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FULL ARTICLE

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Protective effects of dietary carnosine during in-vitro digestion of pork differing in fat content and cooking conditions

Yi Yao Li[1,2] | Varoujan Yaylayan[1] | Marie-France Palin[3] | Brian Sullivan[4] | Frederic Fortin[5] | Simon Cliche[2] | Hassan Sabik[2] | Claude Gariépy[2]

[1]Department of Food Science and Agricultural Chemistry, Macdonald Campus, McGill University, Ste Anne de Bellevue, QC, Canada

[2]Saint-Hyacinthe Research and Development Centre, Agriculture and Agri-Food Canada, Saint-Hyacinthe, QC, Canada

[3]Sherbrooke Research and Development Centre, Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada

[4]Canadian Centre for Swine Improvement, Ottawa, ON, Canada

[5]Centre de développement du porc du Québec, Québec, QC, Canada

Correspondence
Claude Gariépy, Saint-Hyacinthe Research and Development Centre, Agriculture and Agri-Food Canada, 3600 Casavant blvd. West, Saint-Hyacinthe, QC J2S 8E3, Canada.
Email: claude.gariepy@canada.ca

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Abstract

Muscle carnosine represents an important health advantage of meat. Ground pork samples with intrinsic or added carnosine; fat content; and cooked under low or high intensity as a 2 × 2 × 2 factorial were digested in-vitro. Changes in free carnosine and in markers of lipid (hexanal, 4-hydroxynonenal (4-HNE), malondialdehyde (MDA) and protein (protein-carbonyls, thiols) oxidation, and of advanced glycation end-products (AGEs) N^{ε}-(carboxymethyl)lysine (CML) were determined in the saliva, gastric, and duodenal digests. During digestion, the different markers overall indicated increased oxidation and decreased free carnosine. Increasing pork carnosine level significantly reduced protein carbonyls, loss of thiols, and 4-HNE during in-vitro gastric digestion, irrespective of fat and cooking level of the meat. Increased carnosine also significantly reduced hexanal, MDA and CML up to the duodenum phase in moderately cooked lean pork. Besides substantiating the formation of AGEs during digestion, these results show a potentially important role of dietary carnosine occurring in the gastrointestinal tract.

Practical applications

The ailments epidemiologically associated with red meat consumption could be counteracted by ingesting carnosine into meat. The health advantages of dietary carnosine, however, have never been demonstrated during digestion, a unique and complex oxidative environment compounded by the composition and cooking of the meat. The results obtained substantiated that AGEs formation occurred in-vitro in the GIT. They also showed that increased carnosine had an immediate health beneficial role during pork digestion in reducing the formation of different harmful molecules, including AGEs, modulated by the composition and cooking of the meat. However, in exerting this protective role in the GIT, the remaining free level of carnosine, gradually decreased during digestion. Carnosine, as an important meat compositional factor may, depending on the fat content and cooking conditions, change the image of meat from representing a health risk to a health benefit. Carnosine level may also explain discrepancies observed in the literature.

Abbreviations: 4-HNE, 4-Hydroxynonenal; AGEs, advanced glycation end-products; ALEs, advanced lipoxidation end-products; CEL, N^{ε}-(carboxyethyl)lysine; CML, N^{ε}-(carboxymethyl) lysine; GIT, gastrointestinal tract; HCar, high carnosine; HCI, high cooking intensity; HF, high fat; LCar, low carnosine; LCI, low cooking intensity; LF, low fat; LM, longissimus muscles; MDA, malondialdehyde; PC, principal component; PUFA, polyunsaturated fatty acid; RAGE, receptor for advanced glycation end-products; ROS, reactive oxygen species.

KEYWORDS
advanced glycation end-products, carnosine, health, in-vitro digestion, lipid and protein oxidation, meat

1 | INTRODUCTION

Meat contributes to a healthy diet in providing high-quality proteins, minerals, vitamins, and all eight essential amino acids. Epidemiologically however, high consumption of red and processed meat have been associated with a greater likelihood of chronic diseases including type 2 diabetes mellitus, cardiovascular disease, and colorectal cancer (Wolk, 2017). These conditions could be interrelated through a redox state imbalance as a common triggering and/or contributing factor (Liguori et al., 2018).

In vivo, a redox imbalance increases the production of reactive oxygen species (ROS), that in reacting with the amino acids side chains of the protein or its peptide backbone can form stable protein carbonyls, the most common indicator of oxidized proteins. Carbonylated proteins often lose their functions due to misfolding or aggregation which, when in excess, can lead to their cellular accumulation and cytotoxicity caused by impaired proteolysis, as reported by Dalle-Donne et al. (2006). ROS can also react with sugars and lipids to generate reactive carbonyl species that can in turn covalently react with the proteins to form, respectively, advanced glycation end-products (AGEs) and advanced lipoxidation end-products (ALEs).

(Dalle-Donne et al., 2006). N^{ε}-(carboxymethyl)lysine (CML), a common marker of AGEs implicated in the etiology and development of multiple oxidative-associated diseases, can be related to AGEs or ALEs (Vistoli et al., 2013). Further to the formation of protein adducts, AGEs can in addition activate through the receptor for advanced glycation end-products (RAGE), an intracellular signaling cascade that will contribute further to increase ROS production, damage, and inflammation (Ott et al., 2014). Endogenously formed AGEs can be found in increasing amount in tissues as diverse as the eye, kidney, liver, blood vessels, reproductive tissues, muscle, bone, and brain as reported by Fishman et al. (2018).

Oxidized dietary compounds can also contribute to oxidation invivo. In meat, each of fat and protein, altogether with free or bound iron can be involved in an oxidative process that generate the ROS that will further promote oxidative damage to biological macromolecules (Papuc et al., 2017). In the GIT, the pathogenicity of lipid and protein oxidation products from the diet is mainly due to their cytotoxicity and genotoxicity (Esterbauer, 1993) which could in turn result in the development of inflammatory bowel diseases and other conditions (Estévez & Luna, 2017). Following their absorption, these reactive species could have further biological effects in extra GIT tissues. It is documented that cytotoxic and genotoxic dietary secondary lipid oxidation products, such as MDA, a general lipid oxidation marker, and 4-HNE and hexanal, oxidation biomarkers of the n-6 polyunsaturated fatty acids (PUFA) can be absorbed intact (Estévez et al., 2017). They can then contribute to nutritionally mediated oxidative stress and inflammation that can trigger and/or contribute to the propagation of various chronic diseases via nuclear factor Kappa B (Bee et al., 2018; Estévez et al., 2017; Ng et al., 2013). For their part, the absorption of oxidized amino acids or small peptides may lead to aberrant in vivo protein synthesis leading to inflammation in liver and kidney in mice (Li et al., 2014).

The gastrointestinal tract (GIT) can also be exposed to high level of AGEs of dietary origin (dAGEs) from the production, storage and thermal processing of foods (Poulsen et al., 2013; Zamora & Hidalgo, 2005). These may contribute to increasing the circulating AGEs level, oxidative stress and inflammation, although their accumulation in human extra GIT tissues is not yet confirmed as reviewed by Nowotny et al. (2018). In spite of the oxidative environment of the GIT documented to increased oxidative damage to dietary compounds (Nieva-Echevarría et al., 2020; Oueslati et al., 2016), the potential formation of AGEs during the digestion process itself has been recently suggested (DeChristopher, 2017; Hipkiss, 2018) but to the best of our knowledge has not yet been investigated.

Besides their commonly recognized nutrients, meat and muscle foods are unique in also containing carnosine and its derivatives, anserine and sometime ophidine, in species-dependent proportions (Boldyrev et al., 2013). Carnosine is a naturally occurring L-histidine and β-alanine multifunctional dipeptide exclusive to muscle food and discovered over a century ago. Carnosine possesses pH-buffering, metal-ion chelation, carbonyl scavenging and antioxidant activities, along with the ability to interfere with the formation of AGEs and ALEs, conferring altogether the dipeptide with therapeutic potential toward health conditions involving oxidative stress as reported by Boldyrev et al. (2013). Some potential applications of carnosine would include healthy aging, improving cognitive function, preventing diabetes complications, offering some protection during acute kidney failure and showing anti-neoplastic effects as reviewed (Artioli et al., 2019). It has been in fact suggested that carnivore diets containing carnosine and its related peptides might ameliorate pathologies associated with macromolecular glycation and other health conditions (Brownlee, 2001; Hipkiss, 2005; Wu, 2020).

The antioxidant effect of muscle carnosine has been demonstrated in the meat itself (Chan & Decker, 1994). In addition, significant number of investigations of the biochemical and physiological properties of carnosine, including human clinical studies based on carnosine supplement are also available, as reviewed by Boldyrev et al. (2013). However, no study is yet available on the advantages and bio-accessibility of dietary carnosine with particular reference to the potential role that it may exert in the GIT itself upon digestion, as reviewed by Xing et al. (2019). The authors reported that the release of dietary carnosine from its food matrix would be proportional to its amount in the meat and would depend also on the conditions found in the GIT, including the composition of the food.

According to Marcolini et al. (2015), in vitro gastric digestive treatment at pH 2 contributed to increasing the release, and therefore the bio-accessibility of carnosine from a raw lean meat product (cured braseola). However, the authors did not investigate the interactions of carnosine with its meat matrix components that could also affect such release. Meat, however, is mostly consumed cooked and within a meat specie cooking intensity and fat content represent the two most important oxidative factors (Van Hecke et al., 2014, 2015). Indeed, increased cooking intensity has been shown to increase lipid and protein oxidation during digestion of pork (Van Hecke et al., 2014, 2015). Hipkiss (2005) in addition, suggested that any increased intake in animal fat accompanying higher dietary carnosine in carnivores may mask the benefit that carnosine might exert. To the best of our knowledge however, the interactions occurring between dietary carnosine and health detrimental compounds such as AGEs and lipid and protein oxidation products as part of a more complex meat system varying in cooking conditions and composition has never been investigated during digestion. Therefore, the purpose of this study was to assess through the formation of lipid and protein oxidation products, including AGEs, the functional role of dietary carnosine during in-vitro digestion of ground pork containing two levels of carnosine, two levels of fat and cooked under two different cooking intensities.

2 | MATERIALS AND METHODS

All chemicals and enzymes used in this study were from Sigma-Aldrich (MO, USA) except when indicated otherwise. All reagents were of analytical grade, For in-vitro digestion, reagents and enzymes for the digestive fluids preparation included mucin, peroxidase, pepsin, porcine pancreatin, lipase, porcine bile, bovine serum albumin, calcium chloride dehydrate, urea, sodium chloride, glucuronic acid, glucose amine-HCl, 37% HCl, hydrogen peroxide, and monosodium phosphate. Ferrous sulfate heptahydrate was from Thermo Fisher Scientific (MA, USA). The following reagents were used for sample preparation and digest analyses: L-Carnosine, hydrochloric acid, trichloroacetic acid, 2,4-dinitrophenylhydrazin, ethyl acetate, ethanol, 2, 2′-dithiobis (5-nitropyridine), sodium hydroxide, hydrated copper(II) sulfate, potassium sodium tartrate, methanol (≥99.9%), N, O-Bis(trimethylsilyl)trifluoroacetamide (≥99%), O-(2,3,4,5,6-Pentafluorobenzyl)hydroxylamine hydrochloride (98%), 2,2,4-Trimethylpentane (≥99.5%), 4-hydroxy Hexenal-d3 (≥99%) was from Cayman Chemical (MI, USA). Lean fresh *Longissimus* muscle (*LM*) and subcutaneous fat were purchased from a commercial pork processor in Canada (Olymel S.E.C).

2.1 | Meat sample preparation

A stock mixture from two entire pork *Longissimus* muscles (*LM*) was prepared in order to have a constant extrinsic addition of carnosine and fat to reach the targeted levels described below. This mixture served for the preparation of the treatments for each of the four repetitions. This approach was prompted by our earlier report of the large *LM* carnosine variation (from 217 to 410 mg/100 g *LM*) between pigs representative of the Canadian population (D'Astous-Pagé et al., 2017) and ensured no other compositional variables inherent to the pigs were involved. Therefore, 50 gram samples from the anterior, central and posterior portion of the two *LM* were collected, ground twice (first with a 6 mm plate, then with a 3.5 mm plate) and mixed for the determination of their intrinsic carnosine (Mora et al., 2007) and fat content (AOAC method 920.39). The day after, the remaining two *LM* were ground with a 6 mm plate and the minced meat was thoroughly mixed and divided into eight portions corresponding to a $2 \times 2 \times 2$ factorial arrangement of treatments as follow: samples containing either low (intrinsic or 309.8 mg carnosine/100 g meat; LCar) or high level (600 mg carnosine/100 g meat; HCar) of carnosine were mixed with each of two levels of fat (intrinsic or 1.3% fat; LF versus. 10% or high fat; HF) and each subsample was then cooked under either low (65°C for 15 min; LCI) or high cooking intensity (90°C for 30 min; HCI) as shown in Table 1. Four separate bags of mixture for each of the eight treatments were then vacuum packaged and stored at −80°C until used for each of the four digestion repetitions carried out on four different days within a two-month period. We arbitrarily targeted the HCar group to contain 600 mg carnosine per 100 g meat by complementing the intrinsic amount of carnosine (309.8 mg) of the LCar groups with 290.2 mg carnosine/100 g meat. For HF groups, the 10% fat corresponds to the highest amount allowed in extra lean ground meat as per the Canadian regulation (Canadian Food Inspection Agency, 2015) and was obtained by adding external subcutaneous pork fat to complement the intrinsic fat level measured in the meat. For each treatment, the mixture was first thoroughly hand-mixed for one minute and then ground again with a 3.5 mm grid before being transferred in 50 ml Nalgene screw-cap containers with a fitted handmade rubber stopper in the lid for the cooking processes. Containers were filled to the rim

TABLE 1 Preparation of meat sample

Low Carnosine (LCar = 309.8 mg/100 g *longissimus* muscle)[†]				High Carnosine (HCar = 600.0 mg/100 g *longissimus* muscle)[‡]			
Low Fat (LF = 1.3%)[†]		High Fat (HF = 10.0%)[‡]		Low Fat (LF = 1.3%)[†]		High Fat (HF = 10.0%)[‡]	
LCI	HCI	LCI	HCI	LCI	HCI	LCI	HCI

Note: Low cooking intensity (LCI): 65°C for 15 min; High cooking intensity (HCI): 90°C for 30 min.

[†]Intrinsic levels of carnosine and fat in pork.

[‡]Targeted levels of carnosine and fat after extrinsic addition.

and cooked in water bath under the two different conditions described (Table 1). Within each of the four repetitions, cooking was conducted as a batch process with the core temperature being monitored with a thermocouple in one control sample. After cooked meat cooling to room temperature, the entire content of the container was mixed in a food blender at low speed for 2 min. For each treatment combination, 20 g of the ground cooked meat was then vacuum-packaged in individual bag and stored at −80°C until used for digestion experiments.

2.2 | In-vitro digestion

In-vitro digestion mimicking the saliva, gastric, and duodenal milieu were conducted as described by Van Hecke et al. (2014). This model was chosen because it has been adapted for meat digestion from the in-vitro model of Versantfoort et al. (2005) and has also been validated by a series of studies on lipid and protein oxidation in meat digest from pork with different fat content and cooking intensities. Compared with model used for other types of food (Ng & See, 2019), this model from Van Hecke et al. (2014) had additional lipase targeting higher fat content in meat sample, and a larger volume of fluids considering the meat sample is solid instead of liquid extract. Moreover, mucin, oxidants, and antioxidants usually present in digestive fluids were also included and made the system closer to the GIT environment. We further modified it as follow: one tube of the digest was taken after each of the saliva, gastric and duodenal phase. No colon digestion phase was carried out. Each of the three digests was homogenized (10,000 rpm, 1 min) and kept at −80°C until analyses. The saliva and gastric digests were not diluted to the final volume found in the duodenal phase before conducting the analyses but the dilution factors were taken into account in order to report the results with respect to the total final digestive fluid found in the duodenum.

2.3 | Carnosine quantitation

Free carnosine measurement in the meat was carried out as described (Mora et al., 2007). This method was also adapted for the measurement of carnosine in the digests. Accordingly, the saliva phase was first diluted at a 1:1 ratio with HCl 0.01N, then 250 uL volumes of each of the diluted saliva phase and the undiluted gastric and duodenal phases were mixed with 750 µl acetonitrile and centrifuged (10,000 rpm 20 min) at 4°C. HPLC analysis was performed on the sample extracts using a Waters Alliance 2,695 HPLC instrument (with W600 pump, Waters Corporation, MA, USA) equipped with a HILIC Silica pre-column (Atlantis 3.9 × 5 mm, 3 µm; Waters Corporation, MA, USA) and a HILIC Silica column (4.6 × 150 mm, 3 µm; Waters Corporation, MA, USA). The flow was adjusted as follow: the solvent gradient was linear from 0% (solvent B) and 100% (solvent A) to 70% (solvent B) and 30% (solvent A) in 9.1 min and then maintained for 2.0 min followed by a return to 0% (solvent B) and 100% (solvent A) in 2.0 min, and maintained for another 10.0 min.

2.4 | Lipid oxidation markers: hexanal, MDA, and 4-HNE determination

The measurement of free hexanal, MDA, and 4-HNE in the digests was adapted from Tsikas et al. (2016) using Agilent 7890B gas chromatograph coupled to a 5977B quadrupole mass spectrometer (Agilent Technologies, CA, USA) in SIM mode (electron impact ionization). For the first derivatization, 200 µl of digest, 170 µl of PFB-HA (O-(2,3,4,5,6-Pentafluorobenzyl) hydroxylamine hydrochloride) (30 mg/1 ml of H_2O), and 10 µl of HHE-d_3 (20 ng/1 µl of ethanol) were mixed with a vortex and incubated in an ultrasonic bath for 3 min (B1510R-MT, Branson Ultrasonic, USA). Subsequent steps were performed as reported (Tsikas et al., 2016) except for the extraction where centrifugation was carried out at 2,700 rpm for 5 min.

2.5 | Protein carbonyls determination

Protein carbonyls colorimetric determination was carried out according to Ventanas et al. (2006) using 200 µl of digest (0.1 g meat/ml) with 200 µl of 20% trichloroacetic acid (TCA) and 2 ml of 10 mM (in 2N HCl) 2,4-dinitrophenylhydrazine. Centrifugation parameters were changed to 3,000 ×*g* for 5 min and pellet washing was repeated three times and then dissolved with 2.5 ml of 20 mM sodium phosphate buffer containing 6M guanidine hydrochloride (pH = 6.5). Results are expressed as nmol/mg protein.

2.6 | Free thiols determination

Thiols were determined using the 2, 2′-dithiobis (5-nitropyridine) (DTNP) colorimetric method (Martinaud et al., 1997) with the following modifications. Protein concentration of the digest was determined with the biuret method and adjusted to 5 mg/ml with 200 mM phosphate buffer (pH = 7.4). Urea buffer (100 mM phosphate buffer, pH 8.0, containing 8M urea) was used to adjust the protein concentration to 1 mg/ml. The calculated results are expressed as µmol/mg protein.

2.7 | N^{ε}-(carboxymethyl)lysine level

An OxiSelect™ CML competitive enzyme-linked immunosorbent assay (ELISA) kit (Cell Biolabs, Inc. San Diego CA, USA) was used as recommended for the determination of N^{ε}-(carboxymethyl)lysine as a marker of AGE formation. Digested samples were diluted with the PBS buffer as per the instructions. Results are expressed as ng/g meat.

2.8 | Statistical analyses

Statistical analyses of all data were carried out by the MIXED procedure of SAS version 9.4 (SAS, 2002–2012; SAS Institute Inc., Cary, NC, USA) according to a complete 2 × 2 × 2 factorial (two carnosine levels, two fat levels, and two cooking conditions) and using heterogeneous variances when appropriate. The entire protocol was repeated four times. Therefore, the total number of observations was 32 (8 treatments with four repetitions) for each of the salivary, gastric, and duodenal phase. $p < .05$ was considered as significant with tendency defined as $0.05 < p < .10$. Interactions were further analyzed using partitioned analysis (slice option of the LS Means statement). A Principal Component Analysis (PCA) of the markers measured in the digests was also carried out.

3 | RESULTS AND DISCUSSION

3.1 | Carnosine quantitation

The addition of external carnosine (290.2 mg/100 g meat) to complement the intrinsic level measured in pork *LM* (309.8 mg/100 g meat) in order to obtain 600 mg carnosine/100 g meat in the HCar group was arbitrarily selected considering that other species such as poultry and some fishes with comparable muscle glycolytic profile contain even much higher amount of histidine-containing dipeptides (Boldyrev et al., 2013). Compared to their respective level before cooking, the carnosine concentration measured in the cooked meat was 350.4 ± 14.9 mg/100 g meat in the LCar group (without added carnosine), and 438.5 ± 39.0 mg/100 g in the HCar group. The literature on carnosine level after meat cooking is scarce and contrasted. Both increased (Park et al., 2005) and decreased (Peiretti et al., 2012) carnosine concentration were reported after cooking, which in the latter case was attributed to the potential but unmeasured loss in cooking juice considering the hydrophilic nature of carnosine. However, the reason why the level of free carnosine in the HCar group was lower than expected is not clear. It has been shown (Miwako et al., 2015) that no significant heat degradation of carnosine occurs at the cooking intensities used herein. It is, therefore, possible that the partial disruption of cellular membranes that occurs upon mincing (Honikel, 2014) maintained some level of protection or shelter that may have reduced the interacting potential of the intrinsic carnosine with its undigested matrix compared to the added one. As result for the in-vitro digestion assay, the HCar group contained 25% higher free carnosine than the LCar treatment. Such a difference between the two carnosine treatments is in the range naturally found in *LM* carnosine level between pigs (D'Astous-Pagé et al., 2017).

The effect of digestion on carnosine levels in the digests expressed as both absolute values and percent of their respective amount measured in the cooked meat before digestion is shown in Table 2. A progressive decrease in carnosine reaching over 50% in some treatments occurred from the saliva to the gastric and duodenal phase with absolute carnosine level from HCar treatment remaining significantly greater ($p < .05$) in all three digestion phases. No single effect of fat or cooking treatments were observed in the saliva and gastric phases ($p > .05$), but carnosine level remained greater in the LF-LCI duodenal digest as result of a fat x cooking interaction ($p = .0077$). Increased fat and cooking intensity of the meat are two important factors that contribute to its oxidation during digestion (Van Hecke et al., 2014, 2015) and may account in part for the observed decrease in carnosine most likely due to the involvement of its antioxidant capacity without precluding, however, the potential implication of its other properties such as pH buffering, carbonyl scavenging and metal ions chelation capacity.

3.2 | Hexanal

Results on hexanal formation, an aldehyde commonly used as a lipid oxidation marker, in each of the three digestive phases are presented in Table 3. Globally, the increase in hexanal occurred throughout digestion from either saliva to gastric or gastric to duodenal or both depending on the treatment and was paralleled with the observed decrease in free carnosine (Table 2). Significant interactions between carnosine level and fat content were found in the saliva ($p < .0001$) and gastric ($p < .0001$) with a tendency also observed in the duodenal phase ($p = .0729$). These interactions showed that enhanced carnosine level decreased hexanal formation in the LF meat, which suggests also that the efficacy of carnosine in limiting lipid oxidation depends on its proportion relative to the fat content of the meat. The capacity of carnosine to decrease hexanal formation in solution has been reported by Zhou and Decker (1999). An interaction of carnosine with cooking intensity was also observed in each of the saliva ($p < .0001$), gastric ($p < .0001$), and duodenal ($p = .0492$) phase, indicating that less hexanal was formed in HCar-LCI meat compared to the other treatments. Compared to undigested raw and cooked meat, the oxidative effects of increased fat content and cooking intensity on increased hexanal formation after duodenal digestion has also been reported by Van Hecke et al. (2014, 2015). An interaction between fat and cooking intensity ($p < .0001$) was found in both saliva and gastric phases, indicating a synergistic pro-oxidative effect of the two factors. As result, higher hexanal levels were found in HF-HCI samples in the saliva and gastric digest.

3.3 | 4-HNE

Results for 4-HNE, a n-6 PUFA peroxidation product, are reported in Table 3. Contrary to hexanal, which showed an increase throughout digestion, the 4-HNE level generally increased from saliva to gastric and decrease thereafter in the duodenal phase except in the two LF-LCI groups and the HCar-LF-HCI group. A reduction of 4-HNE in the duodenum has earlier been reported (Steppeler et al., 2016) and was explained by the ability of 4-HNE to react further with other compounds such as DNA and protein. Enhanced 4-HNE formation

TABLE 2 Effects of carnosine level, fat content and cooking intensity on carnosine content during in-vitro digestion of ground pork

		Low Carnosine				High Carnosine
		Low Fat		High Fat		Low Fat
	Phase	Low cooking intensity (LCI)	High cooking intensity (HCI)	Low cooking intensity (LCI)	High cooking intensity (HCI)	Low cooking intensity (LCI)
Carnosine (mg/100 g cooked meat before digestion)		354.22±9.01	346.21±7.41	338.42±14.45	362.87±9.81	458.39±28.87
Carnosine (μg/ml digest)	Salivary	1,258.87 (79.69%)	1,221.30 (79.18%)	1,265.98 (86.27%)	1,131.51 (71.40%)	1,620.88 (80.33%)
	Gastric	885.50 (56.34%)	1,049.66 (68.20%)	1,056.05 (72.02%)	1,051.09 (66.35%)	1,197.08 (59.22%)
	Duodenal	765.76 (48.68%)	617.43 (40.12%)	466.58 (31.67%)	586.87 (37.05%)	1,094.32 (54.45%)

Note: Values for carnosine in cooked meat before digestion are presented as: Mean ± *SD*; For all other variables, Results are presented as LS Mean and (*SEM*); Car: carnosine; F: fat; C: cooking intensities. Percentage is calculated based on each treatment respective amount measured in the cooked meat before digestion. Statistical significance at $p \leq .05$; statistical trend at $0.05 < p < .10$. There was no triple interaction of the treatments ($p > .05$). Bold Indicates significance of (P > .05).

TABLE 3 Effects of carnosine level, fat content and cooking intensity on lipid oxidation markers during in-vitro digestion of ground pork

		Low carnosine				High carnosine
		Low Fat		High Fat		Low Fat
	Phase	Low cooking intensity (LCI)	High cooking intensity (HCI)	Low cooking intensity (LCI)	High cooking intensity (HCI)	Low cooking intensity (LCI)
Hexanal (μg/ml digest)	Salivary	2.35 (0.08)	1.81 (0.07)	2.81 (0.07)	4.39 (0.07)	1.64 (0.08)
	Gastric	2.53 (0.14)	3.33 (0.14)	4.25 (0.14)	6.37 (0.17)	0.89 (0.14)
	Duodenal	3.37 (0.14)	3.41 (0.14)	9.11 (0.49)	10.18 (0.49)	1.82 (0.14)
4-HNE (ng/ml digest)	Salivary	42.6 (1.2)	53.0 (0.6)	98.8 (0.4)	94.8 (3.0)	40.8 (1.8)
	Gastric	37.6 (6.0)	71.6 (6.0)	239.8 (20.2)	233.4 (20.2)	22.8 (6.0)
	Duodenal	24.0 (1.4)	23.8 (1.4)	160.6 (8.4)	114.0 (8.4)	1.4 (1.4)
MDA (μg/ml digest)	Salivary	1.64 (0.11)	1.74 (0.02)	2.28 (0.02)	2.20 (0.05)	1.30 (0.03)
	Gastric	1.93 (0.02)	2.75 (0.09)	4.74 (0.29)	5.08 (0.13)	1.01 (0.01)
	Duodenal	2.29 (0.15)	2.40 (0.15)	4.94 (0.15)	4.60 (0.15)	0.90 (0.15)

Results are presented as LS Mean and (*SEM*). Statistical significance at $p < .05$; statistical trend at $0.05 < p < .10$. There was no triple interaction of the treatments ($p > .05$).

Bold Indicates significance of (p > .05).

Abbreviations: Car, carnosine; F, fat; C, cooking intensities.

occurred in the LF samples cooked at higher intensity as result of a significant interaction between fat content and cooking for each of saliva, gastric, and duodenal phase ($p = .0002$, $p = .0189$, $p < .0001$, respectively). This interaction could be explained by a synergistic increase in heat conductivity from HCI combined with that from the higher water content inherent to the LF samples (Ahmed et al., 1995). As a single effect, increased fat content increased the formation of 4-HNE in each digestion phases ($p < .0001$) (Table 3). Increased 4-HNE in duodenal in-vitro digest of HF pork samples has also been observed (Van Hecke et al., 2014). No single or interactive effect of carnosine was found in the saliva ($p > .05$) but in the gastric phase, increased carnosine significantly decreased 4-HNE concentrations in all treatments ($p < .001$). In the duodenum, an interactive effect of carnosine with fat ($p = .0119$) was observed. Increased carnosine led to reduced 4-HNE in LF treatments and particularly in the HCar-LF-LCI one where 4-HNE was reduced below its detection limit. A carnosine by cooking interaction ($p = .0255$) was also observed in the duodenal phase. Low cooking intensity led to increased formation of 4-HNE in the low carnosine group, for which we have no clear explanation. The capacity of increased carnosine in decreasing the level of 4-HNE in the gastric phase and also in inhibiting its duodenal formation in low oxidative sample such as the LF-LCI one is in accordance with the demonstrated ability of carnosine to quench 4-HNE in phosphate buffer (Aldini et al., 2002).

3.4 | MDA

In Table 3, MDA generally increased from the saliva to the gastric phase. Afterward, a decrease from gastric to duodenum was generally observed except in the two LCar-LCI groups where MDA increased further. Similar to 4-HNE, the decreased MDA levels between the stomach and duodenum phases may also be due to the known capacity of MDA to react further with other molecules

				p values					
	High Fat								
High cooking intensity (HCI)	Low cooking intensity (LCI)	High cooking intensity (HCI)	*SEM*	Car	F	C	Car × F	Car × C	F × C
409.56±6.10	404.84±14.29	481.36±7.17	/						
1587.25 (88.46%)	1599.13 (89.61%)	1646.95 (78.27%)	45.86	**<0.001**	0.7332	0.2354	0.3617	0.1640	0.9061
1,141.57 (63.86%)	1,265.12 (71.01%)	1,319.93 (62.94%)	96.63	**0.0036**	0.1389	0.5674	0.7877	0.5640	0.8315
502.17 (27.81)	814.16 (45.74%)	809.37 (38.37%)	104.08	**0.0137**	0.3141	**0.0443**	0.2373	0.0652	**0.0077**

			p values					
	High Fat							
High cooking intensity (HCI)	Low cooking intensity (LCI)	High cooking intensity (HCI)	Car	F	C	Car × F	Car × C	F × C
2.13 (0.07)	2.70 (0.07)	5.05 (0.07)	0.4683	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
2.39 (0.14)	4.30 (0.14)	7.61 (0.14)	0.0052	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
3.16 (0.14)	8.76 (0.49)	1.06 (0.49)	0.1087	**<0.0001**	**0.0003**	0.0729	**0.0492**	0.1379
60.8 (7.2)	98.6 (2.4)	91.4 (3.8)	0.7915	**<0.0001**	0.0511	0.3148	0.4905	**.0002**
31.2 (6.0)	206.2 (7.0)	175.4 (7.0)	**<0.0001**	**<0.0001**	0.8712	0.2613	0.1282	**.0189**
25.6 (1.4)	165.2 (8.4)	134.4 (8.4)	0.8032	**<0.0001**	**0.0042**	**0.0119**	**0.0255**	**<0.0001**
1.50 (0.11)	2.15 (0.04)	2.15 (0.06)	**0.0005**	**<0.0001**	0.2233	**0.0403**	0.3459	0.0562
2.07 (0.09)	4.75 (0.33)	4.93 (0.08)	**0.0013**	**<0.0001**	**<0.0001**	**0.0061**	0.8774	**.0095**
1.53 (0.15)	4.43 (0.15)	4.41 (0.15)	**<0.0001**	**<0.0001**	0.3820	**0.0010**	0.0516	**.0156**

(Steppeler et al., 2016). In the HCar-LF-LCI, being the treatment with the lowest oxidative potential, MDA decreased throughout digestion. Significant interactions between fat level and cooking intensity were obtained in gastric ($p = .0095$) and duodenal ($p = .0156$) digests along with a trend in the saliva phase ($p = .0562$), all indicating, compared with HF samples, that HCI increased MDA formation in the LF ones, similar to what was reported for 4-HNE and attributed to the potentially higher heat conductivity of these samples. As single effects, increased fat contributed to increased MDA levels in each of the three phases ($p < .0001$), as observed by Steppeler et al. (2016) during in-vitro digestion of beef with increased fat level. Cooking of meat also increased MDA in the gastric phase ($p < .0001$). With respect to its raw state, increased MDA has been reported after cooking, with the highest intensity generating the highest MDA level (Hernandez et al., 1999). Increased carnosine however, reduced MDA formation in the LF groups as revealed by the carnosine x fat interactions in each of the saliva ($p = .0403$), gastric ($p = .0061$) and duodenal ($p = .0010$) digests. The capacity of carnosine to reduce the formation of MDA is well known (Colzani et al., 2015) and its addition (0.5%–1.5% carnosine) to ground pork has been reported to reduce TBARS in the cooked products (Decker & Crum, 1993). Carnosine can also react with MDA and prevent MDA-induced protein cross-linking and oligomerization (Hipkiss & Chana, 1998).

In the present study, the capacity of increased carnosine to reduce lipid oxidation during digestion was limited to the moderately cooked lean pork. Therefore, the potential protection of dietary carnosine against lipid oxidation in the GIT would be relative to its concentration with respect to the fat content and cooking conditions of the meat, among others. Given the further intestinal absorption of each of hexanal, 4-HNE and MDA and their respective health implications, further studies involving different combinations of pro-and antioxidant factors as found in meat-based meals are warranted to

better understand the conditions limiting or favoring the role of dietary carnosine.

3.5 | Protein carbonyls

Protein carbonyl levels expressed per mg of protein in the digest are presented in Table 4 which shows an increase from saliva to duodenal phase irrespective of the treatments. Since the oxidative degradation in the uncooked meat generally proceeds further during its digestion up to the duodenal phase (Rysman et al., 2016), the lower level of protein carbonyls measured in the saliva might reflect in part the protein carbonyl formation that occurred in the cooked meat prior to its digestion considering the short duration passage in this phase. The increased protein carbonyl level would be explained by the activity of oxidative enzymes along with other oxidants present the GIT as reported by Rysman et al. (2016) for pork and beef when compared before and after their in-vitro digestion. In the saliva phase, increased cooking intensity increased the formation of protein carbonyls in the LCar-LF group leading to the triple interaction of the treatments ($p = .0441$). This interaction could be explained by the reduced protection of LCar combined with the plausible extra heat conductivity of the LF samples as explained earlier for 4-HNE. Regardless, increased protein carbonyls has been reported in digest of pork cooked at 90°C for 30 min compared to 65°C for 15 min. (Van Hecke et al., 2015). In the gastric phase, cooking had no further effect on protein carbonyl ($p > .05$) but increased fat content increased their formation ($p < .0001$), which was slightly but significantly decreased by the increased level of carnosine in the meat ($p = .0257$). No further effect of carnosine toward protein carbonyls was observed in the duodenal phase. Instead, protein carbonyls increased with increased fat level ($p < .0001$). A similar increased duodenal formation of protein carbonyls has been reported by Van Hecke et al. (2014) who explained that in attaching to amino acid residues, MDA and 4-HNE can cause the indirect oxidation of proteins. In support, it has been shown during digestion of lean pork that lipid oxidized faster than protein (Bax et al., 2012). The carnosine reduction of protein carbonyl compounds in the gastric phase could, therefore, be attributable to its carbonyls scavenging capacity and/or its antioxidant activity toward lipid oxidation products (Boldyrev et al., 2013).

3.6 | Free thiols

The gradual decrease in thiol groups from the saliva to the duodenal phase irrespective of the treatments (Table 4), supports further the increase in protein oxidation occurring during digestion. When compared to the level measured before digestion, a similar decrease in free thiols has been reported in the duodenal digest of raw pork (Rysman et al., 2016). A continuous decrease in free thiols observed during in-vitro digestion of myofibrillar protein from gastric to intestinal phase has been attributed to the formation of disulfide bonds between oxidized sulfhydryl groups of cysteine (Oueslati et al., 2016). In the saliva phase, increased fat level ($p < .0001$) and cooking intensity ($p = .0164$) significantly decreased the level of free thiols. As reported, fat can contribute to decrease free thiols through their reactions with lipid oxidation products such as 4-HNE (Esterbauer et al., 1991) and MDA (Antonio et al., 2014). Heat, for its part can rupture the weak intramolecular forces of protein including disulfide bonds, leading first to unfolding and denaturation followed by the formation and rearrangement of new disulfide bridges upon further increase in temperature (Yu et al., 2017). This process can lead to aggregation and cross-linking with other polypeptides and potentially increase the digestion time of the meat (Bax et al., 2012). Meat with increased level of carnosine however, maintained a significantly higher ($p = .0005$) level of free thiols in the saliva in line with its antioxidant capacity. This carnosine protective effect could have occurred through the scavenging of ROS or other radicals or through reduced lipid oxidation or both as discussed earlier. In the gastric phase, the same pro-oxidant effect of increased fat ($p < .0001$) and antioxidant effect of increased carnosine ($p = .0261$) in, respectively, decreased and increased free thiols were observed. However,

TABLE 4 Effects of carnosine level, fat content and cooking intensity on protein carbonyls and free thiols during in-vitro digestion of ground pork

		Low carnosine				High carnosine	
		Low Fat		High Fat		Low Fat	
	Phase	Low cooking intensity (LCI)	High cooking intensity (HCI)	Low cooking intensity (LCI)	High cooking intensity (HCI)	Low cooking intensity (LCI)	High cooking intensity (HCI)
Protein carbonyls (nmol/mg protein)	Salivary	2.21 (0.18)	3.38 (0.18)	2.80 (0.18)	2.51 (0.18)	2.37 (0.18)	2.73 (0.18)
	Gastric	2.93 (0.24)	3.11 (0.24)	4.42 (0.24)	3.83 (0.24)	2.54 (0.24)	2.98 (0.24)
	Duodenal	3.24 (0.40)	3.84 (0.30)	4.89 (0.76)	5.07 (0.06)	2.58 (0.19)	3.43 (0.20)
Free Thiols (µmol/mgprotein)	Salivary	44.87 (1.77)	38.92 (1.77)	30.90 (2.05)	25.46 (1.77)	46.51 (1.77)	47.04 (1.77)
	Gastric	21.96 (2.42)	23.50 (2.42)	11.91 (2.42)	12.90 (2.42)	25.90 (2.42)	26.19 (2.42)
	Duodenal	6.38 (1.65)	11.78 (1.65)	8.18 (1.65)	6.60 (1.65)	5.10 (1.65)	8.31 (1.65)

Data are presented as LS Mean and (*SEM*). Statistical significance at $p < .05$; statistical trend at $0.05 < p < .10$.
Bold Indicates significance of ($p > .05$).
Abbreviations: Car, carnosine; F, fat; C, cooking intensities.

these effects of fat and carnosine observed in the saliva and gastric phases were no longer present in the duodenum ($p > .05$), whereas increased cooking intensity surprisingly led to increased thiols level ($p = .0272$), except in the LCar-HF group. We have no clear explanation for the reoccurrence of the cooking effect on increased thiols after the gastric phase. Nevertheless, the overall increase in protein oxidation during digestion and amplified to different extents by both increase fat content and cooking intensity is consistent with other reports (Van Hecke et al., 2014, 2015; Traore et al., 2012). In our study, the capacity of carnosine to reduce dietary protein oxidation in reducing protein carbonyls and increasing free thiols was observed in the saliva and gastric digests but not in the duodenum ones in spite of the remaining amount of free carnosine remaining in these digests (Table 2). This aspect warrants further research.

3.7 | N^{ε}-(carboxymethyl)lysine

Levels of CML, a commonly used marker of AGEs formation from carbonyls of either reducing sugar or lipid oxidation products are shown in Table 5. Compared to the lipid and protein oxidation markers, the increase in CML throughout the digestion phases and irrespective of the treatments was the most remarkable. It has been reported that the use of the ELISA approach can overestimate the CML content due to possible reactions of the antibody with some food components such as fat globules, oxidized lipids (Nowotny et al., 2018; Poulsen et al., 2013) and also N^{ε}-(carboxyethyl)lysine (CEL), another AGEs marker. Although the kit used herein is designed by the manufacturer to be more specific for protein-bound CML, we cannot rule out that any interaction might have occurred between the antibody and oxidized lipids. However, irrespective of the treatments, the exponential increase in CML during digestion being several folds higher than that of the lipid oxidation markers suggests that increased free amines, peptides and amino acids upon protein hydrolysis became available for reaction with carbonyls, and support some legitimate formation of AGEs during digestion. Recently, suggestions were formulated regarding the possibility that AGEs might be formed during digestion (DeChristopher, 2017; Hipkiss, 2018) and DeChristopher (2017) presented the perspective that the source of serum and urinary AGEs associated with dietary AGEs would be the intestine, not the food. To the best of our knowledge however, no report showing the formation of AGEs in the GIT is yet available and the results presented herein represent the first substantiation of this possibility. Yet, further validation with more direct measurement of CML and other AGEs is warranted.

In the saliva phase where the measured CML probably reflects at least in part some formation that occurred in the cooked meat before its digestion, as presented for the previous lipid and protein oxidation markers, a triple interaction of the treatments was found ($p < .0001$) (Table 5). This triple interaction is explained by the effect of each of increased fat content and cooking intensity in raising CML more importantly in the low carnosine group as revealed by the partitioned analyses (not shown). Thus, compared to the other treatments, the highest level of CML formation occurred in the most oxidative combination; the LCar-HF-HCI group. Oxidized lipids and higher processing temperature are known factors that affect the rate and amount of AGEs and ALEs formation during cooking (Poulsen et al., 2013). The capacity of carnosine to inhibit the formation of AGEs and ALEs has been reviewed (Freund et al., 2018; Ghodsi, 2019) in line with its capacity to scavenge ROS, inhibit glycation through its reaction with reducing sugars or with unsaturated aldehydic lipid oxidation products, and reverse through "transglycation" the early reaction between glucose and protein. In addition to prevent further degradation of the glycated protein, carnosine can also block the AGEs-induced cross-linking. From a structure-activity relationship, the imidazole ring of histidine is required for the antioxidant, metal chelation, and pH buffering activities of carnosine. However, both beta-alanine and histidine residues need to act synergistically in the inhibiting effect toward AGEs and ALEs as reviewed by Boldyrev et al. (2013).

In the gastric phase, another significant triple interaction of treatments ($p = .0265$) resulted this time in the lowest CML formed in the gastric digest of the HCar-LF-LCI group being the least oxidative

		p values						
High Fat								
Low cooking intensity (LCI)	**High cooking intensity (HCI)**	**Car**	**F**	**C**	**Car × F**	**Car × C**	**F × C**	**Car × F ×C**
2.86 (0.18)	2.87 (0.18)	0.8921	0.5134	**0.0240**	0.0906	0.3302	**0.0018**	**.0441**
3.29 (0.24)	3.84 (0.24)	**0.0257**	**<0.0001**	0.4150	0.3878	0.0555	0.3461	0.2111
4.90 (0.51)	5.15 (0.13)	0.3717	**<0.0001**	0.0937	0.3032	0.7682	0.3525	0.8681
34.77 (1.77)	32.38 (1.77)	**0.0005**	**<0.0001**	**0.0164**	0.8420	0.0753	0.6427	0.5087
15.50 (2.42)	18.91 (2.42)	**0.0261**	**<0.0001**	0.3709	0.6671	0.8659	0.7093	0.5960
4.00 (1.65)	7.97 (1.65)	0.1194	0.3140	**0.0272**	0.6827	0.4787	0.1956	0.1110

TABLE 5 Effects of carnosine level, fat content and cooking intensity on N^{ϵ}-(carboxymethyl)lysine (CML) formation during in-vitro digestion of ground pork

		Low carnosine				High carnosine	
		Low Fat		High Fat		Low Fat	
	Phase	Low cooking intensity (LCI)	High cooking intensity (HCI)	Low cooking intensity (LCI)	High cooking intensity (HCI)	Low cooking intensity (LCI)	High cooking intensity (HCI)
CML (ng/g meat)	Salivary	17.08 (2.44)	19.04 (3.83)	25.82 (2.77)	68.13 (3.24)	13.83 (1.23)	17.66 (0.91)
	Gastric	101.17 (10.02)	125.20 (24.22)	74.28 (2.84)	148.92 (16.19)	47.61 (11.09)	91.30 (5.50)
	Duodenal	635.79 (21.51)	832.01 (69.03)	1,033.04 (77.25)	1529.71 (112.53)	596.92 (65.60)	741.41 (93.62)

Data are presented as LS Mean and (*SEM*). Statistical significance at $p < .05$; statistical trend at $0.05 < p < .10$.
Bold Indicates significance of ($p > .05$).
Abbreviations: Car, carnosine; C, cooking intensities; F, fat.

combination of treatments. This triple interaction differing from that found in the saliva could be due to the reduced formation of AGEs which are not favored in acidic condition (Ott et al., 2014) such as found in the stomach. It could also be hypothesized that apart from its potential involvement as antioxidant, antiglycant, and radical scavenger, part of the available carnosine was also potentially involved as a pH buffer in the acidic condition of the stomach, resulting this way with significant carnosine protection occurring only in the HCar group under low oxidative condition. This is in agreement with increased carnosine level in the gastric phase being more efficient in reducing each of the three lipid oxidation products in the LF groups as presented earlier. Observably, each of hexanal, 4-HNE and MDA were, similar to CML, numerically lowest in the gastric digest of HCar-LF-LCI samples (Tables 3). Considering the low level of carbohydrate in meat, CML formation potentially occurred through the production of either glyoxal or methylglyoxal from lipid oxidation (Vistoli et al., 2013). It has been shown that the intense oxygenated free radical production in the stomach caused a large and rapid increase of myofibrillar lipid oxidation during in-vitro gastric digestion of meat (Oueslati et al., 2016).

In the duodenum, a carnosine × fat interaction ($p = .0123$) revealed that increased carnosine level in the meat was necessary to reduce CML formation in HF groups. An increased CML formation observed under high intensity cooking ($p = .0013$) was more prevalent in the duodenal LCar digest as per the statistical trend for the carnosine × cooking interaction ($p = .0561$). Although there is still debates on the health consequences of dietary AGEs in humans (Nowotny et al., 2018; Poulsen et al., 2013), Tessier et al. (2016) have clearly shown that protein-bound CML from the diet are found in high concentration in the kidney, intestine and lung of mice. This first report of the potential of carnosine to reduce AGEs formation in the GIT and particularly in the duodenum where they are the most prevalent (as measured by CML) and potentially absorbable will deserve more study but emphasizes further how carnosine in meat could be health beneficial in a low oxidative diet.

3.8 | Principal component analysis

In our study, the average carnosine level after duodenal digestion was 40.5% of the original level measured before digestion. This decrease in free or bioaccessible carnosine can be explained by the sum of its interaction with the components and oxidative state of the meat matrix during digestion. A higher level of bioaccessible carnosine, which correspond to the carnosine proportion released from the meat into the GIT, has been reported during in-vitro digestion of Bresaola (a lean and raw cured beef product) by Marcolini et al. (2015). The authors observed a decrease in accessible carnosine after the chewing and subsequent acidification in the gastric phase from an average 93%, compared to the amount in the raw undigested sample, to an average 85% following duodenal digestion for 3 hr. This decrease was explained by the extent to which the meat matrix could release carnosine into the soluble fraction. The higher complexity associated with the composition and preparation of the meat matrices used herein can explain the lower level of free carnosine measured compared to that of Marcolini et al. (2015). Results of the principal component analysis (Figure 1) provide an overview of the relations that occurred among the components during digestion. In this model, percent of variance from principal component 1 (60.1%) and 2 (22.5%) accounted for over 82% of the total variance. The positive association among the antioxidant compounds (carnosine and free thiol groups) in the upper left quadrant suggests their potential in sparing each other. In the upper right quadrant, the positive association between lipid oxidation products (hexanal, 4-HNE,

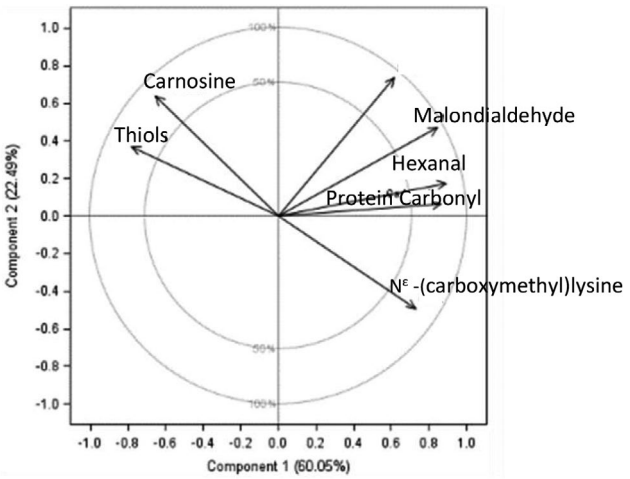

FIGURE 1 Principal component analysis

		p values						
High Fat								
Low cooking intensity (LCI)	High cooking intensity (HCI)	Car	F	C	Car × F	Car × C	F × C	Car × F × C
12.48 (0.50)	15.30 (0.95)	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
121.21 (12.48)	108.07 (28.08)	0.0857	0.0668	0.**0090**	0.0505	0.1465	0.8918	0.**0265**
854.11 (102.56)	924.52 (79.06)	0.**0012**	**<0.0001**	0.**0013**	0.**0123**	0.0561	0.3435	0.1265

and MDA) and protein carbonyls supports the interaction between lipid and protein oxidation as discussed. The negative correlation between the antioxidants (or reducing compounds) and oxidized compounds on PC 1 portrays the effective contribution of carnosine and thiols in reducing the formation of different oxidation markers. However, in absence of some other markers, it is not clear if the negative correlation between CML and oxidation markers on PC 2 is a direct or an indirect effect. For instance, considering the influence of increased fat on CML formation (Table 5), measuring glyoxal as a degradation product of lipid oxidation could have supported further, in our context, the possibility that CML derived mainly from glyoxal reacting with lysine side chains of proteins (Vistoli et al., 2013). Any possible contribution by the Maillard reaction per se cannot be ruled out, however. Nevertheless, the results obtained support a potentially important postprandial role that dietary carnosine can play immediately in the GIT in reducing the formation hence, the potential absorption of health deleterious compounds.

4 | OVERALL PERSPECTIVE AND LIMITATIONS OF THE STUDY

Recently, questions on whether or not carnosine from cooked beef with HF content could by itself efficiently prevent oxidation during digestion, or if a high-meat diet could efficiently reduce AGEs formation were raised by Van Hecke et al. (2017) and Freund et al. (2018), respectively. Here, we are reporting for the first time that a 25% increase in free carnosine in ground pork significantly reduced protein carbonyls, loss of free thiols and 4-HNE during in-vitro gastric digestion irrespective of fat and cooking level of the meat, and reduced hexanal, MDA and CML up to the duodenum phase in moderately cooked lean pork. This protective role of carnosine, however, reduced its accessibility, indicating the importance of maintaining or even improving the redox homeostasis in the meat itself and by extension in the diet for health benefits (Kanner et al., 2017). Additional research will be necessary before extrapolating these results to other types of meat due to the variation of inherent proportions of pro- and antioxidants in different species, including not only carnosine, but also its derivatives anserine, ophidine and their ratios, free fatty acid profile, and iron content. We have shown earlier the potential to attain a comparable higher intrinsic level (25%) of carnosine through genetic selection in pigs (D'Astous-Pagé et al., 2017) but it is not known yet if or to what extent a similar increase could be achieved in other species.

Although in-vitro digestion systems are useful alternatives to animal and human models for the study of oxidative processes (Bohn et al., 2018), the health-related benefits reported herein will need further validations, including human clinical studies. Our results bring forward a potentially important and previously unidentified role of carnosine occurring during digestion with a likelihood of protection first for the GIT itself considering among others the reported antiproliferative effect of carnosine on gastric and colorectal cancer cells (Cheng et al., 2019; Lee et al., 2018). The results, in addition, raise the possibility that the effects of carnosine in the GIT could potentially contribute further outside the GIT. Indeed, it has been reported (Houjeghani, Kheirouri, Faraji, & Jafarabadi, 2018; Houjeghani, Kheirouri, Faraji, Asghari Jafarabadi, & Jabbari, 2018), apart from reducing fasting blood glucose and glycated hemoglobin in type 2 diabetic patients, that carnosine supplement taken after a meal reduced serum level of MDA, protein carbonyls and CML compounds, part of which in the light of our results could have occurred earlier in the GIT. Such potentially important extra GIT benefit of dietary carnosine role in the GIT is further emphasized by the clinical results of Yubero-Serrano et al. (2015). The researchers orally administered sevelamer carbonate, an unabsorbable compound that can bind AGEs and appear to reduce their intestinal absorption, to fully treated T2DM patients with kidney disease. Compared to the controls, they observed reduced level of circulating and cellular AGEs (CML), restored levels of several innate defenses, improved inflammation and reduced chronic oxidative stress. A wider systemic potential of dietary carnosine in reducing CML formation and other lipid and protein oxidation products in the GIT is also consistent with the increased substantiation that dietary AGEs restriction could reduce oxidative stress and inflammation as reviewed by Nowotny et al. (2018).

Increased bioaccessible carnosine level has, therefore, the potential to counteract or at least interfere with some of the causes of the health conditions epidemiologically associated with red meat

consumption in some studies. Carnosine could in fact represent a very significant and unique health advantage of consuming meat as part of a balanced diet but more research is needed to understand the effect of carnosine in a larger meal context, including its composition, preparation and cooking process, and its overall redox status.

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CONFLICT OF INTEREST

The authors declared that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Investigation; Methodology; Writing-original draft; Writing-review & editing: **Li**. Resources; Supervision; Writing-review & editing: **Yaylayan**. Writing-review & editing: **Palin**. Funding acquisition; Writing-review & editing: **Sullivan**. Funding acquisition; Writing-review & editing: **Fortin**. Investigation; Methodology; Writing-review & editing: **Cliche**. Methodology; Writing-review & editing: **Sabik**. Conceptualization; Resources; Writing-review & editing: **Gariépy**.

ORCID

Claude Gariépy https://orcid.org/0000-0001-7205-9122

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