ORANGE OIL AS A PRESERVATIVE FOR FISH SAUSAGE

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Fish sausage is ground fish meat, or a mixture primarily of ground fish with ground pork, together with vegetable oil, permissible preservatives, seasoning and starch. It is packed in a casing and processed. It is a semi-processed food, the time and temperature of processing of the product being not sufficient to kill all microorganisms. Thermotolerant and spore-forming bacteria survive the processing and bring about spoilage. Consequently, chemical preservatives are used to prevent the growth of these. Preservatives such as nitrofurazone, sorbic acid and tylosin1,2) used in other countries for preservation of fish sausage, are not permitted in our country. The use of an easily available, natural preservative, with effective preservation action, will therefore, help in the development of fish sausage industry in the country.

The germicidal activities of several essential oils possessing remarkable lethal action against a series of pathogenic and nonpathogenic bacteria and fungi have been reported3,4). The disinfectant power of lemon and orange essences in aqueous solution against spore-bearing organisms was found to be greater than that of phenol5). Cold-pressed (undistilled) orange oil (0.1%) exerted a toxic effect upon Streptococcus faecalis6). Orange oil and d-limonene exhibited lethal properties against Zygosaccharomyces major at 0.02% concentration in aqueous solution and at 0.1% in orange juice, respectively7). d-Limonene was more effective than orange oil in both water and orange juice. The study further indicated that orange oil and d-limonene in concentration as low as 0.02% increased the preservative effect of sodium benzoate. Brown mustard powder or volatile oil of mustard and orange oil were inhibitory to yeast8). Oxidized d-limonene shifted the lag-phase in the growth of the yeast culture. A preservative emulsion for pickles from acetic acid, orange oil and powders of brown mustard and turmeric has been prepared9). This prevented the growth of microorganisms in pickles even when the concentration of salt or acid, or both was low, without significantly altering the taste and flavour of the pickles. Addition of cinnamon, clove and mustard oil improved the keeping quality of fresh water fish-fillets10). The preservative effect of orange oil or other essential oils in the case of fish sausage has not been studied. The object of the present investigation was, therefore, to study the preservative effect of orange oil on fish sausage.

Experimental

For the purpose of the study, fish sausage was prepared in two groups. The 1st
group consisted of 2 batches of 3 samples each, one sample with orange oil (1000 ppm) another with sorbic acid (2000 ppm) and the third one without preservative.

Similarly, the 2nd group of fish sausages were made in 2 batches of 3 samples each, one with orange oil (1000 ppm) another with tylosin (10 ppm) and the 3rd one without preservative. Fish sausages (with 3 percent salt) were prepared as described in an earlier paper except that the natural spice-mixture was replaced by a mixture of oleoresins to reduce the microbial load. The mixture of oleoresins consisting of the essences of pepper (4 ml), clove (0.2 ml), nutmeg (2 drops), cardamom (0.2 ml), cinnamon (1 ml), mace (0.2 ml), chilli (2 ml), ginger (2 ml), and cumin (1 ml) was incorporated into 2.5 kg. of sausage meat. The orange oil ("Naarden" brand) (10 gm) together with mustard (50 gm), gum acacia (40 gm) and water (100 gm) was emulsified in a glass Waring blender and the blend mixed thoroughly with the fish sausage. Sausages were packed in sealed synthetic casings and processed at 85°C for not less than 50 min. and cooled. Prepared samples of fish sausage were examined individually.

pH was determined directly with a Beckman pH meter. Total volatile base was determined by extracting the fish sausage material with 15% trichloroacetic acid and estimating ammonia in 1 ml. of the extract by CONWAY microdiffusion method.

Sampling and preparation of the product for microbiological examination were carried out according to recommended procedures. Total plate counts were carried out in duplicate. Coliforms were enumerated by plating in duplicate on desoxycholate lactose-agar. To obtain atypical colonies, the plates were covered with a second layer of the same medium after the first layer of medium solidified and then incubated. Yeast and moulds were determined by plating in duplicate on acidified potato-dextrose agar. Sausages were examined for coagulase positive-staphylococci (presumptive) and Clostridium botulinum by standard procedures. Aerobic spore formers were determined by plating on standard plate-count agar after heat-shocking the sample for 20 min. (plus come-up-time) in a thermostatically controlled water bath at 80°C ± 0.5°C.

The sausage samples were stored at room temperature (28°C) and R. H. 50~60% and changes in pH, microbial load and total volatile base (TVB) were noted. Organoleptic evaluation was done on a point system of grading for colour, flavour, elasticity, texture and overall acceptance by a panel of 10 persons who were habitual fish eaters.

Results and Discussion

Samples of fish sausage without preservatives spoiled in a short time and so the results are not reported. Changes in pH, microbial load and TVB of fish sausage preserved with orange oil and sorbic acid are represented in Figure 1. (mean). The changes with orange oil practically followed the same pattern as with sorbic acid. There were no significant differences in the organoleptic properties also. There was neither gas production nor swelling of the sausage. Texture was firm and somewhat
elastic. There was no sweating, spot formation or softening of the sausage. Except for slight discolouration, there were no other apparent signs of spoilage. There was not any loss of taste at the end of storage (25 days).

Though sorbic acid has been considered to be more effective in acid medium, in Japan it has been used in fish sausage (pH 6.5 to 7.0) as a singly permitted preservative\(^1\).\(^{13}\).\(^{16}\). It appears that at present the Japanese fish sausage industry uses sorbic acid only in combination with other preservatives. However, it will be seen from the above results that sorbic acid effectively preserved fish sausage. This may probably due to the combined effect of sorbic acid, mixture of oleoresins and other ingredients in the fish sausage.

The comparative preservative effect (mean) of orange oil and tylosine is shown in Table 1. The total microbial load and aerobic spore formers were slightly higher

![Fig. 1. Variations in pH, microbial counts and TVB of fish sausage with orange oil and sorbic acid as preservatives.](image)

<table>
<thead>
<tr>
<th>Storage period (days)</th>
<th>pH</th>
<th>Total microbial count/g.</th>
<th>Aerobic spore formers/g.</th>
<th>Total volatile bases g N/100 g (TVB)</th>
<th>Storage behaviour</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.3</td>
<td>2.2 x 10(^6) 2.5 x 10(^5)</td>
<td>2.2 x 10(^4) 1.8 x 10(^3)</td>
<td>36 32</td>
<td>No gas production. No swelling. Same as of sausage; firm and elastic texture. No. 1.</td>
</tr>
<tr>
<td>20</td>
<td>6.6</td>
<td>2.2 x 10(^6) 4.3 x 10(^5)</td>
<td>3.2 x 10(^6) 5.2 x 10(^8)</td>
<td>54 57</td>
<td>Neither gas production nor swelling. Same as of sausage. Texture same as No. 1. what firm and elastic. No sweating of sausage. Neither softening nor spot formation. Slight discoloration but no other signs of spoilage.</td>
</tr>
</tbody>
</table>
in the case of orange oil than with tylosin. There were, however, not any significant
difference either in TVB or apparent storage behaviour.

Coliforms, yeasts and moulds, coagulase-positive staphylococci and \textit{Clostridium botulinum} were absent in all cases both before and after storage.

\textsuperscript{15} Tanikawa has reported that fish sausages with microbial counts of $45 \times 10^7$ for aerobic bacteria and $67 \times 10^3$ for anaerobic bacteria were quite edible and could be safely sold. They observed that, provided the surviving bacteria did not become active, the meat did not show any abnormal appearance and did not putrify and was quite edible. Microbial loads in the sausages studied (Fig. 1 and Table 1) are less than those reported for edible sausage. Amounts of TVB for edible fish sausages have not been reported. In the present case, however, there were no significant differences in TVB of sausages preserved with orange oil, sorbic acid and tylosin. The sausages were edible at the end of storage. When deep fried, these had good puffing properties and were acceptable except that their flavours were not as agreeable as that of fish sausage containing natural spice mixture. Organoleptic tests combined with other laboratory tests to indicate safety, seem to be the best method of judgement of fish sausage.

The present data show that orange oil compares well with sorbic acid and tylosin in its preservative action on fish sausage. The mechanism of the preservative action of orange oil is not known. It is likely that oxygenated derivatives of \textit{d}-limonene, which is the principal component of orange oil, act as antimicrobial agent.

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\textbf{References}

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