DETERMINATION OF THE CONTENT OF COENZYMES Q_9 AND Q_{10} 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 Q_9 and Q_{10} 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 CoQ_9 and CoQ_{10}.

The results showed that the breed influenced significantly the mean content of CoQ_9 (0.70 mg 100 g^{-1}) and CoQ_{10} (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 (CoQ_9 and CoQ_{10}) and that, among the muscles evaluated, the muscle *Semimembranousus (Sm)* showed higher concentrations of CoQ_{10}.

Keywords: chromatographic analysis, coenzyme Q_9, coenzyme Q_{10}, 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 Q_9 and Q_{10}, 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 Q_{10} (2,3-dimethoxy-5-methyl-6-decaprenylbenzoquino-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 Q), as for example, CoQ_1 CoQ_2, CoQ_4, CoQ_6, CoQ_7, CoQ_8, CoQ_9 and CoQ_{10}. The Q_{10} is the most common form found in humans and most mammals, while coenzyme Q_9 is most often found in rats and guinea pig. On the other hand, the Q_6, Q_7 and Q_8 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 determinate the content of coenzymes Q_9 and Q_{10} 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 *Longissumus 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 to 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 proceded 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 Q_9 and Q_{10} 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 Q_9 and Q_{10} 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 Q_9 and Q_{10};
- Influence of muscle (*Ld* and *Sm)* in the content of coenzymes Q_9 and Q_{10};
- Influence of the interaction between muscle and breed.

Results and Discussion

Validation of analytical procedures

Determination of standard curves coenzymes Q_9 and Q_{10}

In order to proceed to quantification of the content of coenzymes in the samples, were prepared two solutions of coenzyme Q_9 and Q_{10}, 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 Q_9 and 8.065 minutes to Q_{10}.

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

Limits of detection and quantification of the process obtained

Type of coenzyme	LOD	LOQ
Coenzyme Q_9, mg 100 g^{-1}	0.343	1.040
Coenzyme Q_{10}, mg 100 g^{-1}	0.067	0.203

Determination of recovery rate

The calculus of the recovery rate is done using the peak obtained for the sample with 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

Recovery rates obtained in the process

Type of coenzyme	Recovery Rate
Coenzyme Q_9, mg 100 g^{-1}	50.00
Coenzyme Q_{10}, mg 100 g^{-1}	69.77

Simultaneous determination of coenzymes Q_9 and Q_{10} in pork meat

Coenzyme Q_9

Statistical analysis undertaken to the results of the determination of coenzyme Q_9 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 Q_9 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 Q_9 higher (0.70±0.21 mg 100 g^{-1}) than samples of the animals *LW×LR* (0.52±0.19 mg 100 g^{-1}).

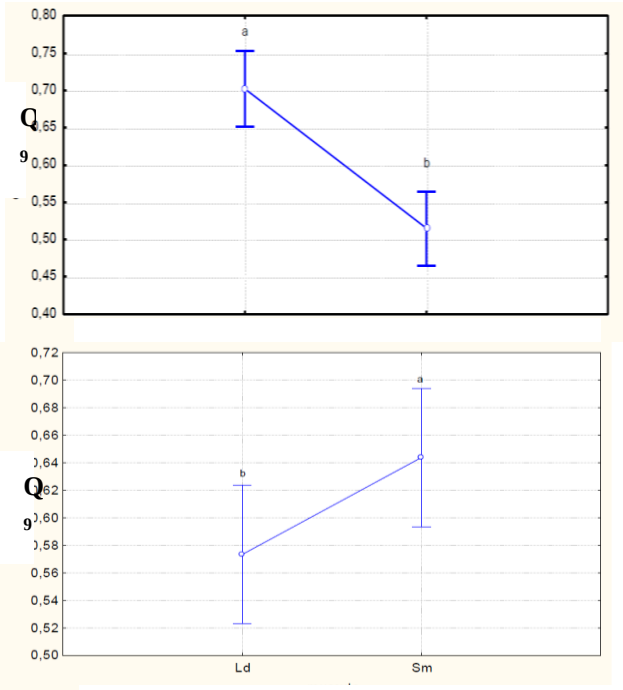

Figure 1. Influence of breed and muscle on the pork meat

With respect to the influence of the muscle type in the amount of Coenzyme Q_9, 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* CoQ_9 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 Q_9 in pork meat.

Coenzyme Q_{10}

In analyzing the results (ANOVA) of the content of CoQ_{10} 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 CoQ_{10} ($p<0.0001$).

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 Q_{10} 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.

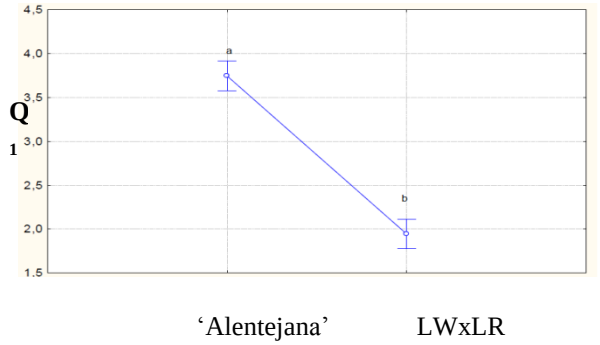

Figure 2. Influence of breed and muscle on pork meat (coenzyme Q_{10})-

This difference that occurred between muscles, as already mentioned in the analysis of CoQ_9, 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.

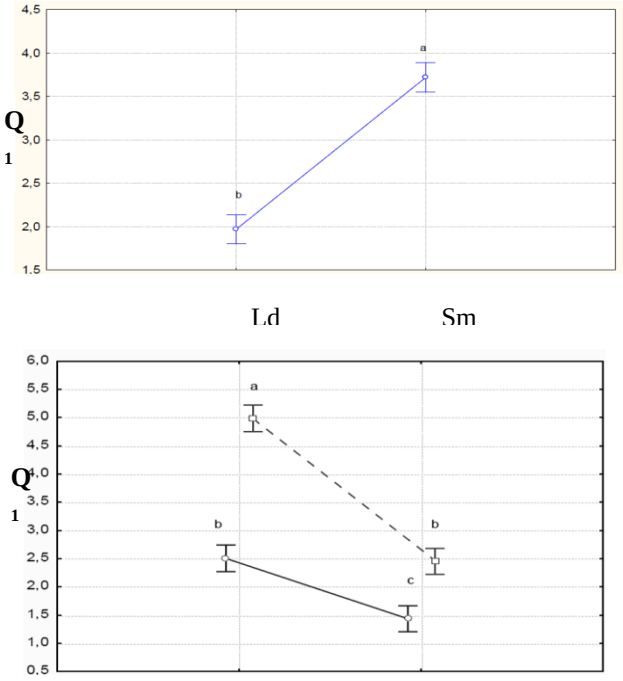

Figure 3. Influence of the interaction of breed with muscle in pork meat (coenzyme Q_{10})

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

1. Araujo W. M. C., Montebello N. de P. (2006) *Carne & cia* (Vol 1). Senac.
2. Toldrá F. (2010) *Handbook of Meat Processing*. John Wiley & Sons.
3. Souchet N., Laplante S. (2007) Seasonal variation of Co-enzyme Q10 content in pelagic fish tissues from Eastern Quebec. *Journal of Food Composition and Analysis*, Vol. 20, No. 5, p. 403–410. doi:10.1016/j.jfca.2006.09.004
4. Sauer H., Shah A. M., Laurindo F. R. M. (2010). *Studies on Cardiovascular Disorders*. Springer.
5. Santos S. (2011). *Aspectos Bioquímicos e Moleculares da Coenzima Q10*. Universidade de Aveiro; Departamento de Biologia, Aveiro. Obtido de https://ria.ua.pt/bitstream/10773/7981/1/246586.pdf
6. Lunceford A. L. (2008). *The Effect of Coenzyme Q(10) Supplementation on Aging and Disease*. ProQuest.
7. Linnane A. W., Kios M., Vitetta L. (2007). Coenzyme Q10 – Its role as a prooxidant in the formation of superoxide anion/hydrogen peroxide and the regulation of the metabolome. *Mitochondrion*, *7*, *Supplement*, S51–S61. doi:10.1016/j.mito.2007.03.005.