



Antioxidative and antihypertensive activities of pig meat before and after cooking and *in vitro* gastrointestinal digestion: Comparison between Italian autochthonous pig Suino Nero Lucano and a modern crossbred pig



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ABSTRACT

The aim of this study was to evaluate and compare antioxidative and antihypertensive activities of *Longissimus dorsi* muscle from Suino Nero Lucano (SNL) and a modern crossbred (CG) pigs, before and after cooking and *in vitro* gastrointestinal digestion. Pig meat showed antioxidative and antihypertensive activities, heat treatment decreased the thiols content but at the same time increased angiotensin I-converting enzyme (ACE) inhibitory activity, and *in vitro* gastrointestinal digestion enhanced the biological activity of meat. Autochthonous SNL meat showed a higher nutraceutical quality compared to CG meat, highlighting a greater potential beneficial physiological effect on human health. The results of this study indicate that the pig meat, in particular autochthonous pig meat, may be considered a functional food since it is a good source of antioxidative and antihypertensive peptides.

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1. Introduction

In recent years consumer attention has been increasingly oriented towards foods that exert a positive physiological effect in the body, beyond that of nutrition (Cencic & Chingwaru, 2010). Consequently, to date, many studies reported the biological activities, such as antihypertensive, antithrombotic, opioid, immunomodulatory, antimicrobial, and antioxidant activities of food derived-peptides (Samaranayaka & Li-Chan, 2011). Angiotensin I-converting enzyme (ACE) is an enzyme of the rennin-angiotensin system responsible for regulation of blood pressure (Schmieder, Hilgers, Schlaich, & Schmidt, 2007). In particular, ACE catalyzes the conversion of Angiotensin I into Angiotensin II, a potent vasoconstrictor, and inactivates bradykinin, a vasodilator (Ondetti & Cushman, 1977). Elevated ACE activity could lead to higher Angiotensin II level, giving rise to an increase in blood pressure (Escudero, Sentandreu, Arihara, & Toldrá, 2010). Many reports (Iwaniak, Minkiewicz, & Darewicz, 2014; Wijesekara & Kim, 2010) have demonstrated that moderate hypertension may be controlled by intake of food proteins that have ACE inhibitory peptides. Oxidative stress is implicated in many degenerative diseases including Alzheimer, Parkinson, emphysema, cirrhosis,

and diabetes (Chen, Guo, & Kong, 2012); it is the consequence of an imbalance between prooxidants and antioxidants in the body (Rahal et al., 2014). Thiols, like glutathione and protein thiol groups, play an essential role as antioxidants, since they can act as free radical scavengers and chelators of metal ions. Such compounds are extraordinarily efficient antioxidants protecting cells against oxidative damage caused by reactive oxygen species (ROS), due to their ability to react with the ROS converting them to a relatively less toxic state (Perna, Intaglietta, Simonetti, & Gambacorta, 2015). Erel (2004) reported that in human serum samples, –SH protein groups contribute for 52.9% to total antioxidant capacity in healthy subjects. Natural antioxidant supplement may be used to help the human body to reduce the oxidative damage (Samaranayaka & Li-Chan, 2011). Albeit meat currently suffers of a bad nutritional image due to association between saturated fatty acids and cholesterol content and cardiovascular diseases, it represents a high quality protein source that provides also bioactive components which have been demonstrated to have an antihypertensive, antioxidative and anti-inflammatory activity (Ahhmed & Muguruma, 2010). Increasing awareness of potential positive health consequences associated with intake of meat bioactive compounds is a great opportunity to improve the qualitative image of meat. Moreover, consumers are increasingly oriented towards the intake of meat obtained from an ancient autochthonous genetic type (AAGT) reared under outdoor system.

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The value-added of this meat is due to the respect for the animal welfare, high feed quality, animals capacity to express the natural behavior of the species, and at the same time to the traceability of product and the qualitative and organoleptic traits of meat. Suino Nero Lucano (SNL) is an autochthonous pig breed of southern Italy (Basilicata region), included in the National Pedigree Register (<http://www.anas.it/html/homew.htm>), and it represents an example of the connection among local breed, territory and typical product. Today, this breed is much appreciated for high quality of its product both fresh and seasoned. Fresh meat is almost always cooked before consumption. It has been widely shown that the thermal treatments accelerate greatly the protein oxidation and decrease the antioxidant content (Gatellier, Kondjoyan, Portanguen, & Santé-Lhoutellier, 2010; Santé-Lhoutellier, Astruc, Marinova, Greve, & Gatellier, 2008; Soladoye, Juárez, Aalhus, Shand, & Estévez, 2015). In fact, during cooking the bioactive components, like thiols, react with free radicals or they form intermolecular disulfide bridges (Promeyrat et al., 2010; Santé-Lhoutellier et al., 2008). The antihypertensive and antioxidative activities of bioactive components, which are encrypted and inactive within meat proteins, increase when these are released during gastrointestinal digestion (Escudero et al., 2010; Samaranyaka & Li-Chan, 2011). These bioactive peptide sequences are usually 2–20 amino acid residues in length and, once released, they can be absorbed through intestine to enter the blood circulation or they can produce local effect within the gastrointestinal tract (Arihara, 2006). Although several studies have evaluated the antioxidative and antihypertensive activities of commercial genotypes meat, no information was reported on autochthonous pig meat.

The aim of this study was to evaluate and compare antioxidative and antihypertensive activities of *Longissimus dorsi* muscle from Suino Nero Lucano (SNL) and a modern crossbred (CG) pig, before and after cooking and *in vitro* gastrointestinal digestion, in order to evaluate the effective biological activity of meat and to give a value-added to consumption of autochthonous meat.

2. Materials and methods

2.1. Materials

This study was carried out on 100 castrated male pigs of two different pig breeds, 50 from purebred Suino Nero Lucano (SNL) pigs and 50 from modern crossbred (CG) pigs. SNL pigs were randomly selected from 5 farms of the Potenza province (southern Italy), where there were 2 breeding pigs for farm, without exchanges of breeding pigs among farms. CG pigs were randomly selected from 2 farms of the Potenza province (southern Italy), and they resulting from the mating of Landrace (LR) × Large White (LW) females with LR sires, so the final percentages of each breed were LR 75% and LW 25%. All pigs during growing phase, at about 90 days of age, have been located in the same farm, in different pens according to their genotype, and raised under a semi-wild system. They were fed the same diet until their slaughter. Feed and water were provided *ad libitum*. The amount of concentrate fed to animals was calculated according to their daily requirements of maintenance, growth, more a portion for ambulation and equal to 70% of the estimated daily needs. The composition of the concentrate fed was: corn 30%, field beans 25%, barley 25%, bran and residues from processing of cereals 20%, administered as grains mixture in the form of crushed. The pigs were slaughtered in November 2014, at a live weight of about 140 kg and 540 days of age for SNL pigs and at 160 kg of live weight and 480 days of age for CG pigs. The carcasses were kept at 4 °C for 3 days and at day 4 postmortem, *Longissimus dorsi* (LD) muscle, between 2° and

5° lumbar vertebra of the right side was removed from each carcass (n = 50 for SNL pigs and n = 50 for CG pigs) and brought to the laboratory.

2.2. Experimental design

From each of the LD muscle samples, two pieces of approximately 150 g (10 cm × 15 cm × 1.5 cm) were cut from the central portion, labelled, and distributed in two groups (raw and cooked). Cooking process was performed by placing the one piece of each meat sample in a convection-steam oven (Küppersbusch CPE 110, Küppersbusch Großküchentechnik GmbH, Gelsenkirchen, Germany) set to 120 °C. The heating treatment was considered complete when all the meat samples had reached a temperature of 75 ± 3 °C at the center of the muscles (approximately 20 min). The internal temperature of the muscles was monitored using a thermometer connected to the oven, which was placed in the central part of each muscle. After thermal treatment, the muscles were immediately cooled in an ice batch. The raw and cooked pieces were minced, vacuum packed and stored at –20 °C until analyzed. All raw and cooked meat samples were analyzed for dry matter (DM), fat, protein and ash content. All cooked meat samples were submitted to *in vitro* gastrointestinal digestion. Antioxidative and antihypertensive activity was evaluated in raw, cooked and digested-cooked meat samples.

2.3. Analytical methods

2.3.1. Proximate composition

DM, protein, intramuscular fat (IMF), and ash contents of raw and cooked meat samples were determined according to AOAC (1995) methods. For all samples, each determination was performed in triplicate.

2.3.2. Preparation of water-soluble extracts of raw and cooked meat for biological activities

Raw and cooked meat samples (2 g) were homogenized with 6 mL of distilled water by a Polytron (PT-MR 2100, Kinematica AG, Littau, Luzern, Switzerland) at 13,500 rpm for 15 s. The homogenates were placed in an ultrasound (US) water bath apparatus (Elma Transsonic 460/H, Singen, Germany) for 10 min at 25 °C and centrifuged at 5000×g at 4 °C for 20 min. The supernatant was filtered through a 0.45 µm cellulose acetate membrane filter (Sigma-Aldrich, Milan, Italy), and it was frozen and kept a –55 °C until analysis.

2.3.3. *In vitro* gastrointestinal digestions

The *in vitro* gastrointestinal digestion of cooked meat proteins was simulated using pepsin and pancreatin according to the method of Laparra, Vélez, Montoro, Barberá, and Farré (2003), with some modifications. Five gram of cooked meat was mixed with 50 mL of bidistilled water and human chewing was simulated by using Stomacher (Steward Stomacher 400 Lab Blender, London, UK) for 1 min. After that, the pH was adjusted to 2 (model PHM 92, Radiometer, Copenhagen, Denmark) with 3 M HCl (Sigma-Aldrich, Milan, Italy) and stomach phase was simulated by adding pepsin (Sigma-Aldrich P6887, Milan, Italy) at a 1:100 (enzyme:substrate) ratio. After 2 h of digestion at 37 °C and continuous stirring, the enzyme was inactivated by adjusting the pH to 7.2 with 1 M NaHCO₃. Then, pancreatin (Sigma-Aldrich P3292, Milan, Italy) was added at a 1:50 (enzyme:substrate) ratio to simulated intestinal phase. After 3 h of digestion at 37 °C, enzyme activity was terminated by heating for 10 min at 95 °C. The reaction mixture was centrifuged at 5000g for 20 min at 4 °C (CR 412, Jouan, Saint-Herblain, France) to remove large particles, the supernatant

was filtered through a 0.45 µm cellulose acetate membrane filter (Sigma-Aldrich, Milan, Italy), and it was frozen and kept at -55°C until analysis.

2.3.4. Free thiol assay

The number of free thiol groups of raw, cooked and digested-cooked meat was determined according to [Ellman's method \(1959\)](#), with some modifications. Two hundred fifty microliter of each samples were mixed with 2.5 mL of 0.1 M sodium phosphate buffer (containing 1 mM ethylenediaminetetraacetic acid (EDTA); pH 8.0, reaction buffer; Sigma-Aldrich, Milan, Italy) and 50 µL of 5–5'-dithiobis(2-nitrobenzoic acid) (DTNB) reagent solution (4 mg in 1 mL of sodium phosphate buffer; Sigma-Aldrich, Milan, Italy). After the solution was mixed and allowed to stand at room temperature (25°C) for 30 min, absorbance was read at 412 nm, using UV–vis Spectrophotometer 1204 (Shimadzu, Kyoto, Japan). Reaction buffer was used instead of sample, as a reagent blank. A molar extinction coefficient of $14.150\text{ M}^{-1}\text{ cm}^{-1}$ was used to calculate moles of thiol groups. Thiol groups are expressed as nanomoles of free thiol groups per milligram of protein. Each determination and measurement was made in triplicate.

2.3.5. Angiotensin-converting enzyme inhibitory assay

ACE-inhibitory activity of raw, cooked and digested-cooked meat was measured by the spectrophotometric assay of [Hernandez-Ledesma, Miralles, Amigo, Ramos, and Recio \(2005\)](#), with some modifications. Briefly, 20 µL of each sample was added to 110 µL of 0.1 M sodium borate buffer (pH 8.3; Sigma-Aldrich, Milan, Italy) containing 0.3 M NaCl (Sigma-Aldrich, Milan, Italy) and 5 mM hippuryl-histidyl-leucine (HHL, Sigma-Aldrich H1635, Milan, Italy). After that, 20 µL ACE (EC 3.4.15.1, 5.1 units/mg; Sigma-Aldrich, Milan, Italy) was added, and the reaction mixture was incubated at 37°C for 30 min. The enzymatic reaction was stopped by the addition of 250 µL of 1 M HCl. The hippuric acid formed was extracted with 1.5 mL of ethyl acetate (Sigma-Aldrich, Milan, Italy), heat-evaporated at 95°C for 10 min (Hei-VAP Advantage Rotary Evaporator; Heidolph, Schwabach, German), redissolved in 5 mL of 1 M NaCl, and measured spectrophotometrically at 228 nm. ACE inhibitory activity was calculated as follows:

$$\text{Inhibition (\%)} = [(C - S)/(C - B)] \times 100$$

S: absorbance of sample; C: absorbance of control (buffer for sample); B: absorbance of blank (hydrochloric acid was added before ACE). The activity of each sample was tested in triplicate.

2.4. Statistical analysis

Data were analyzed according to the following linear model ([SAS Institute, 1996](#)):

$$Y_{ijk} = \mu + \alpha_i\beta_j + \varepsilon_{ijk}$$

where y_{ijk} is the observation; μ is the overall mean; α_i is the fixed effect of the i th genetic type ($i = 1, 2$); β_j is the fixed effect of the j th meat state ($j = 1, 2, 3$); and ε_{ijk} is the random error. Before setting the values, expressed in percentage terms, they were subjected to arcsine transformation. Student's test was used to determine the effect of genetic type (SNL vs CG) nested within raw, cooked, and digested-cooked meat samples or to compare the meat state (within raw, cooked, and digested-cooked) nested within each genetic type. Results are presented as mean \pm standard deviation (SD). Differences between means at the 95% ($P < 0.05$) confidence level were considered statistically significant.

3. Results and discussion

3.1. Proximate composition

Chemical composition of SNL and CG raw meat samples was evaluated ([Table 1](#)). No statistically significant differences ($P > 0.05$) were found for DM, protein and ash content, whereas IMF content was significantly higher in SNL (7.21 g/100 g meat) than in CG muscles (6.53 g/100 g meat). The higher IMF content detected in SNL muscle is due to the high adipogenic potential, which is a typical characteristic of autochthonous pig breeds ([Pugliese & Sirtori, 2012](#)). Chemical composition of SNL pigs meat was in line with what was previously detected by [Perna, Simonetti, Intaglietta, and Gambacorta \(2015\)](#). The cooking process of meat influenced all considered parameters ([Table 1](#)), since cooking treatment led to a loss of water content and, consequently, an increase of others parameter. As regard to two studied genetic types, cooked meat samples showed the same differences detected in raw meat ones.

3.2. Antioxidant activity

In this study, the antioxidant activity of raw, cooked and digested-cooked meat from SNL and CG pigs was calculate measuring the number of thiol groups ($-\text{SH}$). In particular, this assay measures sulfhydryl groups with the thiol reagent DTNB, which forms the 5–thionitrobenzoic acid and a mixed disulfide. Under conditions of oxidative stress, free sulfhydryl decreases and disulfides increase ([Prakash, Shetty, Tilak, & Anwar, 2009](#)). This assay reflects the ability of thiols to eliminate free radicals and chelate prooxidative metals, in fact, the loss of free $-\text{SH}$ groups in muscle proteins is widely used as indicator of protein oxidation ([Rysman et al., 2014](#)).

Overall, raw meat showed antioxidant activity ([Table 2](#)), which is a desirable characteristic that enhance the therapeutic values of meat. This is due to both presence of $-\text{SH}$ groups in amino acid sequences and muscle proteolytic degradation by endogenous enzymes that could expose reactive thiol groups. As regard to genetic type, SNL raw meat showed a higher thiols content (83.37 nmol SH-groups/mg protein) compared to CG meat (75.10 nmol SH-groups/mg protein; $P < 0.05$). [Nieto, Jongberg, Andersen, and Skibsted \(2013\)](#) detected, in commercial pig meat, a thiols content of 60 nmol SH-groups/mg protein, whereas no information was reported in literature on thiols content in autochthonous pig meat. The higher thiols content of SNL meat compared to CG meat could be due to both amino acid composition and sequence of muscle proteins and higher calpain and cathepsin

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 (g/100 g meat)				P
		SNL		CG		
		Mean	SD	Mean	SD	
No of pigs		50	–	50	–	–
Dry Matter	Raw	30.48 ^a	1.68	28.98 ^a	2.09	0.068
	Cooked	41.35 ^b	2.66	39.71 ^b	0.98	0.087
IMF	Raw	7.21 ^a	0.51	6.53 ^a	0.64	0.017
	Cooked	9.78 ^b	0.82	8.68 ^b	0.51	0.005
Protein	Raw	22.12 ^a	1.19	21.33 ^a	1.58	0.135
	Cooked	30.01 ^b	1.81	29.04 ^b	0.80	0.120
Ash	Raw	1.15 ^a	0.05	1.12 ^a	0.05	0.284
	Cooked	1.56 ^b	0.09	1.49 ^b	0.09	0.100

^{a,b}Values in the same column, for each parameter, with different superscripts were significantly different ($P < 0.001$).

Table 2

Antioxidant activity, measured as thiols content (nmol SH groups/mg protein), of raw, cooked, and digested-cooked meat from Suino Nero Lucano (SNL) and a modern crossbred genotype (CG).

	Thiols content (nmol SH groups/mg protein)				P
	SNL		CG		
	Mean	SD	Mean	SD	
Raw meat	83.37 ^a	3.77	75.10 ^a	8.57	0.013
Cooked meat	51.45 ^b	3.68	39.82 ^b	1.22	<0.001
Digested-cooked meat	159.80 ^c	7.73	97.03 ^c	4.88	<0.001

^{a–c}For each genetic type, different superscripts within a column indicate a statistical difference ($P < 0.001$).

activity, important proteolytic enzymatic systems involved in *post-mortem* muscle proteolysis, which are more active in SNL meat due to higher temperature of autochthonous meat resulting from prolonged anaerobic metabolic activity (D'Alessandro, Marrocco, Zolla, D'Andrea, & Zolla, 2011; Ohlendieck, 2010). Also, Damon, Wyszynska-Koko, Vincent, Héroult, and Lebret (2012) detected that calpastatin gene (CAST), an inhibitor of calpain, was highly expressed in commercial than in autochthonous pigs meat; while Murgiano et al. (2010) reported in autochthonous Casertana pigs meat the presence of higher levels of antioxidant enzymes which might play a role in protecting cathepsins and calpains during *post-mortem* proteolysis.

After heat treatment, the average thiols content in muscles of both genetic types decreased (Table 2; $P < 0.001$), in agreement with what reported by other authors (Haak, Raes, Van Dyck, & De Smet, 2008; Santé-Lhoutellier et al., 2008). Many studies reported that the heat treatment, still within the normal range of heat process (70–125 °C), decreases –SH and increases S–S content, whereas a mild heat treatment (<50–70 °C) of muscle protein may increase –SH for reduction of pre-existent disulfide bridges or for access of the DTNB to internal free thiols due to the thermal denaturation of proteins (Gatellier et al., 2010; Promeyrat et al., 2010; Soladoye et al., 2015). The cooking process has also been noted to trigger the generation of ROS (Soladoye et al., 2015), and thiols, like cysteine and methionine, due to the high reaction susceptibility of their sulfur group, are particularly prone to react with these free radicals (Gatellier et al., 2010; Santé-Lhoutellier et al., 2008). Santé-Lhoutellier et al. (2008) reported that the decrease of thiols could be due to the oxidation of accessible free thiol groups from cysteine residues located at the protein surface, whereas the inside cysteine residues are protected against free radical attack even during a long heating time. At last, the lower thiols content in cooked meat could be also due to the losses in the cooking juice. After cooking process, SNL meat showed a higher thiols content (51.45 nmol SH-groups/mg protein) compared to CG meat (39.82 nmol SH-groups/mg protein; $P < 0.001$), corresponding to respective losses of 38 and 50% for SNL and CG cooked meat, respectively. In particular, CG cooked meat showed a loss of thiols content about 1.32-fold higher than SNL cooked meat, this indicates a better oxidative stability of SNL meat and, consequently, a higher antioxidant capacity compared to CG meat. Many authors (D'Alessandro et al., 2011; Damon et al., 2012; Xu et al., 2009) observed that muscle equal to the autochthonous pig breeds (Casertana, Meishan, and Basque pigs) showed a higher level of oxidative fibers compared to modern crossbred pigs. The oxidative fiber, during cooking process, was less sensitive to the combined effects of heat and oxidants than glycolytic fiber (Promeyrat, Daudin, Astruc, Danon, & Gatellier, 2013), due to high level of heat shock proteins (HSP) which can be associated with myofibrillar proteins, limiting oxidation process (Oishi et al., 2001).

The digestion of cooked meat with gastrointestinal enzymes increased the thiols content ($P < 0.001$). In fact, enzymatic hydrolysis leads to the formation of many bioactive peptides with antioxidant activity (Samardi & Ismail, 2010). Taylor, Martinez-Torres, Romano, and Layrisse (1986) reported that during meat digestion cysteine-containing peptides, like glutathione, are released. These peptides are stabler in the gastrointestinal tract than the free amino acid, since the thiol group remaining unoxidized and the cysteine in this peptide form is active even at intestinal pH. SNL digested-cooked meat showed a higher thiols content (159.80 nmol SH-groups/mg protein) compared to CG digested-cooked meat (97.03 nmol SH-groups/mg protein; $P < 0.001$). In particular, free –SH content was increased about two and a half times in CG digested-cooked meat and about threefold in SNL digested-cooked meat, showing a greater susceptibility of SNL meat to the proteases action. Santé-Lhoutellier et al. (2008) reported that the susceptibility of cooked meat proteins to the action of digestive enzymes depends on the oxidation state of proteins at the digestion time. In fact, a mild oxidation will induce a partial unfolding of the protein structure, enhancing its protease susceptibility, whereas, a high oxidative environment will lead to a greater protein-unfolding and a massive aggregation as well as modification of protease-active sites, with a decreased proteolytic susceptibility (Gatellier & Santé-Lhoutellier, 2009; Promeyrat et al., 2010; Santé-Lhoutellier et al., 2008). Chao, Ma, and Stadtman (1997) reported that during oxidation process the formation of covalent bonds, such as disulfide and dityrosine bonds, induces proteins to cluster and precipitate. At last, the reduced protein digestion rate would have a negative impact on human health because non hydrolyzed proteins are fermented by colonic flora into phenol and p-cresol, which are mutagenic products, increasing the risk of colon cancer (Evenepoel et al., 1998).

3.3. Antihypertensive activity

The ACE inhibitory activity of raw, cooked and digested-cooked meat from SNL and CG pigs is reported in Table 3. Overall, raw meat of both genetic type showed antihypertensive activity (2.89 and 3.01% for SNL and CG pigs, respectively), due to the proteolytic degradation of muscle that, after rigor mortis, leads to the production of biologically active small peptides (Mullen et al., 2000). In fact, bioactive peptides are inactive or latent in the parent protein but can be released in an active form by endogenous enzymes during *postmortem*, meat processing or after proteolytic digestion (Lafarga & Hayes, 2014).

After cooking process, ACE inhibitory activity of meat increased slightly ($P < 0.001$), in agreement with Bauchart et al. (2006) who found that the bioactive peptides content was lower in fresh beef muscle (*M. pectoralis profundus*) and increased during cooking. Cooking process, and the concomitant protein denaturation

Table 3

Angiotensin converting enzyme (ACE) inhibitory activity (%) of raw, cooked, and digested-cooked meat from Suino Nero Lucano (SNL) and a modern crossbred genotype (CG). Values are means \pm SD (standard deviation).

	ACE inhibitory activity (%)				P
	SNL		CG		
	Mean	SD	Mean	SD	
Raw meat	2.89 ^a	0.34	3.01 ^a	0.28	NS
Cooked meat	3.67 ^b	0.29	3.88 ^b	0.45	NS
Digested-cooked meat	78.92 ^c	4.91	69.21 ^c	2.42	<0.001

^{a–c}For each genetic type, different superscripts within a column indicate a statistical difference ($P < 0.001$).

caused, induces the release of peptides because of drastic changes in meat structure, such as myofibril fragmentation, gel formation of sarcoplasmic proteins or shrinkage and solubilization of the connective tissue (Palka, 2003). There was no significant difference for ACE inhibitory activity between SNL and CG pigs for both raw and cooked meat. Meat is mainly taken after cooking, therefore to evaluate the effective biological activity of meat, the analysis of ACE inhibitory activity of denatured protein and their hydrolysates with digestive enzymes was needed. After *in vitro* gastrointestinal digestion, ACE inhibitory activity of cooked meat has increased greatly ($P < 0.001$; Table 3), in agreement with what reported by other authors (Ahmed & Muguruma, 2010; Katayama et al., 2003). In particular, Ahmed and Muguruma (2010), found an antihypertensive activity of meat hydrolysate (from pork loin steaks) 27 times greater than in undigested meat from the same muscle. In fact, the most common way to produce bioactive peptides is through enzymatic hydrolysis of protein, since the bioactive sequences into proteins are inactive until cleaved by some proteases, and many bioactive peptides, like ACE inhibitor peptides, are released only by proteolytic digestion (Arihara, 2006). In this study, SNL digested-cooked meat showed a higher ACE inhibitor activity (78.92%) compared to CG digested-cooked meat (69.21%; $P < 0.001$), with an increase of about 21% compared to cooked meat and about 26% compared to raw meat, whereas the activity of CG digested-cooked meat was about 17% greater than cooked meat and about 22% greater than raw meat. In this case too, the highest ACE inhibitory activity of SNL digested-cooked meat could be associated to higher oxidative stability of cooked meat proteins, since the protein oxidation during cooking process decreased the protein susceptibility to peptidase activity (Gatellier & Santé-Lhoutellier, 2009). An intense proteolysis during gastrointestinal digestion, instead leads to the production of many peptides to low molecular weight with biological activity (Ahmed & Muguruma, 2010; Arihara, 2006). Structure-activity correlations among different peptide ACE inhibitors indicate that binding to the enzyme is strongly influenced by the C-terminal tripeptide sequence of the substrate (Norris & FitzGerald, 2013). In particular, ACE appears to prefer substrates or competitive inhibitors containing hydrophobic (aromatic or branched side-chains) amino acid residues at each of the three C-terminal positions (Li, Le, Shi, & Shrestha, 2004). Also, Kawakami and Kayahara (1993) suggested that most of ACE inhibitory peptides that are released by proteolytic digestion contain Pro, Lys or aromatic amino acid residues, and these peptides are particularly stable because are not further degraded by intestinal peptidases (Balti, Nejar-Arroume, Bougateg, Guillochon, & Nasri, 2010).

4. Conclusion

The results of our research indicate that the pig meat may be considered a functional food since it is a good source of antioxidative and antihypertensive peptides. The changes in protein physicochemical state during cooking decreased the thiols content but in the same time increased the ACE inhibitory activity. Furthermore, gastrointestinal digestion enhanced the biological activity of the meat. As regard to genetic type, autochthonous SNL meat, before and after cooking process showed a higher thiols content and, consequently, a higher oxidative stability of proteins than modern CG meat. The *in vitro* gastrointestinal digestion enhanced the nutraceutical quality of SNL meat that showed a higher antioxidative and antihypertensive activity compared to CG meat, highlighting a greater potential beneficial physiological effect on human health. However, further studies are necessary to identify and assess the efficacy, dose response and safety *in vivo* of bioactive peptides derived from autochthonous pig meat.

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References

- Ahmed, A. M., & Muguruma, M. (2010). A review of meat protein hydrolysates and hypertension. *Meat Science*, 86, 110–118.
- AOAC (1995). *Official methods of analysis* (16th ed.). Arlington, VA: Association of Official Analytical Chemists.
- Arihara, K. (2006). Strategies for designing novel functional meat products. *Meat Science*, 74, 219–229.
- Balti, R., Nejar-Arroume, N., Bougateg, A., Guillochon, D., & Nasri, M. (2010). Three novel angiotensin I converting enzyme (ACE) inhibitory peptides from cuttlefish (*Sepia officinalis*) using digestive proteases. *Food Research International*, 43, 1136–1143.
- Bauchart, C., Remond, D., Chambon, C., Mirand, P. P., Savary-Auzeloux, I., Reynes, C., & Morzel, M. (2006). Small peptides (b5 kDa) found in ready-to-eat beef meat. *Meat Science*, 74(4), 658–666.
- Cencic, A., & Chingwaru, W. (2010). The role of functional foods, nutraceuticals, and food supplements in intestinal health. *Nutrients*, 2, 611–625.
- Chao, C. C., Ma, Y. S., & Stadtman, E. R. (1997). Modification of protein surface hydrophobicity and methionine oxidation by oxidative systems. *Proceedings of the National Academy of Sciences of the United States of America*, 94(7), 2969–2974.
- Chen, X., Guo, C., & Kong, J. (2012). Oxidative stress in neurodegenerative diseases. *Neural Regeneration Research*, 7(5), 376–385.
- D'Alessandro, A., Marrocco, C., Zolla, V., D'Andrea, M., & Zolla, L. (2011). Meat quality of the longissimus lumborum muscle of Casertana and Large White pigs: Metabolomics and proteomics intertwined. *Journal of Proteomics*, 75, 610–627.
- Damon, M., Wyszynska-Koko, J., Vincent, A., Hérault, F., & Lebret, B. (2012). Comparison of muscle transcriptome between pigs with divergent meat quality phenotypes identifies genes related to muscle metabolism and structure. *PLoS One*, 7(3), e33763.
- Ellman, G. L. (1959). Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics*, 82, 70–77.
- Erel, O. (2004). A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clinical Biochemistry*, 37, 277–285.
- Escudero, E., Sentandreu, M. A., Arihara, K., & Toldrà, F. (2010). Angiotensin I-converting enzyme inhibitory peptides generated from *in vitro* gastrointestinal digestion of pork meat. *Journal of Agricultural and Food Chemistry*, 58, 2895–2901.
- Evenepoel, P., Claus, D., Geypens, B., Maes, B., Hiele, M., Rutgeerts, P., & Ghoo, Y. (1998). Evidence for impaired assimilation and increased colonic fermentation of protein related to gastric acid suppression therapy. *Alimentary Pharmacology and Therapeutics*, 12, 10–11.
- Gatellier, P., Kondjoyan, A., Portanguen, S., & Santé-Lhoutellier, V. (2010). Effect of cooking on protein oxidation in n-3 polyunsaturated fatty acids enriched beef. Implication on nutritional quality. *Meat Science*, 85(4), 645–650.
- Gatellier, P., & Santé-Lhoutellier, V. (2009). Digestion study of proteins from cooked meat using an enzymatic microreactor. *Meat Science*, 81, 405–409.
- Haak, L., Raes, K., Van Dyck, S., & De Smet, S. (2008). Effect of dietary rosemary and α -tocopheryl acetate on the oxidative stability of raw and cooked pork following oxidized linseed oil administration. *Meat Science*, 78, 239–247.
- Hernandez-Ledesma, B., Miralles, B., Amigo, L., Ramos, M., & Recio, I. (2005). Identification of antioxidant and ACE-inhibitory peptides in fermented milk. *Journal of the Science of Food and Agriculture*, 85, 1041–1048.
- Iwaniak, A., Minkiewicz, P., & Darewicz, M. (2014). Food-originating ACE inhibitors, including antihypertensive peptides, as preventive food components in blood pressure reduction. *Comprehensive Reviews in Food Science and Food Safety*, 13, 114–134.
- Katayama, K., Fuchu, H., Sakata, A., Kawahara, S., Yamauchi, K., Kawamura, Y., & Muguruma, M. (2003). Angiotensin I-converting enzyme inhibitory activities of porcine skeletal muscle proteins following enzyme digestion. *Asian-Australian Journal of Animal Science*, 16, 417–424.
- Kawakami, A., & Kayahara, H. (1993). Synthesis of Leu-Lys-Tyr derivatives and their interaction with angiotensin converting enzyme. *Japan Society of Nutrition and Food Science*, 46, 425–428.
- Lafarga, T., & Hayes, M. (2014). Bioactive peptides from meat muscle and by-products: Generation, functionality and application as functional ingredients. *Meat science*, 98, 227–239.
- Laparra, J. M., Vélez, D., Montoro, R., Barberá, R., & Farré, R. (2003). Estimation of arsenic bioaccessibility in edible seaweed by an *in vitro* digestion method. *Journal of Agricultural and Food Chemistry*, 51(20), 6080–6085.
- Li, G. H., Le, G. W., Shi, Y. H., & Shrestha, S. (2004). Angiotensin I-converting enzyme inhibitory peptides derived from food proteins and their physiological and pharmacological effects. *Nutrition Research*, 24, 469–486.

- Mullen, A., Stoeva, S., Laib, K., Gruebler, G., Voelter, W., & Troy, D. (2000). Preliminary analysis of amino acids at various locations along the *M. longissimus dorsi* in aged beef. *Food Chemistry*, 69, 461–465.
- Murgiano, L., D'Alessandro, A., Egidio, M. G., Crisà, A., Prosperini, G., Timperio, A. M., ... Zolla, L. (2010). Proteomics and transcriptomics investigation on longissimus muscles in Large White and Casertana pig breeds. *Journal of Proteome Research*, 9(12), 6450–6466.
- Nieto, G., Jongberg, S., Andersen, M. L., & Skibsted, L. H. (2013). Thiol oxidation and protein cross-link formation during chill storage of pork patties added essential oil of oregano, rosemary, or garlic. *Meat Science*, 95, 177–184.
- Norris, R., & FitzGerald, R. J. (2013). Antihypertensive peptides from food proteins. In B. Hernandez-Ledesma (Ed.), *Bioactive food peptides in health and disease* (pp. 42–46). Rijeka: InTech.
- Ohlendieck, K. (2010). Proteomics of skeletal muscle glycolysis. *Biochimica et Biophysica Acta*, 1804(11), 2089–2101.
- Oishi, Y., Taniguchi, K., Matsumoto, H., Ishihara, A., Ohira, Y., & Roy, R. R. (2001). Muscle type-specific response of HSP60, HSP72, and HSC73 during recovery after elevation of muscle temperature. *Journal of Applied Physiology*, 92, 1097–1103.
- Ondetti, M. A., & Cushman, D. W. (1977). Design of specific inhibitors of angiotensin-converting enzyme: New class of orally active antihypertensive agents. *Science*, 196, 441–444.
- Palka, K. (2003). The influence of post-mortem ageing and roasting on the microstructure, texture and collagen solubility of bovine semitendinosus muscle. *Meat Science*, 64, 191–198.
- Perna, A., Intaglietta, I., Simonetti, A., & Gambacorta, E. (2015). Donkey milk for manufacture of novel functional fermented beverages. *Journal of Food Science*, 80, S13532–S13539.
- Perna, A., Simonetti, A., Intaglietta, I., & Gambacorta, E. (2015). Fatty acids composition, cholesterol and vitamin E contents of *Longissimus dorsi* and *Semitendinosus* muscles of Suino Nero Lucano pigs slaughtered at two different weights. *Animal Production Science*, 55, 1037–1043.
- Prakash, M., Shetty, M. S., Tilak, P., & Anwar, N. (2009). Total Thiols: Biomedical importance and their alteration in various disorders. *The Online Journal of Health and Allied Sciences*, 8(2), 2–9.
- Promeyrat, A., Daudin, J. D., Astruc, T., Danon, J., & Gatellier, P. (2013). Kinetics of protein physicochemical changes induced by heating in meat using mimetic models: (2) effects of fibre type, peroxides and antioxidants. *Food Chemistry*, 138, 2283–2290.
- Promeyrat, A., Gatellier, P., Lebret, B., Kajak-Siemaszko, K., Aubry, L., & Sante-Lhoutellier, V. (2010). Evaluation of protein aggregation in cooked meat. *Food Chemistry*, 121, 412–417.
- Pugliese, C., & Sirtori, F. (2012). Quality of meat and meat products produced from southern European pig breeds. *Meat Science*, 90, 511–518.
- Rahal, A., Kumar, A., Singh, V., Yadav, B., Tiwari, R., Chakraborty, S., & Dhama, K. (2014). Oxidative stress, prooxidants, and antioxidants: The interplay. *BioMed Research International*, 761264, 1–19.
- Rysman, T., Jongberg, S., Van Royen, G., Van Weyenberg, S., De Smet, S., & Lund, M. N. (2014). Protein thiols undergo reversible and irreversible oxidation during chill storage of ground beef as detected by 4,4'-dithiodipyridine. *Journal of Agricultural and Food Chemistry*, 62, 12008–12014.
- Samaranayaka, A. G. P., & Li-Chan, E. C. Y. (2011). Food-derived peptidic antioxidants: A review of their production, assessment, and potential applications. *Journal of Functional Foods*, 3, 229–254.
- Samardi, B. H., & Ismail, A. (2010). Antioxidative peptides from food proteins: A review. *Peptides*, 31(10), 1949–1956.
- Santé-Lhoutellier, V., Astruc, T., Marinova, P., Greve, E., & Gatellier, P. (2008). Effect of meat cooking on physicochemical state and in vitro digestibility of myofibrillar proteins. *Journal of Agricultural and Food Chemistry*, 56(4), 1488–1494.
- SAS Institute (1996). *SAS user's guide: Statistics. Version*. Cary, NC: SAS Inst. Inc.
- Schmieder, R. E., Hilgers, K. F., Schlaich, M. P., & Schmidt, B. M. W. (2007). Renin-angiotensin system and cardiovascular risk. *Lancet*, 369(9568), 1208–1219.
- Soladoye, O. P., Juárez, M. L., Aalhus, J. L., Shand, P., & Estévez, M. (2015). Protein oxidation in processed meat: Mechanisms and potential implications on human health. *Comprehensive Reviews in Food Science and Food Safety*, 14, 106–122.
- Taylor, P. G., Martínez-Torres, C., Romano, E. L., & Layrisse, M. (1986). The effect of cysteine-containing peptides released during meat digestion on iron absorption in humans. *The American Journal of Clinical Nutrition*, 43, 68–71.
- Wijesekara, I., & Kim, S. K. (2010). Angiotensin-I-converting enzyme (ACE) inhibitors from marine resources: Prospects in the pharmaceutical industry. *Marine Drugs*, 8, 1080–1093.
- Xu, Y. J., Jin, M. L., Wang, L. J., Zhang, A. D., Zuo, B., Xu, D. Q., ... Xiong, Y. Z. (2009). Differential proteome analysis of porcine skeletal muscles between Meishan and Large White. *Journal of Animal Science*, 87, 2519–2527.