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Water-soluble precursors of beef flavour. Part II: Effect of *post-mortem* conditioning

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Abstract

Changes in glycolytic metabolites, nucleotide degradation products, free amino acids and other amino compounds were monitored in beef muscle (*M. longissimus lumborum*), stored for 21 days at 4 °C, in order to evaluate how *post-mortem* conditioning may affect flavour formation in beef. The major effects observed in sugar-related substances were the dephosphorylation of the phosphates of glucose, fructose and mannose, to yield their free sugars, as well as the breakdown of inosine 5′-monophosphate, to give a sixfold increase in ribose. Total reducing sugars increased by only 15% during conditioning, while glycogen levels remained unchanged from 2 days post-slaughter. Free amino acids increased during conditioning, particularly between days 7 and 14. Phenylalanine, methionine, lysine, leucine and isoleucine were the amino acids showing the greatest increase with conditioning time, with methionine, in particular, showing a sevenfold increase during the conditioning period. The effects of these precursor changes on cooked beef flavour are discussed.

Keywords: Beef; *Post-mortem* conditioning; Water-soluble flavour precursors; Sugars; Amino acids; Inosine 5′-monophosphate; Flavour

1. Introduction

Small molecular weight water-soluble compounds, such as amino acids, peptides, non-amino acid nitrogenous compounds and carbohydrates, contribute to the formation of meat flavour upon heating (Hornstein & Crowe, 1960; Kramlich & Pearson, 1958; Macy, Naumann, & Bailey, 1964), through their participation in the Maillard reaction. These flavour precursors are affected by several meat production factors, such as species, diet, breed and post-slaughter treatment (conditioning). Wasserman (1972) emphasised the importance of conditioning, particularly of beef, in the formation of flavour precursors, and suggested that microbial and enzymatic changes in the muscle alter the flavour profile of the meat. Enzymatic changes in *post-mortem* muscle, which affect the water-soluble fraction, include the breakdown of ribonucleotides to yield free ribose, hypoxanthine and phosphate; the increase in free amino acids and peptides through proteolysis; and the depletion of glycogen, to yield a pool of low molecular weight, sugar-related metabolites (Lawrie & Ledward, 2006). Heating these products of enzymatic action together gives rise to meaty aroma (Dwivedi, 1975).

Investigations on model Maillard reaction systems have identified ribose and ribose 5-phosphate, and the free amino acid cysteine, as potent precursors of meat flavour volatiles. In particular, alkylthiazoles, acylthiazoles, thiols, pyrazines, pyridines and thiophenes have been isolated as volatile products of reaction systems comprising ribose and cysteine, with or without the incorporation of lipids (Farmer, Mottram, & Whitfield, 1989; Mottram & Whitfield, 1995). The roles of these volatile compounds in meat aroma are well established (Macleod & Seyyedain-Ardebili, 1981).

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The water-soluble compounds in meat not only act as flavour precursors but also possess taste properties. MacLeod (1994) suggests that, in beef, sugars may contribute to its sweetness, while organic acids provide sour taste. Bitterness may be due to peptides and hypoxanthine, while saltiness is due to inorganic salts. Free amino acids may contribute sweetness, sourness and bitterness, while their salts may contribute to saltiness and the umami taste, a characteristic savoury quality. Important contributors to umami taste are glutamic acid and its sodium salt (MSG), as well as hypoxanthine, inosine 5′-monophosphate (IMP), guanosine 5′-monophosphate and certain peptides.

A number of publications have demonstrated the importance of conditioning on the enhancement of certain meat flavour attributes. Daszkiewicz, Wadja, and Matusevičius (2003) concluded that the process of ageing had a positive effect on the organoleptic properties of beef *M. longissimus lumborum* conditioned at 0–2 °C. In particular, samples stored for 10 and 14 days were characterised by much better taste than those stored for 3 and 7 days. Miller et al. (1997) also concluded that ageing of beef for 14 days compared to 7 days increased flavour intensity, as measured by a trained panel, while initial differences, attributed to location of production and quality grade, disappeared. Campo, Sañudo, Panea, Alberti, and Santolaria (1999) investigated the effects of breed and ageing time on sensory characteristics of beef strip loin steaks and reported highly significant effects of ageing in all of the sensory properties studied, including overall odour/flavour intensity, liver odour/flavour intensity, acid flavour intensity, tenderness and juiciness. The authors attributed the increase in the flavour intensity to the accumulation of water-soluble flavour precursors, and demonstrated that significant changes in flavour attributes were related to extended *post-mortem* conditioning times. Jeremiah and Gibson (2003) concluded that ageing of beef ribs or short loins for up to four weeks appeared to increase tenderness, flavour intensity and desirability. Gorraiz, Beriain, Chasco, and Insausti (2002) also concluded that ageing of beef for 7 days resulted in an increase in the characteristic flavour and aftertaste. The authors related these changes in flavour to the increased amount of volatile compounds derived from fatty acid degradation products that were produced, as a result of lipolytic enzyme activity during the period of conditioning and upon heating.

Most of the studies on *post-mortem* conditioning related to flavour are focused on quality changes, as determined by either volatile and/or sensory analysis. Few authors have included correlations with individual meat components, while quantitative data on the changes of the concentration of water-soluble flavour precursors in meat, as a result of conditioning, are scarce. The present study was aimed to investigate the changes in the concentrations of the major sugars, nucleotides, amino acids and other amino compounds in beef *M. longissimus lumborum* during conditioning.

2. Materials and methods

2.1. Experimental design

Sixteen Charolais steers were raised on a concentrate diet at the Institute of Grassland and Environmental Research, Aberystwyth, and were slaughtered in an EU-licensed abattoir at the University of Bristol, according to EU slaughter and dressing regulations. Immediately after bleeding the carcasses were subjected to electrical stimulation across the whole body with 90 V DC for 1 min. Carcasses were then chilled to below 7 °C.

The *longissimus lumborum* muscle was removed, vacuum packed and stored at 4 °C prior to sampling for analysis after different storage times. Samples (approximately 1.5–2 cm-thick steaks) were cut from the muscle after 1, 3, 7, 14 and 21 days of conditioning. For the determination of glycogen, samples were obtained after 2, 4, 7, 14 and 21 days. All samples were blast frozen and stored at −18 °C until analysis.

2.2. Materials

All reference compounds (amino acids, sugars, nucleotides, creatinine, creatine and carnosine) were purchased from Sigma–Aldrich Company, Ltd. (Poole, Dorset, UK). Anhydrous cyclohexane, anhydrous dimethyl sulfoxide, hexamethyldisilazane, trimethylchlorosilane, ammonium molybdate tetrahydrate, potassium antimonyl tartrate, phosphoric acid, disodium hydrogen phosphate and sodium tetraborate were of analytical grade from Sigma–Aldrich Company, Ltd. Reaction vials were purchased from Perbio Ltd. (Chester, UK).

2.3. Extraction

Meat samples were extracted using cold water, followed by centrifugation at 4 °C, and isolation of the low molecular weight components, using 3000 Da molecular weight cut-off ultrafiltration tubes (Centriplus® YM-3; Millipore Corporation, Bedford, MA) as described by Koutsidis et al. (2007).

2.4. Silylation and gas chromatography–mass spectrometry (GC–MS) of sugars and sugar phosphates

Aliquots of the aqueous extracts (0.5 ml) and standards were freeze dried and silylated, according to a modification of the method developed by Leblanc and Ball (1978), and analysed by GC–MS as described by Koutsidis et al. (2007).

2.5. Determination of nucleotides, carnosine, creatine, creatinine and arginine

Nucleotides were determined using the method developed by Uhrová, Deyl, and Suchánek (1996). Carnosine,

creatine, creatinine and arginine were determined by a modification of the method of Flores, Aristoy, Spanier, and Toldrá (1997), as described by Koutsidis et al. (2007). Both methods used capillary electrophoresis with diode-array detection.

2.6. Determination of free amino acids by GC–MS

The free amino acid content of the meat samples, with the exception of arginine, was determined using the EZ-Faast amino acid derivatisation technique for GC–MS (Phenomenex, Torrance, CA), as described by Koutsidis et al. (2007).

2.7. Determination of free phosphate

Free phosphate was determined in an aliquot (0.2 ml) of the ultrafiltrate of the aqueous meat extracts, using the ascorbic acid method described by Clesceri, Greenberg, and Eaton (1998).

2.8. Determination of glycogen

Five grams of homogenised tissue was accurately weighed and extracted with 10 ml of cold 8% perchloric acid (PCA) in a 50 ml Teflon centrifuge tube. Tubes were shaken for 5 min using a Microid flask shaker (Griffin & George Ltd., London), and subsequently centrifuged at 10,000*g* for 10 min at 4 °C. The residue was re-extracted with 5 ml of 8% PCA. The two extracts were combined and centrifuged at 15,000*g* for 5 min at 4 °C. Glycogen was determined, according to the method described by Dreiling, Brown, Casale, and Kelly (1987).

2.9. Statistical analysis

Statistical analysis of the results was performed by two-way analysis of variance, using SPSS version 14.0.0 (SPSS Inc., Chicago, IL). Pearson correlations were also calculated using this software.

3. Results and discussion

3.1. Effect of conditioning on muscle glycogen

Glycogen levels, after 48 h of conditioning, were in the range of 0.58–2.86 g/kg, with an average value of 1.87 g/kg (Table 1), and were virtually the same after 21 days. The relative standard deviations within the sample population over the conditioning period ranged from 31.5% to 42.3%. Immonen and Puolanne (2000) studied the variation of glycogen in beef and reported that at pH < 5.75 the glycogen concentrations varied from 10 to 83 mmol/kg (expressed as glucose). These variations were attributed to stress-related factors. However, Lahucky, Palanska, Mojto, Zaujek, and Huba (1998) showed that, despite the fact that non-stressed animals had significantly higher glycogen levels *ante mortem* and after 3 h *post-mortem*, the glycogen concentrations after 48 h were virtually the same in the muscles of all animals, while pH after 48 h was significantly higher in stressed animals. Bodwell, Pearson, and Spooner (1965) also demonstrated that after 24 h there is little further degradation of glycogen in beef *longissimus* muscle conditioned at 3–4 °C.

Table 1
Concentration of glycogen (g/kg) in beef *M. longissimus lumborum* during conditioning at 4 °C

Conditioning (days)	Mean	RSD[a] (%)	Range
2	1.87	42.3	0.58–2.86
4	1.86	38.1	0.62–2.58
7	1.97	35.2	0.99–3.03
14	1.98	31.5	0.75–2.76
21	1.85	39.1	0.86–3.02

Values are means of analyses of eight replicates.
[a] Relative standard deviation.

Post-mortem glycolysis could potentially yield a major pool of hexoses, trioses and their corresponding phosphates which could subsequently participate in the Maillard reaction during cooking, leading to the formation of flavour volatiles. However, the yields of these metabolites will be dependent on both the initial glycogen concentration and the subsequent enzymatic processes. In this present study, degradation of glycogen after 48 h of conditioning did not occur.

3.2. Effect of conditioning on sugars and sugar phosphates

The level of total reducing sugars after 24 h, determined as the sum of the sugars and sugar phosphates, was 24.8 mmol/kg, while after 21 days of conditioning the total reducing sugar content increased to 28.5 mmol/kg (Table 2). The total hexose content, i.e., mannose, glucose and fructose, increased linearly during storage, while the decrease of the phosphates of these sugars was much slower (Fig. 1). Some of the differences between the changes in the concentrations of the hexoses and the hexose 6-phosphates could probably be explained by the conversion of fructose 6-phosphate to fructose 1,6-biphosphate by phosphofructokinase and glucose 1-phosphate to glucose 6-phosphate by phosphoglucomutase. Unfortunately, neither glucose 1-phosphate nor fructose 1,6-diphosphate could be determined with the method used.

In the present study, glucose 6-phosphate increased slightly between 1 and 3 days of conditioning, suggesting that there was some conversion of glucose 1-phosphate to glucose 6-phosphate, possibly related to glycogen breakdown after 24 h. Degradation of glycogen after 48 h, as stated earlier, did not occur. All of the hexose 6-phosphates followed a similar pattern of variation during conditioning. The marginal increase in samples conditioned for 14 days, compared to samples conditioned for 7 days, may be related to the uneven distribution of precursors across the muscle, as postulated by Dreiling et al. (1987). This hypothesis is supported by the fact that glycogen levels in

Table 2
Concentrations of sugars and sugar phosphates (mmol/kg) in beef *M. longissimus lumborum* during *post-mortem* conditioning at 4 °C

	Conditioning time (days)[a]				
	1	3	7	14	21
Mannose	1.21 ± 0.06 a	1.46 ± 0.06 a	2.00 ± 0.12 b	2.37 ± 0.12 c	2.93 ± 0.14 d
Fructose	1.81 ± 0.17 a	1.98 ± 0.13 a	2.66 ± 0.18 b	3.18 ± 0.21 c	3.81 ± 0.18 d
Glucose	7.33 ± 0.20 a	7.73 ± 0.25 a	7.80 ± 0.26 a	9.18 ± 0.36 b	10.3 ± 0.36 c
Mannose 6-phosphate	3.02 ± 0.16 a	3.08 ± 0.20 a	2.34 ± 0.14 b	2.44 ± 0.17 b	2.05 ± 0.12 b
Fructose 6-phosphate	2.06 ± 0.12 a	2.06 ± 0.15 a	1.57 ± 0.10 b	1.73 ± 0.14 ab	1.46 ± 0.09 b
Glucose 6-phosphate	8.79 ± 0.51 a	8.83 ± 0.62 a	6.74 ± 0.44 b	7.05 ± 0.53 b	6.06 ± 0.41 b
Ribose	0.25 ± 0.02 a	0.43 ± 0.02 b	0.76 ± 0.04 c	1.26 ± 0.06 d	1.67 ± 0.09 e
Ribose 5-phosphate	0.04 ± 0.003	0.04 ± 0.003	0.04 ± 0.004	0.04 ± 0.003	0.04 ± 0.003
Ribulose 5-phosphate	0.07 ± 0.004	0.08 ± 0.006	0.07 ± 0.005	0.07 ± 0.005	0.06 ± 0.006
Xylulose 5-phosphate	0.17 ± 0.01	0.17 ± 0.01	0.15 ± 0.01	0.15 ± 0.01	0.13 ± 0.01
Myo-inositol	0.27 ± 0.01 a	0.28 ± 0.02 a	0.33 ± 0.01 b	0.34 ± 0.02 b	0.43 ± 0.02 c
Free phosphate	26.0 ± 0.90 a	29.1 ± 0.89 b	32.1 ± 0.85 c	33.1 ± 1.09 cd	35.3 ± 0.69 d
Total 5-phosphates	0.28 ± 0.02	0.29 ± 0.02	0.25 ± 0.02	0.26 ± 0.02	0.23 ± 0.02
Total reducing sugars[b]	24.8 ± 0.97 a	25.9 ± 1.19 ab	24.1 ± 0.98 a	27.5 ± 1.25 b	28.5 ± 1.12 b

Values are means of 16 replicates ± standard errors of the means.
[a] Different letters in the same row indicate significant difference at $p < 0.05$.
[b] Sum of mannose, glucose, fructose, fructose 6-phosphate, glucose 6-phosphate, mannose 6-phosphate, ribose 5-phosphate, ribulose 5-phosphate and xylulose 5-phosphate.

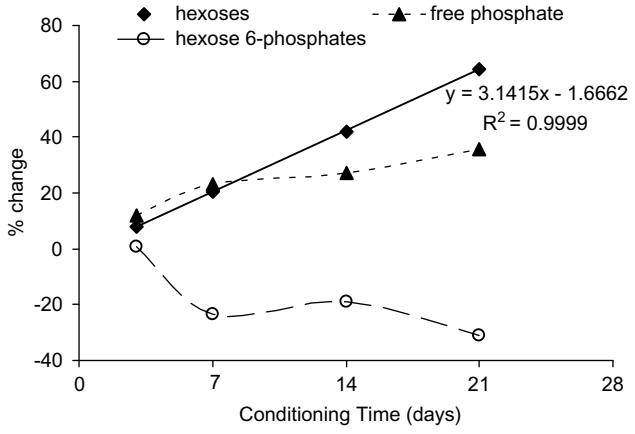

Fig. 1. Change (%) of total hexoses, hexose 6-phosphates and free phosphate in beef *M. longissimus lumborum* during conditioning at 4 °C.

muscles sampled in the same way (Table 1) showed a similar pattern of fluctuation.

As there is no increase in the concentration of total reducing sugars during the first 7 days of conditioning but a significant increase thereafter, there could be another pool of metabolites, which could yield sugars upon enzymatic hydrolysis. Free amino acids showed a similar pattern of increase, which could suggest an interaction between the accumulation of free sugars and amino acids, probably as a result of enzymatic hydrolysis of glycoproteins. This is further supported by the fact that the decrease in the total hexose phosphates does not necessarily correspond with the linear increase in the total free hexoses (Fig. 1). Total sugar phosphate concentrations fell by 31.0%, whereas free phosphate accumulation in the muscle (Table 2) increased by 35.6% over 21 days of conditioning. Bodwell et al. (1965) reported an approximate 30% increase in the free phosphate of beef between 48 h and 20 days of conditioning.

An interesting observation is the increase in free *myo*-inositol after 7 days of conditioning. *Myo*-inositol is an important component of phospholipids and in particular, phosphotidylinositol and glycosyl phosphotidylinositols present in cell membranes (Kuksis, 2003). Cleavage of these phospholipids by phospholipase D yields free inositol, while cleavage by phospholipase C yields inositol phosphate that could be subsequently dephosphorylated by phosphatases (Kuksis, 2003). It is therefore possible that the increase in *myo*-inositol is directly related to the cleavage of phospholipids.

Large variations between animals were observed for the concentrations of sugars and related substances. The relative standard deviations for the concentrations of individual sugars and sugar phosphates ranged from 11% to 43%.

3.3. Nucleotide degradation and liberation of ribose

The degradation of inosine 5′-monophosphate (IMP), as well as the accumulation of its enzymatic breakdown products hypoxanthine, inosine and ribose, was almost linear over the period of conditioning (Fig. 2). After 21 days there was still sufficient IMP to produce ribose. However, the rate of inosine and ribose accumulation tended to decrease with time, indicating that the enzymatic reactions involved were probably limited. In particular, the rate of IMP degradation was highest during the first week of conditioning (Table 3). The fact that the levels of inosine remained almost unchanged after 14 days of conditioning while hypoxanthine was still increasing could be an indication that IMP, rather than being directly dephosphorylated to inosine, is probably hydrolysed to hypoxanthine and ribose 5-phosphate (the latter being subsequently dephosphorylated to free ribose). It is likely that during conditioning some of the enzymes involved in the degradation of IMP

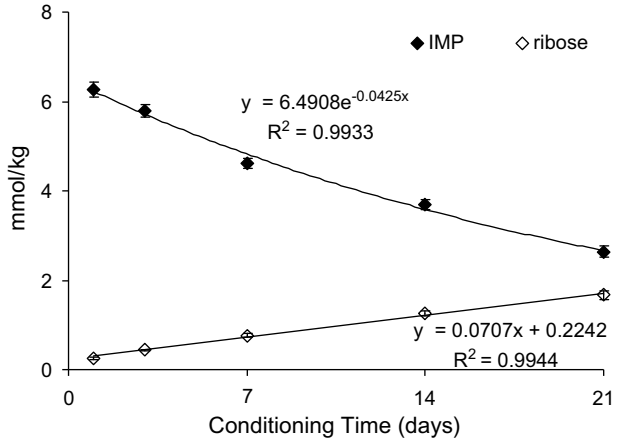

Fig. 2. Degradation of IMP and formation of ribose in beef *M. longissimus lumborum* during conditioning at 4 °C.

are becoming gradually inactivated, since proteolytic activity in *post-mortem* muscle affects other enzymatic processes, i.e., glycolysis (Lametsch, Roepstorff, & Bendixen, 2002). Alternatively, the accumulation of degradation products in the muscle may also limit the rate of IMP breakdown.

Watanabe, Tsuneishi, and Takimoto (1989) determined the IMP content of beef rib-eye muscle, over a period of 10 days at 4 °C, and reported a linear decrease. Dannert and Pearson (1967) observed a similar effect over 28 days at 0.5–1.5 °C. Their results were similar to those presented here (Table 3).

There are only limited data in the literature on the changes in free ribose concentrations during *post-mortem* conditioning. Freedholm (1960) reported that the ribose concentration in beef *M. gracilis* muscle, stored at 1–4 °C, increased from 0.07 mmol/kg after 2 days to 0.8 mmol/kg after 16 days. In this present study, the ribose content of beef muscle increased linearly over the ageing period, from 0.25 mmol/kg at 24 h *post-mortem* to 1.67 mmol/kg at 21 days.

The concentration of ribose 5-phosphate did not change during ageing. However, initial experiments showed that it was possible for ribose 5-phosphate to be enzymatically converted to both ribulose 5-phosphate and xylulose 5-phosphate during aqueous extractions. Such interconversions have also been shown to take place in animal tissues (Ashwell & Hickman, 1957; Dickens & Williamson, 1956). The formation of ribose 5-phosphate is attributed to the enzymatic hydrolysis of IMP through the direct action of nucleosidases (Lee & Newbold, 1963). As previously stated, one of the major changes that affect the concentrations of sugars in meat during conditioning is the dephosphorylation of the sugar phosphates, to yield the parent sugar. Therefore, ribose 5-phosphate is an intermediate product between the enzymatic hydrolysis of IMP and the sugar dephosphorylation processes occurring during conditioning. Moreover, any differences in the concentrations of ribose 5-phosphate during ageing would be probably related to differences in the activities of the enzymes involved.

The importance of ribose and ribose 5-phosphate in flavour formation was demonstrated by Mottram and Nobrega (2002) who heated three reaction systems, each containing cysteine and IMP, ribose 5-phosphate or ribose. Although the volatile products obtained varied substantially, depending on the pH and type of buffer used in the systems, it was clear that both ribose and ribose 5-phosphate were far more reactive than IMP. Hence, the increase in ribose during conditioning is likely to have a major effect on flavour formation.

3.4. Effect of conditioning on free amino acids and other amino compounds

After 21 days of conditioning the total free amino acid content of beef *M. longissimus lumborum* had increased by 58% to 18.8 mmol/kg; at 14 days the level of free amino acids was 16.2 mmol/kg, and at 7 days 11.9 mmol/kg (Table 4), which agrees well with Field and Chang (1969), who reported a total free amino acid content in beef aged for 7 days of 12.0 mmol/kg.

Table 3
Concentration of nucleotide degradation products, carnosine, creatinine and creatine (mmol/kg) in beef *M. longissimus lumborum* during *post-mortem* conditioning at 4 °C

	Conditioning time (days)[a]				
	1	3	7	14	21
Hypoxanthine	0.84 ± 0.02 a	1.13 ± 0.03 b	1.52 ± 0.03 c	2.09 ± 0.04 d	2.66 ± 0.05 e
Xanthine	0.28 ± 0.02 a	0.28 ± 0.02 a	0.29 ± 0.02 a	0.34 ± 0.02 a	0.42 ± 0.03 b
Inosine	1.14 ± 0.04 a	1.36 ± 0.06 b	1.47 ± 0.07 b	1.87 ± 0.09 c	1.91 ± 0.09 c
Uridine	0.05 ± 0.002 a	0.07 ± 0.003 b	0.09 ± 0.003 c	0.11 ± 0.004 d	0.12 ± 0.003 e
Uric acid	0.13 ± 0.009 a	0.11 ± 0.007 a	0.09 ± 0.005 b	0.08 ± 0.006 bc	0.06 ± 0.008 c
Guanosine 5′-monophosphate	0.11 ± 0.005 a	0.10 ± 0.006 ab	0.09 ± 0.005 b	0.08 ± 0.004 c	0.06 ± 0.004 d
Inosine 5′-monophosphate	6.27 ± 0.17 a	5.79 ± 0.14 b	4.61 ± 0.10 c	3.70 ± 0.11 d	2.63 ± 0.12 e
Creatine	40.5 ± 0.70 a	42.3 ± 1.78 ab	43.1 ± 1.14 b	36.9 ± 1.32 ac	38.8 ± 0.99 ac
Creatinine	0.65 ± 0.02 a	0.72 ± 0.03 b	0.87 ± 0.02 c	0.86 ± 0.03 c	0.99 ± 0.03 d
Carnosine	33.8 ± 0.93 a	32.7 ± 0.74 a	31.1 ± 0.89 ab	30.5 ± 0.55 b	28.5 ± 0.44 c

Values are means of 16 replicates ± standard errors of the means.
[a] Different letters in the same row indicate significant difference at $p < 0.05$.

Table 4
Concentrations of free amino acids[a] (mmol/kg) in beef *M. longissimus lumborum* during *post-mortem* conditioning at 4 °C

	Conditioning time (days)				
	1	3	7	14	21
Alanine	1.82 ± 0.14 a	1.76 ± 0.12 a	1.88 ± 0.07 a	2.40 ± 0.08 b	2.52 ± 0.10 b
Arginine	0.76 ± 0.07 a	0.81 ± 0.04 b	0.94 ± 0.04 c	1.19 ± 0.04 d	1.37 ± 0.09 e
Asparagine	0.05 ± 0.006 a	0.05 ± 0.004 a	0.06 ± 0.004 a	0.10 ± 0.006 b	0.13 ± 0.007 c
Aspartic acid	0.02 ± 0.001 a b	0.01 ± 0.001 a	0.01 ± 0.001 a	0.02 ± 0.002 b	0.04 ± 0.006 c
Cysteine	0.05 ± 0.005 a	0.06 ± 0.008 a	0.07 ± 0.009 a	0.12 ± 0.02 b	0.16 ± 0.02 b
Glutamic acid	0.43 ± 0.04 a	0.39 ± 0.02 a	0.49 ± 0.02 a	0.79 ± 0.04 b	0.97 ± 0.05 c
Glutamine	0.98 ± 0.21	1.02 ± 0.21	0.86 ± 0.12	0.92 ± 0.13	0.94 ± 0.15
Glycine	0.53 ± 0.02 a	0.53 ± 0.02 a	0.58 ± 0.02 b	0.66 ± 0.01 c	0.73 ± 0.02 c
Histidine	0.55 ± 0.02 a	0.61 ± 0.02 b	0.68 ± 0.02 c	0.85 ± 0.02 d	0.93 ± 0.03 e
Isoleucine	0.16 ± 0.01 a	0.17 ± 0.01 a	0.22 ± 0.01 a	0.41 ± 0.02 b	0.57 ± 0.03 c
Leucine	0.43 ± 0.03 a	0.46 ± 0.03 a	0.59 ± 0.03 a	1.29 ± 0.07 b	1.75 ± 0.11 c
Lysine	0.31 ± 0.02 a	0.30 ± 0.02 a	0.36 ± 0.02 a	0.62 ± 0.03 b	0.79 ± 0.05 c
Methionine	0.05 ± 0.005 a	0.07 ± 0.005 b	0.11 ± 0.007 c	0.24 ± 0.01 d	0.35 ± 0.02 e
Ornithine	0.07 ± 0.005	0.07 ± 0.004	0.06 ± 0.004	0.06 ± 0.004	0.08 ± 0.008
Phenylalanine	0.25 ± 0.01 a	0.29 ± 0.01 a	0.39 ± 0.02 b	0.74 ± 0.03 c	1.00 ± 0.05 d
Proline	0.21 ± 0.01 a	0.20 ± 0.01 a	0.21 ± 0.01 a	0.26 ± 0.01 b	0.29 ± 0.01 b
Serine	0.16 ± 0.06 a	0.16 ± 0.02 a	0.20 ± 0.03 a	0.42 ± 0.05 b	0.56 ± 0.05 b
Threonine	0.09 ± 0.008 a	0.09 ± 0.008 a	0.11 ± 0.008 a	0.20 ± 0.015 b	0.26 ± 0.022 c
Tryptophan	0.04 ± 0.004 a	0.04 ± 0.004 a	0.05 ± 0.005 a	0.08 ± 0.008 b	0.11 ± 0.011 c
Tyrosine	0.17 ± 0.01 a	0.18 ± 0.01 a	0.24 ± 0.01 b	0.46 ± 0.02 c	0.59 ± 0.03 d
Valine	0.22 ± 0.02 a	0.22 ± 0.01 a	0.26 ± 0.01 a	0.49 ± 0.02 b	0.64 ± 0.04 c
β-Alanine	2.34 ± 0.13	2.34 ± 0.18	2.29 ± 0.14	2.48 ± 0.12	2.41 ± 0.18
Total amino acids	10.8 ± 0.59 a	11.0 ± 0.52 a	11.9 ± 0.36 a	16.2 ± 0.52 b	18.9 ± 0.58 c

Values show means and standard error of the means of 16 replicates.
[a] Different letters in the same row indicate significant difference at $p < 0.05$.

The concentration of total free amino acids did not change during the first 7 days of conditioning, while there was a significant increase thereafter. This observation is in agreement with Feidt, Petit, Bruas-Regnier, and Brun-Bellut (1996), who concluded that the increase in the free amino acid pool in beef was more evident between 10 and 14 days than between 3 and 10 days. Daszkiewicz et al. (2003) also concluded that the proportion of soluble protein and non-protein nitrogen in beef was higher between 7 and 14 days than between 3 and 7 days.

Glutamine, ornithine and β-alanine remained at the same levels throughout conditioning, while proline increased only marginally. However, there were significant changes in the concentrations of all of the other free amino acids, with methionine, leucine, isoleucine and phenylalanine showing the largest increases. Locker (1960) reported an increase in free amino acids in beef of 22–33% over 14 days of conditioning at 2 °C. In his study, the largest increases were observed for serine, threonine, leucine or isoleucine, methionine or valine or tryptophan, while alanine, β-alanine, glycine and glutamine remained relatively stable.

The precursors of volatile sulfur-containing compounds are formed from sulfur-containing amino acids, which break down during the cooking of meat. Methanethiol and hydrogen sulfide are reactive intermediates formed from the breakdown of methionine and cysteine, respectively (Davídek, Velíšek, & Pokorný, 1990). Methionine increased by approximately seven times its original concentration over the ageing period, the largest increase of any of the free amino acids. Methional, methanethiol, dimethyl disulfide and dimethyl trisulfide all increased in cooked salmon to which methionine had been added (Methven, Tsoukka, Oruna-Concha, Parker, & Mottram, 2007); all of these compounds were reported as important contributors to cooked beef aroma (Kerscher & Grosch, 2000).

The concentration of cysteine increased threefold during storage, which may also be responsible for increased flavour intensity in aged meat, particularly when considered alongside the sixfold increase in ribose. The reaction between cysteine and ribose when meat is cooked results in the formation of potent sulfur-containing compounds, such as 2-methyl-3-furanthiol, 2-furanmethanethiol and various thiophenes. These compounds, which have been reported as being crucial in cooked meat aroma (Mottram, 1998), are likely to be present at elevated concentrations in meat cooked after 21 days conditioning. Leucine, isoleucine, serine, threonine, valine and phenylalanine all increased by a factor of at least 3 in beef conditioned for 21 days. These amino acids are important in flavour formation, providing Strecker aldeyhdes, such as 2- and 3-methylbutanals, and other aroma compounds such as pyrazines.

Other amino compounds showed small changes during conditioning (Table 3). Carnosine remained stable during the first 7 days of conditioning with a small but significant decrease thereafter, while creatine showed little change. Locker (1960) demonstrated little degradation of these compounds in beef muscle during conditioning, while similar results were obtained for pork (Moya, Flores, Aristoy, and Toldrá (2001)).

4. Conclusions

The investigation of the effect of conditioning on the concentrations of water-soluble meat flavour precursors revealed some significant changes that occur during the storage of beef *M. longissimus lumborum* at refrigerated temperatures, which could potentially affect the formation of flavour compounds upon heating of the meat. Quantitatively, the most significant changes observed amongst the small water-soluble flavour precursors during conditioning were the increases in the concentrations of ribose and methionine, while the threefold increase in cysteine is also considered to be important. All of the above compounds have been shown to participate in the formation of potent meat flavour volatiles in model systems upon heating. After conditioning for 21 days, the increase in the total free amino acid pool was greater than that observed for the sugars and related substances. Such a change is likely to result in an increase in Maillard reaction-derived flavour compounds, such as pyrazines and Strecker aldehydes.

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