The Effects of Koji and Histidine on the Formation of Histamine in Anchovy Sauce and the Growth Inhibition of Histamine Degrading Bacteria with Preservatives

Jung Min LEE¹, Dong Chul LEE², and Sang Moo KIM¹ *

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Abstract

Koji and histidine have been applied to accelerate the fermentation of fish sauce for the purpose of shortening the manufacturing period and cost. The histamine contents of anchovy sauces fermented at 15 and 25 °C for 12 months were 3.7-3.8 mg/mL. The histamine content increased up to 6 months of ripening and then decreased, where at 25 °C was higher than at 15 °C at 6 months of ripening, but similar at 12 months. Koji decreased the histamine formation at 3 months at 15 °C, but no significant difference at 12 months, whereas histidine increased at 6 months at 15 °C, but no great difference at 25 °C. Histamine degrading bacteria isolated from the anchovy was identified to Staphylococcus xylosus. Optimal pH for the growth of S. xylosus was neutral. The growth of S. xylosus was strongly inhibited as the concentration of NaCl, benzoic acid, and sorbic acid increased.

Keywords: Anchovy sauce; Histidine; Histamine degrading bacteria; Koji; Staphylococcus xylosus

1. Introduction

Fish sauce, the liquid part of fermented fishes, has long been used as a condiment for rice dishes. The manufacturing procedure for fish sauce consists of mixing small uneviscerated fishes with NaCl at high concentration (>25%) and then storing the mixture in sealed vessels at ambient temperatures or underground for a period of 1-5 years. Because most microorganisms, except for some halophiles, cannot grow and endogeneous enzymes also cannot hydrolyze fishes very well at high salt conditions, it usually takes a long fermentation period to manufacture the fish sauce (Kim et al., 2002).
Biogenic amines have been found to be produced during processing of foods which include fishery products and other fermented foods (Sanceda et al., 1999). Biogenic amines including histamine are produced as a result of microbial decarboxylation of dietary amino acids in animal, plants, and microorganism, and are reported to be toxic to humans (Sanceda et al., 1999). It has been reported that high amounts of biogenic amines (500 mg/kg of histamine and 100-800 mg/kg of tyramine in foods) are hazardous to human health (Askar et al., 1993). Common toxic symptoms of biogenic amines in humans are nausea, respiratory distress, hot flushes, sweating, heart palpitation, headache, a bright red rash, oral burning, and hypertension as well as hypotension (Mah et al., 2003).

There are few reports on the inhibition of histamine formation in fish sauce; rice bran nuka (Kuda and Miyawaki 2010) and starter culture (Mah and Hwang 2009; Zaman et al., 2011). Koji (Baek et al., 1996) and histidine (Sanceda et al., 1996) have been applied to accelerate the fermentation of fish sauce for the purpose of shortening its manufacturing period.

Therefore, the objectives of this study were to analyze the effects of koji and histidine on the histamine formation, to isolate and identify the histamine degrading bacteria, and to investigate the influence of preservatives on the production of histamine and the growth of histamine degrading bacteria in anchovy sauce.

2. Materials and methods

2.1 The manufacture of anchovy sauce
Anchovy (Engraulis japonicus) caught in the eastern coastal area of the Korean peninsula in September in 2009 was used to manufacture its sauce by mixing 30% of sun-dried salt per weight, packaging by 1 kg in a plastic vessel (10 cm in diameter and 20 cm in length), and then fermenting at different temperatures (15 and 25 ℃). In order to inhibit the histamine formation during the fermentation of anchovy sauce, each 0.25 % of histidine (L-α-Amino-β-[imidazoiy]propionic acid) and 0.5% of Aspergillus oryzae koji (Chungmu Fermentation Co., Busan) were added. In addition, eight kinds of commercial anchovy sauce were purchased at a local market in Gangneung, Korea, to compare the histamine content.

2.2 Analysis of proximate composition
Proximate compositions were determined according to the AOAC method (AOAC. 2000).

2.3 Determination of histamine contents
Sulfosalisylic acid (50 mg/mL) was added to anchovy sauce in order to precipitate high molecule weight of protein, and then centrifuged at 5,000 g for 30 min. The supernatant was filtered with 0.45 μm filter paper (Gelman Laboratory Co., Ann Arbor, MI, USA). Histamine content was determined by the HPLC method (Gill and Thompson 1984). The operation condition of HPLC (Gilson, Middleton, WI, USA) was: column, Asahipak ODP-50 4E (Shoko Co., Tokyo, Japan); wavelength, 210 nm; mobile phase, 0.25 M ammonium sulfate; flow rate, 0.7 mL/min. Histamine concentration was determined from the standard curve by calculating the ratio of peak area between the sample and the control.
2.4 Isolation and identification of histamine degrading bacteria

Histamine degrading bacteria were isolated from the 9 months-fermented anchovy sauce at 15 °C. One mL of anchovy sauce was added to 20 mL of a selective broth {trypticase soy broth (TSB) supplemented with 1% L-histamine-HCl (TSBH) and 0.0005% pyridoxal-HCl} and then cultivated at 37 °C for 24 hrs. One mL of each cultured broth was inoculated in brain heart infusion (BHI) agar and then cultivated at 37 °C for 24 hrs. Histamine degrading bacteria were identified on the bases of Gram stain, endospore stain, catalase and oxidase reactions. API Staph Kit (Biomerieux Co., Marcy l’Etoile, France) was used for further identification, and also compared with the standard data from Bergey’s Manual of Systematic Bacteriology (Murray et al., 1986).

2.5 Cultivation of histamine degrading bacteria

One mL of selective broth with histamine degrading bacteria was inoculated to 20 mL of selective TSBH broth and precultured at 37 °C for 24 hrs in a shaking incubator (Vision Scientific Co., Incheon, Korea) at 50 rpm, and then subcultured same as above. Total viable cell number of histamine degrading bacteria was 1.2 × 10^8 CFU/mL.

2.6 Determination of histamine degradation

Histamine degradation by histamine degrading bacteria was determined according to the modified method of Leuschner et al., (1998). One mL of subcultured broth with histamine degrading bacteria (1.2 × 10^8 CFU/mL) was added to 10 mL of 0.05 M sodium phosphate buffered(pH 7.0) with 0.5 mM histamine. The cell suspension was incubated in a 100 mL Erlenmeyer flask at 30 °C for 24 hrs under shaking at 200 rpm, and then centrifuged at 5,000 g for 30 min. The supernatant was filtered with 0.45 μm filter paper (Gelman Laboratory Co. Ann Arbor, MI, USA). Histamine content was determined same as above.

2.7 Growth inhibition of Staphylococcus xylosus

In order to determine the effects of pH and preservatives (NaCl, citric acid, D-sorbitol, ethyl alcohol, and benzoic acid) on the growth of S. xylosus, one mL of the TSBH broth with S. xylosus (1.2 × 10^8 CFU/mL) was added to 50 mL of TSBH broth at different pHs and preservative concentrations. One mL of TSBH broth was inoculated in BHI agar and then cultivated at 37 °C for 24 hrs to determine the total viable cell count.

2.8 Statistical analysis

Statistical analysis was performed using SPSS Ver. 11.0 program (SPSS, Chicago, IL, USA) with Duncan’s multiple comparison test at p<0.05.

3. Results and discussion

3.1 Proximate composition and histamine contents of commercial anchovy sauces

Proximate compositions and histamine contents of eight different commercially available anchovy sauces with similar manufacturing are shown in Table 1. The contents of moisture, crude protein, crude lipid, ash, and carbohydrate were in the ranges of 65.1-67.3, 21.4-24.2, 0.2-0.5, 7.5-10.4, and 0.2-2.5%, respectively with significantly differences in the contents of crude protein, ash, and carbohydrate (p<0.05). In addition, histamine contents, 434.2-797.3 mg/100 mL, were also significantly different (p<0.05) regardless of the protein contents in anchovy sauces. It is
considered that the significant differences in crude protein and histamine contents might be due to the different raw materials for anchovy sauce and manufacturing processes. Brillantes and Samosorn (2001) reported that the histamine content of Nampla, a Thai fish sauce, was in the range of 100-1,000 ppm depending on different kinds of fish sauce. Most of Korean commercial anchovy sauces exceeded this range in histamine content (Table 1).

### Table 1 Proximate composition and histamine concentration of commercial anchovy sauces

<table>
<thead>
<tr>
<th>Sample</th>
<th>Moisture (%)</th>
<th>Crude protein (%)</th>
<th>Crude ash (%)</th>
<th>Crude lipid (%)</th>
<th>Carbohydrate (%)</th>
<th>Histamine (mg/100 mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>67.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>575.2&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>B</td>
<td>66.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.0&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>470.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C</td>
<td>66.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.4&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>648.4&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>D</td>
<td>65.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.5&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>574.6&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>E</td>
<td>66.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>747.5&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>F</td>
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<td>24.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>622.4&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>G</td>
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<td>21.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>797.3&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>H</td>
<td>65.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>434.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a-d</sup> Means in the same column with the different superscripts are significantly different (p<0.05)

Although histamine poisoning outbreak by fish sauce has not been reported, there are very strong regulations in seafood; 50 mg/L in USA (FDA. 2001) and 200 mg/L for enzyme ripened products including fish sauce in Canada (CFIA. 2009). However, large amount of histamine was formed in red-muscle fishes including anchovy, if the temperature was not managed properly (Russell et al., 1986; Taylor et al., 1989). Sanceda et al., (1996) reported that histamine contents of Patis, Nampla, Shottsuru, and Korean anchovy sauce (Ekjeot) were 4, 43, 37, and 138 mg/mL, respectively, in which Korean anchovy sauce contained the highest histamine content. Kang and Park (1984) reported that histamine was formed in the early period of putrefaction in mackerel processing and fermentation. Therefore, the handling of raw fishes after catching is considered to be very important especially in the processing of red muscle fish products.

### 3.2 The effects of koji and histidine on the histamine formation of anchovy sauce

The changes in the histamine content of the anchovy sauce added with histidine and koji at different fermentation temperatures and periods are shown in Fig. 1. The histamine content of the anchovy sauce fermented at 15 °C rapidly increased up to 6 months of storage and then decreased. At 3 months of storage, the histamine content of the control anchovy sauce was highest followed by histidine and koji group in order, whereas at 6 months of storage, the reverse was true.
Fig 1. The histamine content in the anchovy sauces fermented at 15 °C (A) and 25 °C (B), and different fermentation periods

The anchovy sauce with histidine fermented for 6 months had highest histamine content (Fig. 1), thus might be due to the putrefactive bacteria such as *Proteus morganii*, *Hafnia alvei*, and *Kelebsiella pneumoniae* producing histidine decarboxylase utilized histidine effectively for histamine production (Sanceda et al., 1996; Taylor et al., 1979). Histamine content of anchovy sauce with koji fermented at 25 °C was higher than those of the control and histidine group up to 6 months of storage, but there were no significant differences at 12 months of storage (p < 0.05). The histamine content of anchovy sauce with histidine was lowest in up to 3 months of storage, but no significantly difference at 6 and 12 months of storage. The histamine content of anchovy sauce fermented at 15 °C was lower than at 25 °C. Hence, low temperature is better to decrease the histamine content in anchovy sauce, which is similar to the result of Edmunds and Eitenmiller (1975) and also the same as the results of Frank et al., (1981) in which the growth of histamine forming bacteria and histamine formation were accelerated at higher temperatures. Koji (Kim et al., 2002) and histidine (Sanceda et al., 1996) have been used to accelerate the fermentation of fish sauce for shortening the fermentation period. Especially, Sanceda et al., (1996) reported that the addition of histidine to the fish mixture during fermentation did not increase the histamine content of sardine fish sauce for up to 4 months of storage, which was similar at 25 °C in this study. One of the reasons the histamine content of the anchovy sauce with koji fermented at 25 °C rapidly and increased up to 6 months of storage is that koji produced various proteases including histidine decarboxylase and histidine by hydrolyzing anchovy meat protein effectively. Su et al., (1981) reported that soy sauce koji produced protease and amylase, which was adversely affected by the addition of NaCl. The difference in the histamine content of anchovy sauce with histidine between at 15 and 25 °C might be that histamine forming bacteria could hydrolyze anchovy protein effectively at 25 °C to produce enough free histidine for histidine decarboxylase. The addition of histidine might promote the production of histamine by histamine forming bacteria or activate hisditine decarboxylase in raw materials, resulting in increasing the content of histamine in this study, which was different from the result of Sanceda et al., (1996). In addition, high salt
concentration in anchovy sauce might also inhibit histamine forming bacteria effectively at 25 and 15 °C. Histamine forming bacteria generates histamine even if their growth was inhibited by the secretion of histidine decarboxylase (Baranowski et al., 1985), which was similar to this study where the content of histamine increased up to 6 months of fermentation at high salt condition. Therefore, the decrease of histamine content along with increase of a storage period might be due to the inhibition on the growth of histamine forming bacteria and the activity of histidine decarboxylase. Choi et al., (1982) reported that histamine formation during the fermentation of soybean increased rapidly and then decreased as the fermentation period increased, which was similar to this study. However, Brillantes et al., (2002) reported that histamine was generated in a fresh fishes meat and suddenly increased at the first month of anchovy sauce fermentation (small scale) at 5-15 °C, and maintained a constant level or decreased thereafter, whereas it increased a little in a commercial manufacturing process (large scale) of anchovy sauce fermented at 32-35 °C. There was also a very close correlation between the histamine content in raw materials and histamine contents after ripened. Furthermore, histamine can be produced before fermentation by histidine decarboxylase existing in fresh fishes (Brillantes et al., 2002). Kang and Park (1984) reported that the addition of salt decreased the histamine content of mackerel more effectively at 5 °C than at 25 °C. Because the salt concentration of anchovy sauce was 25-30%, the growth of putrefactive bacteria should be inhibited effectively. Amino acid histidine, arginine, lysine and tyrosine concentration decreased at different rates during fish sauce fermentation with starter cultures, Staphylococcus carnosus and Bacillus amyloliquefaciens, as they were converted into their respective amines, in which the concentration of biogenic amines namely histamine, putrescine, cadaverine, and tyramine increased throughout fermentation. However, their concentrations were markedly lower than the control (without starter culture) (Zaman et al., 2011).

3.3 Isolation and identification of histamine degrading bacteria
Two bacteria were isolated from the anchovy sauces fermented at 15 and 25 °C for 9 months, respectively, and identified to the same species of Gram positive bacteria. Based on taxonomic characteristics in Table 2, histamine degrading bacteria were identified to Staphylococcus xylosus by 99.9% (T=0.39) (Murry et al., 2003). The optimal pH for the growth of S. xylosus (1.5 × 10⁹ CFU/mL) was 7, but its growth decreased in alkali and acidic condition (Fig. 2). In the processes of fish paste fermentation, Staphylococcus spp. are dominant populations among halophilic and halotolerant bacteria (Mah et al., 2003; Um and Lee 2002). Among them, S. xylosus is known to possess not only the greatest capability to degrade biogenic amines (Mah and Hwang 2009; Martuscelli et al., 2002), but to form them at lower level as well (Martuscelli et al., 2002).
Table 2 Characteristics of *S. xylosus* compared with the isolated microorganism from the anchovy sauce

<table>
<thead>
<tr>
<th>Characteristics</th>
<th><em>Staphylococcus xylosus</em></th>
<th>Isolated microorganism</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Glucose (GLU)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-Fructose (FRU)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-Mannose (MNE)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-Maltose (MAL)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-Lactose (LAC)</td>
<td>d</td>
<td>+</td>
</tr>
<tr>
<td>D-Trehalose (TRE)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-Mannitol (MAN)</td>
<td>d</td>
<td>+</td>
</tr>
<tr>
<td>Xylitol (XLT)</td>
<td>-w</td>
<td>+</td>
</tr>
<tr>
<td>D-Melibiose (MEL)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Potassium nitrate (NIT)</td>
<td>d</td>
<td>+</td>
</tr>
<tr>
<td>β-Naphthyl phosphate (PAL)</td>
<td>d</td>
<td>+</td>
</tr>
<tr>
<td>Sodium pyruvate (VP)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D-Raffinose (RAF)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D-Xylose (XYL)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-Saccharose (SAC)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Methyl-α-D-glucopyranoside (MDG)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>N-Acetyl-glucosamine (NAG)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>L-Arginine (ADH)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Urea (URE)</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Symbols: +, 90% or more strains positive; -, 90% or more strains negative; d, 11-89% strains positive; w, weak reaction; -w, negative to weak reaction; ND, test not determined.

3.4 Determination of histamine degradation

Biogenic amines can be degraded through oxidative deamination catalyzed by amine oxidase with the production of aldehyde, ammonia and hydrogen peroxide (Yamashita et al., 1993). *S. xylosus* isolated from anchovy sauce degraded the histamine content by about 38%, which was higher than 27.7 and 15.4% by *S. carnosus* FS19 and *B. amyloliquefaciens* FS05 isolated from fish sauce, respectively (Zaman et al., 2011), but much lower than 62-65% of *S. xylosus* 0538, *Bacillus coagulans* 0038 and 01051 isolated from a salted and fermented anchovy (Mah and Hwang 2009). *S. xylosus* S142 also decreased tyramine and histamine content of fermented sausages by 63 and 47%, respectively (Martuscelli et al., 2000). The presence of biogenic amine-degrading oxidase activity has been described in different microbial groups; *Micrococcus* spp. for tyramine (Leuschner and Hammes 1998a), *Brevibacterium linens* for histamine and tyramine (Leuschner and Hammes 1998b), *Lactobacillus sakei* for histamine (Dapkevicius et al., 2000), *Lactobacillus casei* and *Lactobacillus plantarum* for tyramine (Fadda et al., 2001), and *S. xylosus* for histamine and tyramine (Martuscelli et al., 2000). *S. xylosus* 0538 degraded histamine significantly, but tyramine slightly (Mah and Hwang 2009). Most of *S. xylosus* had monoamine oxidase activity and ability to degrade histamine and tyramine (Martuscelli et al., 2000). On the other hand, Mah and Hwang (2009)
reported that *S. xylosus* 0538 of a salted and fermented anchovy produced bacteriocin-like substances to inhibit the growth of *Bacillus licheniformis*, a powerful histamine forming bacteria from the same sample, which can be used as a starter culture for reducing biogenic amines in a salted and fermented anchovy.

### 3.5 The growth inhibition of *S. xylosus*

The growth of *S. xylosus* was inhibited as the NaCl concentration increased (Fig. 2). The number of *S. xylosus* decreased from $1.2 \times 10^8$ CFU/mL at 0% NaCl to $7.8 \times 10^7$ CFU/mL at 5% and $4.5 \times 10^5$ CFU/mL at 10%.

Kang and Park (1984) reported that histamine content and histidine decarboxylase activity decreased as the NaCl concentration increased, which was similar to this study (Fig. 2). The number of *S. xylosus* decreased rapidly as the concentrations of carboxylic acid increased where sorbic and benzoic acid were more effective than citric acid (Fig. 3).

![Fig 2. Effects of pH and NaCl on the growth of *S. xylosus*.](image)

Kang and Park (1984) reported that histamine content and histidine decarboxylase activity decreased as the NaCl concentration increased, which was similar to this study (Fig. 2). The number of *S. xylosus* decreased rapidly as the concentrations of carboxylic acid increased where sorbic and benzoic acid were more effective than citric acid (Fig. 3).

![Fig 3. Effects of acids and alcohols on the growth of *S. xylosus*.](image)
Benzate (C₆H₅COOH) and sorbate (C₅H₇COOH) are widely used as preservatives in food, beverage, cosmetics, etc. in which benenate is more effective in pH 2.5-4.0 whereas sorbate does in > pH 6.5 (Luca et al., 1995). Histamine forming decreased by citric acid, malic acid, and succinic acid because organic acid lowered pH, which inhibited the growth of histamine forming bacteria (Kang and Park 1984). The growth of S. xylosus decreased rapidly as the concentration of D-sorbitol increased (Fig. 3). D-sorbitol, glucose, and sucrose inhibited the formation of histamine by forming sugar-alcohol complex (Ota et al., 1984). Glycine and sorbic acid also decreased the histamine content of mackerel (Kang and Park 1984). Sorbate was completely effective against Escherichia coli and Bacillus cereus, whereas benenate was the most effective against Staphylococcus aureus when used at pH < 6 (Thomas et al., 1993). Ethyl alcohol also inhibited the growth of S. xylosus more moderately than others. Ethanol at concentrations up to 1.25% did not inhibit growth of Listeria monocytogenes, but growth was strongly inhibited in the presence of 5% ethanol (Oh and Marshall 1993), which was very similar to this study. In addition, the growth of Lactobacillus hilgardii and Oenococcus oeni were inhibited by 10% ethanol (Arena et al., 1993). The growth inhibition of Zymomonas mobilis at ethanol concentration above 20 g/L was caused by the reduction of the transketolase activity, which in turn provided less precursors for the cell growth (Park et al., 1988). Koji protease activity was still active or not greatly inhibited in the products supplemented with less than 10% ethanol and koji enzymes were comparatively less affected by ethanol than were populations of molds, yeasts, and lactic acid bacteria (Chiou et al., 1999). Ethanol would appear an effective additional barrier to inhibit fungal growth in food products and represent an interesting alternative to the use of preservatives (Dantigny et al., 2005). Therefore, the use of preservatives in the manufacture of fish sauce might accumulate the histamine by inhibiting histamine degrading bacteria. Hence, the use of histamine degrading bacteria including S. xylosus is an alternative starter culture to prevent the accumulation of histamine in fish sauces.

4. Conclusion

The histamine content of anchovy sauce was lower at a lower fermentation temperature. The addition of koji decreased the histamine production, while the reverse was true for histidine. The preservatives inhibited the growth of S. xylosus, a histamine degrading bacteria, in anchovy sauce. Therefore, the use of preservatives in the manufacture of fish sauce might accumulate the histamine by inhibiting histamine degrading bacteria. Hence, the addition of histamine degrading bacteria as a starter culture with no addition of preservatives is an alternative way to prevent the formation of histamine in fish sauce.

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