Reduction of Warmed-Over Flavor Volatiles from Freeze-Dried Lean Beef by Supercritical CO$_2$ Extraction

A. Thongwong, L.N. Fernando, I.U. Grün, and A.D. Clarke

ABSTRACT

Supercritical carbon dioxide (SC-CO$_2$) was used to extract warmed-over flavor (WOF) volatiles from cooked, freeze-dried beef at 40°C and pressures of 10.3 MPa or 30 MPa after 2 days storage. WOF markers, hexanal, heptanal, octanal, nonanal, and 2,3-octanedione, were identified in the volatiles profile of precooked beef by dynamic headspace extraction and gas chromatography-mass spectrometry before and after CO$_2$ extraction. TBARS and levels of WOF markers increased over 2 days storage. There was a reduction in WOF markers at both extraction pressures. The higher pressure led to greater reduction of WOF markers (e.g., hexanal: 73.5%) than the lower pressure (e.g., hexanal: 60.3%), notably for heptanal and nonanal. SC-CO$_2$ may be applicable for reducing WOF volatiles from precooked meats.

Key Words: GC-MS, dynamic headspace, supercritical CO$_2$, warmed-over flavor, beef

INTRODUCTION

PRECOOKED, READY-TO-EAT MEAT PRODUCTS ARE USUALLY stored frozen or at refrigerator temperatures prior to being purchased, reheated, and consumed, giving rise to warmed-over flavor (WOF) development. This term has long been accepted to describe the rancid or stale flavor which usually is noted in cooked meats within 48h of refrigeration at 4°C. This deterioration is in contrast to rancidity in raw meats, fatty tissues, lard or rendered fat which becomes evident after prolonged freezer storage (Pearson and Gray, 1983). The mechanism of WOF development is not fully understood. Research suggests that WOF is associated with iron and autoxidation of polyunsaturated fatty acids, mostly in phospholipids (Pearson et al., 1977; Gray and Pearson, 1987).

Means to reduce WOF may include prevention of its development or removal of WOF by methods such as supercritical fluid extraction. Supercritical carbon dioxide (SC-CO$_2$) extraction provides advantages over other procedures because CO$_2$ is low cost, nontoxic, nonflammable, and the moderate temperatures of SC-CO$_2$ extraction are desirable for food applications. Rizvi et al. (1986) reported that SC-CO$_2$ extraction was useful in the food industry because of its potential for extracting lipids and flavors. SC-CO$_2$ extraction has been reported by many researchers to extract cholesterol and lipids from meat products (King et al., 1989, 1993; Chao et al., 1991, 1993; Um et al., 1992; Wehling et al., 1992). This method may be more effective with precooked meat products due to the reduced moisture content than in fresh meat products (King et al., 1993). Our objective was to investigate extraction conditions for reducing WOF volatiles in beef using SC-CO$_2$.

MATERIALS & METHODS

Sample preparation

Three batches of beef, one for each experimental replication, were selected. Denuded beef top round (~3-4 kg) was obtained for each batch from the Meat Laboratory. The beef was ground through a 9.4-mm plate and then through a 3.1-mm plate. Three samples (350g from each batch) were weighed, vacuum-packaged, and stored at −18°C prior to use. Single samples were allowed to thaw overnight at 4°C and then cooked in a convection oven at 190°C for 15 min. Analysis for thiobarbituric acid-reactive substances (TBARS) was performed in duplicate to determine oxidative rancidity on the freshly cooked sample. A fraction of the sample was freeze-dried and analyzed for WOF volatiles using dynamic headspace GC-MS analysis. These analyses represented 0 days storage. The bulk of the cooked sample was stored in a refrigerator at 4°C to develop WOF for 48h and all subsequent analyses refer to 2 days storage for this sample. After chilled storage, each sample was tested for TBARS (in duplicate) and the remaining sample was freeze-dried. The freeze-dried material was divided into 3 portions. One portion was immediately analyzed for WOF volatiles using dynamic headspace GC-MS analysis. The second and third portions were extracted by SC-CO$_2$, either at 10.3 MPa or 30 MPa, prior to determination of volatiles.

TBARS analysis

The conventional distillation method was used to determine malonaldehyde (MA) and other TBARS in the meat samples. TBARS values, reported as mg malonaldehyde/kg meat, were determined on duplicate samples of cooked beef on day 0 and after 2 days refrigerated storage at 4°C.

Freeze-dried meat

Prior to GC-MS analysis or SC-CO$_2$ extraction, all cooked samples (80g) were dried in a freeze-drier (Model Lyph-Lock 12, Labconco, Kansas City, MO). Samples were removed from the freeze-drier when they had a water activity of 0.2 or less as measured with a Model CX-2 water activity meter (Decagon Devices, Pullman, WA).

SFE extraction procedures

A supercritical fluid extractor model SFX 2-10 with syringe pump model 260D (Isco Inc., Lincoln, NE) and CO$_2$ (99.5% purity, Airgas, Radnor, PA) were used for all extractions. Freeze-dried sample (1 g) was extracted with SC-CO$_2$ at 10.3 MPa or 30 MPa at 40°C. The samples were extracted statically for 5 min and then a dynamic extraction step was carried out until 10 mL CO$_2$ had passed through the sample. The WOF volatiles of the extracted samples were collected using dynamic headspace analysis.

Dynamic headspace analysis (DHA)

Each freeze-dried sample or SC-CO$_2$ extracted sample (0.5g) was placed in a 6-mL headspace vial (22 × 38 mm) (Supelco Inc., Bellefonte, PA). The headspace vial was crimped with a 20-mm aluminum seal, with star springs for pressure release, containing a PTFE/ coated silicone rubber septum (Perkin-Elmer Co., Norwalk, CT). The authors are affiliated with the Dept. of Food Science & Human Nutrition, 21 Agriculture Building, Univ. of Missouri-Columbia, Columbia, MO 65211. Direct inquiries to Dr. A. D. Clarke.
volatiles were purged at 80°C for 30 min with N2 flow 30 mL/min (99.99% purity, Midstates Airgas, Kansas City, MO) and trapped on previously conditioned (275°C for 30 min with He flow 13–14 mL/min) Tenax TA (20:30 mesh) packed in a 4 mm × 11.5 cm sorbent tube (Dynatherm Analytical Instrument, Inc., Kelton, PA). The trapped volatiles were analyzed by GC-MS.

Gas chromatography-mass spectrometry (GC-MS) analysis

The Tenax tube was transferred to a thermal desorption system (Model ACE900, Dynatherm Inc., Kelton, PA) connected to a GC-MS (Varian 3400CX GC and Varian Saturn 2000 MS; Varian Associates, Inc., Sugar Land, TX). The adsorbed volatile compounds were thermally desorbed at 250°C for 5 min and separated on a fused silica capillary column (DB-5, 60 m × 0.25 mm, J & W Scientific, Folsom, CA) using He (Praxair, Des Moines, IA) with a split ratio of 1:23 and a flow rate of 0.67 mL/min. The column temperature of 35°C was held 5 min and then increased at 8°C/min to 220°C, then at 2°C/min to 250°C and held for 15 min. MS conditions were ion trap temperature 150°C, ionization voltage 70 eV, and ion mass range 41 to 350 m/z. The WOF markers were identified using the 1992 National Institute of Standard and Technology library of mass spectra.

Statistical analysis

TBARS values and concentrations of WOF volatiles were analyzed by ANOVA using a Randomized Complete Block Design using the replicate batches of beef as the block. Storage time and SC-CO2 extraction pressure were the two treatment effects evaluated. Fisher’s least significant difference (LSD) test was used to determine treatment differences with significance defined at P<0.05. All data were analyzed using SAS (SAS Institute, Inc., 1990).

RESULTS & DISCUSSION

A RAPID INCREASE OF TBARS VALUES AND CONCENTRATION OF LOW-MOLECULAR-WEIGHT VOLATILES IN COOKED MEAT DURING STORAGE AT 4°C has been reported (Bailey et al., 1987; Dupuy et al., 1987). The TBARS test has been used frequently to measure lipid oxidation during short-term storage of non-freeze-dried cooked meats. Storage of our samples for 48h at 4°C resulted in an increase (P<0.01) in mean TBARS from 0.152 at 0 day to 2.396 mg malonaldehyde/kg sample. This confirmed that lipid oxidation had occurred.

SC-CO2 has been reported to extract cholesterol and lipids from meat products (King et al., 1989, 1993; Chao et al., 1991, 1993; Um et al., 1992; Wehling et al., 1992). We observed that SC-CO2 at 30 MPa extracted some fat, while no fat was extracted at 10.3 MPa. This confirmed data of Chao et al. (1991) and Um et al. (1992) who reported that higher pressure conditions were more effective in extracting lipids. Furthermore, King et al. (1989) reported that high pressure removed fat much more rapidly than low pressure. Bott (1982) indicated that higher pressure resulted in higher density of the SC-CO2 resulting in higher solubility of lipids.

In addition to pressure, other parameters may be important in optimizing SC-CO2 extraction. Moisture reduction of the sample has been necessary to increase extraction of various compounds from meats using SC-CO2 (Clarke, 1997). Cooking of meats would reduce moisture content but it increases the likely development of undesired WOF volatiles. King et al. (1993) showed that freeze-drying of beef patties prior to SC-CO2 extraction increased removal of fat and cholesterol. In addition to lipids compounds, Um et al. (1992) observed that beef volatiles were also extracted by SC-CO2. Our preliminary work showed that freeze-drying also improved extraction of volatiles from meats using SC-CO2. However, freeze-drying did not improve the oxidative stability of meats (Nakhost and Karel, 1984) and comparisons of WOF volatiles from SC-CO2 extracted and nonextracted samples need to be made on freeze-dried samples.

A dynamic headspace method was used to collect volatile compounds from cooked, freeze-dried beef. This method was highly-effective because the sample contained little or no water. The volatiles hexanal, heptanal, 2,3-octanone, octanal, and nonanal, common markers of WOF, were identified by GC-MS with retention times of 11.37, 14.10, 16.07, 16.54, and 18.73 min, respectively (Table 1). The peak area counts of hexanal, heptanal, octanal, and nonanal after 2 days storage were greater (≈13, 5, 4, and 3 times greater, respectively) than those of 0 day storage. The increase in hexanal confirmed results of Bailey et al. (1987) who considered that hexanal was one of the most important volatiles produced during WOF development in cooked meat. The diketone 2,3-octanone was not detected in the freshly cooked, freeze-dried beef (0 day); however, it was detected after 2 days storage.

The SC-CO2 extraction at either pressure decreased (P<0.05) all WOF volatiles (Table 1) as shown by reduction in peak area counts compared to 2 day values prior to SC-CO2 extraction. Higher extraction pressure (30 MPa) decreased all WOF volatiles more than the lower extraction pressure (10.3 MPa). The difference in WOF reduction between the two pressure treatments was significant (P<0.05) only for heptanal and nonanal. For example, at 30 MPa, hexanal was reduced by 73.5% from the original level, while at 10.3 MPa it was only reduced by 60.3%. Um (1992) showed that higher extraction pressures (34.5 MPa) removed more WOF aldehydes than lower pressures (20.7 MPa) based on residue analysis of SC-CO2 extracted beef fat. This trend was seen for several other non-WOF compounds (Um et al., 1992). While their work was performed at 50°C and ours at 4°C, the density of CO2 at our high-pressure treatment was comparable. One may need to consider the effects of CO2 density on solubility of compounds in any application for removal of WOF volatiles.

Results showed that SC-CO2 at 30 MPa could reduce WOF volatiles to near the values for 0-day samples. Specifically, hexanal, octanal and nonanal could be reduced to values that were not different (P>0.05) from 0-day samples. While heptanal and 2,3-octanone values were still greater after SC-CO2 extraction than those on day 0, they were considerably reduced from the 2-day values (64 and 80%, respectively).

The total volatile concentration, including WOF and other volatiles detected by GC-MS, revealed that SC-CO2 at both pressures reduced volatiles (P<0.05). The reduced volatile content was not different (P>0.05) from those of freshly cooked and freeze-dried meat. This suggests that the process of extraction was useful for altering flavor of precooked meat products by removing several WOF volatiles. Development of WOF volatiles is one aspect of overall meat flavor deterioration (MFD), which is more inclusive in describing the poor quality associated with precooked meat (Spanier et al., 1988). SC-CO2 extraction may also cause reduction of desirable flavor compounds such as Maillard reaction products. Because this

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Table 1—Mean peak area counts (and standard deviations) of WOF markers in cooked, freeze-dried beef stored at 4°C

<table>
<thead>
<tr>
<th>Days of storage</th>
<th>Extracted with SC-CO2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Hexanal</td>
<td>24.9 ± 15.95</td>
</tr>
<tr>
<td>Heptanal</td>
<td>2.7 ± 2.27</td>
</tr>
<tr>
<td>2,3-Octanone1</td>
<td>nd1</td>
</tr>
<tr>
<td>Octanal</td>
<td>2.4 ± 1.60</td>
</tr>
<tr>
<td>Nonanal</td>
<td>31.0 ± 15.95</td>
</tr>
<tr>
<td>Total volatiles</td>
<td>420.6 ± 142.16</td>
</tr>
</tbody>
</table>

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1Peak area counts ×10^5.

bMeans across columns with different superscripts are different (P<0.05). (n-9).
experiment did not investigate the impact on volatiles other than the WOF markers, further work is needed to determine whether desirable compounds are affected by SC-CO₂.

CONCLUSIONS

A SIMPLE AND RAPID DYNAMIC HEADSPACE GC-MS ANALYSIS WAS used for monitoring WOF volatiles. SC-CO₂ extraction was effective in reducing WOF volatiles from cooked, freeze-dried beef, with 30 MPa more effective than 10.3 MPa. Results suggest that SC-CO₂ might be applicable for removing WOF volatiles from precooked meats.

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