Research Article

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Effect of carnosine on nitrosamine formation in gastric-simulated aqueous and lipid environments

Yi Yao Li,[a,b] Varoujan Yaylayan,[a] Marie-France Palin,[c] Tania M Ngapo,[b] Simon Cliche,[b] Fleur Gagnon[b] and Claude Gariépy[b*]

Abstract

Background: Nitrite salts are frequently utilized as meat additives to improve the quality and safety of processed meat products. However, these salts are associated with the formation of carcinogenic nitrosamines. Given its potential regulating effect on the formation of intermediate molecules, such as nitric oxide, it is hypothesized that carnosine, a meat constituent possessing antioxidant activity and other multiple health benefits, could dampen the formation of nitrosamines. The current study therefore assessed the effect of carnosine on nitrosamine formation in both a monophasic aqueous system and a biphasic water–lipid system simulating a gastric environment.

Results: In the monophasic system, relatively high levels of carnosine were required to significantly reduce the formation of different species of nitrosamine compared with the control (no carnosine). While higher levels of some nitrosamines were generated in both phases of the biphasic system, low carnosine concentrations significantly suppressed nitrosamine formation in the aqueous phase, while in the lipid phase, intermediate levels of carnosine were required. At higher carnosine levels, further reduction in nitrosamines was observed in the lipid phase.

Conclusions: This study demonstrates the capacity of carnosine to reduce nitrosamine formation in aqueous and lipid environments and suggests the potential of dietary carnosine to lower the risks associated with the consumption of processed meat products.

Keywords: carnosine; secondary amine; nitrosamine; aqueous environment; lipid environment

INTRODUCTION

Processed meat products, such as bacon and sausages, are consumed on a daily basis by many North American[1] and European consumers.[2] Consumption of processed meat products has been associated with various health issues such as oxidative stress-related diabetes and cardiovascular diseases.[3] Different types of cancer have also been linked with carcinogenic nitrosamines induced by the use of nitrite salts.[4-8] The general mechanism by which nitrite salts (nitrite) can lead to the formation of harmful nitrosamines was detailed by Toldrá[9] as presented in Fig. 1.

Nitrite is an important and frequently used ingredient in many meat products as a color- and flavor-improving agent and as a preservative owing to its antimicrobial and antioxidant functions.[10] Owing to its versatility, nitrite as a meat additive cannot be replaced by any single molecule, in particular, for its important contribution to food safety because it can protect consumers against *Clostridium botulinum*, which produces lethal neurotoxins.[11] It is, therefore, important to be able to suppress nitrosamine formation in order to reduce the risks associated with the use of nitrites while also maintaining the advantages. Among a variety of compounds inhibiting nitrosamine formation, those naturally found in the diet, such as ascorbic acid, can easily be utilized with few safety risks.[12,13] Carnosine (*β*-alanyl-L-histidine), a naturally occurring molecule in meat, has been suggested to have the potential to reduce nitric oxide,[14] and therefore by deduction could also potentially reduce nitrosamine formation.

* *Correspondence to: C Gariépy, Saint-Hyacinthe Research and Development Centre, Agriculture and Agri-Food Canada, 3600 Casavant Boulevard West, Saint-Hyacinthe, Quebec, J2S 8E3, Canada, E-mail: claude.gariepy@agr.gc.ca*

a *Department of Food Science and Agricultural Chemistry, Macdonald Campus, McGill University, Montreal, Quebec, Canada*

b *Saint-Hyacinthe Research and Development Centre, Agriculture and Agri-Food Canada, Saint-Hyacinthe, Quebec, Canada*

c *Sherbrooke Research and Development Centre, Agriculture and Agri-Food Canada, Sherbrooke, Quebec, Canada*

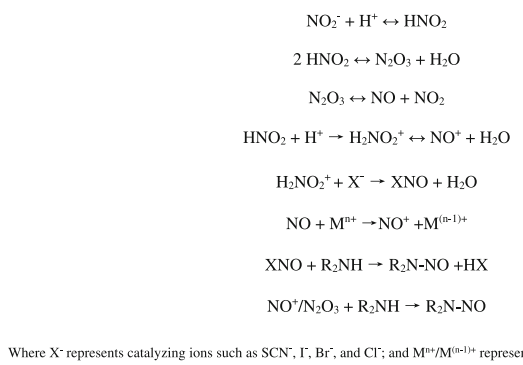

Figure 1. Nitrosamine formation as detailed by Toldrá.[9] Nitrite contributes to the generation of dinitrogen trioxide (N_2O_3) and the nitrosonium cation (NO^+), which subsequently reacts with secondary amines and produces nitrosamines.

The hypothesis that carnosine could affect nitrosamine formation is based on indirect observations. For instance, as part of its antioxidant activity, carnosine could potentially lower the levels of nitrosating agents, such as peroxynitrite, through its superoxide scavenging capacity. Superoxide is one of the precursors of peroxynitrite[14,15] which can generate nitrogen dioxide radicals that could take part in the formation of N_2O_3, a direct precursor of nitrosamines.[15] Furthermore, the L-histidine moiety of carnosine could potentially form adducts with nitric oxide (NO) or nitrogen dioxide (NO_2), subsequently reducing the formation of nitrosamine precursors, NO^+ and N_2O_3.[16] And, it was also shown by Caruso *et al.*[17] that carnosine could suppress NO generation *in vivo*, which may contribute to the control of nitrosamine formation *in vivo*. Other researchers, however, have suggested that carnosine may enhance nitrosamine formation *in vivo* by stimulating the activity of NO synthase, an inference based on the changes brought about by carnosine on NO formation,[14] which is not a direct precursor of nitrosamine. Information on the potential of carnosine to reduce nitrosamine formation, let alone its effects during the digestion process, is inconclusive and has never been clearly demonstrated. The study reported here, therefore, aimed to determine the effect of different carnosine concentrations on the formation of several nitrosamines in an aqueous model and, given that the stomach environment has been suggested to contribute to nitrosamine formation,[18] in a lipid–water model simulating a gastric environment.

MATERIALS AND METHODS

All chemicals used for the models and sample extractions were obtained from Sigma-Aldrich (Saint Louis, Missouri, USA) except for *N*-nitrosodimethylamine-d_6 (NDMA-d_6; CDN Isotopes, Pointe-Claire, Quebec, Canada) and QuEChERS products Bond Elut EMR-Lipid dSPE and Bond Elut EMR-Lipid Polish tubes (Agilent Technologies, Santa Clara, California, USA).

Monophasic system for assessment of carnosine's inhibiting potential

The monophasic (aqueous) model was based on that of Combet *et al.*[19] with the following modifications: (i) seven secondary amines that could contribute to the formation of seven detectable nitrosamines previously reported in foodstuffs[20] were selected; (ii) higher levels of each secondary amine precursor and a longer incubation time were used to form sufficient nitrosamines for quantification, based on Hinuma *et al.*[18] and data from a pre-test; and (iii) the sodium nitrite used was equivalent to the amount observed in digestive fluids.[21] Similar to Combet *et al.*,[19,22] no digestive enzymes were used since nitrosamine formation is a non-enzymatic chemical process and carnosine hydrolysis only occurs through the activity of carnosinase, which has only been reported in tissue and serum in humans.[14] A control (no carnosine) and seven carnosine levels (60, 120, 200, 300, 500, 1000 and 1300 μg mL^{-1}) were used, covering the range of possible carnosine levels that can be found in meat digests, given that meat is a natural source of this functional molecule.

Briefly, 10 mmol L^{-1} of each precursor secondary amine (dimethylamine (DMA), dibutylamine (DBA), diethylamine (DEA), dipropylamine (DPA), pyrrolidine (PYR), morpholine (MOR) and piperidine (PIP)), 1 mmol L^{-1} EDTA, 1 mmol L^{-1} potassium thiocyanate, 300 μmol L^{-1} sodium nitrite and different levels of carnosine were prepared in 0.1 mol L^{-1} HCl (pH 1.5; 3 mL; a simulated gastric pH) in a 5 mL sealed snap cap polypropylene tube and incubated for 4 h in a shaking water bath at 37 °C (50 rpm).[19]

Aqueous–lipid model for assessment of carnosine's inhibiting potential

Based on the method of Combet *et al.*,[19,22] glycerol tributyrate was chosen as the representative triacylglycerol for the lipid phase, and the ratio between water and lipid phases was 10:1 to simulate that in the stomach.[19] A control (no carnosine) and five levels of carnosine were used (120, 300, 500, 1000 and 1300 μg mL^{-1}).

The aqueous phase (3 mL) of the biphasic model was prepared following the same protocol as for the monophasic aqueous model described above, modifying the carnosine concentrations to 1.1 times those of the aqueous model (0, 132, 330, 550, 1100 and 1430 μg mL^{-1}) given that the final total volume of this water–lipid system was 3.3 mL. Glycerol tributyrate (0.3 mL) containing 10 mmol L^{-1} of each precursor secondary amine (DMA, DBA, DEA, DPA, PYR, MOR and PIP) was prepared as the lipid phase. The two phases were mixed in a 5 mL snap cap polypropylene tube and incubated for 4 h in a shaking water bath at 37 °C (50 rpm).

Analysis of nitrosamines in the monophasic model

The sample extraction method of Combet *et al.*[19] was followed with two modifications. The only internal standard used was 0.1 mg mL^{-1} NDMA-d_6, and the extract was concentrated to 50 μL final total volume after evaporation for better quantification. In brief, sample (1 mL) and internal standard (10 μL) were mixed with 0.08 mol L^{-1}M HCl saturated sodium chloride solution containing 50 mg mL^{-1} sulfamic acid (0.5 mL). Two consecutive extractions with dichloromethane–diethyl ether (45:55 v/v, with 25 μg mL^{-1} 2,6-di-*tert*-butyl-4-methylphenol to avoid interference from oxidation during extraction) were carried out. The top layer was collected and evaporated to 50 μL under a gentle nitrogen stream before being injected into a gas chromatography (GC)/mass spectrometry (MS) system. Calibration curves were prepared with different concentrations of the targeted nitrosamines in 0.1 mol L^{-1} HCl (pH 1.5; 1 mL) and mixed with the internal standard following the same extraction and evaporation processes as for the samples. The targeted nitrosamines were *N*-nitrosodimethylamine (NDMA), *N*-nitrosodibutylamine (NDBA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosodipropylamine (NDPA),

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N-nitrosopyrrolidine (NPYR), *N*-nitrosomorpholine (NMOR) and *N*-nitrosopiperidine (NPIP). All samples and calibration curves were undertaken in duplicate. Some adjustments to the GC/MS instrument were also made based on the information from Combet *et al.*[19] and Sieira *et al.*[23] The GC/MS system comprised an Agilent 7890B gas chromatograph coupled to a 5977B quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) with a VF-WAXms column (60 m × 0.32 mm × 0.50 μm; Agilent Theologies, CA, USA) in electron impact ionization and single ion monitoring modes. The oven temperature was programmed as follows: 37 °C for 1 min then increased to 220 °C at 8 °C min^{-1} where it was held for 7 min. Helium was used as carrier gas at a flow rate of 1 mL min^{-1}. The ions selected for different molecules were 74 *m/z* (NDMA, RT (retention time) = 14.77 min), 80 *m/z* (NDMA-d_6, RT = 14.76 min), 102 *m/z* (NDEA, RT = 16.18 min), 70 and 130 *m/z* (NDPA, RT = 18.53 min), 158 *m/z* (NDBA, RT = 21.37 min), 114 *m/z* (NPYR, RT = 22.11 min), 100 *m/z* (NPIP, RT = 22.55 min) and 116 *m/z* (NMOR, RT = 23.24 min).

Analysis of nitrosamines in the biphasic model

The two phases of the model were separated and the samples from the aqueous phase were extracted and analyzed as for the samples from the monophasic model (Section Analysis of nitrosamines in the monophasic model above). Samples from the lipid phase (200 μL) were mixed with 0.1 mg mL^{-1} NDMA-d_6 in acetonitrile (2 μL) and the nitrosamines were extracted and purified using a modified EMR – Lipid QuEChERS technique from Agilent.[24] In brief, 200 μL of this mixture was mixed with 10 mL of water by vortexing for 30 s, then incubated for 30 min. Acetonitrile (10 mL) was added and treated with buffered QuEChERS (9.75 g for method EN 15662). The following steps were as described by Sheshadri *et al.*[24] using two series of products, Agilent Bond Elut EMR-Lipid dSPE material and Agilent Bond Elut EMR-Lipid Polish material, to obtain the final extracts, which were concentrated threefold by evaporation and then analyzed by GC/MS. The oven temperature program was as follows: 55 °C for 1.5 min, then increased to 200 °C at 12 °C min^{-1}, held for 13 min, and then increased to 220 °C at 25 °C min^{-1} and held for 7 min. Although ions for different nitrosamines were all the same, retention times differed as follows: RT = 10.91 min for NDMA; RT = 11.02 min for NDMA-d_6; RT = 11.92 min for NDEA; RT = 13.46 min for NDPA; RT = 15.55 min for NDBA; RT = 16.34 min for NPYR; RT = 16.33 min for NPIP; and RT = 17.41 min for NMOR.

Statistical analysis

Statistical analysis of the data was carried out with the one-way analysis of variance procedure followed by the Tukey honestly significant difference test for multiple comparisons (SAS version 9.4, 2002–2012; SAS Institute Inc., NC, USA) with 8 carnosine levels (including the blank) for the aqueous model and 6 carnosine levels for each of the aqueous and lipid phases of the biphasic model. The entire protocol was repeated four times. Differences were considered significant at $P \leq 0.05$. All results are presented as LS Means with SEM.

RESULTS AND DISCUSSION

Effect of carnosine on nitrosamine formation in the monophasic system

Results from the aqueous model are presented in Table 1. Although the amount of each of the seven types of secondary amines used was the same, the corresponding nitrosamines formed to different extents. Indeed, NMOR reached the highest concentrations, ranging from about 85 to 850 times that of the other measurable nitrosamines, NDMA, NPYR, NPIP and NDBA. Signals for NDEA and NDPA were observable, but too small to be quantified.

Since unprotonated amines are more prone to nitrosation, the ammonium ion pK_a values of the secondary amines in such an acidic environment can partially explain the differences in nitrosamine formation – the less basic the amines and, therefore, the smaller the pK_a values, the higher the rate of formation will be, yielding more nitrosamines when incubated in the same environment.[25] Among the seven amines used in the current study, MOR, the secondary amine generating NMOR which was found at the highest nitrosamine levels, possesses the lowest pK_a (values reported by Perrin[26]; Table 2). Furthermore, the basic DMA with an intermediate pK_a value led to the third highest level of the nitrosamines, NDMA, and DEA, DPA, PIP and PYR alkylamines, with pK_a values from 11.0 to 11.3,[26] produced the lowest amounts of corresponding nitrosamines. However, the second highest nitrosamine level, NDBA, is derived from the alkylamine DBA that

Table 1. Effect of carnosine on the formation of nitrosamines (LS Mean with SEM) in a monophasic aqueous model

	Nitrosamine concentrations				
Carnosine level (μg mL^{-1})	NDMA (ng mL^{-1})	NPYR (ng mL^{-1})	NPIP (ng mL^{-1})	NDBA (μg mL^{-1})	NMOR (μg mL^{-1})
0 (control)	60.337^{a}	25.427^{a}	18.421^{a}	0.184^{a}	15.636^{a}
60	58.936^{ab}	24.665^{ab}	15.736^{ab}	0.162^{ab}	14.974^{ab}
120	56.412^{ab}	22.785^{ab}	15.038^{b}	0.146^{ab}	13.867^{ab}
200	55.872^{ab}	22.381^{ab}	15.157^{ab}	0.145^{ab}	14.183^{ab}
300	56.228^{ab}	22.177^{ab}	15.282^{ab}	0.146^{ab}	13.681^{ab}
500	53.410^{ab}	21.921^{ab}	14.048^{b}	0.141^{b}	13.580^{ab}
1000	52.284^{ab}	21.033^{b}	13.167^{b}	0.148^{ab}	13.982^{ab}
1300	49.399^{b}	21.266^{b}	13.208^{b}	0.130^{b}	12.871^{b}
SEM	3.000	1.000	1.000	0.010	0.500
P	**0.0077**	**0.0138**	**0.0028**	**0.0156**	**0.0226**

Values with different superscripts within the same column denote significant differences ($P \leq 0.05$).

shares (with PYR) the highest pK_a value of 11.3. Given that DBA, DMA and DPA have similar pK_a values, proton affinity cannot explain the differing levels of nitrosamines generated and further research is required.

Irrespective of the different concentrations of the various nitrosamines, it was generally observed that less nitrosamine was generated with the addition of carnosine. Among the five quantified nitrosamines, NDMA, NPYR and NPIP are usually found in meat products.[27] NDMA has one of the simplest nitrosamine structures and has been widely studied, not only in meat-related research, but also in medicine and other fields.[28] In the current study, only the highest carnosine level (1300 μg mL^{-1}) caused a significant decrease in the final NDMA concentration. Such high levels of carnosine were observed in the meat gastric digests following *in vitro* digestion of pork enhanced in carnosine,[21] of which the enhancement corresponded to levels found in different types of meat.[14] Although the overall decrease in NDMA was minor, with no more than 0.011 μg mL^{-1}, during the 4 h incubation, carnosine in the system was still efficient in showing its potential for lowering this nitrosamine formation. Likewise, the highest carnosine level (1300 μg mL^{-1}) also significantly suppressed NPYR and NPIP formation compared with the control group. Furthermore, carnosine levels of 120, 500 and 1000 μg mL^{-1} resulted in a significant decrease ($P < 0.05$) in NPIP formation, and 1000 μg mL^{-1} in NPYR formation compared with the control.

The efficiency of carnosine in reducing nitrosamine formation was also observed for nitrosamines whose concentrations were in the μg mL^{-1} range. For instance, carnosine at 500 and 1300 μg mL^{-1} exerted an inhibiting effect on the formation of NDBA ($P < 0.05$) and at 1300 μg mL^{-1} on NMOR generation. This high level of carnosine can be achieved during the digestion of carnosine-rich meat.[21] Overall, in this aqueous environment where carnosine is well dissolved, carnosine showed a capacity to inhibit the formation of different types of nitrosamines, consistent with the capacity of carnosine to form carnosine–NO and carnosine–NO_2 adducts as reported by Nicoletti *et al.*[16]

Table 2. The pK_a values of the secondary amines used in the current study[26]

Secondary amine ammonium ion	pK_a value
Dibutylamine (DBA)	11.3
Diethylamine (DEA)	11.0
Dimethylamine (DMA)	10.7
Dipropylamine (DPA)	11.0
Morpholine (MOR)	8.3
Piperidine (PIP)	11.1
Pyrrolidine (PYR)	11.3

Effect of carnosine on nitrosamine formation in the biphasic system

Data from the biphasic aqueous–lipid model are reported in Table 3. Except for NDPA, whose peak was below the quantification limit, nitrosamines from all other secondary amines studied were detected in each of the water and lipid phases of this biphasic system. In both the aqueous and lipid phases of the biphasic system, most of the measured nitrosamines were at higher levels than those observed in the monophasic aqueous system. The levels of nitrosamines observed in the biphasic model confirmed that the amounts of sodium thiocyanate, sodium nitrite and different secondary amines used were sufficient for nitrosamine formation and suggest that the low levels of nitrosamines observed in the aqueous model were not caused by an insufficient amount of reactants.

With the exception of NMOR, the lipid phase provided a better environment for the formation of all nitrosamines, leading to significantly higher levels ($P < 0.0001$). Similar observations were found by Combet *et al.*[19] who reported that the monophasic

Table 3. Effect of carnosine on nitrosamine formation (LS Mean with SEM) in the aqueous and lipid phases of the biphasic model

		Nitrosamine concentrations (μg mL^{-1})					
Phase	Carnosine level (μg mL^{-1})	NDMA	NDEA	NDBA	NPYR	NPIP	NMOR
Aqueous	0 (control)	0.195^{a}	0.238^{a}	0.178	0.204^{a}	0.179^{a}	13.673
	120	0.146^{b}	0.173^{b}	0.158	0.180^{ab}	0.143^{ab}	13.109
	300	0.147^{b}	0.173^{b}	0.156	0.154^{b}	0.139^{ab}	13.140
	500	0.143^{b}	0.168^{b}	0.154	0.151^{b}	0.136^{b}	13.062
	1000	0.144^{b}	0.156^{b}	0.141	0.137^{b}	0.137^{ab}	12.912
	1300	0.122^{b}	0.145^{b}	0.136	0.135^{b}	0.133^{b}	12.479
	SEM	0.008	0.015	0.010	0.011	0.010	0.402
	P	**0.0002**	**<0.0001**	0.0970	**0.0019**	**0.0283**	0.4898
Lipid	0 (control)	2.609^{a}	1.084^{a}	10.416^{a}	1.353^{a}	2.054^{a}	8.982
	120	2.395^{a}	1.006^{ab}	9.304^{ab}	1.211^{ab}	1.704^{ab}	7.763
	300	2.003^{b}	0.962^{ab}	8.734^{ab}	1.131^{ab}	1.770^{ab}	7.783
	500	1.842^{bc}	0.869^{abc}	8.782^{ab}	1.113^{bc}	1.540^{bc}	7.624
	1000	1.740^{bc}	0.788^{bc}	8.805^{ab}	1.014^{bcd}	1.215^{cd}	7.506
	1300	1.516^{c}	0.612^{c}	7.822^{b}	0.863^{d}	1.079^{d}	7.265
	SEM	0.115	0.080	0.532	0.055	0.087	0.412
	P	**<0.0001**	**<0.0001**	0.0637	**<0.0001**	**<0.0001**	0.1169

Values with different letters in the same column differ significantly ($P \leq 0.05$).

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aqueous model had less NDMA, NDEA and NPIP than the water phase from the biphasic model, and there were more NDMA and NPIP formed in the lipid phase than in both the monophasic and the aqueous phase from the biphasic model.

Compared with a monophasic aqueous environment, the higher concentrations of nitrosamines in the biphasic system could be mainly attributed to better solubilities of nitric oxide and oxygen in the lipid environment and higher reaction rates (up to 300 times higher) for the production of N_2O_3, which reacts with secondary amines to form nitrosamines.[29] The higher nitrosamine levels found in the lipid phase of the biphasic system may, therefore, be partially explained by this difference in N_2O_3 generation. The affinity of different nitrosamines for water or lipid needs to be considered, as Combet *et al.*[19] pointed out. For instance, being more hydrophilic than NDBA,[30] there might be less NMOR in the water phase for the potential diffusion into the lipid phase, resulting in lower NMOR than NDBA concentrations in lipid samples. This effect of solubility might also explain the concentrations of NDEA and NDBA in both water and lipid phases (according to the solubility reported by the European Medicines Agency[30]). As a result of the alkalinity of precursor amines, more NDEA than NDBA was observed in the aqueous environment. However, the higher lipophilicity of NDBA than NDEA[30] might explain why more NDBA was observed in the lipid phase.

Despite the biphasic system favoring nitrosamine formation, beneficial effects of carnosine were observed in the aqueous and lipid phase where NMOR and NDBA were the highest nitrosamines respectively formed in each of these phases. Compared to their respective controls, a high level of carnosine (1300 μg mL^{-1}) significantly decreased NDBA ($P < 0.05$) in the lipid phase, with a tendency also observed for decreased NDBA ($P = 0.074$) in the aqueous phase. A high level of carnosine (1300 μg mL^{-1}) also showed a tendency towards reduction of NMOR in the lipid phase ($P = 0.079$). Given the lower concentration of the other four types of nitrosamines, lower levels of carnosine were required to significantly reduce their formation ($P < 0.05$).

In the aqueous phase, all carnosine treatments significantly impeded NDMA and NDEA formation compared with the control group. Interestingly, even though the five carnosine levels studied here represent a relatively large range of concentrations (120 to 1300 μg mL^{-1}), the impact on the formation of these two nitrosamines did not differ with carnosine concentration ($P > 0.1$). Similarly, carnosine treatments from 300 to 1300 μg mL^{-1} all led to less NPYR than samples containing no carnosine, but increasing carnosine concentration did not further decrease the level of NPYR ($P > 0.1$). The carnosine treatments containing 500 and 1300 μg mL^{-1} significantly suppressed NPIP formation compared with the control group ($P < 0.05$). No significant differences were observed among the NPIP levels with added carnosine.

Although carnosine is a water-soluble molecule, its effect was also observed in the lipid phase. Different carnosine treatments led to less nitrosamine formation than the control group, and also brought about differences in concentrations for each of NDMA, NDEA, NPYR and NPIP. Carnosine treatments from 300 to 1300 μg mL^{-1} decreased the level of NDMA in the lipid phase compared with both the control ($P < 0.0001$) and the 120 μg mL^{-1} group ($P < 0.05$), which were not significantly different from each other. The highest carnosine level (1300 μg mL^{-1}) even further reduced the amount of NDMA when compared with the treatment containing 300 μg mL^{-1} carnosine ($P < 0.05$). These observations not only confirmed the ability of carnosine to decrease nitrosamine formation, but also indicated a greater capacity of carnosine to inhibit NDMA formation at a higher carnosine level. For NDEA, higher carnosine levels (1000 and 1300 μg mL^{-1}) were required to achieve significantly lower concentrations than the control group ($P < 0.0001$). The 1300 μg mL^{-1} treatment also led to significantly less NDEA than the 120 and 300 μg mL^{-1} treatments ($P < 0.05$). The observations in NPYR and NPIP among different carnosine levels also supported a dose-dependent nitrosamine-reducing property of carnosine. Significant decreases (compared with the control group) in both NPYR and NPIP levels were obtained with the 500, 1000 and 1300 μg mL^{-1} treatments. Furthermore, compared to that at 500 μg mL^{-1} carnosine treatment, the production of NPYP and NPIP was further decreased when the carnosine concentration was increased to 1300 μg mL^{-1} ($P < 0.05$), again demonstrating the potential dose-dependent response of carnosine on nitrosamine formation in a lipid environment.

Since carnosine is a hydrophilic antioxidant, its effect on nitrosamine concentrations in the lipid environment is likely attributed to carnosine reducing the amount of nitrosating agents in the aqueous phase. Fewer nitrosating agents available in the aqueous phase would result in fewer being available in the lipid phase for further reactions. For instance, when carnosine forms carnosine–NO and carnosine–NO_2 complexes in the aqueous environment,[16] the balance ($N_2O_3 \leftrightarrow NO + NO_2$) shifts increasing the consumption of N_2O_3 and reducing its availability to participate in nitrosamine formation. Simultaneously, since NO is prone to migrate to the lipid phase, the binding of NO by carnosine in water may lead to less NO diffusing into the lipid phase and, therefore, decrease the availability of NO for further reaction with oxygen, yielding less N_2O_3[29] for nitrosamine formation in the lipid phase.

Interestingly, instead of occurring in the aqueous phase (where carnosine could act directly), it was in the lipid phase that the dose-dependent nitrosamine-reducing potential of carnosine was mainly observed. The hypothetical explanation could be related to nitrosating agents other than the NO-derived N_2O_3 in the water phase. For instance, the NaSCN included in the acidic model could easily react with HNO_2 and generate the nitrosating agent ON-SCN ($HNO_2 + H^+ \rightarrow H_2NO_2^+$; $H_2NO_2^+ + SCN^- \rightarrow$ ON-SCN + H_2O).[31] This ON-SCN, in such a situation (containing different nitrosating agents), could be more electrophilic than N_2O_3 and exhibit a higher reaction rate for nitrosamine formation.[31] Therefore, the ability of carnosine to react with NO could only partially affect the nitrosamine generated by NO^+/N_2O_3, with little control in the nitrosamine derived from the reactions between ON-SCN and secondary amines. More studies supporting this hypothetical explanation and providing information about the mechanisms involved are necessary.

The current study directly demonstrates the inhibitory ability of carnosine on nitrosamine formation, and suggests that dietary carnosine may also bring this benefit in the digestive environment. Considering that the carnosine levels in meat can be enhanced through breeding and nutrition,[32,33] it is expected that beneficial effects of carnosine might be obtained by the consumption of a meal that contains carnosine-rich meat. However, the models used in the current study were relatively simple and there is still a lack of information on the complex environment associated with daily diets that may interfere with the possible carnosine nitrosamine-reducing ability. For instance, oxidation and nitrosation are related to each other. The formation of

oxidative peroxynitrite ($O2^{\bullet-}$ + NO → $ONOO^-$) might leave fewer nitrosating agents, such as nitric oxide, for nitrosamine formation.[34,35] Based on the results reported in this study and the potential mechanisms involved, muscle carnosine could directly contribute to reducing nitrosamine formation in meat products, even during their consumption, thus warranting further study.

CONCLUSION

Although absorption of nitrosamine from food is limited, increased endogenous nitrosamines due to a high intake of nitrite have been associated with an increase in cancer risk, particularly gastric and esophageal cancers. The results of this study demonstrate, for the first time, the potential of dietary carnosine to reduce the formation of nitrosamines in a simulated gastric environment. Additional studies are required to evaluate potential interactions among carnosine at dietary levels, nitrite and other molecules, such as additional antioxidants and secondary amines at concentrations generated during the production of different processed meat products, as well as the global effects of cooking, diet composition, and digestion processes, which could all impact the bioaccessibility of carnosine and, ultimately, its ability to inhibit nitrosamine formation. It is also essential to determine if the level of reduction of nitrosamines brought about by carnosine is sufficient to bring about practical health benefits. Indeed, given the exploratory nature of the current study, it is imperative to further explore these significant preliminary findings that show potential health benefits of carnosine toward the reduction of nitrosamine formation in the gastrointestinal environment.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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