SOURCES OF VARIABILITY IN THE ANALYSIS OF MEAT NUTRIENT COENZYME Q_{10} FOR FOOD COMPOSITION DATABASES

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ABSTRACT

Coenzyme Q_{10} (CoQ_{10}) or ubiquinone (2,3-dimethoxy-5-methyl-6-multiprenyl-1,4-benzoquinone) is an endogenous hydroxybenzoquinone liposoluble compound which plays important physiological roles that makes it to be considered as a bioactive compound that may be used for clinical practices and as food supplement. The purpose of this work was to analyse CoQ_{10} in three muscles with different oxidative patterns and determine its variability in different animal species (pork, beef, lamb and rabbit). The content of CoQ_{10} ranged from 4.3 to 30.9 µg/g meat with the highest content in those muscles with oxidative pattern. So, more specific data on type of meat cut and proportion of muscles must be given for this nutrient when reporting its content in food composition databases.

Keywords: coenzyme Q_{10}, meat nutrients, meat composition, food composition, food databases, muscle metabolism

INTRODUCTION

Meat constitutes a food with relevant nutritional properties. Its content in nutrients can be found in many food composition databases even though the large natural variability in meat nutrients is not well reflected in such databases. In fact, the identification of the meat source is usually incomplete because only the animal species and type of cut, that may include several different muscles, are given. However, there are relevant nutrient substances in meat that are affected by intrinsic factors of the animal like its genetics, age and type of muscle (Reig, Aristoy & Toldrá, 2013). For instance, the analysis of specific nutritional substances like carnosine, anserine, taurine, glutamine, carnitine, myoglobin, creatine and creatinine show a large dependence on the type of muscle. Meat cuts are usually composed of various skeletal muscles which contain various types of fibres of different metabolic type. The feed also exerts a relevant effect, not only in the amount of fat but also on its composition in fatty acids. This is important when considering the amount of nutrients in meat for healthier purposes (Toldrá and Reig, 2011).

All these sources of variability must be taken into account when including such data in composition databases because it may give very different values. This work shows the variability in the analysis of specific meat nutrients depending on the type of assayed meat and how they may affect the general food composition databases.

Coenzyme Q_{10} (CoQ_{10}) or ubiquinone (2,3-dimethoxy-5-methyl-6-multiprenyl-1,4-benzoquinone) is an endogenous hydroxybenzoquinone liposoluble compound which plays an important role as electron carrier in the mitochondrial respiratory chain and favour ATP generation (Overvad, Diamant, Holm, Holmer, Mortensen, Stender, 1999). The consequence of this action is an antioxidant activity which makes the CoQ_{10} a protector of lipoproteins against oxidative damage, not only of the mitochondria membrane, but also in the rest of cell membranes. In the same way, it plays an important role in regenerating other antioxidants such as vitamin E (Bentinger, Brismar & Dallner, 2007). Lately, CoQ_{10} has been recognised as a potent gene regulator (Groneberg, Kindermann, Althammer, Klapper, Vormann, Littarru, Döring, 2005). These properties make CoQ_{10} to be considered as a bioactive compound which has been targeted for clinical practices and prescribed as food supplement (Overvad et al., 1999, Litarro & Tiano, 2010). Some studies have reported a reduction in human LDL

cholesterol oxidation after oral supplementation with CoQ_{10} (Kaikkonen, Nyyssonen, Porkkala-Saratho, Poulsen, Metsa-Ketela, Hayn, Salonen, 1997) while others reported an improvement the cardiac function in those patients suffering cardiac muscle weakness (Turunen, Olsson & Dallner, 2004) or heart failure (Singh, Devaraj & Jialal, 2007).

CoQ_{10} was named as ubiquinone because it is ubiquitous (present everywhere). The highest content is found in meat and fish tissues and viscera due to their high levels of mitochondria (Mattila & Kumpulainen, 2001). The effect of cooking on the content of CoQ_{10} in meat has resulted in some losses. So, there are reported losses of about 15 to 32 % after frying pork cutlets (Weber, Bysted & Holmer, 1997) and about 15% after grilling of beef (Purchas, Busboom & Wilkinson, 2006) while, on the contrary, some increase was reported after slow cooking (90 min at 70ºC) of lamb (Purchas, Rutherfurd, Pearce, Vather & Wilkinson, 2004). The reported losses were higher than 50% after 10 months of dry-curing (Marusic, Aristoy & Toldrá, 2013).

The purpose of this work was to analyse CoQ_{10} in different animal muscles, evaluate the methodology and determine the influence of the type of muscle metabolism in its content in different animal species (pork, beef, lamb and rabbit). The final goal is to evaluate the information that must be given for meat when reporting its CoQ10 content in food composition databases.

MATERIALS AND METHODS

Samples

Meat samples of 5 animals from each pork, beef, lamb and rabbit were obtained from Vaquero Meat industry (Madrid, Spain) and excised for specific muscles: *Masseter*, *Longissimus dorsi* and *Biceps femoris*. Pork and lamb were also excised for *Trapezius.* Samples were kept under frozen storage at -80ºC until analysis.

Chemicals and Solvents

HPLC grade isopropyl alcohol, ethanol and n-hexane 96% were purchased from Scharlau (Scharlab, Barcelona, Spain). Pyruvate, oxalacetate, NADH, sodium dodecyl sulphate (SDS) and CoQ_{10} standard were from Sigma (Sigma-Aldrich, St Louis, Mo, USA). Tris-(hydroxymethyl)-aminomethane (Tris-HCl), magnesium chloride, Ethylene

diamine tetracetic acid (EDTA) and sodium chloride were from Panreac (Panreac Química S.A., Barcelona Spain)

Muscles characterisation

Lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) activities were assayed in the presence of NADH which is cleaved by oxidation to NAD^+. The LDH and MDH activity present in sample, and consequently, the disappearance of NADH in the reaction medium, was measured along 3 min by continuously monitoring, each 20 s, the decrease in absorbance at 340 nm. For these analyses, sample (1g) was extracted with 20 mM Tris-HCl buffer, pH 7.5 (10 mL), using a Polytron homogeniser (Kinematica, Barcelona, Spain) while the sample was maintained in an ice bath (Lin, Wang, Feng, Huang, Xu, Jin, Li, Jiang &, Zheng, 2011). After centrifugation (10.000 rpm, 4º C for 30 min), the supernatant was filtered through glass wool and diluted 1/50 with the buffer. The activity was defined as the amount of pyruvate (for LDH) or oxalacetate (MDH) which is reduced to lactate per minute and per gram of muscle.

Total myoglobin was analysed as described by Lin et al. (2011). Thus, sample (1 g) was extracted with 75 mM Tris-HCl, pH 7.2, containing 3 mM magnesium chloride and 5 mM EDTA, (5 mL) using a Polytron homogenizer. After centrifugation (10.000 rpm, 4º C for 30 min), the supernatant was filtered through glass wool and then followed by a 0.2 µm nylon membrane filter. The optical density of the filtrate was measured at 576 nm (Bentinger, Brismar & Dallner, 2007).

Determination of CoQ_{10}

CoQ_{10} was analysed as described by Mattila et al. (2000) with some modifications. Thus, 1 g of fresh meat sample was mixed thoroughly with a mixture of 5 ml of 0.5 M sodium chloride and 5 mL of 0.1M SDS. 2 mL of ethanol and 5 mL of *n*-hexane were added to 1 mL of sample aliquote for CoQ_{10} liquid-liquid extraction (by shaking for 2 min). After centrifugation (5.000 rpm, 4º 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 stream. Dry extracts were dissolved in 500 µL of isopropyl alcohol and centrifuged (10.000 rpm, 4º C for 3 min) before HPLC analysis.

The chromatographic analysis was accomplished in an Agilent 1100 series (Agilent Technologies, Palo Alto, CA, USA), with diode array detection (fixed at 275 nm). Sample (20 µL) was injected into an Ultrabase C18 reversed-phase column (2.5 µm particle size and 100 x 4 mm) (Análisis Vínicos, Tomelloso, Spain) maintained at 40 ºC and isocratically eluted at 1.0 mL/min using methanol:ethanol:isopropyl alcohol (70:15:15) as mobile phase.

The analytical method was validated (linearity, repeatability, reproducibility and recovery) and the LOD and LOQ were determined from the average of five replicate calibration standard curves resulting in 0.9 µg/g and 2.9 µg/g, respectively.

Statistical analysis

All data obtained from experiment were subjected to variance analysis and differences between mean values were evaluated by Duncan's multiple range test with SPSS statistical software (SPSS Inc., Chicago, version 20) for windows. The results were presented as mean values ± standard deviation.

RESULTS AND DISCUSSION

Muscle fibers are generally categorised as types I (slow twitch, predominantly oxidative), IIA (fast-twitch, oxido-glycolytic) or IIB (fast-twitch, glycolytic) and each muscle contains different proportions of these types of fibers (Lawrie & Ledward, 2006). Tissue lactate dehidrogenase (LDH) activity represents the glycolytic potential while malate dehydrogenase (MDH) activity represents the oxidative potential (Lin et al., 2011). The muscle LDH and MDH activities cannot be directly compared among animal species because they are also affected by the type of breed, sex, age and also type of feeding (Turunen, Olsson & Dallner, 2004; Lin et al., 2011; Singh, Devaraj, & Jialal, 2007). This is why the ratio MDH/LDH is usually reported as a better indication of the type of muscle metabolism.

The LDH and MDH enzyme activities, and the myoglobin and CoQ10 content were analysed in the muscles *Masseter* as representative of oxidative metabolism, *Trapezius* as an intermediate metabolism and *Biceps femoris* and *Longissimus dorsi*, as

representative of glycolytic metabolism, from pork, rabbit, lamb and beef. *Masseter* muscle, which is considered as a model representative of oxidative muscle due to its rich content in fibers type I, exhibited the lowest LDH activity and the highest MDH activity, with a MDH/LDH ratio much higher than 1 for all the assayed species (see table 1). On the other hand, *Biceps femoris and Longissimus dorsi*, which are predominantly glycolytic due to their high content in fibers type IIB, showed a reverse trend with higher LDH and lower MDH, having a MDH/LDH ratio below 1 also for all the species (see table 1). The content of CoQ_{10} has been directly related with the mitochondria content which is more abundant in the oxidative red-type fibres I (Purchas & Busboom, 2005). Souchet and Laplante (2007) observed 5 times higher concentration of CoQ_{10} in mackerel red flesh as compared with white flesh which was explained mainly by the higher abundance of mitochondria in red flesh. Other factors like the production system were also reported to affect the CoQ_{10} content (Purchas & Busboom, 2005). The muscle type was also reported to have the greatest effect in dairy cow meat, with two time smore CoQ_{10} in *Gluteus medius* muscle than in *Longissimus dorsi*, while the age of cows and the carcass weight did not show any significant influence (Roseiro, Santos, Gonçalvez et al, 2014). In our work, there was a significantly ($p<0.05$) higher content of coenzyme Q10 in oxidative muscle *Masseter* as compared to the other assayed muscles. This effect is noticeable not only in pork but also in other animal species like rabbit, lamb and beef (see Table 2). Similar effect is observed on the content of myoglobin, the heme iron meat protein which is the protein responsible of meat colour and excellent contributor of heme iron (Table 3). The content of myoglobin was found more abundant in the oxidative muscles and lowest in the glycolytic ones as already reported for pork (Aristoy & Toldrá, 1998).

When analyzing the results obtained with the oxidative metabolic patterns of the assayed species, a direct relationship between MDH activity and myoglobin with the CoQ_{10} content was observed (see tables 1-3). Similarly, an inverse relationship between LDH activity and CoQ_{10} content was also observed. In fact, the highest content ($p<0.05$) of CoQ_{10} and the lowest LDH activity ($p<0.05$) was observed in the *Masseter* muscle which is predominantly oxidative while the lowest CoQ_{10} content ($p<0.05$) and highest LDH activity ($p<0.05$) was detected in the *Biceps femoris* and *Longissimus dorsi* muscles which are predominantly glycolytic. Intermediate MDH and LDH activity as

well as CoQ_{10} and myoglobin content was observed in the *Trapezius* muscle of pork and lamb that has an intermediate oxidative pattern.

In summary, the analysis of muscles with different oxidative pattern indicates that those muscles with higher oxidative pattern have a significantly ($p<0.05$) higher content of myoglobin and CoQ_{10} than those with glycolytic pattern. This trend is observed for all the assayed animal species. So, more specific data on type of meat cut and a somehow defined proportion of muscles is required when reporting nutrient content in food composition databases.

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References

Aristoy MC, Toldrá F (1998) Concentration of free amino acids and dipeptides in porcine skeletal muscles with different oxidative patterns. Meat Sci. 50: 327-332

Bentinger M, Brismar K, Dallner G (2007) The antioxidant role of coenzyme Q. Mitochondrion 7 (Suppl.): 41–50

Flores, M, Aristoy, M.C, Spanier, A M, Toldrá, F. (1997). Non-volatile components effects on quality of Serrano dry-cured ham as related to processing time. J. Food Sci., 62: 1235-1239.

Groneberg DA, Kindermann B, Althammer M, Klapper M, Vormann J, Littarru GP, Döring F (2005) Coenzyme Q_{10} affects expression of genes involved in cell signaling, metabolism and transport in human CaCo-2 cells. Int J Biochem Cell Biol, 37**:**1208–1218

Kaikkonen J, Nyyssonen K, Porkkala-Saratho E, Poulsen HE, Metsa-Ketela T, Hayn M, Salonen J T (1997) Effect of oral coenzyme Q_{10} supplementation on the oxidation resistance of human VLDL+LDL fraction : absorption and antioxidative properties of oil an d granule-based preparations. Free Rad. Biol. Med. 22: 1195-1202

220 Kikkawa K, Takchara I, Miyakoshi T, Miyawaki H (2007) Safety of high dose
221 supplementation of coenzyme Q10 in healthy human adults. Jpn. J. Food Chem. 14: 76-
222 81
223 Lawrie RA, Ledward DA (2006) Lawrie´s meat science. 7th edition, pp. 75-127,
224 Cambridge, UK: Woodhead Pub. Ltd
225 Lin YQ, Wang GS, Feng J, Huang JQ, Xu YO, Jin SY, Li YP, Jiang ZR, Zheng YC
226 (2011) Comparison of enzyme activities and gene expression profiling between yak and
227 bovine skeletal muscles. Livestock Sci. 135: 93-97
228 Litarru GP, Tiano L (2010) Clinical aspects of coenzyme Q_{10}: An update. Nutrition 26:
229 250-254
230 Marusic, N., Aristoy, M.C., Toldrá, F. (2013) Nutritional meat compounds as affected
231 by ham dry-curing. Meat Sci. 93: 53-60
232 Mattila P, Kumpulainen J (2001). Coenzyme Q_9 and Q_{10}: contents in foods and dietary
233 intake. J Food Comp Anal 14:409-417
234 Mattila P, Lehtonen M, Kumpulainen J (2000) Comparison of in-line connected diode
235 array and electrochemical detectors in the high-performance liquid chromatographic
236 analysis of coenzymes Q_9 and Q_{10} in food materials. J. Agric. Food Chem. 48: 1229–
237 1233
238 Overvad K, Diamant B, Holm L, Holmer G, Mortensen SA, Stender S (1999)
239 Coenzyme Q_{10} in health and disease. Eur. J. Clin. Nutr. 53: 764-770
240 Paliakov EM, Crow BS, Bishop MJ, Norton D, George J, Bralley J (2009) A. Rapid
241 quantitative determination of fat-soluble vitamins and coenzyme Q-10 in human serum
242 by reversed phase ultra-high pressure liquid chromatography with UV detection. J.
243 Chromatogr. B 877: 89-94
244 Purchas RW, Busboom JR, Wilkinson BH (2006) Changes in the forms of iron and in
245 concentrations of taurine, carnosine, coenzyme Q10, and creatine in beef longissimus
246 muscle with cooking and simulated stomach and duodenal digestion. Meat Sci. 74: 443-
247 449
248 Purchas RW, Rutherfurd SM, Pearce PD, Vather R, Wilkinson BHP (2004)
249 Concentrations in beef and lamb of taurine, carnosine, coenzyme Q_{10}, and creatine.
250 Meat Sci. 66: 629-637

Purchas RW, Busboom JR (2005) The effect of production system and age on levels of iron, taurine, carnosine, coenzyme Q_{10} and creatine in beef muscles and liver. Meat Sci. 70: 589-596

Reig, M., Aristoy, M.C., Toldrá, F. (2013) Variability in the contents of pork meat nutrients and how it may affect food composition databases. Food Chem. 140: 478-482.

Reynafarge B (1963) Simplified method for the determination of myoglobin. J. Lab. Clin. Med. 61: 138-145

Roseiro, L.C., Santos, C., Gonçalves, H., Moniz, C., Afonso, I., Tavares, M., da Ponte, D.J.B. (2014) Concentration of antioxidants in two muscles of mature dairy cows from Azores. Meat Sci 96: 870-875.

Ruusunen M, Puolanne E (1997) Comparison of histochemical properties of different pig breeds. Meat Sci. 45: 119-125

Singh U, Devaraj S, Jialal I (2007) Coenzyme Q_{10} supplementation and heart failure. Nutr. Rev. 65: 286-293

Souchet N, Laplante S (2007) Seasonal variation of Co-enzyme Q_{10} content in pelagic fish tissues from Eastern Quebec. J. Food Comp. Anal. 20: 403-410

Toldrá, F. & Reig, M. (2011) Innovations for healthier processed meats. Trends Food Sci. Technol., 22, 517-522.

Turunen M, Olsson J, Dallner G (2004) Metabolism and function of coenzyme Q. Biochim Biophys Acta 1660: 171–199

Vestergaard M, Oksbjerg N, Henckel P (2000) Influence of feeding intensity, grazing and finishing feeding on muscle fibre characteristics and meat colour of semitendinosus, longissimus dorsi and supraspinatus muscles of young bulls. Meat Sci. 54: 177-185

Weber C, Bysted A, Holmer G. (1997) Coenzyme Q_{10} in the diet – daily intake and relative bioavailability. Mol. Aspects Med. 18 (Suppl.) S251–S254

Table

Table 1.- Malate (MDH) and lactate (LDH) dehydrogenase activity mean values (expressed as U/g) and MDH/LDH ratios in oxidative muscle *Masseter*, intermediate muscle *Trapezius* and glycolytic muscles *Biceps femoris and Longissiumus dorsi* of the assayed animal species.

Animal species	Muscle type	LDH (U/g)	MDH (U/g)	MDH/LDH ratio
Pork	*Masseter*	150[a] ±20	390[a] ±50	2.6
	Trapezius	590[b] ±75	240[b] ±20	0.4
	L. dorsi	970[c] ±85	100[c] ±10	0.1
	B. femoris	720[b] ±90	120[c] ±15	0.2
Lamb	*Masseter*	130[a] ±10	380[a] ±45	2.9
	L. dorsi	630[b] ±75	290[b] ±30	0.5
	B. femoris	300[c] ±25	220[b] ±35	0.7
Rabbit	*Masseter*	140[a] ±15	510[a] ±60	3.6
	L. dorsi	1010[b] ±90	150[b] ±20	0.1
	B. femoris	760[c] ±95	80[c] ±9	0.1
Beef	*Masseter*	70[a] ±5	310[a] ±35	4.4
	L. dorsi	890[b] ±95	230[b] ±25	0.3
	B. femoris	840[b] ±85	200[b] ±25	0.2

[a]Different letters within a same column for a given animal species indicate statistical significant difference ($p<0.05$).

Table 2.- Content of Coenzyme Q10 expressed as µg/g muscle (mean values ± SD) in oxidative muscle *Masseter (M)* and glycolytic muscles *Biceps femoris (B) and Longissiumus dorsi (L)* of the assayed animal species.

Animal species	*Masseter* X± SD	*Longissimus dorsi* X± SD	*Biceps femoris* X± SD
Pork	16.8^{a} ±2.5	5.3^{b} ±0.4	6.3^{b} ±0.5
Lamb	17.4^{a} ±1.6	7.2^{b} ±0.5	7.3^{b} ±0.5
Rabbit	30.9^{a} ±1.6	6.8^{b} ±0.8	4.3^{c} ±0.3
Beef	28.8^{a} ±1.0	9.9^{b} ±0.4	12.2^{c} ±0.7

[a]Different letters within a same row indicate statistical significant difference ($p<0.05$).

Table 3.- Content of myoglobin expressed as nmol/g muscle (mean values ± SD) in oxidative muscle *Masseter (M)* and glycolytic muscles *Biceps femoris (B) and Longissiumus dorsi (L)* of the assayed animal species.

Animal species	*Masseter* X± SD	*Longissimus dorsi* X± SD	*Biceps femoris* X± SD
Pork	180.5^{a} ±13.5	44.2^{b} ±6.8	27.5^{c} ±2.8
Lamb	365.7^{a} ±32.3	154.7^{b} ±12.2	134.3^{b} ±14.3
Rabbit	350.4^{a} ±34.6	66.5^{b} ±9.1	55.0^{b} ±4.4
Beef	336.5^{a} ±29.9	198.1^{b} ±14.5	153.1^{c} ±13.3

[a]Different letters within a same row indicate statistical significant difference ($p<0.05$).

Highlights

High variability in the composition of meat in certain specific nutrients

Meat composition in databases may not reflect the real situation for specific nutrients

Adequate labeling for meat including type/proportion of muscles in cuts is necessary