Occurrence of 1-(methylthio)propane producing off-flavour in fresh beef meat

Sara Corral⁽¹⁾ and Mónica Flores*

Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC) Avda. Agustín Escardino 7, 46980 Paterna, Valencia, Spain. (1) Present address: Sociedad Cooperativa Esperanza del Jiloca (CEJI), Calamocha (Teruel), Spain.

Short title: Off-flavour in fresh beef meat

*Correspondence to: Mónica Flores. Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC) Avda. Agustín Escardino 7, 46980 Paterna, Valencia, Spain.
E-mail: mflores@iata.csic.es

Abstract

The identification of compounds producing a characteristic off-flavour in fresh beef meat obtained from different locations is essential to avoid consumer rejection. The volatile compounds of fresh beef samples were extracted by solid phase micro extraction (SPME) and their odour was evaluated by using olfactometry analysis (GC-O). The compound responsible for the characteristic off-flavour was identified by using GC-MS and quantified in different beef samples obtained from different locations. The sniffing analysis revealed eight odour active zones being four of them identified as 2,3-butanedione, allyl methyl sulfide, 1-(methylthio)propane and 1-(methylthio)-1-propene (Z). The most potent odorant was 1-(methylthio)propane described as garlic, cabbage, oxidized iron, solvent and rancid odour. All panellists attributed the characteristic off-flavour detected in fresh beef to the odour of 1-(methylthio)propane. A chromatographic method was validated to quantify 1-(methylthio)propane in fresh beef. Several beef samples were characterized by this off-flavour although its origin was not elucidated.

Keywords: fresh beef; 1-(methylthio)propane; off-flavour; meat.

Introduction

Flavour, tenderness and juiciness are crucial criteria for acceptability and palatability of beef as they can compromise consumer purchasing ^[1]. Beef flavour is developed from water soluble components and lipids. In addition, lipid concentration and compositional differences lead to specific modifications in flavour ^[2]. The differences among animal's digestive systems (ruminants and non-ruminants), produce diverse deposition of fatty acids. Lamb or beef muscles have lower levels of polyunsaturated fatty acids than poultry and pork. In addition, the most important environmental factor influencing meat flavour is feeding due to variations in fatty acid composition by the feeding prior to slaughter. However, other factors influencing lipid and fatty acid content are breed, animal age or supplements given during feeding ^[3].

The thermal oxidation of long chain fatty acids can contribute to undesirable flavours ^[1]. In the last years, several research works have been conducted to elucidate the origin of beef off-flavour such as grassy, gamey, rancid and especially livery off-flavour. In general, these studies have been focused on the effect of fatty acid profile; however no direct relationship of fatty acid composition/oxidation and incidence of grassy, rancid or gamey off-flavour were reported ^[1,4,5]. On the other hand, livery off-flavour could not be related to any single factor ^[6]. Livery off-flavour was related to specific unsaturated fatty acids and volatile compounds, sodium content and myoglobin concentration ^[6,7] while iron, heme iron content and pH were not related to this off-flavour ^[5].

However, these studies have been carried out in cooked beef since beef flavour is thermally derived while fresh meat has little flavour and a blood like taste ^[2]. Nevertheless, original fresh beef flavour should be taken into account in contrast to cooked beef where hundreds of volatile compounds have been identified ^[8]. In addition,

the presence of off-flavours in fresh beef meat may produce a rejection in processing plants due to their impact in sensory characteristics. Bacterial population has been related to the generation of volatile compounds ^[9] in spoilage meat while their generation depends on the different storage conditions. Although the presence of sulphur compound in fresh meat is scarcely detected ^[9] except in inoculated fresh meat. Therefore, the aim of this study was to identify the compounds related to an off-flavour present in fresh beef meat obtained from different geographical origins.

Experimental

Samples

Fourteen fresh beef samples (*semimembranosus* muscle) from different geographical origins in Europe; country 1 (samples 1-10), country 2 (samples 11-12), country 3 (sample 13) and country 4 (sample 14) were obtained from vacuum packaged meat in collaboration with a local meat processor. All samples were vacuum stored for 14 d. The samples were wrapped in aluminium foil, vacuum packaged and frozen at -20 °C until volatile analysis. The beef samples were smelled by a trained panel consisting of 4 panelists trained in olfactometry analysis which reported the presence of an unusual odour in several of these samples.

Extraction of volatile compounds

For each experiment, 1g of minced fresh beef was weighted into a 20 ml headspace (HS) vial, 0.5 mg butylated hydroxytoluene (BHT) and 80 ng 1,3-dichlorobenzene (internal standard) (Sigma-Aldrich, St. Louis, USA) were added. The vial was kept at 40°C for 10 min to equilibrate its HS. Then, the SPME fiber (StableFlex

85µm carboxen/Polydimethylsiloxane CAR/PDMS, Supelco, Bellefonte, PA) was exposed to the HS while maintaining the sample at 40°C for 5 min. The compounds extracted were desorbed into the injection port (splitless mode) of the gas chromatograph-olfactometry (GC-O) or gas chromatograph-mass spectrometry (GC-MS) for 5 min at 240°C.

Olfactrometry analysis

Gas chromatograph with flame ionization detector (GC-FID) (Agilent 6890, USA) equipped with a sniffing port (ODP3, Gerstel, Mülheim an der Ruhr, Germany) was used to analyse aroma compounds as described by Corral et al. ^[10] using SPME technique. Four trained panelists evaluated the odour eluted from the GC-effluent of two beef different samples. The panelists were selected among the personal of the institute based on their ability to detect and recognize odours. First, several sessions were carried out at room temperature to evaluate ortho-nasal aroma (smell) of individual volatile compounds and to train them in the identification and recognition of odours. In the training assessment, panel members were then individually trained in the use of the GCO device to check their ability to identify and described different odours compounds. For this, different mixtures containing volatile compounds were analysed and the reproducibility of the panel members was evaluated. The detection frequency method was used to determine the aromatic impact of each volatile compound ^[11] being eight the maximum detection frequency value (DF) for each compound.

Compounds were identified by comparison with mass spectra, linear retention indices ^[12] of authentic standards injected in the GC-MS and GC-O with a minimum concordance of ± 5 between two LRIs, and by coincidence of the assessors's descriptors with those described in the Fenaroli's Handbook of Flavor Ingredients ^[13].

GC-MS analysis

An Agilent HP 7890 series II GC (Hewlett-Packard, Palo Alto, CA) with an HP 5975C mass selective detector (Hewlett-Packard) equipped with Gerstel MPS2 multipurpose sampler (Gerstel, Germany) was used. The volatile compounds extracted by SPME were separated using a DB-624 capillary column J&W Scientific (Agilent Technologies, USA). The GC oven temperature program began at 38°C, held for 10 min, ramped to 120°C at 5°C min⁻¹ and then to 210°C at 10°C min⁻¹ and held 5min at 210°C. Mass spectra were obtained by electron impact at 70ev and data acquired across the range 29-400 amu (scan mode). The compounds were identified by comparison with mass spectra from the library database (Nist'05), and as indicated above.

Quantification of the off-flavour compound (1-(methylthio)propane)

A stock solution of 1-(methylthio)propane (TCI, Japan) and 1,3-dichlorobenzene (internal standard) were prepared in methanol (7500 and 2300 ppm, respectively) and diluted to different concentrations using methanol. The identified off-flavour compound 1-(methylthio)propane was quantified by means of the internal standard method. Beef sample without off flavour was selected using trained panelists who identified those samples without a detectable off-odour. Trained panelists were those participating in the olfactometry study who perceived off-odours as those related to cabbage, rotten and rancid odours. Then, raw beef without off flavour was minced and 1g was used as matrix. The minced meat was weighted into a 20 ml headspace (HS) vial with 0.5 mg butylated hydroxytoluene (BHT) and 80 ng 1,3-dichlorobenzene (internal standard). For calibration different amounts of 1-(methylthio)propane from 0.5-10 µg were added to the vials and analysed by GC-MS. The MS was operated in SIM mode and the ions

monitored were m/z 61 and 146 for 1-(methylthio)propane and 1,3-dichlorobenzene, respectively. A calibration curve for 1-(methylthio)propane was constructed by desorption of SPME fibre into GC-MS instrument. The area ratio of the analyte (m/z 61) to the internal standard (m/z 146) was plotted against 1-(methylthio)propane quantity ($\mu\text{g/g}$ meat). The results were expressed as μg of 1-(methylthio)propane per g fresh beef. All experiments were performed in triplicate.

Method validation

The analysis of 1-(methylthio)propane ratio in fresh beef was validated in terms of linearity, intra-day, inter-day and method precision, accuracy and sensitivity (LOD and LOQ). The LOD was defined as $3\sigma_b/m$ and LOQ as $10\sigma_b/m$ where σ_b is the standard deviation of the y-intercept and m is the slope, in accordance with IUPAC ^[14]. Linearity was checked using standard solutions of 1-(methylthio)propane at seven concentration levels (0.5 to 10 $\mu\text{g g}^{-1}$). Each concentration level was repeated three times. Accuracy, in terms of recovery (%), was tested at three different levels (50, 100 and 150 % using 2.5, 5 and 10 $\mu\text{g/g}$) and three independent analyses were repeated for each concentration. The regression coefficients of these standard calibrations were calculated. Instrumental precision, in terms of relative standard deviation (%) was evaluated by multiple injection ($n=6$) of standard solutions. Intra-day and inter-day precision of the method was checked by injecting ($n=3$) individual solutions of standards at three concentration levels (90, 100 and 110%) at the same day and different days, respectively.

Results and discussion

Several samples of fresh beef from different geographical origins were supplied from a local market. The beef samples smelled by the trained panel reported the

presence of unusual odour in several of them. This off-flavour was characterized by unpleasant, cabbage odour notes and absence of fresh odour; therefore a GC-O analysis was performed to elucidate the compounds responsible for this undesirable odour (Table 1). The sniffing analysis revealed eight odour active zones (Table 1 and Figure 1). Four of them were identified as 2,3-butanedione, allyl methyl sulfide, 1-(methylthio)propane and 1-(methylthio)-1-propene but this last compound was tentatively identified and the odour could be due to any of its forms (Z or E). Furthermore, other four were not identified and numbered as unknown compounds (Table 1). The most potent odorant with the highest detection frequency value (Table 1) was 1-(methylthio)propane described as garlic, cabbage, oxidized iron, solvent and rancid odour. All panellists attributed the characteristic off-flavour detected in fresh beef to the odour of 1-(methylthio)propane. However, other compounds such as allyl methyl sulphide and 1(methylthio)-1-propene may produce a synergic effect. Other compounds present in raw beef (table 1) like aliphatic and aromatic hydrocarbons (hexane, heptane, toluene, octane...) might be originated from chemical changes that take place during storage or as a consequence of their presence in animal feedstuffs and diet ^[15]. However, these compounds did not play a role in the overall flavour of the beef samples as no one has an odour itself.

Therefore, a method was optimized and validated to detect and quantify the presence of 1-(methylthio)propane in fresh beef samples from different locations. For the concentration range examined, the calibration curve was linear being the regression coefficient satisfactory ($R^2=0.997$) (Table 2). The limit of detection (LOD) and limit of quantification (LOQ) for 1-(methylthio)propane were calculated from the calibration curve regression statistics and were 0.33 $\mu\text{g/g}$ and 1.10 $\mu\text{g/g}$, respectively (Table 2).

In order to evaluate the accuracy of the method, a recovery study was carried out at three concentrations levels of 1-(methylthio)propane corresponding to 50, 100, 150% of the concentration used for the linearity study (2.5, 5, 10 µg/g). The mean recovery for 1-(methylthio)propane ranged from 94.61 to 98.29 % while the percentage of the relative standard deviation (%RSD) for recovery was 7.58-15.58 (Table 2). Therefore, these results indicated the accuracy of the method to quantify 1-(methylthio)propane in fresh beef. Instrumental precision was checked by multiple injections of 1 µg and its RSD was 11.3 %, which is agreement with the precision criteria for repeatability (Table 2). The precision of the method based on intra-day (within-day) and inter-day (between-day) repeatability at three concentrations level (9, 10, 11 µg) for 1-(methylthio)propane was assessed. The relative standard deviation for each concentration ranged from 5.5 to 16% intraday and from 13.7 to 29.8% interday (Table 2). Therefore, the method for 1-(methylthio)propane quantification in fresh beef is accurate but the precision interday was high.

The validated method was applied to quantify the presence of 1-(methylthio)propane in 14 fresh beef samples from different geographical origins (Table 2). The samples from country 1 (samples 1-5) and country 2, 3 and 4 (samples 11-14) presented concentrations below LOD while samples from country 1 (samples 6-9) showed high levels of 1-(methylthio)propane and sample 10 was below LOQ agreeing with those that evoked off-flavour. During meat storage the olfactory impact can lead to rejection of the products in case the concentrations increase significantly as a result of microbial activity [9]. Volatile compounds found in vacuum packaged meat have been associated to enterobacteria. Among them alcohols were the most abundant group and several ketones were related to spoiled vacuum packaged meat; like acetoin (3-hydroxy-2-butanone), 2-butanone, 2-decanone and propanone [9]. In the analysed off-flavour beef, 3-hydroxy-2-

butanone and 2-butanone were present but an odour was not detected by GCO as probably they were below threshold. Regarding sulphur compounds in vacuum packaged meat, dimethyl sulphide, dimethyl disulphide, dimethyl trisulfide and S-methyl thioacetate have been reported ^[9] but no reports about the presence of 1-(methylthio)propane have been found. So far, only Raes et al^[16] reported the presence of the compound 1-(methylthio)propane at low concentration in grilled beef. However, the presence of high concentrations of 1-(methylthio)propane in fresh beef evoked off-flavour, as shown in this study. The probably origin of this compound can be the feeding due to its high impact on beef aroma ^[1]. In a study done to evaluate the products formed during propylene glycol fermentation in the rumen, the presence of 1-(methylthio)-propane was also reported ^[17]. These authors indicated that this compound is produced when sugars and polyols are administered to ruminants for therapeutic or nutritional purposes. A common practice in animal production industry is the use of diet supplements for beef with propylene glycol to prevent ketosis ^[18]. On the other hand, another source of this compound can be truffles as 1-(methylthio)-propane was also detected in truffle aroma ^[19]. Therefore, different feeding practices can be the source of this off-flavour compound. These results reflect those of Stelzeni & Johnson ^[1] who indicated that short-term realimentation practices affect the perception of off-flavours.

Conclusion

In summary, the identification of a beef off-flavour characterized by a garlic-cabbage aroma, was attributed to the presence of 1-(methylthio)propane. A method was validated to quantify 1-(methylthio)propane by SPME-GC-MS analysis in fresh beef from different geographical origin. Several samples were characterized by this odour

although its origin was not elucidated. However, caution should be taken in animal production systems as a probable source could be the feeding practices of cows.

Acknowledgements

Financial support from AGL2015-64673-R from MINEICO (Spain) and FEDER funds are fully acknowledged.

References

1. Stelzlen, A M and Johnson DD. Effect of days on concentrate feed on sensory off-flavor score, off-flavor descriptor and fatty acid profiles for selected muscles from cull beef cows. *Meat Sci.*, 2008, 79: 382-393.
2. Song S, Zhang X, Hayat K, Liu P, Jia C, Xia S, Xiao Z, Tian H, and Niu Y. Formation of the beef flavour precursors and their correlation with chemical parameters during the controlled thermal oxidation of tallow. *Food Chem.*, 2011, 124: 203-209.
3. Calkins C R and Hodgen JM, A fresh look at meat flavor. *Meat Sci.*, 2007, 77: 63-80.
4. Stelzleni A M and Johnson DD, Benchmarking sensory off-flavor score, off-flavor descriptor and fatty acid profiles for muscles from commercially available beef and dairy cull cow carcasses. *Livestock Sci.*, 2010, 131: 31-38.
5. Wadhwani R, Murdia LK and Cornforth DP, Effect of muscle type and cooking temperature on liver-like off-flavour of five beef chuck muscles. *Int. J. Food Sci. Tech.*, 2010, 45: 1277-1283.
6. Yancey EJ, Grobbel JP, Dikeman ME, Smith JS, Hachmeister KA, Chambers IEC, Gadgil P, Milliken GA and Dressler EA, Effects of total iron, myoglobin,

- hemoglobin, and lipid oxidation of uncooked muscles on livery flavor development and volatiles of cooked beef steaks. *Meat Sci.*, 2006, 73: 680-686.
7. Stetzer AJ, Cadwallader K, Singh TK, McKeith FK and Brewer MS. Effect of enhancement and ageing on flavor and volatile compounds in various beef muscles. *Meat Sci.*, 2008, 79: 13-19.
 8. Elmore JS, Mottram DS, Enser M and Wood JD, Effect of the polyunsaturated fatty acid composition of beef muscle on the profile of aroma volatiles. *J. Agric. Food Chem.*, 1999, 47: 1619-1625.
 9. Casaburi, A., Piombino, P., Nychas, G.-J., Villani, F., & Ercolini, D. (2015). Bacterial populations and the volatilome associated to meat spoilage. *Food Microbiology*, 45, 83-102.
 10. Corral S, Salvador A and Flores M, Salt reduction in slow fermented sausages affects the generation of aroma active compounds. *Meat Sci.*, 2013, 93: 776-785.
 11. Pollien P, Ott A, Montigon F, Baumgartner M, Muñoz-Box R and Chaintreau A. Hyphenated Headspace-Gas Chromatography-Sniffing Technique: Screening of Impact Odorants and Quantitative Aromagram Comparisons. *J. Agric. Food Chem.*, 1997, 45: 2630-2637.
 12. Van den Dool H and Dec. Kratz, P. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *Journal of Chromatography*, 1963, II, 463-471.
 13. Burdock GA, *Fenaroli's handbook of flavor ingredients* (4th ed.). Boca Raton, Florida, CRC Press Inc. 2002.
 14. Long GL and Winefordner JD. Limit of detection: A closer look at the IUPAC definition. *Anal. Chem.*, 1983, 55: 712-724.

15. Ansorena, D., Gimeno, O., Astiasaran, I., Bello, J. Analysis of volatile compounds by GC-MS of a dry fermented sausage: chorizo de Pamplona. *Food Res. Int.* 2001, 34, 67-75.
16. Raes K, Balcaen A, Dirinck P, De Winne A, Claeys E, Demeyer D, and De Smet S. Meat quality, fatty acid composition and flavour analysis in Belgian retail beef. *Meat Sci.*, 2003, 65: 1237-1246.
17. Trabue S, Scoggin K, Tjandrakusuma S, Rasmussen MA and Reilly PJ Ruminal fermentation of propylene glycol and glycerol. *J. Agric. Food Chem.*, 2007, 55: 7043-7051.
18. Nielsen NI and Ingvarsen KL, Propylene glycol for dairy cows: A review of the metabolism of propylene glycol and its effects on physiological parameters, feed intake, milk production and risk of ketosis. *Animal Feed Sci. Tech.*, 2004, 115: 191-213.
19. Pelusio F, Nilsson T, Montanarella L, Tilio R, Larsen B, Facchetti S and Madsen JØ Headspace solid-phase microextraction analysis of volatile organic sulfur compounds in black and white truffle aroma. *J. Agric. Food Chem.*, 1995, 43: 2138-2143.

FIGURE LEGEND

Figure 1. Chromatogram of the volatile compounds identified in fresh beef without (sample 12) (A) and with off-flavour (sample 8) (B).

Table 1. Odour active compounds identified in fresh beef with and without off-flavour.

Compound	LRI std	LRI ^a GC-MS		RI ^b	LRI GC-O	Descriptor	DF ^c
		Off-flavour beef (sample 8)	Beef (sample 12)				
Hexane	600	600	600	a			
2,3-Butanedione	632	628	628	a	635	Butter, oxidized iron, toasted	7
2-Butanone	638	632	633	a			
Heptane	700	698	698	a			
Acetic acid	706	707	708	a			
Allyl methyl sulfide	718	717	-	a	717	Rotten, cured, oxidized iron	6
2-Ethylfuran	719	- ^d	719	a			
2-Pentanone	730	-	732	a			
1-(methylthio)propane	734	734	-	a	731	Garlic, cabbage, oxidized iron, solvent, rancid	8
1-Penten-3-ol	737	-	740	a			
3-Hexanone		-	742	b			
1-(methylthio)-1-propene (E)		744	-	b			
1-(methylthio)-1-propene (Z)		760	-	b	753	Garlic, cabbage, oxidized iron, rancid	5
3-Hydroxy-2-butanone	782	780	780	a			
2-Ethylthiophene		784	-	b			
Toluene	789	789	789	a			
Octane	800	800	800	a			
2-Octene	812	820	-	a			
Hexanal	839	-	841	a			
Butanoic acid	876	879	880	a			
Nonane	900	900	900	a			
Unknown 1				a	935	Cabbage, oxidized iron, rancid	6
2-Heptanone	933	936	936	a			
Unknown 2				a	1023	Stable, dung, burnt plastic, solvent	6
Butyrolactone	1021	1028	1028	a			
Limonene	1048	1050	1050	a			
Dimethyl sulfone	1060	1061	1061	a			
Hexanoic acid	1064	1064	1064	a			
Unknown 3				a	1134	Spicy, herbal, paprika	7
4-Methylphenol	1190	1192	1193	a			
Dodecane	1200	1200	1200	a			
Unknown 4				a	1240	Toasted, cured, tasty	4
Tridecane	1300	1300	-	a			

^a Linear retention index (LRI) of the compounds eluted from the GC-MS using a DB-624 column capillary column (30m x 0.25mm i.d. x 1.4µm film thickness), LRIstd: LRI of standard compounds in the GC-O-FID.

^b RI: reliability of identification: a, identification by mass spectrum and by coincidence with the LRI of an authentic standard; b, tentatively identification by mass spectrum.

^c DF Detection frequency value reported in off-flavour beef.

^d Not detected

Table 2. Method validation and quantification ($\mu\text{g/g}$ beef) of 1-(methylthio)propane in fresh beef samples from different origins.

Method validation		
Instrument response	Slope	28.29
	Intercept	58.82
	R^2	0.997
Range	$\mu\text{g/g}$	0.5-10.0
Accuracy	2.5 $\mu\text{g/g}$ %	98.29
	% RSD	7.58
	5 $\mu\text{g/g}$ %	94.61
	% RSD	15.58
	10 $\mu\text{g/g}$ %	96.78
	% RSD	15.43
LOD	μg	0.33
LOQ	μg	1.10
Precision	Repeatability (n=6) % RSD	11.3
	Precision intraday (n=3)	
	9 $\mu\text{g/g}$ Area (% RSD)	16.0
	10 $\mu\text{g/g}$ Area (% RSD)	12.7
	11 $\mu\text{g/g}$ Area (% RSD)	5.5
	Precision interday (n=3)	
	9 $\mu\text{g/g}$ Area (% RSD)	29.8
	10 $\mu\text{g/g}$ Area (% RSD)	26.7
	11 $\mu\text{g/g}$ Area (% RSD)	13.7
Application method		
Fresh beef	1-(methylthio)propane ($\mu\text{g/g}$)^a	
Sample 1	nd ^b	
Sample 2	nd	
Sample 3	nd	
Sample 4	nd	
Sample 5	nd	
Sample 6	2.3 $\pm$ 0.5	
Sample 7	2.1 $\pm$ 0.8	
Sample 8	3.0 $\pm$ 1.1	
Sample 9	6.0 $\pm$ 0.6	
Sample 10	< LOQ	
Sample 11	nd	
Sample 12	nd	
Sample 13	nd	
Sample 14	nd	

^aMean $\pm$ SD (n=3)

^bnd: not detected (below LOD).