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Fat effect on physico-chemical, microbial and textural changes through the manufactured of dry-cured foal sausage Lipolysis, proteolysis and sensory properties

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ABSTRACT

The effect of fat content on chemical traits related to dry-curing process (pH, moisture and water activity), color and textural properties and changes of free fatty acids and amino acids compositions during the processing of foal dry-cured sausages were studied. For this purpose, three batches (20 units per batch) of dry fermented sausages with different pork back fat content (5%, 10% and 20%) were manufactured; low fat (LF), medium fat (MF) and high fat (HF), respectively. Samples at 0 days (mix before stuffing), and after 7, 14, 28, 42 and 49 days of ripening were taken.

The fat level affected color and textural parameters at the end of the process, showing dry-cured foal sausage with the higher level of fat, the highest values of luminosity and the least hardness. No significant differences ($P>0.05$) among batches were detected on total viable counts, lactic acid bacteria and *Micrococcaceae* during the process. Regarding lipolysis and lipid oxidation it can be deduced that the increase in the fat level encouraged the production of free fatty acids and 2-thiobarbituric acid reactive substances. At the end of the ripening individual free fatty acids followed this order: oleic, palmitic, linoleic and stearic acid, representing 82–95% of the total free fatty acids. Final level of TBARS index was in the worst case of 1.23 mg MDA/kg of sausage. On the contrary, the batch with lesser fat content showed the highest levels of free amino acids at the beginning and at the end of the process, showing final values of 1.6%.

1. Introduction

Horsemeat consumption is marginal when compared with other conventional types of meat such as beef, chicken or pork, which are much more important in the human diet (Lombardi-Boccia, Lanzi, & Aguzzi, 2005). Horsemeat has excellent nutritional properties, i.e. it is of low fat, rich in iron and it has a favorable dietetic fatty acid profile with a high content of unsaturated fatty acids and vitamin B (Badiani, Nanni, Gatta, Tolomelli, & Manfredini, 1997; Franco, Rodríguez, Purriño, Bermudez, & Lorenzo, 2011; Lorenzo, Fuciños, Purriños, & Fraco, 2010; Sarriés & Beriain, 2005; Sarriés, Murray, Troy, & Beriain, 2006; Tateo, De Palo, Ceci, & Centoducati, 2008). However, the acceptance of horsemeat as an appealing food for humans has changed along the centuries due to changes in attitude from aversion to qualified approval of this meat (Sarriés et al., 2006).

The horse meat production in Spain has increased in the recent years, being the fourth major producer of horse meat in the U.E. in 2009 with a quantity of 6400 tonnes (FAOSTAT, 2009). However the consumption in Spain is limited, a high percentage is exported to other countries, mainly to Italy (MERCASA, 2011). In 2009, the worldwide horse meat production amounted over 750 thousand tonnes. The major producers were Asia, with 45% of worldwide production, followed by Europe (19%), South America (12%), Central America (11%), and North America (6%). The greatest importers of horse meat were Italy, Belgium, Russia and France, and the most important exporters were Argentina, Belgium, Canada and Poland (FAOSTAT, 2009).

There is a growing demand for low fat meat products among the health conscious consumers, because of its relation with cardiac diseases and obesity related foods. Dry fermented sausages are high fat content meat products, which is visible in the sliced product. Dry sausages made according to a standard recipe have fat contents around 30% directly after manufacture, but during the first week these values rise to about 40%, as a result of drying, and after 4 weeks to about 40–50% (Wirth, 1988). Fat is responsible for various properties of dry fermented sausages. First, from a physiological point of view, fat acts as a source of essential fatty acids and fat soluble vitamins and constitutes the most concentrated source of energy in the diet (9 kcal/g) (Mela, 1990). Second, fat contributes to the flavor, texture, mouth feel, juiciness and lubricity, which determine the quality and acceptability of dry sausages. Finally, the granulated fat has a further technological function in the manufacture of these products. It helps to loosen up the sausage mixture in order to facilitate the continuous

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release of moisture from the inner layer of the sausage; this process is absolutely necessary for undisturbed fermentation and flavor development (Wirth, 1988). Consequently, the reduction of fat can affect the acceptability of these products (Giese, 1996). Thus, the aim of the present study was to investigate the effect on fat content on physicochemical, microbial, textural, proteolysis, lipolysis and sensory changes occurring during the manufacture of dry-cured foal sausage.

2. Materials and methods

2.1. Dry fermented sausages

In order to carry out this study, three batches (20 units per batch) of dry fermented sausages with different pork back fat contents (5%, 10% and 20%) were manufactured; low fat (LF), medium fat (MF) and high fat (HF), respectively in the pilot plant of the Meat Technology Center of Galicia. The lean foal (from the hind quarter, composed principally by *Gluteus medius*, *Semitendinosus* and *Semimembranosus* muscles) and the pork back fat were ground through a 10 mm diameter mincing plate and vacuum minced with 50 g per kg of supplement "542 Salchichón" from Laboratorios Ceylamix (Valencia, Spain) composed, in unknown proportions, of sugar, salt, dextrin, spices (black and white pepper and nutmeg), milk protein, monosodium glutamate (E_{621}), phosphates (E_{450i} and E_{451i}), sodium erythorbate (E_{316}), potassium nitrate (E_{252}) and colorant (E_{120}). No starter culture was added. The meat mixture was maintained at 3–5 °C for 24 h and then was stuffed into collagen casings (Fibran, S.A., Girona, España, 55–60 mm diameter) being the final weight of each sausage of around 550 g. Conditions of stuffed were: 20 °C and relative humidity of 80% for 48 h. The sausages were transferred to a drying–ripening chamber where they were kept for 49 days at 11 °C and 75–80% of relative humidity.

Samples at 0 days (mix before stuffing), and after 7, 14, 28, 42 and 49 days of ripening were taken. Each sample point consisted of three entire units of foal sausage. In order to prepare the samples for analysis, after removing and discarding the outer casing of each dry-cured foal sausage unit, the edible part was ground in a Moulinette micer (Moulinex/Swan Holding Ltd., Birmingham, England) until a homogeneous mass was obtained. After determining the moisture content and pH, the samples were stored in airtight bottles, frozen at −80 °C, for no longer than 4 weeks prior to further analysis. Sensory analysis was carried out at the end of the process (after 49 days of ripening) in 6 sausage units.

2.2. Analytical methods

2.2.1. Reagents

Fatty acid methyl esters (FAMEs) standard mixtures and nonadecanoic acid methyl ester were acquired from Supelco Inc. (Bellefonte, PA, USA). Analytical grade and liquid chromatographic grade chemicals were purchased from Merck Biosciences (Darmstadt, Germany). Boron trifluoride (14% solution in methanol) was obtained from Panreac (Castellar del Vallès, Barcelona, Spain). AccQ.Fluor reagent kit (AQC, borate buffer) and AccQ.Tag Eluent A concentrate were acquired from Waters (Milford, MA, USA). Acetonitrile (MeCN), disodium ethylenediaminetetraacetic acid (EDTA), phosphoric acid, sodium acetate trihydrate, and sodium azide were supplied from Baker (Phillipsburg, PA, USA); triethylamine (TEA) was purchased from Aldrich (Milwaukee, WI, USA). Amino acid standards (AA-18), taurine and hydroxyproline were from Sigma (St. Louis, MO, USA).

2.2.2. Physico-chemical analysis (pH, color, moisture, TBARS and water activity)

The pH of samples was measured using a digital pH-meter (Thermo Orion 710 A+, Cambridgeshire, UK) equipped with a penetration probe. Color measurements were carried out using a CR-600 colorimeter (Minolta Chroma Meter Measuring Head, Osaka, Japan). Each sausage was cut and the color of the slices was measured three times for each analytical point. CIELAB space (CIE, 1976): lightness, (L^*); redness, (a^*); yellowness, (b^*) were obtained. Before each series of measurements, the instrument was calibrated using a white ceramic tile.

Moisture percentage was calculated by weight loss experimented by the sample (5 g) maintained in an oven (Memmert UFP 600, Schwabach, Germany) at 105 °C, until constant weight according to the ISO recommended standards 1442:1997 (ISO (International Organization for Standardization), 1997). The 2-thiobarbituric acid (TBARS) assay was carried out according to the extraction method described by Vyncke (1975) with a few modifications: the meat sample (2.0 g) was homogenized (Ultra Turrax T-25, Janke & Kunkel IKA-Labortechnik, Staufen, Germany) with 10 mL of a 5% trichloroacetic acid (TCA) for 2 min at 4500 rpm (Allegra X-22R, Beckman, Fullerton, CA, USA), and the homogenate was centrifuged for 10 min at 3500 rpm (Allegra X-22R, Beckman, Fullerton, CA, USA) and then filtered through 0.45 μm (Filter Lab, Spain). The extract (5.00 mL) was mixed with 0.2 M thiobarbituric acid (5.00 mL) and heated in a 97 °C water bath (JP Selecta, Precisdg, Barcelona, Spain) for 40 min followed by cooling in ice-water for 5 min. The absorbance was measured on a spectrophotometer (Agilent 8453, Waldbronn, Germany) at 532 nm against a blank consisting of 5 mL of the same homogenizing solution plus 5 mL of TBA solution. Thiobarbituric acid reactive substances (TBARS) values were calculated from a standard curve of malonaldehyde (MA) and expressed as mg MA/kg sample. Water activity was determined using a Fast-lab (Gbx, Romans sur Isére Cédex, France) water activity meter, previously calibrated with sodium chloride and potassium sulphate.

2.2.3. Texture profile analysis (TPA)

The Texture Analyzer (TA-XT.plus, Stable Micro Systems, Vienna Court, UK) was used to conduct texture profile analysis (Bourne, 1978). Dry fermented sausage slices of 1×1×2 cm (height×width×length) were used for texture analysis. Textural parameters were measured by compressing to 60% with a compression probe of 19.85 cm^2 of surface contact. Force–time curves were recorded at a crosshead speed of 3.33 mm/s. Hardness (kg/cm^2), cohesiveness, springiness, gumminess (kg/cm^2) and chewiness (kg) were obtained using the available computer software (TEE32 Exponent 4.0.12. Stable Micro Systems, Vienna Court, UK).

2.2.4. Microbial analysis

In each sausage, after aseptically removing and discarding the outer plastic, 10 g of the product were aseptically taken and homogenized with 90 mL of a sterile solution of 0.1% (w/v) peptone water (Oxoid, Unipath, Basingtoke, UK), at 20.25 °C, containing 0.85% NaCl and 1% Tween 80 as emulsifier, for 2 min in a sterile plastic bag in a Masticator blender (IUL Instruments, Barcelona, Spain), thus making a 1/10 dilution. Decimal dilutions were prepared by mixing 1 mL of the previous dilution with 9 mL of 0.1% (w/v) sterile peptone water.

Total viable count (TVC) was determined on Plate Count Agar (PCA, Oxoid), incubated at 30 °C for 48 h; lactic acid bacteria (LAB) on MRS Agar (Oxoid), incubated anaerobically in 6% CO_2, at 30 °C for 2–3 days; and *Micrococcaceae* (MICR) on mannitol salt phenol-red agar (MSA, Oxoid), incubated at 30 °C for 2 days.

2.2.5. Free fatty acid

Total intramuscular lipids were extracted from 5 g of ground meat sample, according to Folch, Lees, and Stanley (1957) procedure. Free fatty acids were separated using NH_2-aminopropyl mini-columns as described by García-Regueiro, Gilbert, and Díaz (1994). Fifty milligrams of the extracted lipids was transesterified with a solution of boron trifluoride (14%) in methanol, according to Carreau and Dubacq (1978) and the FAMEs were stored at −80 °C until chromatographic analysis.