KMITL Sci. Tech. J. Vol. 7 No. S2 Nov. 2007

STUDY OF PROTEASE ENZYME AND AMINO ACID CONTENTS IN SOY SAUCE PRODUCTION 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. The aim of this work is to analyse the amino acid contents in soy sauce produced from the mixture of peagion pea and soy bean compared with the original production process. In this study the protease enzyme was also monitored to evaluate the fermentation process. The soy sauces were prepared from 6 formulas by varying the ratios between peagion pea and soy bean : 100 : 0 (F1) , 80 : 20 (F2), 60 : 40 (F3), 40 : 60 (F4) , 20 : 80 (F5) and 0 : 100 (F6) (control). After the koji process, those koji molds were fermented with saline 20 % (w/v) for 90 days. The protease enzyme was analysed between the fermentation process at 0, 10, 30, 60 and 90 days by spectrometry. The amino acid contents were analysed by high performance liquid chromatography. The result showed that F5 moromi gave the highest content of glutamic acid and the content decreased as the ratio of soy bean decreased. The protease content in all formulas also increased as fermentation time increased. The peagion pea which was cultivated for animal food could be used as the replacement of soy bean to produce the value added product like soy sauce.

KEYWORDS: soy sauce, soy bean, peagion pea, koji, *Aspergillus oryzae***,** protease, amino acid

1. INTRODUCTION

Soy sauce is used as flavoring agent. It is probably the most well known soy food. Soy sauce is one of the world's oldest condiments and has been used in China for over 2,500 years. It is made from the fermenting mixture of mashed soybeans, wheat flour, salts and microorganisms like *Aspergillus oryzae* or *Aspergillus sozae* [1]. The authentic soy sauces are made from whole soy bean, but cheaper soy sauces are made from hydrolysed soy protein instead [2]. In China, other grain

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was also mixed with soy bean in soy sauce production. The raw material such as peagion pea could be used for the production of soy sauce. Peagion pea (*Cajanus cajan mill* sp.) is also known as Anglola pea or Congo pea [3]. In Thailand, peagion pea was used only as an animal food but it was used as a human food in other countries [4]. Peagion pea contains higher carbohydrate content than soy bean. Soy sauces produced from soy bean were studied and reported by many investigators [5-7]. However, there was no report on the soy sauce production from peagion pea [8]. Thus, in this study soy sauces were prepared from 6 formulas by varying the ratios between peagion pea and soy bean: 100 : 0 (F1) , 80 : 20 (F2), 60 : 40 (F3) , 40 : 60 (F4) , 20 : 80 (F5) and 0 : 100 (F6) (control). Then amino acid contents in each soy sauce was analysed by high performance liquid chromatography and protease enzyme content was also monitored during moromi fermentation to evaluate the production of soy sauce.

2. MATERIALS AND METHODS

2.1 Preparation of soy sauce [1]

The peagion pea (from Nan Province in the northern part of Thailand) and soy bean (Kaset Brand) were placed into water for 10 hours, then cooked and weighed with the following ratios.

	Peagion pea	:	Soy bean
Formula 1	1.00	:	0.00
Formula 2	0.80	:	0.20
Formula 3	0.60	:	0.40
Formula 4	0.40	:	0.60
Formula 5	0.20	:	0.80
Formula 6 (control)	0.00	:	1.00

All formulations were mixed with wheat flour (Bua Deang Brand) by 1: 1 ratio. *Aspergillus oryzae* (from Thailand Institute of Scientific and Technological Research, TISTR) was inoculated and spreaded through the mixing component. The inoculated materials called koji were then covered with wet cheesecloth and incubated at room temperature for 5 days, then transferred to the jar and fermented with 20% (w/v) NaCl solution for 3 months (moromi process).

2.2 Analysis of protease content [9]

The fermented formulas were sampled at 0, 10, 30, 60 and 90 days of fermentation for analysis of protease enzyme contents. Those fermentated formulas were mixed with 352 µl of phosphate buffer (pH 6.8) and 625 µl of deionised water and then incubated at 40 ^{o}C for 30 mins. 50% w/v of trichloroacetic acid (Aldrich Chemical, AR grade) were added to each solution and centrifuged at 10,000 rpm for 5 mins. The clear filtrate (1ml) was mixed with 0.5 mL of diluted Folin-Ciocalteu reagent and incubated at room temperature for 30 mins. The absorbance of the samples were measured at 660 nm. The protease activity was calculated by comparison with standard soy protein.

2.3 Analysis of amino acid content [10]

Standard solutions of amino acids (Sigma Chemicals, chromatographic grade): Alanine (Ala), Arginine (Arg), Asparagine (Asp), Cystine (Cys), Glutamine (Glu), Glycine (Gly), Histidine (His), Isoleucine (Lue), Leucine (Ile), Lysine (Lys), Methionine (Met), Phenylalanine (Phe), Proline (Pro), Serine (Ser), Theonine (Thr), Tyrosine (Tyr), Valine (Val) were prepared. Each amino acid was prepared from 20 mM in milli Q deionised water. Then 10 µl of each amino acid was mixed with 20 µl of AccQ Fluor reagent. Mixed components were incubated at 55 ^{o}C in heating block for 10 mins. The mixing solution (1 ml) was added into test tube with distilled water (9 ml) and mixed by vortex mixer. The solution (0.2 ml) was transferred to the centrifugation tube, mixed

with 0.5 ml of 5% trichloroacetic acid. and centrifuged at 10,000 x g at 4°C for 10 mins. The clear filtrates were collected and filtered through millipore membrane filter prior to injection to C_{18} column of HPLC. The system of mobile phase was controlled by gradient elution mode with mixing AccQ Taq (A), acetonitrile (B) and nanopure distilled water (C). The conditions for separation are shown in Table 1. The signal of the chromatographic system was detected by fluorescence detector at 250 nm for the excitation wavelength and 395 nm for the emission wavelength.

Table 1 The condition for HPLC experiment

Time, min	A, %	B, %	C, %
0	100	0	0
0.5	99	1	0
21.0	95	5	0
22.0	91	9	0
32.5	83	17	0
38.0	0	60	40
46.0	100	0	0
55.0	100	0	0

3. RESULTS AND DISCUSSIONS

The contents of protease enzyme in each moromi of all formulas are shown in Figure 1. The protease enzyme contents of F3 showed the highest content at the initial time of fermentation. However, the protease enzymes in all formulas trend to increase as fermentation time increases. In control (F6), an acitivity of fermentation decreased after 60 days of fermentation. This showed that after 90 days of fermentation the activity of protease declined for constant rate. In F1-F5, the activity of protease enzyme still increased with little change in F3-F5 at 60-90 days. Thus the mixing of peagion peas for soy sauce production has shown a good potential since their protease enzyme contents were higher than the control. The amino acid contents in each formula were analysed by HPLC technique and the standard amino acid chromatogram was presented in Figure 2. The amino acid contents of each moromi were compared with the standard calibration curve presented in Figures 3-5.

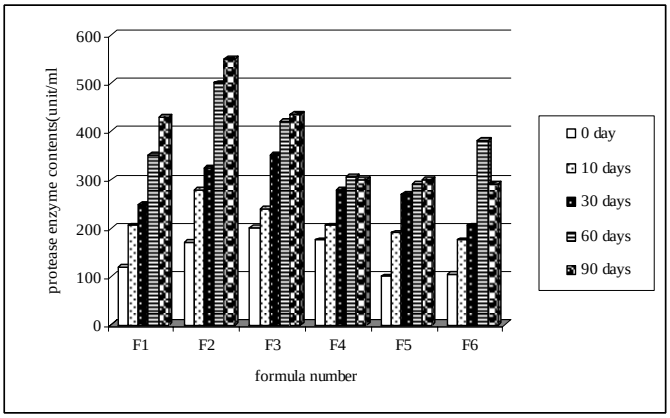

Figure 1 Contents of protease enzyme in each moromi at each interral fermentation

Note: F1 = moromi contains peagion pea: soy bean = 1.00:0.00
F2 = moromi contains peagion pea: soy bean = 0.80:0.20
F3 = moromi contains peagion pea: soy bean = 0.60:0.40
F4 = moromi contains peagion pea: soy bean = 0.40:0.60
F5 = moromi contains peagion pea: soy bean = 0.20:0.80
F6 = moromi contains peagion pea: soy bean = 0.00:1.00 (control)

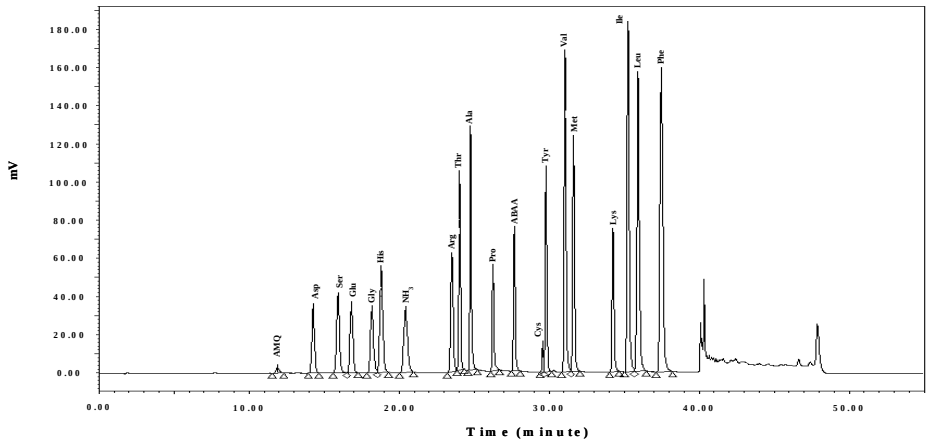

Figure 2 Chromatogram of standard amino acids

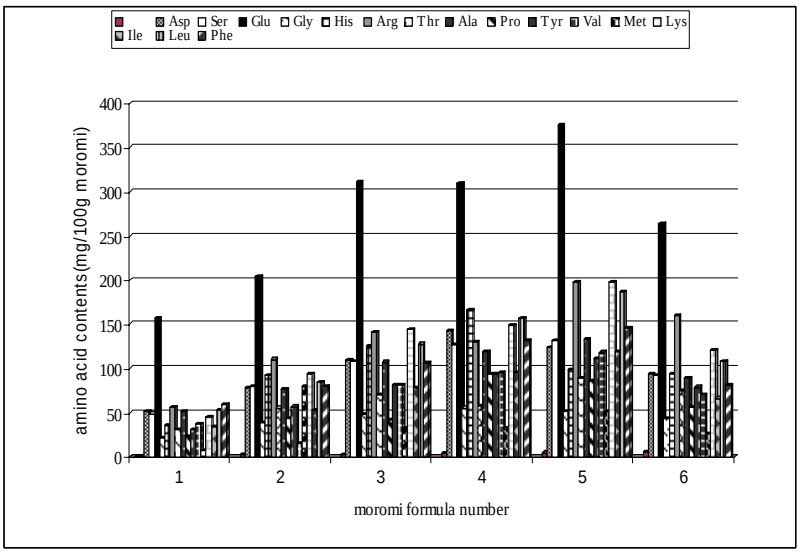

Figure 3 Amino acid contents of each moromi at initial day of fermentation

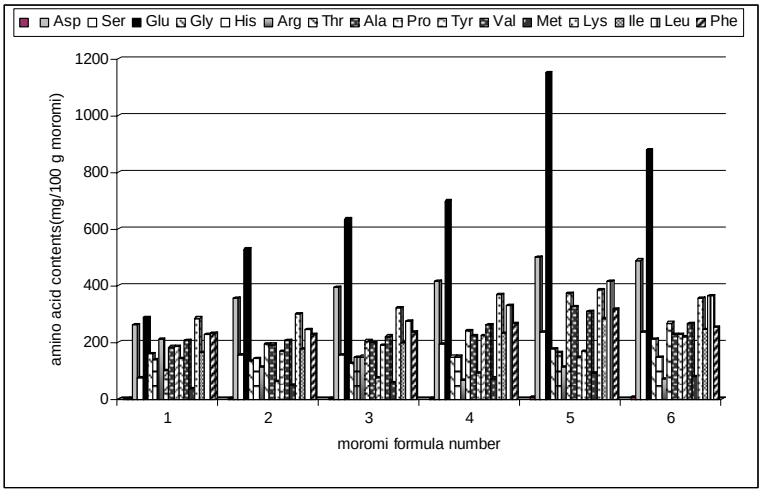

Figure 4 Amino acid contents of each moromi at 60 days of fermentation

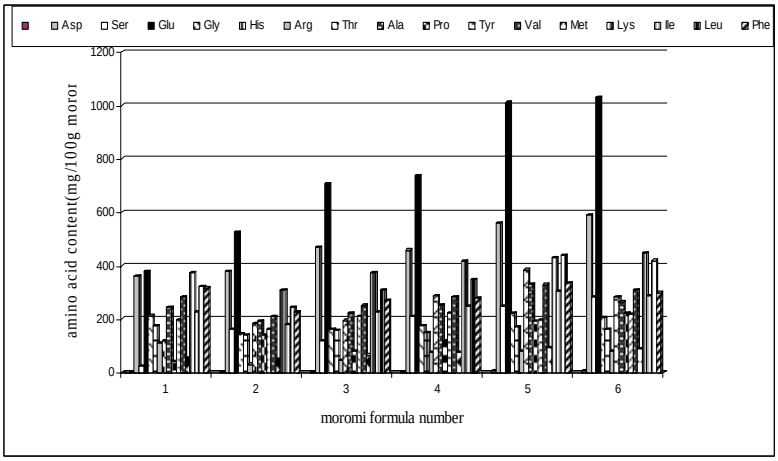

Figure 5 Amino acid contents of each moromi at 90 days of fermentation

Table 2 Total amino acid contents in each moromi

Fermentation time (day)	Total amino acid contents in each moromi formula (mg/100g)					
	F1	F2	F3	F 4	F5	F6
0	741.89	1240.63	1716.25	1950.41	2214.67	1517.18
60	2900.12	3244.47	3576.81	3980.82	5157.99	4545.15
90	3431.23	3275.59	3855.45	4334.58	5324.03	5179.66

The amino acid contents in all moromi at initial day of fermentation were 741.89-2214.67 mg/100 g moromi. After fermentation for 90 days the total amino acid content increased to the range of 3275.59-5324.03 mg/100 g moromi as shown in Table 2. However, among all the amino acid contents glutamic acid showed the highest content in each moromi and in all period of fermentation time. This showed the good taste of soy sauce. Moromi F5 gave the highest glutamic acid content. It could be explained that the formulated moromi that contained high ratio of soy bean also showed high glutamic acid. From the nutritional value of soy bean it is claimed that soy bean contains more protein than peagion pea [2]. From the result, it showed that the content of protease enzyme did not correlate with the content of total amino acid, because the protease enzyme contents at 90 days of fermentation (F1 to F3) were higher than the protease contents in control. The reason for this may be due to the anatomy of peagion pea which has thicker coat seed [3, 5]. Eventhough there was a high protease enzyme in the moromi that contained high peagion pea, it was hard to digest all of the component of peagion pea. Thus, the total amino acid in pure soy bean moromi or the formulas that contained high soy bean showed the high amino acid content.

4. CONCLUSIONS

This work proved that soy sauce could be produced from the other raw materials such as the peagion pea. The content of protease enzyme showed that there were the activity of this enzyme for protein digestion during the period of fermentation for 90 days. The F5 showed the highest total amino acid contents after the fermentation for 90 days. All formulas contained high content of glutamic acid. Peagion pea could be used as the part or as a whole part of raw material for soy sauce production. The use of peagion pea as raw material for soy sauce production could add more values to the pea than using the pea for animal feed.

5. ACKNOWLEDGEMENTS

The author would like to thank Mrs Lawan from Thailand (Institute of Scientific and Technological Research, TISTR) for providing *Aspergillus oryzae* for this work. This work was financially supported by the Foundation of Faculty of Science, Srinakharinwirot University.

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