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Research Article

Development of healthy soy sauce from pigeon pea and soybean

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Abstract

Soy sauce is a popular flavouring agent which is produced by the fermentation of *Aspergillus oryzae* with a combination of soybean, wheat flour and salt. In general, the salt that is used in the fermentation process acts as a preservative for the mixing component. The objective of this research was to develop low salt soy sauce from a combination of pigeon pea and soybean. The work was divided into two parts. In the initial part, soy sauce was prepared from 6 different formulas by varying the ratios of pigeon pea and soybean as; 100:0 (F1), 80:20 (F2), 60:40 (F3), 40:60 (F4), 20:80 (F5) and 0:100 (F6 - control). These koji moulds were fermented with 20% (w/v) sodium chloride for 90 days. The best formula was selected for further study in the second part. In 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 the moromi fermentation broth and soy sauce products were analysed. From the first part, the formula having the highest potential was selected as being pigeon pea : soybean at 6:4 (F3), and this formula was used as the control formula for the second part of the research. All moromi fermentation broths in the second part were also monitored for amylase activity, lactic acid and acetic acid in the time periods of 0, 10, 20, 30, 45, 60, 75and 90 days. The results from this monitoring presented the same trends as found in the initial part of the experiment, i.e., that the reduced salt in the fermentation process did not affect

the process itself. The quality checks conducted on the reduced salt soy sauce still showed a high content of lactic acid and no acetic acid. The F3 formula comprising pigeon pea and soybean at a ratio of 6:4 and using 12% sodium chloride in the moromi fermentation proved to be the best model for production of healthier soy sauce.

Keywords: salt content, moromi fermentation, Thailand

Introduction

Soy sauce is one of the popular fermented flavouring products, especially in Asia. It is also one of the world's oldest condiments and has been used in China for over 3,000 years [1]. From the original formula, this soy sauce used soybean mixed with wheat flour, salt, water and microorganisms such as *Aspergillus oryzae* or *Aspergillus zozae* [2]. In Thailand, soy sauce is also used as a seasoning for food, as a flavouring agent or marinade, or as a component in cooking. Soybean is an important raw material in production of soy sauce and it is also expensive, but necessary for authentic soy sauce. There have been a number of reports on the use of other legumes in the production of sauce, such as corn mixed with soy bean [2] and with lentil bean [3]. In this work, the pigeon pea (*Cajanus canja* [L] Mill *sp*.) has been selected as a potential new raw material to be used as one component or whole component in the production of sauce. While pigeon pea is commonly grown in Thailand, particularly in Nan province, application for value added products remains quite low. Pigeon pea typically contains 61.86 – 65.81% carbohydrate, 21.44 -23.64% protein and 1.60 – 2.47% fat [4, 5]. However, protein content remains approximately 50% lower than soy bean, so pigeon pea is only suitable to supplement or partially replace the more expensive soy bean. A formulation of soy sauce from pigeon pea has already been tested using 20% sodium chloride in the fermentation process [1]. However in these more health conscious days, some people avoid salty food since they develop symptoms such as diabetes, hypertension and high blood pressure. Thus the aim of this work is to study a new formulation for soy sauce from pigeon pea with lower salt content. The salt generally acts as a preservative for the other components in the fermentation mix. In Thailand, 20-23% salt is normally used in the production of soy sauce [3], while in Japan closer to 17-19% is used [6]. After the initial fermentation for 3 months, natural evaporation removes some of the water and the salt brine must be gradually topped up to progress the fermentation. For this reason, when the soy mash is filtered out and the sauce pasteurized, the salt will concentrate so the flavour of the soy sauce becomes too salty. During production some manufacturers may add some sugar to dilute the salty flavour. In this work, the reduction of NaCl salt content was undertaken in the fermentation process of the system using a mixture of both of soybean and pigeon pea. The moromi of all formulas were sampled between a range of fermentation periods to analyse the amylase activity and the occurrence of organic acids. The important organic acid is lactic acid and it is a good indicator for detection of the progression of the fermentation process, as well as being an indicator of the flavor of soy sauce. This work also analysed the final content of protein, sugar and salt to indicate the quality of soy sauce.

Methodology Part 1

Preparation of soy sauce from pigeon pea and soy bean

Formulation of sauce from pigeon pea and soybean [1]

Soybean (Kaset brand, purchased from Foodland Supermarket) and pigeon pea (from Borklua, Nan, Thailand) were soaked in clean water and left for 15 hours at room temperature, then they were streamed and mixed together as per the following ratios:

	Pigeon pea	:	Soy bean	
F1	1.0	:	0	
F2	0.8	:	0.2	
F3	0.6	:	0.4	
F4	0.4	:	0.6	
F5	0.2	:	0.8	
F6	0.0	:	1.0	(control formula)

All of the components in each formula were mixed with wheat flour (Bua Daeng brand, purchased from Foodland Supermarket) with the ratio of soybean/pigeon pea : wheat flour as 1 : 1, or equal parts. Pure *Aspergillus oryzae* (from Thailand Institute of Scientific and Technological Research, TISTR) was spread on the mixed components. One kg of the mixed component used 3 g of *Aspergillus oryzae.* The mixed component systems were then sprayed with clean water and covered with gauze cloth. The koji of each formula was incubated at room temperature for 5 days or as observed from the growth of *Aspergillus oryzae*. Kojis were transferred into jars and mixed with 20% w/v of NaCl (Prungthip brand) to ferment for 3 months. During the fermentation process, the moromis were sampled at 0, 10, 20, 30, 45, 60, 75 and 90 days between the fermentations to monitor the amylase enzyme activity, the organic acids and the microorganism. The soy mash was filtered out of the raw sauce and the sauces pasteurized at 80-85^{o}C for 15 minutes, and then stored for study in the second stage.

Properties of moromi and soy sauce

Analysis of amylase enzyme activity [7]

The moromi sample 2.00 g was diluted with 9.0 ml of distilled water and the solution prepared for 1 : 200 dilution. The starch solution of 2 ml (1 % w/v) was added to the 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 then heated in a water bath for 10 min. and diluted again with water to 4 ml. The coloured solution was measured with an absorbance at 540 nm and compared with the absorbance of standard glucose solution to calculate the amylase activity unit.

Analysis of organic acid content

The moromi was blended to fine particles and 1 ml of liquid moromi sample was transferred to a microcentrifuge, diluted with 1 ml of water and centrifuged for 5 min. The filtrate was filtered again through 0.45 μm cellulose membrane filter and injected into aminex HPX-87H (BioRad, Hercules, Calif., USA) column which was attached to HPLC (water 410) and eluted with 5 mM H_2SO_4 as mobile system. The chromatogram of samples were recorded and compared with calibration graphs of standard lactic acid and acetic acid.

Analysis of microorganisms

The microorganisms as bacteria, yeast and fungi were analysed by pour plate technique [8]. Moromi was sampled during this period in ten-fold dilution and spread on the agar for bacteria test and potato dextrose agar for yeast and fungi test. These plates were incubated at 30^{o}C for 48 hours. The colony of total microorganisms were counted and reported as CFU/g.

Analysis of protein content

The protein in the soy sauce samples was analysed as per AOAC methods by acid base titration. The soy sauce sample 1.40 ml was diluted with distilled water to 50 ml, then the 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 for 5 min before being 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 the amount of protein content in soy sauce calculated.

Analysis of sugar content

The sugar in soy sauce samples 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 was attached to HPLC (water 410) and eluted with 5 mM H_2SO_4 as mobile system. The chromatogram of samples was recorded by refractive index detector and compared with calibration graphs of standard glucose to calculate the sugar concentration.

Analysis of salt content

The salt content in soy sauce samples was analysed according to AOAC methods by using the Volhard titration method. Soy sauce 3 ml. was diluted with 50 ml. of distilled water, then transferred to a conical flask. 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 and 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 the concentration of sodium chloride content in soy sauce calculated.

Methodology Part 2

Analysis of new formulation of reduced salt soy sauce

Formulation of low salt sauce from pigeon pea and soybean

Using the results from the first stage, the best formula was chosen to prepare low salt soy sauce. The soybean and pigeon pea were treated as described in Part 1, however the salt content was varied in the moromi fermentation as follows: F1 used 20% (control formula), F2 used 18%, F3 16%, F4 14%, F5 12% and F6 used 10%. During the moromi fermentation, the amylase activities and organic acid content were monitored between 90 days as per the method described above. After pasteurization, the soy sauces were stored for further analysis

Chemical properties of low salt moromi and low salt soy sauce

All formulas, F1 – F6 were analysed using the methods described in Part 1.

Results and Discussion

The original soy sauce selected from Part 1 was prepared in 6 formulas using F6 as the control. The amylase activity in all moromi fermentations was monitored and is shown in Figure 1.

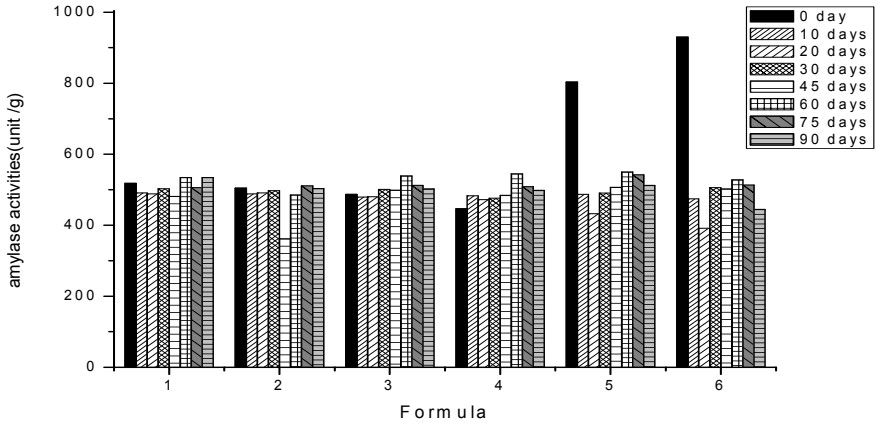

Figure 1. Amylase activity in moromi of each formula.

In each set of moromi, enzyme amylase showed 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 control formula which was made from 100% soybean as the raw material which is the normal formula used in the production soy sauce. The amylase activity in each formula underwent the same changes as the F6 control formula from 10 – 90 days of fermentation. This indicates that the replacement of pigeon pea as some part of the raw material does not adversely affect the fermentation process.

Results of the analysis of organic acids such as lactic acid are shown in Figure 2.

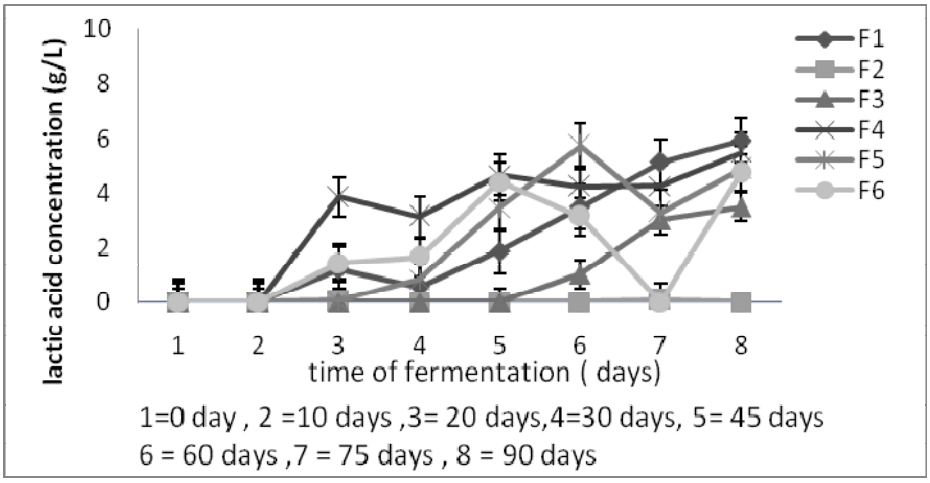

Figure 2. Lactic acid content in moromi of each formula.

From Figure 2, the moromi fermentation period shows the occurrence of lactic acid fermentation which in turn gave the important product of lactic acid. This acid contributes to the good flavour of soy sauce [1, 9]. In this particular set of fermentation, the lactic acid tended to increase during the course of fermentation.

The results of analysis of acetic acid are shown in Figure 3.

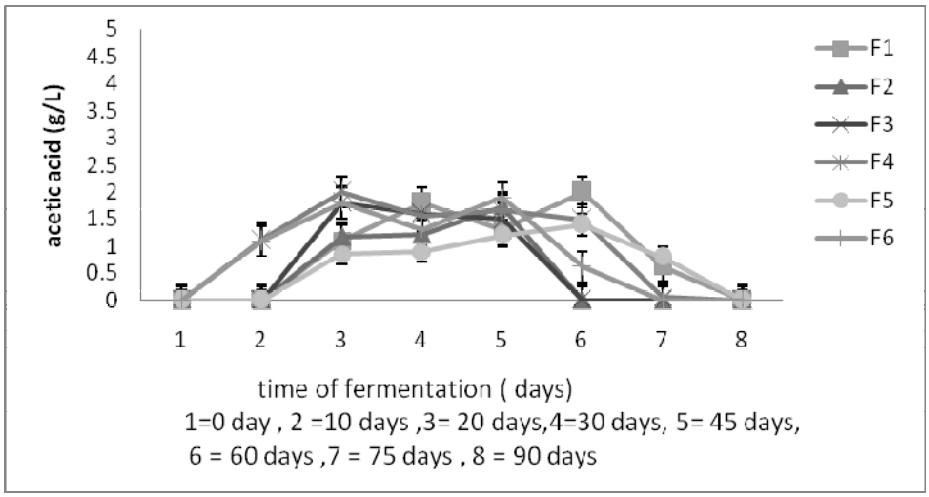

Figure 3. Acetic acid content in moromi of each formula.

In Figure3, the acetic acid product from fermentation in moromi showed the highest content between 20 -60 days of fermentation, then at 90 days of fermentation it decreases to zero. This demonstrated that acetic acid should not be found in pasteurized soy sauce.

The analysis of bacteria, yeast and fungi is shown in Figures 4 and 5.

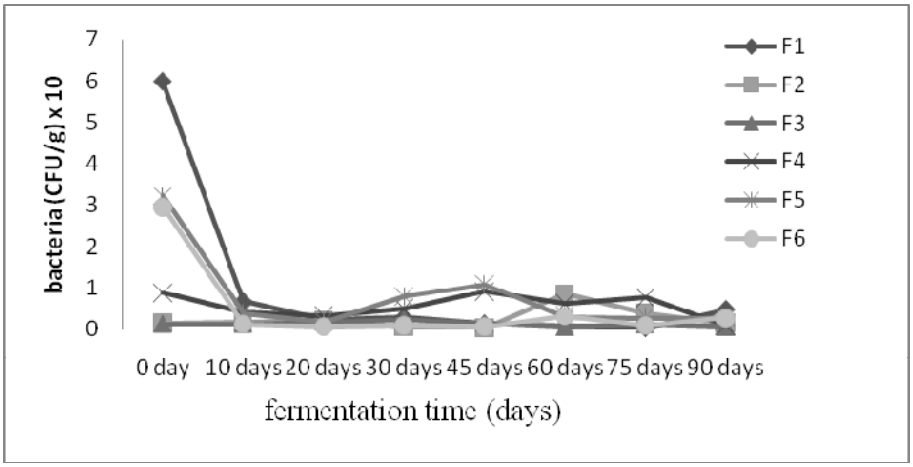

Figure 4. Bacteria content in moromi fermentation.

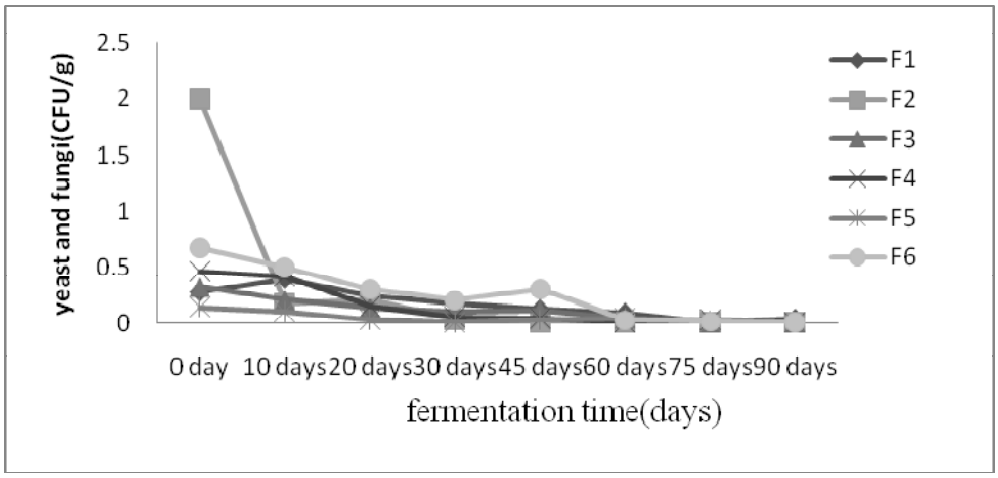

Figure 5. Yeast and fungi content in moromi fermentation.

The bacteria, yeast and fungi tended to decrease after fermentation for 90 days. These microorganisms grew fast at the initial stage of fermentation and this suggests that all microorganisms can survive in the moromi fermentation broth. This result also corresponded with the amylase enzyme activity.

The 6 moromi formulas which were fermented to produce soy sauce showed the following characteristics presented in Table 1.

Table 1. Physical characteristics of soy sauces.

Formula no.	Colour	Odor	Taste
1	pale brown	pale	salty
2	medium brown	pale	salty
3	medium brown	strong	salty with sweet
4	medium brown	medium	salty with sweet
5	medium dark brown	pale	salty
6	dark brown	normal	salty

The soy sauces exhibited pale brown to dark brown colour, the control formula F6 especially showing the darkest brown. The sauce made from 100% pure pigeon pea (F1) showed the palest brown colour. Sauces made from pigeon pea and soybean showed the medium brown colour. Thus, the colour of sauce depends on the content of each pea in the formula.

The results from the analysis of protein, glucose and salt are shown in Table 2.

Table 2. Protein, sugar and salt content in soy sauces.

Formula no.	Contents		
	% protein	% sugar	% salt as NaCl
1	6.98	0.12	23.74
2	6.00	0.09	23.54
3	6.50	0.19	21.90
4	5.80	0.18	23.10
5	5.45	0.13	22.05
6	6.10	0.13	22.05

From the results in Table 2, it can be seen that F3 contained the lowest salt content and showed the highest sugar content, exhibiting the same trend as in Table 1. Thus, the F3 formula was selected to be a new model in reduced salt soy sauce for further study in Part 2.

From Part 2 of the study, the moromi of formulas F1-F6 contained the amylase activities as shown in Figure 6.

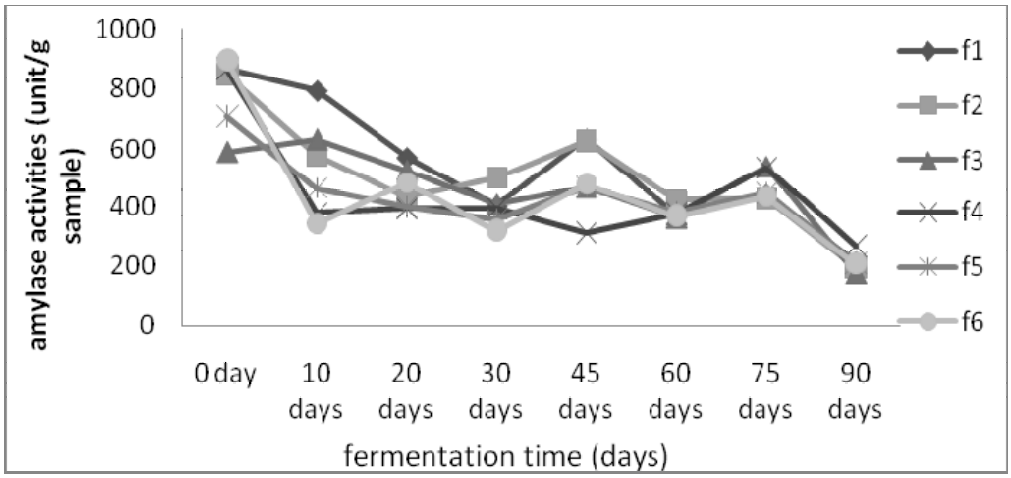

Figure 6. Amylase activity in moromi on fermentation periods.

The moromi in each formula showed the highest activity of amylase enzyme at the initial stage of fermentation and decreased until 90 days. This shows similarity with the results from Part 1 and demonstrates that the reduction in salt concentration in the moromi fermentation broth did not affect the activities of the amylase enzymes.

Following examination of amylase activity, the lactic acid and ascetic acid contents were determined, as shown in Figures 7 and 8.

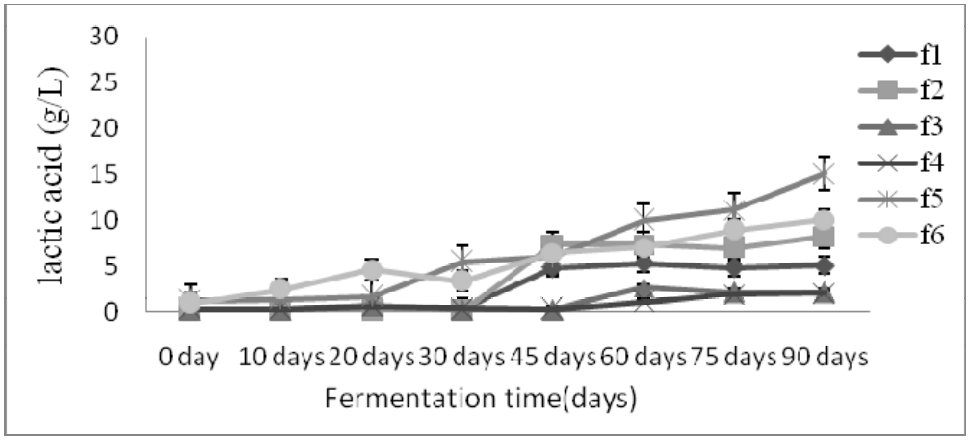

Figure 7. Lactic acid contents in moromi during fermentation periods.

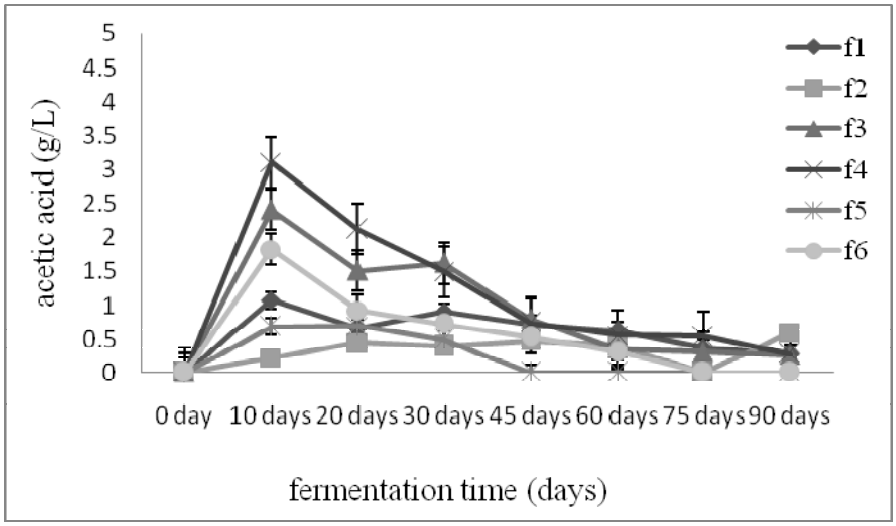

Figure 8. Acetic acid contents in moromi during fermentation periods.

From the results displayed in Figures 7 and 8, it can be seen that ascetic and lactic acid contents were very similar to the results obtained with the set of moromi fermentations in Part 1, providing further evidence that the salt reduction process gave good flavour from lactic acid but may contain a small amount of acetic acid in some moromi formulas.

Following preparation of the new reduced salt soy sauce sets, these sauces were further analysed for lactic acid and acetic acid content, as presented in Figure 9.

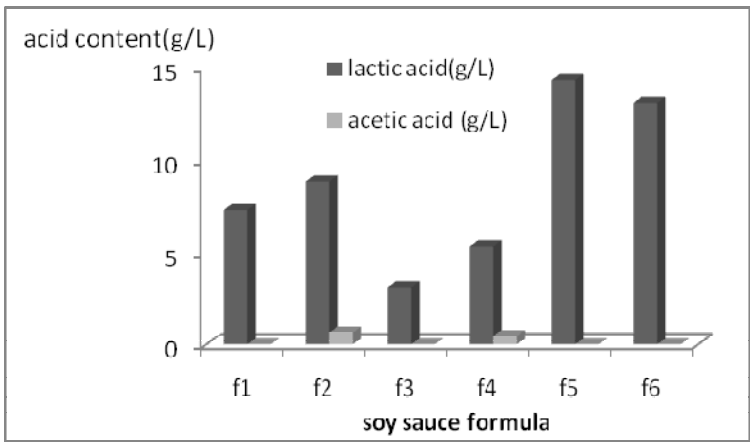

Figure 9. Acid contents in soy sauces.

Lactic acid content in each soy sauce was predominant, being consistently higher than acetic acid. The F5 formula contained 15 g/L of lactic acid and no acetic acid, thus giving the best flavour of soy sauce, superior to F1 which was the control formula.

The protein, sugar and salt contents of each formula were analysed and these are presented in Table 3.

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

Formula	Contents		
	% protein	% sugar	% salt as NaCl
1	6.50	0.19	22.00
2	6.45	1.19	21.54
3	6.50	1.11	20.48
4	6.42	1.40	20.80
5	6.50	1.52	16.27
6	6.30	1.55	11.45

From the results shown in Table 3, it can be seen that F5 and F6 had the lowest salt (as NaCl) content, while from Figure 7 it can be seen that F5 contained the highest lactic acid content and no acetic acid, providing the preferred flavour. This demonstrates that healthier soy sauce can be produced with reduced NaCl salt content in the fermentation process by using about 12% salt. The formula F5 also exhibited a sweeter taste than the control formula.

Conclusion

From Part 1, the ratio of pigeon pea to soybean with the best potential was selected. This 60:40 soy sauce was then used as the control formula in Part 2 of the research. All moromi fermentations in Part 2 were monitored for amylase activity, lactic acid and acetic acid in the time periods of 0, 10, 20, 30, 45, 60, 75and 90 days. The monitored results exhibited a similar trend as the experiment in Part 1, demonstrating that the reduced salt in the fermentation did not adversely affect the fermentation process. The

checks for quality of the reduced salt soy sauce still showed a high content of lactic acid and no acetic acid. The final tests for salt and sugar contents showed that F5, which was made up of 60% pigeon pea and 40% soybean, using 12% salt in the fermentation, should be the best to be a model for the production of healthier soy sauce. F5 showed the salt content in the sauce to be about 16.27%. According to the standard specifications for soy sauce, salt content should be about 20% [7]. Thus the formulas F3 and F4 would also pass the standard requirements, even though both formulas exhibited a higher salt content than F5 and F6.

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