DEVELOPMENT OF HEALTHY SOY SAUCE FROM PEAGION PEA AND SOY BEAN

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ABSTRACT

Soy sauce is one of the flavoring agent product which was produced by the fermentation process of *Aspergillus oryzae* with a combination of soy bean, wheat flour and salt. In general the salt content that used in the fermentation process acts as the preservative for the mixing component. The objective of this research was to develop low salt soy sauce from Pigeon Pea and Soy Bean. The work was divided into 2 parts; part 1 soy sauces were prepared from 6 formulas by variation the ratios between peagion pea and soy bean as 100:0 (F1), 80:20 (F2), 60:40 (F3), 40:60 (F4), 20:80 (F5) and 0:100 (F6) (control). Those koji molds were fermented with 20% (w/v) sodium chloride for 90 days. The best formula was selected to further study in part 2. This part, the soy sauces were prepared by the variation of sodium chloride content as 18%, 16%, 14%, 12% and 10%. In this work, the chemical change of organic acid content, sodium chloride content, amylase enzyme activities, glucose content and in moromi fermentation broth and soy sauce products were analysed. From part 1, the interested soy sauce should be used peagion pea : soy bean as 6:4 and this formula was a control formula in part 2. All moromi fermentation broths in part 2 was also monitored amylase activity, lactic acid and acetic acid in the period time of 0, 10, 20, 30, 45, 60, 75 and 90 days. The results also presented the same trend as the experiment in part 1, this means that the reduced salt in fermentation process did not affect on fermentation process. The quality checked in reduced salt soy sauce still showed the high content of lactic acid and no acetic acid. The f5 which compose of peagion pea : soy bean ratio as 6:4 with used 12% salt in fermentation should be the best to be a model in production healthy soy sauce. The f5 contained the salt about 16.27% content in sauce. The healthy soy sauce should prepared from peagion pea : soy bean as 60:40 and 12% of sodium chloride in moromi fermentation.

Keywords: soy sauce, peagion pea, soy bean

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INTRODUCTION

Soy sauce is one of the fermented products from the Asian countries. It is also one of the world’s oldest condiments which has been used for appetizer in China for over 3000 years (Groff, 1919). From the original formula, this soy sauce used soybean mixed with wheat flour, salt, water and microorganisms like an Aspergillus oryzae or Aspergillus sojae (Impoolsup, et al., 1981). In Thailand, soy sauce is also used as seasoning food for flavoring agent or as component in cooking. However, soybean is an important raw material in production soy sauce and also expensive too but give the authentic soy sauce. There were many reports to use the other plant in production sauce such as corn mixed with soy bean (Impoolsup, et al., 1981), lentil bean (Lebumfacil, 1967) In this work, the peagion pea is the new raw material that used as the one component or whole component in production sauce. Peagion pea has a scientific name as Cajanus canja(L) Mill sp. There were a lot of peagion pea in Nan province in the Northern part of Thailand. An application of this peagion pea still quite low, but this pea contains 61.86 – 65.81 % carbohydrate, 21.44 -23.64 % protein and 1.60 – 2.47 %fat, (Yong. and Wood, 1974a; Raniah, and Satyanaragana, 1938). However, this pea contains approximately 50 % protein lower than soy bean, so the peagion pea should replace some part of soy bean. The formulation of soy sauce from peagion was tried out, by using 20 % of sodium chloride in fermentation process (Groff, 1919). Nowadays, some people cannot eat salty food since they have symptom of adiabetes, hypertension high blood pressure. Thus the aim of this work is to study the new formulation soy sauce from peagion pea which lower salt content. The salt acts as the preservation the mixing component. In Thailand 20-23 % salt (Lebumfacil, 1967) was normally used in production sauce, in Japan normally used 17 -19 % salt (Lockwood, 1947). After the fermentation for 3 months, the evaporation process remove out of some water, the salt brine must be gradually filled to progress the fermentation. From the above reason, when the soy mash was filtered out from the sauce and pasteurized the soy sauce, the salt in soy sauce will concentrate so the flavour of soy sauce are too salty. In the production soy sauce some producer may added some sugar to brake the salty flavour. In this work, the reduction on NaCl salt content was treated in the fermentation process of the system mixed both of soy bean and peagion pea. The moromi of all formulas treatment were sampled between the range of fermentation period to analyse the amylase activity, the occurrence on organic acids. The important organic acid as lactic acid is a good indicator for detection the progression of fermentation process, and also indicate the flavor of soy sauce. This work also analysed the final content of protein, sugar and salt in sauce to indicate the quality of soy sauce.

MATERIALS AND METHODS

Part1 Preparation of soy sauce from peagion pea and soy bean

1.1 Formulation of sauce from peagion pea and soy bean (Groff, 1919)

Soy bean (Kaset brand, purchased from Foodland super market) and peagion pea (from amphur bokhlkur, Nan province, Thailand) were placed into clean water and left for 15 hours at room temperature, then they were streamed and mixed together as the following ratio:
All those component in each formula was mixed with wheat flour (Bua deang brand, purchased from Foodland super market) with ratio soy bean with peagion pea : wheat flour as 1 : 1. The pure Aspergillus oryzae (from Thailand Institute of Scientific and Technology Research, TISTR) were spreaded on the mixed component. The mixed component 1 kilogram used 3 g of Aspergillus oryzae. Those mixed component systems were sprayed with clean water and covered with gross clothes. The koji of each formula was incubated at room temperature for 5 days or observed from the growth of Aspergillus oryzae. Kojis were tranfered into jars and mixed with 20 % w/v of NaCl (prungthip brand) for 3 months. In fermentation process, the moromis were sampled at 0, 10, 20, 30, 45, 60, 75 and 90 days between the fermentation to monitor the amylase enzyme activity, the organic acids, the microorganism. The soy mash were filtered out of the raw sauce. The raw soy sauces were pasteurized at 80-85 °C for 15 minutes, and stored to study in the second step.

2.1 Study the properties of moromi and soy sauce

2.1.1 Analysis of amylase enzyme activity Udo, 1932

The moromi sample 2.00 g. was diluted with a 9.0 ml of distilled water. Then, the solution was prepared for 1 : 200 dilution. The starch solution 2 ml (1 % w/v) was added to sample solution. The mixed solution was left at room temperature for 10 min. and 0.5 ml of 3,5-dinitrosalicylic acid (Analytical grade, purchased from fluka) was added to the solution. The mixed solution was heated on waterbath for 10 min. and diluted again with water to 4 ml. The color solution was measured an absorbance at 540 nm. and compared an absorbance with the absorbance of standard glucose solution to calculate the amylase activity unit.

2.1.2 Analysis of organic acid content

The moromi was blended to finely particles. The 1 ml of liquid moromi samples were transferred to microcentrifuge, diluted with 1 ml of water and centrifuged 5 min. The filtrate was filtered again through 0.45 μm cellulose membrane filter and injected to aminox HPX-87H (BioRad, Hercules, Calif., USA) column which attached to HPLC (water 410) and eluted with 5 mM H₂SO₄ as mobile system. The chromatogram of samples were recorded and compared with calibration graph of standard lactic acid and acetic acid.

2.1.3 Analysis of microorganism

The microorganism as bacteria, yeast and fungi was analysed by pour plate technique[8]. The moromi sample were sampled in the period and ten fold dilution and sprade on the agar for bacteria test and potato...
dextrose agar for yeast and fungi test. Those plate were incubated at 30 °C for 48 hours. The colony of total microorganism were count and reported as CFU/g.

2.1.4 Analysis of protein content

The protein in soy sauce sample was analysed as AOAC method by acid base titration. The soy sauce sample 1.40 ml was diluted with distilled water to 50 ml. Then the soy sauce solution was pipetted 12.5 ml and transferred to conical flask. The 14.00 ml of 37% v/v of formaldehyde solution was added and left 5 min before titrated with 0.5N sodium hydroxide solution using phenolphthalein as an indicator for end point detection. The volume of sodium hydroxide solution was recorded and calculated the amount of protein content in soy sauce.

2.1.5 Analysis of sugar content

The sugar in soy sauce sample was analysed by HPLC technique. The soy sauce 1 ml was diluted with 1 ml of distilled water. The solution sample was filtered through 0.45 μm membrane filter and injected to aminex HPX-87H column which attached to HPLC (water 410) and eluted with 5 mM H₂SO₄ as mobile system. The chromatogram of samples were recorded by refractive index detector and compared with calibration graph of standard glucose to calculated the sugar concentration.

2.1.6 Analysis of salt content

The salt in soy sauce sample was analysed as AOAC method by Volhard titration method. The soy sauce 3 ml. was diluted with 50 ml of distilled water. The soy sauce 10 ml was transferred to conical flask. The 20 ml of 0.1 N of silver nitrate solution was added to the flask. Then, 5 ml of 3.0 N of nitric acid solution, 0.5 ml of ferrous alum solution were added to the sample solution. The total solution was titrated with 0.1 N of potassium thiocyanate solution till end point. The volume of potassium thiocyanate solution was recorded and calculated to concentration of sodium chloride content in soy sauce.

Part 2 Study the new formulation of reduce salt soy sauce

2.1 Formulation of low salt sauce from peagon pea and soy bean

The result from part 1, the best formula was chosen to prepare low salt soy sauce. The soy bean and peagon pea were treated as part 1 but the salt content was varied in fermentation of moromi as following: f1 used 20% (controlled formula), f2 used 18%, f3 used 16%, f4 used 14%, f5 used 12% and f6 used 10%. In the moromi fermentation, the amylase activities, organic acid content were monitored between 90 days as the method in part 2.1.1 – 2.1.2. After pasteurization, the soy sauces were stored to analyse in 2.2.

2.2 Study the chemical properties of low salt moromi and low salt soy sauce

All formula f1 – f6 were analysed as the method in 2.1.1 – 2.1.6 of part 1.

RESULTS AND DISCUSSION

From part 1, the original soy sauce were prepared 6 formulas by F6 was the controlled. The amylase activity in all moromi were monitored as showed in figure 1.
In each set of moromi, enzyme amylase showed the good activity for the range of fermentation time, however amylase enzyme showed the highest activity in F6 at the first day of fermentation. F6 was the controlled formula which made of 100 % soy bean as a raw material which was the normally formula that used in production soy sauce. The amylase activity in each formula changed in the same as F6 from 10 – 90 days of fermentation. This mean that the replacement of peagion pea as some part of raw material not effect on the fermentation process.

The analysis result of organic acids such as lactic acid showed in figure 2

From figure 2, in moromi fermentation period showed the occurrence of lactic acid fermentation which gave the important product as lactic acid. This acid made the good flavor of soy sauce Groff, 1919, Sakaguchi, 1958). So that in this set of fermentation, the lactic acid trend to increase as all time of fermentation.
The analysis of acetic acid as showed the result in figure 3.

**Figure 3** Acetic acid content in moromi of each formula

In figure 3, the acetic acid product from fermentation in moromi showed the highest content between 20 - 60 days of fermentation, then at 90 days of fermentation its decrease to zero. This presented that the acetic acid should not be found in pasteurized soy sauce.

The analysis of bacteria, yeast and fungi showed in figure 4 and 5.

**Figure 4** Bacteria content in moromi fermentation
The bacteria, yeast, and fungi trended to decrease after fermentation for 90 days. Those microorganism grew fast at the initial of fermentation, this means that all microorganism can survive in the moromi fermentation broth. This result also corresponding with the amylase enzyme activity.

After those 6 moromis were prepared to soy sauce in 6 formulas and presented the physical characteristics as in Table 1.

**Table 1 Physical characteristics of soy sauces**

<table>
<thead>
<tr>
<th>Formula no</th>
<th>color</th>
<th>odor</th>
<th>taste</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>pale brown</td>
<td>Pale</td>
<td>salty</td>
</tr>
<tr>
<td>2</td>
<td>medium brown</td>
<td>Pale</td>
<td>salty</td>
</tr>
<tr>
<td>3</td>
<td>medium brown</td>
<td>Strong</td>
<td>Salty with sweet</td>
</tr>
<tr>
<td>4</td>
<td>medium brown</td>
<td>medium</td>
<td>Salty with sweet</td>
</tr>
<tr>
<td>5</td>
<td>Medium dark brown</td>
<td>pale</td>
<td>salty</td>
</tr>
<tr>
<td>6</td>
<td>Dark brown</td>
<td>normally</td>
<td>salty</td>
</tr>
</tbody>
</table>

The soy sauces had pale brown to dark brown color, especially in control formula F6 showed the dark brown. The sauce that made of 100% pure peagion pea as F1 showed pale brown color. The sauce that made of peagion pea and soy bean showed the medium brown color. Thus, the color of sauce depend on the content of each pea in the formula. The result in analysis of protein, glucose and salt showed in Table 2.
Table 2  Protein , sugar and salt content in soy sauces

<table>
<thead>
<tr>
<th>formula</th>
<th>% protein</th>
<th>% sugar</th>
<th>% salt as NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.98</td>
<td>0.12</td>
<td>23.74</td>
</tr>
<tr>
<td>2</td>
<td>6.00</td>
<td>0.09</td>
<td>23.54</td>
</tr>
<tr>
<td>3</td>
<td>6.50</td>
<td>0.19</td>
<td>21.90</td>
</tr>
<tr>
<td>4</td>
<td>5.80</td>
<td>0.18</td>
<td>23.10</td>
</tr>
<tr>
<td>5</td>
<td>5.45</td>
<td>0.13</td>
<td>22.05</td>
</tr>
<tr>
<td>6</td>
<td>6.10</td>
<td>0.13</td>
<td>22.05</td>
</tr>
</tbody>
</table>

From the results in table 2, showed F3 contained the lowest salt content and showed the highest sugar content had same trend of table 1. Thus, the F3 was selected to be a new model in reduce salt soy sauce to study in part 2.

In part 2, the moromi of those f1-f6 contained the amylase activities as in figure 6.

![Figure 6 amylose activity in moromi on fermentation periods](image)

The moromi in each formula showed the highest activity of amylase enzyme at the initial of fermentation and decrease till the 90 days of fermentation. This similarity with the result from part 1. This work presented that the reduction in salt concentration in the moromi fermentation broth, did not effect on the activities of amylase enzyme.

However, after the determination of the organic acid as lactic acid and acetic acid as in figure 7 and 8.
From the above result in figure 7 and 8, showed the same result as the moromi fermentation set in experimental part 1, so this part also proved that the salt reduction process gave the good flavor from lactic acid but may contained a few acetic acid in some moromi formula. However, after the preparation of the new reduced salt soy sauce sets, those sauce were analysed lactic acid and acetic acid as presented the result in figure 9.
The lactic acid content in each soy sauce predominant, more content than acetic acid. The f5 contained 15 g/L of lactic acid and no acetic acid, thus formula gave the best flavor of soy sauce and better than f1 which was the controlled formula.

The protein, sugar and salt were analysed in those soy sauce as present in table 3.

### Table 3 Protein, sugar and salt content in reduced salt soy sauces

<table>
<thead>
<tr>
<th>formula</th>
<th>% protein</th>
<th>% sugar</th>
<th>% salt as NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.50</td>
<td>0.19</td>
<td>22.00</td>
</tr>
<tr>
<td>2</td>
<td>6.45</td>
<td>1.19</td>
<td>21.54</td>
</tr>
<tr>
<td>3</td>
<td>6.50</td>
<td>1.11</td>
<td>20.48</td>
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<tr>
<td>4</td>
<td>6.42</td>
<td>1.40</td>
<td>20.80</td>
</tr>
<tr>
<td>5</td>
<td>6.50</td>
<td>1.52</td>
<td>16.27</td>
</tr>
<tr>
<td>6</td>
<td>6.30</td>
<td>1.55</td>
<td>11.45</td>
</tr>
</tbody>
</table>

The result from table 3, showed that the salt content in f5 and f6 contained NaCl about 11.45 -16.27 %, and from figure 7 the f5 contained the highest lactic acid content and no acetic acid. So this presents that, the healthy soy sauce can be produced from the reduce NaCl salt content in the fermentation process by apply about 12 % salt. However, the reduced salt soy sauce f5 gave the more sweet taste than controlled formula.

**CONCLUSION**

From part 1, the interested soy sauce should used peagon pea : soy bean as 6 :4 and this formula was used as a control formula in part 2. All moromi fermentation in part 2 was monitored amylase activity,
lactic acid and acetic acid in the period time of 0, 10, 20, 30, 45, 60, 75 and 90 days. The monitored results also had same trend as the experiment in part 1, this means that the reduced salt in fermentation did not affect the fermentation process. The quality checked in reduced salt soy sauce still showed the high content of lactic acid and no acetic acid. The last checked in salt content and sugar content showed that f5 which compose of pigeon pea: soy bean ratio as 6:4 and used 12% salt in fermentation should be the best to be a model in production healthy soy sauce. The f5 showed the salt content in sauce about 16.27%. However, the standard specification of soy sauce should contain salt about 20%[7], thus the f3 and 4 may passed the standard gain but both formula use the higher salt content than f5 and 6.

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