Influence of household cooking methods on amino acids and minerals of Barrosã-PDO veal

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A B S T R A C T
The effect of commonly household cooking methods (boiling, microwaving and grilling) on amino acid and mineral (Fe, Mg, K and Zn) contents was investigated in the longissimus lumborum muscle of Barrosã-PDO veal. Fifteen Barrosã purebred calves at 7–8 months of age and an average weight of 177 ± 37 kg were slaughtered. Cooking had a strong effect (P < 0.05) on yield, being higher (67.5%) in boiling compared to microwave and grilling (64.0% and 64.5%, respectively). Grilling increased most of the percentage retention of individual amino acids (>100%), in particular for leucine. No significant differences (P > 0.05) were observed for iron and zinc retentions among the cooking methods, while the retention of magnesium and potassium was strongly affected, mainly after boiling. Our findings indicate that the different cooking methods clearly affect the chemical composition and nutritional value of meat, which may have a strong impact on the intake of essential nutrients.

1. Introduction

Red meat is generally recognized as valuable food with relevant nutritional properties due to its content of high-quality proteins, with a balanced content in amino acids, particularly in essential amino acids. In fact, from the twenty amino acids constituting proteins, eight have to be supplied by the diet (isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine) in order to ensure an adequate physical development and well-being (Aristoy & Toldrá, 2009; Wu, 2009). Amino acid content and composition play an important role in meat quality by providing nutritive value and amino acids. In fact, from the twenty amino acids constituting proteins, eight have to be supplied by the diet (isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine) in order to ensure an adequate physical development and well-being (Aristoy & Toldrá, 2009; Wu, 2009). Amino acid content and composition play an important role in meat quality by providing nutritive value and flavor (Cai et al., 2010). Meats is also a major dietary source of complex B vitamins, especially vitamin B12, zinc, selenium, phosphorus and iron, thus contributing significantly to the daily intake of these essential micronutrients (Cabrera, Ramos, Saadoun, & Brito, 2010). The nutritional importance of meat also lies in its essential trace element content, specifically in iron, which is present in the form of heme (hemoglobin and muscle myoglobin), accounting for its high bioavailability in red meats (Alegria, Barberà, Lagarda, & Farré, 2009). Moreover, iron has a crucial role in human health. Iron deficiency is one of the biggest nutrient deficiency concerns in Europe, which can lead to anemia as well as to disturbances in child growth and development (Lozoff & Georgieff, 2006).

Meat becomes more edible and digestible when subjected to cooking (Alfaia, Lopes, & Prates, 2013). During cooking, meat undergoes both physical and chemical changes, such as a decrease in the nutritional value, which are strongly dependent on protein denaturation and water loss (Mora, Curti, Vittadini, & Barbanti, 2011; Tornberg, 2005). It is well known that an increase in core temperature in meat will promote collagen shrinkage, reduce water holding capacity and increase cooking loss that influences its final quality and acceptability (Chiavaro, Rinaldi, Vittadini, & Barbanti, 2009). Cooking loss is a combination of liquid and soluble matter and with increasing temperature, water content decreases while fat and protein contents increase, thus indicating that the main part of cooking loss is water (Heymann, Hedrick, Karrasch, Eggeman, & Ellersieck, 1990). Heating time, temperature, cooking method and muscle composition are all important variables, which may influence the final desirable characteristics of the meat (Alfaia et al., 2010; Christensen, Purslow, & Larsen, 2000). Although meat changes induced by cooking have been studied for many years and extensively discussed (Offer, 1984; Tornberg, 2005), only few reports have specifically dealt with the influence of different cooking conditions on the amino acid and mineral contents (Wilkinson, Lee, Purchas, & Morel, 2014). Moreover, the nutrient composition of cooked meats available in Food Composition databases is quiet limited.

Consumers are increasingly aware of the relationships between diet, health and well-being resulting in choices of foods which are healthier and more nutritious (Banovic, Grunert, Barreira, & Fontes, 2010). Beyond the choice of healthy foods, there is also a concern for healthy cooking. In fact, a better understanding of the complexity of heat treatments used to cook meat and its influence on their nutrients may increase consumer’s expectations and acceptance of more convenient and healthy cooking choices (Font-i-Furnols, & Guerrero, 2014). Meats
with Protected Designation of Origin (PDO) are recognized and valued by the consumers due to their distinctiveness and quality. The PDO policy intends to guarantee to consumers a trustful supply that respects both sanitary rules and the features perceived by consumers as signs of quality (Monteiro et al., 2013). In line with this, the maintenance of meat quality throughout cooking is particularly important for PDO meats.

Therefore, the present study assessed the effect of three cooking methods (microwave oven, boiling and grilling) on amino acid and mineral (iron, magnesium, potassium and zinc) contents of Barrosã-PDO veal. The influence of cooking methods, widely used in household processing, on amino acids and minerals of meat is compared on the basis of nutrient retention. Finally, the contribution of meat cooked under different methods for the recommended daily intake of amino acids and minerals was also evaluated.

2. Materials and methods

2.1. Animals and meat sampling

Fifteen Barrosã purebred calves produced in the Northwest of Portugal were kept according to the traditional production pasture-based system following the rules established in the Barrosã-PDO product specifications (Commission Regulation no. 1263/96 of 01/07, EEC). After weaning, the animals were slaughtered between January and February 2008, at 7–8 months of age and an average weight of 177 ± 37 kg. Meat samples were taken from the longissimus lumborum (LL) muscle, between the L1 and L4 ribs, two to three days after slaughter (+1 °C), and vacuum packed and frozen at −80 °C until application of heat treatments.

2.2. Cooking methods and preparation of samples

Frozen muscle samples were thawed overnight at 4 ± 2 °C. Once the meat was thawed, each sample was sliced into cuts with 1.5 cm thickness (5 × 5 × 1.5 cm), weighed (around 38 g) and subjected to each of the following cooking methods: microwaving, boiling and grilling, while the raw cuts were sampled directly as the uncooked control.

Microwave oven (Mod. AVM 559, Whirlpool, USA) samples were placed in a Pyrex container. Cooking was performed in a 2450 MHz at 750 W using two heating cycles of 4.5 s and turned over between cycles. Boiling was performed in a water bath with samples completely submerged for 40 min at 81 °C. In electric grill (Mod. ES/FOFTES), the samples were placed on the center of the thermal resistance and approximately 4 cm from heat source and cooked for 30 min at 225 °C. The samples were turned every 5 min. The cooking temperature and time used for each method resulted from a series of tests, which showed the best value for temperature/time cooking samples without a trace of blood. The final cooking temperature was determined beforehand by inserting thermocouples (Type K – Lufft C100 Series Digital Instruments, USA) into the approximate geometric center of each steak.

2.3. Determination of yield

After cooking and cooling (30 min at 20–22 °C), the samples were wiped with a paper towel to remove visible exudates and the cooking yield was calculated by the weight difference before and after cooking.

2.4. Determination of amino acids

2.4.1. Hydrolysis and derivatization of samples

Meat cooked samples were lyophilized (−60 °C and 2.0 hPa) until constant weight using a lyophilisator Edwards Moduloy (Edwards Hight Vacuum International, UK). Dry samples (0.2 g) were hydrolysed with 5 mL of HCl 6 N solution (containing 1% phenol, w/v) for 24 h at 110 °C according to the procedure described by Pellet and Young (1980). After hydrolysis, 25 mL of HCl 0.01 M was added and the extract was filtered and evaporated in a rotary evaporator (R110 BUCHI) at 60 °C to a final volume of 5 mL. Then, 500 μL was withdrawn and the pH was adjusted by the addition of approximately 1 mL of NaOH 1 N. Then, 100 μL of internal standard (norleucine, 1 mg/mL) and 800 μL of ethanol were added to 100 μL of the extract. The extracts were centrifuged at 14,500 rpm for 2 min. The supernatant was led to dryness at 70 °C under a nitrogen stream. The derivatization was carried out using 250 μL of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) plus 1% trimethylchlorosilane (TMCS), during 2.5 h at 130 °C (Multi-Blok Heater Lab-line). After derivatization, 300 μL of acetonitrile was added (Quaresma et al., 2003).

2.4.2. Gas chromatography analysis

The separation of amino acids was achieved by gas chromatography (Hewlett 5890 Packard Series II) with flame ionization detection. A DB-17 fused-silica capillary column (Agilent Technologies, Palo Alto, CA, USA) with 30 m × 0.25 mm was used. The analysis conditions were as follows: column temperature programmed from 80 °C (3 min), up to 210 °C with a rate of 5 °C/min; injector and detector temperatures, 275 °C and 300 °C, respectively. Helium was the carrier gas (70 kPa). The amino acids were identified by their retention times and by comparison with a standard amino acid solution (Sigma-Aldrich AA-S-18). Quantification was based on the internal standard method.

2.5. Determination of minerals

The determination of ash content was carried out according to the procedures described in AOAC (2000). The mineral elements (iron, magnesium, potassium and zinc) were determined by atomic absorption spectrophotometry (PU 9100X Philips) based on the method proposed by Hermida, Gonzalez, Miranda, and Rodríguez-Otero (2006).

2.6. Determination of retention

The retention of amino acids and minerals was calculated according to Murphy, Criner, and Gray (1975), using the following equation: TR (%) = (A × B / C × D) × 100, where A – nutrient content (g) of cooked meat; B – meat weight (g) after cooking; C – nutrient content (g) of raw meat and D – weight (g) of raw meat.

2.7. Statistical analysis

As variance heterogeneity was detected for most parameters, the data were analyzed using the PROC MIXED with variance heterogeneity analysis of SAS (2009) software package (version 9.2; Statistical Analysis Systems Institute, Cary, NC, USA). The statistical model evaluated included the treatment effect as repeated measure. Data were reported as mean ± standard error (SE). Least squares means (LSMEANS), with the option PDIFF adjusted with Tukey–Kramer, were determined to compare groups. Differences were considered significant at a P-value below 0.05.

3. Results and discussion

3.1. Final cooking temperature and cooking yield

Meat is considered cooked when the temperature reached inside the sample is kept at 65–70 °C for 10 min (Bender, 1992). Furthermore, the end of the cooking process is generally indicated by change of the meat color and by the development of flavors. In current study, the internal endpoint temperature of meat was significantly different (P < 0.001) among the three cooking processes (Table 1). The highest value was observed for microwaving (93.9 ± 0.32 °C), followed by boiling (74.1 ± 0.34 °C) and grilling (70.0 ± 1.21 °C). This internal endpoint temperature for each heating treatment enabled the meat to attain a medium
Moisture content results (Table 2) were in agreement with the cooking yield values of each treatment. Regarding amino acid composition in normalized terms (each amino acid as a percentage of total amino acids) and in decreasing order, the foremost amino acids in raw and cooked meat were glutamic acid (17–19%), leucine (12%) and aspartic acid (10–11%), except for grilling. In grilled veal-PDO, the predominant amino acids were leucine (23%), followed by glutamic and aspartic acids (16% and 9.2%, respectively). In contrast, methionine, glycine, proline and tyrosine had the lowest values (<2.8%). Cystine, asparagine, glutamine and tryptophan were not detected, which is in agreement with the findings of Moughan (2003), who stated that these amino acids are destroyed by thermal hydrolysis. A similar trend was observed in pork, where glutamic and aspartic acid were the amino acids present at a higher level, whereas proline, serine, glycine and alanine showed the lowest values (e.g. Okrouhlá et al., 2006; Purchas, Morel, Janz, & Wilkinson, 2009). From the 15 amino acids analyzed, only 6 changed significantly as a result of cooking, namely glutamic acid (P < 0.05), leucine (P < 0.001), methionine (P < 0.001), serine (P < 0.01), threonine (P < 0.05) and valine (P < 0.01). Recently, Wilkinson et al. (2014) showed that 12 of the 17 amino acids measured in samples of pork longissimus muscle changed significantly with cooking, with all amino acids increasing, except for histidine and taurine. Throughout cooking, meat proteins are denatured by the heat with subsequent loss of water-holding capacity of the proteins (Tornberg, 2005), but most of the loss appears to be water enhanced higher contents of other components in cooked meat.

### 3.3. Effect of cooking methods on ash and minerals

The values of ash for raw and cooked LL muscle from Barrosã-PDO veal are shown in Table 3. The ash value, an indicator of total mineral content, obtained for raw meat (0.98 g/100 g) is in agreement with the results (0.6–1.1 g/100 g) of Farfán and Sammán (2003). However, Hoffman, Kroucamp, and Manley (2007) found higher levels in longissimus dorsi muscle of springbok meat (1.1 to 1.4 g/100 g). Cooking led to significant differences in ash content among treatments (P < 0.001). Microwaving (1.4 g/100 g) and grilling (1.8 g/100 g) increased the ash content of meat, while boiling decreased its content compared with the raw meat. Since microwave cooking and grilling proceed in the absence of water (dry cooking methods), these cooking methods allowed for a high retention of ash than boiling (wet cooking method). Dal Bosco, Castellini, and Bernardini (2001) also found a

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Microwaving</th>
<th>Boiling</th>
<th>Grilling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>61.8 ± 0.736</td>
<td>62.9 ± 0.799</td>
<td>59.4 ± 0.727</td>
</tr>
<tr>
<td>Total amino acids</td>
<td>31.3 ± 2.27</td>
<td>31.3 ± 1.82</td>
<td>37.2 ± 2.19</td>
</tr>
</tbody>
</table>

### Table 1

Final internal temperature and yield of longissimus lumborum muscle from Barrosã-PDO veal after three different cooking methods (n = 15).

The cooking yields of LL muscle of Barrosã-PDO veal are also shown in Table 1. Cooking yields were also affected by the cooking process used (P < 0.05). Boiling had a higher cooking yield (67.5%) compared to grilling and microwave oven (64.5% and 64.0%, respectively). Temperature and sample size are generally accepted to be key factors connected with weight loss. Bengtsson et al. (1976) observed that as internal temperature increased, cooking yields decreased. Our results also showed that cooking yields decreased with increasing endpoint temperatures due to moisture loss (e.g., evaporation, moisture drip) or fat gains/losses during cooking (Roseland et al., 2012). Offer and Knight (1988) reported that cooking involves heat and mass transfer by diffusion of water through the meat and evaporation from the meat surface, and through physical expulsion caused by contriction of muscle bundles. The authors hypothesized that meat surface dehydration led to the development of crust formation in grilling and microwave heating with less pronounced loss of liquids, as was previously suggested by other authors (Vittadini, Rinaldi, Chiavaro, Barbanti, & Massini, 2005).

### 3.2. Effect of cooking methods on amino acid content and composition

The total content (g/100 g meat) and composition (% of total amino acids) of fifteen amino acids in raw and cooked LL muscle from Barrosã-PDO veal are presented in Table 2. The data showed that amino acid content in Barrosã-PDO veal increased significantly (P < 0.001) after cooking, which was the result of water loss. Cooking significantly decreased moisture content (P < 0.001), as expected, from 75% in raw state up to 59% and, consequently, to a significantly higher amino acid contents, with no significant differences (P > 0.05) among treatments.

Regarding amino acid composition in normalized terms (each amino acid as a percentage of total amino acids) and in decreasing order, the foremost amino acids in raw and cooked meat were glutamic acid (17–19%), leucine (12%) and aspartic acid (10–11%), except for grilling. In grilled veal-PDO, the predominant amino acids were leucine (23%), followed by glutamic and aspartic acids (16% and 9.2%, respectively). In contrast, methionine, glycine, proline and tyrosine had the lowest values (<2.8%). Cystine, asparagine, glutamine and tryptophan were not detected, which is in agreement with the findings of Moughan (2003), who stated that these amino acids are destroyed by thermal hydrolysis. A similar trend was observed in pork, where glutamic and aspartic acid were the amino acids present at a higher level, whereas proline, serine, glycine and alanine showed the lowest values (e.g. Okrouhlá et al., 2006; Purchas, Morel, Janz, & Wilkinson, 2009). From the 15 amino acids analyzed, only 6 changed significantly as a result of cooking, namely glutamic acid (P < 0.05), leucine (P < 0.001), methionine (P < 0.001), serine (P < 0.01), threonine (P < 0.05) and valine (P < 0.01). Recently, Wilkinson et al. (2014) showed that 12 of the 17 amino acids measured in samples of pork longissimus muscle changed significantly with cooking, with all amino acids increasing, except for histidine and taurine. Throughout cooking, meat proteins are denatured by the heat with subsequent loss of water-holding capacity of the proteins (Tornberg, 2005), but most of the loss appears to be water enhanced higher contents of other components in cooked meat.

### Table 2

Effect of cooking methods on moisture content (g/100 g meat), amino acid content (g/100 g meat) and composition (% total amino acids) of longissimus lumborum muscle from Barrosã-PDO veal (n = 15).

The values of ash for raw and cooked LL muscle from Barrosã-PDO veal are shown in Table 3. The ash value, an indicator of total mineral content, obtained for raw meat (0.98 g/100 g) is in agreement with the results (0.6–1.1 g/100 g) of Farfán and Sammán (2003). However, Hoffman, Kroucamp, and Manley (2007) found higher levels in longissimus dorsi muscle of springbok meat (1.1 to 1.4 g/100 g). Cooking led to significant differences in ash content among treatments (P < 0.001). Microwaving (1.4 g/100 g) and grilling (1.8 g/100 g) increased the ash content of meat, while boiling decreased its content compared with the raw meat. Since microwave cooking and grilling proceed in the absence of water (dry cooking methods), these cooking methods allowed for a high retention of ash than boiling (wet cooking method). Dal Bosco, Castellini, and Bernardini (2001) also found a
decrease in ash content of rabbit meat after boiling and roasting. Similar ash values for cooked meat (1.3 to 1.9 g/100 g) were reported by Farfán and Sammán (2003).

The mineral (iron, magnesium, potassium and zinc) contents for raw and cooked LL muscle from Barrosã-PDO veal are also presented in Table 3. The major minerals present in raw and cooked meats were potassium (129–289 mg/100 g), followed by magnesium (13.0–30.1 mg/100 g), zinc (3.44–5.79 mg/100 g) and iron (1.69–3.14 mg/100 g), which altogether represented about 31% of total ash. The mean values for potassium, magnesium, zinc and iron in raw meat varied greatly among the species (Gerber, Scheeder, & Wenk, 2009). The mean potassium content in raw Barrosã-PDO veal (289 mg/100 g) was higher than that found in pork (172–175 mg/100 g), poultry (248–259 mg/100 g) and mutton (275 mg/100 g) (Sainsbury, Schonfeldt, & Van Heerden, 2011), but inside of the range (299 to 350 mg/100 g) described for beef (275 mg/100 g) (Sainsbury, Schonfeldt, & Van Heerden, 2011). The mean values of zinc and magnesium in Barrosã-PDO veal increased significantly after boiling, probably due to the leaching of minerals into cooking water. As reported by Badiani et al. (2002), boiling leads to some leaching of nutrients from meat to the cooking medium, while in dry cooking methods, such as grilling and microwave, the water loss by evaporation induces a concentration of minerals. Gerber et al. (2009) also showed that cooking processes involving water, such as boiling, affected mineral content the most.

3.4. Retention of moisture, amino acids, ash and minerals

In order to evaluate the increase or loss/degradation of meat components during cooking, the retention of nutrients was calculated. The effect of cooking methods on moisture and minerals retention values is shown in Table 4. Water showed the greatest loss (retention of 51–56%) during cooking compared to minerals. The retention of moisture was significantly affected by heating methods (P < 0.01) being significantly lower in grilling (51%) than in boiling (56%). Boiling showed the lowest water loss due to the incorporation of water during the cooking process. As expected, all the amino acids were retained (above 100%) after cooking due to water loss but without any statistically significant differences (P > 0.05) among heat treatments (104–108%, except for leucine (data not shown). Leucine was significantly higher for grilled meat than for both microwave and boiled meat (P < 0.001). Since grilling proceeded at a higher temperature (225 °C during 30 min) and in the absence of water, this cooking method probably allowed for a high retention of amino acids due to a higher water loss. A hundred percent retention is expected, unless amino acids are partially degraded (protein denaturation) or lost in the cooking juice. Recently, Wilkinson, Lee, Purchas & Morel (2014) found that the amino acid retention in samples of pork longissimus muscle was generally less than 90% cooked to either 60 °C or 75 °C in a water bath for 90 min, with slightly higher values at the lower cooking temperature.

Table 4 also shows the influence of cooking on retention of ash and minerals from LL muscle of Barrosã-PDO veal. The heat treatments changed the ash retention from 46.5% to 105%. The ash retention was higher in grilling (105%) than in microwaving (89.9%) and boiling (46.5%), which was expected given the former’s superior cooking yield. Dal Bosco et al. (2001) and Maranési et al. (2005) found similar ash retention values (72–98%) for cooked rabbit and lamb meats, respectively. Serrano, Librelotto, Cofrades, Sáchès-Muniz, and Jiménez-Colmenero (2007) also reported that ash retention in cooked beef steak ranged from 73.0% to 93.4% in microwaving and from 82.3% to 97.7% in electric grill. Minerals were reasonably well retained by cooking, except potassium. The retention values of iron and zinc varied between 78.9–105% and 88.9–97.0%, for microwaving and grilling, respectively. Lombardi-Boccia et al. (2005) also found that retention of iron and zinc varies from 83 to 88% in beef. Although only a few studies have been performed on mineral retention of cooked meats, a broad
agreement exists that zinc, copper and iron largely remain in the meat sample during cooking (Gerber et al., 2009; Lombardi-Boccia et al., 2005). In our study, no significant differences were detected for iron and zinc retentions among the cooking methods (P > 0.05). In contrast, the retention of magnesium and potassium were significantly affected by cooking (P < 0.001) mainly after boiling. Thus, the dry cooking methods (microwave and grilling) allowed for a higher retention of the mineral content in cooked meats, which is in agreement with data described by Lombardi-Boccia et al. (2005) and Gerber et al. (2009). Previously, Lassen and Ovesen (1995) stated that, when properly used, microwave cooking does not affect the mineral contents of food to a larger extent than conventional heating. There is a tendency towards greater retention of many micronutrients with microwaving, which tends to make microwave cooking healthier, probably due to the reduced preparation time.

3.5. Dietary reference intake contribution

Generally, the consumption of 100 g of raw bovine meat would be enough to supply the daily adult’s requirement of amino acids and minerals. However, nutrient requirements vary in specific groups of people, as for example in children and/or in pregnant or lactating women, because they have added needs (WHO/FAO/UNU, 2007). In modern societies, meat is usually cooked prior to consumption, and the effect of cooking on these nutrients is poorly documented (Gatellier et al., 2007). In order to preserve its nutritional quality. This information is of particular relevance for added value meats, such as PDO meats.

4. Conclusions

The data indicate that amino acid contents of meat were more affected by cooking methods than amino acid composition. The increase in amino acid contents in cooked veal is a consequence of water loss during the heating process. In contrast, only amino acid composition was influenced by the different cooking methods. Through cooking, it was demonstrated that modifications were dependent on the heating method and on the time/temperature evolution. The major amino acids found in raw and cooked veal were glutamic acid, leucine and aspartic acid. Moreover, glutamic acid, leucine, methionine, serine, threonine and valine were the amino acids mostly affected by heating. Among the cooking methods, grilling seems to be the treatment that changed the most amino acid composition. The percentage retention of individual amino acids was all above 100%, although with a value particularly high for leucine after grilling.

Furthermore, the results indicate that cooking conditions also induce changes in mineral composition, affecting the bioavailability and content of these micronutrients in meat. Potassium was the mineral most affected by cooking. No significant differences were observed for iron and zinc retentions among the cooking methods, while the retention of magnesium and potassium was deeply affected, mainly after boiling. It is suggested that heating processes involving water allow for a low retention of the mineral content in cooked meats. However, the magnitude of mineral loss is dependent on the cooking medium and on the utilization of drip following cooking.

Overall our data indicate how the different cooking methods affect the chemical composition and nutritional value of meat. This information may help consumers to choose the best procedure to cook meat in order to preserve its nutritional quality. This information is of particular relevance for added value meats, such as PDO meats.

Conflict of interest

There is no conflict of interest.

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