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Additional Information

1        **SOURCES OF VARIABILITY IN THE ANALYSIS OF MEAT NUTRIENT**  
2        **COENZYME Q<sub>10</sub> FOR FOOD COMPOSITION DATABASES**

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17 **ABSTRACT**

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

28

29

30 **Keywords:** coenzyme Q<sub>10</sub>, meat nutrients, meat composition, food composition, food  
31 databases, muscle metabolism

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## 35 INTRODUCTION

36 Meat constitutes a food with relevant nutritional properties. Its content in nutrients can  
37 be found in many food composition databases even though the large natural variability  
38 in meat nutrients is not well reflected in such databases. In fact, the identification of the  
39 meat source is usually incomplete because only the animal species and type of cut, that  
40 may include several different muscles, are given. However, there are relevant nutrient  
41 substances in meat that are affected by intrinsic factors of the animal like its genetics,  
42 age and type of muscle (Reig, Aristoy & Toldrá, 2013). For instance, the analysis of  
43 specific nutritional substances like carnosine, anserine, taurine, glutamine, carnitine,  
44 myoglobin, creatine and creatinine show a large dependence on the type of muscle.  
45 Meat cuts are usually composed of various skeletal muscles which contain various types  
46 of fibres of different metabolic type. The feed also exerts a relevant effect, not only in  
47 the amount of fat but also on its composition in fatty acids. This is important when  
48 considering the amount of nutrients in meat for healthier purposes (Toldrá and Reig,  
49 2011).

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

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

67 cholesterol oxidation after oral supplementation with CoQ<sub>10</sub> (Kaikkonen, Nyysönen,  
68 Porkkala-Saratho, Poulsen, Metsa-Ketela, Hayn, Salonen, 1997) while others reported  
69 an improvement the cardiac function in those patients suffering cardiac muscle  
70 weakness (Turunen, Olsson & Dallner, 2004) or heart failure (Singh, Devaraj & Jialal,  
71 2007).

72 CoQ<sub>10</sub> was named as ubiquinone because it is ubiquitous (present everywhere). The  
73 highest content is found in meat and fish tissues and viscera due to their high levels of  
74 mitochondria (Mattila & Kumpulainen, 2001). The effect of cooking on the content of  
75 CoQ<sub>10</sub> in meat has resulted in some losses. So, there are reported losses of about 15 to  
76 32 % after frying pork cutlets (Weber, Bysted & Holmer, 1997) and about 15% after  
77 grilling of beef (Purchas, Busboom & Wilkinson, 2006) while, on the contrary, some  
78 increase was reported after slow cooking (90 min at 70°C) of lamb (Purchas,  
79 Rutherford, Pearce, Vather & Wilkinson, 2004). The reported losses were higher than  
80 50% after 10 months of dry-curing (Marusic, Aristoy & Toldrá, 2013).

81 The purpose of this work was to analyse CoQ<sub>10</sub> in different animal muscles, evaluate the  
82 methodology and determine the influence of the type of muscle metabolism in its  
83 content in different animal species (pork, beef, lamb and rabbit). The final goal is to  
84 evaluate the information that must be given for meat when reporting its CoQ<sub>10</sub> content  
85 in food composition databases.

86

## 87 **MATERIALS AND METHODS**

### 88 *Samples*

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

### 93 *Chemicals and Solvents*

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

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

#### 100 *Muscles characterisation*

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

112 Total myoglobin was analysed as described by Lin et al. (2011). Thus, sample (1 g) was  
113 extracted with 75 mM Tris-HCl, pH 7.2, containing 3 mM magnesium chloride and 5  
114 mM EDTA, (5 mL) using a Polytron homogenizer. After centrifugation (10.000 rpm, 4°  
115 C for 30 min), the supernatant was filtered through glass wool and then followed by a  
116 0.2 µm nylon membrane filter. The optical density of the filtrate was measured at 576  
117 nm (Bentinger, Brismar & Dallner, 2007).

#### 118 *Determination of CoQ<sub>10</sub>*

119 CoQ<sub>10</sub> was analysed as described by Mattila et al. (2000) with some modifications.  
120 Thus, 1 g of fresh meat sample was mixed thoroughly with a mixture of 5 ml of 0.5 M  
121 sodium chloride and 5 mL of 0.1M SDS. 2 mL of ethanol and 5 mL of *n*-hexane were  
122 added to 1 mL of sample aliquote for CoQ<sub>10</sub> liquid-liquid extraction (by shaking for 2  
123 min). After centrifugation (5.000 rpm, 4° C for 3 min.), the upper (hexane) layer was  
124 removed and the extraction was repeated twice with 3 mL of hexane, respectively. The  
125 hexane extracts were pooled and afterwards evaporated under N<sub>2</sub> stream. Dry extracts  
126 were dissolved in 500 µL of isopropyl alcohol and centrifuged (10.000 rpm, 4° C for 3  
127 min) before HPLC analysis.

128 The chromatographic analysis was accomplished in an Agilent 1100 series (Agilent  
129 Technologies, Palo Alto, CA, USA), with diode array detection (fixed at 275 nm).  
130 Sample (20 µL) was injected into an Ultrabase C18 reversed-phase column (2.5 µm  
131 particle size and 100 x 4 mm) (Análisis Vínicos, Tomelloso, Spain) maintained at 40 °C  
132 and isocratically eluted at 1.0 mL/min using methanol:ethanol:isopropyl alcohol  
133 (70:15:15) as mobile phase.

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

137

#### 138 *Statistical analysis*

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

143

## 144 **RESULTS AND DISCUSSION**

145

146 Muscle fibers are generally categorised as types I (slow twitch, predominantly  
147 oxidative), IIA (fast-twitch, oxido-glycolytic) or IIB (fast-twitch, glycolytic) and each  
148 muscle contains different proportions of these types of fibers (Lawrie & Ledward,  
149 2006). Tissue lactate dehydrogenase (LDH) activity represents the glycolytic potential  
150 while malate dehydrogenase (MDH) activity represents the oxidative potential (Lin et  
151 al., 2011). The muscle LDH and MDH activities cannot be directly compared among  
152 animal species because they are also affected by the type of breed, sex, age and also  
153 type of feeding (Turunen, Olsson & Dallner, 2004; Lin et al., 2011; Singh, Devaraj, &  
154 Jialal, 2007). This is why the ratio MDH/LDH is usually reported as a better indication  
155 of the type of muscle metabolism.

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

159 representative of glycolytic metabolism, from pork, rabbit, lamb and beef. *Masseter*  
160 muscle, which is considered as a model representative of oxidative muscle due to its  
161 rich content in fibers type I, exhibited the lowest LDH activity and the highest MDH  
162 activity, with a MDH/LDH ratio much higher than 1 for all the assayed species (see  
163 table 1). On the other hand, *Biceps femoris* and *Longissimus dorsi*, which are  
164 predominantly glycolytic due to their high content in fibers type IIB, showed a reverse  
165 trend with higher LDH and lower MDH, having a MDH/LDH ratio below 1 also for all  
166 the species (see table 1). The content of CoQ<sub>10</sub> has been directly related with the  
167 mitochondria content which is more abundant in the oxidative red-type fibres I (Purchas  
168 & Busboom, 2005). Souchet and Laplante (2007) observed 5 times higher concentration  
169 of CoQ<sub>10</sub> in mackerel red flesh as compared with white flesh which was explained  
170 mainly by the higher abundance of mitochondria in red flesh. Other factors like the  
171 production system were also reported to affect the CoQ<sub>10</sub> content (Purchas & Busboom,  
172 2005). The muscle type was also reported to have the greatest effect in dairy cow meat,  
173 with two time smore CoQ<sub>10</sub> in *Gluteus medius* muscle than in *Longissimus dorsi*, while  
174 the age of cows and the carcass weight did not show any significant influence (Roseiro,  
175 Santos, Gonçalves et al, 2014). In our work, there was a significantly ( $p<0.05$ ) higher  
176 content of coenzyme Q10 in oxidative muscle *Masseter* as compared to the other  
177 assayed muscles. This effect is noticeable not only in pork but also in other animal  
178 species like rabbit, lamb and beef (see Table 2). Similar effect is observed on the  
179 content of myoglobin, the heme iron meat protein which is the protein responsible of  
180 meat colour and excellent contributor of heme iron (Table 3). The content of myoglobin  
181 was found more abundant in the oxidative muscles and lowest in the glycolytic ones as  
182 already reported for pork (Aristoy & Toldrá, 1998).

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



191 well as CoQ<sub>10</sub> and myoglobin content was observed in the *Trapezius* muscle of pork and  
192 lamb that has an intermediate oxidative pattern.

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

199

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203

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278

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 *Longissimus dorsi* of the assayed animal species.

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

<sup>a</sup>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  $\mu\text{g/g}$  muscle (mean values  $\pm$  SD) in oxidative muscle *Masseter* (*M*) and glycolytic muscles *Biceps femoris* (*B*) and *Longissimus dorsi* (*L*) of the assayed animal species.

| Animal species | <i>Masseter</i><br>X $\pm$ SD | <i>Longissimus dorsi</i><br>X $\pm$ SD | <i>Biceps femoris</i><br>X $\pm$ SD |
|----------------|-------------------------------|--|-------------------------------------|
| Pork           | 16.8 <sup>a</sup> $\pm$ 2.5   | 5.3 <sup>b</sup> $\pm$ 0.4             | 6.3 <sup>b</sup> $\pm$ 0.5          |
| Lamb           | 17.4 <sup>a</sup> $\pm$ 1.6   | 7.2 <sup>b</sup> $\pm$ 0.5             | 7.3 <sup>b</sup> $\pm$ 0.5          |
| Rabbit         | 30.9 <sup>a</sup> $\pm$ 1.6   | 6.8 <sup>b</sup> $\pm$ 0.8             | 4.3 <sup>c</sup> $\pm$ 0.3          |
| Beef           | 28.8 <sup>a</sup> $\pm$ 1.0   | 9.9 <sup>b</sup> $\pm$ 0.4             | 12.2 <sup>c</sup> $\pm$ 0.7         |

<sup>a</sup>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  $\pm$  SD) in oxidative muscle *Masseter* (*M*) and glycolytic muscles *Biceps femoris* (*B*) and *Longissimus dorsi* (*L*) of the assayed animal species.

| Animal species | <i>Masseter</i><br>X $\pm$ SD | <i>Longissimus dorsi</i><br>X $\pm$ SD | <i>Biceps femoris</i><br>X $\pm$ SD |
|----------------|-------------------------------|--|-------------------------------------|
| Pork           | 180.5 <sup>a</sup> $\pm$ 13.5 | 44.2 <sup>b</sup> $\pm$ 6.8            | 27.5 <sup>c</sup> $\pm$ 2.8         |
| Lamb           | 365.7 <sup>a</sup> $\pm$ 32.3 | 154.7 <sup>b</sup> $\pm$ 12.2          | 134.3 <sup>b</sup> $\pm$ 14.3       |
| Rabbit         | 350.4 <sup>a</sup> $\pm$ 34.6 | 66.5 <sup>b</sup> $\pm$ 9.1            | 55.0 <sup>b</sup> $\pm$ 4.4         |
| Beef           | 336.5 <sup>a</sup> $\pm$ 29.9 | 198.1 <sup>b</sup> $\pm$ 14.5          | 153.1 <sup>c</sup> $\pm$ 13.3       |

<sup>a</sup>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