (around 6.07 AU x 10^6), followed by pentanal (around 4.84 AU x 10^6), β-thujene (around 3.52 AU x 10^6), hexanal (around 3.5 AU x 10^6), 1-octen-3-ol (around 3.40 AU x 10^6), gamma-terpinene (around 3.20 AU x 10^6) and heptanal (around 3.15 AU x 10^6), and the rest individual volatile compounds showed less than 3 AU x 10^6. In previous studies in cooked sausages, hexanal has been identified as the most abundant volatile compound, followed by heptanal, pentanal and volatiles compounds from the chemical families of alcohols (1-pentanol and 1-octen-3-ol) and terpenes (limonene, β-myrcene, and gamma-terpinene) (Chevance and Farmer, 1999b; Chevance *et al.*, 2000; Estévez *et al.*, 2005), which is quite in agreement with the findings of the present study. However, in these previous works α-thujene and β-thujene were identified but with lower AU than in the present work. These compounds are associated with spicy flavor and have been found in a wide variety of medicinal herbs, essential oils, flavorings and spices such as nutmeg [72], therefore, its abundance in the present study could be related to the addition of spices in the meat product formulation.

In C-SAU, the addition of Mo and Mu fish oil microcapsules significantly influenced the volatile compounds of most chemical families (Table 3), excluding terpenes and pyrazines, finding significant differences in 27 volatile compounds. Higher abundance was found in 2 volatile compounds in C-SAU-Mo (1-pentanol and 2-decenal) than in C-SAU-Co and C-SAU-Mu. 9 volatile compounds (hexane, heptane, decane, tridecane, 1-hexanol, phenyl ethyl alcohol, acetic acid, nonanoic acid, methyl propanoate and methyl propanoate) obtained higher AU in C-SAU-Mu than in C-SAU-Co and C-SAU-Mo. On the contrary, C-SAU-Mu showed lower abundance for 2-octene, nonane, 1-octen-3-ol, 2-methyl-propanal, 3-heptanone, 2-buthyl-furan and octanoic acid than C-SAU-Co and C-SAU-Mo. In comparison to the enriched batches, C-SAU-Co showed higher AU for 6 volatile compounds (pentanal, 2-heptanone, 2-methyl-furan, pentanoic acid, β-thujene and α-thujene) and lower for 3 (dodecane, 4-hexen-1-ol and 1-heptanol). Score plot of PCA of volatile compounds data from the C-SAU samples is shown in Figure 3.b. The PC1 accounted for 54.78% of the total variance, and the PC2 comprised 34.19%. The score plot allowed a clear separation of the samples: those with high positive PC1 scores (C-SAU-Mu), those with high positive PC2 scores (C-SAU-Mo) and those with negative PC2 scores (C-SAU-Co). 2-decenal, 2-buthylfuran, 3-heptanone, 1-pentanol, 4-hexen-1-ol, 1-octen-3-ol, dodecane, nonane and methyl-propanoate were grouped and allocated in the upper quadrants (left and right), which corresponds to the C-SAU-Mo batch, while 2-heptanone, pentanal, 2-methylpropanal, β-thujene, α-thujene, 2-methylfuran and hexane, heptane, decane, 1-hexanol, 1-heptanol, phenyl-ethyl-alcohol were in the lower left and right quadrants, related to C-SAU-Co and C-SAU-Mu, respectively. Thus, in C-SAU, Mo enriched batches also showed a close relation with volatile compounds from lipid oxidation, such us 2-decenal, a characteristic volatile compound of ω-3 PUFA oxidation, and in 1-pentanol, 4-hexen-1-ol and 1-octen-3-ol, described as typical lipid oxidation products [50,53]. 2-decenal has been related in previous studies to rancid odors in fish oil enriched mayonnaise [55] and fish oil microcapsules [20]. This volatile compounds has a fatty and fishy aroma [71] with a low odor threshold, around 10 μg kg^{-1} oil [73], which may detriment the acceptability of the meat products added with Mo. However, as occurred in D-SAU, similar scores in the acceptability analysis have been found by [28] in the three batches of C-SAU, not being reflected the differences in the profile volatile compounds by the sensory results.

It is worth noting that the differences found in the present study in the profile volatile compounds depending on the type of fish oil microcapsules added. Anyway, it could be indicated a major protection of the microencapsulated fish oil against lipid oxidation when Mu are added. This can be ascribed to the different wall material in Mo (maltodextrine) and Mu (chitosan plus maltodextrine). Thus, the multilayer structured of chitosan-maltodextrine may protect the encapsulated material more effectively than the simple coating of maltodextrine. Indeed, chitosan improves the emulsion stability, increasing the electrostatic force and viscosity of the layers, and can also act as an antioxidant [63,64]. Moreover, a high oxidative stability has been found in microcapsules with chitosan [74]. So, although no marked effect on sensory or oxidation stability have been previously found [28], differences in the volatile compounds should be considered since they could release to unhealthy oxidized products [75]. This can be the case of furans, such us 2-ethylfuran, 2-butylfuran, 2-acetylfuran, 2-pentylfuran, 2-furfural and furfural alcohol, which have been found in different fish products [76,77], and are revealed toxicity in animals and humans [78,79]. In fact, 2-ethylfuran and 2-buthylfuran have been closely related to the D-SAU-Mo and C-SAU-Mo samples in this study. Considering this aspect, more studies should be claimed in this sense, for evaluating the formation of contaminants in different omega-3 enriched meat products.

4 CONCLUSIONS

The type of fish oil microcapsules influences on its profile of volatile compounds and on that of the enriched meat products. The use of multilayered microcapsules with chitosan-maltodextrine walls may be more protective to the formation of lipid oxidation products, especially from omega-3 fatty acids, than microcapsules with a maltodextrine layer. Thus, the use of multilayered fish oil emulsions to elaborate omega-3 microcapsules for enriching meat products may be indicated,

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AUTHOR CONTRIBUTIONS

Conceptualization, T. Antequera. and T. Pérez-Palacios.; Methodology, JC. Solomando, A. Martín, T. Pérez-Palacios and T. Antequera.; Software, A. Martín; Validation, JC. Solomando, T. Pérez-Palacios. and T. Antequera.; Formal Analysis, JC. Solomando; Investigation, T. Pérez-Palacios and JC. Solomando; Resources, T. Pérez-Palacios; Data Curation, JC. Solomando; Writing – Original Draft Preparation, JC. Solomando.; Writing – Review & Editing, T. Pérez-Palacios and T. Antequera; Visualization, JC. Solomando; Supervision, A. Martín, T. Pérez-Palacios; Project Administration, T. Pérez-Palacios; Funding Acquisition, T. Pérez-Palacios.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Schunck, W.H.; Konkel, A.; Fischer, R.; Weylandt, K.H. Therapeutic potential of omega-3 fatty acid-derived epoxyeicosanoids in cardiovascular and inflammatory diseases. *Pharmacol. Ther.* 2018.

2. Horrobin, D.F. Seafoods and fish oils in human health and disease. *Int. J. Cardiol.* **1989**, doi:10.1016/0167-5273(89)90287-8.

3. Ruxton, C.H.S.; Reed, S.C.; Simpson, M.J.A.; Millington, K.J. The health benefits of omega-3 polyunsaturated fatty acids: A review of the evidence. *J. Hum. Nutr. Diet.* 2004.

4. Schmidt, E.B.; Arnesen, H.; De Caterina, R.; Rasmussen, L.H.; Kristensen, S.D. Marine n-3

polyunsaturated fatty acids and coronary heart disease: Part I. Background, epidemiology, animal data, effects on risk factors and safety. *Thromb. Res.* 2005.

5. Yashodhara, B.M.; Umakanth, S.; Pappachan, J.M.; Bhat, S.K.; Kamath, R.; Choo, B.H. Omega-3 fatty acids: A comprehensive review of their role in health and disease. *Postgrad. Med. J.* 2009.

6. Lee, S.; Faustman, C.; Djordjevic, D.; Faraji, H.; Decker, E.A. Effect of antioxidants on stabilization of meat products fortified with n - 3 fatty acids. *Meat Sci.* **2006**, doi:10.1016/j.meatsci.2005.05.022.

7. Simopoulos, A.P. The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed. Pharmacother.* 2002, *56*, 365–379.

8. Garg, M.L.; Wood, L.G.; Singh, H.; Moughan, P.J. Means of delivering recommended levels of long chain n-3 polyunsaturated fatty acids in human diets. *J. Food Sci.* 2006, *71*.

9. European Food Safety Authority Reglamento (UE) 116/2010 que modifica al Reglamento (CE) 1924/2006 en lo relativo a la lista de declaraciones nutricionales. **2010**, 16–18.

10. International Society for the Study of Fatty Acids and Lipids *6th congress of the International society for the study of fatty acids and lipids*; 2004; Vol. 43;.

11. EU Commision Regulation (EU) N°116/2010 of 9 February 2010 amending Regulation (EC) N° 1924/2006 of the European Parliament and of the Council with regard to the list of nutrition claims. *Off. J. Eur. Union* **2010**, 16–18.

12. Godfray, H.C.J.; Aveyard, P.; Garnett, T.; Hall, J.W.; Key, T.J.; Lorimer, J.; Pierrehumbert, R.T.; Scarborough, P.; Springmann, M.; Jebb, S.A. Meat consumption, health, and the environment. *Science* 2018.

13. Nuernberg, K.; Fischer, K.; Nuernberg, G.; Kuechenmeister, U.; Klosowska, D.; Eliminowska-Wenda, G.; Fiedler, I.; Ender, K. Effects of dietary olive and linseed oil on lipid composition, meat quality, sensory characteristics and muscle structure in pigs. *Meat Sci.* **2005**, *70*, 63–74, doi:10.1016/j.meatsci.2004.12.001.

14. Jiménez-Colmenero, F. Healthier lipid formulation approaches in meat-based functional foods. Technological options for replacement of meat fats by non-meat fats. *Trends Food Sci. Technol.* **2007**, *18*, 567–578, doi:10.1016/j.tifs.2007.05.006.

15. Josquin, N.M.; Linssen, J.P.H.; Houben, J.H. Quality characteristics of Dutch-style fermented sausages manufactured with partial replacement of pork back-fat with pure, pre-emulsified or encapsulated fish oil. *Meat Sci.* **2012**, *90*, 81–86, doi:10.1016/j.meatsci.2011.06.001.

16. Cáceres, E.; García, M.L.; Selgas, M.D. Effect of pre-emulsified fish oil - as source of PUFA n-3 - on microstructure and sensory properties of mortadella, a Spanish bologna-type sausage. *Meat Sci.* **2008**, *80*, 183–193, doi:10.1016/j.meatsci.2007.11.018.

17. Delgado-Pando, G.; Cofrades, S.; Ruiz-Capillas, C.; Solas, M.T.; Triki, M.; Jiménez-Colmenero, F. Low-fat frankfurters formulated with a healthier lipid combination as functional ingredient: Microstructure, lipid oxidation, nitrite content, microbiological changes and biogenic amine formation. *Meat Sci.* **2011**, *89*, 65–71, doi:10.1016/j.meatsci.2011.03.022.

18. Miyashita, K.; Uemura, M.; Hosokawa, M. Effective Prevention of Oxidative Deterioration of Fish Oil: Focus on Flavor Deterioration. *Annu. Rev. Food Sci. Technol.* **2018**, *9*, 209–226, doi:10.1146/annurev-food-030117-012320.

19. Onwulata, C.I. Microencapsulation and functional bioactive foods. *J. Food Process. Preserv.* **2013**, *37*, 510–532, doi:10.1111/j.1745-4549.2012.00680.x.

20. Jónsdóttir, R.; Bragadóttir, M.; Arnarson, G. Oxidatively Derived Volatile Compounds in Microencapsulated Fish Oil Monitored by Solid-phase Microextraction (SPME). *J. Food Sci.* **2005**, doi:10.1111/j.1365-2621.2005.tb11465.x.

21. Jimenez-Alvarez, D.; Giuffrida, F.; Golay, P.A.; Cotting, C.; Destaillats, F.; Dionisi, F.; Keely, B. Profiles of volatile compounds in milk containing fish oil analyzed by HS-SPME-GC/MS. *Eur. J. Lipid Sci. Technol.* **2008**, doi:10.1002/ejlt.200700148.

22. Yang, K.M.; Cheng, M.C.; Chen, C.W.; Tseng, C.Y.; Lin, L.Y.; Chiang, P.Y. Characterization of volatile compounds with HS-SPME from oxidized n-3 PUFA rich oils via rancimat tests. *J.*

Oleo Sci. **2017**, doi:10.5650/jos.ess16157.

23. Jiménez-Martín, E.; Pérez-Palacios, T.; Carrascal, J.R.; Rojas, T.A. Enrichment of Chicken Nuggets with Microencapsulated Omega-3 Fish Oil: Effect of Frozen Storage Time on Oxidative Stability and Sensory Quality. *Food Bioprocess Technol.* **2016**, *9*, 285–297, doi:10.1007/s11947-015-1621-x.

24. Lorenzo, J.M.; Munekata, P.E.S.; Pateiro, M.; Campagnol, P.C.B.; Domínguez, R. Healthy Spanish salchichón enriched with encapsulated n − 3 long chain fatty acids in konjac glucomannan matrix. *Food Res. Int.* **2016**, *89*, 289–295, doi:10.1016/j.foodres.2016.08.012.

25. Aquilani, C.; Pérez-Palacios, T.; Sirtori, F.; Jiménez-Martín, E.; Antequera, T.; Franci, O.; Acciaioli, A.; Bozzi, R.; Pugliese, C. Enrichment of Cinta Senese burgers with omega-3 fatty acids. Effect of type of addition and storage conditions on quality characteristics. *Grasas y Aceites* **2018**, *69*, 235, doi:10.3989/gya.0671171.

26. Serfert, Y.; Drusch, S.; Schwarz, K. Sensory odour profiling and lipid oxidation status of fish oil and microencapsulated fish oil. *Food Chem.* **2010**, *123*, 968–975, doi:10.1016/j.foodchem.2010.05.047.

27. Felix, P.H.C.; Birchal, V.S.; Botrel, D.A.; Marques, G.R.; Borges, S.V. Physicochemical and Thermal Stability of Microcapsules of Cinnamon Essential Oil by Spray Drying. *J. Food Process. Preserv.* **2017**, doi:10.1111/jfpp.12919.

28. Solomando, J.; Antequera, T.; Perez-Palacios, T. Evaluating the use of fish oil microcapsules as omega-3 vehicle in cooked and dry-cured sausages as affected by their processing, storage and cooking. *Meat Sci.* **2020**, *162*, doi:https://doi.org/10.1016/j.meatsci.2019.108031.

29. Solomando, J.C.; Antequera, T.; Ruiz-Carrascal, J.; Pérez-Palacios, T. Improvement of encapsulation and stability of EPA and DHA from monolayered and multilayered emulsions by high-pressure homogenization. *J. Food Process. Preserv.* **2019**, doi:10.1111/jfpp.14290.

30. Pelser, W.M.; Linssen, J.P.H.; Legger, A.; Houben, J.H. Lipid oxidation in n - 3 fatty acid

enriched Dutch style fermented sausages. *Meat Sci.* **2007**, *75*, 1–11, doi:10.1016/j.meatsci.2006.06.007.

31. Solomando, J.C.; Antequera, T.; González-Mohíno, A.; Perez-Palacios, T. Fish oil/lycopene microcapsules as a source of eicosapentaenoic and docosahexaenoic acids: a case study on spreads. *J. Sci. Food Agric.* **2020**, *100*, 1875–1886, doi:10.1002/jsfa.10188.

32. Pérez-Palacios, T.; Ruiz-Carrascal, J.; Jiménez-Martín, E.; Solomando, J.C.; Antequera, T. Improving the lipid profile of ready-to-cook meat products by addition of omega-3 microcapsules: effect on oxidation and sensory analysis. *J. Sci. Food Agric.* **2018**, *98*, 5302–5312, doi:10.1002/jsfa.9069.

33. Jiménez-Martín, E.; Gharsallaoui, A.; Pérez-Palacios, T.; Carrascal, J.R.; Rojas, T.A. Suitability of using monolayered and multilayered emulsions for microencapsulation of ω-3 fatty acids by spray drying: effect of storage at different temperatures. *Food Bioprocess Technol.* **2014**, *8*, 100–111, doi:10.1007/s11947-014-1382-y.

34. Garcia-Esteban, M.; Ansorena, D.; Astiasarán, I.; Ruiz, J. Study of the effect of different fiber coatings and extraction conditions on dry cured ham volatile compounds extracted by solid-phase microextraction (SPME). *Talanta* **2004**, doi:10.1016/j.talanta.2004.03.007.

35. Perea-Sanz, L.; López-Díez, J.J.; Belloch, C.; Flores, M. Counteracting the effect of reducing nitrate/nitrite levels on dry fermented sausage aroma by Debaryomyces hansenii inoculation. *Meat Sci.* **2020**, doi:10.1016/j.meatsci.2020.108103.

36. Cano-García, L.; Rivera-Jiménez, S.; Belloch, C.; Flores, M. Generation of aroma compounds in a fermented sausage meat model system by Debaryomyces hansenii strains. *Food Chem.* **2014**, doi:10.1016/j.foodchem.2013.11.051.

37. Olivares, A.; Navarro, J.L.; Flores, M. Effect of fat content on aroma generation during processing of dry fermented sausages. *Meat Sci.* **2011**, doi:10.1016/j.meatsci.2010.10.021.

38. Olivares, A.; Navarro, J.L.; Flores, M. Establishment of the contribution of volatile compounds to the aroma of fermented sausages at different stages of processing and storage. *Food*

Chem. **2009**, doi:10.1016/j.foodchem.2009.01.083.

39. Perea-Sanz, L.; Montero, R.; Belloch, C.; Flores, M. Nitrate reduction in the fermentation process of salt reduced dry sausages: Impact on microbial and physicochemical parameters and aroma profile. *Int. J. Food Microbiol.* **2018**, doi:10.1016/j.ijfoodmicro.2018.06.004.

40. Corral, S.; Salvador, A.; Flores, M. Salt reduction in slow fermented sausages affects the generation of aroma active compounds. *Meat Sci.* **2013**, doi:10.1016/j.meatsci.2012.11.040.

41. Marco, A.; Navarro, J.L.; Flores, M. The influence of nitrite and nitrate on microbial, chemical and sensory parameters of slow dry fermented sausage. *Meat Sci.* **2006**, doi:10.1016/j.meatsci.2006.03.011.

42. Corral, S.; Belloch, C.; López-Díez, J.J.; Flores, M. Lipolysis and aroma generation as mechanisms involved in masking boar taint in sodium reduced fermented sausages inoculated with Debaryomyces hansenii yeast. *J. Sci. Food Agric.* **2018**, doi:10.1002/jsfa.8694.

43. Jiménez-Martín, E.; Gharsallaoui, A.; Pérez-Palacios, T.; Ruiz Carrascal, J.; Antequera Rojas, T. Volatile compounds and physicochemical characteristics during storage of microcapsules from different fish oil emulsions. *Food Bioprod. Process.* **2015**, *96*, 52–64, doi:10.1016/j.fbp.2015.07.005.

44. Pop, F. Chemical stabilization of oils rich in long-chain polyunsaturated fatty acids during storage. *Food Sci. Technol. Int.* **2011**, doi:10.1177/1082013210368738.

45. Ahn, D.U.; Olson, D.G.; Jo, C.; Love, J.; Jin, S.K. Volatiles production and lipid oxidation in irradiated cooked sausage as related to packaging and storage. *J. Food Sci.* **1999**, doi:10.1111/j.1365-2621.1999.tb15870.x.

46. Chevance, F.F.V.; Farmer, L.J. Identification of major volatile odor compounds in frankfurters. *J. Agric. Food Chem.* **1999**, doi:10.1021/jf990515d.

47. Chevance, F.F.V.; Farmer, L.J. Release of volatile odor compounds from full-fat and reduced-fat frankfurters. *J. Agric. Food Chem.* **1999**, doi:10.1021/jf9905166.

48. Chevance, F.F.V.; Farmer, L.J.; Desmond, E.M.; Novelli, E.; Troy, D.J.; Chizzolini, R. Effect of some fat replacers on the release of volatile aroma compounds from low-fat meat products. *J. Agric. Food Chem.* **2000**, doi:10.1021/jf991211u.

49. Jo, C.; Ahn, D.U.; Byun, M.W. Irradiation-induced oxidative changes and production of volatile compounds in sausages prepared with vitamin E-enriched commercial soybean oil. *Food Chem.* **2002**, doi:10.1016/S0308-8146(01)00276-X.

50. Estévez, M.; Ventanas, S.; Ramírez, R.; Cava, R. Influence of the addition of rosemary essential oil on the volatiles pattern of porcine frankfurters. *J. Agric. Food Chem.* **2005**, doi:10.1021/jf051025q.

51. Carrapiso, A.I. Effect of fat content on flavour release from sausages. *Food Chem.* **2007**, doi:10.1016/j.foodchem.2006.07.037.

52. Yoo, S.S.; Kook, S.H.; Park, S.Y.; Shim, J.H.; Chin, K.B. Physicochemical characteristics, textural properties and volatile compounds in comminuted sausages as affected by various fat levels and fat replacers. *Int. J. Food Sci. Technol.* **2007**, doi:10.1111/j.1365-2621.2006.01402.x.

53. Sun, W.; Zhao, Q.; Zhao, H.; Zhao, M.; Yang, B. Volatile compounds of Cantonese sausage released at different stages of processing and storage. *Food Chem.* **2010**, doi:10.1016/j.foodchem.2009.12.031.

54. Feng, C.H.; Li, C.; García-Martín, J.F.; Malakar, P.K.; Yan, Y.; Liu, Y.W.; Wang, W.; Liu, Y.T.; Yang, Y. Physical Properties and Volatile Composition Changes of Cooked Sausages Stuffed in a New Casing Formulation Based in Surfactants and Lactic Acid During Long-Term Storage. *J. Food Sci.* **2017**, doi:10.1111/1750-3841.13641.

55. Jacobsen, C.; Hartvigsen, K.; Lund, P.; Adler-Nissen, J.; Hølmer, G.; Meyer, A.S. Oxidation in fish-oil-enriched mayonnaise. *Eur. Food Res. Technol.* **2000**, doi:10.1007/s002179900070.

56. Roberts, D.D.; Pollien, P.; Milo, C. Solid-phase microextraction method development for headspace analysis of volatile flavor compounds. *J. Agric. Food Chem.* **2000**, doi:10.1021/jf991116l.

57. Venkateshwarlu, G.; Let, M.B.; Meyer, A.S.; Jacobsen, C. Chemical and Olfactometric Characterization of Volatile Flavor Compounds in a Fish Oil Enriched Milk Emulsion. *J. Agric. Food Chem.* **2004**, doi:10.1021/jf034833v.

58. Giogios, I.; Grigorakis, K.; Nengas, I.; Papasolomontos, S.; Papaioannou, N.; Alexis, M.N. Fatty acid composition and volatile compounds of selected marine oils and meals. *J. Sci. Food Agric.* **2009**, *89*, 88–100, doi:10.1002/jsfa.3414.

59. Jiménez-Martín, E.; Antequera Rojas, T.; Gharsallaoui, A.; Ruiz Carrascal, J.; Pérez-Palacios, T. Fatty acid composition in double and multilayered microcapsules of ω-3 as affected by storage conditions and type of emulsions. *Food Chem.* **2016**, *194*, 476–486, doi:10.1016/j.foodchem.2015.08.046.

60. Suh, J.H.; Niu, Y.S.; Hung, W.L.; Ho, C.T.; Wang, Y. Lipidomic analysis for carbonyl species derived from fish oil using liquid chromatography–tandem mass spectrometry. *Talanta* **2017**, doi:10.1016/j.talanta.2017.03.023.

61. Rørbæk, K. Oxidation and flavours in fish oil, Technical University of Denmark, 1994.

62. Medina, I.; Satué-Gracia, M.T.; Frankel, E.N. Static Headspace Gas Chromatographic Analyses to Determine Oxidation of Fish Muscle Lipids during Thermal Processing. *JAOCS, J. Am. Oil Chem. Soc.* **1999**, doi:10.1007/s11746-999-0223-z.

63. Klinkesorn, U.; Sophanodora, P.; Chinachoti, P.; Decker, E.A.; McClements, D.J. Encapsulation of emulsified tuna oil in two-layered interfacial membranes prepared using electrostatic layer-by-layer deposition. *Food Hydrocoll.* **2005**, *19*, 1044–1053, doi:10.1016/j.foodhyd.2005.01.006.

64. Ngo, D.H.; Kim, S.K. Antioxidant effects of chitin, chitosan, and their derivatives. In *Advances in Food and Nutrition Research*; 2014.

65. Van Gemert, L.J. *Odour thresholds*; 2003; ISBN 9789081089418.

66. Zhu, J.C.; Chen, F.; Wang, L.Y.; Niu, Y.W.; Xiao, Z.B. Evaluation of the synergism among volatile compounds in Oolong tea infusion by odour threshold with sensory analysis and E-

nose. *Food Chem.* **2017**, doi:10.1016/j.foodchem.2016.11.002.

67. Karahadian, C.; Lindsay, R.C. Evaluation of compounds contributing characterizing fishy flavors in fish oils. *J. Am. Oil Chem. Soc.* **1989**, doi:10.1007/BF02682616.

68. El Sheikha, A.F.; Bakar, J. Fermented meat products. In *Microorganisms and Fermentation of Traditional Foods*; 2014 ISBN 9781482223095.

69. Škrlep, M.; Čandek-Potokar, M.; Atorek-Lukač, N.B.; Tomažin, U.; Flores, M. Aromatic profile, physicochemical and sensory traits of dry-fermented sausages produced without nitrites using pork from krškopolje pig reared in organic and conventional husbandry. *Animals* **2019**, doi:10.3390/ani9020055.

70. Reiners, J.; Grosch, W. Odorants of Virgin Olive Oils with Different Flavor Profiles. *J. Agric. Food Chem.* **1998**, doi:10.1021/jf970940b.

71. Morales, M.T.; Luna, G.; Aparicio, R. Comparative study of virgin olive oil sensory defects. *Food Chem.* **2005**, doi:10.1016/j.foodchem.2004.06.011.

72. Sirisoma, N.S.; Höld, K.M.; Casida, J.E. α- and β-thujones (herbal medicines and food additives): Synthesis and analysis of hydroxy and dehydro metabolites. *J. Agric. Food Chem.* **2001**, doi:10.1021/jf001445+.

73. Kalua, C.M.; Allen, M.S.; Bedgood, D.R.; Bishop, A.G.; Prenzler, P.D.; Robards, K. Olive oil volatile compounds, flavour development and quality: A critical review. *Food Chem.* **2007**, doi:10.1016/j.foodchem.2005.09.059.

74. Jiménez-Martín, E.; Gharsallaoui, A.; Pérez-Palacios, T.; Carrascal, J.R.; Antequera, T.A. Suitability of using monolayered and multilayered emulsions for microencapsulation of ω-3 fatty acids by spray drying: effect of storage at different temperatures. *Food Bioprocess Technol.* **2014**, doi:10.1007/s11947-014-1382-y.

75. Ye, A.; Cui, J.; Taneja, A.; Zhu, X.; Singh, H. Evaluation of processed cheese fortified with fish oil emulsion. *Food Res. Int.* **2009**, doi:10.1016/j.foodres.2009.05.006.

76. Giri, A.; Osako, K.; Ohshima, T. Identification and characterisation of headspace volatiles of

fish miso, a Japanese fish meat based fermented paste, with special emphasis on effect of fish species and meat washing. *Food Chem.* **2010**, doi:10.1016/j.foodchem.2009.10.036.

77. Pérez-Palacios, T.; Petisca, C.; Melo, A.; Ferreira, I.M.P.L.V.O. Quantification of furanic compounds in coated deep-fried products simulating normal preparation and consumption: Optimisation of HS-SPME analytical conditions by response surface methodology. *Food Chem.* **2012**, doi:10.1016/j.foodchem.2012.05.100.

78. Sujatha, P.S. Monitoring cytotoxic potentials of furfuryl alcohol and 2-furyl methyl ketone in mice. *Food Chem. Toxicol.* **2008**, doi:10.1016/j.fct.2007.08.008.

79. Arts, J.H.E.; Muijser, H.; Appel, M.J.; Kuper, C.F.; Bessems, J.G.M.; Woutersen, R.A. Subacute (28-day) toxicity of furfural in Fischer 344 rats: A comparison of the oral and inhalation route. *Food Chem. Toxicol.* **2004**, doi:10.1016/j.fct.2004.03.014.

FIGURE CAPTIONS

Figure 1. Volatile compounds of monolayered (Mo) and multilayered (Mu) fish oil microcapsules classified according to chemical families. Values are expressed as the average percentage of each family.

Figure 2. Volatile compounds of dry-cured (a) and cooked (b) sausages as affected by enrichment with ω-3 PUFA (control: dark gray; enriched with multilayered fish oil microcapsules: medium gray; enriched monolayered fish oil microcapsules: light gray) classified according to chemical families. Values are expressed as the average percentage of each family. Bars with different letters (a,b,c) within the same formulation show significant differences ($p < 0.05$) due to enrichment effect (Co vs Mo vs Mu).

Figure 3. Principal component analysis (PCA) of the significant volatile compounds in dry-cured (a) and cooked (b) sausages. The plots represent, for the two first principal components, the loading of each volatile compound and the average scores of each one of batches. Control (▷); enriched with monolayered fish oil microcapsules (○); enriched with multilayered fish oil microcapsules (◊).

Effect of omega-3 microcapsules addition on the profile of volatile compounds in enriched meat products

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13	repositorio-aberto.up.pt Internet	25 words — < 1%
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Technology, 03/2008
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16 kb.psu.ac.th
Internet
22 words — < 1%

17 Juan Carlos Solomando, Teresa Antequera, Trinidad Pérez-Palacios. "Study on fish oil microcapsules as neat and added to meat model systems: Enrichment and bioaccesibility of EPA and DHA", LWT, 2020
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22 Trinidad Pérez-Palacios. "Influence of pre-cure freezing on the profile of volatile compounds during the processing of Iberian hams", Journal of the Science of Food and Agriculture, 2010
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24 worldwidescience.org
Internet

16 words — < 1%

25 M.J. Andrade, M. Rodríguez, E.M. Casado, E. Bermúdez, J.J. Córdoba. "Differentiation of yeasts growing on dry-cured Iberian ham by mitochondrial DNA restriction analysis, RAPD-PCR and their volatile compounds production", Food Microbiology, 2009
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26 onlinelibrary.wiley.com
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27 Marianthi Sidira, Panagiotis Kandylis, Maria Kanellaki, Yiannis Kourkoutas. "Effect of immobilized Lactobacillus casei on the evolution of flavor compounds in probiotic dry-fermented sausages during ripening", Meat Science, 2015
Crossref
13 words — < 1%

28 Gonzalo Delgado-Pando. "CHARACTERISTICS OF HEALTHIER LIPID COMBINATION OIL-IN-WATER EMULSIONS PREPARED WITH VARIOUS PROTEIN SYSTEMS. AN APPROACH FOR DEVELOPMENT OF FUNCTIONAL MEAT PRODUCTS", European Journal of Lipid Science and Technology, 04/19/2010
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29 Jinhyuk Park, J. Alex Thomasson, Cody C. Gale, Gregory A. Sword, Kyung-Min Lee, Timothy J. Herrman, Charles P.-C. Suh. "Adsorbent-SERS Technique for Determination of Plant VOCs from Live Cotton Plants and Dried Teas", ACS Omega, 2020
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30 T. Pérez-Palacios, C. Petisca, A. Melo, I.M.P.L.V.O. Ferreira. "Quantification of furanic compounds in coated deep-fried products simulating normal preparation and consumption: Optimisation of HS-SPME analytical conditions by response surface methodology", Food Chemistry, 2012
Crossref
12 words — < 1%

31 Sakhi Ghelichi, Bahareh Shabanpour, Parastoo Pourashouri. " Properties of Fish Sausages Containing Common Carp ( ) Roe Oil and Defatted Roe Protein Hydrolysate during Refrigerated Storage ", Journal of Aquatic Food Product Technology, 2018
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32 es.scribd.com
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35 Rosario Ramírez, Ramón Cava. "Volatile Profiles of Dry-Cured Meat Products from Three Different Iberian X Duroc Genotypes", Journal of Agricultural and Food Chemistry, 2007
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36 www.pubfacts.com
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37 Ledan Huang, Zufang Wu, Xiaoqian Chen, Peifang Weng, Xin Zhang. "Characterization of flavor and volatile compounds of fermented squid using electronic nose and HPMS in combination with GC-MS", International Journal of Food Properties, 2018
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38 "Springer Handbook of Odor", Springer Science and Business Media LLC, 2017
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39 B. Dave Oomah. "Volatile Compounds of Dry Beans (Phaseolus

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40 Rossawan Intarasirisawat, Soottawat Benjakul, Wonnop Vissessanguan, Sajid Maqsood, Kazufumi Osako. "Skipjack roe protein hydrolysate combined with tannic acid increases the stability of fish oil upon microencapsulation", European Journal of Lipid Science and Technology, 2015
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41 library.wur.nl
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9 words — < 1%

42 www.trv130.com
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43 Kourtney Gardner, Jerrad F Legako. "Volatile flavor compounds vary by beef product type and degree of doneness1", Journal of Animal Science, 2018
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9 words — < 1%

44 Zeng, Xuefeng, Wei Zhang, and Qiujin Zhu. "Effect of starter cultures on the quality of Suan yu , a Chinese traditional fermented freshwater fish", International Journal of Food Science & Technology, 2016.
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9 words — < 1%

45 Genovese, Alessandro, Nicola Caporaso, Lucia De Luca, Antonello Paduano, and Raffaele Sacchi. "Influence of Olive Oil Phenolic Compounds on Headspace Aroma Release by Interacting with Whey Proteins", Journal of Agricultural and Food Chemistry
Crossref

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46 epdf.tips
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47 Henryk H. Jeleń, Małgorzata Obuchowska, Renata Zawirska-Wojtasiak, Erwin Wąsowicz. "Headspace Solid-Phase Microextraction Use for the Characterization of Volatile Compounds in Vegetable Oils of Different Sensory

9 words — < 1%

Quality", Journal of Agricultural and Food Chemistry, 2000
Crossref

48 orbi.uliege.be
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49 Stefania Vichi, Lorena Pizzale, Lanfranco S. Conte, Susana Buxaderas, Elvira López-Tamames. "Solid-Phase Microextraction in the Analysis of Virgin Olive Oil Volatile Fraction: Modifications Induced by Oxidation and Suitable Markers of Oxidative Status", Journal of Agricultural and Food Chemistry, 2003
Crossref
9 words — < 1%

50 Zhou, Feibai, Mouming Zhao, Guowan Su, and Weizheng Sun. "Binding of Aroma Compounds with Myofibrillar Proteins Modified by a Hydroxyl-Radical-Induced Oxidative System", Journal of Agricultural and Food Chemistry
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51 res.mdpi.com
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52 Venkateshwarlu, Gudipati, Mette B. Let, Anne S. Meyer, and Charlotte Jacobsen. "Modeling the Sensory Impact of Defined Combinations of Volatile Lipid Oxidation Products on Fishy and Metallic Off-Flavors", Journal of Agricultural and Food Chemistry, 2004.
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53 www.scientific.net
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54 Olivares, Alicia, Kseniya Dryahina, José Luis Navarro, David Smith, Patrik Španěl, and Mónica Flores. "SPME-GC-MS versus Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) Analyses for the Study of Volatile Compound Generation and Oxidation Status during Dry Fermented Sausage Processing", Journal of Agricultural and Food Chemistry, 2011.
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55 epdf.pub
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56 Mar Roldan, Teresa Antequera, Monica Armenteros, Jorge Ruiz. "Effect of different temperature–time combinations on lipid and protein oxidation of sous-vide cooked lamb loins", Food Chemistry, 2014
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8 words — < 1%

57 Fabienne F. V. Chevance, Linda J. Farmer, Eoin M. Desmond, Enrico Novelli, Declan J. Troy, Roberto Chizzolini. "Effect of Some Fat Replacers on the Release of Volatile Aroma Compounds from Low-Fat Meat Products", Journal of Agricultural and Food Chemistry, 2000
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58 Juan Carlos Solomando, Teresa Antequera, Trinidad Perez-Palacios. "Lipid digestion and oxidative stability in ω-3-enriched meat model systems: effect of fish oil microcapsules and processing or culinary cooking", Food Chemistry, 2020
Crossref
8 words — < 1%

59 Els Vossen, Katleen Raes, Danny Van Mullem, Stefaan De Smet. "Production of docosahexaenoic acid (DHA) enriched loin and dry cured ham from pigs fed algae: Nutritional and sensory quality", European Journal of Lipid Science and Technology, 2016
Crossref
8 words — < 1%

60 Ruiz, Jorge, Jesús Ventanas, and Ramón Cava. "New Device for Direct Extraction of Volatiles in Solid Samples Using SPME", Journal of Agricultural and Food Chemistry, 2001.
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66 www.science.gov
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Crossref
7 words — < 1%

68 Pérez-Palacios, T., C. Petisca, R. Henriques, and I.M.P.L.V.O. Ferreira. "Impact of cooking and handling conditions on furanic compounds in breaded fish products", Food and Chemical Toxicology, 2013.
Crossref
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69 M Jesús Andrade, Juan José Córdoba, Beatriz Sánchez, Eva M Casado, Mar Rodríguez. "Evaluation and selection of yeasts isolated from dry-cured Iberian ham by their volatile compound production", Food Chemistry, 2009
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7 words — < 1%

70 Arkadiusz Szpicer, Anna Onopiuk, Andrzej Półtorak, Agnieszka Wierzbicka. "Influence of tallow replacement by oat β-glucan and canola oil on the fatty acid and volatile compound profiles of low-fat beef burgers", CyTA - Journal of Food, 2019
Crossref
7 words — < 1%

71 Trinidad Pérez-Palacios, Jorge Ruiz-Carrascal, Juan Carlos Solomando, Teresa Antequera. "Strategies for Enrichment in ω-3 Fatty Acids Aiming for Healthier Meat Products", Food Reviews International, 2019
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7 words — < 1%

72 Jimenez-Colmenero, F.. "Healthier lipid formulation approaches in meat-based functional foods. Technological options for replacement of meat fats by non-meat fats", Trends in Food Science & Technology, 200711
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73 José M. Lorenzo, Daniel Franco, Javier Carballo. "Effect of the inclusion of chestnut in the finishing diet on volatile compounds during the manufacture of dry-cured “Lacón” from Celta pig breed", Meat Science, 2014
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