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# Effect of the use of chestnuts (*Castanea sativa* Miller) in the finishing diet of Celta pig breed on the shelf-life of meat refrigerated and frozen

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ABSTRACT

The effect of the use of commercial feed or chestnuts (*Castanea sativa* Miller) in the finishing diet of Celta pig on the microbiological counts, physicochemical parameters (pH and color), myoglobin state, lipid and protein oxidation, and sensory attributes of *Longissimus dorsi* muscle packaged in modified atmospheres during the refrigerated storage (28 days at 4 °C) was investigated. Also, the effect on the physicochemical parameters (pH and color), myoglobin state, lipid and protein oxidation, and sensory attributes of meat during frozen storage (180 days at −20 °C) was studied.

The use of chestnuts in the finishing diet significantly increased the counts of the different microbial groups (total aerobic mesophilic bacteria, total aerobic psychrotrophic bacteria, lactic acid bacteria, and Enterobacteriaceae) during refrigerated storage of meat. However the use of chestnuts did not affect significantly the values of pH, color parameters, percentages of the myoglobin forms, lipid and protein oxidation or sensory attributes of the meat during the refrigerated or frozen storage. The microbiological and oxidative parameters indicated an acceptable quality of meat after 28 days of refrigerated storage; however, judges found the sensory attributes modified after 12 or 20 days of storage.

## 1. Introduction

The main chestnut forests in Europe are concentrated in France and Italy, followed by Spain, Portugal, and Switzerland (Conedera, Manetti, Giudici, & Amorini 2004), being *Castanea sativa* Miller, the most commonly cultivated chestnut species. This traditional cultivation is oriented to timber production (coppice and high forest), as well as to fruit production (Conedera et al. 2004). Chestnuts are used as human and animal foods (Ferreira-Cardoso, Sequeira, Torres-Pereira, Rodrigues, & Gomes 1999). In the northwest of Spain, chestnuts are traditionally considered as valuable fodder for livestock. The composition of chestnut (Mataix, García, Mañas, Martínez, & Llopis 2003) makes it a suitable food for maintaining and fattening adult animals, in which establishment of a correct balance between protein and energy in the diet is not as critical as in young growing animals. Chestnuts could be used to feed autochthonous pigs, as another way of valorization of this vegetal resource thus maintaining reasonable production costs and increasing the quality of the meat and meat products.

In previous works, it has been observed that the use of chestnuts in the diet of the pigs increases the degree of unsaturation of the fat in the different locations in the carcases and in the meat products manufactured (Bermúdez, Franco, Franco, Carballo, & Lorenzo 2012; Domínguez, Martínez, Gómez, Carballo, & Franco 2015; Pugliese et al. 2013). Given the greater sensitivity to the autooxidation of unsaturated fatty acids, a greater degree of self-oxidative fat rancidity would be expected in meat and meat products. However, this circumstance has not been observed in previous studies (Pugliese et al. 2009; Sirtori et al. 2005). Moreover, some authors even observed lower TBA index values in fresh meat (Díaz, Ros, Veiga, & Cobos 2009) and in meat products (Cobos, Veiga, & Díaz 2008) from pigs fed chestnut, attributing this circumstance to the antioxidant substances present in this fruit. Chestnut fruits contain several antioxidant compounds, particularly phenolic acids (ellagic and gallic acid), flavonoids (rutin, quercetin and apigenin) and tannins (De Vasconcelos et al. 2010; Vázquez et al. 2012).

There is, however, no scientific evidence to date that the use of chestnuts in the diet significantly and constantly increases the concentration of antioxidants in the body tissues of the pigs fed in this way.

Microbial spoilage and oxidative processes, including the myoglobin oxidation, are the main causes of meat deterioration; these processes in fact lead to negative changes in the color, flavour, texture and nutritional quality of meat (Karakaya, Bayrak, & Ulusoy 2011). Lipid and

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protein oxidations in meat are closely related because protein oxidation also occurs via interactions between proteins and lipid hydroperoxydes or secondary lipid oxidation products such as aldehydes (Baron 2010). As a result of the oxidation processes, several compounds having a negative effect on the sensory characteristics of meat and meat products are produced (Frankel 2012; Jakobsen & Bertelsen 2000).

Pugliese et al. (2013) studied the effect of chestnut inclusion in the finishing diet of Cinta Senese pigs during different times of fattening: 1 and 3 months. In both cases the diet led to more coloured meat due to higher lightness, redness and yellowness values. For fatty acid composition, the animals fed during 3 months with chestnut showed the highest level of unsaturation, due to the highest percentage of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). Nevertheless, a negative effect of chestnut on meat quality traits was found in the case of 3 months, showing the highest values of drip loss and cooking loss. Temperán, Lorenzo, Castiñeiras, Franco, and Carballo (2014) studied the effect of partially or totally replacing commercial compound feed with chestnut in the finishing diet on the carcass and meat quality traits of Celta pigs; no significant effects were observed on the carcass and meat quality traits except for the increase of the fat unsaturation.

The effect of the inclusion of chestnuts in the finishing diet of pigs on the meat quality attributes has been studied. However, no studies were carried out on the effect of this practice on the shelf-life of meat. The aim of the present work was to study the effect of the use of the chestnuts (*Castanea sativa* Miller) in the finishing diet of Celta pigs on the shelf-life of meat stored both refrigerated and frozen.

## 2. Materials and methods

### 2.1. Animal rearing and preparation and storage of pork samples

A total of 18 Celta pigs (10 males and 8 females) were used in this study. Piglets, which were vaccinated and deparasitised according to the standard protocols, were suckled until 40 days. Males and females were castrated by a veterinary surgeon at the age of two and three months, respectively; castration was carried out according to the Council Directive 2008/120/EC (European European Union, 2008) under anaesthesia and additional prolonged analgesia. All pigs were reared and fattened until the age of 8 months in an extensive regime, with a livestock density of 12 animals per hectare. After weaning, the pigs were fed a commercial compound feed. At the age of 8 months, pigs were randomly divided into two different groups each comprising 9 animals: group A (5 males and 4 females) was fed commercial compound feed (3 kg animal$^{-1}$ d$^{-1}$) for another 4 months prior to slaughter; group B (5 males and 4 females) was fed a mixed diet (commercial compound feed/chestnuts; 1.5 kg commercial compound + 3 kg chestnuts animal$^{-1}$ d$^{-1}$) for a month (until the age of 9 months), and then a diet of only chestnuts (6 kg animal$^{-1}$ d$^{-1}$) in the remaining three months prior to slaughter. The gross and fatty acid composition of chestnuts and commercial compound feed are those indicated by Gómez, Fonseca, Cachaldora, Carballo, and Franco (2017). During the last four months (finishing period), groups A, B were kept in different pieces of land, and there was not any other vegetation which pigs have possibly consumed. Pigs were transported to a commercial slaughterhouse (Frigolouro, Porriño, Pontevedra, Spain) located 80 km from the experimental land, and were kept for 12 h with full access to water but not to food. Pigs were electrically stunned, exanguinated, scalded, skinned, eviscerated and chilled according to standard commercial procedures. Immediately, carcasses were chilled at 4 ± 1 °C in a cold chamber for 24 h.

After the refrigeration period, the *Longissimus dorsi* muscles were obtained from the carcasses, and steaks (1.5 cm thickness) were obtained from the middle part of the muscles (30 cm). For the study of the shelf-life during refrigerated storage, steaks were placed in low oxygen permeable polyethylene terephthalene (PET)/polyethylene (PE) trays (3 steaks per tray). Trays were then filled with a gas mixture ($CO_2$, 80%; $O_2$, 20%) using a vacuum-sealing unit (LARI/PN-VG; CAVECOR, Palazzolo, Italy) equipped with a gas mixer (KM 100-2 ME PA; Witt-Gasetechnik GmbH & Co. KG, Witten, Germany), and stored at 4 ± 1 °C in a refrigerator (GKPv 503; LIEBHERR, Bulle, Switzerland). Four trays were prepared from each pig. At 0, 4, 8, 12, 20 and 28 days of storage, three trays belonging to three different pigs were randomly collected from each group (commercial feed or chestnuts) and analyzed. In each of the three trays per feeding group taken in each storage time, after aseptically taking the 25 g necessary to make the microbiological analysis, the steaks were subjected to sensory analysis and next the three steaks were independently analyzed for the chemical parameters.

For the study of the shelf-life during frozen storage, steaks were vacuum packaged in bags (3 steaks per bag) and stored at −20 ± 1 °C in a freezer (MDF-U537; Sanyo, Osaka, Japan). Four bags were prepared from each pig. At 0, 45, 90, 135, and 180 days of storage, three bags belonging to three different pigs were randomly collected from each feeding group (commercial feed or chestnuts) and samples were thawed at 4 °C prior analysis. In each of the three bags per feeding group taken in each sampling time, the steaks were subjected to sensory analysis and next the three steaks were independently analyzed for the chemical parameters.

### 2.2. Microbiological analysis

From each replicate at each sampling time, twenty five g of meat were aseptically taken and placed into a sterile stomacher bag and 100 mL of a sterile 1% trisodium citrate solution were added. The mixture was then homogenized in a *Masticator Classic* (IUL Instruments, Barcelona, Spain) for 2 min at room temperature. The homogenate was filtered through a sterile gauze, and appropriate serial decimal dilutions were prepared in 0.1% of peptone water (Oxoid, Unipath Ltd., Basingstoke, UK). From each sample, one mL of appropriate dilutions were poured in duplicate on different culture media. Total aerobic mesophilic bacteria were enumerated in Standard Plate Count Agar (PCA; Oxoid, Unipath Ltd., Basingstoke, UK) after incubation at 30 °C for 48 h (ICMSF 1978); total psychrotrophic aerobic bacteria on Standard Plate Count Agar (PCA; Oxoid, Unipath Ltd., Basingstoke, UK) after incubation at 7 °C for 10 days (ICMSF 1978); lactic acid bacteria on the de Man Rogosa Sharpe (MRS) agar (Oxoid, Unipath Ltd., Basingstoke, UK) acidified to pH 5.7 after incubation at 30 °C for 5 days (De Man, Rogosa, & Sharpe 1960); and *Enterobacteriaceae* on Violet Red Bile Glucose (VRBG) agar (Merck, Darmstadt, Germany) after incubation at 37 °C for 24 h (Mossel, Mengerink, & Scholts 1962). Before incubation, plates of MRS agar and VRBG agar were covered with a layer of the same culture medium in order to limitate the oxygen diffusion. After incubation, plates with 30–300 colonies were counted. All microbiological counts were expressed as the logarithm of the colony forming units (cfu) per gram.

### 2.3. Chemical analysis

#### 2.3.1. Physicochemical analysis: pH and color parameters

The pH of samples was measured directly in the steaks using a digital pH-meter (Hanna Instrument HI 99163, Eibar, Spain) equipped with a penetration probe. Color was measured on the surface of the pork steaks using a portable CR-400 colorimeter (Konica Minolta Sensing Inc., Osaka, Japan). The results were expressed in the CIELAB space (CIE (Commission Internationale de l'Eclairage) 1978) as lightness (L*), redness (a*), and yellowness (b*). Color was measured at four different points in each steak. The relative content of myoglobin (MYO), metmyoglobin (MET) and oxymioglobin (OX) at the surface of the steaks is based on measurements of reflex attenuance of incident light at the isobestic points 572, 525, 473 and 730 nm (Krzywicki 1979). The values obtained for the different pigments were expressed as percentage

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