JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2003,** *51,* 4185−4190 **4185**

Solid Phase Microextraction−Gas Chromatography for Quantifying Headspace Hexanal Above Freeze-Dried Chicken Myofibrils

CAROLYN F. GOODRIDGE,*,† RANDOLPH M. BEAUDRY,‡ JAMES J. PESTKA,† AND DENISE M. SMITH§

Department of Food Science and Human Nutrition and Department of Horticulture, Michigan State University, East Lansing, Michigan 48824, and Department of Food Science and Toxicology, University of Idaho, Moscow, Idaho 83844

A method using solid phase microextraction (SPME) combined with gas chromatography/mass spectrometry (GC/MS) was developed and used to determine the oxidation of freeze-dried chicken myofibrils spiked with methyl linoleate. Freeze-dried chicken myofibrils were found to act as a significant reservoir for hexanal. Recovery of hexanal emissions from the headspace above spiked myofibrils was 95% using a 5 min sampling time, with a total analysis time of ~12 min/sample. The SPME-GC/MS working linear response was from 0.01 to 10 mg hexanal/L ($r^2 = 0.995$). Freeze-dried chicken myofibrils with added methyl linoleate (0.6 mmol/g of protein) were stored at 50 °C at water activities of 0.30 and 0.75 for 0, 12, 27, and 50 h. Lipid oxidation was determined using SPME-GC/MS to measure headspace hexanal concentration, the thiobarbituric acid reactive substances assay (TBARS) to quantify malonaldehyde, and a conjugated diene assay. Lipid oxidation was influenced by storage time and water activity. A strong correlation ($r = 0.938$) existed between SPME-GC/MS and TBARS. The use of SPME−GC/MS was a sensitive and rapid method for detecting hexanal as an indicator of lipid oxidation in chicken myofibrils.

KEYWORDS: Lipid oxidation; solid phase microextraction; hexanal; chicken

INTRODUCTION

Lipid oxidation has long been recognized as a leading cause of quality deterioration in muscle foods and is often the decisive factor in determining food product storage life (*1*). All muscle foods are susceptible to lipid oxidation; however, the muscle foods of most concern are those with high concentrations of unsaturated fatty acids, such as chicken (*2*). The oxidative deterioration of food lipids involves autoxidative reactions of unsaturated fatty acids, accompanied by various secondary reactions. The major initial products of lipid oxidation are hydroperoxides, which degrade into a complex mixture of low molecular weight alkanes, alkenes, aldehydes, and ketones. These compounds have objectionable odors and reduce consumer acceptance of meat products (*3*). Associated changes in quality are manifested by deterioration in texture, nutritional quality, and the production of potentially toxic compounds (*4*).

From the perspective of the meat industry, detection of lipid oxidation needs to be accomplished using a specific, sensitive, and rapid method. A wide spectrum of lipid oxidation tests exists, ranging from simple organoleptic evaluations to more complex chemical methods. The most commonly used method for measuring lipid oxidation is the 2-thiobarbituric acid reactive substances test (TBARS), which was originally thought to quantify only malonaldehyde, a secondary product of lipid oxidation (*5*). Criticisms of this test arise from its lack of sensitivity and specificity to malonaldehyde.

Hexanal has become a popular indicator of lipid oxidation in foods. Hexanal is one of the dominant volatile secondary products formed during the oxidation of linoleic acid. Hexanal is the only aldehyde formed from both the 9- and the 13-hydroperoxide of linoleate (*6*). The odor of hexanal is often described as "grassy" and contributes to undesirable off-odors created during lipid oxidation as it also has a low odor threshold. Gas chromatography (GC) was used to establish strong correlations between hexanal content, sensory scores, and TBARS values in a number of meat products, including chicken (*6*), beef (*8*), and irradiated pork (*9*).

Several GC techniques have been employed for the analysis of hexanal. Static headspace techniques are simple and rapid but lack sensitivity as insufficient quantities of hexanal accumulate in the incubation chamber headspace (*10*). To overcome the decreased sensitivity of the static method, dynamic headspace or purge-and-trap techniques utilize an adsorbent material to collect volatiles over a period of time. While these

* To whom correspondence should be addressed. Tel: (519)888-4567 ext. 5123. Fax: (519)746-0435. E-mail: cgoodrid@scimail.uwaterloo.ca.
† Department of Food Science and Human Nutrition, Michigan State University.
‡ Department of Horticulture, Michigan State University.
§ Department of Food Science and Toxicology, University of Idaho.

10.1021/jf0260646 CCC: $25.00
Published on Web 06/21/2003

dynamic headspace techniques are sensitive, disadvantages include excessive preparation time, difficulty of use, and inconvenience when analyzing a large number of samples quickly (*11*).

Solid phase microextraction (SPME) is an adsorption technique that has gained widespread acceptance in analysis of volatile compounds as it overcomes the difficulties experienced with traditional headspace methods (*11*). SPME involves the extraction of analytes from the headspace above a sample onto a fused silica fiber; the analytes are then desorbed into a GC for analysis. Using SPME-GC, analytes were calculated to be within the same concentration range as those reported using headspace techniques, and the SPME method displayed high reproducibility (*11*). SPME has been used to detect volatile compounds in a number of products, including tomato and strawberry fruit (*12*), cheese (*13*), milk (*14*), and meat products such as cooked pork (*15*) and ham (*16*, *17*). Recently, SPME fibers have been used for the quantitative measurement of pentanal and hexanal in cooked turkey as indicators of lipid oxidation (*18*).

The goal of this study was to develop a method to monitor lipid oxidation using SPME combined with GC/mass spectrometry (MS) to detect hexanal in freeze-dried chicken myofibrils. Freeze-dried chicken myofibrils were selected as a lipid-free system in order to model the quantification of hexanal during lipid oxidation. To induce lipid oxidation, myofibrils were spiked with methyl linoleate (ML) at a ratio of 1:5.9, equal to the ratio of fat to protein typical in chicken meat (*19*). The specific objectives were (i) to develop a precise and accurate GC/MS method for the quantification of hexanal, (ii) to study hexanal distribution in chicken myofibrils incubated with hexanal, and (iii) to establish a correlation between hexanal concentration in chicken myofibrils determined by SPME-GC/MS and malonaldehyde concentration determined by TBARS.

MATERIALS AND METHODS

Preparation of Chicken Myofibrils. Chicken breast was obtained from a local supermarket and ground in a Hobart Kitchen-Aid food grinder with a 4.5 mm plate (model KF-A, Troy, OH). Myofibrils were isolated as described by Smith (*20*). Ground chicken was blended for 90 s in 4 volumes of 0.1 M NaCl and 0.05 M potassium phosphate buffer at pH 7.0 at maximum speed in a Waring blender (model 1120, Winsted, CT). The slurry was stirred for two 1 h intervals at 4 °C. Following each interval, the myofibrils were centrifuged at 2000*g* for 15 min. The final pellet was washed twice in 10 volumes of distilled water and centrifuged at 6000*g* to precipitate the protein. If required for an experiment, ML (Sigma Chemical Co., St. Louis, MO) was stirred in at 0.6 mmol/g of protein. Protein content in the final pellet was determined using the Bradford Protein Assay (*21*). The myofibrils were freeze-dried (Labconco, Kansas City, MO), vacuum-packaged in polyethylene-laminated pouches (Butcher and Pack Supply, Detroit, MI), and stored at −80 °C until needed.

SPME-GC/MS Procedure. A SPME fiber assembly (57310-U, Supelco Co., Bellefonte, PA) coated with 100 μm poly(dimethylsiloxane) was inserted into a fiber holder for manual sampling (57330U, Supelco) and preconditioned at 250 °C for 1 h prior to initial use. For all experiments, the fiber was introduced into the headspace of a clear glass screw top vial through a Mininert valve (33301, Supelco) screw cap fitted for the vial. The sampling time of spiked myofibrils was 5 min at ambient temperature (23 °C), which was sufficient to permit the establishment of near equilibrium for hexanal (data not shown). Following sampling, the fiber was retracted and the desorption time in the glass-lined, splitless injection port of the GC (HP-6890, Hewlett-Packard Co., Wilmington, DE) was 2 min.

A Supelcowax −10 capillary GC column (15 m × 100 μm, film thickness of 0.25 mm; 24077, Supelco) and helium carrier gas at a flow rate of 0.6 mL/min were used for volatile detection. The first 20 cm of the column was cooled with liquid nitrogen to cryofocus the volatiles from the fiber. Following volatile desorption, cryofocusing was removed, and the oven heating and mass spectra collection were initiated simultaneously. Oven temperature was increased from 40 to 250 °C at a rate of 60 °C/min and held for 3 min. The flow was maintained at 0.6 mL/min, the GC/MS transfer line was 200 °C, the inlet temperature was 250 °C, and the injection temperature was 250 °C. Volatiles were detected using time-of-flight MS, with electron impact ionization (Pegasus II, Leco Corp., St. Joseph, MI) and an ionization energy of 70 eV. Mass spectra were collected at a rate of 40 spectra/s over the mass range of m/z 33−250. Hexanal was identified by comparison of retention times and mass spectra to authenticated hexanal and by comparison of the mass spectra to that of hexanal in the National Institute for Standards and Technology (Search Version 1.5). Data were analyzed using LECO deconvolution software (Pegasus Version 1.33). Total analysis time was about 12 min/sample, and hexanal was not found to coelute with other compounds.

The hexanal vapor standard was prepared by diluting liquid hexanal (Sigma) in squalene (Sigma) to a concentration of 83 mg/L. An aliquot of the diluted hexanal was applied to a piece of filter paper and placed into a 4.4 L Erlenmeyer flask, fitted with a Mininert valve (Supelco). The flask was sealed, and the hexanal was allowed to vaporize to provide a headspace concentration of 0.9 mg hexanal/L. The hexanal vapor standard was analyzed by GC/MS as above, and the peak area of the standard was determined.

Limit of Detection and Response Linearity of SPME-GC/MS. Hexanal standards of 0.001, 0.01, 0.1, 1.0, 5.0, and 10.0 mg hexanal/L were prepared in squalene. To prepare a standard curve, an aliquot of each standard (1.5 mL) was added to a 15 mL clear glass screw top vial (27162, Supelco) sealed with a Mininert valve (33301, Supelco) and allowed to equilibrate for 5 min at ambient temperature. Each standard was then sampled and analyzed as previously described. Mean response and standard deviation were calculated for each standard from six replicate experiments.

Hexanal Loading and Repeated Sampling of Spiked Chicken Myofibrils. Emission of hexanal from myofibrils was determined by incubating freeze-dried chicken myofibrils over 0.01, 0.1, 1, and 10 mg hexanal/L (**Figure 1A**) in triplicate. Briefly, 1.5 mL of each hexanal standard was added to a 15 mL screw top vial (27162, Supelco) containing 2 g of glass beads (33201, Fisher Chemicals, Pittsburgh, PA). A 0.03 g sample of myofibrils was weighed in a 4 mL screw top vial (27111, Supelco), and these vials were nested into the glass beads in the larger 15 mL vials. A Mininert valve (33301, Supelco) was used to seal the 15 mL screw top vial and allow for sampling. The headspace in the vial was sampled at time 0, 1, 2, 3, 4, 5, and 24 h at ambient temperature over a 24 h incubation period using GC/MS.

Following 24 h of incubation, the myofibrils were removed, placed in new 4 mL screw top vials, sealed with a Mininert valve (33300, Supelco), and sampled hourly over a 6 h period (**Figure 1B**) using the SPME procedure described above, 5 min sampling time at ambient temperature followed by a 2 min desorption time into the GC/MS. Hexanal emission from the spiked myofibrils was determined by comparing the initial hexanal concentration with the concentration of hexanal recovered from the myofibrils following 24 h of incubation. Differences in vial volumes were taken into consideration when calculating hexanal concentration.

Hexanal Distribution Between Headspace and Myofibrils. Aliquots of 1.5 mL of each hexanal standard (0.01−10 mg hexanal/L) in squalene were added to 15 mL screw top vials containing glass beads. The vials were sealed with a Mininert valve and allowed to equilibrate for 24 h at ambient temperature. To a second set of vials, 1.5 mL of each standard was added to a 15 mL glass vial with glass beads. Small 4 mL screw top vials containing 0.03 g of myofibrils were nested into the glass beads; the vial was sealed with a Mininert valve and allowed to equilibrate for 24 h. Experiments were run in triplicate. Following 24 h of incubation, each vial was sampled using the SPME-GC/MS procedure previously described. The coefficients of variation were determined for each concentration. Hexanal distribution was calculated by dividing the peak area of the hexanal alone by the peak area of the hexanal at the same concentration incubated with myofibrils.

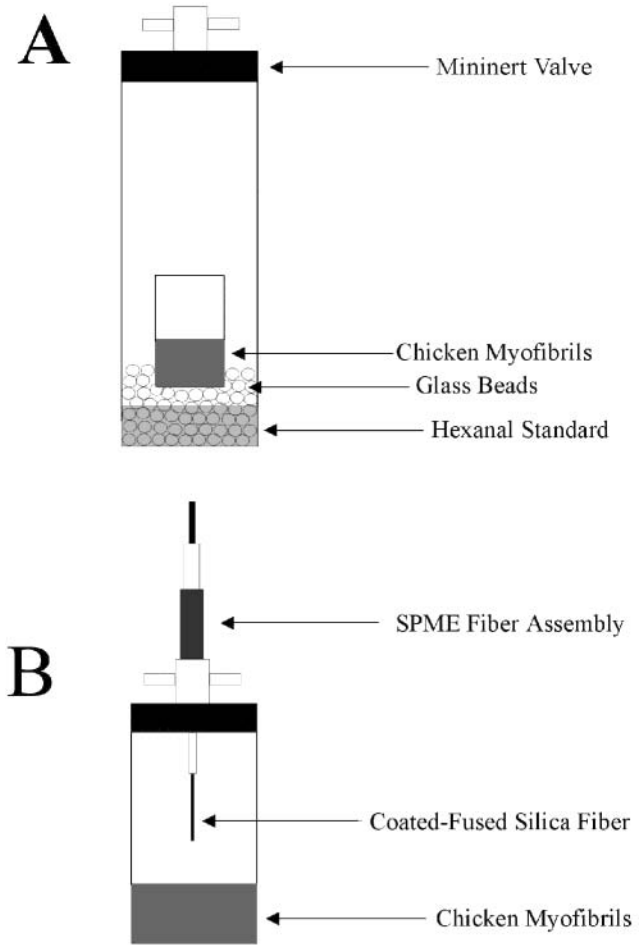

Figure 1. Hexanal loading and hexanal emission collection from chicken myofibrils. (**A**) Hexanal loading: 0.03 g of myofibrils incubated over 1.5 mL of hexanal standard. (**B**) Hexanal emission collection from spiked myofibrils following 24 h of incubation.

Accelerated Oxidation Study. Freeze-dried chicken myofibrils−ML were in sealed chambers in the dark at water activities (a_w) of 0.30 and 0.75 at 50 °C, an accelerated storage temperature typical of military provision storage studies (*22*). Saturated salt solution (10 mL) was added to the bottom of each 1 L Teflon chamber sealed with a screw lid (Berghof of America, Coral Spring, FL). Saturated salt solutions used for humidity control were sodium chloride ($a_w = 0.75$) and magnesium chloride ($a_w = 0.30$) and were prepared at 50 °C (*23*). Duplicate chambers were used for each storage time and a_w. A 0.5 g sample and a 0.05 g sample of myofibrils were weighed out, evenly distributed on an aluminum weigh dish, and transferred to the chamber. The samples were preweighed to account for any changes in weight that may have occurred during storage at the different water activities.

At each time point, 0, 12, 27, and 50 h, the storage chambers were opened and 0.05 g of myofibrils was removed and assayed for conjugated dienes (CD; see below). Also, at each sampling time, 0.5 g of myofibrils was transferred to a 15 mL glass screw top vial and sealed with a Mininert valve, and the hexanal was analyzed using the SPME-GC/MS procedure and quantified using a the hexanal vapor standard. Results of the SPME-GC/MS procedure were expressed as μg hexanal/g myofibril. This same sample was then analyzed using the TBARS assay.

CD Assay. CDs were quantified using the method of van Ruth et al. (*24*) except with a smaller sample size. In this method, 0.05 g of myofibrils was mixed with 5 mL of cyclohexane and centrifuged for 3 min at 1500*g*, after which the absorbance of the supernatant was read at 234 nm (Agilent UV/Visible Spectrophotometer, Agilent Technologies, Palo Alto, CA). Hydroperoxide concentration was calculated as mmol per g protein, using a molar absorptivity of 26 000 for linoleate peroxides (*25*). Each sample was tested in triplicate, and results were expressed as mmole CD/g myofibril.

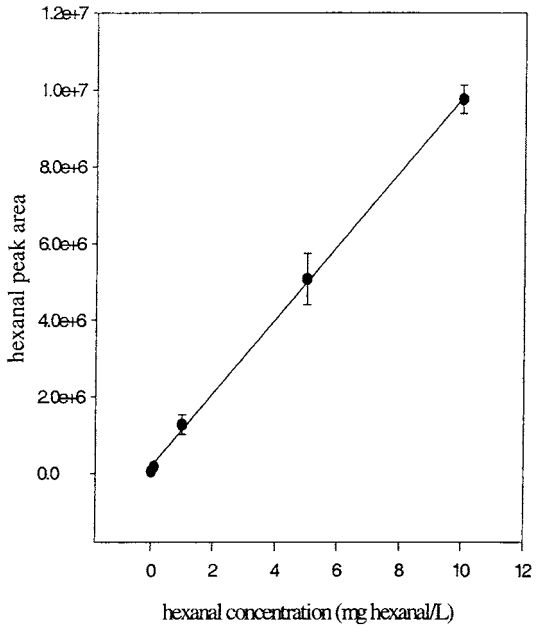

Figure 2. Linearity of SPME-GC/MS response to hexanal concentrations ($r^2 = 0.995$). Standards were prepared in squalene and sampled using SPME. Line represents a fitted regression line ($y = 963\,142x + 168\,333$). Determinations were made in triplicate.

TBARS. The TBARS were determined using the distillation procedure of Tarladgis et al. (*26*) except that a smaller sample size and antioxidant was added during homogenization. Myofibrils (0.5 g) were homogenized for 1 min with 48.75 mL of distilled water. Tenox 2 antioxidant (100 μg/mL; Eastman Chemical Company, Roebuck, SC) was added to minimize lipid oxidation during distillation. Hydrochloric acid (4 N), glass beads, and antifoam (Arthur H. Thomas Company, Philadelphia, PA) were added, and 15 mL of distillate was collected. A 5 mL sample of distillate was reacted with 5 mL of 0.02 M thiobarbituric acid and held in a boiling water bath for 35 min. After they were removed and cooled for 10 min, absorbance of the solution was read at 532 nm. The malonaldehyde standard curve was prepared using $0-9 \times 10^{-6}$ M tetramethoxypropane (TEP), and total recovery was determined by mixing TEP standard with the myofibrils. The TBARS were expressed as mg malonaldehyde/kg myofibril. Each sample was tested in triplicate.

Accelerated Oxidation Study Data Analysis. Accelerated oxidation study results were analyzed using SAS (Version 6.1; SAS Institute, Inc., Cary, NC). All samples were analyzed in triplicate, and the experiment was conducted in triplicate. Using time and treatment as factors, a two way analysis of variance on the log transform of the CD, TBARS, and GC/MS data was conducted.

Pairwise differences and interactions between the two main factors were tested, and correlation coefficients were determined between GC/MS, TBARS, and CD. The significance level was defined as $p < 0.05$.

RESULTS AND DISCUSSION

Validation of the GC/MS Procedure. The response of the GC/MS procedure to hexanal concentration was linear from 0.01 to 10 mg hexanal/L ($r^2 = 0.995$) (**Figure 2**), which was within the range of hexanal determined in meat systems during lipid oxidation (*6*). The limit of detection for this method was 0.01 mg hexanal/L. This value is comparable to the 0.007 mg hexanal/L limit of detection reported in turkey (*18*).

The precision of the GC/MS procedure as reflected by the coefficients of variation of hexanal peak areas ranged from 1 to 13%, with the majority of responses falling below 10%. The higher coefficient of variation was at the lowest hexanal concentration, agreeing with previous papers (*27*, *28*). The precision of this GS/MS−SPME method was comparable to the

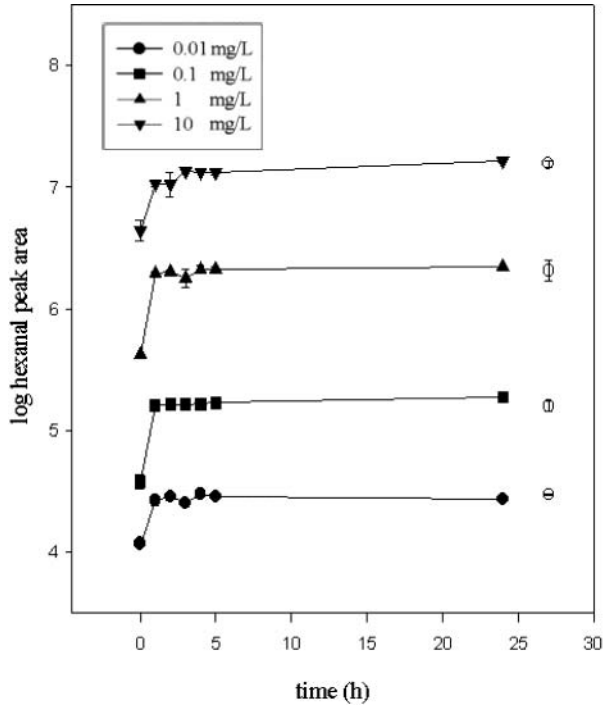

Figure 3. Achievement of steady state equilibrium in chicken myofibrils during hexanal loading. Myofibrils were incubated over hexanal standard (0.01−10 mg hexanal/L) prepared in squalene. The headspace over the myofibrils in the chamber was sampled every hour up to 5 h and following 24 h of incubation at 23 °C. Open symbols represent the first desorption of hexanal from spiked chicken myofibrils following transfer from hexanal loading vials to 4 mL vials. Determinations were made in triplicate.

SPME method developed for orange juice, in which coefficients of variation for 17 common flavor volatiles ranged from 1 to 18%, with the majority of volatiles falling below 10% deviation (*11*).

The SPME-GC/MS procedure used a 23 °C sampling temperature. It is important to use a low temperature to minimize oxidation and subsequent hexanal production during sampling. Other published SPME procedures describe the use of higher temperatures (40−60 °C) to maximize desorption of hexanal (*17*, *18*, *29*). Ruiz et al. (*17*) studied the effect of extraction time and temperature on volatile detection in ham, observing that the chromatographic area of hexanal increased with longer extraction times and higher extraction temperature (60 °C). These researchers suggested that the increase in chromatographic area may be attributed not only to the enhancement in extraction but also to the formation of hexanal during extraction due to the higher temperature.

Incubation of Myofibrils with Hexanal. No lipid oxidation was observed in myofibrils containing no ML. The headspace concentration of hexanal increased rapidly during the first hour of incubation when chicken myofibrils were nested over hexanal in squalene (**Figure 3**). An equilibrium within the chamber was reached after 1 h of incubation, with no significant differences observed between data points, and maintained up to 24 h. When foods undergo lipid oxidation, the gas phase is often not constrained and a true equilibrium is not attained (*30*). The quantification of hexanal using the SPME-GC/MS method indicated the concentration of free hexanal in the system at that time point.

Following 24 h of incubation, chicken myofibrils were removed and sampled to determine whether hexanal sorbed into the myofibrils and to measure the amount of hexanal that would be reemitted. Emission of hexanal from spiked myofibrils at the first sampling time (immediately after transfer) ranged from 91 to 95.3% of that detected in the incubation vial, indicating that the myofibrils acted as a significant reservoir for hexanal. The hexanal concentration in the myofibrils decreased with repeated sampling as it was desorbed onto the SPME fiber and was removed from the system. Hexanal losses ranged from 29.2 to 56.7% over six sampling times. Results indicated that the spiked myofibrils reached equilibrium in the vial during the 5 min sampling time, and the first sampling time resulted in the greatest emission of hexanal from the spiked myofibrils.

Table 1. Log Peak Area of Hexanal Alone and Hexanal Incubated with Chicken Myofibrils Following Equilibrium[a]

hexanal concn (ppm)	hexanal log peak area following 24 h incubation	% CV	hexanal plus myofibril hexanal log peak area following 24 h incubation	% CV	% loss in hexanal due to the addition of myofibrils in incubation vial
0.01	4.5 ± 0.031	7.1	4.4 ± 0.05	1.1	19.3
0.1	5.3 ± 0.034	7.8	5.25 ± 0.02	5.5	10.6
1	6.4 ± 0.02	4.1	6.39 ± 0.003	0.62	3.3
10	7.2 ± 0.007	1.6	7.19 ± 0.004	0.90	1.8

[a] Hexanal standards (1.5 mL) were incubated either alone in a 15 mL vial or with chicken myofibrils (0.03 g) for 24 h at ambient temperature and sampled.

Hexanal Distribution between Headspace and Myofibrils. Hexanal distribution refers to the distribution of hexanal between the gaseous phase of the vial (headspace) and the myofibrils. Hexanal distribution was examined by comparing the hexanal concentration found in the headspace above the chicken myofibrils to that found above hexanal standards following 24 h of incubation (**Table 1**). The concentration of hexanal in the vial headspace was affected by the percentage of hexanal retained by the myofibrils. In the 0.01 mg hexanal/L standard, a greater percentage difference (19.3%) in the headspace was observed between the peak area of hexanal alone as compared to the peak area of hexanal with chicken myofibrils, indicating that more hexanal was being retained by the myofibrils as compared to the standard alone. As the hexanal concentration was increased, the percentage concentration of hexanal in the headspace of the vial decreased. In the 10 mg hexanal/L, a 1.8% difference existed between the headspace of hexanal alone and the hexanal plus the myofibrils. Thus, while the myofibrils still sorbed hexanal and removed it from the headspace, the percentage of hexanal removed from the system was lower at higher hexanal concentrations. This may be due to the saturation of the limited number of hexanal binding sites (*31*). Because of the initial binding of hexanal to the myofibrils, the use of a headspace GC method may provide a more accurate means of assessing lipid oxidation when greater concentrations of hexanal are present.

The distribution of hexanal vapors into the chicken myofibrils may be attributed to specific binding and sorption. While several studies in the packaging area have examined the relationship between hexanal and its sorption into different polymer films (*32*, *33*), few studies have studied the sorption of hexanal by a food matrix. Gremli (*34*) conducted a study using a high vacuum shell freezing system to determine whether different flavor compounds reacted reversibly or irreversibly with soy protein. In a 5% soy protein solution, 37−44% of hexanal was found to be reversibly bound, while less than 5% was irreversibly bound. In a later study, Gutheil and Bailey (*28*) examined the relationship of hexanal with the proteins myosin and actin. At 100−1600 mg hexanal/L, myosin was reported to bind less than 10% of the hexanal. When studying the binding of actin to

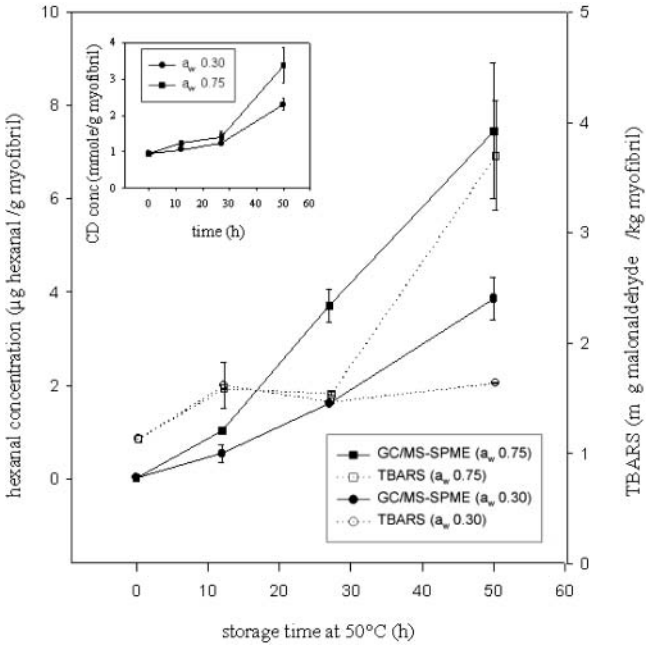

Figure 4. Comparison of the hexanal concentration determined by SPME-GC/MS and TBARS concentration of chicken myofibrils stored for 50 h at different water activities at 50 °C. Chicken myofibrils contained 0.6 μmol methylinoleate/g protein. Results of GC/MS are expressed as μg hexanal/g myofibril, and TBARS are expressed as mg malonaldehyde/kg myofibril. Inset: Effect of water activity and length of storage at 50 °C on the production of CDs during the same 50 h storage period. Determinations were made in triplicate.

hexanal, an average of 25−30% of the added hexanal (200−1000 mg hexanal/L) was bound to the protein. In determining binding of hexanal to protein, the published studies all used a hexanal solution, while this study was the first to examine binding using hexanal vapors.

Accelerated Oxidation Study. Hexanal, CDs, and TBARS increased ($p < 0.05$) during the 50 h accelerated oxidation study of freeze-dried myofibrils containing ML (**Figure 4**), suggesting that lipid oxidation was occurring. All three tests indicated that lipid oxidation proceeded more slowly ($p < 0.05$) at an a_w of 0.30 rather than at 0.75. Smith et al. (*35*) found that TBARS values were lower in freeze-dried chicken myofibrils stored at a_w of 0.22 and 0.43 than at an a_w above or below these values. The theoretical monolayer moisture content of freeze-dried chicken myofibrils is a_w 0.28, and a_w is 0.18 for myofibrils plus ML (*35*), which represents a moisture content at which dried food is least susceptible to oxidation (*23*).

The headspace hexanal concentration of myofibrils was greater ($p < 0.05$) at 0.75 a_w than at 0.30 a_w at all times during the 50 h study. The hexanal concentration in the headspace reached 7.5 μg hexanal/g myofibril at a_w 0.75 and 3.0 μg hexanal/g myofibril at a_w of 0.30 following 50 h of storage.

The CD concentration increased more rapidly over the 50 h study at 0.75 a_w than 0.30 a_w. CDs reached 3.39 mmol/g myofibril at a_w 0.75 and 2.3 mmol/g myofibril at a_w 0.30 after 50 h of storage. The a_w did not affect ($p < 0.05$) TBARS values until 27 h of storage. TBARS were 2.4-fold greater at a_w 0.75 than a_w of 0.30 after 50 h of storage ($p < 0.05$), with final TBARS of 1.5 and 3.7 mg malonaldehyde/kg myofibril for a_w 0.30 and 0.75, respectively.

Similar values were reported by other researchers. In freeze-dried pork and beef, TBARS were reported to increase to 0.70 and 0.75 mg malonaldehyde/kg sample, respectively, over a week of storage at 45 °C (*35*). Sun et al. (*22*) followed TBARS in freeze-dried beef patties and found that TBARS values increased over a 25 h storage period at 49 °C.

Table 2. Correlation Coefficient Matrix (*r*) between Methods Used to Measure the Extent of Lipid Oxidation in Freeze-Dried Chicken Myofibrils over 50 h of Storage at 50 °C

method	SPME-GC/MS	TBARS
TBARS	0.938[a]	
CD	0.975[a]	0.952[a]

[a] Significant at $p < 0.05$. Methods used were GC/MS–SPME, TBARS, and CD.

Hexanal formation was correlated to TBARS ($r = 0.94$) and CDs ($r = 0.98$) ($p < 0.05$) during the 50 h storage period (**Table 2**). The correlation of TBARS to CD formation was 0.95 ($p < 0.05$).

A greater change in hexanal concentration was observed between 12 and 27 h than in TBARS during the same time period. In cooked ground pork, Shahidi et al. (*37*) used hexanal analysis, TBARS, and sensory evaluation as indicators of oxidative stability and flavor acceptability. Following 2 days of storage at 4 °C, little difference between TBARS values was reported, whereas differences were detected using hexanal analysis and sensory evaluation. The researchers suggested that hexanal content is a better measure of oxidative state than TBARS in meats during the early stages of storage.

The application of SPME for lipid oxidation detection in meat in the food industry is feasible. Future studies of SPME use in lipid oxidation detection involve the application of SPME to detect lipid oxidation products in a fresh meat system. In addition, sensory analysis needs to be conducted in order to verify the relationship of SPME detection with sensory changes in the food. As a note, SPME coupled with GC alone may be used for hexanal detection, as only one compound is being analyzed. SPME coupled with GC may be more practical for the food industry laboratories as GC capabilities are more affordable than GC/MS equipment.

Hexanal content has been reported to be a sensitive and reliable indicator for the evaluation of the oxidative status of meat products. A precise and accurate SPME-GC/MS method for the rapid quantification of hexanal was described in this study. In a short term study of chicken myofibrils containing ML, the SPME-GC/MS method for hexanal appeared to be more sensitive than TBARS during the initial stages of lipid oxidation. As early detection of lipid oxidation is crucial in the food industry, this method shows promise as a fast and accurate alternative to the traditional methods of hexanal analysis.

ACKNOWLEDGMENT

The manuscript is based on the Ph.D. dissertation of Carolyn (Ross) Goodridge.

LITERATURE CITED

(1) Frankel, E. N. In search for better methods to evaluate natural antioxidants and oxidative stability in food lipids. *Trends Food Sci. Technol.* **1993**, *4*, 220−225.

(2) Tamura, H.; Kitta, K.; Shibamoto, T. Formation of the reactive aldehydes from fatty acids in an Fe^{+2}/H_2O_2 oxidation system. *Am. Chem. Soc.* **1991**, *39*, 439−442.

(3) Ramarathnam, N.; Rubin, L. J.; Diosady, L. L. Studies on meat flavor. 2. A quantitative investigation of the volatile carbonyls and hydrocarbons in uncured and cured beef and chicken. *J. Agric. Food Chem.* **1991**, *39*, 1839−1847.

(4) Kanner, J. Oxidative processes in meat and meat products: Quality implications. *Meat Sci.* **1994**, *36*, 169−189.
(5) Ladikos, D.; Lougovois, V. Lipid oxidation in muscle foods: A review. *Food Chem.* **1990**, *35*, 295−314.
(6) Shahidi, F.; Pegg, R. B. Hexanal as an indicator of the flavor deterioration of meat and meat products. In *Lipid Oxidation in Food*; St. Angelo, A. J., Ed.; ACS Symposium Series 500; American Chemical Society: Washington, DC, 1994; pp 105−119.
(7) Ahn, D. U.; Jo, C.; Olson, D. G. Analysis of volatile components and the sensory characteristics of irradiated raw pork. *Meat Sci.* **2000**, *54*, 209−215.
(8) Spanier, A. M.; Miller, J. A.; Bland, J. M. Lipid oxidation: Effect on meat proteins. In *Lipid Oxidation in Food*; St. Angelo, A. J., Ed.; ACS Symposium Series 500; American Chemical Society: Washington, DC, 1992; pp 105−110.
(9) Ahn, D. U.; Nam, K. C.; Du, M.; Jo, C. Volatile production in irradiated normal, pale softexudative (PSE) and dark firm dry (DFD) pork under different packaging and storage conditions. *Meat Sci.* **2001**, *57*, 419−426.
(10) Reineccius, G. A. Instrumental means of monitoring the flavor quality of foods. In *Chemical Markers for Processed and Stored Foods*; Lee, T. C., Kim, H. J., Eds.; American Chemical Society: Washington, DC, 1996; pp 241−251.
(11) Steffen, A.; Pawliszyn, J. Analysis of flavor volatiles using headspace solid-phase microextraction. *J. Agric. Food Chem.* **1996**, *44*, 2187−2193.
(12) Song, J.; Fan, L.; Beaudry, R. M. Application of solid-phase microextraction and gas chromatography/time-of-flight mass spectrometry for rapid analysis of flavor volatiles in tomato and strawberry fruits. *J. Agric. Food Chem.* **1998**, *46*, 3721−3726.
(13) Peres, C.; Viallon, C.; Berdague, J. Solid-phase microextraction -mass spectrometry: A new approach to the rapid characterization of cheese. *Anal. Chem.* **2001**, *73*, 1030−1036.
(14) van Aardt, M.; Duncan, S. E.; Bourne, D.; Marcy, J. E.; Long, T. E.; Hackney, C. R.; Eisey, C. Flavor threshold for acetaldehyde in milk, chocolate milk, and spring water using solid phase microextraction gas chromatography for quantification. *J. Agric. Food Chem.* **2001**, *49*, 1377−1381.
(15) Elmore, J. S.; Mottram, D. S.; Hierro, E. Two-fibre solid-phase microextraction combined with gas chromatography−mass spectrometry for the analysis of volatile aroma compounds in cooked pork. *J. Chromatogr. A* **2000**, *905*, 233−240.
(16) Sen, N. P.; Seaman, S. W.; Page, B. D. Rapid semiquantitative estimation of *N*-nitrosodibutylamine and *N*-nitrosodibenzylamine in smoked hams by solid-phase microextraction followed by gas chromatography-thermal energy analysis. *J. Chromatogr. A* **1997**, *788*, 131−140.
(17) Ruiz, J.; Cava, R.; Ventanas, J.; Jensen, M. T. Headspace solid-phase microextraction for the analysis of volatiles in a meat product: Dry-cured Iberian ham. *J. Agric. Food Chem.* **1998**, *46*, 4688−4694.
(18) Brunton, N. P.; Cronin, D. A.; Monahan, F. J.; Durcan, R. A comparison of solid-phase microextraction (SPME) fibres for measurement of hexanal and pentanal in cooked turkey. *Food Chem.* **2000**, *68*, 339−345.
(19) USDA. *Composition of Foods, Poultry Products: Agriculture Handbook 805*; U.S. Department of Agriculture: Washington, DC, 1979.
(20) Smith, D. M. Functional and biochemical changes in deboned turkey due to frozen storage and lipid oxidation. *J. Food Sci.* **1987**, *52*, 22−27.
(21) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248−254.
(22) Sun, Q.; Faustman, C.; Senecal, A.; Wilkinson, A. L.; Furr, H. Aldehyde reactivity with 2-thiobarbituric acid and TBARS in freeze-dried beef during accelerated storage. *Meat Sci.* **2001**, *57*, 55−60.
(23) Labuza, T. P. Determination of moisture sorption isotherms. In *Moisture Sorption: Practical Aspects of Isotherm Measurement and Use*; American Association of Cereal Chemists: St. Paul, MN, 1984; pp 64−66.
(24) van Ruth, S. M.; Roozen, J. P.; Jansen, F. J. H. M. Aroma profiles of vegetable oils varying in fatty acid composition vs concentrations of primary and secondary lipid oxidation products. *Nahrung* **2000**, *44*, 318−322.
(25) AOAC. *Official Methods of Analysis*, 14th ed.; Association of Official Chemists: Arlington, VA, 1992.
(26) Tarladgis, B. G.; Watts, B. M.; Younathan, M. T.; Dugan, L. R. A distillation method for the quantitative determination of malonaldehyde in rancid foods. *J. Am. Oil Chem. Soc.* **1960**, *37*, 44−48.
(27) Elmore, J. S.; Erbahadir, M. A.; Mottram, D. S. Comparison of dynamic headspace concentration of tenax with solid-phase microextraction for the analysis of aroma volatiles. *J. Agric. Food Chem.* **1997**, *45*, 2638−2641.
(28) Gutheil, R. A.; Bailey, M. E. A method for determining binding of hexanal by myosin and actin using equilibrium headspace sampling gas chromatography. In *Food Science and Human Nutrition: Developments in Food Science*, 29; Charalambous, G., Ed.; Elsevier Science Publishers: Amsterdam, Netherlands, 1992; pp 783−815.
(29) Nielsen, J. H.; Sorensen, B.; Skibsted, L. H.; Bertelsen, G. Oxidation in pre-cooked minced pork as influenced by chill storage of raw muscle. *Meat Sci.* **1997**, *46*, 191−197.
(30) Taylor, A. J. Physical chemistry of flavor. *Int. J. Food Sci. Technol.* **1998**, *33*, 53−62.
(31) O'Keefe, S. F.; Wilson, L. A.; Resurreccion, A. P.; Murphy, P. A. Determination of the binding of hexanal to soy glycinin and β-conglycinin in an aqueous model system using a headspace technique. *J. Agric. Food Chem.* **1991**, *39*, 1022−1028.
(32) Hernandez-Munoz, P.; Catala, R.; Gavara, R. Effects of sorbed oil on food aroma loss through packaging materials. *J. Agric. Food Chem.* **1999**, *47*, 4370−4374.
(33) Hernandez-Munoz, P.; Catala, R.; Hernandez, R. J.; Gavara, R. Food aroma mass transport in metallocene ethylene-based copolymers for packaging applications. *J. Agric. Food Chem.* **1998**, *46*, 5238−5243.
(34) Gremli, H. A. Interaction of flavor compounds with soy protein. *J. Am. Oil Chem. Soc.* **1974**, *51*, 95A−97A.
(35) Smith, D. M.; Noormarji, S. H.; Price, J. F.; Bennink, M. R.; Herald, T. J. Effect of lipid oxidation on the functional and nutritional properties of washed chicken myofibrils stored at different water activities. *J. Agric. Food Chem.* **1990**, *38*, 1307−1312.
(36) King, V. A. E.; Chen, J. F. Oxidation of controlled low-temperature vacuum dehydrated and freeze-dried beef and pork. *Meat Sci.* **1998**, *48*, 11−19.
(37) Shahidi, F.; Yun, J.; Rubin, L. J. The hexanal content as an indicator of oxidative stability and flavor acceptability in cooked ground pork. *Can. Inst. Food Sci. Technol. J.* **1987**, *20* (2), 104−106.

Received for review October 24, 2002. Revised manuscript received April 18, 2003. Accepted April 24, 2003. Research was supported in part by the Michigan Agricultural Experiment Station and the Michigan State University Crop and Food Bioprocessing Center.

JF0260646