Lipid digestion products in meat derivatives enriched with fish oil microcapsules

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ABSTRACT

This study analyzes the potential application of monolayered (Mo) and multilayered (Mu) fish oil microcapsules as EPA and DHA vehicles in cooked (C-SAU) and dry-cured (D-SAU) meat products and the bioaccesibility of their fatty acids. The quantities of EPA and DHA in all batches (44–64 mg EPA + DHA/100 g sample) exceeded the level established by the European Union to label a food as "source of ω-3 fatty acids" (40 mg EPA + DHA/100 g product). The highest percentages of released fat were observed in the intestinal phase. The amount of EPA and DHA bioaccesible was higher in C-SAU-Mu and D-SAU-Mu batches (0.35 and 0.33 mg EPA + DHA per gram of sample digested), in contrast to C-SAU-Mo and D-SAU-Mo batches (0.25 and 0.24 mg EPA + DHA per gram of sample digested). Therefore, the types of microcapsule of fish oil do not influence the EPA + DHA enrichment, but it did in their bioaccessibility, being better when using Mu.

1. Introduction

The addition of bioactive compounds, such as eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3), to food has become increasing important in the last years (Fernandez-Avila, Arranz, Guri, Trujillo, & Corredig, 2016; Tocher, Betancor, Sprague, Olsen, & Napier, 2019). This is mainly due to two reasons, the well-known beneficial effects of these fatty acids (Kris-Etherton, Harris, & Appel, 2002; Swanson, Block, & Mousa, 2012; Zhang, Xu, Wang, & Xue, 20019) and the insufficient consumption of fish, seafood or algae to reach the recommended intake EPA plus DHA, which is around 0.25 g per person and day (EFSA, 2016). Besides, 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 (EU, 2010).

Meat and meat products are valuable foods with high quality proteins, vitamins (especially vitamin B6 and B12) and minerals (iron, selenium and zinc) (Santos, Hoz, Cambero, Cabeza, & Ordóñez, 2008). However, these products are sometimes questioned because of their lipid profile, especially in relation to their high to moderate percentage of saturated fatty acids (SFA) and low polyunsaturated FA (PUFA) contents (Nuernberg et al., 2005). On the other hand, the consumption of meat products is around 3–4 times per week (OMS, 2015), which is associated to the growing intake of "ready-to-eat" products.

Consequently, different strategies have been tested in well-accepted meat products to increase the content of EPA and DHA. The inclusion of fish and algae oils, as bulk or emulsified, has been principally reported. However, these approaches have a detrimental influence on some sensory attributes and lipid oxidation stability, even when antioxidants are used in some cases (Bolger, Brunton, & Monahan, 2017; Lee et al., 2006; Valencia, Ansorena, & Astiasarán, 2007). This is due to the undesirable off-flavor and off-odor of fish and algae oils and to the high suceptibiliy of EPA and DHA to oxidation, leading to unhealthy secondary oxidation products (Fetterman & Zdanowicz, 2009; Shahidi & Zhong, 2010).

The addition of fish oil microcapsules to meat products has also been tested, not finding marked negative effects (Trinidad Pérez-Palacios, Ruiz-Carrascal, Solomando, & Antequera, 2019). In fact, the microencapsulation is based on creating a physical barrier between the encapsulated compounds and the environment, reducing the contact and reactivity with water, oxygen, iron and other oxidizing promoters (Miyashita, Uemura, & Hosokawa, 2018; Onwulata, 2013). Different types of fish oil microcapsules have been used to enrich meat products: commercials (Josquin, Linssen, & Houben, 2012; Pelser, Linssen, Legger, & Houben, 2007) and from spray-dried monolayered (Lorenzo,

Munekata, Pateiro, Campagnol, & Domínguez, 2016) and multilayered emulsions (Aquilani et al., 2018; Jiménez-Martín, Pérez-Palacios, Carrascal, & Rojas, 2016) . All these studies have evaluated the influence of adding fish oil microcapsules on the proximal composition of the enriched products, the lipid oxidation stability, sensory attributes and on the percentage of EPA and DHA. However, the content of these fatty acids expressed in mg per g of sample has not been determined, not being possible to know if the amount of microcapsules added to the food products is enough to label the enriched meat products as "source of omega-3 fatty acids" or "rich in omega-3" according to European regulations (EU, 2010).

The gastrointestinal stability and bioavailability of the bioactive compounds added to the products should be one of the major concerns in the omega-3 enrichment studies. When new vehicles of bioactive lipid compounds are developed, it is necessary to know their behavior during the digestion process (Dimagno, Go, & Summerskill, 1973) as well as to their bioaccessibility. This information will allow to evaluate the fraction of the ingested compound available to be absorbed (Fernández-García, Carvajal-Lérida, & Pérez-Gálvez, 2009). A bioaccessibility study requires the use of an adequate in vitro digestion model that reliably simulates human digestion. The usual models described in the literature for lipids are generally hydrolytic (Dahan & Hoffman, 2006; Kaukonen, Boyd, Porter, & Charman, 2004), static (Porter et al., 2004; Sek, Porter, Kaukonen, & Charman, 2002) and with distinction of the digestive compartment and enzymes at all stages (Guerra et al., 2012). (Chatterjee & Judeh, 2016) have evaluated microencapsulated and un-encapsulated fish oil under simulated gastrointestinal condition, determining the percentage of released oil and finding a major stability and delivery with the microcapsules. However, there are no published data on the gastrointestinal release of EPA and DHA from microcapsules, neither meat products enriched with fish oil microcapsules

Considering all these aspects, the objective of this work was evaluating the bioaccesibility of the fatty acids, especially EPA and DHA of the different meat products enriched with fish oil microcapsules.

2. Material and methods

2.1. Biological material

Fish oil from cod liver was kindly provided by Biomega Nutrition (Galicia, Spain). Soybean lecithin (Across Organics, Madrid, Spain), chitosan with 95% of deacetylation (Chitoclear FG 95, kindly provided by Trades, Murcia, Spain), maltodextrin with a dextrose equivalent of 12% (Glucidex 12, kindly provided Roquette, Lestrem, France), and food-grade glacial acetic acid (Scharlau, Barcelona, Spain) were used for the preparation of the emulsions. Iberian pork and chicken meat, water, pork fat, salt, dextrose, soy protein, pork plasma, stabilizer (E-450 and E-451), flavors, vegetable fiber, spices, spice extracts, smoke flavor, antioxidant (E-316), preservative (E-250 and E-252) enhancer flavor (E-621) and colorant (E-120) used for the elaboration of cooked and dry-cured products were kindly provided by a factory (remain anonymous).

2.2. Reagents

Sulphuric acid, methanol, sodium metal and hexane 96% (Scharlau) were used for the transesterification of fatty acids. For the simulated digestion, α-amylase from *Aspergillus oryzae* (30U/mg), pepsin from porcine gastric mucose (2500U/mg protein) and pancreatin from porcine pancreas (4 × USP specifications) (Sigma, St Louis, MO, USA), sodium chloride, potassium phosphate and hexane 96% (Scharlau) were used.

2.3. Experimental design

Monolayered and multilayered emulsions of fish oil were spray-dried to obtain their corresponding microcapsules (Mo and Mu, respectively). Two different meat products were elaborated, cooked (C-SAU) and dry-cured sausages (D-SAU), which were added with Mo (C-SAU-Mo and D-SAU-Mo) and 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). Moreover, all batches were analyzed at time 0 (T0) and after 4 months (T4) of storage at refrigeration (0–5 °C). The six meat products were elaborated in triplicate and analyzed by fatty acid composition and digestibility. The analyses were carried out in triplicate.

2.4. Preparation of emulsions and microcapsules

Emulsion and microcapsules of this study were prepared following the methodology of (Jiménez-Martín, Gharsallaoui, Pérez-Palacios, Carrascal, & Rojas, 2014) with slight modifications.

Fish oil (20 g) and lecithin (6 g) 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-3 passes for Mo and 1100–2 passes for Mu (J. C. Solomando, Antequera, Ruiz-Carrascal, & Pérez-Palacios, 2019).

The homogenized primary emulsion was blended with 200 g of water, in the case of Mo, and with 200 g of 1% of chitosan (w/w) 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) to obtain the feed emulsion.

Feed emulsions (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 (Jiménez-Martín et al., 2014).

2.5. Elaboration of meat products.

Formulation and manufacture of meat products were made in a meat industry (remain anonymous), following their procedures.

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, in the case of the enriched batches, the corresponding microcapsules, which were added in the knead phase. All C-SAU batches were pasteurized in a water bath at 85 °C during 30 min, vacuum packed and stored at refrigeration temperature (0–5 °C). All batches were analyzed at time 0 (T0) and after 4 months (T4) of storage at refrigeration (0–5 °C).

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, mixed for 3 min and kept at 4 °C until stuffed. No starter culture was added. The obtained dough was stuffed into collagen casings (40 cm length × 60 mm diameter). The sausages followed a dry-cured process under controlled conditions of 4 °C and 82% of relative humidity for 3 days. Then, the

Table 1

Fatty acid composition (mg FAMEs/g sample) on cooked sausages (C-SAU) as affected by enrichment with fish oil microcapsules (*p*E) and storage (*p*S)*.

Fatty acids		Co	Mo	Mu	*p*E	*p*S Co	Mo	Mu
C14:0	T0	0.72 ± 0.01	0.67 ± 0.09	0.52 ± 0.19	0.081	0.569	0.201	0.056
	T4	0.70 ± 0.07	0.71 ± 0.08	0.70 ± 0.06	0.204			
C16:0	T0	2.49 ± 0.23	2.51 ± 0.17	2.65 ± 0.11	0.065	0.057	0.041	0.035
	T4	2.83 ± 0.18	2.87 ± 0.09	3.21 ± 0.18	0.044			
C16:1n-7	T0	0.67 ± 0.09	0.79 ± 0.03	0.72 ± 0.10	0.081	0.486	0.136	0.569
	T4	0.62 ± 0.12	0.87 ± 0.09	0.79 ± 0.09	0.256			
C17:0	T0	0.39 ± 0.03	0.37 ± 0.05	0.25 ± 0.06	0.209	0.105	0.236	0.436
	T4	0.27 ± 0.8	0.33 ± 0.10	0.21 ± 0.03	0.296			
C17:1n-7	T0	0.20 ± 0.01	0.20 ± 0.02	0.20 ± 0.03	0.130	0.786	0.852	0.866
	T4	0.21 ± 0.02	0.20 ± 0.01	0.20 ± 0.02	0.169			
C18:0	T0	3.94 ± 0.22	3.86 ± 0.19	3.97 ± 0.17	0.471	0.411	0.285	0.058
	T4	4.06 ± 0.26	3.99 ± 0.27	4.21 ± 0.19	0.302			
C18:1n-9	T0	5.87 ± 0.16	6.09 ± 0.17	6.14 ± 0.14	0.089	0.044	0.156	0.039
	T4	5.62 ± 0.10[b]	5.87 ± 0.23[a]	5.93 ± 0.13[a]	0.024			
C18:2n-6	T0	4.92 ± 0.09	5.02 ± 0.16	5.06 ± 0.13	0.103	0.368	0.589	0.758
	T4	4.96 ± 0.05	5.09 ± 0.07	5.03 ± 0.09	0.052			
C18:3n-6	T0	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.00	0.682	0.032	0.029	0.044
	T4	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.00	0.166			
C18:3n-3	T0	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.147	0.041	0.625	0.758
	T4	0.01 ± 0.00	0.02 ± 0.01	0.01 ± 0.00	0.222			
C20:0	T0	0.16 ± 0.01	0.14 ± 0.04	0.18 ± 0.01	0.179	0.215	0.113	0.352
	T4	0.14 ± 0.02	0.15 ± 0.02	0.16 ± 0.03	0.331			
C20:1n-9	T0	0.92 ± 0.04	0.88 ± 0.24	0.85 ± 0.06	0.382	0.425	0.154	0.102
	T4	0.90 ± 0.12	0.86 ± 0.28	0.91 ± 0.09	0.101			
C20:2n-6	T0	0.39 ± 0.02	0.33 ± 0.09	0.31 ± 0.02	0.294	0.536	0.125	0.253
	T4	0.40 ± 0.11	0.36 ± 0.04	0.36 ± 0.03	0.421			
C20:3n-6	T0	0.08 ± 0.00	0.08 ± 0.02	0.06 ± 0.00	0.208	< 0.001	0.246	< 0.001
	T4	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.00	0.165			
C20:3n-3	T0	0.21 ± 0.00	0.27 ± 0.07	0.21 ± 0.00	0.789	0.136	0.045	0.255
	T4	0.16 ± 0.06	0.17 ± 0.06	0.19 ± 0.03	0.352			
C20:4n-6	T0	0.06 ± 0.00	0.06 ± 0.01	0.06 ± 0.00	0.183	0.456	0.256	0.225
	T4	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.0	0.096			
C20:5n-3	T0	Nd[b]	0.18 ± 0.01[a]	0.19 ± 0.01[a]	< 0.001	–	0.022	0.042
	T4	Nd[b]	0.14 ± 0.02[a]	0.15 ± 0.03[a]	< 0.001			
C24:0	T0	0.06 ± 0.02	0.09 ± 0.04	0.06 ± 0.00	0.244	0.365	0.106	0.126
	T4	0.06 ± 0.01	0.06 ± 0.02	0.06 ± 0.01	0.120			
C22:6n-3	T0	Nd[c]	0.41 ± 0.02[b]	0.44 ± 0.02[a]	< 0.001	–	0.289	0.469
	T4	Nd[c]	0.37 ± 0.06[b]	0.43 ± 0.07[a]	< 0.001			
Σ SFA	T0	7.90 ± 0.42	8.00 ± 0.33	7.89 ± 0.24	0.275	0.098	0.063	0.356
	T4	7.56 ± 0.23	7.55 ± 0.29	7.76 ± 0.31	0.296			
Σ MUFA	T0	7.66 ± 0.29	7.96 ± 0.44	7.91 ± 0.52	0.078	0.059	0.328	0.279
	T4	7.35 ± 0.48[b]	7.80 ± 0.29[a]	7.83 ± 0.22[a]	0.023			
Σ PUFA	T0	5.70 ± 0.51[b]	6.37 ± 0.94[a]	6.36 ± 0.78[a]	0.019	0.371	0.289	0.211
	T4	5.63 ± 0.44[b]	6.25 ± 0.56[a]	6.27 ± 0.20[a]	< 0.001			
n3	T0	0.23 ± 0.09[b]	0.88 ± 0.21[a]	0.86 ± 0.14[a]	< 0.001	0.106	0.067	0.088
	T4	0.17 ± 0.14[b]	0.70 ± 0.11[a]	0.79 ± 0.17[a]	< 0.001			
n6	T0	5.47 ± 0.54	5.52 ± 0.96	5.51 ± 0.66	0.410	0.597	0.492	0.438
	T4	5.46 ± 0.71	5.56 ± 0.49	5.49 ± 0.84	0.346			
n6/n3	T0	23.78 ± 0.33[a]	6.49 ± 0.27[b]	6.48 ± 0.09[b]	< 0.001	< 0.001	< 0.001	0.003
	T4	32.12 ± 0.36[a]	8.06 ± 0.29[b]	7.04 ± 0.28[c]	< 0.001			
SFA/UFA	T0	0.59 ± 0.11	0.56 ± 0.13	0.55 ± 0.06	0.976	0.542	0.393	0.601
	T4	0.58 ± 0.08	0.54 ± 0.08	0.55 ± 0.11	0.685			
MUFA/PUFA	T0	1.34 ± 0.25	1.25 ± 0.62	1.24 ± 0.37	0.749	0.375	0.788	0.690
	T4	1.31 ± 0.45	1.25 ± 0.44	1.25 ± 0.41	0.428			
Σ EPA + DHA	T0	Nd[c]	0.59 ± 0.06[b]	0.64 ± 0.09[a]	< 0.001	–	0.067	0.079
	T4	Nd[c]	0.51 ± 0.07[b]	0.58 ± 0.05[a]	< 0.001			

* Not enriched (Co) and enriched with monolayer (Mo) and multilayered microcapsules (Mu): samples analyzed before and after the refrigeration storage for 4 months (T0 and T4, respectively). Bars with different letters (a, b, c) within the same formulation show significant differences (p < 0.05) due to enrichment effect. Nd: not detected. Myristic acid (C14:0); palmitic acid (C16:0); palmitoleic acid (C16:1n-7); margaric acid (C17:0); margaroleic acid (C17:1n-7); stearic acid (C18:0); elaidic acid (C18:1n-9); linoleic acid (C18:2n-6); γ-Linolenic acid (C18:3n-6); α-Linolenic acid (C18:3n-3); arachidic acid (C20:0); eicosenoic acid (C20:1n-9); eicosadenoic acid (C20:2n-6); dihomo-γ-linolenic acid (C20:3n-6); 5,8,11-eicosatrienoic acid (C20:3n-3); arachidonic acid (C20:4n-6); eicosapentaenoic acid (C20:5n-3); lignoceric acid (C24:0); docosahexaenoic acid (C22:6n-3).

product was 21 days in the drying-curing chamber at 8 °C and 80% of relative humidity, and finally they rested in a cellar at 5 °C and 85% humidity until reaching a percentage of weight loss around 38–40%. D-SAU were analyzed at time 0 (T0) and after 4 months (T4) of storage at room temperature (0–5 °C)

In both products, the quantity of Mo and Mu added was 3 and 5 g per 100 g of dough, respectively. These amounts 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 (EU, 2010).

2.6. Measure of lipid content.

Fat content was determined gravimetrically, following the method

Table 2

Fatty acid composition (mg FAMEs/g sample) on dry-cured sausages (D-SAU) as affected by enrichment with ω-3 PUFA (pE) and storage (pS).*

Fatty acids		Co	Mo	Mu	pE	pS		
						Co	Mo	Mu
C14:0	T0	0.40 ± 0.08	0.37 ± 0.9	0.40 ± 0.08	0.816	0.041	0.137	0.169
	T4	0.26 ± 0.06	0.26 ± 0.04	0.31 ± 0.02	0.360			
C16:0	T0	8.33 ± 0.14	8.46 ± 0.17	8.38 ± 0.06	0.371	0.269	0.331	0.369
	T4	8.11 ± 0.17	8.37 ± 0.14	8.29 ± 0.33	0.480			
C16:1n-7	T0	1.12 ± 0.05	1.20 ± 0.16	1.07 ± 0.03	0.220	0.086	0.346	0.038
	T4	1.28 ± 0.10	1.23 ± 0.06	1.24 ± 0.10	0.466			
C17:0	T0	0.26 ± 0.05	0.28 ± 0.04	0.29 ± 0.04	0.563	0.038	0.021	0.030
	T4	0.17 ± 0.02	0.17 ± 0.03	0.16 ± 0.06	0.774			
C17:1n-7	T0	0.13 ± 0.03	0.12 ± 0.05	0.13 ± 0.03	0.152	0.097	0.249	0.101
	T4	0.09 ± 0.01	0.08 ± 0.02	0.09 ± 0.01	0.883			
C18:0	T0	3.50 ± 0.29	3.71 ± 0.41	3.42 ± 0.19	0.167	0.498	0.204	0.274
	T4	3.49 ± 0.33	3.65 ± 0.26	3.58 ± 0.24	0.276			
C18:1n-9	T0	10.05 ± 0.32[b]	11.22 ± 0.56[a]	10.21 ± 0.68[b]	< 0.001	0.069	0.034	0.019
	T4	9.41 ± 0.45[c]	10.78 ± 0.22[b]	11.65 ± 0.28[a]	< 0.001			
C18:2n-6	T0	8.11 ± 0.75	7.94 ± 1.02	8.22 ± 0.69	0.067	< 0.001	0.031	0.019
	T4	6.55 ± 0.38	6.24 ± 0.29	6.50 ± 0.53	0.081			
C18:3n-6	T0	0.07 ± 0.01	0.07 ± 0.02	0.08 ± 0.01	0.926	0.075	0.136	0.247
	T4	0.05 ± 0.01	0.05 ± 0.01	0.07 ± 0.01	0.104			
C18:3n-3	T0	0.02 ± 0.00	0.03 ± 0.01	0.03 ± 0.00	0.432	0.671	0.111	0.257
	T4	0.02 ± 0.00	0.01 ± 0.00	0.03 ± 0.01	0.090			
C20:0	T0	0.10 ± 0.01	0.12 ± 0.00	0.12 ± 0.00	0.653	0.002	< 0.001	0.051
	T4	0.06 ± 0.01	0.06 ± 0.03	0.11 ± 0.04	0.075			
C20:1n-9	T0	0.66 ± 0.04	0.71 ± 0.03	0.87 ± 0.15	0.236	< 0.001	< 0.001	< 0.001
	T4	0.39 ± 0.06	0.42 ± 0.15	0.40 ± 0.05	0.092			
C20:2n-6	T0	0.25 ± 0.03	0.25 ± 0.06	0.28 ± 0.05	0.874	< 0.001	0.090	0.064
	T4	0.14 ± 0.02	0.23 ± 0.05	0.14 ± 0.08	0.141			
C20:3n-6	T0	0.12 ± 0.01	0.11 ± 0.03	0.11 ± 0.04	0.907	< 0.001	0.233	0.307
	T4	0.06 ± 0.01	0.08 ± 0.02	0.10 ± 0.03	0.186			
C20:3n-3	T0	0.35 ± 0.09	0.32 ± 0.08	0.34 ± 0.14	0.788	< 0.001	< 0.001	0.186
	T4	0.16 ± 0.04	0.17 ± 0.03	0.24 ± 0.09	0.225			
C20:4n-6	T0	0.04 ± 0.02	0.06 ± 0.02	0.08 ± 0.01	0.108	0.136	0.003	0.001
	T4	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.054			
C20:5n-3	T0	Nd[b]	0.15 ± 0.03[a]	0.14 ± 0.03[a]	< 0.001	–	0.057	0.121
	T4	Nd[b]	0.11 ± 0.01[a]	0.11 ± 0.02[a]	< 0.001			
C24:0	T0	0.07 ± 0.00	0.07 ± 0.00	0.08 ± 0.00	0.549	0.169	0.834	0.256
	T4	0.06 ± 0.00	0.07 ± 0.00	0.07 ± 0.00	0.081			
C22:6n-3	T0	Nd[b]	0.31 ± 0.02[a]	0.30 ± 0.02[a]	< 0.001	–	0.213	0.351
	T4	Nd[b]	0.27 ± 0.01[a]	0.29 ± 0.01[a]	< 0.001			
Σ SFA	T0	12.59 ± 0.81	12.81 ± 1.23	12.69 ± 0.45	0.304	0.099	0.132	0.375
	T4	12.15 ± 0.58	12.58 ± 0.35	12.52 ± 1.09	0.080			
Σ MUFA	T0	11.96 ± 0.39	13.25 ± 0.74	12.28 ± 0.21	0.286	0.095	0.048	0.024
	T4	11.37 ± 0.17[b]	12.71 ± 0.21[a]	13.58 ± 1.08[a]	0.031			
Σ PUFA	T0	8.96 ± 0.25	9.01 ± 0.27	9.57 ± 0.24	0.105	0.057	< 0.001	0.033
	T4	7.00 ± 0.33[c]	7.17 ± 0.09[b]	7.50 ± 0.18[a]	0.039			
n3	T0	0.37 ± 0.02[b]	0.81 ± 0.02[a]	0.81 ± 0.05[a]	< 0.001	0.020	0.041	0.029
	T4	0.18 ± 0.01[b]	0.56 ± 0.10[a]	0.66 ± 0.03[a]	< 0.001			
n6	T0	8.59 ± 0.63	8.23 ± 0.42	8.77 ± 0.49	0.087	< 0.001	< 0.001	< 0.001
	T4	6.82 ± 0.17	6.62 ± 0.15	6.84 ± 0.16	0.361			
n6/n3	T0	23.22 ± 1.35[a]	10.55 ± 1.04[b]	10.96 ± 0.95[b]	< 0.001	< 0.001	0.088	0.164
	T4	37.89 ± 2.07[a]	12.04 ± 0.66[b]	10.36 ± 1.33[b]	< 0.001			
SFA/UFA	T0	0.60 ± 0.03	0.58 ± 0.26	0.58 ± 0.12	0.595	0.107	0.253	0.365
	T4	0.66 ± 0.27	0.63 ± 0.14	0.59 ± 0.09	0.468			
MUFA/PUFA	T0	1.33 ± 0.28	1.47 ± 0.08	1.28 ± 0.45	0.180	0.070	0.088	< 0.001
	T4	1.62 ± 0.17	1.77 ± 0.24	1.81 ± 0.13	0.422			
Σ EPA + DHA	T0	Nd[b]	0.46 ± 0.02[a]	0.44 ± 0.02[a]	< 0.001	–	0.044	0.127
	T4	Nd[b]	0.38 ± 0.08[a]	0.40 ± 0.04	< 0.001			

* Not enriched (Co) and enriched with monolayer (Mo) and multilayered microcapsules (Mu): samples analyzed before and after the refrigeration storage for 4 months (T0 and T4, respectively). Bars with different letters (a, b, c) within the same formulation show significant differences (p < 0.05) due to enrichment effect. Nd: not detected. See the caption of the table 1 for the names of the fatty acids.

of (Pérez-Palacios, Ruiz, Martín, Muriel, & Antequera, 2008).

2.7. Analysis of fatty acids

Firstly, the Fatty Acid Methyl Esthers (FAMEs) of fat (10 mg) were prepared by acidic transesterification, as described by (Sandler, Karo, Sandler, & Karo, 1992). In the case the digestion phases, the total amount of extracted fat was esterified, adjusting the volume of the methylation reagents. FAMEs were analyzed by gas chromatography (GC) using a Hewlett–Packard HP-5890A gas chromatograph, equipped with an on-column injector and a flame ionization detector, using a polyethylene glycol capillary column (Supelcowax-10, Supelco, Bellefonte, PA, USA) (60 m × 0.32 mm i.d. × 0.25 μm film thickness). The GC oven program temperature was as follows: initial temperature of 180 °C that increased at 5 °C/min to 200 °C, being maintained 40 min at this temperature; thereafter, it increased at 5 °C/min to 250 °C, and then kept for an additional 21 min. The injector and detector temperatures were 250 °C. The carrier gas was helium at a flow rate of
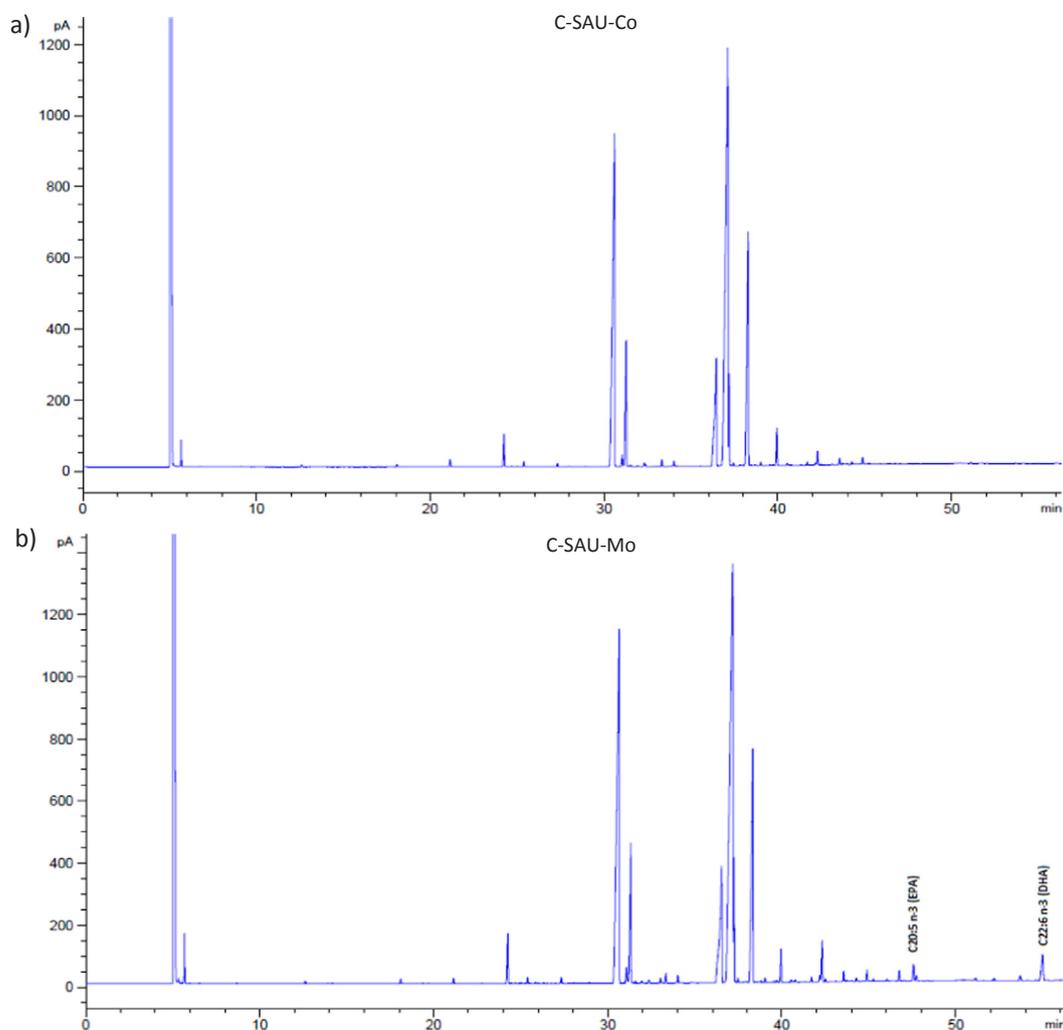
Fig. 1. Chromatograms of fatty acid methyl esters peaks from control (a) and enriched (b) cooked sausages.

0.8 mL/min. Individual FAME peaks were identified by comparison of their retention times with those of standards (Sigma, St. Louis, MO, USA). Peak areas were measured and FAMEs were expressed as mg FAMEs per g of sample, by using internal standard (tridecanoic acid, C13) and calibration curves of FAMEs (Supelco 37 component FAME mix, PA, USA).

2.8. Simulated digestion

The release of fatty acids was evaluated under simulated digestion conditions following the methodology of (Wang, Gong, Huang, Yu, & Xue, 2009) and (Werner & Böhm, 2011) with slight modifications. Firstly, the oral, gastric and intestinal solutions were prepared with 20 mg amylase in 1 mL water at pH 6.5, 3.2 g/L pepsin in 2 g/L NaCl at pH 1.5, and 10 g/L pancreatin in 0.05 mol/L KH_2PO_4 at pH 7.4. Then, the sample (2 g) was weighed, mixed with the oral fluid (0.5 mL) by vortex during 1 min, and stirred at 300 rpm during 5 min at 37 °C. The supernatant was separated from the residue by extraction with hexane (5 mL) and centrifugation (4000 rpm, 20 min). The residue was added with the gastric fluid (8 mL) and mixed by vortex. The mixture was incubated at 37 °C with shaking at 300 rpm during 2 h. Again, the supernatant was extracted with hexane, and the residue was incubated with the intestinal fluid (10 mL) at 37 °C with stirring at 300 rpm during 3 h. At each time point of 1, 2 and 3 h, three tubes were taken out to extract the supernatant. All supernatants were extracted in weighted glass tubes. After evaporating the solvent of each digestion stage, the

lipid content was calculated gravimetrically and their fatty acid composition was analyzed by GC-FID, as previously described. Results were expressed as mg of FAMEs per gram of sample released at each digestion stage. Besides, the percentage of released fat in relation the initial fat content (before digestion) was also calculated.

2.9. Sampling replication and statistical analysis

The effects of fish oil microcapsules addition and storage on composition and bioaccessibility of fatty acids were analyzed by one-way analysis of variance (ANOVA). When a significant effect ($p < 0.05$) was detected, paired comparisons between means were conducted using the Tukey's test. The statistics were run using the program IBM SPSS Statistics v.22 (IBM Co., New York, USA).

3. Results and discussion

3.1. Lipid composition of dry-cured and cooked sausages as affected by enrichment with fish oil microcapsules and storage

The addition of ω-3 fish oil microcapsules did not affect the percentages of fat in C-SAU and D-SAU, being around 17.59–17.84% and 26.31–26.87%, respectively. These results are expected result since the amount of microcapsules added in the enriched batches is quite small (3 and 5 g per 100 g of meat product in Mo and Mu respectively).

Tables 1 and 2 show the fatty acid composition of C-SAU and D-SAU

batches, respectively, as affected by the addition with fish oil microcapsules and refrigeration storage for four months. In the case of C-SAU, the sum of saturated (SFA) and monounsaturated fatty acids (MUFA) showed similar quantities, which were higher than the sum polyunsaturated fatty acids (PUFA). In these batches, oleic acid (C18:1n-9) showed the highest quantity, followed in decreasing order by, linoleic (C18:2n-6), stearic (C18:0) and palmitic (C16:0) acids, the rest of fatty acids showed concentrations lower than 1 mg FAMEs/g sample. This fatty acid profile reflected the fatty acid composition of the ingredients used to manufacture this product, mainly made with chicken meat and pork fat. Moreover, the fatty acid composition of C-SAU is quite in agreement with that found in other previous studies on similar meat products (Pereira, Tarley, Matsushita, & de Souza, 2000; Yilmaz, Şimşek, & Işikli, 2002).

In relation to the effect of enrichment with fish oil microcapsules, as example, Fig. 1 shows the FAME peak chromatograms from C-SAU-C and C-SAU-Mo bathes, evidencing the existence of EPA and DHA FAME peaks in the enriched batches but not in the control ones. In C-SAU, the addition of Mo and Mu lead to significant differences in some fatty acids (Table 1). As expected, EPA and DHA quantities significantly increased from Co (not detected) to the enriched batches ($p < 0.001$), with no significant differences between Mo and Mu in the case of EPA at T0 (0.18 and 0.19 mg EPA/g sample, respectively) and at T4 (0.14 and 0.15 mg EPA/g sample, respectively). However, the batches enriched with Mu showed higher quantities of DHA at T0 and at T4 (0.44 and 0.43 mg DHA/g sample, respectively) than Mo (0.41 and 0.37 mg DHA/g sample, respectively). This could be explained by the additional layer of chitosan on wall of Mu, which avoids contacts and reactivity with water, oxygen, iron and other oxidant promoters during cooking process and storage, minimizing the oxidation of ω-3 PUFA encapsulated (Jiménez-Martín et al., 2014; Klinkesorn, Sophanodora, Chinachoti, Decker, & McClements, 2005). Thus, C-SAU enriched with Mo and Mu exceeded the minimum level established by the European Union legislation to label a food as "source of ω-3 fatty acids" (EU, 2010), with values of 0.59 and 0.64 mg EPA + DHA/g sample, respectively, at T0 and of 0.51 and 0.58 mg EPA + DHA/g sample, respectively, at T4. The addition of Mo and Mu microcapsules also significantly increased the quantities of oleic acid (C18:1n-9) (5.87 and 5.93 mg C18:1n-9/g sample, respectively) in comparison with Co (5.62 mg C18:1n-9/g sample) at T4 ($p = 0.024$), and this tendency was also observed at T0. These differences could be ascribed to the moderate percentage of oleic acid in fish oil (15.81 g/100 g FAMEs) (Jiménez-Martín, Antequera Rojas, Gharsallaoui, Ruiz Carrascal, & Pérez-Palacios, 2016). As consequence of these effects, the n-6/n-3 ratio significantly diminished from Co to Mo and Mu at T0 ($p < 0.001$) of (23.78, 6.49 and 6.48 in Co, Mo and Mu, respectively) and at T4 ($p < 0.001$) (32.12, 8.06 and 7.04 in Co, Mo and Mu, respectively). This effect has also been previously reported by (Aquilani, Pérez-Palacios, Jiménez Martín, et al., 2018) in pork burgers enriched with fish oil microcapsules.

The effect of storage at refrigeration of C-SAU for four months significantly influence on some fatty acids, leading to a slight decrease ($p < 0.05$) from T0 to T4 of oleic acid (C18:1n-9), γ-linolenic acid (C18:3n-6), α-linolenic acid (C18:3n-3), eicosatrienoic acid (C20:3n-3) and EPA with independence of the experimental batches (Co, Mo or Mu). Nevertheless, the sum of SFA, MUFA, PUFA and EPA + DHA was similar at T0 and T4. This may indicate the appropriateness of both of wall materials of both types of microcapsules tested in the present study (maltodextrin in the case of Mo and chitosan-maltodextrin in the case of Mu) to protect the fatty acids of the encapsulated fish oil to oxidation during the refrigeration storage for four months. Besides, this finding is quite in concordance with previous studies in similar dry-cured sausages (Rubio, Martínez, García-Cachán, Rovira, & Jaime, 2008; Summo, Caponio, & Pasqualone, 2006), which showed scarce differences in the profile of fatty acid after storage.

Table 2 exposes the fatty acid composition of D-SAU batches. As occurred in C-SAU, SFA and MUFA showed similar quantities and

higher than the sum PUFA. Again, oleic acid (C18:1n-9) was the major fatty acid, followed in decreasing order by palmitic (C16:0), linoleic (C18:2n-6), stearic (C18:0) and palmitoleic (C16:1n-7) acids, and the rest of fatty acids showed concentrations lower than 1 mg FAMEs/g sample. This fatty acid profile is in concordance with the fatty acid composition of the ingredients used to manufacture this product, mainly made with Iberian pork meat and fat. Besides, it agrees with other previous studies on similar dry-cured sausages (Bañón, Bedia, Almela, & Martínez, 2010; Navarro, Nadal, Izquierdo, & Flores, 1997). The enrichment effect by the addition of Mo and Mu fish oil microcapsules in D-SAU significantly increased the quantities of EPA and DHA ($p < 0.001$) from Co (not detected) to Mo (0.15 and 0.11 mg EPA/g sample and 0.31 and 0.27 mg DHA/g sample at T0 and T4, respectively) and Mu batches (0.14 and 0.11 mg EPA/g sample and 0.30 and 0.29 mg DHA/g sample at T0 and T4, respectively). No significant differences were found between the enriched batches. Previous studies in pork fermented sausages and pork Spanish salchichon (Lorenzo et al., 2016; Muguerza, Ansorena, & Astiasarán, 2004) have reported the increase in the percentage of EPA and DHA in the batches enriched with bulk and microencapsulated fish oil extract respectively. Nevertheless, these works did not show quantification results, not being possible to compare their results with that obtained in present study. The addition of Mo and Mu microcapsules also significantly increased the quantities of oleic acid (C18:1n-9) ($p = 0.034$ and $p = 0.019$, respectively), being also higher at T0 and at T4 in the enriched batches (11.22 and 10.78 mg/g sample for Mo, respectively and 10.21 and 11.65 mg/g sample for Mu, respectively) in comparison to the control one (10.05 and 9.41 mg/g sample, respectively). As previously explained, these results may be ascribed to the high content of oleic acid in the fish oil. A significant decrease in the n6/n3 ratio has also been observed at T0 ($p < 0.001$) and at T4 ($p < 0.001$) from Co batches (23.22 and 37.89, respectively) to Mo (10.55 and 12.04, respectively) and Mu ones (10.96 and 10.36, respectively). This means a decrease factor around 13 in the n6/n3 ratio, higher than that found in previous studies in Spanish salchichon (Lorenzo et al., 2016), dry-fermented sausages (Muguerza et al., 2004) and Dutch-style fermented sausages (Josquin et al., 2012; Pelser et al., 2007), which showed decreased factors around of 2–6, 8–11, 5–12 and 7–9, respectively. The n6/n3 ratio is considered an important health parameter associated with cardiovascular illness, cancer and inflammatory and autoimmune diseases (Simopoulos, 2002; Wood et al., 2004), and their decreased by the addition of fish oil microencapsulates in D-SAU may be a notable aspect from a healthy point of view.

The effect of the storage at refrigeration for four months on D-SAU significantly decreased the quantities of most fatty acids and of the sum MUFA, PUFA and n-3 in most samples. However, the quantity of EPA and DHA were not influenced by storing, in concordance with previous studies in similar dry-cured sausages (Rubio et al., 2008; Summo et al., 2006). The general maintenance of EPA and DHA quantities in C-SAU and D-SAU batches may point out the protection effect of Mo and Mu during processing, storing and cooking. This supports the findings of a previous study showing low levels of primary and secondary oxidation products in control and enriched C-SAU and D-SAU with Mo and Mu. Besides, similar oxidation values were observed among these batches (Solomando, Antequera, & Perez-Palacios, 2020).

3.2. Fat and fatty acids released through in vitro digestion of cooked and dry-cured sausages as affected by enrichment with fish oil microcapsules

Regarding to the in vitro digestion assay on meat products, Fig. 2.a exposes the percentages of released fat in C-SAU and D-SAU. In general, in both meat products, the highest percentages of released fat were found in the intestinal phase (around 34 and 71%, respectively) followed in decreasing order by oral (around 8 and 19%, respectively) and gastric phases (around 3 and 7%, respectively). These results are in concordance with previous fat digestion studies, which have shown
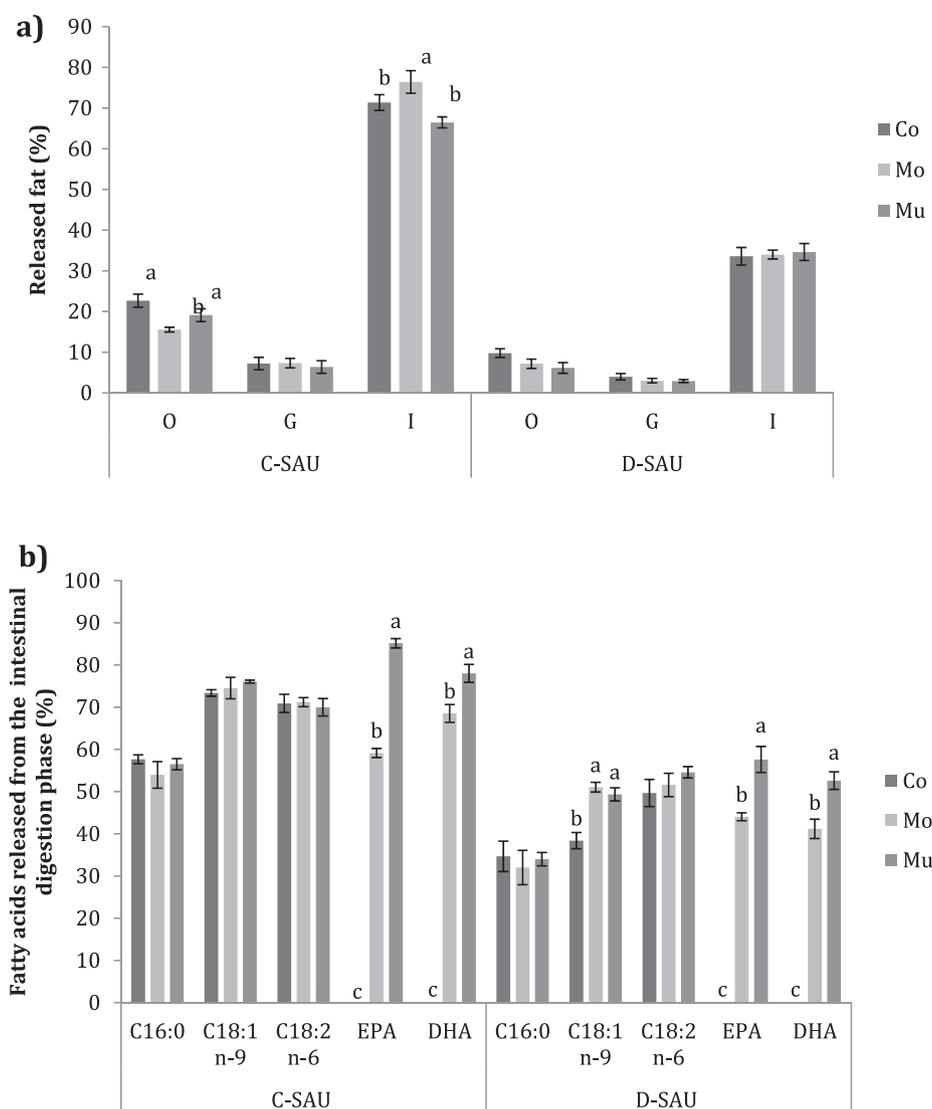
Fig. 2. Results on released of fat (%) through the in vitro digestion phases (O: oral, G: gastric and I: intestinal) (a) and of individual fatty acids (%) in the intestinal digestion phase (b) of cooked (C-SAU) and dry-cured sausages (D-SAU) as affected by enrichment with fish oil microcapsules *.Sausages not enriched (Co, dark gray), enriched with multilayered (Mu, medium gray) and monolayer fish oil microcapsules (Mo, light gray). Bars with different letters (a,b,c) show significant differences (p < 0.05) due to enrichment effect (Co vs Mo vs Mu).

percentages of hydrolysis around 10–30% in the stomach phase and 50–90% in the intestine phase, depending on the nature of the lipid compound (Friedman & Nylund, 1980; Gunstone, 2001; Lairon, 2009). This is normal since all digestive enzymes, including lipolytic enzymes (pancreatic lipase, phospholipase and sterol esterase), are secreted at the intestinal level, producing most of the hydrolyzed lipid compounds.

The microcapsules addition led to significant differences in C-SAU, with samples added with Mo showing lower percentage of released fat in the oral phase (15.54%) and higher in intestinal phase (76.42%) in comparison to Co (22.66 and 71.36%, respectively) and Mu (19.11 and 66.47%). However, no significant differences were found between D-SAU batches. It is also noted that the percentage of released fat from C-SAU is higher than from D-SAU at each phase of the digestion, being the total released fat around 100 and 45% for C-SAU and D-SAU, respectively. It is described that the presence of solid fat and low areas of exposure to lipolytic enzymes leads to a lower lipolysis rate (Golding & Wooster, 2010; McClements, Decker, & Park, 2009). This may explain the results of this study since the chopping process of the ingredients until obtain a fine paste in C-SAU decrease the size of the fat globules (Barretto, Pacheco, & Pollonio, 2015), facilitating the action of lipolytic enzymes.

Table 3 shows the quantity of fatty acids released in the different phases of the in vitro digestion of C-SAU. In Co batches, the highest quantities of fatty acids were found in the intestinal phase, followed in decreasing order by oral and gastric phases. This is in concordance with the results on the percentage of fat released, previously explained. In general, the major fatty acid released was oleic acid (C18: 1n-9), following in decreasing order by palmitic (C16: 0) and linoleic acids (C18: 2n-6), and stearic acid (C18: 0), with the rest of fatty acids having less than 0.5 mg/g sample digested. This behavior agrees with the profile of fatty acids described in Table 1 for C-SAU. These results are hardly comparable since there are no previous studies of in vitro digestibility of enriched meat products. Nevertheless, the effects of digestibility on the amount and type of fatty acids have been studied in millet (Annor, Marcone, Corredig, Bertoft, & Seetharaman, 2015), prawns fed with diets enriched with different oils (cod liver, olive, coconut, sesame, canola, flaxseed and fish) (Glencross, Smith, Thomas, & Williams, 2002), red hybrid tilapia fed with palm oil (Bahurmiz & Ng, 2007) and broiler chickens fed with a commercial diet (Tancharoenrat, Ravindran, Zaefarian, & Ravindran, 2014). All these investigations showed that the digestibility of total lipids was not affected by the composition of fatty acids, except when the levels of PUFA in the diet exceeded 17 g kg^{-1}.

Table 3

Fatty acids released through in vitro digestion of cooked sausages as affected by the addition of different types of fish oil microcapsules: monolayered (Mo) and multilayered (Mu)*.

Fatty acids	Co			Mo			Mu			pE		
	O	G	I	O	G	I	O	G	I	O	G	I
C14:0	0,09 ± 0.01	0,03 ± 0.00	0,29 ± 0.05	0,08 ± 0.00	0,03 ± 0.00	0,28 ± 0.03	0,08 ± 0.01	0,02 ± 0.00	0,31 ± 0.07	0.321	0.562	0.196
C16:0	2,66 ± 0.24	0,58 ± 0.05	5,24 ± 0.16	2,54 ± 0.12	0,58 ± 0.02	5,58 ± 0.31	2,39 ± 0.09	0,51 ± 0.03	5,74 ± 0.40	0.207	0.126	0.097
C16:1n-7	0,25 ± 0.07	0,08 ± 0.01	0,82 ± 0.10	0,25 ± 0.04	0,08 ± 0.02	0,91 ± 0.22	0,20 ± 0.04	0,07 ± 0.01	0,83 ± 0.13	0.321	0.436	0.224
C17:0	0,06 ± 0.01	0,02 ± 0.00	0,19 ± 0.06	0,06 ± 0.00	0,02 ± 0.00	0,21 ± 0.04	0,06 ± 0.00	0,02 ± 0.00	0,23 ± 0.03	0.789	0.825	0.510
C17:1n-7	0,03 ± 0.00	0,01 ± 0.00	0,10 ± 0.01	0,02 ± 0.00	0,01 ± 0.00	0,09 ± 0.00	0,02 ± 0.00	0,01 ± 0.00	0,10 ± 0.00	0.621	0.987	0.778
C18:0	0,89 ± 0.13	0,33 ± 0.03	2,07 ± 0.26	0,86 ± 0.09	0,35 ± 0.02	2,20 ± 0.37	0,75 ± 0.05	0,31 ± 0.06	1,97 ± 0.29	0.109	0.204	0.326
C18:1n-9	2,27 ± 0.41	0,71 ± 0.11	7,37 ± 0.56[b]	2,30 ± 0.34	0,76 ± 0.09	8,46 ± 0.76[a]	1,94 ± 0.08	0,62 ± 0.02	7,96 ± 0.58[b]	0.153	0.065	0.041
C18:2n-6	1,83 ± 0.22	0,58 ± 0.10	5,95 ± 0.84[b]	1,59 ± 0.13	0,53 ± 0.09	5,84 ± 0.56[b]	1,56 ± 0.08	0,50 ± 0.06	6,41 ± 0.43[a]	0.231	0.069	0.037
C18:3n-6	0,02 ± 0.00	Nd	0,05 ± 0.00	0,01 ± 0.00	Nd	0,05 ± 0.00	0,02 ± 0.00	Nd	0,06 ± 0.01	0.458	–	0.495
C18:3n-3	Nd	Nd	0,01 ± 0.00	0,01 ± 0.00	Nd	0,02 ± 0.00	0,01 ± 0.00	Nd	0,02 ± 0.00	0.105	–	0.079
C20:0	0,02 ± 0.00	0,01 ± 0.00	0,07 ± 0.01	0,02 ± 0.00	0,01 ± 0.00	0,09 ± 0.02	0,02 ± 0.00	0,01 ± 0.00	0,09 ± 0.01	0.462	0.945	0.101
C20:1n-9	0,15 ± 0.03	0,05 ± 0.00	0,48 ± 0.09	0,15 ± 0.02	0,05 ± 0.01	0,54 ± 0.08	0,17 ± 0.04	0,05 ± 0.00	0,68 ± 0.11	0.174	0.906	0.206
C20:2n-6	0,06 ± 0.01	0,02 ± 0.00	0,18 ± 0.03	0,05 ± 0.00	0,02 ± 0.00	0,19 ± 0.03	0,05 ± 0.01	0,02 ± 0.00	0,22 ± 0.04	0.398	0.864	0.422
C20:3n-6	0,03 ± 0.00	0,01 ± 0.00	0,09 ± 0.01	0,02 ± 0.00	0,01 ± 0.00	0,08 ± 0.01	0,02 ± 0.00	0,01 ± 0.00	0,09 ± 0.02	0.378	0.789	0.736
C20:3n-3	0,08 ± 0.03	0,02 ± 0.00	0,26 ± 0.06	0,07 ± 0.01	0,02 ± 0.00	0,24 ± 0.07	0,06 ± 0.00	0,02 ± 0.00	0,27 ± 0.04	0.208	0.836	0.501
C20:4n-6	0,01 ± 0.00	Nd	0,03 ± 0.00[b]	0,01 ± 0.00	Nd	0,05 ± 0.00[a]	0,02 ± 0.01	Nd	0,06 ± 0.00[a]	0.471	–	< 0.001
C20:5n-3	Nd[b]	Nd[b]	Nd[c]	0,04 ± 0.00[a]	0,02 ± 0.00[a]	0,08 ± 0.04[b]	0,03 ± 0.00[a]	0,02 ± 0.00[a]	0,12 ± 0.04[a]	< 0.001	< 0.001	< 0.001
C24:0	0,02 ± 0.00	Nd	0,05 ± 0.02	0,01 ± 0.00	Nd	0,05 ± 0.00	0,02 ± 0.01	Nd	0,06 ± 0.02	0.169	–	0.071
C22:6n-3	Nd[b]	Nd[b]	Nd[c]	0,06 ± 0.00[a]	0,05 ± 0.00[a]	0,17 ± 0.04[b]	0,06 ± 0.00[a]	0,02 ± 0.00[a]	0,23 ± 0.07[a]	< 0.001	< 0.001	< 0.001
Σ SFA	2.84 ± 0.21	0.89 ± 0.17	9.21 ± 0.55	2.67 ± 0.16	0.68 ± 0.10	9.81 ± 0.62	2.61 ± 0.20	0.77 ± 0.08	10.38 ± 0.49	0.324	0.269	0.276
Σ MUFA	2.70 ± 0.18	0.80 ± 0.15	8.57 ± 0.71[c]	2.62 ± 0.21	0.85 ± 0.11	9.29 ± 1.06[b]	2.24 ± 0.63	0.75 ± 0.07	9.98 ± 0.84[a]	0.096	0.061	< 0.001
Σ PUFA	2.02 ± 0.13	0.64 ± 0.07	6.57 ± 0.46	1.85 ± 0.27	0.61 ± 0.11	6.80 ± 0.33	1.82 ± 0.21	0.58 ± 0.08	7.47 ± 1.13	0.065	0.116	0.084

Data are expressed as mg FAMEs / g sample digested.

Bars with different letters (a,b,c) within the same digestion phase show significant differences (p < 0.05) due to enrichment effect.

See the caption of the Table 1 for the names of the fatty acids.

* O, G, I: oral, gastric and intestine digestion phases, respectively. Nd: not detected.

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The enrichment with Mo and Mu did not lead to marked differences in the quantities of fatty acid released throughout in vitro digestion. As for the quantities of EPA and DHA released, they followed the same trend as the rest of fatty acids, with higher values in the intestinal than in the oral and gastric phases. This may indicate than the encapsulated fatty acids within the meat matrix are digested as the rest of fatty acids of the products, and point out the appropriateness of the wall materials of both types microcapsules (maltodextrin in combination or not with chitosan) to protect against the acidity of the gastric environment, which is supported by results on previous studies on different wall material (Aranaz et al., 2012; Klinkesorn, Sophanodora, Chinachoti, & McClements, 2004; Yongsheng et al., 2008). Moreover, the type of fish oil microcapsules significantly influence on the quantities of these fatty acids that are released in the intestinal phase, being higher when Mu are added to C-SAU in comparison to Mo, for both EPA (0.08 and 0.12 mg EPA/g sample digested, respectively) and DHA (0.17 and 0.23 mg DHA/g sample, respectively). This result can be attributed to the combination of the protective effect of the maltodextrin wall together with the chitosan in Mu, since their electrostatic characteristic depends on the relative pH. At pH between 1 and 3 the chitosan tends to charge positively, having a high electrostatic attraction with the anionic molecules, however the chitosan losses its positive charge at above 6.5, not having electrostatic interaction (Hur, Lim, Decker, & McClements, 2011; McClements & Li, 2010). Thus, during the acid digestion in the stomach, it may take place a high electrostatic interaction between the chitosan and the lipid drops, decreasing the amount of exposed lipid surface, but in the intestinal digestion phase (pH between 6 and 7.5) that electrostatic interaction diminishes, which may favor the release of the lipid molecules to be digested by the pancreatic lipase enzyme.

In the case of the batches enriched with Mo the protection of the ω-3 PUFA is lower than Mu because the fish oil is only covered by a single wall of maltodextrin and, although the polysaccharides disperse easily and quickly in water, the compact molecular structure of maltodextrin prevents rapid absorption of water from the food, providing a partial protection against oxidizing agents in the early stages of the gastrointestinal tract. At the gastric level, the decrease in pH catalyzes the hydrolysis of the glycosidic bonds that bind the monosaccharide molecules, which induce the beginning of the microcapsule wall degradation. However, the microcapsule may not be completely digested until the intestine by the action of pancreatin (Damodaran, Parkin, & Fennema, 2008).

According to (Calvo, Lozano, Espinosa-Mansilla, & González-Gómez, 2012), the amount of oil released is related to the efficiency of the microencapsulation and therefore with the external and internal oil content of the microcapsules. This statement could also be related to the higher quantities of EPA and DHA released when Mu are added, since the external fat of Mu is higher than of Mo (Jiménez-Martín, Gharsallaoui, Pérez-Palacios, Carrascal, & Antequera, 2014).

Results on the quantities of fatty acids released throughout the in vitro digestion of D-SAU batches are shown in Table 4. As occurred in C-SAU, the major release of fatty acids took place in the intestinal phase and the major fatty acids are the most released ones. However, no significant differences were found between D-SAU samples enriched with Mo and Mu in the quantity of EPA and DHA, whereas this effect was observed in C-SAU. Moreover, comparing results from Table 3 and 4, it is observed a higher quantity of fatty acid released from C-SAU than from D-SAU. This is in concordance with results on the percentage of fat released (Fig. 2a), which were previously discussed.

Once quantified the fatty acids of C-SAU and D-SAU batches as well as their released throughout the different phases of the in vitro digestion, the bioaccessibility of major fatty acids (palmitic acid (C16:0), oleic acid (C18:1n-9), linoleic acid (C18:2n-6)) and of added fatty acids (EPA and DHA) has been calculated (Fig. 2.b), in order to know about the percentage of the ingested fatty acids available for absorption. As can be seen, the type of fish oil microcapsules significantly influenced on the bioaccessibility of EPA and DHA, being lower when added Mo

than Mu in C-SAU (59 and 85% EPA, 68 and 78% DHA, respectively) and in D-SAU (44 and 57% EPA, 41 and 52% DHA, respectively). Moreover, the addition of fish oil microcapsules significantly increased the bioaccessibility of oleic acid (C19:1n-9) (38, 51 and 49% in Co, Mo and Mu, respectively), which is an extra but positive effect from a nutritional point of view. As occurred with the quantities of fatty acid released, their bioaccesibility was also higher in C-SAU than in D-SAU, which can be ascribed to the differences in the meat matrix, as previously explained. Moreover, these results are in agreement with those found by Shen, Apriani, Weerakkody, Sanguansri, and Augustin (2011) who added microencapsulated tuna oil powder to orange juice, yogurt and cereal bar with. These authors found a higher lipolysis extent of omega-3 PUFA in orange juice and yogurt samples than in cereal bar ones, which was explained by the larger lipid droplets in the digest of the cereal bar and, hence, to the low total surface area available for lipase attack. This statement could be the reason behind the findings of this study. It is also noted differences in the bioaccesibility between fatty acids in all analyzed batches, being lower for palmitic acid (C16:0) (around 60 and 40% in C-SAU and D-SAU, respectively) in comparison to oleic acid (C18:1n-9), linoleic acid (C18:2n-6), EPA and DHA (around 70 and 50% in C-SAU and D-SAU, respectively). These differences could be related to the specific position (sn-1, sn-2 or sn-3) at which the different fatty acids are sterified, since the pancreatic lipase has a high specificity of for fatty acids esterified at the sn-1 and sn-3 positions of triacylglycerols (Shen & Wijesundera, 2006)

4. Conclusions

The addition of fish oil microcapsules made of lecithin + maltodextrin and lecithin + chitosan-maltodextrin to cooked and dry-cured meat products achieves the enrichment in EPA and DHA of these products, not being influenced by the refrigeration storage but susceptible to be labelled as "source of ω-3 fatty acids" according to European Union legislation. Besides, the enrichment with these types of vehicles of omega-3 fatty acids did not influence on the lipid composition of the analyzed meat products.

The quantity of fatty acid released at the different phases of the in vitro digestion of meat products added with microcapsules is firstly described in the present study. The release of fat and fatty acids of the meat products is not affected by the addition of fish oil microcapsules, but it influenced on the bioaccesibility of EPA and DHA. Moreover, the type meat matrix seems to be a significant effect on the released of fat and fatty acids. In this way, the addition of fish oil microcapsules of lecithin + chitosan-maltodextrin as wall material to cooked sausages should be more appropriate than fish oil microcapsules of lecithin + maltodextrin and dry-cured sausages to maximize the percentage of EPA and DHA available for absorption. Therefore, it could be pointed out the importance of analyzing not only the quantity of EPA and DHA in the enriched foods, but also the bioavailability of these bioactive compounds in most of the possible products. These could be used to develop functional foods that provide healthier lipid profiles and promote health and welfare.

CRediT authorship contribution statement

Juan Carlos Solomando: Validation, Formal analysis, Investigation, Data curation, Writing - original draft. **Teresa Antequera:** Methodology, Resources, Writing - review & editing. **Trinidad Perez-Palacios:** Conceptualization, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

None.

Table 4

Fatty acids released through in vitro digestion of dry-cured sausages as affected by the addition of different types of fish oil microcapsules: monolayered (Mo) and multilayered (Mu)*.

Fatty acids	Co			Mo			Mu			pE		
	O	G	I	O	G	I	O	G	I	O	G	I
C14:0	0.07 ± 0.01	0.03 ± 0.00	0.28 ± 0.07	0.06 ± 0.01	0.04 ± 0.00	0.33 ± 0.09	0.04 ± 0.00	0.03 ± 0.00	0.27 ± 0.06	0.352	0.263	0.195
C16:0	0.55 ± 0.06	0.10 ± 0.03	0.71 ± 0.31[b]	0.57 ± 0.06	0.16 ± 0.02	1.12 ± 0.19[a]	0.54 ± 0.05	0.15 ± 0.02	1.18 ± 0.14[a]	0.236	0.046	< 0.001
C16:1n-7	0.06 ± 0.00	0.03 ± 0.00	0.26 ± 0.11	0.07 ± 0.00	0.04 ± 0.00	0.39 ± 0.08	0.06 ± 0.01	0.04 ± 0.00	0.38 ± 0.04	0.411	0.569	0.102
C17:0	0.04 ± 0.00	0.02 ± 0.00	0.15 ± 0.03	0.03 ± 0.00	0.02 ± 0.00	0.18 ± 0.06	0.02 ± 0.00	0.01 ± 0.00	0.13 ± 0.03	0.201	0.236	0.302
C17:1n-7	0.02 ± 0.00	0.01 ± 0.00	0.08 ± 0.01	0.02 ± 0.00	0.01 ± 0.00	0.10 ± 0.02	0.02 ± 0.00	0.01 ± 0.00	0.11 ± 0.03	0.732	0.653	0.721
C18:0	0.38 ± 0.08	0.17 ± 0.04	1.06 ± 0.29	0.36 ± 0.07	0.22 ± 0.04	1.16 ± 0.41	0.34 ± 0.07	0.20 ± 0.05	1.09 ± 0.57	0.521	0.098	0.085
C18:1n-9	0.56 ± 0.11	0.25 ± 0.06	2.25 ± 0.43[b]	0.56 ± 0.13	0.34 ± 0.07	3.11 ± 0.67[a]	0.53 ± 0.18	0.31 ± 0.14	3.03 ± 0.54[a]	0.186	0.092	0.039
C18:2n-6	0.47 ± 0.09	0.21 ± 0.01	2.69 ± 0.47	0.46 ± 0.11	0.28 ± 0.06	2.49 ± 0.21	0.43 ± 0.10	0.26 ± 0.04	2.76 ± 0.39	0.236	0.312	0.142
C20:0	0.02 ± 0.00	0.01 ± 0.00	0.06 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.07 ± 0.01	0.02 ± 0.00	0.01 ± 0.00	0.09 ± 0.02	0.421	0.721	0.206
C20:1n-9	0.09 ± 0.01	0.04 ± 0.00	0.35 ± 0.07	0.08 ± 0.00	0.05 ± 0.00	0.44 ± 0.11	0.07 ± 0.01	0.04 ± 0.00	0.45 ± 0.13	0.645	0.695	0.158
C20:2n-6	0.04 ± 0.00	0.02 ± 0.00	0.15 ± 0.02	0.03 ± 0.00	0.02 ± 0.00	0.16 ± 0.03	0.03 ± 0.00	0.02 ± 0.01	0.16 ± 0.04	0.462	0.430	0.671
C20:3n-6 + C21	0.01 ± 0.00	Nd	0.03 ± 0.00	0.01 ± 0.00	Nd	0.04 ± 0.00	0.01 ± 0.00	Nd	0.03 ± 0.00	0.853	–	0.409
C20:3n-3	0.02 ± 0.00	0.01 ± 0.00	0.08 ± 0.02	0.03 ± 0.00	0.02 ± 0.01	0.12 ± 0.03	0.02 ± 0.00	0.01 ± 0.00	0.11 ± 0.04	0.512	0.301	0.096
C20:4n-6	0.01 ± 0.00	Nd	0.02 ± 0.00	0.01 ± 0.00	Nd	0.03 ± 0.00	0.01 ± 0.00	Nd	0.03 ± 0.01	0.802	–	0.723
C20:5n-3	Nd[b]	Nd[b]	Nd[b]	0.03 ± 0.00[a]	0.01 ± 0.00[a]	0.08 ± 0.03[a]	0.02 ± 0.00[a]	0.01 ± 0.00[a]	0.11 ± 0.05[a]	< 0.001	< 0.001	< 0.001
C24:0	0.01 ± 0.00	Nd[b]	0.02 ± 0.00[c]	0.01 ± 0.00	0.01 ± 0.00[a]	0.04 ± 0.01[a]	0.01 ± 0.00	Nd[b]	0.03 ± 0.00[b]	0.326	< 0.001	< 0.001
C22:6n-3	Nd[b]	Nd[b]	Nd[b]	0.05 ± 0.01[a]	0.03 ± 0.00[a]	0.16 ± 0.06[a]	0.04 ± 0.01[a]	0.02 ± 0.00[a]	0.23 ± 0.07[a]	< 0.001	< 0.001	< 0.001
Σ SFA	0.76 ± 0.13	0.33 ± 0.08	3.03 ± 0.59	0.74 ± 0.15	0.45 ± 0.06	3.97 ± 0.71	0.67 ± 0.23	0.40 ± 0.08	4.10 ± 0.39	0.098	0.075	0.106
Σ MUFA	0.74 ± 0.08	0.32 ± 0.08	2.94 ± 0.09[b]	0.74 ± 0.09	0.45 ± 0.05	3.95 ± 0.82[a]	0.68 ± 0.11	0.41 ± 0.06	4.16 ± 0.74[a]	0.136	0.083	< 0.001
Σ PUFA	0.55 ± 0.06	0.27 ± 0.04	2.19 ± 0.33[b]	0.59 ± 0.21	0.36 ± 0.07	3.16 ± 0.51[a]	0.55 ± 0.11	0.33 ± 0.07	3.35 ± 0.27[a]	0.263	0.102	< 0.001

Data are exposed as mg FAMEs / g sample digested.

Bars with different letters (a,b,c) within the same digestion phase show significant differences (p < 0.05) due to enrichment effect. See the caption of the Table 1 for the names of the fatty acids.

* O, G, I: oral, gastric and intestine digestion phases, respectively. Nd: not detected.

Acknowledgements

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