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A comparison of solid-phase microextraction (SPME) fibres for measurement of hexanal and pentanal in cooked turkey

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Abstract

Static headspace solid-phase microextraction (SPME) was evaluated for the quantitative measurement of hexanal in cooked turkey samples. Three fibre materials Carboxen/PDMS, PDMS/DVB and Carbowax/DVB with fibre thickness of 75, 65 and 65 μm, respectively, were compared for linearity, limit of detection, and repeatability of response to hexanal in cooked turkey samples. In terms of reproducibility and linearity of response the PDMS/DVB fibre was the most effective of the three fibres studied while Carboxen/PDMS was the most sensitive, with a limit of detection for hexanal of 2 ng/g. The use of 2-methyl pentanal as an internal standard facilitated the accurate measurement of hexanal and pentanal in oxidised turkey homogenates. In fresh and oxidised cooked turkey samples a high correlation between both hexanal and pentanal levels determined by SPME/GC and lipid oxidation measured as 2-thiobarbituric reactive substances (TBARS) was obtained.

1. Introduction

Lipid oxidation leading to the development of warmed-over flavour (WOF) is a significant deteriorative problem in pre-cooked meats and pre-prepared meals. It is generally accepted that the development of lipid oxidation in cooked meats is due to the autoxidation of muscle lipids especially the phospholipids (Igene & Pearson, 1979; Wu & Sheldon, 1988). In a comparison of muscle from five species Wilson, Pearson and Shorland (1976) found turkey muscle to be most susceptible to WOF development.

Measurement of 2-thiobarbituric acid reactive substances (TBARS) is a widely used method for the determination of the oxidative status of muscle foods. The TBARS measurement is relatively non-specific and does not measure volatile compounds that specifically contribute to WOF (Pearson, Love & Shorland, 1977). Hexanal is one of the major products of oxidation of fats (Frankel, Neff & Selke, 1981) and has been used to follow the course of lipid oxidation and off-flavour development in cooked foods (Dupuy, Bailey, St Angelo, Legendre & Vercelotti, 1987). Shahidi, Yun, Rubin and Wood (1987) reported a linear relationship between hexanal content and sensory scores in cooked ground pork and St Angelo et al. (1987) established a similar relationship for cooked beef. Kerler and Grosch (1997) using aroma extract dilution analysis (AEDA) reported that the development of off-flavours in refrigerated stored chicken was associated with a seven-fold increase in hexanal. In a study of cooked turkey Wu and Sheldon (1988) found hexanal to be a major volatile component and to increase with refrigerated storage. The authors also obtained good correlations between hexanal values and other measures of off-flavour development, such as TBARS values and sensory scores.

Solid-phase microextraction is a versatile new sample preparation technique (Arthur & Pawliszyn, 1990) that has, among other applications, been used to measure the volatile flavour profiles of foodstuffs (Yang & Peppard, 1994). In comparison to well established techniques for analysing the volatile constituents of foodstuffs, such as solvent extraction, simultaneous distillation extraction and purge and trap methodology, SPME is inexpensive, solvent free and convenient (Zhang & Pawliszyn, 1993). In addition, because relatively mild sampling conditions can be used, i.e. systems at equilibrium at temperatures less than 50°C, SPME gives a better quantitative estimate of the flavour profile as perceived by the nose.

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A limited range of chemistries was initially available in the commercial fibres including the widely used polydimethylsiloxane (PDMS) fibre coatings of varying thickness (7, 30 and 100 μm). The more recent introduction of a number of new fibres with a potentially greater range of selectivities prompted us to evaluate the performance of these materials for the quantitative measurement of hexanal in the headspace of cooked turkey. The fibres examined included 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB) for polar volatiles and 65 μm Carbowax/DVB for extraction of polar analytes such as alcohols from solution and for measuring volatile profiles (Popp & Pasckhe, 1997). In contrast to PDMS which is essentially a high viscosity rubbery liquid and extracts analytes by absorption, the latter are mixed coating porous polymer solids which function by an adsorption rather than an absorption mechanism. A theory for analyte extraction by these materials has recently been developed by Górecki, Yu and Pawliszyn (1999) which does not apply to Carboxen/PDMS, the third fibre evaluated in the present study. This fibre (thickness, 75 μm) is coated with Carboxen 1006 adsorbent, a porous carbon with a surface area of 1200 m^2/g and is particularly useful for trapping low molecular weight volatiles such as solvents.

2. Materials and methods

2.1. Reagents

Hexanal, 2-methyl pentanal, pentanal, heptanal, 2-(E)-hexenal, nonanal, 2-(E)-nonenal and butylated hydroxy anisole (BHA) were obtained from Sigma (Poole, Dorset, UK). SPME fibres [Carbowax/divinylbenzene (DVB), Carboxen/polydimethylsiloxane (PDMS) and PDMS/DVB] were obtained from Supelco Ltd., Supelco Park, Bellefonte, PA, USA.

2.2. Preparation of cooked turkey

Turkey breast muscle was obtained from Kerry Foods (Glenealy, Co. Wicklow, Ireland). Samples (500 g) were cooked to an internal temperature of 85°C in a domestic oven set at 190°C. Following cooking the samples were rapidly cooled to 4°C on ice and stored at 4°C prior to analysis.

2.3. Evaluation of fibres

Muscle homogenates (16.6% w/w) were prepared by homogenising 5 g freshly cooked turkey in 25 g distilled water. Aliquots (3 ml) of the homogenates were dispensed into 5 ml vials fitted with polytetraflouroethylene (PTFE) lined silicone septa. Hexanal was added to the vials from a stock solution of hexanal in water (100 μg/g) using a 10 μl syringe to give homogenate samples with added hexanal in the range 0.1–6 μg/g muscle. BHA (10 mM) was included in each vial to minimise hexanal production from the cooked muscle samples during fibre evaluation. After mixing the contents thoroughly vials were placed in a water bath set at 40°C and allowed to equilibrate for 5 min. For each analysis the SPME fibre was introduced into the vial headspace and held for different time periods to determine the effect of duration of sampling on hexanal uptake. The fibre was then removed from the headspace and desorbed at the GC injection port set at the recommended temperature for each fibre (250°C for Carbowax/DVB, 300°C for Carboxen/PDMS, 260°C for PDMS/DVB). To evaluate the effect of other aliphatic aldehydes, representing a range of volatilities, on the trapping efficiency of hexanal a mixture of aqueous standards (0.5 μg/g) was prepared from stock soloutions (100 μg/g) of the following: pentanal, hexanal, 2-(E)-hexenal, heptanal, nonanal, and 2-(E)-nonenal. Warming to 40°C with shaking for a few minutes facilitated complete dissolution of the aldehydes. Vials containing 3 ml of the aqueous standard solution were placed in a water bath at 40°C and allowed to equilibrate for 5 min. The SPME fibre was then introduced and allowed to sample as described above.

GC analyses were performed on a Pye Unicam series 204 gas chromatograph using a Quadrex 15 m×0.53 mm i.d. bonded phase fused silica column coated with Carbowax 20M (film thickness, 1 μm) and an FID detector. Hydrogen at 2 ml/min was used as the carrier gas and the column temperature was programmed to increase from 60 to 160°C at 3°C/min from sample injection. An 11 cm borosilicate glass tube (6 mm o.d.×1 mm i.d.) internally deactivated by treating with trimethylchlorosilane (TMCS) at 300°C and fitted with a stainless steel Swagelok reducing union (1/4″–1/16″) and graphite ferrules, was used to connect the column to the injection head. Peak areas were measured using a Spectra Physics Chrom Jet computing integrator.

2.4. Analysis of hexanal and pentanal in cooked turkey during storage

The hexanal concentrations in cooked turkey breast were measured immediately after cooking and after 1, 2, 4 and 6 days of storage at 4°C using the PDMS/DVB fibre. 2-Methyl pentanal was included as an internal standard during homogenisation at a concentration of 1 μg/g muscle. The response factor (peak area hexanal or pentanal/peak area internal standard) was determined by including 2-methyl pentanal and hexanal/pentanal (1 μg/g each) in both raw and unoxidised freshly cooked turkey muscle homogenates containing BHA (10 mM) to inhibit lipid oxidation (St Angelo, Crippen, Dupuy &

James, 1990). The same conditions, i.e. 5 min equilibration at 40°C and 20 min headspace sampling, were used to measure the response factors as were employed in the analysis of the meat samples.

2.5. TBARS measurement

Lipid oxidation in cooked turkey samples was monitored immediately after cooking and following storage at 4°C for up to 6 days using the 2-thiobarbituric acid procedure of Siu and Draper (1978). Results were expressed as TBARS in mg of malonaldehyde/kg muscle.

3. Results and discussion

3.1. Evaluation of fibres

The influence of sampling time on the uptake of hexanal from turkey homogenates spiked with the aldehyde (1 μg/g muscle) is shown in Fig. 1. Maximum recoveries for the two porous polymer fibres, Carbowax/DVB and PDMS/DVB, were achieved after 10 and 20 min respectively. On a peak area basis the capacity of the latter after 20 min was approximately 6 times greater than that of the Carbowax/DVB. The adsorptive fibre Carboxen/PDMS had a much greater capacity than the other two with saturation not being reached even after a sampling time of 40 min. The effect of the presence of a mixture of C_5–C_9 aldehydes (0.5 μg/g each) on hexanal recovery using the three fibres is presented in Table 1. For both the PDMS/DVB and Carboxen/PDMS fibres the peak areas of the individual aldehydes were virtually identical whether sampled alone or in a mixture with the others. By contrast, the recoveries of all the aldehydes were lower by a factor of around 7 in the mixture compared to individual sampling when using the Carbowax/DVB fibre.

Two important requirements for quantitative analysis using SPME are (a) good reproducibility for replicated samples and (b) a linear response (peak area versus headspace concentration) over the range of concentration at which the analyte is expected to occur in the sample. Reproducibility, assessed by measuring hexanal peak areas in triplicated analyses of a 0.5 μg/g muscle hexanal standard was excellent for the PDMS/DVB fibre with a relative standard deviation (rsd) of 1.2%, satisfactory for Carbowax/DVB (rsd 4.4%), but rather poor for Carboxen/PDMS (rsd 7.8%). This feature of the performance of the latter fibre, which has also been noted by other authors (Chai & Pawliszyn, 1995; Jelen, Walzly, Wasowicz & Kaminski, 1998; Popp & Pasckhe, 1997), is not unexpected in the light of the special properties of this material. Fig. 1 shows that while Carboxen/PDMS is clearly the most sensitive fibre saturation was not reached under the conditions used. Górecki, Yu and Pawliszyn (1999) used the Langmuir isotherm model to consider the surface of porous polymer adsorbents as being covered by, at most, a monomolecular layer of analyte molecules which allows equilibrium conditions to be quickly established. They suggested that, by contrast, capillary condensation can occur within the small

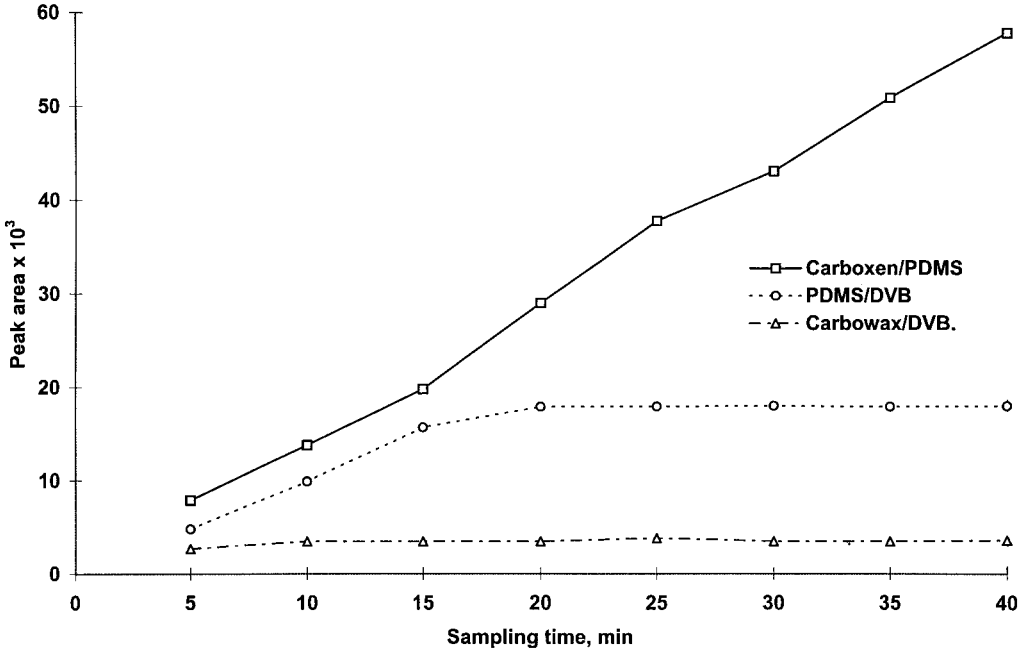

Fig. 1. Effect of sampling time on hexanal uptake by SPME fibres at 40°C from the headspace of a turkey homogenate (16.6%) spiked with hexanal (1 μg/g muscle).

Table 1
Recoveries of volatile aldehydes from headspace of aqueous standards (0.5 μg/g) at 40°C (A) single compound, (B) mixture of six

Compound	Carbowax/DVB (peak area×10²)		PDMS/DVB (peak area×10²)		Carboxen/PDMS (peak area×10²)	
	A	B	A	B	A	B
Pentanal	1.2	0.25	2.8	2.7	23.0	23.0
Hexanal	5.8	0.95	18.9	17.9	28.9	28.9
Heptanal	5.2	0.86	19.0	18.0	16.5	16.8
2-Hexenal	2.7	0.48	14.9	15.0	22.0	22.0
Nonanal	10.2	1.65	10.4	10.4	15.8	15.6
2-Nonenal	3.5	0.64	27.0	26.9	22.0	21.9

pores of Carboxen greatly inhibiting the attainment of an equilibrium under similar conditions. The present reproducibility data for the porous polymer adsorbents are somewhat better than those reported in a recent study (Song, Fan & Beaudry, 1998) using hexanal standards in water where repeatability of peak areas was found to be better for PDMS/DVB than Carbowax/DVB (rsd 6.7 and 8.8%, respectively) but not as good as the more widely used PDMS fibre (film thickness, 100 μm) where the rsd was 2.3%. The uptake of hexanal as a function of concentration in spiked homogenates for the three fibres is presented in Fig. 2. Excellent linearity up to a concentration of 4 μg/g muscle ($r^2 = 0.9981$) was found for the PDMS/DVB fibre confirming its suitability for the monitoring of hexanal in extensively oxidised turkey samples where the hexanal concentrations in 16.6% homogenates rarely exceeded this level. By contrast, the low capacity Carbowax/DVB was only linear to around 1 μg/g muscle ($r^2 = 0.99$), making it unsuitable for the analysis of highly oxidised samples. While the Carboxen/PDMS fibre has a higher capacity than the other fibres, resulting in a somewhat greater linear range (Fig. 2), its linearity for muscle hexanal concentrations up to 4 μg/g ($r^2 = 0.971$) was somewhat inferior to that of the PDMS/DVB fibre.

The high sensitivity of SPME for hexanal analysis was demonstrated by measuring the limit of detection (LOD) for the three fibres. The LOD was defined as three times the standard deviation of baseline noise and was determined by spiking serially diluted hexanal standards into a fresh 16.6% turkey homogenate containing BHA. The LOD values for Carboxen/PDMS, PDMS/DVB and Carbowax/DVB were 2, 7 and 12 ng/g muscle, respectively. Song et al. (1998) found the limit of quantitation (defined as concentration of analyte that produces a signal ten times that of noise) to be very similar for both Carbowax/DVB and PDMS/DVB (33 and 36 ng/g respectively). By determining the partition coefficents (K) using the method of Zhang and Pawliszyn (1993), Song et al. also found that the PDMS/DVB fibre had a K value for hexanal approximately one order of magnitude higher than that of the apolar 100 μm PDMS fibre and almost 4 times higher than that of the Carbowax/DVB. This observation in conjunction with the performance characteristics found in the present study confirms PDMS/DVB as the fibre of choice for quantitative analysis of hexanal in food products. However, we would urge caution in the application of the method to a more heavily oxidised food system than that examined in the present study. It is important to ensure that the hexanal concentration in the sample falls within a range over which there is a linear relationship between the amount of the analyte extracted by the fibre and its concentration in the headspace (Fig. 2). Moreover, if the headspace contains appreciable amounts of other volatile compounds, the possible effects of these on the efficiency of hexanal uptake should be considered.

Possible losses of sample leading to impaired reproducibility in routine SPME/GC analysis can arise through small leaks occurring in both the septum of the sampling vial as well as in that of the GC injection head. Thus, the use of an internal standard is highly desirable in this type of analysis. For monitoring both hexanal and pentanal in oxidised turkey samples we have found 2-methyl pentanal, which has not been reported to occur in turkey breast volatiles, to be an excellent internal standard. It elutes between the two aldehydes just after pentanal, from which it is baseline resolved, giving response factors determined by SPME of 0.954 for hexanal and 0.207 for pentanal using the PDMS/DVB fibre. Moreover, while the peak areas of both hexanal and the internal standard were reduced by about 30% when spiked into BHA treated raw or freshly cooked turkey breast homogenates relative to the values obtained in water, the response factor remained unaltered in all three media. In addition, the baseline levels of hexanal in raw or freshly cooked homogenates prepared using BHA were below the detection limit of the present method, which was of the order of 0.02 μg/g of muscle. Although there is a 25°C difference in the boiling points of pentanal and hexanal (103 versus 128°C), the large variation in the partition coefficients of the two as reflected in the observed response factors, was somewhat surprising.

3.2. Measurement of hexanal in cooked turkey during storage

Hexanal levels measured in 16.6% w/w muscle homogenates (sampling time, 20 min, 40°C) using SPME/GC and the PDMS/DVB fibre increased from levels of 0.8 μg/g immediately after cooking to 2.25 μg/g after 1 day storage and 4.01 μg/g after 6 days storage at 4°C (Fig. 3). These values are lower than those reported by Morrissey and Apte (1988) using a HPLC method. After 4 days storage in air Alfawaz, Smith and Jeon (1994) found hexanal and pentanal values of 6.8 μg/g muscle and 0.53 μg/g muscle in cooked ground beef. Again these values are higher than those reported here (3.72 and 0.211 μg/g, respectively) although in the present study the turkey breast was not comminuted but stored as whole

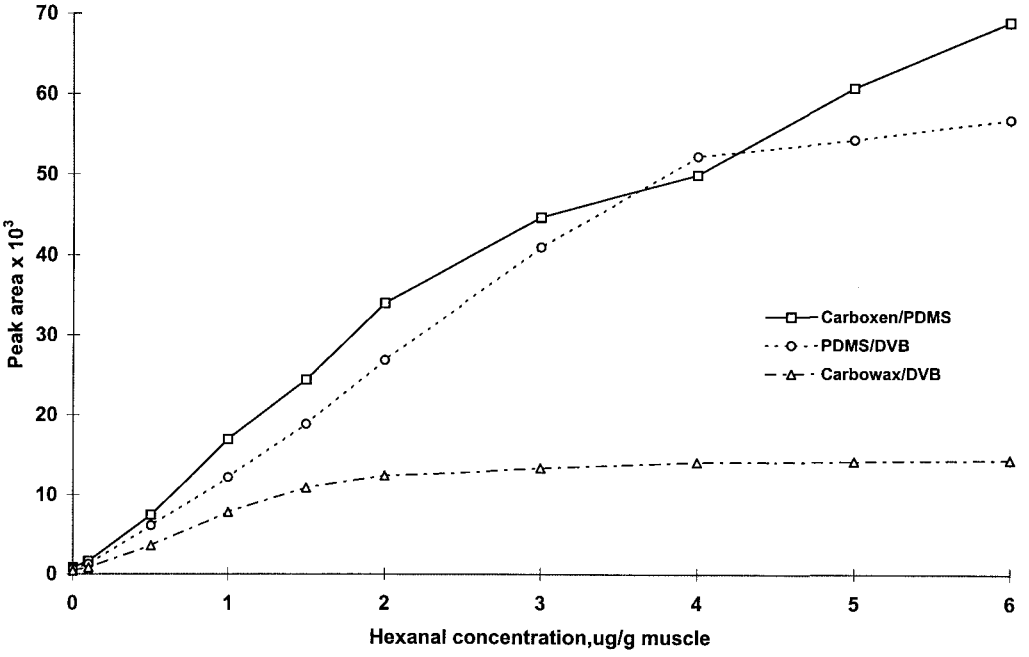

Fig. 2. Recovery of hexanal (0.1–6 μg/g muscle) from the headspace of 16.6% turkey homogenates containing 10 mM BHA.

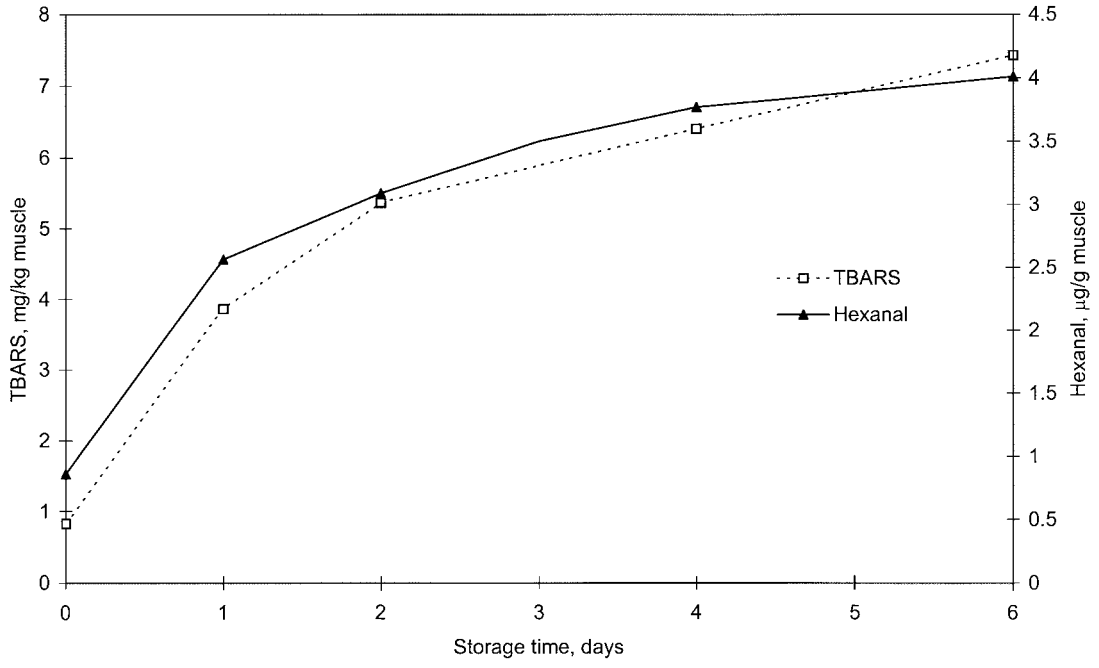

Fig. 3. Time course of lipid oxidation in a 16.6% turkey homogenate as measured by TBARS and headspace hexanal (SPME/GC).

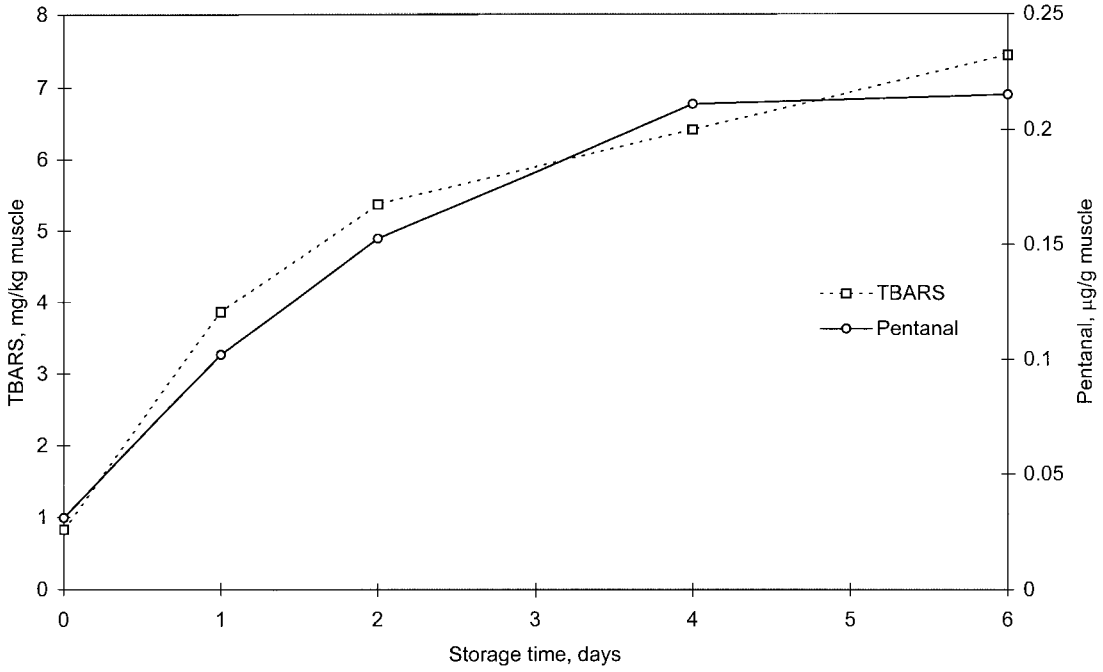

Fig. 4. Time course of lipid oxidation in a 16.6% turkey homogenate as measured by TBARS and headspace pentanal (SPME/GC).

pieces. As shown in Fig. 3 the lipid oxidation profile as a function of storage time monitored by the TBARS procedure was similar to that obtained using hexanal determined by SPME/GC with an excellent correlation between the two methods ($r^2 = 0.994$). Lipid oxidation as measured by accumulation of pentanal (Fig. 4) also agreed well with a slightly lower correlation coefficient ($r^2 = 0.9851$). Using a purge and trap method to measure volatiles in broiler breast patties Su, Ang and Lillard (1991) reported significantly lower correlations between both hexanal and pentanal and TBARS (0.8558 and 0.7296, respectively). Ahn, Sell, Chen, Wu and Lee (1998) using steam distillation to concentrate the volatiles in pork patties obtained correlation coefficients between TBARS and hexanal and pentanal of 0.93 and 0.94 respectively. However, they suggested that hexanal represented the lipid oxidation status of the meat better than any other volatile component. Jensen, Flensted-Jensen, Skibsted and Bertelsen (1998) using a static headspace technique to quantify hexanal in precooked pork patties reported a correlation coefficient of 0.83 between hexanal production and TBARS. As far as we are aware the correlation between hexanal and TBARS obtained in this study is the highest reported to date in the literature for cooked meats.

4. Conclusions

For the quantitative determination of hexanal in cooked turkey SPME represents a highly attractive alternative to other headspace methods. The PDMS/DVB fibre displayed the best combination of attributes in terms of reproducibility, sensitivity and linearity and the quality of the analysis was further enhanced by the use of 2-methyl pentanal as an internal standard. Good agreement between the established TBARS method of measuring lipid oxidation and SPME/GC quantification of hexanal in the headspace of cooked and stored turkey was obtained.

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