Research Note

Antioxidative/Antimicrobial Effects of Galangal and α-Tocopherol in Minced Beef

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ABSTRACT

The antioxidant and microbial stabilities of galangal (Alpinia galanga) extract in raw minced beef were examined at 4 ± 1°C. Raw minced beef containing galangal extracts (0 to 0.10%, wt/wt) were prepared. Lipid oxidation during refrigerated storage was assessed by monitoring malonaldehyde formation, using the thiobarbituric acid reactive substances method. In minced beef, added galangal extract improved oxidative stability. Galangal extract at higher concentrations of 0.05% and 0.10% (wt/wt) were also found to extend the shelf-life of minced beef. Addition of α-tocopherol (0.02%, wt/wt) to galangal extract (0.05%, wt/wt) were observed to increase the oxidative but not the microbial stability of minced beef during the storage of 7 days. Galangal extract may prove useful in inhibiting lipid oxidation and increasing microbial stability of minced meat.

Microbial growth in fresh meat is one of the primary factors associated with meat quality reduction, spoilage, and economic loss. There are many factors that affect microbial quality including initial microbial quality of raw materials, storage conditions, processing temperature, microbial quality of additives, and postprocessing conditions (1, 10). However, such foods are also very susceptible to lipid oxidation and off-flavor development and present the meat-processing industry with some serious challenges (7, 16).

Increased consumption of meat in many less-developed countries is limited by a number of factors, for example the difficulties and expense of establishing suitable cold chains for the storage and distribution of meat and meat products (14). Thus, to overcome these problems, there is a need for low-cost preservation systems to provide meat that has microbiological and chemical stability at tropical ambient temperatures.

Spices are known for both its antioxidative and antimicrobial properties (13). Currently, a range of substances has been investigated as potential antioxidants including nutritive antioxidants (such as α-tocopherol, β-carotene, and vitamin C), spice extracts, and muscle dipeptides (15). Due to an increasing demand for new ethnic foods, galangal (Alpinia galanga), a rhizome closely related to the ginger family has emerged as one of the popular ingredients (22). Our preliminary experiments showed that aqueous extracts of galangal inhibited lipid oxidation and microbial growth minced beef, although at high concentrations (5 to 10%, wt/wt). In these studies, we selected α-tocopherol to enhance the antioxidative capability of galangal extract as α-

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MATERIALS AND METHODS

Preparation of galangal extract. Fresh galangal was purchased from a local market. The rhizome was peeled, sliced to 2 mm × 2 mm × 2 mm, and dried at 35 ± 2°C (AFOS Mini Smoker model AK 3002/56; Hull, England) to a moisture content of ~10.2% (dry basis). The dried galangal was ground, sieved, and the size fraction 0.87 to 1.00 mm collected. The ground material was extracted with acetone for 6 h at 30 ± 2°C in a stirred vessel, at a liquid-to-solid-ratio of 8:1 (5, 19). The filtrate was concentrated in a rotary evaporator (Buchi Rotavapor R-114) and the reddish brown galangal extract obtained was flushed with nitrogen gas and kept in sealed bottle in the dark until used.

α-Tocopherol and 2-thiobarbituric acid (TBA) were purchased from Sigma. All chemicals used were of analytical grade.

Preparation of minced meat. Beef muscles purchased locally at 1 to 2 days postslaughter were trimmed of all visible extramuscular fat. The fat content, determined by the Soxhlet extraction method (2), in all experiments was 2.2 to 2.5%. The beef was minced (Hobart Chopper model 8415; U.S.) and then divided into several portions for each experiment.

To study the effects of concentration, galangal extracts were added separately to each portion for a final concentration of either 0, 0.02, 0.05, and 0.10% (wt/wt, fat basis). After treatment with respective extracts, the samples were again mixed well using a Moulinex Genius 2000 Blender and transferred into individual trays. Each sample was evenly spread to a thickness of 2 cm, covered with oxygen-permeable film, and stored at 4 ± 1°C.
FIGURE 1. Effect of galangal extracts on TBARs in minced raw beef during storage at 4 ± 1°C. Variations are typically 5 to 10% of the means of four determinations. *: 0% (control); ●: 0.02%; ▲: 0.05%; ◆: 0.10% galangal extract.

To study the combination effects of galangal and α-tocopherol on minced beef, the minced meat was formulated to provide the following treatment: (i) control, 0%; (ii) galangal extract, 0.05%; (iii) α-tocopherol (0.10%); and (iv) galangal extract (0.05%) + α-tocopherol (0.10%). As above, each sample was again mixed well, transferred to a plastic tray, covered, and stored at 4 ± 1°C. During storage at 4 ± 1°C, samples were evaluated at regular intervals over 7 days for 2-TBA-reactive substances (TBARs) and total plate count.

Determination of TBARs. The extent of lipid oxidation was determined by the 2-TBA method of Tarladgis et al. (21). The TBARs is expressed as mg reactive substances per kg sample using a conversion factor of 7.8. In all cases four determinations were carried out.

Total plate count. Meat samples (11 g) were removed aseptically and placed into a sterile bottle containing 99 ml sterile saline solution. The samples were shaken for 1 min and serial dilutions of samples were made. Plate count agar (APHA; Oxoid, Basingstoke, UK) tempered at 40 ± 1°C in a water bath was poured into petri dishes containing the serial dilutions. The medium in petri dishes was allowed to solidify, inverted, and incubated at 37 ± 0.5°C for 48 h. Numbers of CFU were counted and reported as log_{10} CFU per g.

Statistical analyses. Each replication included preparation of control and all treatments at the same time. Two replications of the experiment were conducted. The mean values of the microbial counts of the control and the treated samples were subjected to analysis of variance, and Fisher’s least significant difference was used to determine significant (P < 0.05) difference between treatments.

RESULTS AND DISCUSSION

Figure 1 shows the effect of the addition of increasing concentrations of galangal extracts (0.02, 0.05, and 0.10%, wt/wt) on the oxidative stability of raw minced beef during storage at 4 ± 1°C. No differences in initial TBARs were observed between the control (0%, wt/wt) and galangal-treated samples. TBARs for galangal-treated samples were lower than control (P < 0.05) throughout the storage of 7 days. However, no differences in extent of lipid oxidation were observed among the galangal-treated samples at different concentrations.

The addition of galangal, at all levels, reduced the extent of lipid oxidation, as measured by TBARs, in raw minced beef compared to the control with no added galangal. Several researchers have reported that galangal extracts showed antioxidant activities in model systems. Jitoe et al. (9) showed that all tropical ginger extracts (including galangal) have antioxidative activities in an alcohol/water system. It was pointed out that despite only trace amounts of curcuminoinds being present in galangal extracts, galangal still exhibited antioxidant activity, which suggested the presence of other additional antioxidative substances. Tan (20) and Herrmann (8) further identified the phenolic substances responsible for the antioxidant activity of galangal. The results of the present study not only support the finding that galangal extract possesses antioxidant activity in model systems but also extend the inhibitory effect in a complex system, like meat.

Table 1 shows the effect of the addition of increasing concentration of galangal extracts (0.02, 0.05, and 0.10%, wt/wt) on the microbial stability of raw minced beef during storage at 4 ± 1°C. The total plate count of the galangal-treated minced beef were generally lower than the control

<table>
<thead>
<tr>
<th>Samples</th>
<th>Total plate count (log CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 day</td>
</tr>
<tr>
<td>Control (%)</td>
<td>5.74 ± 0.05 A</td>
</tr>
<tr>
<td>Galangal extract (0.02%)</td>
<td>5.62 ± 0.06 B, C</td>
</tr>
<tr>
<td>Galangal extract (0.05%)</td>
<td>5.68 ± 0.02 A, B</td>
</tr>
<tr>
<td>Galangal extract (0.10%)</td>
<td>5.60 ± 0.03 C</td>
</tr>
</tbody>
</table>

a Values are means for four analyses performed in duplicate. Means in the same column followed by different letters are significantly different (P < 0.05).
Throughout the storage period. On day 4, the microbial count of minced beef with added galangal extract at 0.05% and 0.10% were significantly ($P < 0.05$) lower than the control. All samples showed microbial spoilage on day 7. (Fresh muscle foods were considered as spoilt microbiologically when the count exceeds $10^7$/g (3, 6, 18).)

Studies on the antimicrobial activity of galangal extract have not been reported. Our results showed that galangal extracts at 0.05 and 0.10% extended the shelf-life of minced raw beef up to 4 days of storage at 4 $\pm$ 1°C. Galangal is a rhizome closely related to the ginger family, and the antimicrobial activities of the latter have been well examined. Won et al. (26) found that ether extracts of ginger but not ginger juice were effective in inhibiting growth of bacteria. Similarly, Yamada et al. (27) also demonstrated the antimicrobial activity of acetone extract of ginger. As reported, ginger contains pungent components such as zingerone, gingerol, and shogoal that possess antimicrobial activity (13). It is probable that the antimicrobial activity of galangal extract is due to the presence of similar compounds as phenolics and essential oils from spices are known to inhibit microbial growth. However, further work needs to be carried out to identify these active components.

As galangal extract at 0.05% was found to be effective in extending the oxidative stability (Fig. 1) and shelf-life (Table 1) of minced beef, this concentration was used in the following experiments. TBARS in galangal- and/or $\alpha$-tocopherol-treated samples were apparently lower than the control during the storage period (Fig. 2), the lowest being samples treated with both galangal and $\alpha$-tocopherol.

The addition of galangal or $\alpha$-tocopherol is effective in inhibiting TBARS formation in minced beef with a more pronounced effect from galangal extract. Hence, when galangal extract was used in combination with $\alpha$-tocopherol, the inhibiting effect was more than used singly. Thus, our results indicate that the combination of galangal and $\alpha$-tocopherol had an additive protective effect on oxidative stability in minced beef. Synergism between $\alpha$-tocopherol and an extract has been reported; the study indicates that $\alpha$-tocopherol was regenerated by the rosemary extract. However, $\alpha$-tocopherol and sage showed only very poor synergism (12).

Again, it was observed that the total plate count of galangal-treated minced beef at 0.05% was lower than the control during storage at 4 $\pm$ 1°C (Table 2). However, the microbial stability of minced beef does not improve when used in combination with $\alpha$-tocopherol. Similarly, galangal does not show antimicrobial activity in marinated spiced pork shanks when used in combination with other ingredients (25). Resurrection and Reynolds (17) also reported no differences in surface microbial counts of vacuum-packaged frankfurters containing tocopherol and rosemary stored at 4°C.

Thus, these limited studies showed that galangal extract exhibits antioxidant activity in meat systems when used alone or in combination with $\alpha$-tocopherol. It suggests that there is an additive effect between galangal extract and $\alpha$-tocopherol in inhibiting lipid oxidation. Galangal extracts appear to inhibit microbial growth in minced beef when applied alone, but this effect diminishes when used in combination with $\alpha$-tocopherol. However, it must be remembered that only one combination was used (atocopherol and/or $\alpha$-tocopherol).

### Table 2. The effect of galangal extract and $\alpha$-tocopherol on the mean microbial population of minced raw beef stored at 4 $\pm$ 1°C for up to 7 days

<table>
<thead>
<tr>
<th>Samples</th>
<th>0 day</th>
<th>2 days</th>
<th>4 days</th>
<th>7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.08 ± 0.01 A</td>
<td>6.23 ± 0.09 A</td>
<td>6.86 ± 0.09 A</td>
<td>7.43 ± 0.11 A</td>
</tr>
<tr>
<td>Galangal extract (0.05%)</td>
<td>5.89 ± 0.01 B</td>
<td>6.21 ± 0.08 A</td>
<td>6.68 ± 0.13 B</td>
<td>7.23 ± 0.07 B</td>
</tr>
<tr>
<td>$\alpha$-Tocopherol (0.10%)</td>
<td>6.09 ± 0.03 A</td>
<td>6.49 ± 0.11 B</td>
<td>6.76 ± 0.19 A</td>
<td>7.35 ± 0.03 A,B</td>
</tr>
<tr>
<td>Galangal extract (0.05%) +</td>
<td>6.08 ± 0.04 A</td>
<td>6.35 ± 0.05 A,B</td>
<td>6.85 ± 0.05 A</td>
<td>7.30 ± 0.01 A,B</td>
</tr>
<tr>
<td>$\alpha$-Tocopherol (0.10%)</td>
<td></td>
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</table>

$^a$ Values are means for four analyses performed as duplicate. Means in the same column followed by different letters are significantly different ($P < 0.05$).
[0.02%, wt/wt] and galangal extract [0.05%, wt/wt], and it is possible that other combinations of concentrations may more effectively inhibit microbial growth in the meat system. However, more work needs to be carried out to understand this further.

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REFERENCES