

# FLAVOUR PERCEPTION OF OXIDATION IN BEEF

Campo, M.M.<sup>1,\*</sup>, Nute, G.R.<sup>1</sup>, Hughes, S.I.<sup>1</sup>, Enser, M.<sup>1</sup>, Wood, J.D.<sup>1</sup> and Richardson, R.I.<sup>1</sup>

<sup>1</sup> Division of Farm Animal Science, University of Bristol, Langford, Bristol BS40 5DU, United Kingdom \* Current address: Dept. Animal Production and Food Technology, University of Zaragoza, C/ Miguel Servet 177, 50013-Zaragoza, Spain (marimar@unizar.es)

#### Background

Lipid oxidation limits the storage life of meat (Rhee, 1988; Gray *et al.*, 1996) through the production of rancid/off flavours but is also important in cooking-induced production of flavour volatiles, both directly and as precursors for the synthesis of other components of meat flavour.

Methods for measuring the effect of oxidation on meat flavour are either direct, ie. taste panel, or indirect by determining the degree of lipid oxidation by measuring oxidation products. The former is expensive, baseline perceptions may vary and they depend on prior experience/conditioning. The latter is usually quicker, cheaper and more reproducible within and between laboratories, but on the other hand, only determines chemical compounds which may or may not be directly involved in the oxidised flavour. Furthermore, widely used assays for rancidity, such as the determination of 2-thiobarbituric acid reacting substances (TBARS) measure a range of compounds. Although the chemical determinations are generally related to the deterioration in meat flavour there are few specific investigations of this relationship.

Ruminant meat is relatively stable in terms of lipid oxidation because of its high proportion of saturated fatty acids relative to polyunsaturated fatty acids (PUFA). However, by feeding cattle with lipids protected against rumen biohydrogenation it is possible to obtain meat with an increased content of n-3 PUFA (Scollan *et al.*, 2003) making it more desirable in terms of human nutrition. Protected PUFA with different degrees of unsaturation, such as lipid supplement (C18:2 n-6 + C18:3 n-3 ratio 1:1) or fish oil (C20:5 n-3 and C22:6 n-3) can produce meat with a range in oxidative stability, so that knowledge of the shelf-life and flavour of the modified meat becomes important (Richardson *et al.*, 2003). This meat with a wide range in oxidative potential can be used to assess the relationship between chemically determined oxidation and the organoleptic assessment of flavour as evaluated by a trained taste panel.

## Objectives

The objective of this work was to relate human perceptions of lipid oxidation, as determined by a trained taste panel, to a chemical measurement of oxidation, using meat from animals with a wide range of potential oxidation because of the diet-induced changes in their PUFA composition, and further enhancement by displaying the meat in high oxygen modified atmosphere packages for various lengths of time.

## Materials and methods

Meat was obtained from 73 Angus- and Charolais-cross steers raise for different on-going trials on 10 different diets: grass silage (high in C18:3 *n*-3), cereal concentrate (high in C18:2 *n*-6), three diets with 3% added fat consisting of three levels of protected lipid (high in C18:2 *n*-6 and C18:3 *n*-3, ratio 1:1)(PLS) and a control with Megalac  $\mathbb{R}$  (relatively saturated)(contPLS) and three diets with three levels of inclusion of protected fish oil (high in C20:5 *n*-3 and C22:6 *n*-3) and a constant amount of unprotected fish oil (PFO), as well as an unprotected fish oil control (contPFO).

48 hours after slaughter, the left loin of each carcass was removed, vacuum packaged, and kept at +1 °C. Loins of the grass and concentrate fed animals were kept vacuum packaged for an extra 8 days until reaching 10 days of ageing, whereas loins from the rest of the diets were kept for 11 days until reaching 13 days of ageing according to the protocols of their respective projects. After aging, each loin was cut into 2-cm thick steaks, vacuum packaged, frozen, and kept at -18 °C until analysed.

TBARS and sensory analyses were performed on steaks displayed for 0, 4 or 9 days under simulated retail conditions in modified atmosphere packages (MAP). After frozen storage, samples were thawed at +1 °C for



24 hours. They were transferred onto a polystyrene tray, covered with a permeable film and enclosed in a plastic transparent bag impermeable to oxygen. The atmosphere in the bag was modified to contain CO<sub>2</sub>:O<sub>2</sub> in the ratio of 25:75. Samples were displayed for 4 or 9 days at 4 °C under illumination (700 lux 16 h on) until analysed. Samples of day 0 of display were not displayed in MAP, but instead were analysed immediately after the vacuum was broken after thawing to avoid oxidation. These were considered as non-oxidised control samples. TBARS (thiobarbituric acid reactive substances) were analysed by the steam distillation method of Tarladgis *et al.* (1960) and expressed as mg of malonaldehyde per kg of lean muscle. Steaks for sensory analysis were grilled and turned every three minutes for homogeneous cooking until the internal centre temperature reached 74 °C measured by a hand-held digital thermometer. Uniform cuboids were cut, wrapped in coded aluminium foil, and kept at 60 °C until the sensory evaluation was performed. Sensory assessments were performed by trained taste panel of nine members under controlled conditions, which included booths with red lights to mask colour differences. Panellists received three samples at a time to compare the meat within each animal that had been displayed for 0, 4 and 9 days. Panellists rated typical beef flavour, abnormal and rancid flavours as well as the overall palatability on an unstructured line scale where 0 meant no flavour or 'dislike extremely', and 100 meant very intense flavour or 'like extremely'.

Statistical analysis of panel data was performed by analysis of variance (Genstat 5 Release 3.1) within diet with conditioning time and panellist as factors and panel treated as a block structure. Differences between mean values were assessed post hoc using the least significant difference procedure. TBARS were analysed using the Kruskall-Wallis rank sum test. Spearman's rank correlation coefficients (rho) were calculated on the animal by display means for TBARS and sensory data using Minitab Release 11.

## **Results and discussion**

Table 1 shows the values for TBARS and sensory attributes. Meat oxidation increased throughout the time at display for each of the diets, as shown by the rising TBARS values. The increments were not linear, however, being smaller between days 0 and 4 of display than between days 4 and 9 of display, as lipid oxidation is a free-radical chain reaction (Rhee, 1988). The lowest values and lowest increment in oxidation was produced by meat from the two control diets and the silage-fed animals probably due to the higher proportion of saturated fat in the meat from animals fed the contPLS and contPFO diets, as well as a higher content of vitamin E (Richardson *et al.*, 2004) that acts as a powerful antioxidant. Accordingly, meat from animals fed concentrate diet had a low vitamin E concentration and the highest TBARS values. The greater the inclusion of protected PUFA in the diet of the animals, the higher TBARS values were obtained. This was especially observed in PFO diets, where an extra level of inclusion of protected lipid gave a higher oxidation value. In PLS diets, the oxidation of the meat did not increase from PLS2 to PLS3, because the attempt to increase C18:3 deposition in the muscle above PLS2 failed even with a 25% increase in protected lipid in the feed.

Sensory attributes were also influenced by length of display. Positive attributes, such as typical beef flavour or overall palatability, decreased throughout display, whereas negative attributes, such as abnormal or rancid flavours, increased. Although all diets started with similar values, the biggest reduction in beef flavour occurred in the diets producing meat with the most PUFA, especially in PLS2, PLS3, PFO3 and concentrate fed animals, with the lowest beef flavour intensities at day 9 of display. In general, the decrease in strength of beef flavour was more pronounced between days 4 and 9 of display than between days 0 and 4. Byrne *et al.* (2001) suggested that the disappearance of 'meaty' flavour was related to both the 'meaty' compounds degrading and becoming masked by flavour-affecting compounds derived from lipid oxidation. Concentrate, PLS2, PLS3 and PFO3 fed-groups showed also the highest mean ratings for abnormal and rancid flavours, closely related to their higher values of TBARS. Consequently, they received much lower values for overall palatability than the other diets.

Panellists could barely detect rancidity in unconditioned meat (0 days of display) with mean ratings all being less than 2. The lack of oxygen and the use of intact meat during frozen storage avoided the initiation of lipid oxidation (Spanier and Miller, 1996). However, abnormal flavour was perceived from this early stage onwards. Its value was always higher than those of rancidity, implying that rancid was considered an abnormal flavour, but not the only one of such. Negative flavours such as painty, cardboardy, bitter and sour have been described to increase with post-mortem ageing (Spanier *et al.*, 1997).



The correlations between these attributes were very high (Table 2). TBARS was a good predictor of the perception of rancidity (rho = 0.84). Panellist preferences were related to the presence of the typical beef flavour (rho = 0.93) and to the absence of abnormal (rho = -0.89) and rancid flavours (rho = -0.84). The development of rancid flavour in relation to TBARS value followed a sigmoidal curve:  $y = 0.0553x^3 - 0.0553x^3 1.122x^2 + 7.3962x + 2.3204$  (R<sup>2</sup> = 0.62). The third grade of the curve implied that rancidity is perceived strongly once it reaches the threshold but then, either the perception of the panellists becomes numbed, or the analytical technique fails to discriminate TBARS accurately at high levels of oxidation. The development of beef flavour in relation to TBARS also followed a sigmoidal curve but with a different direction than the previous one:  $y = -0.0359x^3 + 0.8276x^2 - 6.2937x + 24.475$  (R<sup>2</sup> = 0.68). This means that typical beef flavour gets weaker but there is always a residual beef flavour in the meat throughout display, even if the development of other off-flavours becomes more perceivable. The two curves cross at a corresponding TBARS value of 2.3. From this point onwards, the perception of rancid flavour overpowers the perception of beef flavour. This could be considered as the oxidation limit at which meat could be rejected. Tarladgis et al. (1960) and Turner et al. (1954) suggested a TBARS of 0.5-1.0 as the threshold value for the detection of off-odour in pork. However, pork has a milder flavour than beef, and therefore, rancid flavours are probably noted at a lower concentration than in beef. Nevertheless, Melton (1985) suggested that oxidised flavours were detectable in beef at TBA numbers of 1.

## Conclusions

Oxidation produces a decrease in beef flavour and overall palatability, as well as an increase in abnormal and rancid flavours during retail display of beef. Under the experimental conditions used, a TBARS value of around 2 could be considered the limiting threshold for the acceptability of oxidation-altered flavour in beef. This threshold was clearly higher than that of 0.5-1.0 suggested for pork.

#### References

Byrne, D.V., Bredie, W.L.P., Bak, L.S., Bertelsen, G., Martens H. and Martens, M. 2001. Sensory and chemical analysis of cooked porcine meat patties in relation to warmed-over flavour and pre-slaughter stress. Meat Sci., 59, 229-249.

Gray, J.I., Gomaa, E.A. and Buckley, D.J. 1996. Oxidative quality and shelf life of meats. Meat Sci., 43, 111-123.

Melton, S.L. 1983. Methodology for following lipid oxidation in muscle foods. Food Tech., 37, 105-111.

Rhee, K.S. 1988. Enzymic and nonenzymic catalysis of lipid oxidation in muscle foods. Food Tech., 42, 127-132. Richardson, R.I., Enser, M., Whittington, F.W., Hallett, K.G., Wood, J.D. and Scollan, N.D. 2003. Effect of product type and fatty acid composition on shelf life of nutritionally modified beef. Proc. BSAS, 41.

Richardson, R.I., Nute, G.R., Wood, J.D., Scollan, N.D. and Warren, H.E. 2004. Effects of breed, diet and age on shelf life, muscle vitamin E and eating quality of beef. Proc. BSAS, 84.

Scollan, N.S., Enser, M., Gulati, S.K., Richardson, I. and Wood, J.D. 2003. Effects of including a ruminally protected lipid supplement in the diet on the fatty acid composition of beef muscle. Brit.J.Nutr., 90, 709-716.

Spanier, A.M. and Miller, J.A. 1996. Effect of temperature on the quality of muscle food. J. Muscle Foods, 7, 355-375.

Spanier, A.M., Flores, M., McMillin, K.W. and Bidner, T.D. 1997. The effect of post-mortem aging on the meat flavor quality in Brangus beef. Correlation of treatments, sensory, instrumental and chemical descriptors. Food Chem., 59, 531-538.

Tarladgis, B.G., Watts, B.M. and Younathan, M.T. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. J. AOCS, 37, 44-48.

Turner, E.W., Paynter, W.D., Montie, E.J., Bessert, M.W., Struck, G.M. and Olson, F.C. 1954. Use of 2-thiobarbituric acid reagent to measure rancidity in frozen pork. Food Tech., 8, 326.

#### Acknowledgements

Authors are most grateful to Anne Baker for technical assistance. This work was supported by the EU (QLK1-CT 1999-51147), ABP, MLC, Masterfoods, Sainsbury's and Ministerio de Ciencia y Tecnología, Programa Ramón y Cajal.



DIET	days	TBARS y	Beef flav. z	Abnormal flav. z	Rancid flav. z	Ov. Liking z
ContPLS	0	nd	26.5 <sup>c</sup>	11.7 <sup>a</sup>	1.2 <sup>a</sup>	26.8 <sup>c</sup>
	4	0.24	22.5 °	23.1 °	5.4 <sup>a</sup>	19.9 <sup>°</sup>
	9	0.43	17.1 <sup>a</sup>	34.0 °	14.2 <sup>b</sup>	15.9 <sup>a</sup>
	sig/sed	***	1.60	2.91	3.06x	1.91
PLS1	0	nd	25.5 <sup>b</sup>	10.7 <sup>a</sup>	1.3 <sup>a</sup>	29.4 <sup>b</sup>
	4	0.48	22.3 °	19.5 °	4.9 <sup>a</sup>	22.6
	9	3.78	10.8 <sup>a</sup>	42.4 °	17.8 °	8.6 <sup>a</sup>
	sig/sed	***	1.57x	2.65x	3.98x	4.35x
PLS2	0	nd	26.1 °	15.0 <sup>a</sup>	1.7 <sup>a</sup>	25.6 °
	4	0.98	17.5 °	27.9 °	11.0 °	14.6
	9	6.60	7.8 ª	51.1 °	21.3	5.7 ª
	sig/sed	***	<u>3.27x</u>	5.33x	<u>3.97x</u>	<u>3.33x</u>
PLS3	0	nd	26.3 °	12.6 ª	$1.3^{a}$	27.5 <sup>°</sup>
	4	0.82	19.9 °	22.3 <sup>a</sup>	8.0 °	19.6
	9	6.21	10.1 "	45.3 °	18.0 °	8.3 ª
	sig/sed	***	<u>2.79x</u>	5.74x	<u>2.90x</u>	<u>3.21x</u>
ContPFO	0	nd	24.9	13.6 <sup>a</sup>	1.7 ª	$26.7^{\text{b}}$
	4	0.31	23.4 °	16.4 <sup>a</sup>	4.3 <sup>a</sup>	23.2 °
	9	1.61	16.3 °	31.6	12.7 °	13.7 ª
PFO1	sig/sed	***	2.32x	<u>3.59x</u>	<u>3.12x</u>	<u>3.45x</u>
	0	nd	22.7 °	17.3 "	1.5 <sup>u</sup>	21.6°
	4	0.49	21.2 °	16.1 °	4.3 <sup>ab</sup>	19.9 °
	9	2.14	14.1 "	36.3	11.4 °	11.9 *
	sig/sed	***	<u> </u>	3.00	$\frac{3./3x}{2.03}$	$\frac{2.83x}{24.4b}$
PFO2	0	nd	23.6°	13.5 °	$2.0^{a}$	24.4 °
	4	0.56	19.7	20.5	5.2 "	18.3
	9	3.00	12.4 "	39.7°	18.5	10.2 "
	sig/sed	1	1.4/	3.09	<u>4.39x</u>	<u>2.03x</u>
PFO3	0	nd	25.2°	13./ <sup>a</sup>	1.2	27.2°
	4	1.10	20.4	22.4 °	6.9 <sup>±</sup>	19.4 °
	9	5.1/ ***	9.8	49.5	19.0 *	7.0 -
	sig/sea	1	$\frac{2.32x}{25.5 \text{ b}}$	3.01	3.03x	$\frac{2.93x}{2.93x}$
Grass	0	nd	$25.5^{\circ}$	$12.8^{-1}$	$0.9^{-1}$	$25.2^{\circ}$
	4	0.59	18.0 14.4 <sup>a</sup>	24.1 °	5.5 <sup>m</sup>	10./
	9	1.10	14.4	51.2	12.1	11.9
	sig/sea	۰. لي م	$\frac{3.3/x}{25.2^{b}}$	3.92x	$\frac{3.84x}{1.2^{a}}$	$\frac{4.12x}{26.2^{b}}$
Concentrate	1	na 2 15	$23.2^{\circ}$	18.2 28.0 <sup>b</sup>	1.5 0.5 <sup>b</sup>	$20.2^{\circ}$
	4	5.15	12.1 77 <sup>a</sup>	50.0 52 7 °	9.3 21.4 °	10.0 6.05 <sup>a</sup>
	y sig/sod	0.00 ***	/./ 2.81x	55.1 652r	21.4	0.03
	sig/sed		2.01X	$0.32\lambda$	4.09X	J.44X

Table 1. Values for TBARS and sensory attributes.

y, mg malonaldehyde/kg muscle (medians);

z, 0-100 scale (0-very low; 100-very high) (mean values);

nd = not detected;

a, b, c. Mean values with different superscripts within diet in the same column are significantly different (P≤0.05)

sig/sed significance (TBARS column), mean standard error (MSE=sed) (other columns);

x Interaction between conditioning time and assessor, therefore significance and sed were recalculated.

Table 2. Spearman's rank correlation coefficients between TBARS and sensory attributes (n=216).

	TBARS	Beef flavour	Abnormal flavour	Rancid flavour
Beef flavour	-0.80 ***			
Abnormal flavour	0.82 ***	-0.87 ***		
Rancid flavour	0.84 ***	-0.79 ***	0.83 ***	
Overall palatability	-0.84 ***	0.93 ***	-0.89 ***	-0.84 ***

\*\*\* =  $P \le 0.001$