DETERMINATION OF THE CONTENT OF COENZYMES Q9 AND Q10 IN PORK MEAT FROM DIFFERENT BREEDS

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Abstract

Meat contains basic and essential nutrients to humans, and recently has been given a special attention to the presence of bioactive compounds.

The objective of this work was the simultaneous determination of coenzymes Q9 and Q10 in pork meat, using HPLC. For this study were used 122 meat samples from different muscles and different breeds of pork. Besides, it was also evaluated the influence of animal breed and muscle type in the contents of CoQ9 and CoQ10.

The results showed that the breed influenced significantly the mean content of CoQ9 (0.70 mg 100 g−1) and CoQ10 (3.76 mg 100 g−1) (p<0.0001), being this significantly higher in the meat from the breed ‘Alentejano’ when compared to the other breeds (Large White and Landrace). The type of muscle was also responsible for the variation in the levels of coenzymes in the samples analysed (p<0.05) and (p <0.0001) respectively.

From the results obtained it was concluded that the breed ‘Alentejano’ presented the highest contents in both coenzymes (CoQ9 and CoQ10) and that, among the muscles evaluated, the muscle Semimembranousus (Sm) showed higher concentrations of CoQ10.

Keywords: chromatographic analysis, coenzyme Q9, coenzyme Q10, pork meat.

Introduction

The negative image often associated with meat and processed meat products is related to the presence of some components that can cause diseases to the health of the consumer, particularly high salt content, saturated fats, fatty acids and cholesterol responsible for the development of cardiovascular diseases, some cancers and obesity (Leunceford, 2008).

Meat also plays an important role in supplying our diet with minerals, such as iron, zinc, selenium, and vitamins of group B and E. Besides these basic nutritional components, studies have revealed that meat contains several bioactive compounds, such as conjugated linoleic acid (CLA), L-carnitine, carnosine, glutathione, taurine, creatine, coenzyme Q5 and Q10, among others (Toldrá, 2010).

The coenzymes Q contain a chromogenic nucleus (2,3-dimethoxy-5-methyl-1,4-benzoquinone) and a polyisoprenoid side chain in the 6-position which differs only in the number of isoprenoid units (Souchet, Laplante, 2007). The predominant form of ubiquinone in humans, animals, and fish is Co-enzyme Q10 (2,3-dimethoxy-5-methyl-6-decaprenylbenzoquinone-ne), containing 10 isoprenoid units in the side chain (Linnane, 2007).

Coenzyme Q is an essential component of the electron transport chain electrons respiratory complexes I and II to respiratory complex III. Another important function of coenzyme Q is that of serving as a lipid soluble antioxidant protecting lipids, proteins and DNA from damage by reactive oxygen species (Santos, 2011).

In the respiratory chain in the mitochondria there are several coenzymes (coenzyme Q5), as for example, CoQ5, CoQ7, CoQ8, CoQ9, CoQ10, CoQ12, and CoQ10. The Q10 is the most common form found in humans and most mammals, while coenzyme Q9 is most often found in rats and guinea pig. On the other hand, the Q5, Q7 and Q8 coenzymes are found in bacteria and yeasts (Sauer Shah, Laurindo, 2010).

The presence of coenzymes Q9 and Q10 in meat assumes a particular importance given their important roles on the human body as promoting health. However, the contents of these coenzymes may vary greatly according to breed, muscle type, sex, age, seasonal variations, and type of feed, among others.

Given the importance of natural antioxidants as preventing the damaging effect of free radicals formed during the cellular processes that are responsible for e.g. aging, cardiovascular disease and certain cancers, the objective of this work was to determine the content of coenzymes Q9 and Q10 in several samples of pork meat from different breeds by the method of HPL.

Materials and Methods

Sampling

In this study were used samples taken from meat originating from different animal breeds and different muscles. The pork samples corresponded to meat from 61 animals, from different breeds: Large White × Landrace (31 animals) and ‘Alentejana’ (30 animals).

From each animal, samples of muscles Longissimus dorsi (Ld) and semimembranousus (Sm) were removed, summing up a total of 122 samples analyzed.

The samples were homogenized and defatted, vacuum packed and kept frozen at -72 °C to perform the analysis.

Analytical Process

All the methodology inherent in the study and analysis of pork meat from different breeds was based on a technical protocol previously defined by Section of Meat and Meat Products from the National Institute for Agricultural and Veterinary Research (INIAV, 2013). These procedures were previously adapted to the study of meat and used for the analyses.
Extraction
For extraction were weighed 4 g of homogenized muscle sample, and cut into small pieces into glass petri dishes, in duplicate. Then, the Petri dishes were placed for about 24 hours in the lyophilizer (CHRIST BETA 1-8 K). The lyophilized samples were reduced by powder in a mortar placed in plastic centrifuge tube with cap and kept in a dry place until analysis. To the sample is added 10 mL of 0.15 M NaCl solution and then homogenized in a homogenization equipment (Polytron PT 3100) for 1 minute at 3500 rpm. After homogenization, was added 10 mL of absolute ethanol and stirred by vortex for 2 minutes (Vortex Heidolph REAX). Then, to the samples were added 15 mL of n-hexane and centrifuged again for 1 minute (Centrifuge Sorvall Instruments RC5C with SS-34 rotor). The extracts were then centrifuged at 3000 rpm at 4 °C for 5 minutes to separate the phases. The supernatant was removed to another tube, properly identified, and proceeded to re-extraction of the pellet with another 15 mL of n-hexane, followed by further centrifugation. The supernatants from both centrifugations were combined. From this extract, 20 mL were used to evaporate on a rotary evaporator at 40 °C to dryness. The residue obtained was resuspended in 3 mL of 2-propanol and filtered through a syringe of 0.2 mm equipped with a PTFE filter (Acrodisc). Fifty microliters were injected into the HPLC system.

Analysis by high performance liquid chromatography (HPLC)
A Waters HPLC system consisting of a mark separation module Alliance 2487 and a detector Waters 2695 was used for the analyses. Empower Pro software was used to analyze the chromatograms obtained.

The separation of coenzymes Q9 and Q10 was performed using a isocratic phase column Atlantis dC 18 4.6×150 mm, 3 μm, Waters mark at room temperature.

The mobile phase consisting of acetonitrile; tetrahydrofuran: water (55 : 40 : 5, v/v/v) was used at a flow rate of 1.5 mL min⁻¹. The coenzymes were detected in UV detector at 275 nm.

Analysis of Results
To evaluate the influence of the studied factors (breed and muscle) and their interactions on the variation of the levels of coenzyme Q9 and Q10 there an Analysis of Variance was undertaken for 95% confidence level. When differences were significant for the race factor or interactions, the difference between means was assessed using the Tukey test.

The following factors were assessed:
- Influence of breed in the content of coenzymes Q9 and Q10;
- Influence of muscle (Ld and Sm) in the content of coenzymes Q9 and Q10;
- Influence of the interaction between muscle and breed.

Results and Discussion
Validation of analytical procedures
Determination of standard curves coenzymes Q9 and Q10
In order to proceed to quantification of the content of coenzymes in the samples, were prepared two solutions of coenzyme Q9 and Q10, which were injected into the HPLC in the form of various concentrations 0, 2, 5, 10, 20, 30, 50 (μg mL⁻¹), giving rise to the various “peaks” of different heights (AU), being the detection time 6.095 minutes to Q9 and 8.065 minutes to Q10.

With the integrations of “peaks” and the respective known concentrations of coenzymes, it was possible to obtain both standard curves, with their corresponding equations and correlation coefficients (r).

Determination of the Limits of Detection (LOD) and Quantification (LOQ)
The LOD and LOQ were determined by a sequence of analytical calculations to determine the standard deviation(s), using the specific formulas, from which were obtained the values in Table 1.

Table 1

<table>
<thead>
<tr>
<th>Type of coenzyme</th>
<th>LOD</th>
<th>LOQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coenzyme Q9, mg 100 g⁻¹</td>
<td>0.343</td>
<td>1.040</td>
</tr>
<tr>
<td>Coenzyme Q10, mg 100 g⁻¹</td>
<td>0.067</td>
<td>0.203</td>
</tr>
</tbody>
</table>

Determination of recovery rate
The calculus of the recovery rate is done using the peak of the sample without the standard and the peak of the sample without the standard. This determination is made by means of the expression for the calculus of the recovery rate.

The results of the recovery rates made can be seen in Table 2.

Table 2

<table>
<thead>
<tr>
<th>Type of coenzyme</th>
<th>Recovery Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coenzyme Q9, mg 100 g⁻¹</td>
<td>50.00</td>
</tr>
<tr>
<td>Coenzyme Q10, mg 100 g⁻¹</td>
<td>69.77</td>
</tr>
</tbody>
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Simultaneous determination of coenzymes Q9 and Q10 in pork meat
Coenzyme Q9
Statistical analysis undertaken to the results of the determination of coenzyme Q9 in pork meat, demonstrated significant differences between the breeds studied (p<0.0001) and between muscles Ld and Sm (p<0.05). With respect to the interaction between the two factors (Breed × Muscle) it was not found any significant influence.

Among the factors studied, the breed of the animal was that which exerted a significant influence over the content of coenzyme Q9 in pork meat, with F value equal to 27.25.
According to Figure 1, it was possible to assess that there is a significant difference in the mean values (p<0.001). The samples obtained from animals of ‘Alentejana’ breed showed an average content of coenzyme Q9 higher (0.70±0.21 mg 100 g\(^{-1}\)) than samples of the animals LW×LR (0.52±0.19 mg 100 g\(^{-1}\)).

With respect to the influence of the muscle type in the amount of Coenzyme Q9, it was found a significant difference between the two muscles in the study (Ld and Sm). The Sm muscle showed a higher average (0.64±0.23 mg 100 g\(^{-1}\)), when compared with Ld muscle (0.57±0.21 mg 100 g\(^{-1}\)) (Figure 1).

Through the second graph of Figure 1, it was possible to ascertain that the average value for Sm CoQ9 was significantly higher than Ld. A possible reason for this difference between the two types of muscle is due to the fact that muscle Sm belongs to the leg muscles, and the muscles in this location exert a greater physical effort for the animal’s mobility.

The interaction between breed and muscle showed no significant influence on the results of coenzyme Q9 in pork meat.

Coenzyme Q10
In analyzing the results (ANOVA) of the content of CoQ10 in pork meat, it was found that all the factors studied: race, muscle, interaction of breed with the type of muscle, significantly influence the content of CoQ10 (p<0.0001).

Regarding the race factor, it was obtained a value of F equa to 226.78. In the first graph of Figure 2 are presented the average values for coenzyme Q10 in meat from breeds ‘Alentejana’ (3.75±1.44 mg 100 g\(^{-1}\)) and LW×LR (1.96±0.75 mg 100 g\(^{-1}\)). The results demonstrated that it is the meat of the ‘Alentejana’ breed that has a higher content compared with that obtained in pork LW×LR (white pig) (Figure 2).

The muscle factor also exerted a high influence on the result, with a value of F equal to 213.46. It was also found that the Sm muscle (3.70±1.53 mg 100 g\(^{-1}\)) showed a higher mean value compared to Ld muscle (1.96±0.66 mg 100 g\(^{-1}\)). The difference between the two types of muscle examined can be observed in the second graph in Figure 2.

This difference that occurred between muscles, as already mentioned in the analysis of CoQ9, may be due to the type of muscle and breed. In fact, the animal exerts more physical effort with some of their muscles, which causes them to gain a greater oxidative character.

Doing the statistical analysis regarding the interaction of breed with muscle it was found to be a high-examined significant difference (p<0.0001), with value F = 37.0.
Among all the conditions studied, the meat samples belonging to the Sm muscle of ‘Alentejana’ pig breed (Figure 3), exhibited a higher average value. The mean value obtained for the samples of coenzyme Q_{10} in Sm muscle of the ‘Alentejana’ breed was 4.99 mg 100 g^{-1}. The values obtained for meat samples of Sm muscle from LW×LR breed and the values obtained for meat samples of Ld muscle from the ‘Alentejana’ breed showed no significant difference (Figure 3). The Ld samples of animals from LW×LR breed showed the lowest mean level of CoQ_{10} (1.44 mg 100 g^{-1}), also revealing to be different from the medium content of all other conditions studied (Figure 3).

It was further observed that the highest values for CoQ_{9} and CoQ_{10} occurred in the most oxidative muscle (Sm), which belongs to the leg muscle, where a greater physical effort is done having in mind the mobility of the animal. It is also known that the higher is the oxidative nature of the muscle, the largest will be the content of coenzymes Q_{9} and Q_{10}, as found in this study.

Among the breeds analyzed that one which stood out for its high content of coenzyme Q_{10} was the sample of swine of the ‘Alentejana’ breed.

**Conclusion**

This study aimed to determine the coenzymes Q_{9} and Q_{10} in different meat samples. In view of the results obtained, it was evaluated the influence in the concentration of the coenzymes of the factors under study (breed, muscle and interaction between the two). As regards pork meat, the results indicated that factors like breed and type of muscle exerted an influence on the content of coenzyme Q_{9}. However in the case of CoQ_{10} all the factors studied revealed to have a significant influence.

**References**