final report

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Prepared by: Linda Farmer and Terence Hagan
Agri-Food and Biosciences Institute

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Feasibility Study to Evaluate the use of SPME Volatile Collection in Beef for Linkage to Consumer Flavour Evaluation

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Feasibility Study to Evaluate the use of SPME Volatile Collection in Beef for Linkage to Consumer Flavour Evaluation

Linda Farmer and Terence Hagan, March 2010

Agri-Food and Biosciences Institute, Belfast, UK

conducted in collaboration with Texas Technical University, Lubbock, USA

1. Summary

1.1. Background

This project was commissioned by MLA, through Rod Polkinghorne, to establish the feasibility of developing a standardised flavour testing protocol linked to consumer response data comparison, working with Texas Technical University (TTU). The full objectives are listed in Section 3.

1.2. Main Findings

- A 10 minute collection from 15 ml vials appears to be effective for most compounds, though some close to their detection limits may cause difficulties.
- SPME fibres may be held for up to 24 hours with minimum effect on most components. However, it is likely that especially reactive components such as thiols will be lost.
- The variation between collections is higher than desirable and does not depend on fibre number, order of injection, or whether steaks are replicated or not. While this is not abnormal for this type of method, it is likely to be an impediment to analysis of the data. There is some evidence that a strict protocol can reduce this variability.
- Recommendations have been made regarding:
    - Cooling the front of the column with liquid nitrogen or solid CO_2.
    - Preferred end of column pressure.
    - GC-MS eV setting to give highest sensitivity.
    - To minimise variability, collections should be conducted on steaks cooked within a batch of ten, according to the MSA cooking protocol.
- As expected, SPME-GC-MS does not allow the estimation of all those compounds important for beef flavour. Nevertheless, it does provide a mechanism for monitoring representative compounds from a wide range of formation pathways.

- Two short trials demonstrated that the method can be successfully operated in conjunction with MSA consumer panels. Despite the small quantity of data these showed that:
    - There is an apparent relationship between high intramuscular fat and slower release of aroma compounds, with smaller concentrations released of some volatile compounds.
    - There was little evidence of the effect of ageing of grilled rump in the quantities of volatiles detected but the experiment was very small. Rump sometimes does not show a strong effect of ageing and it remains to be seen if the consumer panels detected a difference.

1.3. Conclusions

The feasibility studies conducted have shown that the SPME-GC-MS method is well suited to the collection of volatile aroma compounds from very small samples collected during standard MSA consumer panels. While attention to detail is needed, the method may be operated by appropriately qualified scientists provided that at least one is a trained GC-MS operator.

A protocol for collection of volatile aroma compounds has been drafted. However, some details are expected to change and evolve as further tests are conducted.

The method collects a good quantity of compounds from a very small sample. Nevertheless, there are many aroma compounds which cannot be detected. Therefore, the use of this method will have to focus on the detection of a range of representative compounds from the different flavour formation pathways, rather than on the very small quantities of some of the key aroma compounds themselves.

The variability of the method is the main disadvantage encountered. While this is inherent in such methods, there is some evidence that the use of a reproducible cooking method and precise protocol may minimise this problem.

Preliminary studies alongside consumer panels provide evidence of some differences between treatments. These include a reduction in the release of some volatiles in the presence of high fat levels and some changes with ageing of rump steak. However, these trials were very small and greater numbers require to be analysed to check how these relate to consumer sensory results.

The SPME-GC-MS method shows good promise for the objective measurement of aroma volatiles in steaks subjected to MSA consumer assessment. How these measurements will relate statistically to the consumer assessments will require more data.

2. Background

The flavour of cooked meat comprises both taste and aroma. Taste is caused by water soluble compounds mainly detected in the mouth and on the tongue. These comprise salt, sour, sweet, bitter and "umami" (deliciousness). Aroma is caused by small volatile compounds, which are usually fat soluble, most of which are formed during cooking. Many hundreds of volatile aroma compounds are created during cooking, of which perhaps 20 are believed to have a major impact on flavour. The resulting aroma is detected by receptors in the nose both before eating and during eating, via a passage at the back of the nose.

These aroma compounds are formed by chemical reactions, which occur during cooking, including the Maillard reaction between reducing sugars and amino acids, the thermal oxidation of lipids, the breakdown of vitamins as well as, in some cases, the transfer of chemical compounds from the animal's diet to its muscle tissue. Much research has been conducted on the volatile aroma compounds contributing to the flavour of cooked beef and how these are formed from components of the raw meat. This subject has been reviewed (Farmer 1992, Mottram 2000, Farmer 2009).

The flavour of meat can be affected by a number of factors. Examples that are often cited include diet (grass versus grain fed), intramuscular fat, muscle, ageing and cooking method. In fact these factors operate by very different mechanisms. A grass diet increases the n-3 fatty acid content of the diet which alters the volatile products of lipid oxidation; in addition, certain compounds from the vegetation can be transferred to the flesh. Intramuscular fat is believed to influence the release of flavour more than its formation. Ageing results in proteolysis and other breakdown reactions, increasing the concentration of the precursors of the aroma compounds, such as amino acids and sugars. Cooking method (time and temperature) impacts on the quantities of aroma compounds formed from all of these reactions.

It is known that many of the key aroma compounds of cooked meat are present in very small concentrations and that their importance is due to the extreme sensitivity of the human nose to these compounds. The measurement of these compounds by any instrumental technique is always a challenge.

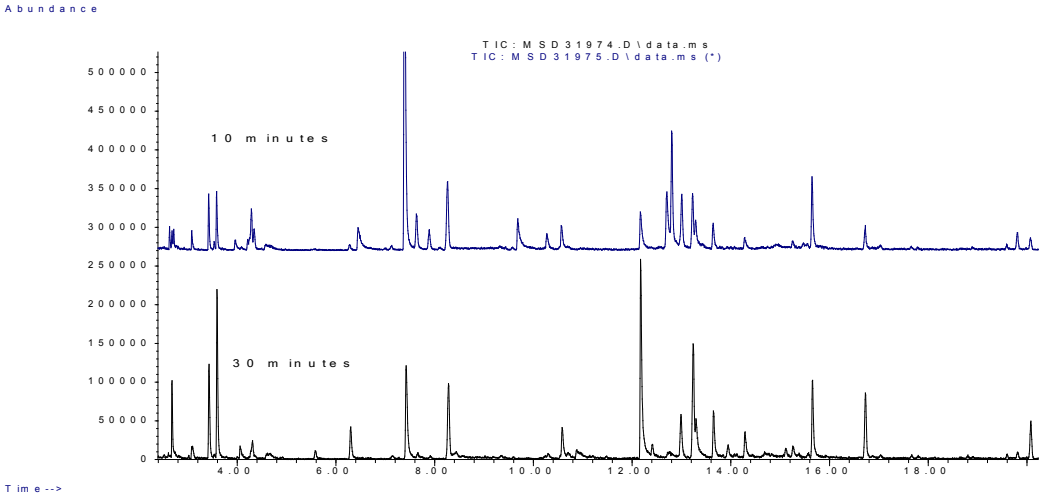
While a number of techniques have been developed for the instrumental measurement of texture, the large number of aroma and taste compounds present in meat, together with the low concentrations of some of these, has always meant that flavour measurement has been a specialised technique. However, the simplification of GC-MS technology and the advent of straightforward volatile collection techniques such as "solid phase micro-extraction" (SPME) has meant that such analyses may now be conducted by many laboratories. Nevertheless, the analysis and interpretation of the data obtained can still cause considerable challenges.

Discussions preliminary to this project focused on the feasibility of developing a technique for measuring some aspects of flavour that might correlate with the sensory quality of the meat. Such a method would have to be transferrable from laboratory to laboratory. The aroma volatiles measured would have to focus on those which lie within the capability of the methodology and be sufficiently well defined for analysis by laboratories unaccustomed to flavour analysis. The intention of this project is to evaluate the feasibility of using the

operationally simple SPME method for the collection of headspace volatiles from steak cooked according to the MLA protocol, followed by analysis by GC-MS.

Example Output for one aspect tested

Figure 2. Effect of time of collection on chromatogram
(black = 30 mins; blue = 10 mins)



3. Sensitivity of SPME / GC-MS for important odour compounds

A major concern with the use of SPME for the routine analysis of grilled beef odour is the relatively low sensitivity of this technique. This is because the technique depends on absorbing odour compounds from the air (headspace) above a sample on to a fine adsorbent fibre. This fibre has limited capacity. Given that the concentrations of many of the important odour compounds are extremely low, there were always doubts whether SPME would have the capacity required.

Consultation of the literature together with our own experience identified 86 compounds contributing to the aroma of cooked beef. Some of these are reported to be "odour impact compounds" through the use of odour dilution studies or odour activity values. These are both techniques that allow a combination of concentration and odour threshold of a compound to be taken into account. The list was narrowed down to 48 compounds which were sought in the beef samples. Table 2 shows these compounds and a preliminary comparison of their detection in beef by several different methods.

Table 2. Compounds reported to contribute to beef aroma and their detection using different methods

Compound[a]	Linear retention index[b] (CPSil 8/BP-5)	SPME-GC-MS[c] (Lubbock)	SPME-GC-MS (SIM)[d] (Lubbock)	SPME-GC-MS[e] (Belfast)	Tenax-GC-MS[f] (Belfast)
Methanethiol	<500	*[g]			

Compound[a]	Linear retention index[b] (CPSil 8/BP-5)	SPME-GC-MS[c] (Lubbock)	SPME-GC-MS (SIM)[d] (Lubbock)	SPME-GC-MS[e] (Belfast)	Tenax-GC-MS[f] (Belfast)
Dimethylsulphide or ethanethiol	<600	**			
2,3-butanedione	595	***	**	***	o
3-Methylbutanal	656	**	**	**	**
3-hydroxy-2-butanone	717	***	***	***	o
Dimethyldisulphide	748	**	**	**	**
2-methylthiophene	776	*	**	*	*
Hexanal	805	***	***	**	**
Methylpyrazine	833	o	**	**	o
2-methyl-3-furanthiol	869	o		o	o
3-mercapto-2-pentanone	901	o	o	o	o
Methional	911	**	**	**	**
2-furanmethanethiol	913	o	o	o	o
2,5/6-dimethylpyrazine	925	**	**	**	**
2-methyl-3-(methylthio)furan	948	o	o	o	o
Dimethyltrisulphide	970	o	*	o	o
1-Octen-3-one	979	o	o	o	*?
2-pentylfuran	994	o	**	**	**
Octanal	1003	**	**	**	**
Trimethylpyrazine	1003	o	**	**	**
3-Hydroxy-4,5-dimethyl-2(5H)-furanone	1010	o	o	o	o
2-Acetylthiazole	1020	o	*	*?	O
Phenylacetaldehyde	1055	**	**	**	**
4-Hydroxy-2,5-dimethyl-3(2H)-furanone	1067	o	*?	O	o
p-Cresol (4-methylphenol)	1074	o	o	o	o
2-Ethyldimethylpyrazines	1082	**	**	**	**
Guaiacol (2-methoxyphenol)	1090	o		o	o
Nonanal	1102	***	***	***	***
2-Acetyl-2-thiazoline	1107	o	*?	*?	O
(E,Z)-2,6-Nonadienal	1155	o	*?	O	o
2,3-Diethyl-5-methylpyrazine	1160	o	*?	*?	*
(E)-2-Nonenal	1160	o	o	o	*?
2-Methyl-3-(methyldithiofuran)	1168	o	o	o	o
2-Decanone	1200	o	o	o	o
(E,E)-2,4-Nonadienal	1217	o	o	o	o
Benzothiazole	1227	o	o	o	*?
3-Acetyl-2,5-dimethylthiophene	1250	o	o	o	o
(Z)-2-decenal	1257	o	o	o	**
(E,Z)-2,4-Decadienal	1295	o	o	o	o
(E,E)-2,4-Decadienal	1318	o	o	o	o

Compound[a]	Linear retention index[b] (CPSil 8/BP-5)	SPME-GC-MS[c] (Lubbock)	SPME-GC-MS (SIM)[d] (Lubbock)	SPME-GC-MS[e] (Belfast)	Tenax-GC-MS[f] (Belfast)
2-Methyl-3-(methyltrithiofuran)			o		
	1383	o		o	o
Vanillin	1410	o	o	o	o
beta-ionone	1493	o	o	o	o
bis (2-methyl-3-furyl) disulphide			o		
	1534	o		o	o
12-Methyltridecanal	1576	o	o	o	*?
Gamma-Dodecalactone	1681	o	o	o	o

a Compounds reported to contribute to the aroma of beef. Those in yellow are especially important. Those in bold were those which were measured at Lubbock and Belfast.
B Linear retention index is a system for referring elution time to that of the alkanes (octane, none etc) to provide a transferrable reference between different instruments.
C Identified in three GC-MS runs from the collections conducted alongside consumer panels.
D Identified in one GC-MS run (so far) from the collections conducted alongside consumer panels (using SIM)
e Identified in three GC-MS runs conducted at Belfast to evaluate effect of fibre storage.
F Identified in only one GC-MS run (so far) conducted at Belfast by collection on to Tenax.
G o = not found; * = occasionally found/hard to identify; ** = routinely found/easily identified; *** = abundant; a space indicates that the instrument settings require adjustment to check for this compound.

Table 2 shows that many of the compounds were not detected by any of the methods compared here. This is as expected as some of these compounds require specialised techniques for their measurement. Nevertheless, surprisingly, SPME has provided more sensitivity than expected. Despite the small fibres, sample sizes and time of collection, the quantity of volatiles collected has proved comparable with a sample previously collected on to traps containing Tenax, which have a greater capacity, though the Tenax was probably not used to maximum capacity. A recent paper (Elmore et al., 200?) reported that SPME collected one tenth of the quantity of volatiles from meat obtained on Tenax traps. The SPME method failed to detect any of the less volatile aroma compounds (after nonanal in Table 2) while the Tenax method did enable the detection of some of these.

The use of single ion monitoring (SIM) can increase the sensitivity by about 10-fold, by focusing the instrument on measuring those ions of specific interest. Evidence of this can be seen in the additional compounds detected in the Lubbock runs when SIM was used.

Thus, as expected, SPME-GC-MS does not allow the estimation of all those compounds important for beef flavour. Nevertheless, it does provide a mechanism for monitoring representative compounds from a wide range of formation pathways.

4. Use of SPME method on beef samples subjected to consumer flavour evaluation

4.1. Use of SPME and GC-MS for comparison of beef with different fat contents

Sensory panels were conducted at TTU, Lubbock on Thursday 10 December. These included sirloins from different US grades of beef and from Waygu cattle, for which the intramuscular fat content had been determined by TTU. A small number of samples (nine) were analysed as shown in Table 3. Collections were by the final method as described in Section 4.3 and GC-MS was conducted with eV = automatic and final pressure = "vacuum".

Table 3. Samples analysed bt SPME and GC-MS, differing in fat content

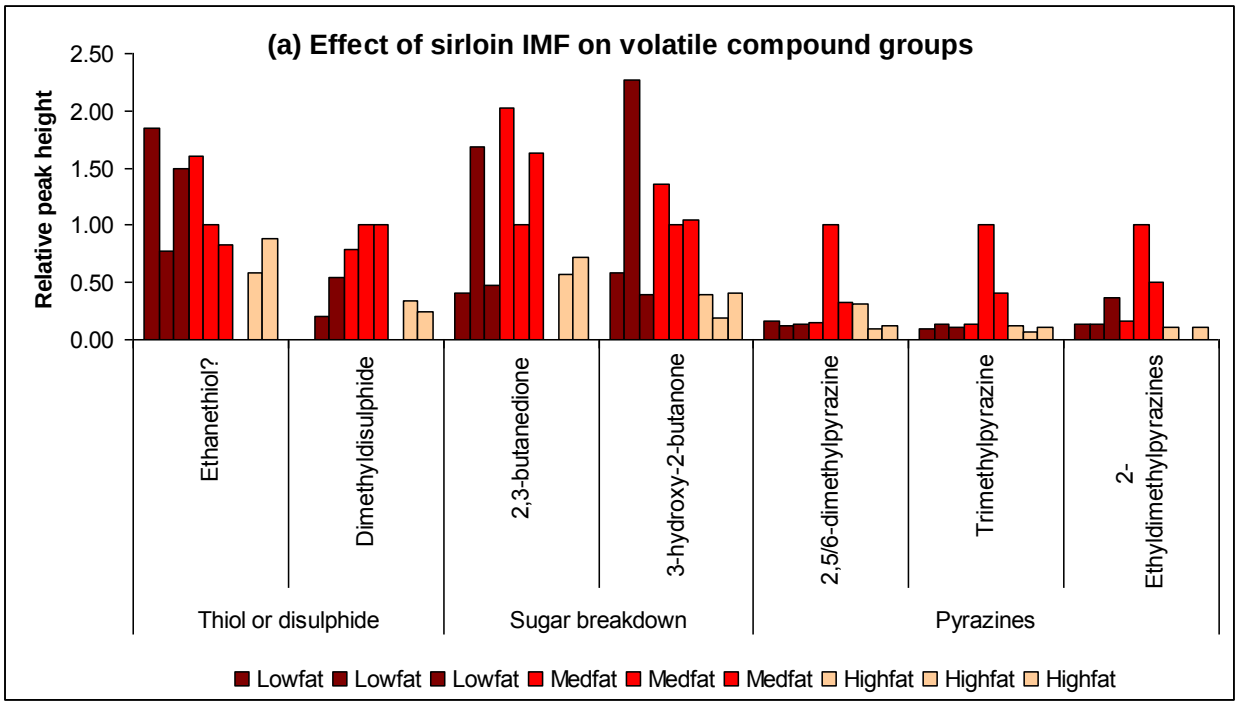
Sample	Fat content	US Grade	IMF	GC-MS Run code
X54B	Low fat			Belfast 028
E79G	Low fat			Belfast 034
P45E	Low fat			Belfast 036
C92U	Medium fat			Belfast 030
D99J	Medium fat			Belfast 033
H91N	Medium fat			Belfast 037
W97Z	High fat			Belfast 029
G38T	High fat			Belfast 032
U87X	High fat			Belfast 035

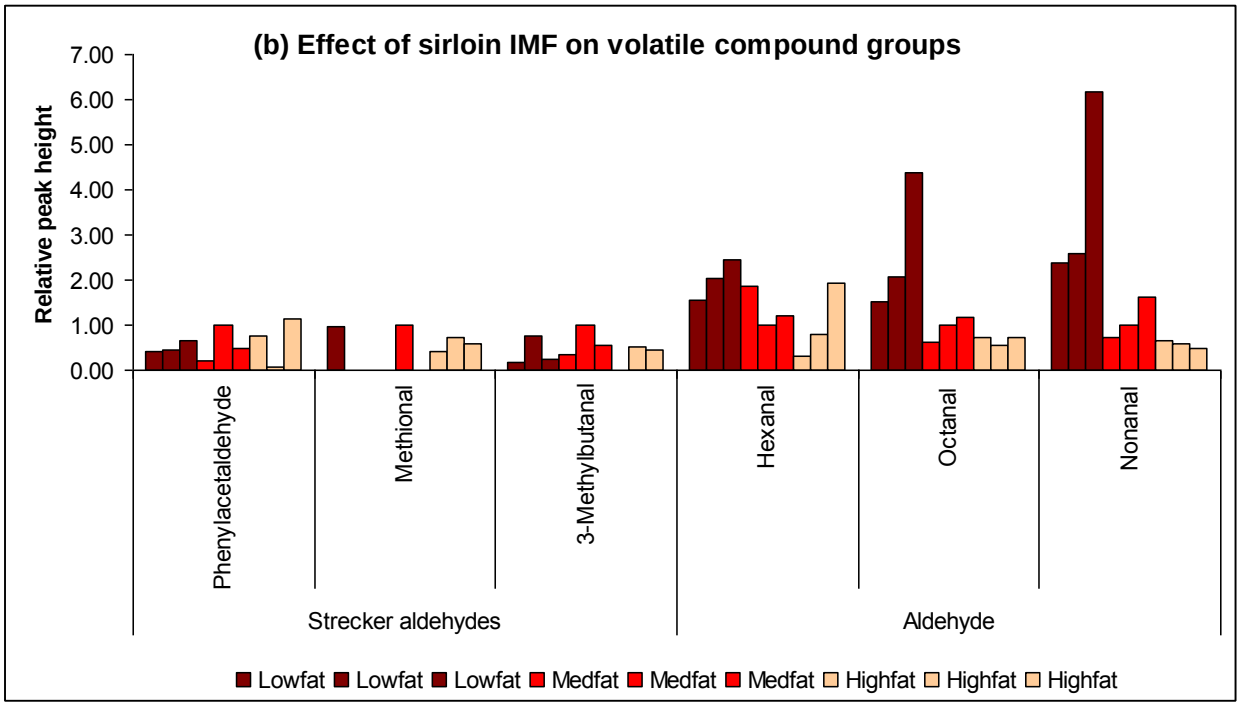
Volatile compounds were selected for analysis from a list of compounds known to contribute to the aroma and flavour of beef, based on those detectable by the methods used. It is expected that additional compounds could become detectable when planned adjustments are made to the instrumentation (new column, higher eV). Nevertheless, the compounds monitored are representative of a number of routes of formation which contribute to flavour formation. Figure 11a and b shows the ion heights for each compound relative to their height in one run (Belfast033.D); this removes the quantitative differences between compounds and allows their comparison in one Figure.

The results shown in Figure 11 show that there was some variation between the individual steak from similar treatments. For this experiment, it is not possible to say if this is due to variability in the method or variability between steaks, as no replication was included. However, the variability is less than for the studies shown in Figures 3 and 4, perhaps due to the fact that ten steaks were cooked each time.

Despite the variability, there is a trend for the steaks from the high fat (Waygu) steaks to give lower quantities of most volatile compounds. This trend is to be expected from the role that intramuscular fat plays in flavour release. The hot fat in cooked meat is believed to act as a solvent in which the fat soluble aroma compounds dissolve preferentially. The flavour is then released gradually during eating, which is desirable. It has been shown that in meat products (Chevance and Farmer 2001?), the higher fat products produce lower quantities of volatile compounds and a more satisfying flavour release.

Figure 11 a and b. Effect of sirloin fat content on selected aroma volatiles (a) thiols/disulphides, sugar breakdown products and pyrazines and (b) Strecker aldehydes and aldehydes





This trend is especially pronounced for the aldehydes which show highest quantities in the low fat steaks and the least in the high fat steaks. The aldehydes are amongst the more abundant aroma compounds that contribute to beef flavour and are formed primarily from

the phospholipids in meat during cooking (Mottram and Edwards 1983). In addition to the action of the intramuscular fat as a solvent for these very fat soluble compounds, the higher ratio of phospholipids to neutral lipids in lower fat beef may also contribute. The differences between the low fat and medium fat samples is less clear and there is considerable variation between samples.

Methional, an important aroma compound in many cooked foods, appears to be detected most consistently in the high fat meat. To determine whether this effect is significant would require further analysis.

Examination of the data was also conducted to check for any effect of individual fibre number or length of storage of fibres before injection. There was no evidence in these results of any consistent effect of these factors.

This very short trial was sufficient to demonstrate the relationship between high intramuscular fat and flavour release, with smaller concentrations released of some volatile compounds.