Carnosine and anserine in four classes of pork

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Abstract

The objective of this study was to determine carnosine and anserine concentrations in the four classes of pork: pale, firm, normal (PFN), red, firm, normal (RFN), red, soft, exudative (RSE) and pale, soft, exudative (PSE). Eighty loins were obtained from a local abattoir and classed into the four quality groups by abattoir staff using visual and tactile indices. Classification was also achieved in the laboratory using pH_{24}, drip $loss_{24}$ and lightness (L*) data. About half of the loins were classed the same. Carnosine and anserine concentrations were measured in meat samples and significant differences were observed for both classification methods, but these differences were not the same. Using the abattoir classification, concentrations of carnosine from PSE and RSE pork were higher than that of PFN pork; no differences in anserine concentrations were observed. In contrast, laboratory classification resulted in higher anserine concentrations in PSE than PFN pork, but no differences in carnosine concentrations. The influence of classification method on the dipeptide concentrations renders relationships with pork quality classes unclear.

Introduction

A quantitative measure of the resistance to pH change is termed buffering capacity and in meat has been correlated with both the rate of pH fall post mortem and ultimate pH, which in turn, are often found to influence, to varying degrees, traits of meat, including colour, drip loss and firmness. In pre-rigor muscle it has been suggested that carnosine and anserine contribute up to 40% of the buffering capacity (Bate Smith, 1938) and while concentrations differ in both species and muscle, it is generally agreed that these dipeptides are important components of buffering capacity. Could the content of these substances therefore explain, at least in part, differences in the quality traits, such as colour, firmness, exudation and ultimate pH that are used to define classes of pork quality? The objective of this study was to determine the differences in carnosine and anserine concentrations from the four pork classes, PFN, RFN, RSE and PSE.

Materials and methods

Chemicals and reagents: All chemicals and reagents were at least analytical grade. Water was deionised.

Meat: Eighty loins of 10 each of PFN, RFN, RSE and PSE pork on each of two days (1st and 16th November 2005) were obtained from a local domestic abattoir at 48 h post-mortem (p.m.). The loins were classed using visual and tactile indices by an abattoir staff member. Cubes (approx 1 cm^3) were cut from a slice of the *M. longissimus dorsi*, frozen in liquid N_2 and stored at -80ºC about 72 h p.m. Drip loss (2 days suspended storage at 4ºC), pH and lightness (CIELAB L*; Minolta Chroma Meter CR-300, Minolta, Japan) were measured at 24 h p.m. The loins used in this study form part of a larger trial.

Sample preparation: Samples were prepared as described by Aristoy & Toldrá (2004). Meat (2.5 g) was homogenised (Polytron PT 3100, Lucerne, Switzerland; 12 mm diam. foam inhibiting saw tooth dispersing aggregate; 4 x 60 s, approx. 15,000 rpm) in water (25 ml). The homogenate was centrifuged (10,000*g*, 4ºC, 20 min) and supernatant filtered using syringe driven filter units (Millex-AP 25 mm filter unit, glass fibre filter; Millipore, Japan). Methanol (900 µl) was added to the supernatant (300 µl), the mix vortexed 5 s, left to stand for 15 min (4ºC) centrifuged (12,000*g*, 4ºC, 3 min) and filtered (Millex-HN 4 mm filter unit, 0.45 µm pore size, nylon filter; Millipore, Japan).

Chromatographic analyses: Chromatographic analyses of carnosine and anserine were adapted from Aristoy & Toldrá (2004). The HPLC system (Waters Corporation, Massachussetts, USA) comprised a 600E Multisolvent Delivery System, a 717 Autosampler, a column heater and a 474 Scanning Fluorescence Detector. Separation was achieved by a Spherisorb 5 µm SCX column (4.6 x 250 mm) with guard column (4.6 x 10 mm) maintained at 45ºC (Ducci et al, 2004). The HPLC system was equilibrated for 15 min with 20% Solvent B (20% acetonitrile in 0.9 mM hydrochloric acid and 0.8 M sodium chloride, pH 3) and 80% Solvent A (20% acetonitrile in 0.8 mM hydrochloric acid, pH 3) at 1 ml/min. Sample (20 µl) was injected and after a further 5 min of isocratic elution, a 10 min gradient to 50% Solvent B was applied to achieve separation, followed by a 10 min washing step using 100% Solvent B. Solvents were filtered (0.45 µm

HVLP Durapore membrane filters, Millipore Corporation, Ireland) and degassed prior to use. Peak development was achieved by addition of pthaldialdehyde (OPA) solution (0.5 ml/min) immediately after the ion exchange column and prior to a reaction coil (0.025 mm i.d. x 200 cm; Waters RXN 1000 coil, Waters Corporation, Massachussetts, USA) connected to the detector. The OPA solution (pH 10.5-11.0) consisted of boric acid (15.5 g) and potassium hydroxide (13.0 g) in water (500 ml) to which was added 30% Brij-35 solution (1.5 ml) and 2-mercaptoethanol (1.5 ml). Finally, OPA (100 mg) in methanol (2.5 ml) was added and the solution mixed, filtered (0.45 μm HVLP Durapore membrane filters, Millipore Corporation, Ireland) and degassed prior to use. The reagent was prepared daily, protected from light and maintained under helium. No reagent deterioration was observed.

The OPA-developed peaks were measured using excitation and emission wavelengths of 340 and 445 nm, respectively, and the chromatograms analysed using the Millenium Version 3.20 software package (Waters Corporation, 1999). Carnosine and anserine peaks were readily identified by both internal and external standards of commercially-obtained purified compounds. Quantification was achieved using standard curves and concentrations were expressed in mg/100 g wet weight tissue. Two samples were analysed from each loin and each sample was chromatographically analysed twice.

Statistical analyses: Effects of meat type and collection day on dipeptide concentrations were analysed by ANOVA and significant differences were determined by least square (LS) means comparisons using the SAS MIXED procedure (SAS, 2007). Bonferroni adjustments were performed and the adjusted p-value used to determine significance for meat type since this factor has more than two levels. As there was no evidence of non-linear relationships between variables, Pearson correlation coefficients were calculated.

Results and discussion

One of the loins classed RSE at the abattoir had pH_{24h} 6.06, drip $loss_{24h}$ 4.49% and L* 45 indicating this muscle was possibly dark, firm and dry (DFD). This loin was therefore rejected giving only 9 RSE loins by abattoir classification. The mobile phase HCl concentrations here were lower than those of Aristoy & Toldrá (2004), achieving a pH within the acceptable range for the column (pH 2-8) and no observed differences in chromatographic output (data not shown).

The pH, drip loss and lightness (L*) of the meat classes are given in Table 1. Of particular note is the relatively high L* indicating an overall pale meat. The average values of these traits are reasonable for the four pork classes using abattoir classification. However, individually the traits of many of these loins suggest a necessity for an alternative classification and hence a 'laboratory' classification of the same loins was made using the categories described in Table 2. These criteria were determined both with reference to literature and a 'natural fit' of the data. Of the 79 loins, 41 were classified the same in the laboratory and abattoir. The smaller standard deviations, in general, of the laboratory classification indicate a greater homogeneity within a meat class for the given traits. Note that five loins were omitted from the laboratory classification to allow better definition of the classes.

Table 1. Loin traits (means and standard deviations, s.d.) for the pork classes by classification method[1]

	PFN		RFN		RSE		PSE	
	Mean	**s.d.**	**Mean**	**s.d.**	**Mean**	**s.d.**	**Mean**	**s.d.**
Abattoir classification								
pH_{24h}	5.53^{ac}	0.05	5.62^{b}	0.08	5.60^{bc}	0.14	5.49^{a}	0.10
$Drip_{24h}$ (%)	5.5^{ab}	1.6	4.1^{a}	1.2	4.6^{a}	2.5	6.8^{b}	2.5
L*	61^{a}	4	56^{b}	3	53^{a}	3	64^{b}	4
Laboratory classification								
pH_{24h}	5.51^{a}	0.06	5.65^{b}	0.11	5.55^{a}	0.05	5.48^{a}	0.09
$Drip_{24h}$ (%)	4.0^{a}	1.1	3.3^{a}	1.0	7.0^{b}	1.6	7.1^{b}	1.8
L*	63^{a}	2	54^{b}	3	56^{a}	3	64^{b}	3

[1]Means in a row with different superscripts are different ($p<0.05$)

Significant differences were observed among mean dipeptide concentrations, but these differences were not the same for a given classification method (Table 3). Using the abattoir classification, carnosine concentrations of PSE and RSE pork were higher than those of PFN pork ($p<0.5$); no differences in anserine concentrations were observed. In contrast, laboratory classification resulted in a higher anserine concentration in PSE than PFN pork, but no differences in carnosine concentrations. Moya *et al*. (2001) also found no differences in carnosine concentrations among the pork classes RFN, RSE and PSE.

Table 2. Laboratory classification criteria for the pork quality classes

	pH_{24}	$Drip_{24}$ (%)	L*
PFN	<6.0	<5.0	≥60
RFN	<6.0	<5.0	<60
RSE	<6.0	≥5.0	<60
PSE	<6.0	≥5.0	≥60

No effect of collection date was observed on dipeptide concentrations for laboratory classification. However, that of the abattoir resulted in lower carnosine and higher anserine concentrations on the 1st than the 16th November. The same person classified the loins both days and those classified the same as in the laboratory were almost equally divided between the two dates indicating consistency in the abattoir method.

Only two significant ($p<0.01$) correlations were observed between the pork traits and dipeptide concentrations, and these were small; the carnosine concentration with L* (r=-0.18) and the anserine concentration with $drip_{24\,h}$ (r=-0.16).

Table 3. Carnosine and anserine concentrations (conc; LS means and standard errors, S.E.)[1]

	Number of loins[2]	Carnosine conc (mg/100g)		Anserine conc (mg/100 g)	
		LS mean	S.E.	LS mean	S.E.
Abattoir classification					
Meat Class					
PFN	20	490[a]	10.2	19.5	0.89
RFN	20	517[ab]	10.2	21.3	0.89
RSE	19	541[b]	10.2	21.5	0.89
PSE	20	545[b]	10.5	20.9	0.90
Collection date					
1st November	39	511[x]	7.3	22.0[x]	0.76
16th November	40	535[y]	7.2	19.5[y]	0.75
Laboratory classification					
Meat Class					
PFN	11 (7)	501	18.6	23.8[a]	0.89
RFN	26 (14)	528	9.4	21.4[ab]	0.89
RSE	17 (7)	538	13.6	20.6[ab]	0.89
PSE	20 (13)	524	10.7	19.9[b]	0.90
Collection date					
1st November	39	515	8.7	21.5	0.76
16th November	35	530	10.5	21.4	0.75

[1]Within a meat class/collection date and classification method, LS means in a column with different superscripts are different ($p<0.05$); [2]Parentheses indicate number of loins in the same meat class as determined by abattoir staff.

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