MODELLING THE ODOUR OF COOKED MEAT *IN VITRO*: RELATIONSHIP BETWEEN LINOLEIC AND α-LINOLENIC ACIDS

Campo, M.M.[1], Elmore, J.S.[2], Nute, G.R.[1], Wood, J.D.[1], Mottram, D.S.[2] and Enser, M.[1]
[1] Division of Food Animal Science, University of Bristol, Langford, Bristol. BS40 5DU, UK.
[2] School of Food Biosciences, University of Reading, Reading. RG6 6AP, UK.

Background

When meat is consumed, the main source of polyunsaturated fatty acids (PUFA) is the intramuscular fat. Linoleic acid (C18:2 *n*–6) and α-linolenic acid (C18:3 *n*–3) are important PUFA in ruminant meat and their relative amounts in muscle can be affected by the composition of an animal's diet. The fatty acid ratio C18:2/C18:3 in the intramuscular fat in ruminants ranges from below 2, associated with animals reared on pastures high in n-3 fatty acids, to over 8, corresponding to animals reared on cereal-based concentrates high in n-6 fatty acids. These differences in composition can lead to differences in flavour (Sañudo *et al.*, 2000).

Objectives

To determine how pasture and cereal diets may alter the aroma of cooked meat, by measuring *in vitro* the effects of altering the C18:2 *n*–6/C18:3 *n*–3 ratio in reaction mixtures containing cysteine, ribose, linoleic acid and α-linolenic acid on aroma volatiles. Cysteine and ribose are important precursors of volatiles with meaty aromas (Mottram, 1998).

Methods

Nine mixtures were prepared, each containing 0.5 mmol of L-cysteine and 0.5 mmol of D(–)-ribose (Sigma Chemical Co.) in 0.2 M sodium pyrophosphate buffer (pH 5.5, similar to the pH of meat). Three sets of three samples were prepared, each containing linoleic and α-linolenic acids. A total of 0.5 mmol of fatty acids in a C18:2 *n*–6/C18:3 *n*–3 ratio of 2, 6 or 10 was present in the first set. The second set consisted of the same C18:2 *n*–6/C18:3 *n*–3 ratios,with a fixed amount of linoleic acid (0.51 mmol). The third set also consisted of the same C18:2 *n*–6/C18:3 *n*–3 ratios with a fixed amount of α-linolenic acid (0.17 mmol). The quantities of the fixed fatty acid in the second and third sets were chosen so that they were similar to the maximum values for C18:2 and C18:3 in the first set of samples. Four replicates were analysed for each model system. Each model system was prepared in 20 ml of buffer in 100-ml capacity screw capped *Duran* borosilicate glass bottles. The reaction mixtures were heated at 140 °C for 30 minutes. For sensory analysis, reaction mixtures were diluted 1/100 in buffer and triangle tests were performed (Campo *et al.*, 2001a). Volatile compounds were extracted using solid phase microextraction and analysed by gas chromatography/mass spectrometry (Elmore *et al.*, 2002).

Results and discussion

Table 1 shows the results of the triangle tests.

(a) *Total fatty acids equal 0.5 mmol.* All 3 reaction mixtures were perceived as being different. However, the number of correct identifications of the odd sample was lower for the comparisons 6 *vs* 10 (26/49) and 10 *vs* 2 (27/49) than for 2 *vs* 6 (35/49, $p<0.001$). Since the amount of total fatty acids was the same in all mixtures, we cannot tell whether these differences were due to the different relation per se or to the different amount of individual fatty acids involved in each relation.

(b) *Amount of linoleic acid constant.* When the C18:2 *n*–6/C18:3 *n*–3 ratio of the reaction mixture was 6, it was significantly different from both of the other reaction mixtures. However, no differences could be determined between the two reaction mixtures with the C18:2 *n*–6/C18:3 *n*–3 ratios of 2 and 10. Campo *et al.* (2001b) also found a lack of difference in flavour intensity between animals fed diets based on linseed oil, which is high in α-linolenic acid, with a C18:2 *n*–6/C18:3 *n*–3 ratio close to 2, and animals fed soya-based concentrates, with high linoleic acid content and a C18:2 *n*–6/C18:3 *n*–3 ratio of almost 10. Ratio 6 was described as 'meaty' on a higher number of occasions than ratios 2 and 10 (Fig. 1).

(c) *Amount of α-linolenic acid constant.* No significant differences were found between any of the reaction mixtures. This suggests that variations in the amount of C18:3 *n*–3 are the key in the discrimination of mixtures with a different proportion of fatty acids and that differences in the amounts of linoleic acid have a smaller effect on odour perception. The number of unsolicited comments describing the odd sample was lower than when C18:2 *n*–6 was constant (Figure 1), due to the lack of differences when α-linolenic acid was constant.

Table 2 shows the volatile compositions of the samples where either C18:2 *n*–6 or C18:3 *n*–3 were constant. Seventy-nine compounds were identified in at least one of the extracts at concentrations greater than 10 ng/100 ml and these compounds are divided into different chemical groups. The main differences were found in sulphur-containing compounds that have been reported to have roasted meat-like, cabbage-like and onion-like odours. When the amount of C18:2 *n*–6 was fixed and the C18:2 *n*–6/C18:3 *n*–3 ratio was 6, significantly higher proportions of sulphur compounds were present in this aroma extract, compared with the other 5 extracts, corresponding to relatively high numbers of "meaty" descriptors from sensory assessors. However, the amounts of C18:2 *n*–6 and C18:3 *n*–3 in this sample (0.51 mmol, 0.085 mmol respectively) were similar to the amounts of C18:2 *n*–6 and C18:3 *n*–3 (0.43 mmol, 0.07 mmol, respectively) in the corresponding sample in the isomolar comparison, where the C18:2 *n*–6/C18:3 *n*–3 ratio was 6. It would be assumed that the proportion of sulphur compounds in these two samples would be similar, although no such effect was observed in the isomolar comparison. Nevertheless, the sensory results appeared to confirm that there were higher levels of sulphur compounds in the sample where the C18:2 *n*–6/C18:3 *n*–3 ratio was 6 when C18:2 *n*–6 was constant. This result will need to be investigated further.

All reaction mixtures showed a reduction in the percentage of identified hydrocarbons as the C18:2 *n*–6/C18:3 *n*–3 ratio increased, showing that most of the hydrocarbons identified came from linolenate metabolism. Straight-chain alcohols and aldehydes are derived from the oxidation of lipids. Aldehydes are considered important aroma contributors to meat flavour due to their low odour thresholds. Both alcohols and aldehydes increased in the aroma extracts, when the amount of linoleic acid present in the reaction mixtures increased, although the increase in aldehydes was not significant. Alcohols arising from the metabolism of linoleic acid included 1-hexanol and 1-octen-3-ol (Grosch, 1987) both identified in the reaction mixtures. In spite of the differences found in the levels of furans when the amount of C18:3 *n*–3 in the reaction mixtures was constant, their odour thresholds are generally high and hence their effects on the sensory properties of cooked meat are likely to be small. Although esters were the major volatiles by peak area, they were methyl esters derived from the fatty acid methyl esters used in the reaction mixtures. Methyl esters such as these are not present in meat, the fatty acids in meat would be attached to triglycerides or phospholipids.

Conclusion

It is possible to create *in vitro* mixtures that imitate the reactions that happen during cooking in the muscle of animals fed different diets high in linoleic or α-linolenic acids. Linoleic acid does not influence sensory perception of cooked meat as much as α-linolenic acid. When the C18:2 *n*–6/C18:3 *n*–3 ratio was 6 and the amount of C18:2 *n*–6 was similar to that of cysteine and ribose, levels of odour-potent sulphur-compounds were proportionately high, although this effect would need further research.

Literature cited

Campo, M.M., Nute, G.R., Wood, J.D., Mottram, D.S. and Enser, M. 2001a. Modelling the odour of cooked beef *in vitro* using different fatty acids. *47th International Congress of Meat Science and Technology, Krakow. Poland.* pp. 194-195

Campo, M.M., Enser, M., Gondou, A., Elmore, J.S., Mottram, D.S., Nute, G.R., Scollan, N.D. and Wood, J.D. 2001b. Effects of diet and time on feed on phospholipid fatty acid composition and beef meat flavour. *Proc. British Society of Animal Science*, Scarborough. p.57.

Elmore, J.S., Campo, M.M., Enser, M. and Mottram, D.S. 2002. The effect of lipid composition on meat-like model systems containing cysteine, ribose and polyunsaturated fatty acids. *Journal of Agricultural and Food Chemistry*, **50** (5), 1126-1132.

Grosch, W. 1987. Reactions of hydroperoxides-products of low molecular weight. In: *Autoxidation of unsaturated lipids*. Ed: H.W-S. Chan. London, Academic Press. pp. 95-139.

Mottram, D.S. 1998. Flavour formation in meat and meat rproducts: a review. *Food Chemistry*, **62**, 415-424.

Sañudo C., Enser, M.E., Campo, M.M., Nute, G.R., María, G., Sierra, I. and Wood, J.D. 2000. Fatty acid composition and sensory characteristics of lamb carcasses from Britain and Spain. *Meat Science*, **54**, 339-346.

Acknowledgements

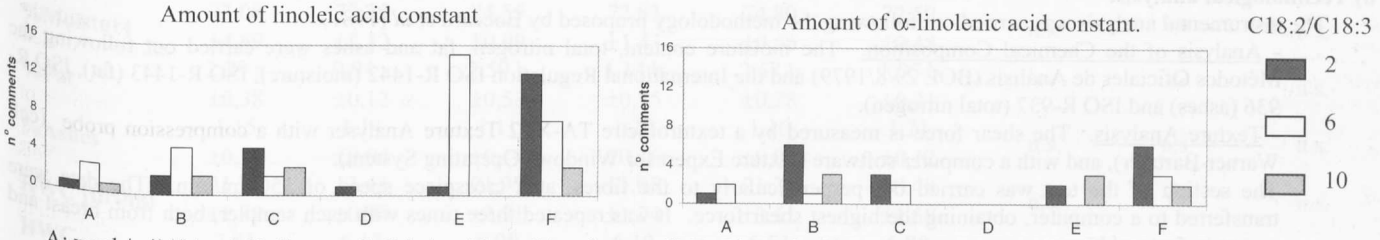
This research is supported by a Marie Curie Fellowship of the European Community programme Quality of Life under contract number QLK1-CT-1999-51147. The authors wish to thank Anne Baker and Sue Hughes for their technical assistance and the British Society of Animal Science for its financial support for presenting this work at 48th ICoMST.

Table 1. *Number of correct answers in triangle tests comparing sets of reaction mixtures containing fatty acids (C18:2 n–6/C18:3 n–3), cysteine and ribose. Ratios of C18:2 n–6/C18:3 n–3 equal 2, 6 and 10.*

Sample set	C18:2 *n*–6/C18:3 *n*–3 ratios of samples compared		
	2 *vs* 6	6 *vs* 10	10 *vs* 2
Total fatty acids equal 0.5 mmol.	35/49 ***	26/49 **	27/49 **
Amount of linoleic acid constant	15/17 ***	14/17 ***	7/17 n.s.
Amount of α-linolenic acid constant	9/18 n.s.	7/18 n.s.	6/18 n.s.

n.s. = no significant; ** = p<0.01; *** = p<0.001

Figure 1. *Number of unsolicited comments given by panellists correctly identifying the odd sample in triangle tests.*



A: weak/mild/bland; B: Strong; C: Oily/cooking oil/sunflower oil; D: Linseed oil/putty; E: Corned beef/beef/meaty; F: others.

Table 2. *Groups of volatiles (per cent total area) in reaction mixtures containing fatty acids (C18:2 n–6/C18:3 n–3), cysteine and ribose. Ratios of C18:2 n–6/C18:3 n–3 equal 2, 6 and 10*

	C18:2 *n*–6/C18:3 *n*–3 ratios of samples							
	Amount of linoleic acid constant				Amount of α-linolenic acid constant			
	2	6	10		2	6	10	
Hydrocarbons	2.89 [a] (0.54)	traces	0.95 [b] (0.15)	***	2.85 [a] (0.52)	1.61 [b] (0.34)	1.61 [b] (0.16)	**
Esters	51.04 (4.76)	47.39 (0.16)	50.14 (6.94)	n.s.	49.70 (4.71)	47.57 (2.23)	44.96 (2.54)	n.s.
Ketones	2.76 (0.37)	2.44 (0.73)	3.18 (0.71)	n.s.	2.72 (0.37)	5.76 (5.27)	5.56 (4.57)	n.s.
Aldehydes	18.55 (4.91)	10.08 (1.60)	19.29 (5.85)	n.s.	15.17 (4.01)	21.47 (7.92)	26.77 (1.08)	n.s.
Alcohols	2.46 (0.42)	2.97 (0.60)	3.24 (0.95)	n.s.	2.11 [b] (0.37)	3.44 [a] (0.58)	4.53 [a] (0.80)	**
Furans	17.50 (2.22)	21.01 (3.84)	19.77 (2.59)	n.s.	22.76 [a] (2.32)	16.31 [b] (2.01)	13.99 [b] (1.10)	**
S-containing	3.89 [b] (0.68)	15.46 [a] (4.40)	2.86 [b] (0.77)	***	4.02 (0.84)	3.31 (1.73)	2.13 (0.58)	n.s.
Miscellaneous	0.72 [a] (0.05)	0.39 [b] (0.04)	0.22 [c] (0.06)	***	0.57 (0.13)	0.40 (0.12)	0.40 (0.05)	n.s.

[a,b,c] Means with different superscripts on the same row have significant differences; n.s. = not significant; ** = p<0.01; *** = p<0.001