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Nutritional pork meat compounds as affected by ham dry-curing

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ABSTRACT

This work is focused on the determination of compounds of nutritional interest that are naturally present in pork meat and how they are affected during the processing of dry-cured ham. Such compounds are creatine, creatinine, coenzyme Q_{10}, glutathione, carnosine, anserine, carnitine, taurine, cystine, cysteine and the essential amino acids. Their antioxidant and antyhipertensive functions were evaluated. Of all the assayed substances, only glutathione decreased totally during processing. Carnosine, creatinine, anserine and glutathione showed antioxidant, while cysteine, glutathione and carnosine showed antyhipertensive activity. So, dry-cured ham constitutes an excellent source of essential amino acids (all essential amino acids exhibited a large increase during processing) and other nutritionally interesting compounds such as cystine, cysteine, carnosine, anserine, taurine, carnitine and coenzyme Q_{10}.

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1. Introduction

Dry-cured ham is a typical meat product produced in the Mediterranean area. Some substances naturally present in meat are of nutritional interest because when consumed they exert antihypertensive, antioxidant or antimicrobial activity among others, with health benefits to the consumer (Toldrá & Reig, 2011). Health effects have been attributed to food-derived peptides which include antimicrobial properties, blood pressure lowering (ACE inhibitory) effect, cholesterol lowering ability, antithrombotic and antioxidative effects, enhancement of mineral absorption, immunomodulatory effects and opioid activities (Hartmann & Meisel, 2007).

Several endogenous antioxidants (e.g., tocopherols, ubiquinone, carotenoids, ascorbic acid, glutathione, lipoic acid, uric acid, spermine, carnosine, anserine) have been studied in meats (Decker, Livisay, & Zhou, 2000). Both carnosine and anserine are antioxidative histidyl dipeptides which are the most abundant antioxidative compounds in meats. These dipeptides help to control oxidation through the prevention of lipid oxidation by inactivating catalysts and/or free radicals in the cytosol. They also perform a buffering function in the muscle, especially the glycolytic muscles where they are present in large amounts. The function they perform is to reduce rancid taste and improve colour stability (Jiménez-Colmenero, Ventanas, & Toldrá, 2010).

L-carnitine is a compound also present in meat which assists the human body in producing energy and in lowering the levels of cholesterol. It is a vitamin-like nutrient essential for energy production and lipid metabolism in many organs and tissues such as skeletal muscle and heart. Even though L-carnitine can be synthesised, most of the carnitine present in human body is provided by food (Demarquoy et al., 2004). It is also known that it helps the body to absorb calcium to improve skeletal strength and chromium picolinate to help build lean muscle mass. A recent study demonstrated that L-carnitine protected skeletal muscle in rats against myopathy due to congestive heart failure (Vescovo et al., 2002). Glutathione (γ -glutamylcysteinylglycine) is an important antioxidant thiol tripeptide providing cellular defense against toxicological and pathological processes. It exists in two forms: reduced as GSH and oxidised as glutathione disulfide (GSSG). Glutathione appears to be involved in several important biological processes, including protection against free radicals formed after exposure to ionising radiation, protection against oxygen toxicity and metabolism of xenobiotics (Winters, Zukowski, Ercal, Matthews, & Spitz, 1995). Due to its numerous and important protective functions, research involving glutathione is increasing (Chauhan, Audhya, & Chauhan, 2012; Fitzpatrick, Jones, & Brown, 2012; Jardim et al., 2012; Janeš, Lisjak, & Vanzo, 2010; Kumaraguruparan, Balachandran, Manohar, & Nagini, 2005).

Coenzyme Q_{10} (also referred as ubiquinone) is a lipid soluble, endogenous hydroxybenzoquinone compound found in the majority of aerobic organisms. It is a key component of the mitochondrial respiratory chain and is mainly known for its role in oxidative phosphorylation (Small, Coombes, Bennett, Johnson, & Gobe, 2012); its presence was then demonstrated in other subcellular fractions and in plasma lipoproteins, where it is endowed with antioxidant properties. CoQ_{10} was also recognised to have an effect on gene expression (Mattila, Lehtonen, & Kumpulainen, 2000). Cardiovascular effects of CoQ_{10} can be ascribed to its bioenergetic role, to its capability of antagonising oxidation of plasma low-density lipoprotein, and to its effect in ameliorating endothelial function (Belardinelli et al., 2006).

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For these reasons Coenzyme Q_{10} can be considered as a bioactive compound.

Creatine and its phosphorylated derivative phosphocreatine are constituents of muscle tissue implicated in energy delivery (Wyss & Kaddurah-Daouk, 2000). Phosphocreatine turns rapidly into creatine early post-mortem. Then, creatine turns into creatinine in muscle tissues due to a non-enzymatic mechanism dependent on pH, time, temperature, and initial creatine concentration (Mora, Sentandreu, & Toldrá, 2008). This conversion happens easily in meat cooking (Del Campo, Gallego, Berregi, & Casado, 1998). Thus, creatine plays an important role in the energy metabolism of skeletal muscle, providing the necessary energy for vigorous muscle contraction. There is also extensive evidence that, under some circumstances, creatine supplements can enhance muscle performance (Demant & Rhodes, 1999).

Essential amino acids are also important compounds that cannot be synthesised and thus, the body can only receive them exogenously. Understanding the importance of essential amino acids is critical, because a failure to eat foods that contain essential amino acids can lead to severe deficiencies and health effects. Taurine is an essential amino acid during lactation and at times of immune challenge (may protect human body from oxidative stress) (Bouckenooghe, Remacle, & Reusens, 2006). It is present in muscle as a free acid rather than as a component of proteins and has been linked with many biological actions.

Due to the interest in meat nutrients, the effect of dry-cured ham processing on the content of bioactive compounds, naturally present in pork meat, like creatine, creatinine, coenzyme Q_{10}, glutathione (GSH and GSSG), carnosine, anserine, carnitine, taurine, cystine, cysteine and essential amino acids has been evaluated and their antioxidant and antyhipertensive activities determined.

2. Materials and methods

2.1. Dry-cured ham preparation

Forty two fresh hams with an average weight of 10.55 ± 0.58 kg from 6-month old pigs (Landrace × Large White) and similar appearance were purchased from a local slaughterhouse. Six of the hams were used to characterise the raw material. The remaining thirty six hams were bled and prepared according to traditional procedures for Spanish dry-cured ham: pre-salting, where hams were exposed to the curing agents (NaCl and nitrifying salts) for 30 min, the salting stage, where hams were completely covered with solid salt and placed in a cold room (1–3 °C and 80–90% relative humidity) for 10 days, post-salting, where salted hams were kept at 3–5 °C and relative humidity in the range 75–85% for 60 days. Finally, hams were ripened-dried at 14–16 °C and lower relative humidity (decreased to 70%). The total length of the curing process was 10 months.

2.2. Samples

Samples from the *Biceps femoris* muscle were taken from six hams at the beginning of the process and at 2, 3.5, 5, 6.5, 9 and 10 months of processing. Excised muscles were vacuum-packaged and stored at −20 °C until the analytical determinations.

2.3. Amino acids and dipeptides analysis

Amino acids and dipeptides (carnosine and anserine) were determined by reversed-phase HPLC as described by Flores, Aristoy, Spanier, and Toldrá (1997), and previous derivatisation with phenylisothiocianate (PITC) (Bidlingmeyer, Cohen, Tarvin, & Frost, 1987). Thus, 5 g of ground muscle was homogenised with 20 mL of 0.01 N HCl using a Stomacher (IUL instruments, Barcelona, Spain) for 8 min at 4 °C and centrifuged at 10,000 g for 20 min. The supernatant was filtered through glass wool and collected for further processing. 250 μL of extract plus 50 μL of nor-leucine (1 mM used as internal standard) were deproteinised by adding 750 μL of acetonitrile, was left to stand for 30 min and centrifuged at 10,000 g for 3 min. The amino acids of the supernatant were derivatised (Bidlingmeyer et al., 1987) and analysed using a 1200 Series Agilent Chromatograph (Agilent Technology, Palo alto, CA, USA) with a photodiode array detector (254 nm). The phenyl isothiocarbamyl derivatives of amino acids were separated using a Novapack C 18 (300 × 3.9 cm) column (Waters, Milford, MA, USA) at 52 °C. The solvent system consisted of solvent A; 0.07 M sodium acetate adjusted to pH 6.55 with glacial acetic acid and containing 2.5% acetonitrile and solvent B; acetonitrile: water: methanol (45:40:15). The flow rate was 1 mL/min and the solvent gradient was as described by Flores et al. (1997).

2.4. Analysis of creatine and creatinine

Creatine and creatinine were analysed by hydrophilic interaction chromatography (HILIC) according to Mora et al. (2008). Thus, 5 g of ground muscle was homogenised at 4 °C with 20 mL of 0.01 N HCl using a Stomacher (IUL) for 10 min and further centrifuged at 4 °C and 10,000 g for 20 min. The supernatant was filtered through glass wool and 250 μL of this solution was deproteinised by adding 750 μL of acetonitrile, standing at 4 °C for 20 min. Finally, the sample was centrifuged at 10,000 g for 5 min at 4 °C and the supernatant was analysed.

The chromatographic separation was achieved in a 1200 Series Agilent Chromatograph with a photodiode array detector using an Atlantis HILIC Silica column (4.6 × 150 mm, 3 μm) with the corresponding HILIC Silica guard column (4.6 × 20 mm, 3 μm), both from Waters. Mobile phases consisted of solvent A, containing 0.65 mM ammonium acetate, pH 5.5, in water/acetonitrile (25:75), and solvent B, containing 4.55 mM ammonium acetate, pH 5.5, in water/acetonitrile (70:30). The separation conditions were a linear gradient from 0% to 100% of solvent B in 15 min at a flow rate of 1.4 mL/min. Ultraviolet detection at 214 nm for creatine, and 236 nm for creatinine were used.

2.5. Determination of CoQ_{10}

CoQ_{10} was analysed as described by Mattila et al. (2000) but with a modification in the first step of extraction for a better recovery (Souchet & Laplante, 2007). Thus, 1 g of ground muscle was homogenised using a Polytron (Kinematica) with a mixture of 5 mL of 0.5 M sodium chloride and 5 mL of 0.1 M SDS. Two mL of ethanol and 5 mL of n-hexane were added to 1 mL of sample aliquot for CoQ_{10} liquid–liquid extraction (by shaking for 2 min). After centrifugation (5000 g, 4 °C for 3 min), the upper (hexane) layer was removed and the extraction was repeated twice with 3 mL of hexane, respectively. The hexane extracts were pooled and afterwards evaporated under N_2. Dry extracts were dissolved in 500 μL of isopropyl alcohol and centrifuged (10,000 rpm, 4 °C for 3 min) before HPLC analysis.

The chromatographic analysis was accomplished in an Agilent 1100 series with diode array detection (fixed at 275 nm) using an Ultrabase C18 reversed-phase column (2.5 μm particle size and 100 × 4 mm) (Análisis Vínicos, Tomelloso, Spain) maintained at 40 °C and isocratically eluted at 1.0 mL/min using methanol:ethanol: isopropyl alcohol (70:15:15) as mobile phase.

2.6. Determination of carnitine

The determination of free L-carnitine was adapted from the method of Arakawa, Ha, and Otsuka, (1989). Briefly, 2 g of ground muscle was homogenised with 15 mL of 6% cold $HClO_4$ using a stomacher (10 min at 4 °C). The homogenate obtained was centrifuged (10,000 g for 10 min, at 4 °C). The pellet was washed with 5 mL of 6% $HClO_4$ and

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