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Suitability of saturated aldehydes as lipid oxidation markers in washed turkey meat

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ABSTRACT

The aim of this study was to evaluate the suitability of saturated aldehydes as lipid oxidation markers in washed turkey muscle, by means of headspace solid phase microextraction-gas chromatography (HS-SPME-GC); the results were compared with the widely used thiobarbituric acid-reactive substances (TBARs) method. Changes in TBARs, propanal and hexanal concentrations were determined over time in a model system consisting of turkey muscle washed with a sodium phosphate buffer (pH 5.6). To stop oxidation from occurring during analysis, an antioxidant mixture (EDTA, trolox and propyl gallate) was added immediately before analyses. After antioxidant addition, propanal and TBARs concentrations did not increase during 8 h of further storage, while an unexpected decrease in hexanal was observed. To determine if aldehydes were interacting with washed turkey muscle, hexanal and propanal were added to either phosphate buffer or washed muscle and concentrations were monitored for 24 h. Neither propanal nor hexanal decreased in the phosphate buffer over time, but the headspace concentration of propanal and hexanal in washed turkey muscle were markedly lower (76% and 96%, respectively) at time zero and continued to decreased up to 24 h of storage. Because of this decrease in headspace aldehyde concentrations, TBARs were found to be a more sensitive and accurate marker of oxidative deterioration in washed turkey muscle.

1. Introduction

Consumption of meat containing high amounts of polyunsaturated fatty acids (PUFA) has increased greatly in the last decade, due to the nutritionists' recommendations to reduce intake of saturated fatty acids (SFA). However, a high degree of unsaturation accelerates oxidative processes, leading to deterioration of meat flavor, color, texture and nutritional value (Fenaille, Visani, Furmeaux, Milo, & Guy, 2003; Goodridge, Beaudry, Pestka, & Smith, 2003; Sanches-Silva, Rodríguez-Bernaldo de Quirós, López-Hernández, & Paseiro-Losada, 2004). Turkey meat has a moderately low fat content and is relatively rich in PUFA (Komprda et al., 2002; Taber, Chiu, & Whelan, 1998), but it is prone to oxidation due to its inefficient accumulation of dietary vitamin E (Mielnik, Olsen, Vogt, Adeline, & Skrede, 2006).

The primary oxidation products for unsaturated fatty acids are the hydroperoxides, highly reactive compounds that decompose rapidly, yielding a complex mixture of non-volatile and volatile compounds, such as hydrocarbons, aldehydes and ketones, which affect the overall quality of the product (García-Llatas, Lagarda, Romero, Abellán, & Farré, 2006). Aldehydes are particular important with respect to flavor alteration and from a toxicological standpoint (Frankel, 1980, 1982, 1993). Hexanal is a specific volatile oxidation product of $n-6$ PUFA, whereas propanal arises from $n-3$ PUFA oxidation (Romeu-Nadal, Castellote, & López-Sabater, 2004). $n-6$ and $n-3$ PUFA correspond to 26% and 3.0% of the total fatty acids in turkey meat, respectively (Taber et al., 1998). During oxidation, both the $n-3$ and $n-6$ fatty acids would be expected to oxidize and generate numerous volatile fatty acid decomposition products, including the saturated aldehydes, propanal and hexanal. Propanal and hexanal are often used as indicators of lipid oxidation in foods, because they can be measured in the sample headspace and their lack of double bonds makes them more oxidatively stable than unsaturated aldehydes.

While most of the current research focuses on the prevention of oxidative deterioration of meats, rapid and inexpensive analytical methods to determine the extent of oxidative rancidity of raw meats are still lacking. Thiobarbituric acid-reactive substances (TBARs) method has been widely used to monitor secondary lipid oxidation in raw and cooked muscle foods, because it is easy to use and relatively fast; however, this method has been largely criticized due to its lack of specificity. Solid phase microextraction (SPME) potentially provides many advantages over conventional

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techniques for the extraction of volatile saturated aldehydes, including easy manipulation and experimental set up, short sampling times, easy automation and high sensitivity. The detection limit of headspace (HS)-SPME can be as low as parts per trillion (Zhang & Pawliszyn, 1993) and has been successfully used to estimate the hexanal content of cooked meats (Nilsen, Sørensen, Skibsted, & Bertlsen, 1997) and raw meat (Ahn, Jo, & Olson, 1999; Nam, Cordray, & Ahn, 2004). HS-SPME is able to extract organic compounds from virtually any matrix, as long as target compounds can be released from the matrix into the headspace (Olivier, Gauch, Mariaca, & Klein, 1995). The main disadvantage of HS-SPME is its inability to recover trace compounds and strongly bound semi volatile compounds. Chemical reactions via covalent and electrostatic reactions are known to be responsible for the irreversible linkage of volatiles to proteins (Fischer & Widder, 1997), which mainly depends on the polarity of proteins (Maier, 1975). This phenomenon can be partly overcome by salt addition (Flores, Gianello, Pérez-Juan, & Toldrá, 2007; Pérez-Juan, Flores, & Toldrá, 2007), which significantly reduces the ability of sarcoplasmic protein to bind branched aldehydes (3-methyl-butanal and 2-methylbutanal), hexanal and methional (Pérez-Juan et al., 2007). For all these reasons, it is necessary to monitor the formation of specific lipid oxidation markers, such as propanal and hexanal, to verify their actual reliability as oxidation indicators in complex food matrices.

The aim of this study was to evaluate the suitability of saturated aldehydes as secondary lipid oxidation markers in washed turkey muscle, by using HS-SPME-gas chromatography (HS-SPME-GC); the results were compared with those obtained with the TBARs method. Changes in TBARs, propanal and hexanal concentrations were determined over time in refrigerated raw washed turkey muscle.

2. Materials and methods

2.1. Reagents and standards

Hexanal, 2-thiobarbituric acid (TBA), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), *n*-propyl gallate, anhydrous monobasic sodium phosphate, pentanal, octanal, and 1,1,3,3-tetraethoxypropane, were purchased from Sigma–Aldrich (Sigma Chemical Co., St. Louis, MO, USA). Trichloroacetic acid (TCA), propanal, heptanal and nonanal were supplied by Acros (Acros Organics, Morris Plains, NJ, USA). Anhydrous dibasic sodium phosphate and potassium ferricyanide were purchased from Fisher (Fisher Scientific, Fair Lawn, NJ, USA). Ethylenediamine tetraacetic acid (EDTA) disodium salt was supplied by Curtin Matheson (Curtin Mahteson Scientific, Inc., Houston, TX, USA). Streptomycin sulfate was purchased from Spectrum Chemical (Spectrum Chemical MFG Corp., Gardena, CA, USA).

2.2. Sample preparation of washed muscle

About 0.5 kg of organic turkey breast meat was purchased in a local supermarket located in Amherst (MA, USA); slaughtering conditions and the postmortem history of the turkey breast meat were not available. The two whole half breasts were trimmed to remove all remaining adipose tissue, cut into small pieces and minced using a commercial blender. The perfectly homogeneous, minced muscle was washed once with distilled deionized water at a 1:3 mince-to-water ratio (w/w) and stirred with a glass rod for 2 min. Subsequently, the mixture was allowed to stand for 15 min before dewatering with two layers of cotton cheesecloth. The mince was then washed twice with 50 mM sodium phosphate buffer (pH 5.6), as described above for the water washing. The washed mince (50 g) was then homogenized using a Tissue Tearor (Biospec Products, Inc. Bartlesville, OK, USA). The homogenized mince was allowed to stand for 15 min and finally centrifuged (15,000*g* for 20 min at 4 °C), using an ultracentrifuge (Sorvall Ultra 80, DuPont, Wilmington, DE, USA). The resulting pellet was vacuum-packed in plastic bags and stored at −80 °C until analysis.

2.3. Experimental set-up

Two sets of experiments were performed, in order to evaluate secondary lipid oxidation products over time (A), and the interaction between aldehydes and washed muscle after antioxidant addition (B).

Each experimental set was repeated twice and three replicates were run per sampling point, using both analytical methods.

2.3.1. Experiment A

Washed turkey muscle (350 mg and 700 mg for TBARs and HS-SPME-GC analysis, respectively) was weighed into 10-mL screw capped tubes. Subsequently, a streptomycin solution (50 μL and 100 μL for TBARs and HS-SPME-GC analysis, respectively, with a final concentration of 300 mg/L) in 2 mM phosphate buffer (pH 5.6), was added to inhibit microbial growth. The tubes were stored at 4 °C for different time periods (0, 12, 36 and 54 h), followed by addition of an aqueous antioxidant mixture (75 μL and 150 μL for TBARs and HS-SPME-GC analysis, respectively) consisting of EDTA (1.0 mM), trolox (0.16 mM) and propyl gallate (0.5 mM).

2.3.2. Experiment B

Samples were prepared as in experiment A, except that the aqueous antioxidants were added after 54 h of storage at 4 °C, followed by HS-SPME-GC and TBARs analysis at 0, 1, 2, 4 and 8 h after antioxidant addition.

In addition, 700 mg of washed turkey muscle were weighed into 10-mL screw capped tubes along with 100 μL of streptomycin solution (final concentration = 300 mg/L). A control consisting of 700 μL of 2 mM phosphate buffer (pH 5.6) was also prepared. Both muscle sample and control were spiked with 2 concentration levels of propanal (0.18 and 0.52 mg/kg) and hexanal (0.02 and 0.06 mg/kg), followed by 150 μL of the aqueous antioxidant mixture. Samples were then analyzed by HS-SPME-GC at different time intervals (0, 6, 12, 18 and 24 h).

2.4. Determination of thiobarbituric acid-reactive substances (TBARs)

This determination was performed according to the modified method of Buege and Aust (1978). Three mL of a solution containing 1.3% TBA dissolved in 50% TCA were added to the samples contained in the tubes, capped, vortexed and incubated for 1 h at 65 °C. Samples were then stored at 4 °C for 1 h, followed by centrifugation at 2000*g* for 10 min. The absorbance of the supernatant was measured at 532 nm with a UV spectrophotometer (UV–Vis mod. UV-1601, Shimadzu, Kyoto, Japan) and TBARs concentration were calculated as mg of malonaldehyde (MDA) using the extinction coefficient of $\epsilon_{532} = 1.56 \times 10^5\ M^{-1}\ cm^{-1}$ (Buege & Aust, 1978). TBARs results were expressed as mg of malonaldehyde/kg meat.

2.5. Determination of volatile aldehydes by HS-SPME-GC/FID

Before analysis, 50 μL of a NaCl solution (3.42 mol/L) were added to the samples contained in the vials, which were capped and placed into the autosampler tray. An AOC-5000 Auto-injector (Shimadzu, Tokyo, Japan) suitable for HS-SPME analysis, was used. The sample vial was transported from the autosampler tray to the incubating chamber and held at 37 °C for 20 min. A 50/30 μm divynilbenzene/carboxen/polydimethylsiloxane (DVB/Carboxen/