Pre-cooked roast beef slices were stored 28 days at 4±2°C in air or 100% N₂, with and without vaporized horseradish essential oil (HEO). Addition of 20 µL HEO/L restricted growth of most spoilage bacteria. Pseudomonas spp. and Enterobacteriaceae were strongly inhibited by HEO. Lactic acid bacteria were more resistant to the antimicrobial effect and dominated spoilage flora. Sensory evaluation and headspace analysis by gas chromatography/mass spectrometry revealed that development of off-flavors and odors derived from fat oxidation products was delayed by HEO. Cooked meat color was also preserved in samples stored under HEO.

Key Words: roast beef, spoilage, horseradish, isothiocyanates

INTRODUCTION

THE SHELF-LIFE OF PRE-COOKED, UNCURED sliced roast beef is limited by the lack of intrinsic barriers to microbial growth and the development of off-odors and flavors due to fat autoxidation (Hintlian and Hotchkiss, 1987a). Processors and retailers therefore rely on the maintenance of chill temperatures and packaging under low oxygen atmospheres to ensure adequate stability during storage and distribution. However, the quality of pre-cooked roast beef is highly variable and microbiological safety is of concern. A survey of commercially available products by Anderson et al. (1989) revealed that microbial numbers on vacuum packaged roast beef slices increased rapidly in storage, particularly at higher temperatures. Although initial contamination consisted of more than 50% Pseudomonas spp., the exclusion of oxygen typically led to development of microflora dominated by Lactobacillus spp. when maintained below 5°C. The dominance of lactic acid bacteria (LAB) was diminished at 10°C however, due to the growth of enteric bacteria, particularly Hafnia spp. and Serratia spp. (Anderson et al., 1989). Several studies have reported the survival of human pathogens in sliced pre-cooked roast beef stored at chill temperatures under different atmospheres (Hintlian and Hotchkiss, 1987a, b; Michel et al., 1991; Hudson et al., 1994). Since temperature abuse is common in meat distribution systems (Greer et al., 1994), additional barriers to microbial growth, including natural preservatives, could improve the stability and safety of pre-cooked meats.

Consumer resistance to undesirable chemicals in foods has encouraged consideration of natural alternatives. Essential oils of Brassica contain isothiocyanates, volatile antimicrobial compounds that impart typical flavor and odor to condiments such as mustard and horseradish (Delaquis and Mazza, 1995). Steam distillation of horseradish root yields an essential oil which comprises up to 90% allyl isothiocyanate (AIT), 4–10% 2-phenethyl isothiocyanate (PEIT) and several minor components (Mazza, 1984). Allyl isothiocyanate is an effective antimicrobial with broad spectrum bacteriostatic and bactericidal activity toward yeast, fungi and bacteria including Salmonella typhimurium, Escherichia coli O157:H7 and Listeria monocytogenes (Delaquis and Sholberg, 1997). Ward et al. (1998) showed that AIT-rich horseradish essential oil had similar activity toward spoilage and pathogenic bacteria on the surface of pre-cooked roast beef incubated at an abusive temperature (12°C). Addition of 20 µL horseradish oil/L air in the headspace above meat prevented growth of most test microorganisms except a strain of Lactobacillus sake.

Use of essential oils or isothiocyanates from Brassicas has been limited due to intense flavors and odors, volatility at room temperature and weak solubilities in aqueous media (Delaquis and Mazza, 1995). However, relatively low concentrations of gaseous AIT inhibits growth of surface-bound microorganisms (Isshiki et al., 1992). This has increased interest in applications for vaporized isothiocyanates as preservative adjuncts in packaged foods. Isothiocyanates could prove useful with sliced pre-cooked roast beef since this product is usually packaged in oxygen impermeable films. Flavor modification also may prove less objectionable than in other foods given the traditional use of horseradish dressings or sauces with roast beef. The objective of our research was to determine the effect of vaporized horseradish essential oil (HEO) on microbial growth, chemical changes and sensory properties of pre-cooked roast beef.

MATERIALS & METHODS

Extraction and characterization of horseradish essential oil

The essential oil from fresh horseradish root grown at the Pacific Agri-Food Research Centre, Summerland, BC, was recovered in a steam distillation apparatus as described by Mazza (1984). The distillate was stored under nitrogen at −40°C in a dark bottle. The chemical composition of the HEO was determined by gas chromatography/mass spectrometry. Suitable dilutions for experiments were prepared in commercial grade canola oil.

Preparation of roast beef samples

Meat slices were prepared from a fully cooked 2 kg cook-in-bag, restructured beef roast obtained from a local processor one day after manufacture. The roast was removed from the packaging in a laminar flow hood, doused with 95% ethanol and ignited. Roast beef slices (0.5 mm thick) were prepared with a clean and sanitized meat slicer (Model 410, Hobart, Don Mills, ON; width setting = 20). Groups of 5 slices were placed in sterile Whirl-pak bags (Canlab, ON) and stored at −30°C until used. The moisture content of the meat was determined by the vacuum oven method (AOAC, 1975); salt content expressed as % NaCl according to the Volhard method as described by Kramlich et al. (1973); pH was measured with a glass electrode; phosphate content (expressed as % P₂O₅) was measured using the titrimetric method (Ko niecko, 1985).

Microbiological studies

Inoculation and storage. One side of the...
thawed roast beef slices was inoculated with microorganisms on a cleaned but unsanitized stainless steel sandwich preparation counter in a cafeteria. This procedure was adopted to simulate natural contamination of roast beef in a food processing environment. Inoculations were performed within 1h after use of the counter. The slices were placed on the counter for ≈10 min. Pieces (6) measuring 4 cm² were then cut from each slice with a scalpel blade and transferred to a single sterile glass plate (20.5 cm × 7 cm), contaminated side up. Individual glass plates were inserted into a series of sterile 2L glass Mason jars which served as model storage systems. The lids were fitted with 2 septa for addition of the distillate, gassing and sampling of the atmosphere. Half of the jars were sealed and prepared for storage, and the remainder were flushed with nitrogen for 5 min using hypodermic needles inserted in the septa. The initial headspace gas composition in nitrogen flushed jars was determined by gas chromatography as described by Moyls et al. (1992). Control jars for the treatments were immediately placed in a low temperature incubator set at 4°C to simulate retail storage. Each of the remaining jars was injected with 400 μL of a 1:10 dilution of HEO in canola oil to yield a headspace concentration of 20 μL HEO/L, assuming complete vaporization. One jar from each treatment and two control jars were removed from the incubator after 0, 1, 3, 7, 14, 21 and 28 days for analysis.

Microbiological analysis. Roast beef pieces (2/jar) were prepared for microbiological analysis by blending with 100 mL sterile 0.1% peptone in a stomacher (Model 400, Canlab, Edmonton, AB; 120 sec., normal speed). Decimal dilutions were prepared in peptone and 0.1 mL samples were spread onto: Plate Count Agar (Difco, Detroit), incubated aerobically for 24h at 30°C for the enumeration of Enterobacteriaceae; and deMan Rogosa Sharpe Agar (Oxoid, New Windsor, NY), were used as the low and high reference points for pink color. Three measurements were recorded for each session (Table 1).

Sample evaluation. Panelists evaluated samples in duplicate according to a completely randomized experimental design. Intensity of each attribute was scored using an unstructured 10 cm line. Low and high reference points were fixed at 1.0 cm and 9.0 cm to permit scoring below and above the references. Physical references were not provided for aroma or flavor. Horseradish distillate dilutions in canola oil were available prior to entering the sensory booth for familiarization with low and high degree of attributes. Color evaluation was conducted under natural light, against a white background. Munsell color standards, 2.5YR 5/1 and 5YR 5/2 (Munsell Book of Color, Tekmar Company, Cincinnati, OH) were used as the low and high reference points for pink color. Tri-stimulus color values (L*, a*, and b*) were also measured immediately after each session with a hand held chroma meter (Model Cr-300; Minolta Canada Inc., Mississauga, ON.). Three measurements were recorded for each of 3 roast beef samples.

Statistical analysis. Analysis of variance was used to examine the effect of sample and tasting time (morning and afternoon) on each of the sensory attributes. Mean scores and Duncan’s multiple range test were calculated on mean scores. All statistical analyses were performed using SAS (SAS Institute, Inc., 1990) procedures.

Headspace volatile analysis
Uninoculated roast beef samples were prepared and stored in the Mason jars as before. Diluted HEO (1:10 dilution in canola oil) was added to several test jars to achieve a headspace concentration of 20 μL horseradish distillate/L, and control jars were incubated without HEO. The jars were placed in a darkened incubator set at 4°C. One control and one test jar were removed from the incubator after 0, 3, 7 and 15 days storage. Three roast beef pieces from each jar were manually diced and placed into a sealed 250 mL Wheaton (Canlab) water jacketed vessel held at 30°C. The sample was purified (Model LSC 2000; Tekmar Company, Cincinnati, OH) for 30 min with helium at a flow rate of 100 mL/min, and trapped volatiles were analyzed by gas chromatography / mass spectrometry. The chromatograph (Model HP5890, Hewlett Packard, Richmond, BC) was fitted with a Supelcowax 10 capillary column (ID=0.25 mm, L=60m) and operated under the following conditions: initial oven temperature 35°C (10 min), increasing oven temperature 3°C/min (45 min), final oven temperature 170°C (0 min), detector temperature 280°C and carrier gas (He) pressure 207 Pa. The mass selective detector (Model HP5970, Hewlett Packard, Richmond, BC) was adjusted to scan over 33–250 mass units with an EM potential of 1800 V and a threshold of 500V.

RESULTS & DISCUSSION

CHEMICAL ANALYSIS OF THE ROAST BEEF yielded a moisture content of 75.22 ± 0.60%, 2.36 ± 0.05% NaCl, pH 6.19, and 0.42 ± 0.04% P₂O₅. Horseradish essential oils recovered by steam distillation of the root con-
tained 90% AIT, 9% PEIT, and trace amounts of minor constituents, including allyl thiocyanate and 1-butane isothiocyanate.

**Microbiological studies**

The antimicrobial effect of vaporized HEO on pre-cooked roast beef stored at 4°C in air or N₂ flushed jars (97% N₂, 2% O₂ and 1% CO₂) was evaluated in 3 separate trials. Replacement of air with N₂ in the headspace above the samples did not affect numbers of total aerobic, *Pseudomonas* spp., enteric or lactic acid bacteria on control or treated samples (p<0.05). The spoilage flora of control samples was dominated by *Pseudomonas* spp. (Fig. 1, 2). Substantial populations of enterics and LAB also developed on the roast beef surface. Growth of all bacteria was slower (p<0.05) in samples stored under HEO in either atmosphere. *Pseudomonas* spp. and enteric bacteria were strongly inhibited throughout storage. In contrast, LAB grew under both storage atmospheres, although at a slower rate in the presence HEO.

Susceptibility to HEO varied between individual groups of spoilage bacteria on pre-cooked roast beef. *Pseudomonas* spp. were strongly inhibited by HEO. This confirmed observations derived from studies carried out with purified AIT, the main active component of HEO, which implied that Gram-negative aerobic bacteria were particularly sensitive to this compound (Delaquis and Mazza, 1995; Delaquis and Sholberg, 1997). LAB proved most resistant to antimicrobial effects of HEO. Resistance of lactic acid bacteria toward AIT has been reported previously (Llanos Palop et al., 1995; Kyung and Fleming, 1997; Ward et al., 1998). Studies have also shown that resistance to AIT is common but highly variable among species of this group (data not shown). Furthermore, inhibition of competing microbial species by LAB in meat has been well documented and is the basis for biopreservation of meat products with selected cultures (Dillon and Cook, 1994). Reduced microbial growth on pre-cooked roast beef stored under HEO may therefore result from direct, though variable, inhibition of all bacterial contaminants, and from the selection of a flora dominated by LAB with enhanced resistance to the antimicrobial effect of AIT.

**Sensory evaluation**

Sensory evaluation revealed aroma, flavor and color differences in pre-cooked, un-cured roast beef exposed to HEO (Table 2). Differences were not observed (p>0.05) between morning and afternoon evaluations. Therefore, means were averaged across sessions and plotted on diagrams (Fig. 3) where the center and distal point of each “axis” represented 0 and 10 respectively. Storage under HEO altered the odor attributes of pre-cooked roast beef. Beef aroma scores were highest in control samples and scores decreased with increasing distillate concentrations, regardless of storage. Conversely, horseradish aroma scores increased with HEO concentration. Differences in this attribute were also observed between the 2 storage periods. Roast beef stored under 4 μL/L and 20 μL/L for 3 days was scored higher than that exposed to equivalent HEO concentrations for 15 days. A similar trend was observed with horseradish irritation aroma. Flavor attributes were also affected by storage under HEO, and beef flavor decreased with increasing concentrations. In addition, roast beef stored for 15 days at 4°C had more beef flavor than that stored for 3 days. Both the horseradish flavor and horseradish irritation flavor attributes increased with increased HEO concentration. For each of these attributes, roast beef exposed to 20 μL/
Roast Beef Preserved with Horseradish . . .

L and stored for 3 days scored higher than roast beef stored at an equivalent HEO concentration for 15 days.

Pink color was the only color attribute assessed by the sensory panel. The typical color of pre-cooked, uncured roast beef degraded rapidly in control samples. In all cases these samples were scored lower than those stored under HEO (Table 2). After 3 days, roast beef stored under 20 μL HEO/L had higher pink color than that stored under 20 μL/L for 15 days.

A graphical representation of results was developed by Principle Component Analysis (Fig. 4). Principle component 1 (factor 1) explained 94.9% of the variability, while factor 2 explained an additional 2.8%, resulting in a total of 97.7%. Heavily loaded on factor 1 in the positive and negative directions were horseradish aroma and flavor, and beef aroma and flavor respectively. The 180° orientation of these two vectors reflected the high relationship between these attributes, as shown by the correlation matrix of 0.99 (Table 3).

Differences between treatments were also clearly separated. The control samples were located to the far left (Fig. 4) characterized by the presence of beef aroma/flavors and the absence of pink color. In contrast, the roast beef samples exposed to 20 μL/L had higher pink color than those stored under HEO (Fig. 4) for 15 days.

Color differences in roast beef stored at

![Fig. 3—Diagrams illustrating intensities of roast beef attributes with respect to level of vaporized horseradish distillate (μL/L) and length of exposure at 4°C: A—3 days; B—15 days. Flavor of controls was not assessed at day 15.](image)

![Fig. 4—Principle Component Analysis diagram illustrating differences in pre-cooked roast beef attributes with respect to level of vaporized horseradish distillate (μL/L) and length of exposure at 4°C.](image)

![Table 3—Pre-cooked roast beef attributes evaluated after storage under horseradish essential oil at 4°C (mean ratings± and standard deviations).](image)

![Table 3—Correlations among descriptive attributes for pre-cooked roast beef.](image)
4±2°C for 3 days and 15 days under three levels of HEO (0 μL/L, 4 μL/L, 20 μL/L) were also measured with a Minolta chroma meter (Table 4). L* values were not affected by treatments, although there were differences in a* values. Roast beef stored under 20 μL/L at 4°C for 3 days had the highest a* value. Control roast beef samples stored for 15 days at 4°C had the lowest. The only significant difference observed for b* values was between control roast beef stored for 15 days and that under 20 μL. HEO/L stored for 3 days. L*, a* and b* values were again monitored after the same roast beef samples had been exposed to air for ≈1.5 h (length of sensory evaluation). As with results for fresh samples, no significant differences were observed in L* values, and a* values were higher for roast beef stored under HEO. Slight differences in b* values between roast beef exposed to 4 μL/L for 15 days and that exposed to 20 μL/L for 3 days were also observed. Note that a* values measured after a 1.5 h exposure to air were lower than those measured in freshly opened samples.

Both sensory evaluation and instrumental measurements provided evidence that the pink color associated with freshly cooked roast beef was preserved in storage under HEO.

The nature of the compound(s) and reaction(s) responsible for this effect are not known. Dymicky et al. (1975) reported that denatured myoglobin can react with several nitrogen compounds to produce pink hemochromes, although neither AIT or PEIT were included. It is possible that the isothiocyanates can chelate iron in the heme portion of the protein to yield such a product. However, the decline in a* values in samples exposed to air suggested that the effect was more likely due to a delay in reactions responsible for cooked meat color degradation.

### Table 4—Mean color values (± standard deviation) for pre-cooked roast beef for 3 and 15 days as related to horseradish essential oil immediately after opening (first measurement) and after 1.5h in air (second measurement) at 4°C

<table>
<thead>
<tr>
<th>Sensory attribute</th>
<th>3 days in storage</th>
<th>15 days in storage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HEO concentration</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 μL/L</td>
<td>4 μL/L</td>
</tr>
<tr>
<td></td>
<td>0 μL/L</td>
<td>4 μL/L</td>
</tr>
<tr>
<td></td>
<td>First measurement</td>
<td></td>
</tr>
<tr>
<td>L*</td>
<td>49.6 ± 0.9 a</td>
<td>49.6 ± 0.7 a</td>
</tr>
<tr>
<td></td>
<td>8.6 ± 0.2 ab</td>
<td>9.5 ± 0.3 bc</td>
</tr>
<tr>
<td></td>
<td>7.8 ± 0.4 ab</td>
<td>7.7 ± 0.4 ab</td>
</tr>
<tr>
<td></td>
<td>Second measurement</td>
<td></td>
</tr>
<tr>
<td>L*</td>
<td>49.5 ± 0.9 a</td>
<td>49.4 ± 1.0 a</td>
</tr>
<tr>
<td></td>
<td>8.1 ± 0.3 ab</td>
<td>9.1 ± 0.3 bc</td>
</tr>
<tr>
<td></td>
<td>7.4 ± 0.4 ab</td>
<td>7.5 ± 0.5 ab</td>
</tr>
</tbody>
</table>

Values within the same row with the same superscript are not significantly different (P>0.05).

Volatile compounds

Sensory evaluation of pre-cooked roast beef revealed that the appearance of cooked meat odors and flavors associated with fat oxidation were delayed by storage under HEO. Volatiles in the headspace above pre-cooked roast beef stored with and without HEO were partially characterized to determine whether release of typical fat oxidation products was altered by storage treatment. Gas chromatograms of volatiles above roast beef samples stored in air (Fig. 5) or HEO (Fig. 6) for 0, 3, 7 and 15 days at 4°C were compared.
and compounds positively identified by mass spectrometry were listed (Table 5). The head-space above roast beef stored in air contained a complex mixture of volatile compounds, including several alcohols, aldehydes and ketones. In comparison, 2-propanone, acetic acid, 3-methyl butanal, pentanal, 2,3-butanedione and hexanal were not detected in samples stored under HEO. The effects on volatile compounds with higher retention time (RT) could not be assessed due to interference by a broad, overlapping peak containing AIT. Furthermore, 3-butenitrile and carbon disulphide were detected in the volatile profiles of roast beef exposed to HEO. There was also a compound with molecular weight 99 and RT = 45.8 which was tentatively identified as allyl thiocyanate. This could be derived from distillation of the horseradish root or reactions between isothiocyanates and meat components.

St. Angelo et al. (1987) reported that the major volatile compounds associated with warmed over flavor and oxidizing fats in re-heated pre-cooked roast beef include pentanal, 3-hydroxy-2-butanone, hexanal, heptanal, nonanal, ethanal, 2-propanone, hexane, acetic acid, 3-methyl butanal, 2,3-butanedione, 1-pentanol and 3-methyl-1-butanol. These compounds were detected in our stored control. However, their concentrations decreased or their synthesis was delayed by storage under an atmosphere containing HEO. Carbonyls such as hexanal and nonanal may be formed via oxidation of unsaturated meat lipids and are normally associated with oxidizing fat in cooked meats (Ramarathman et al., 1991; Kerler and Grosch, 1996). Neither compound was detected in pre-cooked roast beef stored under HEO. This indicated that HEO behaved as an antioxidant which blocked reactions responsible for oxygen-induced degradation of fats in pre-cooked roast beef. Such activity may also account for the effect of HEO on cooked roast beef color. Yamaguchi et al. (1984) documented antioxidant activity in horseradish extracts. Also, Veligouli et al. (1998) showed that HEO had the highest antioxidant activity among 28 natural antioxidant plant products. Since the compounds responsible for this activity were not identified, the mechanism responsible for the antioxidant properties of HEO is unknown.

CONCLUSIONS

ESSENTIAL OIL DISTILLED FROM HORSE-RADISH root showed potent antimicrobial properties. Growth of microorganisms was reduced and composition of spoilage flora was altered on pre-cooked roast beef stored in sealed containers with vaporized HEO. The distillate favored growth of resistant LAB. Storage under HEO also delayed appearance of odors and flavors associated with oxidized fats, and led to retention of typical cooked meat color. This unexpected finding warrants further investigation since these quality defects normally limit shelf-life of refrigerated, pre-cooked roast beef. The effect of HEO on sensory characteristics may preclude the use of horseradish distillate as a conventional preservative.

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Table 5—Volatile compounds identified by mass spectrometry in the head space above pre-cooked, uncured roast beef stored at 4°C in air.*

<table>
<thead>
<tr>
<th>Peak number</th>
<th>Retention time (min)</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>carbon dioxide</td>
</tr>
<tr>
<td>2</td>
<td>4.4</td>
<td>hexane</td>
</tr>
<tr>
<td>3</td>
<td>6.4</td>
<td>2-propanone</td>
</tr>
<tr>
<td>4</td>
<td>8.3</td>
<td>acetic acid</td>
</tr>
<tr>
<td>5</td>
<td>9.4</td>
<td>3-methyl butanal</td>
</tr>
<tr>
<td>6</td>
<td>10.9</td>
<td>ethanol</td>
</tr>
<tr>
<td>7</td>
<td>13.2</td>
<td>pentanal</td>
</tr>
<tr>
<td>8</td>
<td>13.4</td>
<td>2,3-butanedione</td>
</tr>
<tr>
<td>9</td>
<td>18.2</td>
<td>camphene</td>
</tr>
<tr>
<td>10</td>
<td>19.6</td>
<td>hexanal</td>
</tr>
<tr>
<td>11</td>
<td>26.0</td>
<td>di-limonene</td>
</tr>
<tr>
<td>12</td>
<td>26.1</td>
<td>heptanal</td>
</tr>
<tr>
<td>13</td>
<td>31.0</td>
<td>1-pentanol</td>
</tr>
<tr>
<td>14</td>
<td>29.1</td>
<td>3-methyl-1-butanol</td>
</tr>
<tr>
<td>15</td>
<td>33.9</td>
<td>3-hydroxy butane</td>
</tr>
<tr>
<td>16</td>
<td>36.5</td>
<td>3-isothiocyanato propene (AIT)</td>
</tr>
<tr>
<td>17</td>
<td>37.8</td>
<td>nonanal</td>
</tr>
<tr>
<td>18</td>
<td>39.0</td>
<td>2-butoxy ethanol</td>
</tr>
<tr>
<td>19</td>
<td>46.0</td>
<td>carbon disulfide</td>
</tr>
<tr>
<td>20</td>
<td>26.9</td>
<td>3-butenitrile</td>
</tr>
</tbody>
</table>

* Numbers correspond to labeled peaks on chromatograms (Fig. 5, 6).

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