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Contents of creatine, creatinine and carnosine in porcine muscles of different metabolic types

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Abstract

Creatine, creatinine and carnosine have been analyzed by hydrophilic interaction chromatography (HILIC) in seven different pork muscles of different metabolic type (*Semimembranosus, Biceps femoris, Gluteus maximus, Longissimus dorsi, Gluteus medius, Trapezius* and *Masseter*). As reported in previous literature, carnosine contents are related with the type of metabolism, being higher in those muscles with glycolytic metabolism. Creatine and creatinine also showed significantly higher concentrations in glycolytic muscles such as *Semimembranosus, Biceps femoris, Gluteus maximus* and *Longissimus dorsi*. *Masseter*, a red oxidative muscle, was characterized by the lowest contents of creatine, creatinine and carnosine and, finally, *Gluteus medius* and *Trapezius*, both intermediate muscles, had also intermediate contents of these studied compounds. Finally, a correlation between initial content of creatine and creatinine formation after cooking has been verified using pure standards and two different metabolic type muscles, *Longissimus dorsi* and *Masseter*, obtaining slightly higher creatinine amounts in *Longissimus dorsi*, probably due to its higher initial creatine content and its lower pH.

Keywords: HILIC; Hydrophilic interaction chromatography; Muscle; Metabolic type; Carnosine; Creatine; Creatinine

1. Introduction

Carnosine is an histidine-containing dipeptide widely distributed in vertebrate animal tissues, especially skeletal muscle, heart and the central nervous system (Crush, 1970).

Creatine and its phosphorylated derivative phosphocreatine are key components of the energy delivery process in several tissues, particularly those characterized by the transfer of high energy phosphate to ADP in muscle cells (Wyss & Kaddurah-Daouk, 2000). These compounds play an important role in the energy metabolism of skeletal muscle, taking part in the post-mortem biochemical processes occurring immediately after slaughter (Toldrá, 2006). There is also extensive evidence that, under some circumstances, creatine supplements can enhance muscle performance (Demant & Rhodes, 1999).

On the other hand, creatine turns into creatinine in muscle due to a non-enzymatic conversion by the removal of water and the formation of a ring structure. This conversion takes place easily under heating conditions such as meat cooking. The presence of creatine and creatinine in meat has also been associated to negative aspects because creatine and creatinine can constitute important precursors of heterocyclic amines (HAs), which can be formed on the surface of meat when cooked at high temperatures using dry-heat such as in roasting, frying and grilling (Pais, Salmon, Knize, & Felton, 1999).

Skeletal muscles consist of various fibre types, which differ in their contractility, their metabolism and other properties. Contractile and metabolic properties of skeletal muscle may strongly affect the pattern of energy metabolism in live animal, as well as during the *post-mortem* conversion of muscle to meat. Therefore, the metabolic type of the muscle is one of the main factors involved in the variability of meat quality (Karlsson, Klont, & Fernández, 1999; Valin, Touraille, Vigneron, & Ashmore, 1982).

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Several authors have used myoglobin concentration and lactate dehydrogenase activity as an approximation to the glycolytic or oxidative pattern of the muscle (Flores et al., 1996; Leseigneur-Meynier & Gandemer, 1991). The myoglobin content is closely related to the oxidative pattern of the muscles whereas lactate dehydrogenase activity is an indicator of its glycolytic potential (Flores et al., 1996). In this way, porcine *Longissimus dorsi, Semimembranosus* and *Biceps femoris* were classified as glycolytic muscles while *Masseter* and *Trapezius* were assigned to the oxidative metabolism group. On the other hand, Leseigneur-Meynier and Gandemer (1991) consider three types of metabolism in pork: (i) glycolytic, with *Longissimus dorsi*; (ii) oxidative, with *Masseter*; and (iii) an intermediate one including *Trapezius*.

The content of natural dipeptides is significantly lower in oxidative muscles (Aristoy & Toldrá, 1998). These results agree with those reported by Cornet and Bousset (1998), who demonstrated that the content of several amino acids in muscle are closely related to the metabolic type of fibres. Both authors established that the highest carnosine amounts were found in glycolytic muscles, so that the content of the natural dipeptide carnosine constitutes a good indicator of muscle glycolytic activity.

The main objective of the present study was to determine by means of a recently developed methodology consisting of hydrophilic interaction chromatography (HILIC) the content of creatine, creatinine and carnosine on seven different pork muscles (*Semimembranosus, Biceps femoris, Gluteus maximus, Longissimus dorsi, Gluteus medius, Trapezius* and *Masseter*) chosen to represent the three main metabolic types, in order to evaluate the relation between the total content of these compounds and the type of muscle metabolism. In addition, pure standards and *Longissimus dorsi* and *Masseter* pork muscles, with glycolytic and oxidative metabolism, respectively, were cooked and analyzed in order to establish a relation between initial amounts of creatine and the formation of creatinine after cooking.

2. Materials and methods

2.1. Chemicals and reagents

All chemical and chromatographic reagents were of HPLC grade. Acetonitrile, ammonium acetate and glacial acetic acid were purchased from Scharlau (Barcelona, Spain). Creatine and creatinine standards were from Fluka Chemie AG (Buchs, Switzerland).

2.2. Materials

Seven muscles, *Semimembranosus, Biceps femoris, Gluteus maximus, Longissimus dorsi, Gluteus medius, Trapezius* and *Masseter*, obtained each from four different porcine carcasses, from 6-month old female pigs, were used to detect significant differences in creatine, creatinine and carnosine content according to the type of muscle. These muscles were chosen in order to cover a broad range of metabolic types according to the description carried out by Leseigneur-Meynier and Gandemer (1991), Cornet and Bousset (1998) and Karlsson et al. (1999).

2.3. Sample and standards preparation

2.3.1. Standards preparation

The calibration ranges for the assayed compounds were established using a duplicate set of standards. Carnosine and creatinine calibration standards were prepared by diluting a stock solution of 1 mg/mL. A standard solution of creatine at 0.3 mg/mL was prepared because of the low solubility of this compound in the injection solution (0.01 N HCl in water/acetonitrile, (25:75, v/v)). Working standard solutions were prepared over the appropriate concentration range by dilution of stock solutions. All stock solutions were stored at −20 °C until use.

On the other hand, in order to study the influence of initial creatine amounts in the formation of creatinine after cooking, standards of creatine were prepared at three levels, 0.25, 0.5 and 0.75 mg/mL, that correspond to 100, 200 and 300 mg/100 g of muscle, respectively. These concentrations were selected because they covered the usual range of creatine concentration found in pork meat and its derived products (Del Campo, Gallego, Berregi, & Casado, 1998; Kvasnicka & Voldrich, 2000; Macy, Naumann, & Bailey, 1970). The effect of initial creatine amounts on the creatinine formation was studied at 72 °C during 10, 16, 30 and 60 min and during 30 min at 60, 65, 72 and 80 °C. These assays were carried out in 1 mL sealed tubes, introducing the standards in a water bath (Tectron Bio, Selecta, (Barcelona, Spain)) and holding them at 72 °C for the specified times and during 30 min at the temperatures above mentioned. After the heating treatment, the standards were cooled to 0 °C in an ice bath in order to decrease the temperature as soon as possible to stop the creatine conversion to creatinine.

Finally, in order to study the effect of cooking in the creatine conversion to creatinine under controlled conditions of pH, temperature and time, standards of creatine and creatinine were prepared at similar concentrations to those expected in raw *Longissimus dorsi*: 0.875 mg/mL of creatine and 0.02 mg/mL of creatinine. The effect of cooking on the creatinine formation was established under the same cooking conditions described in Section 2.3.3 for *Longissimus dorsi* and *Masseter* muscles.

2.3.2. Pork muscles preparation for analysis

The muscles *Semimembranosus, Biceps femoris, Gluteus maximus, Longissimus dorsi, Gluteus medius, Trapezius* and *Masseter* were excised from four pork carcasses at 24 h post-mortem and immediately processed for further analysis following the method described by Aristoy and Toldrá (1991) with some minor changes. Briefly, 5 g of sample tissue were homogenized with 20 mL of 0.01 N HCl in a