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Effect of cooking method on carnosine and its homologues, pentosidine and thiobarbituric acid reactive substance contents in beef and turkey meat

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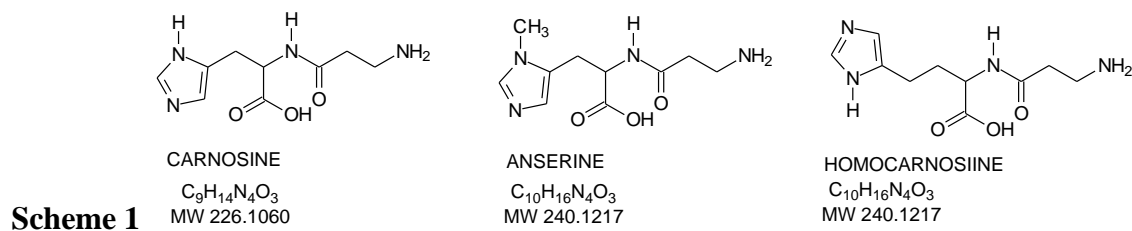
Abstract

Commercial samples of beef and turkey meat were prepared by commonly used cooking methods with standard cooking times: 1) broiled at 200°C for 10 min, 2) broiled at a medium temperature (140°C) for 10 min, 3) cooked by microwave (MW) for 3 min and then grilled (MW/grill) for 7 min, 4) cooked in a domestic microwave oven for 10 min, and 5) boiled in water for 10 min. The raw and cooked meat were then analyzed to determine the carnosine, anserine, homocarnosine, pentosidine and thiobarbituric acid reactive substance (TBARS) contents. It was observed that boiling beef determined a loss of approximately 50% of the carnosine, probably because of the high water solubility of carnosine and its homologues; cooking by microwave determined a medium loss of the anti-oxidants of approximately 20%; cooking by MW/grill led to a reduction in carnosine of 10% approximately. As far as the anserine and homocarnosine contents are concerned, the greater loss was observed for the boiling method (approximately 70%); while for the other cooking methods the value ranged from 30% to 70%. The data oscillates more for the turkey meat: the minimum carnosine decrease was observed in the cases of MW/grill and broiling at high temperature (25%). Analogously, the anserine and homocarnosine contents decreased slightly in the case of MW/grill and broiling at a high temperature (2-7%) and by 10-30% in the other cases. No analyzed meat sample showed traces of pentosidine above the instrumental determination limits. The cooked beef showed an increased TBARS value compared to the raw meat, and the highest values were found when the beef was broiled at a high temperature, cooked by microwave and boiled in water, respectively. The TBARS value of the turkey meat decreased for all the cooking methods in comparison to the TBARS value of the fresh meat.

Key words: Antioxidant; meat; lipid oxidation; heating; AGEs.

1. Introduction

It has been suggested that macromolecular glycation and the associated pathologies induced by sugars, deleterious aldehydes and ketones, (Brownlee, 2001) and glycotoxins produced during cooking (Koschinsky et al., 1997), could be improved by carnivorous diets containing carnosine (β-alanyl-L-histidine), anserine (β-alanyl-L-1-methyl-histidine) and homocarnosine, whose structure, elucidated in a previous paper of us (Peiretti, Medana, Visentin, Giancotti, Zunino & Meineri 2011), is γ-aminopropyl-L-homohistidine (scheme 1).

Scheme 1

CARNOSINE
$C_9H_{14}N_4O_3$
MW 226.1060

ANSERINE
$C_{10}H_{16}N_4O_3$
MW 240.1217

HOMOCARNOSIINE
$C_{10}H_{16}N_4O_3$
MW 240.1217

Krajcovicova-Kudlackova, Sebekova, Schinzel, & Klvanova (2002) found that levels of advanced glycosylation end products (AGEs) were higher in the plasma of vegetarians than those detected in omnivours people, while Hipkiss (2005) suggested that carnivorous diets contain a potential antiglycating agent, carnosine, whilst vegetarians may lack the intake of the dipeptide.

Carnosine and anserine have been found in the skeletal muscle tissue of most vertebrates, and their content has been determined in the muscle of many species (Davey, 1960; Suyama, Suzuki, Maruyama, & Saito, 1970). Carnosine is a major imidazole compound in mammalian animal tissues (Ohkuma & Abe, 1992; Abe, 1995) and it has been reported that a high content of carnosine exists in commercial meat products (Abe & Okuma, 1995).

Chan & Decker (1994) reported that anserine and carnosine could inhibit lipid oxidation through a combination of free radical scavenging and metal chelation. Carnosine could potentially act as a bioactive antioxidant, since it has been suggested to be absorbed intact (without hydrolysis of the peptide bond) through the intestine into the blood (Ferraris, Diamond, & Kwan, 1988).

Carnosine can react with protein carbonyls (Brownson & Hipkiss, 2000) and suppress AGE formation (Hipkiss et al., 1998) and AGE-induced protein modification (Hipkiss & Chana, 1998). It has been shown to inhibit the formation of cross-links induced by reducing sugars and other reactive aldehydes e.g. malondialdehyde and methylglyoxal (Hipkiss & Chana, 1998; Hipkiss & Brownson, 2000).

Heating, by either cooking, roasting, or other means, is very important in food processing with temperatures ranging from 100 to 250 °C. This thermal treatment enhances the formation of Maillard reaction products (MRPs) including Amadori compounds, dicarbonyls, melanoidins, and AGEs (Chuyen, 2006).

Pentosidine is an AGE and protein cross-link that results from the reaction of pentoses with lysine and the arginine residues of proteins (Sell & Monnier, 1989). The combination of these molecules, which results in pentosidine, is favoured by high temperatures of over 100°C for an extended period of time (more than 15'); moreover, the most suitable reagent amount (sugars, proteins) is also necessary in the reaction site. Water also influences the progress of the Maillard reaction: a low water content increases viscosity and renders the encounter of reagents difficult. On the contrary, a water excess hinders the reactions of the drastic dehydration that characterizes the process of non enzymatic browning. It therefore seems likely that pentosidine could also be formed during food processing (Henle, Schwarzenbolz & Klostermeyer, 1997).

In a previous report (Peiretti, Medana, Visentin, Giancotti, Zunino & Meineri 2011) we found that, among different livestock species, beef and turkey meat presented a high thiobarbituric acid reactive substance (TBARS) content, but low and high contents of antioxidant, respectively. In order to clarify whether thermal treatments can affect food protein crosslinking, we here describe the detection and quantification of carnosine, anserine, homocarnosine and pentosidine in beef and turkey meat submitted to different cooking methods. Furthermore, the effect of the contents of carnosine and anserine in beef and turkey meat, submitted to different cooking methods, on their TBARS content has also been investigated.

2. Materials and Methods

2.1. Chemicals

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L-Carnosine, phenylephrine hydrochloride, heptafluorobutanoic acid and trichloroacetic acid were purchased from Sigma-Aldrich (Milan, Italy) and used as received. The HPLC grade water was from MilliQ System Academic (Millipore, Vimodrone, Italy). The HPLC grade acetonitrile was from VWR (Milan, Italy). Pentosidine was synthesized in our laboratory, as reported in literature (Visentin, Medana, Barge, Giancotti, & Cravotto, 2010).

2.2. Sample collection and processing

Commercial samples of beef (fillet) and turkey (breast) (600 g per tray with six slices of meat each) were purchased from two local supermarkets in Turin, Italy. A slice of raw meat (100 g, thickness ~0.4 cm for turkey breast and ~0.6 cm for beef fillet) for each species and from each supermarket was immediately frozen at -20°C and then freeze-dried until analysed. The other meat slices were prepared for standard cooking with commonly used cooking methods: 1) broiled at 200°C for 10 min (5 min per side) in a meat grill (Tefal mod. GC3001) until a core temperature of 100°C was reached, 2) broiled at a medium temperature (140°C) for 10 min (5 min per side) until a core temperature of 70°C was reached, 3) cooked by microwave (3 min) and then grilled (7 min), until a core temperature of 70°C was reached, 4) cooked in a domestic microwave oven (Whirlpool mod. AMW234 WH) with a power setting of 750 W (10 min, 5 min per side), until a core temperature of 100°C was reached, and 5) boiled in water (100°C) for 10 min. The internal temperature was determined using TP 642 thermocouple piercing probes attached to an HD 8464 Delta Ohm digital thermometer.

All the meat samples were immediately frozen after cooking in liquid nitrogen, freeze-dried and then stored at –20°C until analysed. The samples were analyzed in triplicate. Each sample was weighed (50 mg) in a 10 mL polyethylene tube, together with the internal standard (phenylephrine hydrochloride, 2 ng/sample) and treated for 5 minutes with 3 mL of 0.6 M trichloroacetic acid (TCA) in order to denaturate the proteins. After centrifugation (15 minutes, 9000g), 500 µL of supernatant solution was ten-fold diluted using 5 mM heptafluorobutanoic acid and transferred into vials for analysis.

Standard solutions were prepared using the starting eluent as a solvent from 1 g/L water-methanol 1:1 stock solutions at 4 concentration levels (10, 50, 100, 200 mg/L).

2.3. LC-MS analysis (pentosidine, carnosine, anserine and homocarnosine)

The chromatographic separations were run on an Ultimate 3000 HPLC (Dionex, Milan, Italy) coupled to a high resolving power mass spectrometer LTQ Orbitrap (Thermo Scientific, Rodano, Italy), equipped with an atmospheric pressure interface and an ESI ion source. The samples were analyzed using an RP C18 column (Phenomenex Synergi 150 × 2.1 mm, 3 µm particle size) at a flow rate of 200 µL/min. A gradient mobile phase composition was adopted: 95/5 to 40/60 in 25 min, 5 mM heptafluorobutanoic acid/acetonitrile. The injection volume was 5 µL. The tuning parameters adopted for the ESI source were: source voltage 4.5 kV, capillary voltage 17.00 V, and tube lens 45 V. The heated capillary temperature was maintained at 265°C. The mass accuracy of the recorded ions (vs. the calculated ones) was ± 5 mmu (milli-mass units). Analyses were run using both full MS (50-700 m/z range) and MS/MS acquisition in the positive ion mode. The protonated molecular ions were 227.1182 m/z for carnosine, 241.1300 m/z for anserine and homocarnosine and 379.2094 m/z for pentosidine.

2.4. TBARS assay

The TBARS assay was modified from that of Witte, Krause, & Bailey (1970) and was performed for each meat sample in triplicate; 10 g of freeze-dried meat was homogenised for 30 sec at high speed with 20 mL of 10% trichloroacetic acid (TCA) using a Polytron tissue homogenizer

(Type PT 10-35; Kinematica GmbH, Luzern, Switzerland). After centrifugation of the homogenate (600 rpm for 5 min at 4°C), the supernatant was filtered through Whatman #1 filter paper. One millilitre of filtrate was combined with 1 mL of a 0.02 M acqueous 2-thiobarbituric acid solution (TBA), heated in a boiling water bath for 20 min together with a blank containing 1 mL of a TCA/water mix (1/1) and 1 mL of a TBA reagent and subsequently cooled under running tap water. The samples were analysed in triplicate and the results were expressed as mM malonyldialdehyde kg^{-1} DM, using a standard curve that covered the concentration range of 1 to 10 mM 1,1,3,3-tetramethoxypropane (Sigma-Aldrich, Steinheim, Germany). The absorbance was measured at 532 nm with a Helios spectrophotometer (Unicam Limited, Cambridge, UK) against a blank that contained all the reagents, but no meat.

2.5. Statistical analysis

The data were reported as the mean ± standard deviation and subjected to analysis of variance (ANOVA). Differences among the means were determined by means of the Duncan test with significance defined at $P<0.05$.

3. Results and Discussion

3.1 LC-MS analysis

Carnosine and its homologues (anserine and homocarnosine, CRCs) were identified in the meat on the basis of the preliminary separation/fragmentation study and quantified in the two species using calibration curves obtained from the standard carnosine. We used an ion pair reversed phase chromatographic method because of the very low hydrophobicity of these molecules, and we eluting them with heptafluorobutanoic acid in the mobile phase. Detection limits (LODs) (according to the S/N = 3 criterion) were 11 ng/mL for CRCs and 1 ng/mL for pentosidine; CRCs were quantified in the range 1-200 mg/L, so choosing a limit of quantification (LOQ) 1000 fold higher than detection limit. All of the CRC species were quantified using the carnosine calibration curve.

Figure 1 shows the MS^2 chromatograms of carnosine (top line), anserine (second line) and homocarnosine (third line) of the turkey MW cooked meat sample. The anserine and homocarnosine peaks were difficult to resolve by simple LCMS analysis and originate a single asymmetrical peak. In order to obtain a good visible discrimination, the anserine and homocarnosine signals were reported as the arithmetic difference of the main product ions originated by the positional isomeric precursor ions at *m/z* 241. Anserine was evidenced by subtracting the intensity of *m/z* 224 from that of *m/z* 170, while the homocarnosine peak rises were shown by subtracting *m/z* 170-224. This is possible thanks to the different relative intensity of these two ions. The MS^2 spectra were shown in a previous report (Peiretti et al. 2011).

3.2. Pentosidine (Advanced Glycation End Product)

The analysis showed traces of pentosidine above the instrumental determination limits (1 ng/mL equivalent to 20 µg/100 g meat) in all the raw and cooked meat samples.

The lack of pentosidine might be due to several factors. The selected standard cooking methods guaranteed relatively low core temperatures in the meat slices and the Maillard reaction was not able to reach the final stage, with a consequent low formation or even no formation of AGEs. In the same way, as previously reported, the transformations of the Maillard reaction proceed at the chosen time of cooking rather slowly; therefore cooking times of 10 minutes probably prejudiced the final amount of identifiable pentosidine in the meat through LC/MS. The food composition may also influence the formation of pentosidine and other AGEs; for example, a cooking method with added fat (butter, oil) could favour the formation of MRPs. Moreover, the

anti-glycation activity of the carnosine related compounds could have slowed down the synthesis of the AGEs. Chao, Hsu & Yin (2009) found that the interactions of sauce, heating and frying oil markedly enhanced the formation of MRPs in sauce-treated foods, and reported that the content of pentosidine in raw, boiled, fried and baked chicken, pork, beef, salmon and cod was in the range of 10-72 µg/100g, and the process of boiling, frying and baking significantly increased the level of pentosidine. They concluded that, in order to maintain the protein quality of food and decrease the production of MRPs, including pentosidine, it is better to reduce the baking process and/or sauces used for food preparation.

High AGE values were observed by Goldberg, Cai, Peppa, Dardaine, Baliga, Uribarri, & Vlassara (2004); meat and meat-substitute groups exposed to higher temperatures achieved a greater AGE content in the sample. The achieved AGE value trend was ovenfrying>deep frying and broiling>roasting>boiling.

3.3. Carnosine, anserine and homocarnosine

The mean percentages of carnosine and its homologues in the cooked meat compared to their initial values in raw beef and turkey meat are reported in figures 2 and 3, respectively. The final w/w percentages of these antioxidants in the raw and cooked meat of beef and turkey are shown in tables 1 and 2, respectively.

In the case of beef, it can be seen that boiling determined a medium loss of 50% of carnosine compared to the corresponding raw meat sample. A probable cause of this depletion is the high water solubility of carnosine ($LogD_{7.4}$ = -0.23, as reported by Nielsen, Supuran, Scozzafava, Frokiaer, Steffansen, & Brodin, 2002) and the related compounds. Cooking by broiling at medium and high temperatures led to a reduction in carnosine from 40 to 50 % and from 30 to 40 % in the raw meat, respectively. Cooking with a microwave oven with or without a grill determined the loss of less carnosine (mean values of 7% and 19%, respectively) and this confirms that they are good cooking methods. As far as anserine and homocarnosine content is concerned, again the greater loss was observed for boiling (mean values of about 70%); while the decreased order for the latter two molecules followed the sequence: MW/grill (mean value 35%), MW (mean value 43%), broiling at high temperature (mean value 58%) and broiling at medium temperature (mean value 66%). This confirms yet again that boiling led to the maximum antioxidant loss, that microwave heating is the milder method and that grilling at a high temperature induces a corresponding minor antioxidant degradation. This suggests that the formation of a hard surface layer on the meat prevents degradation of the carnosine and the related compounds.

As shown in Table 2, a similar trend was observed for turkey meat, even though the antioxidant loss was undersized compared to that observed for the beef. Cooking did not cause a significant decrease in the homocarnosine content in the turkey meat.

Park, Volpe, & Decker (2005) found that the carnosine concentrations increased from 274 to 277 mg/100 g of meat solids (dry weight basis) for raw and cooked beef, respectively, and suggested that no significant degradation of carnosine occurred during cooking. The authors suggest that the lower carnosine concentration could be due to the higher fat content of the beef used in this experiment. A higher fat content could decrease carnosine concentrations, since carnosine is mainly found in the cytosol of skeletal muscle and the presence of adipose fat could decrease skeletal muscle carnosine concentrations.

Furthermore, heat treatments that remove proteins from untreated extracts may cause protein denaturation by aggregation. Chan, Decker, & Means (1993) suggested that the lack of the complete recovery of carnosine might be due to the destruction of carnosine or an association of carnosine with precipitated proteins during concentration.

3.4. Susceptibility to lipid oxidation

Lipid oxidation was monitored in the two types of meat by measuring TBARS (Tables 1 and 2). The TBARS values were lower in the raw beef than those found in the raw turkey meat and the values were similar to those reported by Peiretti et al (2011) for turkey meat alone, while the TBARS value of the raw beef of the present work was lower than the mean data found by Peiretti et al (2011). Moreover, the latter value was affected by a large standard deviation, indicating a great variability of this parameter in the raw sample. However, a significantly low value of the TBARS measurement could be seen in both the boiled beef and boiled turkey, probably due to the solubilizing properties of water, but which could also be due to reactive substances.

The cooked beef showed TBARS values that increased compared to the raw meat. The highest values were found when the beef was broiled at a high temperature, cooked by microwave irradiation and boiled in water, respectively (Table 1). Lee, Hendricks and Cornforth (1998) found that lipid peroxidation in cooked beef increased with cooking and that carnosine inhibited TBARS formation with a dose-dependent effect. Carnosine (0.5–1.5%) reduced the formation of TBARS in cooked unsalted ground pork after 7 days of storage at 4°C. There was less carnosine antioxidant activity in cooked salted ground pork, with only 1.5% carnosine, which inhibited TBARS formation during refrigerated storage (Decker & Crum, 1993). Chan, Decker, & Means (1993) found that heat treatments, ultrafiltration and demineralization decreased the total iron and hemin concentrations and increased antioxidant activity in a beef extract (2 : 1, water : muscle). A beef extract with high carnosine and low proxidant levels could potentially be used as a natural antioxidant. Decker & Faraji (1990) found that heating carnosine at 100°C for 15 min had no effect on its ability to inhibit the catalysis of lipid oxidation by iron, hemoglobin, lipoxidase and singlet oxygen, therefore they concluded that carnosine has an excellent potential for use as a natural antioxidant in processed foods.

In the present experiment, the TBARS value of turkey meat decreased for all the cooking methods in comparison to the TBARS value of the fresh meat (Table 2). This is probably due to the highest anserine content in the turkey meat than in the beef meat and this suggests that this histidine-related compound also contribute to the major antioxidant effect in the turkey meat than in the beef meat when they were cooked. Boldyrev, Dupin, Pindel, & Severin (1988) demonstrated that carnosine and anserine could decrease the membrane lipid/oxidation by measuring the level of TBARS. Wu, Shiau, Chen, & Chiou (2003), studying the antioxidant activities of carnosine, anserine, some free amino acids and their combinations, found that anserine, with concentrations from 2.5 to 40 mM, exhibited the greatest reducing power among all the studied compounds. Their results revealed that anserine and carnosine are electron donors and could react with free radicals to convert them to more stable products and terminate radical chain reactions.

4. Conclusions

This research has supplied some indications about the preparation methods that could guarantee a low carnosine and homologues degradation without the formation of AGEs; considering the possible toxicity of these molecules, a more detailed study of cooking techniques and the formation of AGEs could become part of a useful alimentary education programme for consumers' health.

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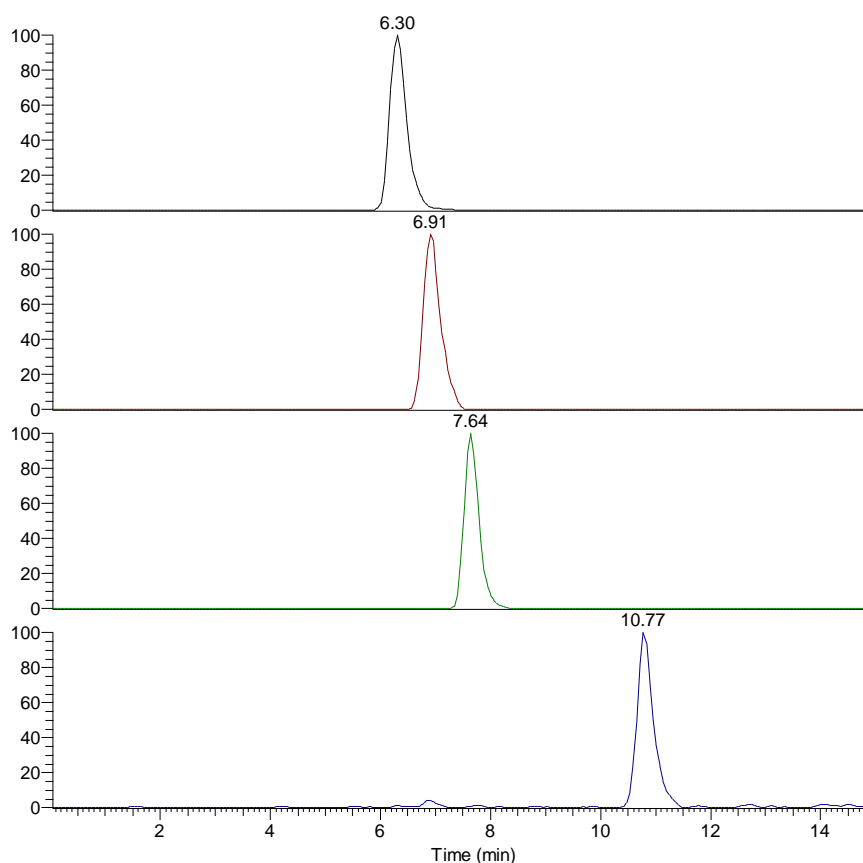
Figure 1. Carnosine (top line), anserine (second line), homocarnosine (third line) and phenylephrine I.S. (fourth line). MS^2 chromatograms of the microwave cooked turkey samples. A selected reaction monitoring of *m/z* 227-110 transition is reported for carnosine. The difference in the signals due to product ions originated by *m/z* 241 precursor ion is reported for isobaric anserine and homocarnosine: anserine *m/z* 170 – 224; homocarnosine *m/z* 224 – 170. Analytes concentrations of this sample were 8.16 (carnosine), 36.34 (anserine), 3.17 (homocarnosine) and 20.0 (phenylephrine) mg/L.

Figure 2. The mean carnosine, anserine and homocarnosine w/w percentage values measured in the beef samples. Raw = raw meat (not cooked); boiled in water 100°C x 10'; BRM = Broiled at a medium temperature 140°C x 10'; BRH = Broiled at a high temperature 200°C x 10'; MW = Microwave irradiation 100°C x 7'; MWG = Microwave irradiation+grill 70°C x 3'+7'.
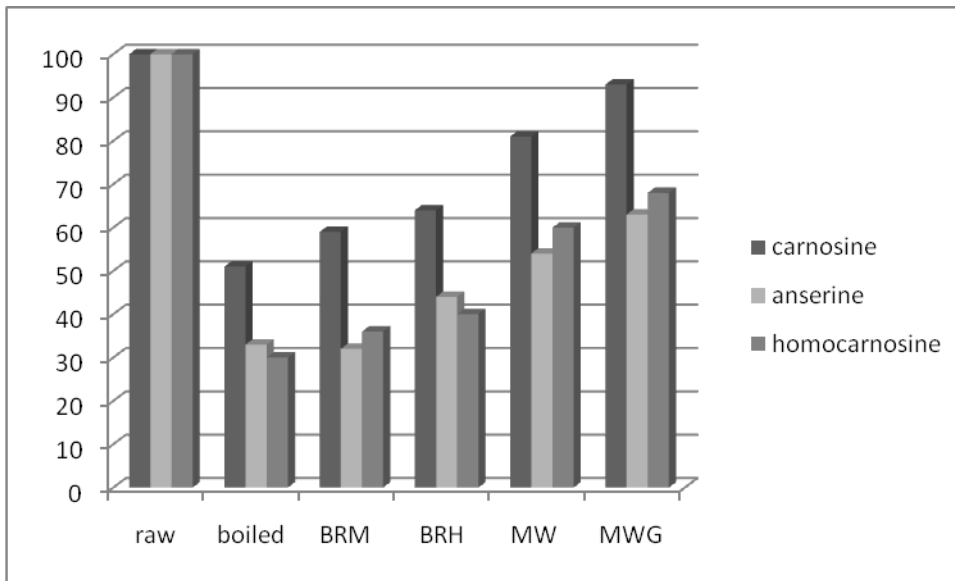
Figure 3. The mean carnosine, anserine and homocarnosine w/w percentage values measured in the turkey samples. Raw = raw meat (not cooked); boiled in water 100°C x 10'; BRM = Broiled at a medium temperature 140°C x 10'; BRH = Broiled at a high temperature 200°C x 10'; MW = Microwave irradiation 100°C x 7'; MWG = Microwave irradiation+grill 70°C x 3'+7'.
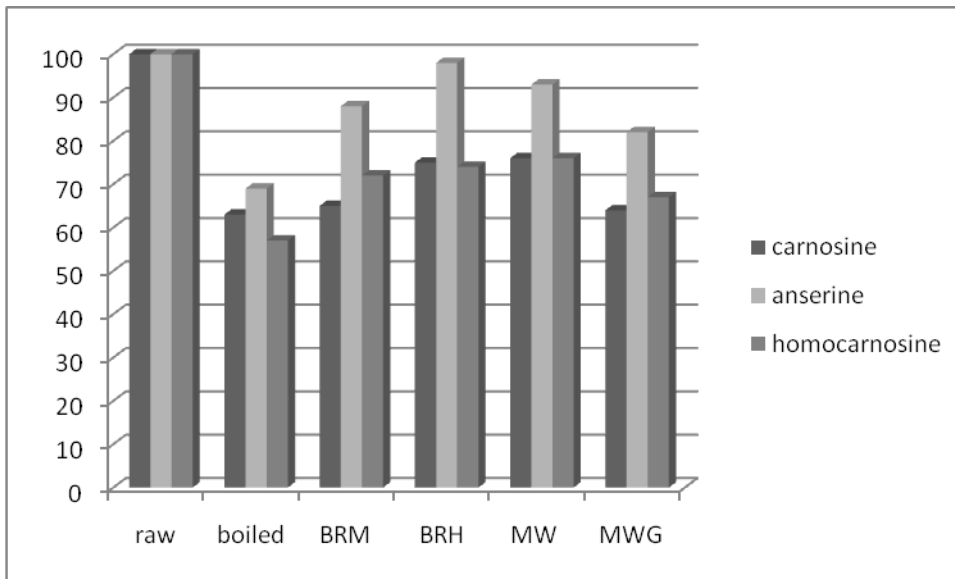
Table 1. Carnosine, anserine and homocarnosine (g/100g freeze dried sample) and TBARS contents (nM malonyldialdehyde/g freeze dried sample) in raw and cooked beef.

Cooking method	Supermarket	Carnosine	Anserine	Homocarnosine	TBARS
Raw	1	1.72 ± 0.28^a	0.16 ± 0.03^a	0.025 ± 0.011^a	11.67 ± 0.25^a
Boil	1	0.90 ± 0.05^d	0.08 ± 0.01^c	0.009 ± 0.002^b	17.90 ± 0.56^b
BRM	1	1.09 ± 0.13^{cd}	0.09 ± 0.01^c	0.011 ± 0.004^b	16.53 ± 0.49^b
BRH	1	1.16 ± 0.06^c	0.11 ± 0.01^b	0.012 ± 0.003^b	25.97 ± 1.46^d
MW	1	1.42 ± 0.10^b	0.12 ± 0.01^b	0.019 ± 0.008^{ab}	16.80 ± 0.44^b
MWG	1	1.61 ± 0.15^{ab}	0.16 ± 0.02^a	0.021 ± 0.010^{ab}	24.43 ± 1.15^c
Raw	2	1.68 ± 0.10^a	0.27 ± 0.03^a	0.032 ± 0.010^a	14.43 ± 0.35^a
Boil	2	0.81 ± 0.05^c	0.09 ± 0.01^{cd}	0.010 ± 0.002^c	20.70 ± 0.44^c
BRM	2	0.86 ± 0.21^c	0.09 ± 0.02^d	0.012 ± 0.007^{bc}	18.53 ± 0.81^b
BRH	2	1.00 ± 0.14^c	0.12 ± 0.02^c	0.015 ± 0.007^{bc}	25.40 ± 0.17^d
MW	2	1.32 ± 0.09^b	0.18 ± 0.01^b	0.022 ± 0.005^{ab}	28.53 ± 0.41^e
MWG	2	1.50 ± 0.11^{ab}	0.18 ± 0.01^b	0.023 ± 0.006^{ab}	21.40 ± 0.20^c

Raw = Raw meat (not cooked); Boil = Boiled in water 100°C x 10'; BRM = Broiled at a medium temperature 140°C x 10'; BRH = Broiled at a high temperature 200°C x 10'; MW = Microwave irradiation 100°C x 7'; MWG = Microwave irradiation+grill 70°C x 3'+7'.

The result are expressed as the mean±standard deviation of three determinations. Different letters in superscript, referring to the same supermarket, mean significant differences ($p < 0.05$).

Table 2. Carnosine, anserine and homocarnosine (g/100g freeze dried sample) and TBARS contents (nM malonyldialdehyde/g freeze dried sample) in raw and cooked turkey meat.

Cooking method	Supermarket	Carnosine	Anserine	Homocarnosine	TBARS
Raw	1	0.88 ± 0.09^a	2.73 ± 0.30^a	0.25 ± 0.11	26.03 ± 6.78^a
Boil	1	0.60 ± 0.04^b	2.00 ± 0.12^c	0.13 ± 0.04	9.40 ± 0.70^c
BRM	1	0.65 ± 0.04^b	2.59 ± 0.16^{ab}	0.18 ± 0.12	16.10 ± 3.48^{bc}
BRH	1	0.60 ± 0.02^b	2.30 ± 0.18^{bc}	0.16 ± 0.11	21.83 ± 1.30^{ab}
MW	1	0.58 ± 0.01^b	2.78 ± 0.11^a	0.20 ± 0.13	16.13 ± 6.79^{bc}
MWG	1	0.64 ± 0.08^b	2.76 ± 0.32^a	0.17 ± 0.11	11.17 ± 0.15^c
Raw	2	0.86 ± 0.04^a	2.87 ± 0.17^a	0.29 ± 0.14	43.33 ± 4.40^d
Boil	2	0.49 ± 0.02^c	1.98 ± 0.09^c	0.19 ± 0.10	15.73 ± 0.23^a
BRM	2	0.65 ± 0.05^b	2.76 ± 0.22^a	0.25 ± 0.14	15.57 ± 5.70^a
BRH	2	0.51 ± 0.05^c	2.41 ± 0.20^b	0.23 ± 0.11	23.23 ± 1.81^b
MW	2	0.53 ± 0.06^c	2.26 ± 0.18^{bc}	0.21 ± 0.11	31.23 ± 5.17^c
MWG	2	0.65 ± 0.04^b	2.86 ± 0.25^a	0.25 ± 0.12	23.07 ± 1.99^b

Raw = Raw meat (not cooked); Boil = Boiled in water 100°C x 10'; BRM = Broiled at a medium temperature 140°C x 10'; BRH = Broiled at a high temperature 200°C x 10'; MW = Microwave irradiation 100°C x 7'; MWG = Microwave irradiation+grill 70°C x 3'+7'.

The result are expressed as the mean±standard deviation of three determinations. Different letters in superscript, referring to the same supermarket, mean significant differences ($p<0.05$).