A Controlled Fermentation Environment for Producing Quality Soya Sauce

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Abstract

Soy sauce fermentation under controlled temperature is a way to shorten the fermentation time. An energy-saving fermentation system was developed to power a heat pump for maintaining the temperature of sauce moromi at 37±1℃ during fermentation. The chemical properties of the sauce moromi and the sensory properties of the soy sauce produced using the controlled fermentation system were evaluated and compared to those of the sauce moromi fermented outdoors without temperature control. The sauce moromi processed using the controlled fermentation system had significantly higher total nitrogen, formal nitrogen, amino nitrogen, reducing sugar and organic acid contents than the moromi fermented outdoor. However, no significant difference was found in overall liking score between two soy sauces. The soy sauce fermented under the control temperature showed higher Brix and salt concentration, but lower pH value than the sauce fermented outdoor. It was possible that the beneficial effects of reducing sugar and organic acid contents were rebuffed by the disadvantage of salt concentration. It was concluded that a controlled fermentation environment deserves the potential to produce a high quality soy sauce.

Keywords: soy sauce, moromi fermentation, quality promotion, controlled environment

1. Introduction

Soy sauce has been the most important seasoning in the Orient [1]. It is prepared from a mixture of steam-cooked soybean and roasted wheat with koji mold Aspergillus oryzae or A. Solace. In sauce moromi fermentation, the proteins and polysaccharides in the moromi are hydrolyzed by the fungal protease and amylase to form different types of small nitrogen compounds and sugars, such as amino acids and reducing sugars. The Maillard reaction between amino acids and reducing sugars during moromi fermentation results in the unique flavor of soy sauce [2, 3]. Cooking raw soy sauce with sugar improves the Maillard reaction and further enriches the sensory properties of the soy sauce.

The fermentation of soy sauce using the traditional method generally takes from six months to one year [4, 5]. The fermentation time can be shortened if the fermentation temperature is control artificially. Young and Wood (1974) reported that if the fermentation temperature was controlled at 35-40℃, it took only 3-4 months to ferment the sauce moromi. Reference [2] stated that 45℃ was an important temperature for sauce moromi fermentation.

Soy sauce making under controlled temperature required energy input. Hence under the consideration of energy saving, we desii was fermented in a pottery tank to preserve the flavor of the sauce, (2) heat pump was 3-4 times more efficient in its use of elecgned an energy-saving fermentation system to transfer solar energy to electric power for a heat pump and used hot water to maintain the temperature of sauce moromi at 37±1℃ during fermentation. The advantages of this system were: (1) the moromtric power than a simple electrical resistance heater, and (3) heat transfer was more efficient by using water as the medium.

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The changes of chemical properties of the sauce moromi in the solar energy system were determined and the sensory properties of the soy sauce made from the system were also assessed to evaluate the potential of suing solar energy to produce a high quality soy sauce.

2. Materials and Methods

2.1. Raw materials and chemicals

Soybean and wheat were purchased from a local farmer in Chiayi City, Taiwan and immediately stored in a refrigerator at 4℃ prior to use. The koji mold (Aspergillus oryzae) was purchased from a supplier (Chuan Feng Microbe Co., Taichung City, Taiwan). All of the chemicals used in the analysis were of analytical grade.

2.2. Koji fermentation

For koji production, raw soybeans were first washed and soaked in water for 24 h at 4℃ and then steamed at 121℃ for 20 min. Wheat was roasted for 30 min at 140℃ and then cracked into 4 or 5 pieces per kernel accompanied by smaller particles of wheat flour. Roasted wheat and steamed soybeans were mixed at a ratio of 1:2 (w/w). The mixture was then inoculated with 0.1% (w/w) of *A. oryzae* (approximately 10^8 spores g^{-1}), and subsequently dispersed onto perforated stainless steel trays (68 x 44 x 3 cm). Each tray was loaded with the mixture to around 3 cm thickness and incubated at 30 ± 3℃. The preparation of koji was completed in 60–72 h when the culture began turning into greenish yellow in color. The koji was stored at 4℃ until use.

2.3. Moromi fermentation

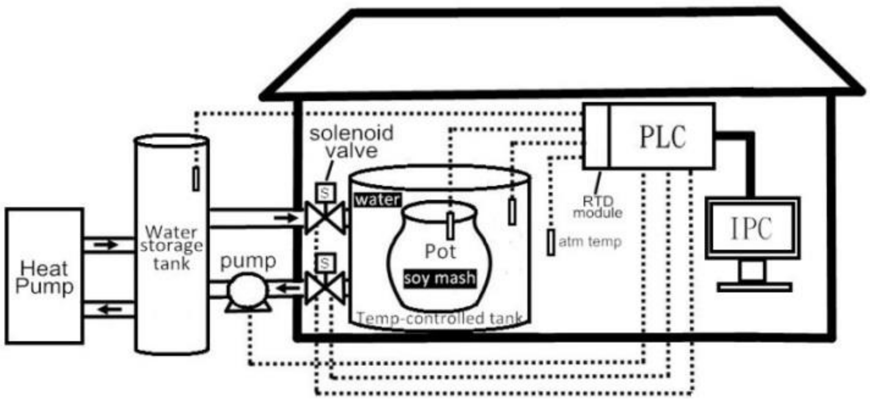


Fig. 1 Setup of the energy-saving soy sauce fermentation system.

Fig. 1 illustrates the structure of the temperature-controlled fermentation system. A 160 L pottery tank that accommodates soy mash is bathed in a stainless tank (diameter 84 cm and height 65 cm) full of circulating water for temperature control of the mash. Temperatures inside and outside of the pottery tank are to be measured for on-line fermentation temperature control and off-line analysis. The hot, circulating water was prepared using a heat pump powered by a solar power generator and energy management system developed in house. The energy system trades with the electricity grid to assure a safe and yet economical power supply for the process. Hot water is to be prepared in the hottest hours of a day for the interest of optimum heat pump efficiency. A programmable logic controller (PLC) performs controls and an industrial personal computer (IPC) executes system monitoring and data acquisition.

Finished koji was put in the pottery tank and then mixed with 18% saline at a weight ratio of 1: 2. A stainless steel cover was made for the mash tank with one hole in the center to the insert thermocouple for measuring the central temperature of the mash, another annulus cover was used to prevent the evaporation of water out of the stainless steel tank. The system was instructed to maintain the central temperature of the mash at 37C±1℃. During the first week of fermentation, the mash was stirred for 3 min once every day and then once a week for the remaining period. The total fermentation period was 13 weeks.

2.4. Microbe analytical methods

Determination of microorganism changes: The 10-fold-concentrated cells were serially diluted (10^0 to 10^{-6}) in 0.85% sterile saline solution, and 100 µl of properly diluted samples were spread by PCA agar media and incubated at 30~35℃ for 1~2 days for the total viable count. Diluted sample (100 µl) were spread by YM agar plate media (BD Difco, Franklin Lakes, NJ, USA), and then incubated for 1 day at 30℃ for the counting of yeast. Whereas MRS agar plate media (BD Difco, Franklin Lakes, NJ, USA) for the counting of lactic acid bacteria, which were incubated for 2–3 days at 30℃ in an anaerobic condition (AnaeroPack, Mitusubishi Kagaku, Kogyo, Tokyo, Japan). The colonies formed (25–250) were calculated as log CFU per milliliter of soy sauce [7].

2.5. Chemical analytical methods

Determination of total nitrogen: Total nitrogen was determined according to the AOAC standard [8] with a little modification. Three milliliters of the sample, 1g of catalyst ($K_2SO_4/CuSO_4.5H_2O$ = 10:1), and 20 mL of concentrated H_2SO_4 were added into a digestion tube and then heated for 6 h at 450℃ for digestion. When the digestion was completed, 25 ml of diluted sample was mixed with 25 mL of 30% NaOH and subjected to distillation. The distillate was absorbed with 25 mL of 0.1 N H_2SO_4. The content of total nitrogen was determined by back-titrating H_2SO_4 with 0.1 N NaOH.

Determination of formal nitrogen and ammonium nitrogen: Formal nitrogen and ammonium nitrogen were determined according to the Chinese National Standard [9]. To determine formal nitrogen, diluted sample and formalin solution were adjusted to pH 8.1, then 20 ml of formalin solution was mixed with 25 ml of diluted samples for 3 min, and titrated to pH 8.1 with 0.01 N NaOH. Amino nitrogen was determined by subtracting ammonium nitrogen from formal nitrogen according to the CNS [9].

Determination of pH: the pH values of the soy sauce samples were measured with a PB-10 pH meter (Sartorius, Göttingen, Germany).

Determination of organic acid content: Organic acid content as lactic acid was determined by alkali titration. To determine total acidity as lactic acid, 25 ml of properly diluted sample was titrated with 0.1 N NaOH to pH 8.1.

Determination of reducing sugar: The reducing sugar (RS) in the samples was determined by the 3,5-dinitrosalicylic acid (DNS) method [10] with a little modification. DNS reagent, which contained (w/v) 1% DNS, 2% NaOH and 30% sodium potassium tartrate, was prepared by dissolving each component in distilled water. One milliliter of the sample diluents were mixed with 1 ml of DNS reagent and then kept in a boiling water bath for 5 min to colorize. After cooling with cold water, 5 ml of distilled water was added and the $OD_{540\ nm}$ was measured by using distilled water as a blank control. The standard glucose solutions were used to make a calibrated curve for quantitative analysis.

Determination of browning: The level of browning was determined using the method of reference [11]. The absorbance of moromi samples was measured at $OD_{555\ nm}$.

Determination of sodium chloride concentration: Sodium chloride content was determined by volumetric titration with $AgNO_3$ using the Mohr's method (Hamilton & Simpson, 1964). A 1 ml of 0.25 M K_2CrO_4 was added into 250 ml flask containing 10 ml of properly diluted sample, then titrated with 0.01 N $AgNO_3$ until the appearance of brownish color. The standard curve of NaCl was prepared with 10–25% (w/v) of NaCl solutions.

2.6. Sensory evaluation

There was a total of 4 different soy sauces under sensory evaluation. The moromi samples from temperature-controlled brewing mash (TCM) and traditional brewing mash (TBM) fermented for 3 months were pressed, and then cooked with sugar (sauce moromi: sugar = 100 g: 10 g) and water (sauce moromi : water =100 g: 40 g) till the added water evaporated out. After cooling down the mixture, ethanol (sauce moromi : ethanol = 100 g: 1 g) was added, filtered with cheese cloth and clarified the mixture additionally by sedimentation. After bottling, the soy sauces were autoclaved at 85℃ for 30 min. These soy sauces were labeled as samples of TCM-3 and TBM-3. A soy sauce made from TCM without the addition of sugar during cooking and without the addition of ethanol was labeled “TCM-3-Raw” as a raw soy sauce. Finally, a commercial soy sauce was served as a standard soy sauce which was selected from a very popular brand in Taiwan.

The color, flavor, taste, savor and overall palatability of soy sauce samples was evaluated by a panel of 60 untrained volunteers. Soy sauce samples (10 ml) were prepared in small white plastic discs bearing 3-digit random numbers and presented them to the panelists in random order. The program started with observation of the color, smell of the flavor and taste of the relish by eating some cool noodle with the samples then scored their preferences based on 9-point hedonic scale.

2.7. Statistical analysis

The data were analyzed by analysis of variance (ANOVA), and significant differences in mean values among data were determined at $p < 0.05$ by Duncan’s multiple-range tests using SPSS 20 (SPSS Inc., Chicago, IL).

3. Results and Discussion

3.1. The changes of microbe counts in the mash during moromi fermentation

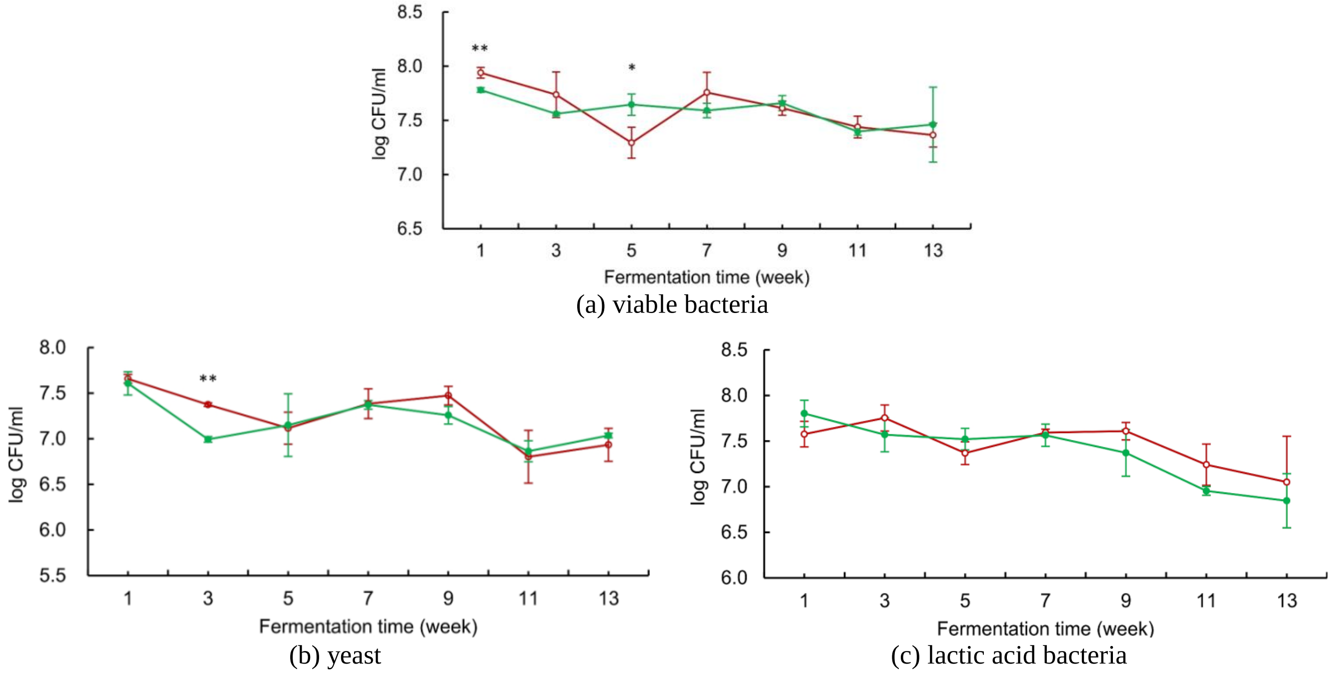


Fig. 2 Changes in total (a), (b), and (c) of traditional brewing mash (TBM) and temperature control brewing mash (TCM) during moromi fermentation.

◯: TBM; ●: TCM. Data are presented as mean±standard deviation (n=3).

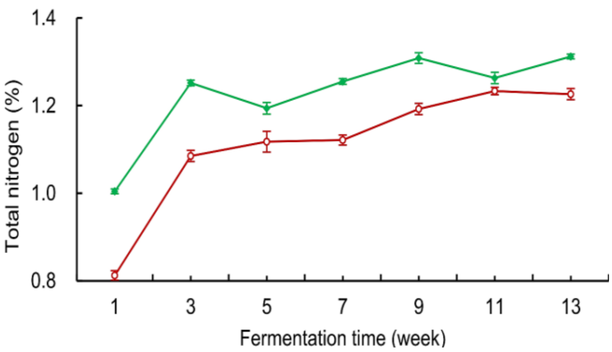
*,** A significant difference between TBM and TCM at the level of $p<0.05$ and $p<0.01$, respectively.

The unique flavor of moromi is attributed to lactic acid bacteria in the first stage of moromi fermentation, resulting in decreased pH in moromi to 6.0 or below, at which yeast starts to propagate [13]. Fig.1 shows the changes of total viable bacteria, yeast, and lactic acid bacteria of traditional brewing mash (TBM) and temperature control brewing mash (TCM) during moromi fermentation. The total viable bacteria and yeast counts in both TBM and TCM went down and then went up, and then decreased gradually with fermentation time (Fig. 2a, Fig. 2b). The lowest total viable bacteria and yeast counts in TBM occurred at 5 weeks, while TCM had the lowest counts at 3 weeks. Similar changes in total viable bacteria and yeast counts in black soybean moromi were reported [14]. The decrease of total viable bacteria and yeast counts in the initial stage of moromi fermentation was due to the inhibitory effects of high salt concentration in the moromi and low pH induced by lactic acid bacteria [15].

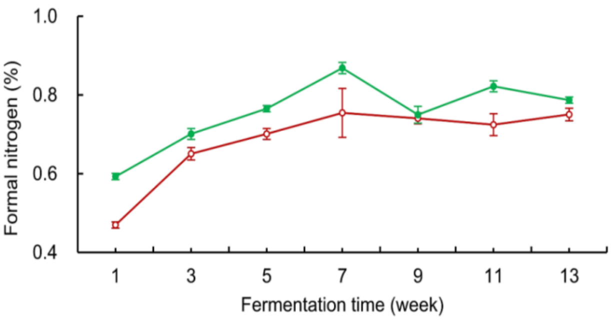
No significant changes in the count of lactic acid bacteria in both TBM and TCM were found from 1 week to 9 weeks, and then the count decreased slightly by fermentation time (Fig. 2c). In general, there were no significant differences in the yeast and lactic acid bacteria counts between TBM and TCM. The results indicated that the moromi fermentation under control temperature at 37℃ did not influence the growths of yeast and lactic acid bacteria compared to the traditional fermentation method that took place at outdoor without temperature control.

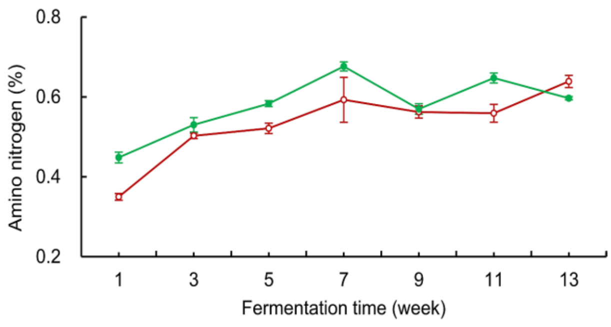
3.2. The changes of chemical properties of the mash during moromi fermentation

During moromi fermentation process, the proteins of soybean and wheat were hydrolyzed by protease of microbes into small fragments of peptides, amino acids and ammonia. Therefore, total nitrogen, formal nitrogen and amino nitrogen contents were often used as quality indexes for sauce moromi [14, 16]. Changes in total nitrogen, formal nitrogen, and amino nitrogen contents of TBM and TCM during moromi fermentation are shown in Fig. 3a It was found that total nitrogen content increased during moromi fermentation in both TCM and TBM, and their marked increases were noted within 3 weeks fermentation (Fig. 3a). TCM showing a higher increase rate compared to TBM indicates that fermentation at 37℃ could promote the protease activities and enhance the rate of protein hydrolysis than traditional fermentation method. Both formal nitrogen and amino nitrogen contents in TCM increased almost linear with fermentation time up to 7 weeks and then leveled off as the fermentation process continue (Fig. 3b, Fig. 3c). Like the total nitrogen content, both formal nitrogen and amino nitrogen contents were higher in TCM than those in TBM. The results suggested that moromi fermentation at the elevated controlled temperature (37℃) would promote the proteases activities to hydrolyze soybean and wheat proteins into different types of small nitrogen compounds. Our data agreed with reference [2], where they stated that high temperature, peculiarly the temperature of 45℃, was a very useful method to promote the proteases in the koji to solubilize and hydrolyze soy proteins efficiently. Reference [17] reported that black soybean sauce fermented at 50℃ had higher total nitrogen content but lower formal nitrogen content compared to the sauce fermented at 30℃, outdoor or indoor. However, reference [18] compared moromi fermentation at 25, 35, 45 and room temperature, and found that fermentation temperature did not affect total nitrogen content.



(a) total nitrogen



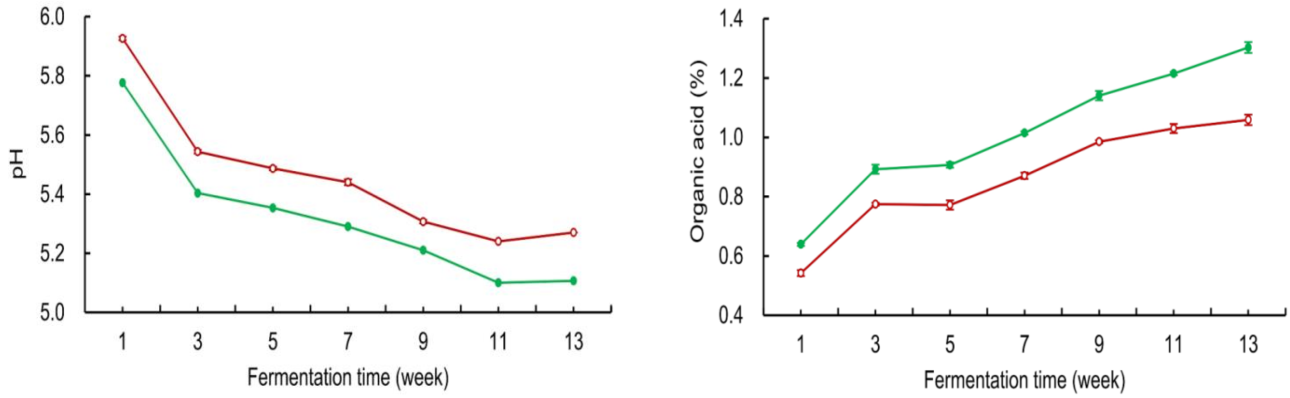


(b) formal nitrogen (c) amino nitrogen

Fig. 3 Changes in (a), (b), and (c) of traditional brewing mash (TBM) andtemperature control brewing mash (TCM) during moromi fermentation.

○: TBM, ●: TCM. Data are presented as mean±standard deviation (n=3).

Fig. 4 shows the changes of pH and organic acid in TBM and TCM. The pH values decreased gradually in both TBM and TCM (Fig. 4a). The rate of decrease in TCM was higher than that in TBM. The mash with a low pH value had the advantage of preventing the contamination of unwanted microbes and maintaining the condition well-suited for the action of yeast. In general, the accumulation of free fatty acids, amino acids, and peptides containing carbolylic side chains as the results of hydrolysis of raw materials, the autolysis of microbial cells, as well as the microbial fermentation of carbohydrates, which may account for the decline of pH during moromi fermentation [3, 11, 19]. The accumulation of organic acid in TCM was greater than that in TBM, and the results agreed with more rapid decrease of pH in TCM than in TBM (Fig. 4b).



(a) pH (b) organic acid

Fig. 4 Changes in (a) and (b) of traditional brewing mash (TBM) and temperature control brewing mash (TCM) during moromi fermentation.

○: TBM, ●: TCM. Data are presented as mean ± standard deviation (n=3).

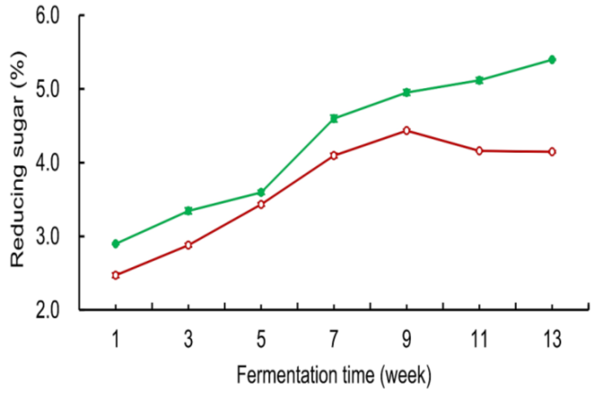


Fig. 5 Changes in reducing sugar content of traditional brewing mash (TBM) and temperature control brewing mash (TCM) during moromi fermentation.

○: TBM, ●: TCM. Data are presented as mean ± standard deviation (n=3).

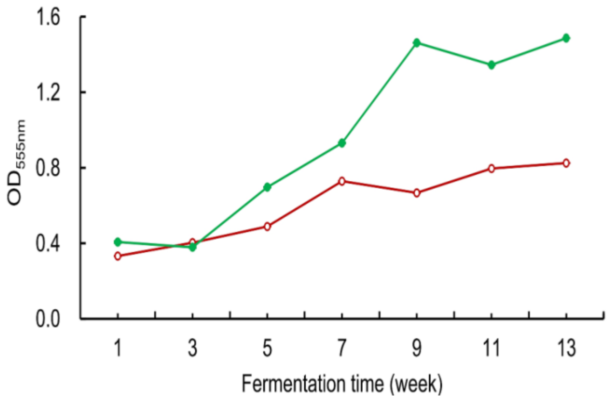


Fig. 6 Change in browning of traditional brewing mash (TBM) and temperature control brewing mash (TCM) during moromi fermentation.The higher the $OD_{555\ nm}$ value, the higher the browning level.

○: TBM, ●: TCM. Data are presented as mean ± standard deviation (n=3).

During moromi fermentation, amylases from the microbes hydrolyze the starch in soybean and wheat moromi to form small sugars [2, 17]. Therefore, reducing sugar can be used as a quality indicator for sauce moromi. Reducing sugar content is also a very important attribute regarding to the sensory properties of soy sauce. It can react with amino acids to form Maillard's compounds and produce unique flavor of soy sauce. The reducing sugar content in TCM increased almost linearly during fermentation; however, the content in TBM increased up to 7 weeks and then leveled off (Fig. 5). TCM had higher reducing sugar content than TBM; therefore, TCM also showed greater browning reaction than TBM after 3 weeks fermentation (Fig. 6).

Our data showed that TCM had higher total nitrogen, formal nitrogen, amino nitrogen, organic acid and reducing sugar contents than TBM. This was because the proteases and amylases showed higher activity at the elevated controlled temperature (37℃) and hydrolyzed the proteins and starch in soybean and wheat moromi to form different types of small nitrogen compounds and sugars. And based on these chemical indexes, it was clear that TCM had higher quality properties than TBM.

3.3. Sensory properties of TCM and TBM soy sauces

The sensory scores of four different soy sauces are shown in Table 1. The color, flavor, texture, savor and overall liking scores in the tested soy sauces showed very high agreement; therefore, the overall liking score alone could be used to describe the overall sensory preference of the tested soy sauces. The overall liking score could be classified into three groups: the commercial soy sauce showing the highest overall liking scores, followed by TBM-3 and TCM-3, and TCM-3-Raw. The commercial product is a very popular blend in Taiwan, and the formulation may include certain ingredients specifically for improving the flavor properties. Therefore, it was not a surprise to see that the commercial product received the highest score in all the sensory properties. For other soy sauces, only additional 10% sugar was added before cooking and then 1% ethanol was added after cooking, while only water was added during the cooking stage and no ethanol was added for TCM-3-Raw.

Table 1. Hedonic rating scores by sensory evaluation

Sample	Colour	Flavour	Texture	Savour	Overall
TBM-3	5.72^{b}	5.32^{b}	5.25^{b}	5.29^{b}	5.31^{b}
TCM-3-Raw	5.49^{b}	4.58^{c}	4.55^{c}	4.71^{bc}	4.63^{c}
TCM-3	5.65^{b}	5.45^{b}	5.22^{b}	5.29^{b}	5.43^{b}
Commercial	6.45^{a}	6.46^{a}	6.51^{a}	6.35^{a}	6.57^{a}

$^{a, b, c,}$ Different letters indicate significant difference in a column ($p<0.05$).

Table 2. Correlation coefficients between chemical properties and the overall sensory score

	Total nitrogen	Formal nitrogen	Amino nitrogen	Salinity	Brix	Reducing sugar	pH	Organic acid	Overall favourite
Total nitrogen	1								
Formal nitrogen	0.09	1							
Amino nitrogen	0.09	1.00	1						
Salinity	-0.26	0.84	0.84	1					
Brix	0.49	-0.51	-0.49	-0.43	1				
Reducing sugar	0.31	-0.90	-0.90	-0.81	0.76	1			
pH	-0.15	0.61	0.64	0.30	-0.59	-0.78	1		
Organic acid	0.67	-0.55	-0.56	-0.56	0.75	0.86	-0.81	1	
Overall favourite	0.32	-0.91	-0.91	-0.86	0.74	0.99	-0.71	0.82	1

TCM-3 and TBM-3 were produced from 3 month-fermented TCM and TBM, respectively, with the addition of 10% sugar to the sauce prior to the cooking process and then 1% ethanol were added after cooling down. Based on the results obtained from the moromi fermentation, it was clear that TCM showed better chemical properties than TBM; however, the overall liking scores between TCM-3 and TBM-3 did not differ significantly. It was possible that different chemical properties contributed differently to human mouth's perception, and the contributions from certain chemical properties were evened by other chemical properties. It was also possible that the addition of sugar prior to cooking and also the addition of ethanol concealed the differences in the chemical properties and resulted in the same level of the overall liking score.

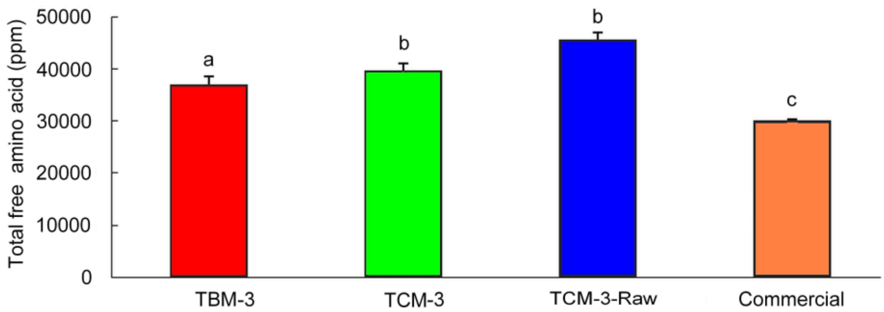
TCM-3-Raw was produced from 3 month-fermented TCM with the addition of only water during cooking. Without extra sugar to promote the Maillard reaction, TCM-3-Raw received a lower overall liking score than TCM-3

The chemical properties of four soy sauces shown in Table 2, and the correlation coefficients of the chemical properties and the overall liking scores are shown in Table 3. Compared to the chemical properties of other soy sauces, the commercial soy sauce had lower formal nitrogen, amino nitrogen and salt concentration but high Brix, reducing sugar and organic acid contents. This agreed with the correlation coefficients between the overall liking score and chemical properties, significant negative coefficients for formal nitrogen content (r = -0.88), amino nitrogen content (r = -0.87) and salt concentration (r = -0.88), but significant positive coefficients for Brix (r = 0.81), reducing sugar content (r = 0.996) and organic acid content (r = 0.76).

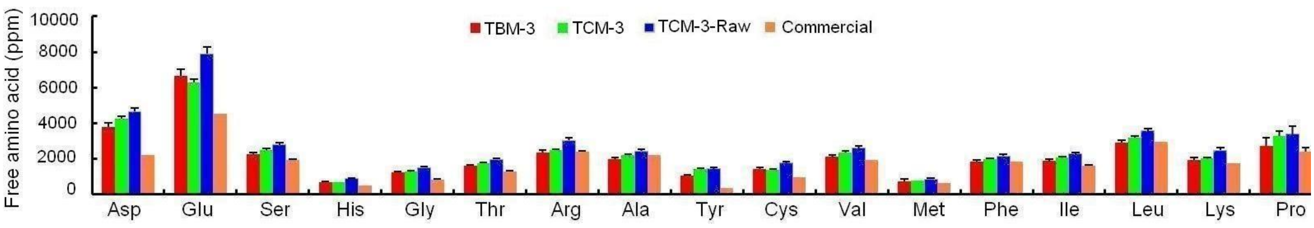
TCM-3 had a higher salt concentration, reducing sugar and organic acid contents than TBM-3; however, there was no significant difference in all the sensory scores. It was possible that the beneficial effects of reducing sugar and organic acid contents were rebuffed by the disadvantage of high salt concentration.

With the addition of sugar and ethanol, TCM-3 showed higher Brix, reducing sugar and organic acid contents than TCM-3-Raw. Thus, TCM-3 was expected to gain higher sensory scores than TCM-3-Raw. During soy sauce making, it is a general practice to add sugar during cooking to promote the Maillard reaction and to enhance the flavor properties of soy sauce.

It is well know that free amino acids are the main sources contributing to the flavor of soy sauce [20]. Free amino acid contents in the four different soy sauces were determined using HPLC and the results are shown in Fig. 7. In all soy sauces, Glu had the highest content (about 14-20%) followed by Asp and Leu (Fig. 7A). The free amino acid profile was similar to the data reported reported data [21]. It was noted that the commercial soy sauce had the lowest Glu, Asp and Leu, while TCM-3-Raw showed the highest contents. The sum of 17 measured amino acid content was calculated and the data indicated that the commercial soy sauce had the lowest total amino acid content, while TCM-3-Raw had high total amino acid content.



(a) total free amino acid contents



(b) individual free amino acid contents

Fig. 7 (a) and (b) in five different soy sauces. TBM-3: TBM at 3 months; TCM-3: TCM at 3 months; TCM-3-Raw: raw soy sauce made from TCM at 3 months without the addition of sugar and ethanol; TCM-3: TCM at 3 months; Commercial soy sauce: a very popular soy sauce in Taiwan. Data are presented as mean + standard deviation (n=2).

Total nitrogen, formal nitrogen and amino nitrogen contents are often served as quality indexes of soy sauces and the decision making in the selection of ending time for the moromi fermentation process. During fermentation, amino acid reacted by reducing sugar to form Maillard compounds, such as pentosidine, carboxymethyllysine and furosine, and these compounds further enriched the flavor of soy sauce [22, 23]. In this study, we found a strong correlation (r = 0.996) between the overall liking score of soy sauces and reducing sugar; however, the correlation was insignificant for total nitrogen content (Table 3). Nevertheless, even negative correlation coefficient was found between the overall liking score and formal nitrogen content (r = -0.88), as well as amino nitrogen content (r = -0.87). Moreover, the total free amino acid content and individual Glu, Asp and Leu all correlated negatively with the overall liking score of the soy sauces.

The question need to be addressed was why formal nitrogen and amino nitrogen contents are considered as quality indexes for soy sauce, but these contents in the tested soy sauce were negative correlated with the sensory scores. It was noted that all 17 measured free amino acid contents in TCM-3 were lower than TCM-3-Raw indicating that it must have a significant factor resulted in such differences in all different types of free amino acids. Since the difference in TCM-3 and TCM-3-Raw was with or without the addition of extra sugar during cooking to promote the Maillard reaction, it was suggested that free amino acids in TCM-3 interacted with sugars to form Maillard compounds and thus the levels of measurable free amino acids decreased in TCM-3 but it also resulted in better sensory properties in TCM-3. Based on the results, it was concluded that the Maillard reaction took place during the cooking process is a critical attribute to sensory properties of soy sauces.

4. Conclusions

Under the considerations of energy saving and hygienic improving, a solar energy system was designed for soy sauce making. Electricity converted from the solar energy was used to power a heat pump that supplies hot circulating water for fermentation temperature control. Hot water was prepared at noon and then stored and circulated (normally at night time) for temperature control. This scheme assures an energy-saving fermentation process. The sauce moromi fermented at the system showed better chemical properties than the traditional brewing moromi. Therefore, the use of solar energy system has the potential to produce a high quality soy sauce under an energy-saving stance.

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