ELISA To Quantify Hexanal−Protein Adducts in a Meat Model System

Tamara L. Zielinski, Stephanie A. Smith, James J. Pestka, J. Ian Gray, and Denise M. Smith*

Department of Food Science and Human Nutrition, Michigan State University, East Lansing, Michigan 48824

Monoclonal antibodies (MAb) were produced to hexanal−bovine serum albumin conjugates. An indirect competitive ELISA was developed with a detection range of 1−50 ng of hexanal/mL. Hexanal conjugated to three different proteins was recognized, whereas free hexanal and the native proteins were not detected. The antibody cross-reacted with pentanal, heptanal, and 2-*trans*-hexenal conjugated to chicken serum albumin (CSA) with cross-reactivities of 37.9, 76.6, and 45.0%, respectively. There was no cross-reactivity with propanal, butanal, octanal, and nonanal conjugated to CSA. The hexanal content of a meat model system was determined using MAb and polyclonal antibody-based ELISAs and compared with analysis by a dynamic headspace gas chromatographic (HS-GC) method and a thiobarbituric acid reactive substances (TBARS) assay. Both ELISAs showed strong correlations with the HS-GC and TBARS methods. ELISAs may be a fast and simple alternative to GC for monitoring lipid oxidation in meat.

Keywords: *Lipid oxidation; hexanal; immunoassay; monoclonal antibodies*

INTRODUCTION

Lipid oxidation can result in objectionable flavors that limit the shelf life of many food products (*1*). Lipid oxidation can also cause undesirable changes in color and nutritional value under a range of storage conditions (*2*). The safety of oxidized products has also been questioned. Malonaldehyde (*3*) and oxidized cholesterol (*4*) have been investigated for their possible roles in disease. Free radicals formed during oxidation are capable of attacking membranes, leading to impairment of membrane function (*5*). Both the safety concerns and economic losses resulting from consumer rejection of products show there is a need for a fast and reliable indicator of oxidation in food.

Hydroperoxides, the primary products of lipid oxidation, are tasteless, colorless, and odorless. Their breakdown produces low molecular weight compounds that result in the characteristic off-odors and flavors associated with oxidized foods (*6*). One of the major secondary products formed from the oxidation of linoleic acid, a common fatty acid in meat, is hexanal (*6*).

Hexanal content has potential for use as an indicator for quality control purposes during the processing and storage of meat products. Hexanal concentration, usually measured by gas chromatography (GC), has been correlated to sensory scores for cooked pork (*7*), cooked beef (*8*), and restructured chicken nuggets (*9*). Unfortunately, static headspace gas chromatographic (HS-GC) techniques for hexanal, although simple, rapid, and reproducible, often lack sensitivity (*10*). Improved sensitivity is achieved by use of dynamic (purge-and-trap) GC techniques that use an adsorbent material to collect volatiles over a period of time. Unfortunately, these are not rapid methods.

* Address correspondence to this author at the Department of Food Science and Toxicology, University of Idaho, Box 442201, Moscow, ID 83844-2201 [telephone (208) 885-7081; fax (208) 885-8937; e-mail dsmith@uidaho.edu].

An ELISA to quantify hexanal would provide a fast and simple test for monitoring lipid oxidation in foods. Immunoassays are rapid, cost-effective, and easy-to-use alternatives to conventional analytical techniques, achieving sensitivity and specificity without requiring highly trained analysts or sophisticated equipment (*11*). In addition, immunoassay kits are portable and can be used for routine surveillance in a processing facility or retail outlet.

This study was undertaken to develop a novel immunoassay for monitoring lipid oxidation in meat and, in particular, to replace more labor-intensive and time-consuming HS-GC methods. Polyclonal antibodies (PAb) have been produced to hexanal−protein conjugates prepared by conjugating hexanal to protein via Schiff base reactions with the lysine side chains (*12*). The PAb were most specific to the aliphatic aldehydes within one carbon length of hexanal but did not recognize branched aldehydes with similar carbon numbers. The limit of detection for the PAb was 7.4 ng of hexanal/mL. Monoclonal antibodies (MAb) may be more sensitive and specific for a particular analyte than a PAb-based assay (*13*).

The specific objectives were to (1) generate hybridomas producing MAbs to hexanal, (2) optimize an ELISA using antibodies from the clone showing the highest sensitivity and specificity, (3) characterize the specificity of the antibodies, and (4) quantitate the hexanal content of an accelerated lipid oxidation meat model system determined using both a PAb-based and a MAb-based indirect competitive (IC) ELISA and a dynamic HS-GC method.

MATERIALS AND METHODS

Materials. Female mice (BALB/c, 6−8 weeks old) were purchased from Charles River Laboratories (Wilmington, MA). Freund's complete and incomplete adjuvants were purchased from Difco Laboratories (Detroit, MI). The myeloma cell line

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P3/NS 1/1-Ag4 (NS-1) (ATCC TIB 18) was obtained from American Type Culture Collection (Rockville, MD). Goat anti-mouse immunoglobulin G conjugated to horseradish peroxidase (GAM−IgG HRP) was from Cappel Laboratories (West Chester, PA). The 3,3′,5,5′-tetramethylbenzidine (TMB) substrate and stopping buffer were from Di-Agra (Sterling Heights, MI). Chicken serum albumin (CSA), bovine serum albumin (BSA), and keyhole limpet hemocyanin (KLH) were from Sigma (St. Louis, MO). Polyclonal antibodies to hexanal-lysine conjugates were the same as those described by Smith et al. (*12*). All other chemicals were of reagent grade or better.

Conjugate Preparation. All conjugates were prepared as described by Smith et al. (*12*), except that the final sodium cyanoborohydride ($NaCNBH_3$) concentration was reduced to 20 mM, eliminating the need for dialysis. BSA, CSA, and KLH were modified by adding 246 μL of hexanal to 9 mL of 16.7 mg/mL protein dissolved in 0.1 M sodium chloride/0.01 M sodium phosphate buffer (PBS), pH 7.4. After mixing, 1 mL of 200 mM $NaCNBH_3$ dissolved in 0.1 N NaOH was added to the hexanal−protein solution to reduce Schiff bases. The final reactant concentrations were 15 mg/mL protein, 200 mM hexanal, and 20 mM $NaCNBH_3$. The unreduced conjugates were prepared the same way except that no $NaCNBH_3$ was added. Conjugates were diluted to 1 mg/mL with PBS, pH 7.2, and frozen at −20 °C.

CSA was modified as described above for cross-reactivity studies with propanal, butanal, pentanal, heptanal, octanal, nonanal, 2-methylpentanal, 2-methylbutanal, 3-methylpentanal, 2-*trans*-hexenal, 2-hexanol, hexyl alcohol, and 2-heptanone. The standard curve used in the ELISA was converted from CSA concentration to aldehyde, alcohol, or ketone concentration to facilitate comparison among the ELISA results.

The difference in reactive amino groups in the conjugates, as compared to the corresponding native protein, was determined by the trinitrobenzenesulfonic acid (TNBS) assay using a standard curve of leucine solutions (*12*). Modification was expressed as the percentage decrease in TNBS-reactive amino groups of native protein. To confirm results, the amino acid contents of hexanal-modified CSA, BSA, and KLH, as well as the native form of these proteins, were determined by reversed-phase high-performance liquid chromatography following pre-column derivatization with phenylisothiocyanate (*14, 15*). Results were expressed as molar percentage for each amino acid.

Production of Monoclonal Antibodies. Five mice were injected subcutaneously with 50 μg of BSA−hexanal-modified protein conjugates in 0.1 mL of 0.8% saline, emulsified with 0.1 mL of Freund's complete adjuvant. Total injection volume was 0.5 mL. Booster injections were given at 2 week intervals using Freund's incomplete adjuvant. Two weeks after the second booster injection, serum titers were determined by indirect ELISA. The two mice whose serum showed high hexanal titers and the lowest recognition of native BSA when tested by an IC ELISA were selected for the fusion. A final injection, containing 50 μg of hexanal-modified protein in 0.1 mL of 0.8% saline, was given 3 days prior to the fusion.

Mouse spleen cells (2×10^8) were fused with 2×10^7 NS-1 cells using 50% polyethylene glycol (*16*). The fusion efficiency of the plates was 89% (425/480). The wells showing growth were tested for antibody production using an IC ELISA. Hybridomas that showed production of antibodies to hexanal−protein conjugates were expanded and cloned twice by limiting dilution (*17*). Supernatant from the cell culture of the final clone was collected, and the antibody was purified by precipitation with 50% ammonium sulfate (*18*), dialyzed in PBS, pH 7.2, and frozen at −20 °C. The immunoglobulin subclass of the antibody secreted by the final clone was determined by using a Sigma Immunotype Mouse Monoclonal Antibody Isotyping Kit.

Titer Determination. An indirect ELISA was performed by coating microtiter plates (Immunolon-2 Removawells, Dynatech Laboratories, Alexandria, VA) overnight at 4 °C with 100 μL of hexanal−KLH conjugate (1 μg of protein/mL), diluted in 0.1 M carbonate buffer, pH 9.6. Plates were washed four times with PBS, pH 7.2, containing 0.05% Tween-20 (PBS-T). Nonspecific binding sites were then blocked by adding 300 μL of 0.5% casein in PBS, pH 7.2, to each well and incubating for 30 min at 37 °C. After four washings with PBS-T, 50 μL of sera, serially diluted in PBS, pH 7.2, was added and incubated for 30 min at 37 °C. After incubation, the plates were again washed four times with PBS-T. To each well was added 100 μL of GAM−IgG HRP diluted 1:500 in PBS with 0.5% casein. After 30 min of incubation at 37 °C, the plate was washed eight times with PBS-T. Peroxidase binding was determined using 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)−H_2O_2 substrate (*19*). Absorbance was measured at 405 nm using a THERMOmax plate reader (Molecular Devices, Menlo Park, CA). The titer of the serum was arbitrarily determined as the dilution showing an absorbance that was twice the absorbance of the preimmune serum at the same dilution.

IC ELISA. The IC ELISA used for screening the mice sera and hybridomas for antibody production was the same as above, except that after the plates had been coated with 100 μL of hexanal-modified CSA or KLH (1 μg of protein/mL) and the nonspecific sites had been blocked, 50 μL of cell culture supernatant or sera was added either with 50 μL of PBS, pH 7.2, as the zero concentration, or with 50 μL of hexanal-modified or native protein at 10 μg of protein/mL as the competitor. The percent inhibition at 10 μg of protein/mL was determined by dividing the absorbance at that concentration by the absorbance at zero concentration and then subtracting from one.

Determination of Antibody Specificity. To determine antibody specificity, the IC ELISA was performed as described above except after the plates had been coated with 100 μL of hexanal−CSA (1 μg of protein/mL) and the nonspecific sites had been blocked, the purified antibody (50 μL of 1.6 μg of protein/mL in PBS, pH 7.2) was added with 50 μL of hexanal-modified or native CSA, BSA, or KLH at concentrations of 0−10 μg of protein/mL and incubated at ambient temperature. Peroxidase binding was determined using TMB buffer at 450 nm.

A standard curve was generated by plotting the concentration of hexanal-modified protein against binding inhibition. Because each protein contained different amounts of lysine, an equivalent hexanal concentration was calculated by using the moles of lysine per mole of protein determined by amino acid analysis based on the percentage modification. Standard curves were generated by plotting the concentration of hexanal against the binding inhibition produced by each protein conjugate.

The antibody was tested for recognition of free hexanal (*12*). Hexanal at concentrations of 0, 0.05, 0.1, 0.25, 0.5, and 1.0 μg/mL was added to a 30% methanol solution and tested using IC ELISA. Unreduced conjugates were also tested for recognition in the IC ELISA at concentrations of 0−5 μg of protein/mL.

Cross-reactivity of the monoclonal antibody was determined by IC ELISA using CSA conjugated to various aldehydes, alcohols, and ketones. The conjugates were added with the antibody at concentrations of 0−100 ng/mL of aldehyde, alcohol, or ketone. Cross-reactivity was calculated using binding inhibition data for each hexanal−protein conjugate (10 μg of protein/mL) and defined as the concentration of hexanal−CSA required for 50% binding inhibition divided by the concentration of aldehyde, alcohol, or ketone conjugate required for 50% binding inhibition multiplied by 100. Intra-assay and interassay precisions of the IC-ELISA were determined using CSA−hexanal conjugates at concentrations of 0, 5, 10, 20, and 40 ng/mL (*13*). The intraassay variability was determined using 16 replicate wells, and the interassay variability was determined using 32 replicate wells from 8 different plates.

Accelerated Lipid Oxidation Meat Model System. Chicken thigh meat with skin attached, purchased from a local retail store, was deboned, vacuum packaged, and frozen at −80 °C. The frozen muscle was mixed with dry ice and pulverized to a fine powder by mixing for 30 s on high speed in a Waring blender (model 1120; Winsted, CT). Three volumes of 0.3 M

NaCl solution was added to the powder that was then blended at high speed for 60 s and strained through two layers of cheesecloth. The homogenate was then diluted in 3.5 volumes of PBS, pH 7.4.

A series of tubes, each containing 22 mL of the diluted homogenate, was held at 50 °C in a water bath, and samples were withdrawn periodically over a 36 h period. Samples withdrawn for analysis by TBARS assay and ELISA were analyzed immediately. A portion of each sample was frozen at −20 °C until prepared for GC analysis. The storage study was performed in triplicate.

One milliliter of oxidized thigh meat homogenate was combined with 1 mL of 160 mM $NaCNBH_3$ and alkylated as previously described (*12*). The hexanal concentration of each sample was determined using the MAb IC ELISA as described above or a PAb-based IC ELISA (*12*).

The method of Buege and Aust (*20*) was used to quantify TBARS in the meat homogenate. The results were calculated as milligrams of malonaldehyde per kilogram of meat using a molar extinction coefficient of 1.56×10^{-5} L mol^{-1} cm^{-1}.

Dynamic Headspace Gas Chromatography. Volatiles from the meat homogenates were absorbed onto Tenax-TA (Alltech, Deerfield, IL) and desorbed using 2-methylbutane (*21*). To determine recovery, hexanal was added to 20 mL of water and subjected to the same isolation and concentration procedure. The recovery was 81.2% ($n = 4$; CV = 4.77%).

A Hewlett-Packard 5890A gas chromatograph (Minneapolis, MN) equipped with a flame ionization detector was used with a DB-225 fused silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness; J&W Scientific Inc., Rancho Cordova, CA) to separate hexanal. The column was held at an initial temperature of 35 °C for 10 min, and then the column temperature was increased at rates of 3 °C/min to 50 °C, 6 °C/min to 90 °C, and 40 °C/min to a final temperature of 200 °C. The injection port and detector temperatures were 225 and 275 °C, respectively. Helium was used as the carrier gas at a flow rate of 40 mL/min with a 3:1 split ratio. Peak integration was performed using Hewlett-Packard 3365 Series II Chem Station software. Retention times of a hexanal reference and sample solutions were compared to identify the elution peaks. Results were calculated as micrograms of hexanal per gram of meat using a standard curve of 3, 6, 20, 60, and 200 μg/mL hexanal in 2-methylbutane.

Statistical Analysis. All experiments were performed in at least triplicate. Standard error of the mean was determined for each curve, at each concentration in the IC ELISA, using one-way analysis of variance (version 6.12, 1996, SAS Institute, Inc., Cary, NC). The general linear model (GLM) procedure was used to perform analysis of variance of the meat homogenate study because the data were unbalanced due to laboratory error. Correlation coefficients were determined between pairs of methods among GC-HS, PAb IC ELISA, MAb IC ELISA, and TBARS.

RESULTS AND DISCUSSION

Hybridoma Production. The BSA conjugate used for immunization of the mice had 97.4% of available lysine residues modified by hexanal. Five weeks after the initial injection, titers for the hexanal−BSA conjugate injected mice ranged from 4.0×10^4 to 5.3×10^4. Sera from all of the mice gave >80% binding inhibition at 100 μg of protein/mL by indirect ELISA. Eight positive hybridomas were produced from two fusions, and three were cloned. The hybridoma that showed the highest binding inhibition in the IC ELISA from the first cloning was cloned a second time. The hybridoma from the second cloning, designated 45P1-D9, showing the highest binding inhibition and the least cross-reactivity with aliphatic aldehydes other than hexanal was used in all subsequent experiments. The isotype of this clone was IgG_1.

Protein Specificity. The antibody did not recognize free hexanal or native protein in the IC ELISA. There was <5% binding inhibition with 5 μg of protein/mL of the native protein or the protein blank (protein that had been through the conjugation reaction but without hexanal). At 10 μg of protein/mL, the hexanal−CSA, hexanal−BSA, and hexanal−KLH conjugates showed 88, 78, and 73% binding inhibition, respectively (Figure 1).

The difference in the amount of binding inhibition for each of the conjugates at the same protein concentration is partially explained by the extent of modification and moles of available lysine in each protein. The percentage modifications of hexanal−CSA, hexanal−BSA, and hexanal−KLH used in the IC ELISA were 99.1, 95.3, and 90.9%, respectively. Taking into account both factors, CSA, BSA, and KLH contained about 700, 800, and 310 ng of hexanal/10 μg of protein on the basis of the percentage modification and expected lysine content of each protein. From this calculation, it was expected that BSA would have the highest binding inhibition and KLH the lowest. Instead, it was found that that the binding inhibition of CSA was greater than that of BSA at the same protein concentration. This finding suggests that the CSA may have contributed to the epitope bound by the antibody. Screening of hybridomas was done using hexanal−CSA, so it is possible that the antibodies may preferentially bind to this protein in the IC ELISA.

Sodium cyanoborohydride was added to reduce the double bond that is formed by the Schiff base reaction between the lysine side chains of the protein and hexanal. It was determined by IC ELISA that the antibody recognized only the conjugates that have had this bond reduced. Relatedly, Klassen et al. (*22*) prepared an MAb that recognized acetaldehyde conjugated to BSA, KLH, or actin but did not recognize unreduced conjugates or unmodified proteins.

ELISA Optimization. The working range of the IC ELISA was 1−50 ng of hexanal/mL. The MAb ELISA was more sensitive for hexanal conjugates than the PAb assay developed by Smith et al. (*12*). The limit of detection of the PAb ELISA was 7.4 ng of hexanal/mL, with a working range up to 740 ng of hexanal/mL. The reproducibility of the MAb-IC ELISA was determined (Table 1). The coefficient of variation ranged from 4.15 to 6.43% for intraassay variability and from 5.07 to 7.71% for interassay variability.

Cross-Reactivity Study. The specificity of the MAb with other aliphatic aldehydes was determined by IC ELISA (Figure 2). Because all of these CSA−aldehyde conjugates were 96−97% modified (Table 2), the differences in cross-reactivity were caused by differences in antibody recognition and not by a difference in the extent of modification.

Cross-reactivity was defined as the concentration of hexanal−CSA required for 50% binding inhibition divided by the concentration of aldehyde−CSA required for 50% binding inhibition multiplied by 100. On the basis of this definition, the antibody did not cross-react with propanal, butanal, and nonanal when conjugated to CSA. Antibody cross-reactivity with heptanal-CSA (76.6%) was almost 2 times that with pentanal−CSA (37.9%).

The antibody also cross-reacted (45.0%) with 2-*trans*-hexenal when conjugated to CSA. 2-*trans*-Hexenal is a six-carbon aldehyde with a double bond between the second and third carbons. The lower cross-reactivity of

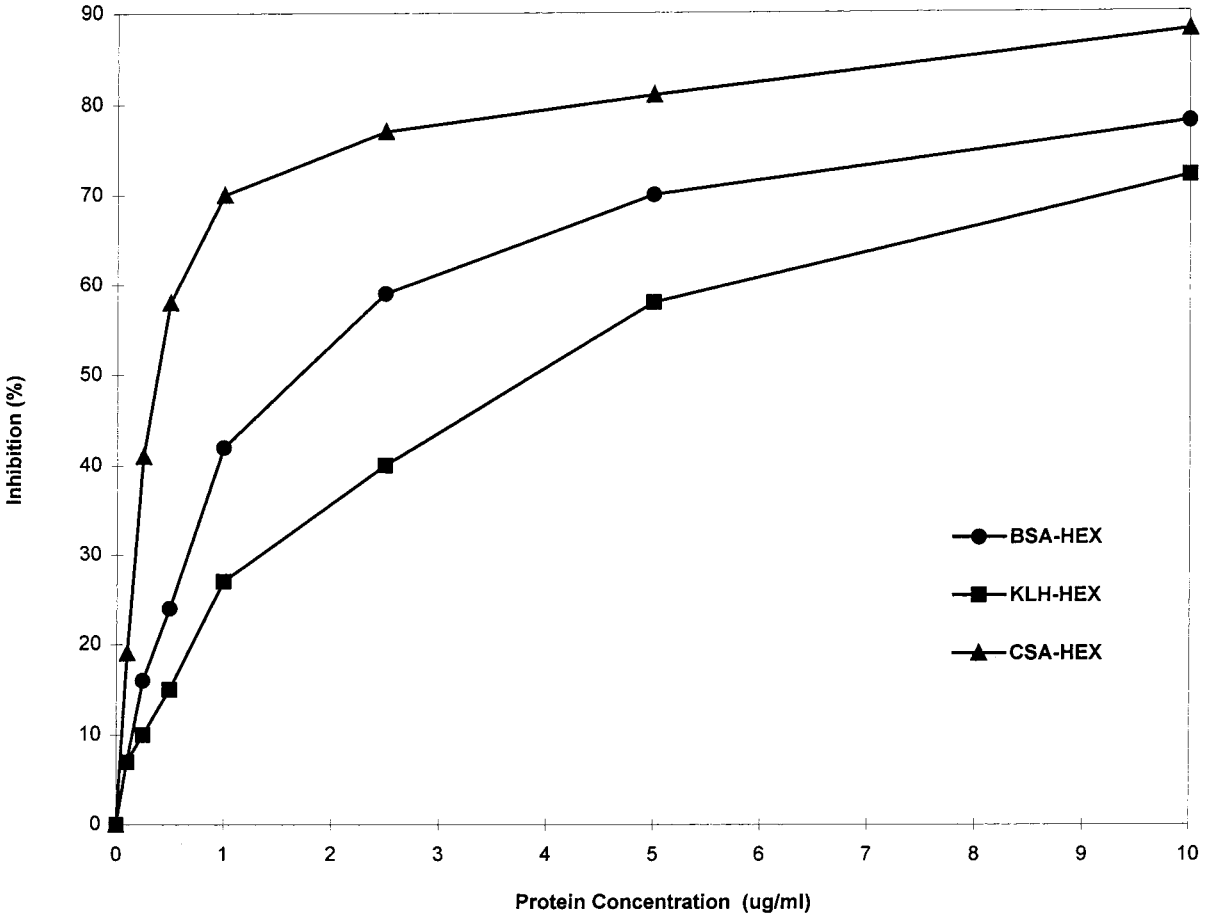

Figure 1. Specificity of MAb to hexanal-modified proteins determined by IC ELISA. Microtiter wells were coated with 1 μg/mL of hexanal-modified CSA. A 1/1000 dilution of antibody (1.6 μg/mL) was incubated with competing native or hexanall-modified CSA, BSA, or KLH. Each data point represents six replicates. The standard errors of the mean were 0.63, 0.78, and 1.43% for hexanal−CSA, hexanal−BSA, and hexanal−KLH, respectively.

Table 1. Reproducibility, Expressed as Coefficient of Variation, of the IC ELISA Determined within a Run (Intraassay) and between Runs (Interassay)

concn (ng of hexanal/mL)	intraassay[a] (%)	interassay[b] (%)
0	4.40	5.07
5	5.60	5.28
10	6.22	6.56
20	6.43	7.71
40	4.15	7.31

[a] Intraassay variability was calculated using 16 replicate wells. [b] Interassay variability was calculated using 32 replicates from 8 different plates run on different days.

the antibody with this conjugate may be due to incomplete reduction of the double bond to form hexanal during conjugation with $NaCNBH_3$ or to the lower modification (87% as measured by TNBS) of the protein, resulting in fewer moles of aldehyde in the conjugate. Smith et al. (*12*) determined that PAbs to hexanal−CSA cross-reacted 100% with a 2-*trans*-hexenal conjugate that was 96% modified.

Strecker degradation compounds, 2-methylbutanal and 2-methylpentanal, may be formed in meat during cooking. These compounds, as well as a structurally related compound, 3-methylbutanal, and 2-heptanone (a seven-carbon ketone) were also checked for cross-reactivity. There was no cross-reactivity with any of these compounds (data not shown), suggesting that conjugates made with branched aldehydes or ketones were not recognized by the antibody.

The antibody was most specific to hexanal−CSA conjugates. Results are similar to those obtained by Smith et al. (*12*) using a PAb prepared against hexanal−protein conjugates. They also observed that cross-reactivity was greatest with hexanal, followed in decreasing order by heptanal and pentanal. The MAbs were more specific than the PAbs in the IC ELISA, because the PAbs also reacted with octanal, butanal, 2-methylpentanal, and 3-methylbutanal. On the basis of its specificity and sensitivity, this ELISA shows applicability for use in food systems. Although the ELISA will detect some pentanal and heptanal, both of these compounds are lipid oxidation products.

Detection of Hexanal in Oxidized Chicken Thigh Homogenate. The hexanal and malonaldehyde equivalent concentrations, as measured by HS-GC and TBARS, respectively, increased in the chicken muscle homogenates over the first 30 h at 50 °C, followed by a decrease in hexanal concentration during longer storage times (Figure 3). The two ELISAs indicated an increase in hexanal content of the thigh homogenate only to 24 h. The decrease in hexanal concentration after prolonged storage may be due to the further oxidation of hexanal to other secondary products (*23*).

Statistically, each method showed a linear rate of change in hexanal content over time. The MAb-based ELISA also showed a quadratic rate of change in hexanal content over time. The linear rate of change for HS-GC was greater than that for PAb-based ELISA. Comparisons were made of hexanal contents determined

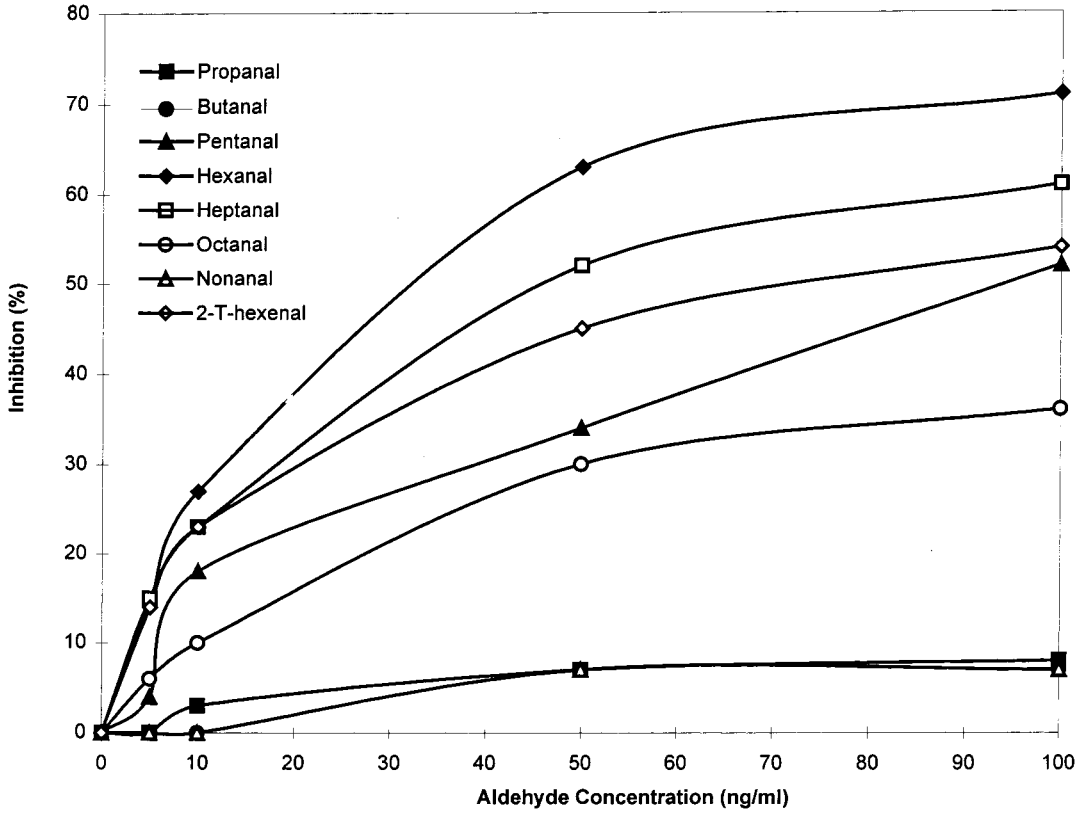

Figure 2. Specificity of MAb to aliphatic aldehydes conjugated to CSA. Microtiter wells were coated with 100 μL of 1 μg/mL of hexanal-modified CSA. A 1/1000 dilution of antibody (1.6 μg/mL) was incubated with aldehyde−CSA conjugates. Each data point represents four replications. The standard errors of the mean were 2.21, 1.52, 0.68, 1.09, 0.73, 0.76, 1.10, and 0.57% for the propanal−, butanal−, pentanal−, hexanal−, heptanal−, octanal−, nonanal−, and 2-*trans*-hexenal−CSA conjugates, respectively.

Table 2. Percentage Modification of Reactive Amino Groups of CSA by Aldehydes during Reductive Alklyation

compound	modification[a] (%)	compound	modification[a] (%)
propanal−CSA	96.1	2-*trans*-hexenal−CSA	81.4
butanal−CSA	97.3	2-methylpentanal−CSA	96.1
pentanal−CSA	97.2	2-methylbutanal−CSA	96.0
hexanal−CSA	97.2	3-methylpentanal−CSA	96.0
heptanal−CSA	97.3		
octanal−CSA	97.4		
nonanal−CSA	97.4		

[a] Defined as percentage decrease in TNBS reactive amino groups of native protein.

by the PAb- and MAb-based ELISAs with HS-GC data at each time point. Hexanal contents determined by MAb ELISA were not different from values determined by HS-GC except at 12 h ($P < 0.05$). Hexanal contents determined by PAb ELISA were different from values determined by HS-GC at all time points ($P > 0.05$). Therefore, on the basis of the data collected in this study, the MAb-based ELISA may be a suitable alternative to HS-GC analysis for hexanal measurement in a meat system.

Each method showed correlation with each of the other methods (Table 3). The highest correlation was between the HS-GC and TBARS ($r = 0.97$) procedures. Ang and Lyon (*24*) reported coefficients of 0.88, 0.90, and 0.92 for cooked and stored broiler thigh, skin, and breast, respectively, comparing values obtained by HS-GC (hexanal content) and TBA methods. Other investigators have also found strong correlations between GC and TBA methods for cooked meats (*7*, *8*, *15*, *26*). Despite the many limitations of TBA assays, they can

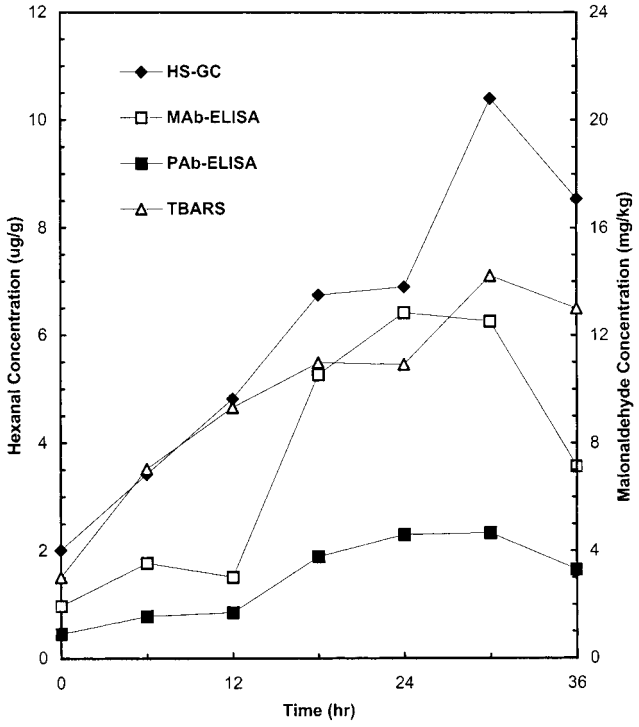

Figure 3. Effect of incubation time on hexanal and malonaldehyde equivalent concentrations of chicken muscle homogenate as measured by dynamic HS-GC, MAb ELISA, PAb ELISA, and TBARS assays. Determinations were made in triplicate.

be used effectively to monitor and evaluate lipid oxidation in meat and other biological tissues when used to assess the extent in general, rather than to quantify

Table 3. Correlation Coefficients between Tests To Measure the Extent of Lipid Oxidation in a Chicken Thigh Homogenate[a]

	HS-GC	TBARS
MAb ELISA	0.81	0.77
PAb ELISA	0.89	0.85
HS-GC		0.97

[a]Methods were headspace gas chromatography (HS-GC), thiobarbituric acid reactive substances (TBARS) assay, and monoclonal (MAb) and polyclonal (PAb) IC ELISAs.

malonaldehyde, or when used in combination with a measurement such as hexanal content (*27*).

The dynamic HS-GC method used in this study, although sensitive, was very tedious and time-consuming. The time required for one sample was ~2.5 h, about the same for the ELISA. However, the GC method was limited by the size of the purge and trap apparatus and by the length of the chromatography run. By ELISA, we were able to run 48 samples including a standard curve, in triplicate, in 2.5 h, using two microtiter plates. Our GC method also required fastidious laboratory practices, including careful cleaning of the glassware and extraction in a cold room to prevent evaporative loss due to the low boiling point of the solvent. Despite the fact that many quality control methods use the dynamic headspace technique (*28*), these methods may not be practical for routine procedures in food-processing plants (*29*). In addition, the occurrence of hexanal−protein binding (*30*−*32*) complicates accurate quantification by HS-GC methods, which measure only volatile compounds (*33*), neglecting those physically or chemically bound in the sample (*34*).

The development of objectionable flavors and odors by oxidation has obvious detrimental consequences on food quality and consumer acceptability. Although hexanal content has been reported to be a sensitive and reliable indicator for evaluation of the oxidative state and flavor quality of meat and meat products, analysis by GC techniques can be time-consuming. We have shown that use of a hexanal-specific antibody-based ELISA to monitor lipid oxidation in chicken thigh homogenate may be a faster and simpler alternative to dynamic HS-GC.

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