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Proteolysis, physicochemical characteristics and free fatty acid composition of dry sausages made with deer (*Cervus elaphus*) or wild boar (*Sus scrofa*) meat: A preliminary study

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Abstract

In order to contribute to typifying delicatessen made with game meat, the proteolysis, physicochemical characteristic and free fatty acid composition were determined in 10 commercial dry sausages, chorizos and saucissons, made with deer or wild boar meat. The a_w and pH values were similar for all the samples; however, the results for dry matter, protein nitrogen, fat, ash, sodium chloride, phosphorus, and sodium nitrite content showed great variation among the samples tested. The myofibrillar protein content was higher than the sarcoplasmic protein content in all samples analysed. The electrophoretic profiles of sarcoplasmic and myofibrillar proteins were different among samples. Principal components analysis, run on the relative density of myofibrillar and sarcoplasmic proteins, separated the chorizo and saucisson samples. Chorizo samples were a homogeneous group in the analysis of myofibrillar proteins, which indicated similar proteolysis effects for all samples. The majority acids were oleic, palmitic, linoleic and stearic in all samples. Chorizos differed from saucissons in the greater quantity ($P < 0.05$) of polyunsaturated fatty acids.

Keywords: Dry sausages; Deer; Wild boar; Physicochemical composition; Proteolysis; Free fatty acids

1. Introduction

Consumers show a growing interest in the quality of meat products and in the system by which they have been produced. First the meat is requested to be safe, meaning that its composition will maintain human health and that no artificial additive has been included in the animal diet or to the product. Furthermore, consumers are increasingly concerned about animal welfare and environmental aspects of animal production systems.

In recent years, there has been a slump in the consumption of traditional meat products and consumer interest in game meat is now increasing. Game is further distinguished but the characteristic texture and taste of the meat, differs from that of poultry and farmyard animals. Game meat is generally darker, stronger tasting and often tougher, according to the type and age of animal.

The meat of game is difficult to prepare; however, today there is a very definite tendency towards processed meat products (sausages, cans, pates) that are ready to eat and suitable for home consumption (Salghetti, 1991).

Cured, fermented and dried products from different game species have recently appeared on the market (Paleari et al., 2000). The market for game products is very restricted, and dependent on the season; for example, for

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deer and wild boar it depends on the hunting season and the culling quotas. In Spain, cured sausages production amounted to 191,844 tons in 2003 (AICE, 2004), and game preparations and canned meat have increased notably in recent years. Game constitutes a rural activity, very important from an economic standpoint in some Spanish regions such as Castilla-La Mancha. On average, the total annual revenue reaches 240 million euros, and hunters take 40,000 big game animals and 3,000,000 small game (Game Producers Association of Castilla-La Mancha, 2004).

During the ripening of fermented sausages, the proteins and lipids experience great changes. Proteolysis influences both texture and flavour development due to the formation of several low molecular mass compounds, including peptides, amino acids, aldehydes, organic acids and amines, which are important flavour compounds or precursors of flavour compounds (Demeyer et al., 1995; Fadda, Vignolo, Aristoy, Oliver, & Toldrá, 2001; Naes, Holck, Axelsson, Andersen, & Blom, 1995). Lipolysis plays an essential role in the development of dry sausage flavour. Lipids are hydrolysed by enzymes, generating free fatty acids, which are substrates for the oxidative changes that are responsible for flavour compounds (Samelis, Aggelis, & Metaxopoulos, 1993; Stahnke, 1995; Verplaetse, 1994). Diet has a great influence on game meat fatty acid composition. Game animals are herbivorous, eating a great variety of indigenous plants, grains and fruits, consequently it is difficult to establish the influence of diet on the lipid composition of their meat.

In recent years, several studies have been carried out on physicochemical characteristics of red deer meat (Peña, Domenech, & Molera, 1993; Semiadi, Barry, & Muir, 1993; Stevenson, Seman, & Littlejohn, 1992; Zomborszky, Szentmihályi, Sarudi, Horn, & Szabó, 1996) and the meat composition of wild boar meat (Zomborszky et al., 1996); to determine the influence of deer feeding on the composition of fatty acids (Volpelli, Valusso, Morgante, Pittia, & Piasentier, 2003), and the sensorial quality (Wiklund, Manley, Littlejohn, & Stevenson-Barry, 2003; Wiklund, Pickova, Sampels, & Lundström, 2001); to analyse the influence of the slaughter method on the quality of the meat (Wiklund et al., 2001) and to study the optimal conditions for packing deer meat (Vergara, Gallego, García, & Landete-Castillejos, 2003). However, very few works have been found on proteolysis, physicochemical characteristics and free fatty acid composition of dry sausages made with meat from deer or wild boar (Cantalejo, 2003; Cruz, 2003; Gómez, 2004; Vioque, Prados, Pino, Fernández-Salguero, & Gómez, 2003).

The aim of this study was to evaluate the proteolysis, physicochemical characteristics and free fatty acids composition of commercial dry sausages, chorizo and saucisson, made with deer or wild boar meat. Chorizo and saucisson are two meat products in a high demand by Spanish consumers. They are elaborated following similar processes and are mainly differentiated by the greater addition of spices in chorizo, particularly paprika, which gives the delicatessen its characteristic red colour.

2. Materials and methods

2.1. Samples

Ten types of sausages made with deer or wild boar meat were purchased from various supermarkets and meat factories in Ciudad Real and Toledo (Spain). The samples were taken, in refrigerated transport, to the laboratory to be analysed. All the samples were made with lean from deer or wild boar, pork belly, salt, natural spices, and nitrifiying salt. Paprika was added to chorizo samples.

Samples were homogenised in a household kitchen blender. Water activity, pH, dry matter, and free fatty acids content were assayed immediately. The remainder of the samples were then placed in hermetically sealed containers and stored at −20 °C for later analysis. Samples were analysed in duplicate.

2.2. Physicochemical analysis

Water activity (a_w) was measured directly using a dew-point hygrometer (Decagon Devices, model CX-2). pH was determined directly, using an Ingold electrode probe connected to a Crison model 2001 pH-meter. Moisture, fat, sodium chloride, sodium nitrite, and ash content were analysed according to AOAC methods (1990). The phosphorus was determined from the ash recovered with hydrochloric acid and the further colour determination as phosphomolybdic acid according to the method of Osborne and Voogt (1986). Total nitrogen (TN) and protein nitrogen (PN) were determined following the Kjeldahl method, with the protein nitrogen being calculated by multiplying the total nitrogen by 6.25 (AOAC, 1990).

2.3. Nitrogen fractions

Water-soluble nitrogen (WSN) and non-protein nitrogen (NPN) were extracted using the procedure put forward by Monin et al. (1997). WSN content was determined on the supernatant obtained following the Kjeldahl method (AOAC, 1990). Precipitation of the proteins was effected using 20% (w/v) trichloroacetic acid and the NPN content was determined according to the Kjeldahl method (AOAC, 1990). The ratio between the NPN and the TN (NPN/TN × 100) was used as the proteolysis index.

2.4. SDS–PAGE

The proteins were extracted following the method described by Hughes et al. (2002). Five grams of sample were homogenised with 35 ml of 0.03 M phosphate buffer (pH 7.4) in an Ultra Turrax (Heidolph RZR 2050) for 2 min at 1200 rpm. The mixture was centrifuged at 10,000*g* for 20 min at 4 °C. The supernatant, containing the sarcoplasmic proteins, was filtered through glass fibre and stored, frozen, at −20 °C pending dialysis. The precipitate was washed with 0.03 M phosphate buffer (pH 7.4) and homogenised with 25 ml of 8 M urea and 1% (v/v) β-mercaptoethanol for 2 min. The mixture was centrifuged at 10,000*g* for 20 min at 4 °C. The supernatant, containing myofibrillar proteins, was filtered through glass fibre and stored frozen at −20 °C pending dialysis. About 100 μl volumes sarcoplasmic and myofribrillar extracts were dialysed through a minidialysis unit (Amersham Pharmacia Biotech) of 7000 Da in distilled water with magnetic stirring for 20 min.

Total proteins of sarcoplasmic and myofibrillar fractions were determined using the procedure suggested by Bradford (1976).

The protein concentration of the myofibrillar fraction was adjusted with water to give a final concentration of 6 mg/ml and diluted with Tris–HCl buffer (pH 8.0) containing 5% (w/v) SDS, 14.5% (v/v) β-mercaptoethanol, 31% (v/v) glycerol, and 0.03% (v/v) bromophenol blue to give a final concentration of 2 mg/ml. The protein concentration of the sarcoplasmic fraction was adjusted directly to 0.7 mg/ml, using the Tris–HCl buffer.

The samples was heated at 100 °C for 4 min prior to electrophoresis.

The proteins were separated by horizontal SDS–PAGE, using a Phast System apparatus (Amersham Pharmacia Biotech). Porosity gradient polyacrylamide gels, PhastGel 8-25 and 10-15 (Amersham Pharmacia Biotech), were used to separate the myofibrillar and sarcoplasmic proteins, respectively. The solid electrode buffer strips comprised 0.2 M tricine, 0.2 M Tris, 0.55% (w/v) SDS, pH 8.1. The protein separations were performed at 15 °C following the recommendations set out in the instruction manual for the equipment used (Amersham Pharmacia Biotech, 1986a).

Gels were stained using one tablet of Coomassie Blue R-350 dissolved in a water:methanol (4:6) mixture and then diluted to 50% with a 20% (v/v) acetic acid solution. Gels were destained twice with methanol:acetic acid: water (3:1:6). Gel consistency was achieved using 13% (v/v) glycerol. Staining and destaining of the gels was performed according to the recommendations set out in the PhastSystem manual (Amersham Pharmacia Biotech, 1986b). The gels were air-dried between two sheets of cellophane soaked in 13% (v/v) glycerol for 48–72 h.

Quantification of the bands was carried out by first scanning the gels using the PhotoPhinish program (TDI, Spain) and then taking densitometric readings, using the 1D-Manager programme (TDI, Spain).

The molecular masses of the protein bands were calculated from the R_f values by interpolation of the calibration curve constructed, using markers of known molecular mass. The markers (Sigma) consisted of the following proteins: myosin (205 kDa), β-galactosidase (116 kDa), phosphorylase *b* (97 kDa), fructose-6-phosphate kinase (84 kDa), albumin (66 kDa), glutamic dehydrogenase (55 kDa), ovalbumin (45 kDa), glyceraldehyde-3-phosphate dehydrogenase (36 kDa), carbonic anhydrase (29 kDa), trypsinogen (24 kDa), trypsin inhibitor (20 kDa), α-lactalbumin (14.2 kDa), and aprotinin (6.5 kDa).

2.5. Free fatty acids analysis

The extraction of total lipids was performed according to Kallio, Lehtinen, Laakso, and Tahvonen (1998). One gram of sample was homogenised in 10 ml methanol in an Ultra Turrax for 2 min at 1200 rpm; 20 ml chloroform were added and the mixture was homogenised for a further 2 min, followed by vacuum-filtration through Whatman No. 1 filter paper (Bligh & Dyer, 1959). The extraction procedure for the residue was repeated and the supernatants combined. The residue was washed with 10 ml methanol and 20 ml chloroform, which were then combined with the supernatants. The extract was first washed with 22.5 ml of 0.88% (w/v) potassium chloride and with 22.5 ml water:methanol (1:1). The organic phase was separated and the solvents were evaporated under vacuum at 40 °C. Free fatty acids were separated from the rest of the neutral and polar lipids by extraction in solid phase using aminopropylsilica minicolumns (500 mg; Supelco). An aliquot of 100 mg of extractable fat was dissolved in 4 ml hexane containing 0.02% (w/v) BHT. Pentadecanoic acid was added to the samples as an internal standard and an aliquot was eluted with 5 ml diethylether with 2% (v/v) glacial acetic acid (García-Regueiro, Gibert, & Díaz, 1994).

The fraction containing the free fatty acids was evaporated to dryness under nitrogen. The free fatty acids of the samples and the calibration solutions were methylated with 1 ml of 10% borum trifluoride in methanol for 20 min at 50 °C (García-Regueiro et al., 1994). Fatty acid methyl esters were extracted with 2 ml hexane for injection into the gas chromatograph.

Analysis of fatty acid methyl esters was carried out in a Perkin–Elmer gas chromatograph, model 8600, equipped with a flame ionization detector at 250 °C. The procedures were controlled with a ChemStation programme (Hewlett–Packard). The capillary column used was a SGL-1000 (50 m × 0.25 mm × 25 μm) (Sugelabor). Helium was used as carrier gas at 1.0 ml/min, inlet pressure 25 psi, and the split ratio in the injector was 1:25. The injector temperature was fixed at 250 °C. The sample

volume injected was 0.6 μl. The oven temperature was held for 35 min at 200 °C. Fatty acids were identified by comparing retention times of the chromatograph peaks with those obtained with the methyl esters from a mixture prepared with fatty acids from Sigma. Quantification of the free fatty acids identified was done by calculating the area under each chromatograph peak that was proportional to the amount of the fatty acid in the sample. The corresponding correction factors were applied for each fatty acid using regression analysis.

2.6. Statistical analysis

Student Newman Keuls test, *t*-test and principal components analysis were performed using SPSS 11.0 for Windows 2000 (SPSS, Inc, Chicago, IL).

3. Results and discussion

3.1. Physicochemical characteristics

Table 1 shows the means and standard deviations achieved for the physicochemical characteristics of chorizos and saucissons made with deer or wild boar meat.

All samples presented similar values for a_w and pH (0.802–0.918 and 4.96–6.03, respectively). These values agree with those obtained by Vioque et al. (2003) in dry sausages prepared with deer meat, which were 0.788–0.905 for a_w and 5.13–5.72 for pH. Cantalejo (2003) found a_w values of 0.827–0.879 and pH in the range of 5.00–5.50 in deer meat chorizos prepared at different dates of the hunting season and ripened under natural conditions and in drying rooms with controlled temperature and relative humidity. Paleari, Moretti, Beretta, Mentasti, and Bersani (2003) obtained mean values of 0.900 for a_w and 6.05 for pH in cured products manufactured with deer or wild boar meat elaborated following the "bresaola" dried meat manufacturing process, values slightly above those presented in this study.

The dry matter values are higher than those achieved by Cantalejo (2003) in deer meat chorizos matured in a natural dryer (34.18–65.82%) and in a dryer with controlled relative humidity and temperature (35.52–69.46%), and those obtained by Paleari et al. (2003) in cured products made with deer (54.2%) and wild boar (51.8%). The variability in the dry matter content shows the different ripening times used by the different producers.

Protein nitrogen values were 32.4–57.5%, referring to dry matter. Vioque et al. (2003) obtained mean values of 34.8% in deer meat saucissons and 39.9% in deer meat chorizos. On the other hand, Paleari et al. (2003) achieved values of 18.9–20.4% in deer and wild boar cured products.

Table 1
Means and standard deviations for the physicochemical characteristics and composition of chorizos and saucissons made with deer or wild boar meat

Type of sausage	Lean	Sample	a_w	pH	Dry matter (%)	Protein nitrogen[a]	Fat[a]	Ash[a]	Sodium chloride[a]	Phosphorus[b]	Sodium nitrite[c]
Chorizo	Deer	1	0.826 ± 0.01	5.46 ± 0.04	70.94 ± 0.39	32.4 ± 2.41	45.42 ± 2.95	6.69 ± 0.38	5.46 ± 0.03	4.56 ± 0.11	3.69 ± 0.05
		4	0.918 ± 0.01	4.96 ± 0.03	60.07 ± 0.52	34.6 ± 0.60	52.59 ± 0.68	7.40 ± 0.23	5.11 ± 0.17	5.63 ± 0.12	5.69 ± 0.14
		6	0.874 ± 0.00	5.42 ± 0.03	66.29 ± 0.12	38.2 ± 0.60	46.62 ± 1.66	7.42 ± 0.81	5.45 ± 0.00	4.37 ± 0.21	6.98 ± 0.07
		8	0.804 ± 0.00	5.41 ± 0.02	70.43 ± 0.47	47.3 ± 2.78	32.58 ± 0.24	9.20 ± 0.69	6.16 ± 0.06	5.22 ± 0.27	6.52 ± 0.12
		9	0.834 ± 0.00	6.03 ± 0.13	65.98 ± 0.31	49.5 ± 0.30	36.07 ± 1.59	10.39 ± 0.11	6.73 ± 0.16	5.54 ± 0.34	5.20 ± 0.34
	Wild boar	5	0.802 ± 0.00	5.46 ± 0.01	69.70 ± 0.15	51.6 ± 1.51	23.25 ± 0.74	8.57 ± 0.03	6.22 ± 0.01	5.38 ± 0.01	2.98 ± 0.01
Saucisson	Deer	3	0.854 ± 0.00	5.39 ± 0.04	65.59 ± 0.50	37.5 ± 1.44	42.20 ± 0.09	8.62 ± 0.32	6.28 ± 0.08	3.92 ± 0.06	3.58 ± 0.01
		7	0.872 ± 0.00	5.60 ± 0.06	71.07 ± 0.20	57.5 ± 0.27	32.71 ± 0.35	5.04 ± 0.35	0.94 ± 0.02	6.18 ± 0.01	18.9 ± 0.21
		10	0.815 ± 0.01	5.93 ± 0.14	67.42 ± 0.11	39.0 ± 0.36	35.07 ± 0.64	10.32 ± 0.37	6.92 ± 0.06	4.82 ± 0.28	13.9 ± 0.19
	Wild boar	2	0.879 ± 0.00	5.62 ± 0.00	58.05 ± 0.08	54.5 ± 1.10	19.54 ± 0.25	10.91 ± 0.01	8.50 ± 0.13	6.04 ± 0.04	18.4 ± 1.42

[a] % expressed as dry matter.
[b] mg/g expressed as dry matter.
[c] ppm expressed as dry matter.

Fat content was higher in the sausages made with deer (32.58–52.59%) than those made with wild boar (19.54–23.25%). Vioque et al. (2003) obtained values of 52.22–58.53%, whereas Cantalejo (2003) found a fat content about 30–40% in deer meat sausages. The fat content depends on the amount added initially as raw material in the production process. Wild boar meat contains more fat than does deer meat (Zomborszky et al., 1996), and this is the reason why producers adds more fat from pork to elaborate deer meat sausages.

Ash contents were 5.04–10.91%, in accordance with reports by Vioque et al. (2003) and Cantalejo (2003), who studied deer meat sausages. Beriain, Peña, and Bello (1993) obtained values of 8–8.7% (referring to raw sample) and Córdoba and Fernández-Salguero (1988) found very different values (11–42% referring to raw sample). Paleari et al. (2003) achieved ash values around 14.5–16.5% expressed as dry matter in cured products from deer and wild boar meat.

Sodium chloride contents were 5.11–6.92%, except for sample 7 that presented a very low content of 0.94% and sample 8, that reached a value of 8.50%. Cantalejo (2003) obtained sodium chloride values of 6–8% in deer meat chorizo, comparable to those achieved by Domínguez, Ferre, and Zumalacarregui (1988) in chorizo from León, and Ferrer and Arboix (1986) in saucisson from Vich.

Phosphorus concentrations were from 3.92 to 6.18 mg/g. Cantalejo (2003) found lower values than those obtained in this study.

Finally, the sodium nitrite content was also highly variable in the results between samples; it was lower than the value achieved by Franco, Prieto, Cruz, López, and Carballo (2002) in their study of cured pork sausages, which oscillated from 40 to 60 ppm.

Principal components analysis was applied to the physicochemical results. Three principal components were achieved, which explained 87.28% of the total variance. Principal component 1 (PC1), with 35.66% of the total variance explained, was correlated with protein nitrogen (0.972), fat (−0.838) and phosphorus (0.828). Principal component 2 (PC2), with 29.52% of the total variance explained, was correlated with sodium chloride (0.961) and ash (0.942). Principal component 3 (PC3), with 22.10% of the total variance explained, was correlated with a_w (0.896) and dry matter (−0.867). Fig. 1 shows the projection of the samples in the plane defined by the first two principal components, which explained 65.18% of the total variance. Chorizos and saucissons made with deer meat were close in space (except for sample 7), with greater disparity appearing between the two types of sausages made with wild boar meat, which had higher positive values for PC1 higher protein nitrogen and phosphorus content and a lower fat content than the sausages prepared with deer meat.

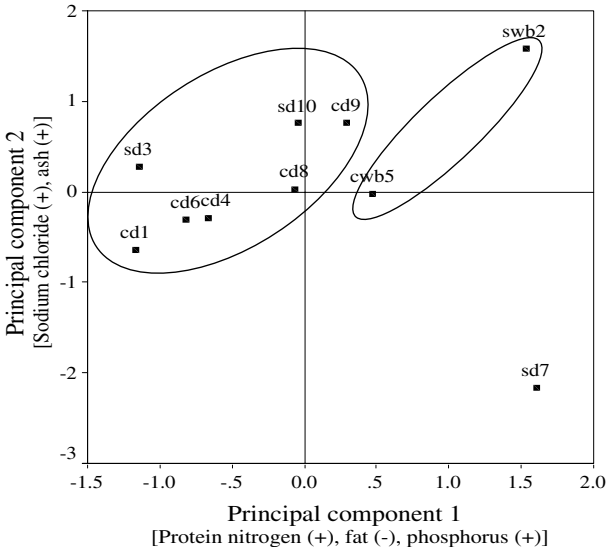

Fig. 1. Projection of the samples in the plane defined by the first two principal components, referring to physicochemical parameters: s, saucisson; c, chorizo; d, deer; wb, wild boar.

3.2. Nitrogen fractions

Table 2 presents the means and standard deviations for the WSN, proteolysis index and total proteins of sarcoplasmic and myofibrillar fractions. The WSN contents were similar for all samples.

The proteolysis indices were from 18.9% to 72.7%, except for sample 1, which showed a very low value. The proteolysis index values found in this study for game meat sausages were higher than those reported for dry sausages elaborated with pork and beef meat, which were from 12% to 20% (De Masi, Wardlaw, Dick, & Acton, 1990; Garriga, Calsina, & Monfort, 1988; Hughes et al., 2002; Johansson, Berdagué, Larson, Tran, & Borch, 1994; Roncales, Aguilera, Beltrán, Jaime, & Peiró, 1991; Toledo, Selgas, Lasas, Ordóñez, & García, 1997). This could be attributable to the different proteolytic activities of the microbial populations during the ripening. The slaughter conditions of wild animals in the field notably increased the microbial contamination of the carcass compared with animals slaughtered at the abattoir.

The total proteins found in the myofibrillar fraction were higher than in the sarcoplasmic fraction in all samples analysed, which was in accordance with Bello, Larralde, and Saenz de Buruaga (1974). The soluble protein concentration depends on the ripening time of the sausage and it even depends on the animal species. The protein solubility decreased during the ripening stage because of the increase of salt concentration, decrease of pH values, and occasionally, the high temperature reached in fermentation and drying stages (Lois, Gutiérrez, Zumalacárregui, & López, 1987). The amount of soluble proteins exerts a great effect on the quality of dry sausages.

Table 2
Means and standard deviations for the water-soluble nitrogen, proteolysis index and total proteins of sarcoplasmic and myofibrillar fractions of the chorizos and saucissons made with deer and wild boar meat

Type of sausage	Lean	Sample	WSN[a]	NPN/TN × 100	Myofibrillar[a]	Sarcoplasmic[a]
Chorizo	Deer	1	0.92 ± 0.04	3.33 ± 1.37	2.96 ± 0.54	0.94 ± 0.04
		4	1.10 ± 0.03	54.0 ± 0.37	6.05 ± 0.23	1.57 ± 0.08
		6	1.15 ± 0.00	72.7 ± 2.72	5.09 ± 0.08	1.39 ± 0.17
		8	1.60 ± 0.03	44.4 ± 0.58	9.43 ± 0.11	0.96 ± 0.06
		9	1.62 ± 0.01	18.9 ± 1.37	9.45 ± 0.32	2.59 ± 0.03
	Wild boar	5	1.83 ± 0.01	30.8 ± 0.84	5.23 ± 0.21	1.54 ± 0.08
Saucisson	Deer	3	0.75 ± 0.01	22.80 ± 2.58	9.50 ± 0.15	1.50 ± 0.08
		7	1.77 ± 0.01	44.5 ± 0.99	8.61 ± 0.78	1.29 ± 0.03
		10	1.46 ± 0.00	32.2 ± 0.35	7.05 ± 0.37	2.00 ± 0.03
	Wild boar	2	1.78 ± 0.00	29.9 ± 1.85	9.33 ± 0.37	3.04 ± 0.12

WSN, water-soluble nitrogen; NPN, non-protein nitrogen; TN, total nitrogen.
[a] % expressed as dry matter.

3.3. SDS–PAGE

3.3.1. Sarcoplasmic proteins

The electrophoretic profile of sarcoplasmic proteins was different for each sample. There were no electrophoretic bands common to all samples.

Some electrophoretic bands found in this study matched the bands identified by Savage, Warriss, and Jolley (1990) and McCormick, Reeck, and Kropf (1988) in pork meat at 24 h postmortem. These authors found the protein pair, phosphorylase *b* and phosphorylase *b* kinase, that migrated together and could not be separated, with an approximate molecular mass of 97 kDa, pyruvate kinase with a molecular mass of about 58 kDa, enolase corresponding to 45–47 kDa, glyceraldehyde-3-phosphate dehydrogenase at 36 kDa, and myoglobin at 16 kDa.

Table 3 presents the means and standard deviations for the relative density (%) of the electrophoretic bands corresponding to sarcoplasmic proteins extracted from dry sausages elaborated with game meat.

The 36 kDa band, corresponding to glyceraldehyde-3-phosphate dehydrogenase, and the 66 kDa polypeptide were the most frequently found (in 8 samples) with a high relative density. Hughes et al. (2002) reported a progressive increase in the amount of a 36 kDa polypeptide during the ripening of salami for 35 days. In addition, a 66 kDa band was identified during the ripening process. Díaz, Fernández, García de Fernando, de la Hoz, and Ordóñez (1997) found an increase in the concentration of 36 and 69 kDa bands at the end of the ripening period (26 days) in dry sausages elaborated with pork and beef meat.

The heaviest polypeptides identified in the samples had molecular masses in the region of 66–145 kDa. The heaviest polypeptide found by Díaz et al. (1997) in pork and beef dry sausages, at the end of the ripening period for 26 days, had a molecular mass of 204 kDa and a low relative density. On the other hand, the heaviest polypeptide identified by Hughes et al. (2002) had a molecular mass of 66 kDa at the end of the salami ripening period for 35 days and a high relative density. These results show the increase of proteolysis when the ripening time is longer.

A great number of poplypeptides of low molecular masses were found in the samples analysed. The lightest polypeptides were from 9 to 16 kDa. Díaz et al. (1997) recorded the appearance of new polypeptides corresponding to 8, 10, 11, 16, 38 and 49 kDa at the end of ripening for 26 days, and the increase of the density of a 13 kDa band. Hughes et al. (2002) found two new bands, corresponding to 14 and 42 kDa, at the end of 35 days of salami ripening.

Garriga et al. (1988) reported an intense proteolysis of sarcoplasmic proteins extracted from pork saucisson ripened for 48 days. Polypeptides smaller than 46 kDa underwent greater changes.

The different electrophoretic profiles for different samples and the great range of molecular masses found for the heaviest and the lightest polypeptides in the different commercial brands showed the variability in ripening conditions applied in the factories that manufacture dry sausages with meat game.

Principal component analysis was run on the relative densities of electrophoresis bands corresponding to sarcoplasmic proteins. The first eight principal components explained 97.3% of the total variance. Fig. 2 shows the projection of the samples in the space defined by the first three principal components, which explained 44.5% of the total variance, and the electrophoretic bands most correlated with each principal component (loading > 0.700). There was a slight tendency to separation into two different groups: the chorizo and saucisson samples.

Table 3
Means and standard deviations for the relative density (%) of the electrophoretic bands for sarcoplasmic proteins extracted from chorizos and saucissons made with deer and wild boar meat

Approximate molecular mass (kDa)	Chorizo						Saucisson			
	Deer					Wild boar	Deer			Wild boar
	1	4	6	8	9	5	3	7	10	2
145	2.72 ± 1.27									
111	3.43 ± 0.12				1.37 ± 0.28		1.45 ± 0.08			1.67 ± 0.81
103		2.83 ± 0.58			2.32 ± 0.46		2.39 ± 2.05		2.65 ± 0.61	1.24 ± 0.14
97					1.76 ± 0.19				3.13 ± 0.29	
86		3.06 ± 0.49	3.37 ± 1.22		2.49 ± 0.54				2.60 ± 1.53	
80			3.07 ± 1.28		4.08 ± 1.42				2.62 ± 0.55	
72		6.00 ± 2.47	10.34 ± 2.77	4.90 ± 1.12	2.08 ± 0.67		1.88 ± 0.92		10.05 ± 0.29	
69						9.52 ± 2.07				
66	42.72 ± 7.58		5.80 ± 1.71		9.50 ± 0.05	9.60 ± 0.77	15.71 ± 2.60	18.02 ± 1.72	7.69 ± 2.65	4.90 ± 1.75
62		24.31 ± 3.25		2.10 ± 0.00				9.34 ± 1.47		2.11 ± 0.74
58			4.91 ± 0.99		3.43 ± 0.06	3.19 ± 0.41		6.49 ± 0.06	12.83 ± 1.44	4.05 ± 0.47
47		3.12 ± 1.04			6.27 ± 1.43			3.86 ± 0.06	6.50 ± 0.16	0.58 ± 0.20
45									16.51 ± 1.21	
43			13.15 ± 1.49	33.51 ± 2.02						
40	8.70 ± 2.76		5.10 ± 1.80		7.07 ± 0.17	17.49 ± 3.11	11.61 ± 0.35	15.13 ± 0.82		5.88 ± 0.65
36	8.74 ± 2.73	11.15 ± 0.52	4.37 ± 6.49	17.69 ± 5.00	10.86 ± 0.06	20.82 ± 1.76	8.18 ± 0.43			4.86 ± 0.62
32	5.62 ± 1.98	7.53 ± 2.93					10.75 ± 4.65	10.70 ± 1.12		3.57 ± 0.23
30	3.81 ± 0.69	7.22 ± 0.68			5.22 ± 0.33	6.43 ± 0.47			9.97 ± 0.40	2.11 ± 0.22
28		4.60 ± 1.34			6.44 ± 0.78				8.11 ± 0.49	
24			8.34 ± 2.37	9.52 ± 2.20	0.93 ± 0.09	11.16 ± 1.17	3.06 ± 0.97	4.68 ± 2.35		4.62 ± 0.60
22	6.28 ± 0.89		5.08 ± 1.10		4.57 ± 0.13		9.00 ± 1.63	4.24 ± 1.28	4.39 ± 0.22	5.97 ± 0.73
21	3.25 ± 1.13	8.10 ± 4.5	3.67 ± 1.35					4.28 ± 1.26	3.71 ± 0.02	4.52 ± 0.30
18			6.64 ± 1.85	11.52 ± 2.55	2.41 ± 0.53			3.59 ± 0.87		4.33 ± 0.32
17					6.48 ± 0.47	6.37 ± 0.30	4.74 ± 0.07	3.36 ± 1.32	2.93 ± 0.23	
16	6.23 ± 2.23				2.72 ± 0.72		7.37 ± 2.31		6.31 ± 0.32	6.94 ± 0.35
14		4.78 ± 0.69	5.26 ± 0.67	6.71 ± 4.02			5.93 ± 0.52			4.40 ± 0.34
12	3.33 ± 0.53		7.47 ± 2.35	11.61 ± 2.43		6.16 ± 0.25	17.94 ± 1.91	8.37 ± 2.12		
11	5.17 ± 1.31	2.40 ± 0.46	6.56 ± 3.24	1.71 ± 0.13		4.33 ± 2.61		7.95 ± 0.44		8.23 ± 2.98
10		7.28 ± 0.33	4.77 ± 1.17	0.92 ± 0.04		4.93 ± 1.14				
9.5		2.04 ± 1.72								
9		5.59 ± 0.51								

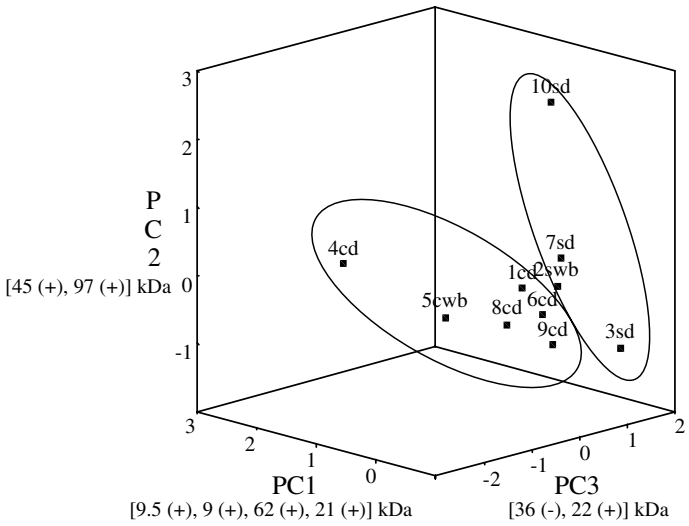

Fig. 2. Projection of the samples in the plane defined by the first three principal components, referring to relative density of sarcoplasmic proteins: s, saucisson; c, chorizo; d, deer; wb, wild boar.

3.3.2. *Myofibrillar proteins*

The electrophoretic profile of myofibrillar proteins was different for each sample. Three polypeptides were found in all samples, which corresponded to 53, 43 and 36 kDa, these polypeptides showed high relative densities.

Matching the electrophoretic bands with myofibrillar proteins was difficult because of the high quantity of bands found in this study. Penny (1980) and Claeys, Uytterhaegen, Buts, and Demeyer (1995) found a band corresponding to tropomyosin with a molecular mass of 51 kDa in their study on beef meat. However, other

workers reported that tropomyosin had a molecular mass of 35 kDa (Mauriello, Casaburi, & Villani, 2002; Porzio & Pearson, 1977). In this study, 53 and 36 kDa polypeptides were found in all samples; both polypeptides could be the tropomyosin. Actin normally appears with a molecular mass of 42 kDa (Monin et al., 1997), 45 or 46 kDa (Garriga et al., 1988; Mauriello et al., 2002; Porzio & Pearson, 1977) and 43 kDa (Fadda et al., 1999), the last one is coincident with the electrophoretic band found in all samples analysed in this study. I-Troponin has been identified with 23 kDa (Díaz et al., 1997; Negishi, Yamamoto, & Kuwata, 1996) and 24 kDa (Porzio & Pearson, 1977). A 23 kDa band was identified in some samples of this study, which could correspond to I-troponin. C-Troponin normally appears at 20 kDa (Negishi et al., 1996; Porzio & Pearson, 1977), coinciding with a band identified in some samples analysed in this study. Myosin light chains I, II and III have been identified with 25, 18 and 15 kDa, respectively (Porzio & Pearson, 1977); the three bands were found in the samples analysed.

Table 4 provides the means and standard deviations for the relative density (%) of the electrophoretic bands corresponding to myofibrillar proteins extracted from dry sausages elaborated with game meat.

The 201 kDa band, corresponding to myosin heavy chain (Alomirah, Alli, Gibbs, & Konishe, 1998), appeared in two samples with a low relative density. Several authors have defined a decrease of the myosin heavy chain concentration during the ripening of dry sausages (García de Fernando & Fox, 1991; Mauriello et al., 2002; Verplaetse, Debosschere, & Demeyer, 1989), and even the complete degradation (Hughes et al., 2002). The myosin has two regions sensitive to different proteinases; hence, enzymes cut the molecule, generating a large number of breakdown products with a wide range of molecular masses (Weeds & Pope, 1977). In the remaining samples, the heaviest polypeptide presented a molecular mass of 130–185 kDa.

The lightest polypeptides found in the samples, generated by an intense proteolysis, showed molecular masses of 10–16.5 kDa. García de Fernando and Fox (1991)

Table 4
Means and standard deviations for the relative density (%) of the electrophoretic bands of myofibrillar proteins extracted from chorizos and saucissons made with deer and wild boar meat

Approximate molecular mass (kDa)	Chorizo						Saucisson			
	Deer					Wild boar	Deer			Wild boar
	1	4	6	8	9	5	3	7	10	2
201			2.48 ± 0.30							3.16 ± 0.21
185		2.32 ± 0.10					4.32 ± 0.25		3.00 ± 0.07	
166							1.27 ± 0.45	1.51 ± 0.60		
154							2.52 ± 1.68			
144							2.77 ± 0.83		3.12 ± 0.80	2.63 ± 0.60
130	5.63 ± 0.20	15.14 ± 3.50	8.61 ± 1.52	10.83 ± 2.09	14.90 ± 3.59	10.47 ± 0.08	4.46 ± 1.02	11.83 ± 1.53	4.31 ± 0.37	
116			2.78 ± 0.76				2.27 ± 1.01		3.47 ± 0.10	3.32 ± 0.01
108	3.13 ± 0.36						4.11 ± 3.48		3.54 ± 1.30	4.17 ± 0.02
94	6.34 ± 1.76	6.43 ± 0.40	5.86 ± 1.82	6.83 ± 0.20	4.30 ± 0.51	7.05 ± 1.68	8.08 ± 0.48		5.67 ± 0.26	
80					6.80 ± 0.77				3.79 ± 1.90	
77	6.46 ± 1.57	4.26 ± 1.00				7.19 ± 2.08				5.55 ± 0.97
72		5.00 ± 1.34	2.75 ± 0.43	5.10 ± 2.17	4.96 ± 0.01	6.81 ± 0.89		3.37 ± 1.30	5.78 ± 0.37	
65	5.03 ± 0.48		2.73 ± 0.39	4.66 ± 0.25				4.44 ± 1.50	5.25 ± 3.50	
60				3.43 ± 0.91		4.96 ± 0.25		3.26 ± 0.45	2.81 ± 2.89	
57	4.16 ± 0.25		2.13 ± 0.04							3.87 ± 0.06
53	3.37 ± 2.77	2.68 ± 0.06	3.76 ± 0.87	4.15 ± 1.11	4.07 ± 1.56	4.92 ± 1.00	3.25 ± 0.32	3.84 ± 0.30	4.47 ± 0.79	5.66 ± 3.23
47		2.53 ± 1.49	4.59 ± 2.35	3.42 ± 2.02	2.67 ± 0.87	5.28 ± 0.14	4.26 ± 0.14	2.46 ± 0.22	6.50 ± 2.10	
43	15.94 ± 3.16	13.15 ± 2.54	11.64 ± 5.56	5.92 ± 0.67	15.01 ± 6.32	5.53 ± 0.60	10.01 ± 0.39	14.85 ± 0.37	5.00 ± 0.27	9.07 ± 0.43
39										6.17 ± 1.82
36	8.15 ± 0.78	11.29 ± 2.44	8.47 ± 0.25	8.5 ± 3.41	5.21 ± 5.16	7.34 ± 1.59	6.74 ± 1.44	11.20 ± 0.53	10.91 ± 1.93	5.84 ± 0.70
32	5.68 ± 0.25	2.44 ± 0.87	9.06 ± 0.86	15.70 ± 1.26	7.05 ± 0.78	4.63 ± 3.76	6.16 ± 0.34	12.46 ± 0.42	4.61 ± 2.44	
29							7.50 ± 0.91		5.30 ± 1.54	4.72 ± 2.05
25							5.48 ± 3.33		5.69 ± 1.70	5.76 ± 1.78
23			8.43 ± 0.18	9.02 ± 10.39	5.95 ± 2.09	4.38 ± 1.97		10.85 ± 1.13		6.76 ± 0.70
20	4.71 ± 0.25	6.78 ± 2.46	5.90 ± 2.24	7.07 ± 0.00		5.21 ± 0.09	8.11 ± 5.75		9.29 ± 0.34	3.70 ± 1.15
19					4.46 ± 0.25					5.53 ± 0.06
18	5.31 ± 0.70	5.12 ± 1.80	5.56 ± 1.23	6.05 ± 1.30	5.62 ± 0.52	7.99 ± 1.95		5.31 ± 1.04		6.97 ± 0.39
16.5	5.69 ± 3.27	4.31 ± 1.42	5.76 ± 0.80	5.46 ± 2.81	5.55 ± 2.66		9.37 ± 0.16	3.46 ± 1.16		
15	5.88 ± 0.14	7.13 ± 0.14	3.98 ± 0.20			6.03 ± 0.53		3.65 ± 2.07		
14										4.40 ± 0.73
13	3.56 ± 1.11		5.52 ± 0.24							12.72 ± 10.09
12					6.18 ± 0.46	6.62 ± 0.05				
11	4.25 ± 1.73	5.77 ± 1.05					8.55 ± 1.63	4.45 ± 0.23	7.50 ± 0.69	
10	6.70 ± 2.00	5.64 ± 0.24			7.25 ± 0.39	5.59 ± 1.87		3.08 ± 0.59		

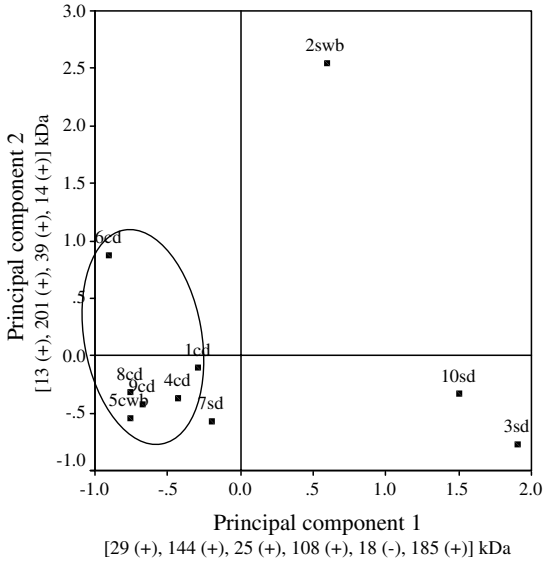

Fig. 3. Projection of the samples in the plane defined by the first two principal components, referring to relative density of myofibrillar proteins: s, saucisson; c, chorizo; d, deer; wb, wild boar.

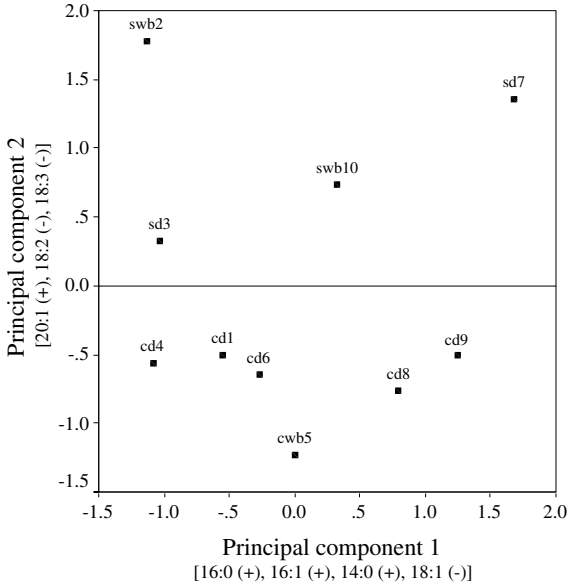

Fig. 4. Projection of the samples in the plane defined by the first two principal components, referring to free fatty acids percentage: s, saucisson; c, chorizo; d, deer; wb, wild boar.

found 8 and 10 kDa polypeptides in pork dry sausages after 41 days of ripening. Verplaetse et al. (1989) reported a slight increase of polypeptides in the region of 10–13 kDa in dry sausages fermented at 22 °C for 3 days and dried at 15 °C for 18 days.

Electrophoretic bands with molecular masses of 144–166 kDa and another two bands of 25 and 29 kDa, were recorded in saucisson samples but not in chorizo samples, perhaps because the formulation of each product was different and there were also slight variations in the elaboration processes.

Principal component analysis was applied to the relative densities of electrophoresis bands corresponding to myofibrillar proteins. The first eight principal components explained 98.2% of the total variance. Fig. 3 presents the projection of the samples in the space defined by the first two principal components, which explained 60.1% of the total variance, and the electrophoretic bands most correlated with each principal component (loading > 0.700). Chorizo samples grouped in the negative part of the principal component 1, showing an homogeneous group. However, saucisson samples were not grouped in the plane; thus saucisson experienced a myofibrillar protein breakdown that was more irregular and variable.

3.4. Free fatty acids composition

Principal component analysis was run on the relative percentage of free fatty acid. The first two principal components explained 85.07% of the total variance. The most positively highly correlated with the first of the principal components (46.66% of the total variance explained) were the percentages of palmitic (C16:0), palmitoleic (C16:1), and myristic (C14:0) acids; the most negatively correlated was the percentage of oleic acid (C18:1). Furthermore, the percentage of 11-eicosenoic (C20:1), linoleic (C18:2), and linolenic (C18:3) acids were strongly correlated with the second of the principal components (34.16% of the total variance). Fig. 4 presents the projection of the samples in the space defined by the first two principal components and the percentages of free fatty acids highly correlated with the principal components (loadings > 0.700). Chorizos and saucissons, regardless of the game species, are well separated. The chorizos are located in the negative part of PC2, i.e., they present higher linoleic (C18:2) and linolenic (C18:3) polyunsaturated fat contents, and lower contents of the monounsaturated 11-eicosenoic fatty acid (C20:1).

Table 5 shows the means and standard deviations of the percentages of free fatty acids with respect to the total free fatty acids (%, w/w) extracted from the chorizos and saucissons made with game meat. For the two types of dry sausage, the larger relative percentages correspond to oleic, palmitic, linoleic and stearic acids. These results are in accordance with Vioque et al. (2003) who studied free fatty acids in commercial saucissons made with deer meat. The amounts of these majority acids are similar to those found in chorizos made with lean pork and beef and fat from pork (Lizarraga, Melgar, & Bello, 1989). In dry sausages made with lean pork and pork belly, similar values were found, except for C18:0, that showed a higher value (13%) (Navarro, Nadal, Nieto, & Flores, 1998). According to Demeyer, Hoozee, and Mesdom (1974), in fermented sausages, there is a tendency to hydrolysis of linoleic, oleic, stearic

Table 5
Means and standard deviations for proportions of free fatty acids (w/w total free fatty acids, %) of extracted lipids from chorizos and saucissons made with deer or wild boar meat

Fatty acid	Chorizo	Saucisson
No. samples	6	4
14:0	1.8 ± 0.5	2.2 ± 0.9
16:0	21.7 ± 5.2	22.4 ± 5.6
16:1	4.6 ± 1.1	4.8 ± 1.2
18:0	6.9 ± 1.3	7.8 ± 1.5
18:1	44.1 ± 5.2	47.6 ± 6.4
18:2 n − 6	*16.7*[a] *± 1.8*	*11.8*[b] *± 3.2*
18:3 n − 3	*1.7*[a] *± 0.5*	*1.0*[b] *± 0.6*
20:1	*0.8*[a] *± 0.1*	*1.2*[a] *± 0.2*
Total SFA	30.2 ± 5.9	32.4 ± 7.6
Total MUFA	49.2 ± 4.9	53.5 ± 5.3
Total PUFA	*20.6*[a] *± 2.9*	*14.1*[b] *± 4.4*

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
[a,b] Means within a row with different letters are significantly different ($P < 0.05$).

and palmitic acids, probably because of the specific lipolysis developed by microbial lipases, that is dependent on position and structural conformation of fatty acids in glycerides (Alford, Smith, & Lilly, 1971).

Table 5 does not include concentrations of the C20:2, C20:3, C20:4 and C22:5 acids because they were not detected in any samples analysed. These free fatty acids were found at percentages below 1.0%.

Paleari et al. (2003) obtained a total saturated fatty acid content of 35.5–44.0%, monounsaturated of 30.3–45.7%, and polyunsaturated of 16.2–19.6%, respectively, in fat extracted from cured products of deer and wild boar from farms. In the present study, the amounts of saturated acids found were lower; however, the monounsaturated acid content was higher. This may be attributed to the fact that, in this study, the dry sausages are made with meat from wild animals bred in freedom. The proportion of unsaturated fatty acids is higher in the muscles of wild animals than in those of feedlots steers. The free access to pasture for grazing, and thus the greater contribution of fresh grass and herbs to the diet of these species, could be responsible for such positive values (Volpelli et al., 2003).

The concentration of free fatty acids in the fat depends on the hydrolytic activity of the lipases, the microbial metabolic processes, and the oxidative reactions that work on the free fatty acids released in the lipolysis. These are directly related to the raw material used to prepare the sausages, ingredients, additives and spices added, and the production process. The commercial chorizos and saucissons used in this study were made with hunted deer or wild boar, and were prepared in the same geographical region, basically being differentiated by the addition of spices. The chorizos had a greater diversity and amount of spices than the saucissons, and included paprika, used as a natural colouring, which is also an antioxidant (Aguirrezábal, Mateo, Domínguez, & Zumalacárregui, 2000). Therefore, paprika is capable of preventing or delaying the chemical oxidation of the unsaturated free fatty acids, including linoleic (C18:2) and linolenic (C18:3), which are particularly sensitive to oxidation reactions, which could justify their greater concentration in chorizos. Linoleic acid (C18:3) belongs to the $n - 3$ polyunsaturated fatty acid family, which is believed to be beneficial for health.

4. Conclusions

Based on the results of this preliminary study, chorizos and saucissons made with deer meat and commercialised in the Spanish market had a higher fat content than those prepared with wild boar meat, and a further two factors that may be differentiating were the protein nitrogen and phosphorus content, which were higher in dry sausages made with wild boar. The proteins found in the myofibrillar fraction were higher in concentration than in the sarcoplasmic fraction extracted from dry sausages elaborated with game meat. There were no electrophoretic profiles of sarcoplasmic and myofibrillar proteins characteristic of game sausages. Chorizos, made with deer or wild boar meat, had higher percentages of polyunsaturated free fatty acids, linoleic and linolenic acids, and lower percentages of the monounsaturated 11-eicosenoic acid, than had the saucissons.

The results obtained in this study contribute to the typification of the delicatessen made with game meat, and are interesting for companies producing these types of products.

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