DETERMINATION OF HEXANAL IN COOKED TURKEY USING SOLID PHASE MICROEXTRACTION (SPME) / GC

Brunton, N.P., Cronin, D.A., Monahan, F.J. and Durcan, R.
Department of Food Science, University College Dublin, Belfield, Dublin 4, Ireland.

BACKGROUND

Hexanal is one of the major products of oxidation of fats (Frankel et al., 1981) and has been used to follow the course of lipid oxidation and off-flavour development in cooked foods. Shahidi et al. (1987) reported a linear relationship between hexanal content and sensory scores in cooked ground pork and St Angelo et al. (1987) established similar relationships for cooked beef. Kerler and Grosch (1997) reported that the development of off flavours in refrigerated stored chicken was due to a seven-fold increase in hexanal. Hexanal contents in the headspace of cooked turkey increased significantly with storage at 4°C and correlated well with other measures of off-flavour development, such as 2-thiobarbituric acid reactive substances (TBARS) values and sensory scores (Wu and Sheldon, 1988).

Solid phase microextraction (SPME) is a relatively new extraction technique and static headspace sampling involving SPME has been used for flavor analysis (Yang and Peppard, 1995). In comparison with other techniques for measuring the volatile profiles of foodstuffs SPME is inexpensive, convenient and solvent free. The extraction conditions used are relatively mild i.e., a low temperature system at equilibrium, and SPME is relatively free of artefact formation representing a better approximation of the volatile profile perceived by the nose. SPME headspace sampling involves exposure of the fibre to the headspace of the sample and, after the system has come to equilibrium, desorption of volatiles from the fibre in the heated injection block of the gas chromatograph.

OBJECTIVE

The objective of our study was to investigate the use of SPME for the quantitative analysis of hexanal in cooked turkey.

METHODS

Reagents. Hexanal, 2-methyl pentanal and butylated hydroxy anisole (BHA) were obtained from Sigma Chemical Co. Ltd., U.K. SPME fibres (Carbowax/DVB, Carboxen/PDMS and PDMS/DVB) were obtained from Supelco Ltd, U.K.

Preparation of cooked turkey. Turkey breast muscle was obtained from Kerry Foods (Gleneally, Ireland) and cooked to an internal temperature of 85°C in a domestic oven set at 190°C. After cooking the samples were cooled to 4°C on ice prior to analysis.

Evaluation of fibre types. Three SPME fibres were evaluated: Carbowax/DVB, Carboxen/PDMS, PDMS/DVB. The comparative trapping efficiencies of the 3 fibres as a function of sampling time were evaluated as was the linearity of response of each over a range of hexanal concentrations. Muscle homogenates (16.6% w/w) were prepared by homogenising 5 g freshly cooked turkey in 25 g distilled water. Before homogenisation the turkey samples were spiked with hexanal from a stock solution in water (100 μg/ml) to give homogenate samples with hexanal concentrations in the range 0 – 6 μg/g muscle. For the fibre evaluation study, BHA (10 mM) was also included to eliminater hexanal production from the cooked muscle samples. Aliquots (3 ml) of the homogenates were dispensed into 5 ml vials each fitted with a PTFE-lined septum. Vials were placed in a water bath set at 40°C and allowed to equilibrate for 5 min. 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 in the GC injection port set at the recommended temperature for each fibre. Analyses were performed on a Pye Unicam series 204 gas chromatograph fitted with a 15 m x 0.53 mm Quadrex bonded phase fused silica column coated with Carbowax 20M and an FID detector. Column temperature was programmed to increase from 60 to 160°C at 3°C/min from injection of the sample.

Quantification of hexanal in cooked turkey by SPME/GC. The hexanal concentrations in cooked turkey breast were analysed immediately after cooking and after 1, 2, 4 and 6 days of storage at 4°C using the PDMS/DVB fibre and the chromatographic conditions outlined above. 2-Methyl pentanal was included as an internal standard during homogenisation at a concentration of 1 μg/g muscle. The response factor for hexanal determination was calculated by including 2-methyl pentanal and hexanal (1 μg/g each) in a turkey muscle homogenate, containing BHA (10 mM) to inhibit lipid oxidation.

Lipid oxidation. 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 2-thiobarbituric acid reactive substances (TBARS) in mg of malonaldehyde/kg muscle.

RESULTS AND DISCUSSION

Evaluation of fibre types. The effect of duration of sampling clearly showed equilibration times for hexanal uptake from the headspace of cooked turkey samples of 10 and 20 min for the Carbowax/DVB and PDMS/DVB fibres, respectively (Figure 1). The absorption capacity of the Carboxen/PDMS fibre was such that even after 40 min the system had not reached equilibrium, making it unsuitable for the present application.

The high absorption capacity of the Carboxen/PDMS fibre was also reflected in its ability to absorb hexanal at concentrations up to 6 μg/g without becoming saturated (Figure 2). The PDMS/DVB fibre demonstrated a linear response to hexanal concentration in the range 0 - 4 μg/g meat.

While the Carbowax/DVB fibre had a low equilibration time for hexanal uptake the fibre became saturated at hexanal concentrations above 2 μg/g muscle, thus making it unsuitable for hexanal determination in highly oxidised samples.

Repeatability of results followed the order: PDMS/DVB > Carbowax/DVB > Carboxen/PDMS with relative standard deviation (rsd) values of 1 – 2%, 3 – 5% and 6 – 9%, respectively. There is conflicting information on the repeatability of the Caboxen/PDMS fibre. Chai and Pawliszyn (1995) found repeatability to be poor while Popp and Paschke (1997) found that rsd values fell within acceptable limits (7 - 10%). The Carboxen/PDMS fibre also suffered from desorption problems with severe peak

spreading in the absence of cryofocussing occurring at concentrations above 1 μg/g muscle even at the maximum recommended desorption temperature (300°C). Both the Carbowax/DVB and PDMS fibres gave excellent peak shapes for hexanal even at high concentrations.

Figures 1 and 2 illustrate that the extraction efficiency of the Carbowax /DVB fibre was the lowest of the three. The limits of detection (LOD) for hexanal for the three fibre types are listed in Table 2. LOD was defined as three times the standard deviation of baseline noise. Again the high extraction efficiency of the Carboxen/PDMS is reflected in its low limit of detection for hexanal. The PDMS/DVB fibre has a limit of detection 50 % higher than the Carboxen/PDMS fibre.

Quantification of hexanal in cooked turkey during storage. Hexanal levels measured using the SPME/GC method increased from levels of 0.8 μg/g immediately after cooking to 2.3 μg/g after 1 day storage and 4.0 μg/g after 6 days storage at 4°C (Figure 3). The values compare favourably with those recently reported by Wen et al (1997). These authors reported that hexanal levels increased from 0.1 to 5 μg/g in cooked turkey stored for 7 days. The time course for lipid oxidation monitored using the TBARS procedure was similar to that obtained using hexanal by SPME/GC (Figure 3). A correlation coefficient of 0.98 was obtained between the two methods.

CONCLUSION

SPME/GC offers a rapid alternative to the non-specific TBARS assay for lipid oxidation and to other GC and HPLC methods for quantitative determination of hexanal. SPME has the advantage of using relatively mild conditions for the generation of a volatile profile similar to that perceived by the nose.

REFERENCES

Chai, M. And Pawliszyn, J. (1995) Environ. Sci. Technol. 29, 693-698.

Frankel, E.N., Neff, W.E. & Selke, E. (1981). Lipids 16, 279-285.

Kerler,J. and Grosch, W. (1997). Zeitschrift-Fur-Lebensmittel-Untersuchung-Und-Forschung. 205, 232-238.

Popp, P. and Pasche, A. (1997). Chromotographia 46, 419-424.

Shahidi, F., Yun, J., Rubin, L.J. & Wood, D.F. (1987). J. Can. Inst. Food Sci. Technol. 20, 104-106.

Siu, G.M. and Draper, H.H. (1978). J. Food Sci. 43, 1147-1149.

St. Angelo, A.J., Vercellotti, J.R., Legendre, M.G., Vinnett, C.H., Kuan, J.W., James, C., Jr. & Dupuy, H.P. (1987). J. Food Sci. 52, 1163-1168.

Wen, J., Morrissey, P.A., Walton, J. and Sheehy, P.J.A. (1997). Ir. J. Ag. Food Res. 36, 75-84.

Wu, T.C.and Sheldon, B.W. (1988). J. Food Sci. 53, 49-54.

Yang, X. and Peppard, T. (1994). J. Agric. Food. Chem.42, 1925-1930.

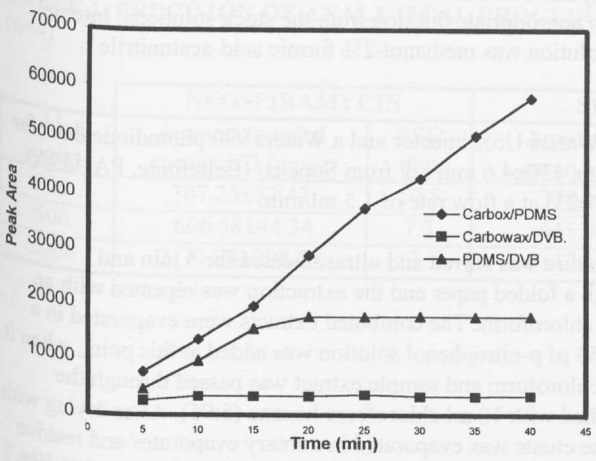

Figure 1. Effect of duration of sampling on hexanal uptake by Carboxen/PDMS, Carbowax/DVB and PDMS/DVB fibers in a 16.6% turkey homogenate spiked with 1.0 ug/g hexanal and containing 10mM BHA.

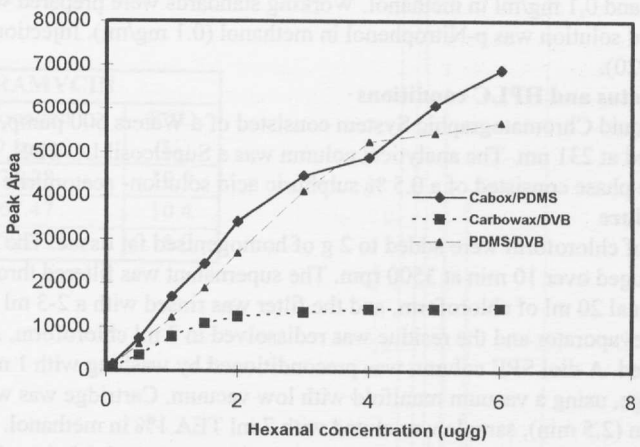

Figure 2. Effect of hexanal concentration in 16.6% turkey homogenates containing 10mM BHA on peak area for hexanal extracted using Carboxen/PDMS, Carbowax/DVB and PDMS/DVB fibers.

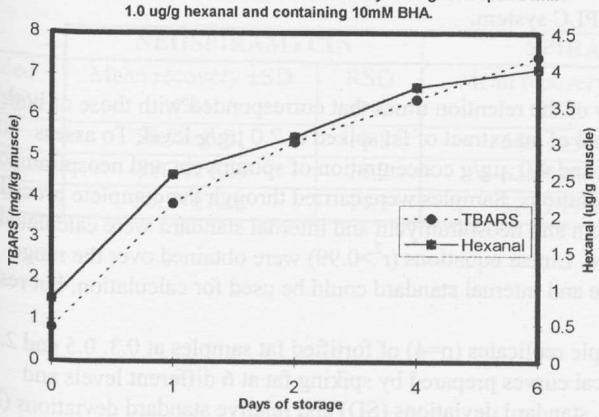

Figure 3. Time course of lipid oxidation in 16.6% turkey homogenate as measured by TBARS and hexanal (SPME/GC quantification.

Fiber Type	*LOD (ng/g)*
Carbowax/DVB	12.2
Carboxen/PDMS	2.2
PDMS/DVB	6.8

Table 1. Limits of detection for Carbowax/DVB, Carboxen/PDMS and PDMS/DVB fibers for hexanal in a 16.6% turkey homogenate.