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Comparison of antioxidant compounds in pig meat from Italian autochthonous pig Suino Nero Lucano and a modern crossbred pig before and after cooking

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ABSTRACT

This study aimed to evaluate and compare the antioxidant compounds of raw and cooked *Longissimus lumborum* muscles from Suino Nero Lucano (SNL) and a modern crossbred (CG) pig. Vitamin E, phenols, histidine-containing peptides, and superoxide dismutase (SOD) activity have been detected in the raw and cooked meat of both genetic types. Cooking process decreased the content of all considered antioxidant compounds ($P < 0.05$). The antioxidant compounds of meat were significantly influenced by genetic type ($P < 0.001$). Autochthonous SNL raw and cooked meat showed a higher endogenous antioxidants content ($P < 0.001$) and SOD activity ($P < 0.02$) compared to CG meat. The results of this research highlighted that the pig meat, in particular autochthonous pig meat, showed good concentrations of endogenous antioxidant compounds that could confer functional properties to the product.

1. Introduction

Today, because of its numerous bioactive compounds, including several endogenous enzymatic and nonenzymatic antioxidant components, the meat may be configured as functional food (Descalzo & Sancho, 2008; Simonetti, Gambacorta, & Perna, 2016). The antioxidants are chemical molecules capable of donating hydrogen to the free radicals to delay lipid oxidation, color loss and microbial growth, extending thus the shelf life of meat without any damage to the sensory properties but improving the nutritional and nutraceutical value of meat (Zinoviadou, Koutsoumanis, & Biliaderis, 2009). Among the non-enzymatic antioxidants, vitamin E is the primary lipid soluble free radical scavenger in biological systems (Young & Woodside, 2001). Several studies (Descalzo & Sancho, 2008) showed that animal diet supplementation with vitamin E inhibits lipids oxidation and sensorial deterioration of meat. Polyphenols are secondary metabolites of plants which, such as vitamins, are incorporated in muscle through the diet, where they improve the shelf life of meat vulnerable to oxidative changes (Kennedy, 2014). In particular, the antioxidant activity of phenolic compounds is carried out through three activity: free-radical scavenging, transition-metal-chelating, and singlet-oxygen quenching (Rice-Evans, Miller, & Paganga, 1997). Bioactive peptides are sequences of between 2 and 30 amino acids in length, inactive within the native protein, that may be generated by endogenous enzymes from meat proteins during hydrolysis, cooking or fermentation (Lafarga, Álvarez, & Hayes, 2014). Many functional peptides with antioxidant activity were described in meat (Liu, Xing, Fu, Zhou, & Zhang, 2016), such as carnosine (β-alanyl-L-histidine) and its methylated analogue anserine (1-methyl carnosine) which are naturally occurring in vertebrate animal tissues, especially in skeletal muscle (Mora, Sentandreu, & Toldrá, 2008). Both these histidine-containing dipeptides play beneficial roles in several physiological functions, including antioxidant activity (Chan & Decker, 1994). Superoxide dismutase (SOD) is an important antioxidant enzymatic compound capable to lower the steady state of superoxide anions (Descalzo & Sancho, 2008) and thereby protecting cells from reactive oxygen species (ROS). In particular, SOD converts superoxide radical to hydrogen peroxide and molecular oxygen which can be counteracted by catalase or glutathione peroxidase reaction thus lowering the cellular damage (Fridovich, 1997). Since that after the animal bleeding, all the cells are in anoxia and depleted of nutrients, antioxidant activity of this enzyme depends only on what remains at the onset of cell death. The presence and the content of these natural endogenous compounds, which depend on animal species, muscle type, animal diet, and breeding system type (Decker, Livisay, & Zhou, 2000; Łukasiewicz et al., 2018), influence the nutraceutical value of meat and, at the same time, they improve the shelf life of meat and its products. However, fresh meat is most often eaten cooked, and it has been widely shown that the cooking process by disrupting cell membranes

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accelerate greatly the ROS generation with a consequent consumption of antioxidants (Gatellier, Kondjoyan, Portanguen, & Santé-Lhoutellier, 2010; Soladoye, Juárez, Aalhus, Shand, & Estévez, 2015). Nowadays, due to greater consumer attention to animal welfare, to high quality meat, and to product traceability, the demand of meat obtained from ancient autochthonous genetic types (AAGTs) reared under outdoor system has increased. It is known that autochthonous pigs reared under outdoor system provide a meat rich of natural endogenous antioxidants (Rey, Lopez-Bote, & Sanz Arias, 1997); despite this, to our knowledge, no information is reported in the literature regarding the antioxidant compounds content in autochthonous pig meat before and after cooking process.

Therefore, the present study was undertaken to evaluate and compare antioxidant compounds of *Longissimus lumborum* muscle from Suino Nero Lucano (SNL), Italian autochthonous black pig breeds reared in southern Italy (Basilicata region; http://www.anas.it/html/homew.htm), and a modern crossbred (CG) pig, before and after cooking process.

2. Materials and methods

2.1. Animals and experimental design

Longissimus lumborum (LL) muscles at the level of the 2nd and 5th lumbar vertebra of the right side of the carcasses from 60 castrated male pigs purebred SNL pigs and 60 CG pigs were collected for this study. All pigs were bred in the same farm and fed with the same diet which was composed of pasture (acorns and natural grasses) and concentrate (corn 30%, barley 25%, field beans 25%, bran and residues from processing of cereals 20%). The pigs at their marketed weight which corresponded to the same physiological phase were slaughtered in a commercial abattoir. In particular, SNL pigs were slaughtered at about 140 kg of live weight and 540 days of age, and CG pigs at 160 kg of live weight and 480 days of age. After stored at 4 °C for 24 h, from each carcass LL muscle was removed and cut into two equal pieces of about 200 g, labelled as raw and cooked. Cooked meat samples were placed in a convection-steam oven (Küppersbusch CPE 110, Küppersbusch Grobküchentechnik GmbH, Gelsenkirchen, Germany) until the core temperature reached 75 ± 3 °C. The raw and cooked minced pieces were then vacuum packaged and stored at −20 °C until analyzed.

2.2. Chemical composition

Standard Official Methods of Analysis (AOAC, 1995) methods were used to determine dry matter (DM; method 950.46), protein (method 990.03), intramuscular fat (IMF; method 920.39), and ash (method 920.153) of raw and cooked meat samples. All samples were analyzed in triplicate.

2.3. High-performance liquid chromatography analysis of antioxidant compounds

2.3.1. Vitamin E analysis

In raw and cooked meat samples, the saponification, extraction and analysis of vitamin E was carried out as described by Perna, Simonetti, Intaglietta, and Gambacorta (2015). The vitamin E standard (Sigma-Aldrich, Milan, Italy; 0.20–1.00 mg/mL) was used for identification and quantification of the peaks; the results were expressed as μg/g meat.

2.3.2. Carnosine and anserine analysis

The extraction and analysis of carnosine and anserine in raw and cooked meat samples was carried out as described by Mora, Sentandreu, and Toldrá (2007). The bioactive peptides analysis was performed in liquid chromatography using an Atlantis HILIC silica column (4.6 × 150 mm, 3 μm) connected with a Atlantis HILIC Silica guard column (10 × 4.0 mm, 5 mm) (Thermo Fischer Scientific). The carnosine and anserine standards (Sigma-Aldrich, Milan, Italy; 0.01–0.50 and 0.01–0.30 mg/mL, respectively) were used for identification and quantification of the peaks; the results were expressed as mg/100 g meat.

2.4. Antioxidant assays

The raw and cooked meat extract preparation was carried out as described by Perna, Simonetti, Grassi, and Gambacorta (2018). The extract was kept a −55 °C until analysis.

2.4.1. Determination of total phenol content (TPC)

TPC in raw and cooked meat was determined using Folin–Ciocalteu reagent, as described by Qwele et al. (2013). Gallic acid standard (Sigma-Aldrich, Milan, Italy; 0–200 mg/L) was used to derive the calibration curve; the results were expressed as mg of gallic acid equivalents (GAE)/ 100 g meat.

2.4.2. Superoxide dismutase (SOD) assay

SOD activity was detected by measuring the inhibition of pyrogallol autoxidation as described by Jin, He, Yu, Zhang, and Ma (2013). The absorbance was read at 340 nm as soon as the reaction started, and the increase in the absorbance was recorded after 2 min. The results were expressed as percentage inhibition (I%) and were calculated by following the equation:

$$I(\%) = [(A_b - -As)/A_b] \times 100$$

where A_b = absorbance of blank sample; As = absorbance of sample.

2.5. Statistical analysis

Differences among meat samples were analyzed using a two-way ANOVA (genetic type and meat state; SAS Institute, 1996). Data are expressed as mean ± standard deviation. A Student's test was used and the results were statistically significant for $P < 0.05$.

3. Results and discussion

3.1. Chemical composition

Regarding the chemical composition of SNL and CG raw meat samples (Table 1), significant differences ($P < 0.05$) were found only for the IMF content which was higher in SNL than in CG muscles. This

Table 1
Chemical composition (g/100 g meat) of raw and cooked meat from Suino Nero Lucano (SNL) and a modern crossbred genotype (CG).

		Chemical composition				*P*
		SNL		CG		
		Mean	SD[1]	Mean	SD	
N° of pigs		60	–	60	–	–
Dry Matter	Raw	31.41[a]	1.76	29.91[a]	1.92	0.066
	Cooked	41.86[b]	1.56	40.31[b]	2.28	0.079
IMF	Raw	7.42[a]	0.31	6.74[a]	0.33	0.012
	Cooked	9.89[b]	0.43	9.08[b]	0.41	0.017
Protein	Raw	22.80[a]	1.83	22,08[a]	2.01	0.097
	Cooked	30.68[b]	1.70	29.76[b]	1.52	0.110
Ash	Raw	1.19[a]	0.21	1.09[a]	0.25	0.181
	Cooked	1.59[b]	0.17	1.48[b]	0.14	0.101

[1] Standard deviation.
[a,b] Values in the same column, for each parameter, with different superscripts were significantly different ($P < 0.05$).

Table 2
Antioxidant compounds of raw and cooked meat from Suino Nero Lucano (SNL) and a modern crossbred genotype (CG).

		Antioxidant compounds				*P*
		SNL		CG		
		Mean	SD[1]	Mean	SD	
N° of pigs		60		60		
Vitamin E, ug/g	Raw	2.75[a]	0.08	2.57[a]	0.06	< 0.001
	Cooked	2.17[b]	0.09	2.01[b]	0.13	< 0.001
Total phenolic, mg of gallic acid equivalents/100 g	Raw	133.62[a]	4.66	122.39[a]	4.17	< 0.001
	Cooked	79.51[b]	2.23	71.39[b]	3.58	< 0.001
Carnosine, mg/100 g	Raw	363.24[a]	10.21	307.19[a]	9.16	< 0.001
	Cooked	284.94[b]	7.45	212.86[b]	8.28	< 0.001
Anserine, mg/100 g	Raw	23.59[a]	1.89	18.69[a]	2.11	< 0.001
	Cooked	15.01[b]	2.11	10.83[b]	1.89	< 0.001
SOD[2], % inhibition	Raw	41.32[a]	1.18	38.87[a]	0.97	0.019
	Cooked	40.03[a]	1.57	35.86[b]	1.16	0.015

[1] Standard deviation.
[2] Superoxide dismutase activity.
[a,b] Values in the same column, for each parameter, with different superscripts were significantly different ($P < 0.05$).

could be due to the high adipogenic potential of autochthonous pigs (Pugliese & Sirtori, 2012). Chemical composition of SNL pig meat was in agreement with what we found in previous studies (Simonetti et al., 2016; Simonetti, Perna, Giudice, Cappuccio, & Gambacorta, 2018). The water loss during cooking process of meat has led to increase of all chemical parameters ($P < 0.05$; Table 1), and regarding studied genetic types, the same differences detected in raw meat were found in cooked meat samples, in line with the findings of our previous study (Simonetti et al., 2016).

3.2. Antioxidant compounds

3.2.1. Raw meat

The content of antioxidant compounds in raw meat from SNL and CG pigs is reported in Table 2. Antioxidant compounds enhance the nutraceutical value and the technological quality of meat and, in this study, both SNL and CG raw meat showed antioxidant activity. Overall, the average vitamin E content in raw meat was 2.66 μg/g meat. Our results were well within the range reported in literature on vitamin E content in meat from pigs fed without vitamin E supplementation (0.2–5.6 mg/kg meat; Szterk, Rogalski, Mikiciuk, Pakuła, & Waszkiewicz-Robak, 2016). Cannon et al. (1996) reported that vitamin E maintained the integrity of muscle cell membranes and the membrane fluidity by preventing the oxidation of membrane lipids during storage, thus inhibiting the passage of sarcoplasmic fluid through the plasma membrane. Moreover, Descalzo and Sancho (2008) reported that the antioxidant action of vitamin E on membrane lipids was about 10^4 times faster than the propagation of lipid peroxidation. The vitamin E content detected in SNL meat was in agreement with what found by Tejerina, García-Torres, Cabeza de Vaca, Vázquez, and Cava (2012) in *Longissimus dorsi* muscle of autochthonous Iberian pigs. The higher vitamin E content detected in SNL meat compared to CG meat could be due to the higher IMF content detected in this meat; in fact, the vitamin E, being a lipophilic molecule, is particularly present in cell membranes and fat deposits, where it exerts its antioxidant function (Kasapidou et al., 2009). Also, the presence of vitamin E in meat is mainly due to its content in feed; in this study, all pigs were raised under a semi-wild system, and several authors (Pugliese & Sirtori, 2012) reported the high adaptability to wild (extensive) system of autochthonous pigs. It is known that the phenolic substances are provided through diet, they enter in the circulatory system, and they are distributed and retained in different animal tissues where inhibit oxidative stress increasing both technological and nutritional quality of meat (Luciano et al., 2011). Overall, the average TPC was 128.00 mg GAE/100 g of raw meat. To our knowledge limited information about the phenolic content in pig meat were reported in literature. However, our finding was in line with what found by Qwele et al. (2013) in goat meat (1.3–1.5 mg of GAE/g), and by Jung et al. (2010) in breast meat from the broiler (1.48 mg GAE/g). Genetic type influenced the TPC, SNL raw meat showed a higher content compared to CG raw meat ($P < 0.001$). The differences observed between the two genetic types could be due to higher efficiency of feed utilization, higher capacity of feed intake, and better ability to select feed of autochthonous pigs than commercial ones. Moreover, Bouarab-Chibane et al. (2018) detected a high binding affinity between polyphenols and proteins and/or peptides which leads to the formation of soluble or insoluble complexes. The Folin-Ciocalteau method, used in this study, detect only the fraction of polyphenols that remains free and exerts antioxidant activity in the meat. The interaction between proteins and/or peptides and phenolic compounds is namely due to non-covalent binding mechanisms that depend on two main factors: the amino acid composition of proteins and the phenols type (Czubinski & Dwiecki, 2017). Prodpran, Benjakul, and Phatcharat (2012) showed that polyphenols can readily combine with sulfhydryl (-SH) and amino groups of proteins decreasing the digestibility and bioavailability of both polyphenols and protein-bound lysine and cysteine. In this study we found a higher TPC in SNL meat despite in an our previous work (Simonetti et al., 2016) we detected a higher thiols content in SNL raw meat (83.37 nmol SH-groups/mg protein) compared to CG meat (75.10 nmol SH-groups/mg protein). Among bioactive peptides of meat, carnosine and anserine are considered precious antioxidants able to chelate transition metals such as cobalt, zinc, and copper, thus reducing oxidative rancidity and stabilizing the meat color (Chan & Decker, 1994). Regardless of the genetic type, raw pig meat showed an average carnosine and anserine content of 335.21 and 21.14 mg/100 g meat, respectively. The carnosine content was in line with what found by Mora et al. (2007, 2008) in *Longissimus dorsi* muscle of Iberian pigs, and by Aristoy and Toldrá (1998) in LL muscle of commercial pigs. However, several authors detected lower carnosine (about 2700 mg/kg meat; Purchas, Rutherfurd, Pearce, Vather, & Wilkinson, 2004) and anserine (7–16 mg/100 g meat; Aristoy & Toldrá, 1998) values in pig meat. Genetic type significantly affected the content of these bioactive peptides ($P < 0.001$), in agreement with what detected by Martino et al. (2014) in pig meat, and by Intarapichet and Maikhunthod (2005) in chicken meat. In particular, SNL raw meat showed carnosine and anserine values approximately 1.2-fold higher than CG raw meat (Table 2). This result is surprising considering that the SNL pig, like all pig AAGTs, is characterized by an oxidative muscular metabolism, while carnosine and anserine concentration is closely related to the glycolytic muscular metabolism since the physicochemical buffering capacity of these biopeptides is more required for muscles characterized by a higher anaerobic metabolism in which increases the lactic acid accumulation (Aristoy & Toldrá, 1998; Mora et al., 2008). In fact, also in the literature a higher carnosine and anserine content was found in LL muscle of domesticated pig breeds characterized by a more pronounced glycolitc metabolism compared to LL muscle of wild pigs (Martino et al., 2014). In this study the lower histidine dipeptide content found in CG meat may be due to the fact that all pigs were raised under a semi-wild system. In fact, Petersen, Henckel, Oksbjerg, and Sorensen (1998) reported that the pigs raised under extensive system with access to large areas showed greater physical activity that could lead to an adaptation of muscle metabolism, with consequently higher capacity for aerobic rather than anaerobic ATP generation. Moreover, Łukasiewicz et al. (2018) reported that carnosine content was affected also by animal age. In this study the pigs were slaughtered at their marketed weight that corresponds to two different ages (540 and 480 days of age for SNL and CG pigs, respectively).

SOD is one of the most important antioxidant enzymes whose activity varies not only according to the species or muscle type, but also among animals of the same species (Hernández, Zomeno, Arino, & Blasco, 2004) with differences in meat oxidative stability. In our study, SNL raw meat showed a significantly higher SOD activity compared to CG raw meat, in agreement with what found by other authors (Hernández et al., 2004) who detected higher SOD activity in meat from autochthonous pigs than commercial pigs; suggesting, therefore, a more efficient protective mechanism of antioxidant compounds in autochthonous meat against superoxide anion radical. Moreover, Renerre, Dumont, and Gatellier (1996) found that SOD activity was higher in oxidative and oxidative-glycolitic muscles.

3.2.2. Cooked meat

The cooking process has lead to a loss of all considered antioxidant compounds ($P < 0.05$; Table 2). Mei, Crum, and Decker (1994) reported that heat treatment causes the destruction of muscle cell structure, the inactivation of enzymatic and nonenzymatic antioxidant compounds, and the release of protein-bound iron, resulting in increased lipid and protein oxidation. In this study, the vitamin E content in cooked meat of both genetic types decreased by about 21%. It has been widely shown that during cooking the vitamins are susceptible to loss from heat, oxygen, water, and light. Moreover, since during cooking the water evaporates and dry mass increases, the data on vitamin E content reported on dry weight basis (875 and 518 μg/100 g DM for SNL raw and cooked meat, and 859 and 499 μg/100 g DM for CG raw and cooked meat) highlighted a real decrease of 41% in both genetic types. Bennink and Ono (1982) detected a loss of 33–44% of the original vitamin E in broiled, roasted and braised beef meat. As regard to two studied genetic types, after heat treatment the SNL meat showed a higher vitamin E content compared to CG meat ($P < 0.001$), thus highlighting a predictably greater oxidative stability and a longer shelf life of autochthonous cooked meat. After cooking process, the average phenolic content, on a dry-matter basis, decreased of 55.35 and 56.72% in SNL and CG cooked meat, respectively. The cooking process, in fact, triggers the generation of ROS (Soladoye et al., 2015), and phenolic compounds are particularly prone to react with these free radicals. Moreover, part of these compounds, in view of their strong water solubility was lost in the cooking juice. Genetic type significantly affected the phenolic content, SNL cooked meat showed a higher content compared to CG one ($P < 0.001$). Studies (Prigent et al., 2003) reported that the binding affinity of phenol–protein decreased as the temperature increased; consequently, we can hypothesize that a higher phenolic content in SNL cooked meat could be due to both a greater phenolic content in its raw meat and a higher content of phenols complexed with proteins in raw meat which were released during cooking. To our knowledge, no information was reported in literature on phenolic content in autochthonous and commercial pig meat after cooking process. The cooking process led to significant decline of both analyzed bioactive peptides ($P < 0.05$; Table 2). In particular, on a dry-matter basis, carnosine content decreased by 41.14 and 48.58% in SNL and CG cooked meat, respectively. This could be due to the high water solubility of carnosine (Peiretti, Medana, Visentin, Dal Bello, & Meineri, 2012; Purchas et al., 2004). Dissimilar results was found in literature concerning the effect of cooking on carnosine content. Some authors reported that the carnosine content in meat is unaffected by cooking (Mora et al., 2008), while other authors found significant differences between raw and cooked meat (Jayasena et al., 2013; Purchas et al., 2004). Our results was in line with what reported by Peiretti et al. (2012) who detected a decrease by 50% of carnosine content in beef meat boiled in water at 100 °C × 10 min. Chan, Decker, and Means (1993) suggested that the loss of carnosine in meat could also be due to its destruction or its association with precipitated proteins during cooking. The anserine content decreased by 52.25 and 57.00% (on dry weight basis) for SNL and CG cooked meat, respectively; in agreement with what reported by Peiretti et al. (2012) in beef and turkey meat and by Jayasena et al. (2013) in chicken meat. The above mentioned authors suggested that the high loss of anserine during cooking could be due to its high water solubility. Genetic type significantly affected the carnosine and anserine content in cooked meat ($P < 0.001$; Table 2). SNL cooked meat showed a higher content of these bioactive peptides than the CG one. After heat treatment, SOD activity decreased by three percentage points in CG meat ($P < 0.05$) and by about one percentage point in SNL meat (Table 2). Mei et al. (1994), in pork and beef meat, detected that SOD activity during cooking did not decrease until the internal temperature of 90 °C was reached. SOD activity of cooked meat was significantly influenced by genetic type; in particular, SNL cooked meat showed higher SOD activity than CG cooked meat, suggesting in the SNL meat a greater tolerance of this enzyme at high temperatures.

4. Conclusion

The results of our research highlighted that the pig meat is a good source of endogenous antioxidants compounds. However, cooking process decreased the content of all considered antioxidant compounds. The genetic type has significantly influenced the content of antioxidant compounds in raw and cooked meat. In particular, autochthonous SNL meat showed a higher antioxidant compounds and SOD activity compared to CG meat, suggesting a greater beneficial effect on health. However, further studies are necessary to determine the availability and amounts of others bioactive compounds in order to improve the qualitative image of meat and, for autochthonous pig, to promote the economic development of areas where they are raised.

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