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Development of Soy Sauce by Using Different Molds during Koji Fermentation

Seav Eng Leng[1], Reasmey Tan[1,2*], Luka Ly[2]

[1] *Faculty of Chemical and Food Engineering, Institute of Technology of Cambodia, Russian Federation Blvd., P.O. Box 86, Phnom Penh, Cambodia*

[2]*Research and Innovation Center, Institute of Technology of Cambodia, Russian Federation Blvd., P.O. Box 86, Phnom Penh, Cambodia*

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Abstract: *Soy sauce is a traditional brown liquid condiment widely used in Asian cuisine to enhance taste and flavor. In Cambodia, the quality of local soy sauce products remains limited. Koji mold is the key microorganism used to improve the quality of soy sauce. This study aimed to improve the quality of soy sauce by using different koji molds including Aspergillus oryzae and Aspergillus sojae. The physicochemical characteristics of koji and enzymatic activities of molds were analyzed during koji fermentation. In addition, the microbiological, and sensory characteristics of soy sauce were also analyzed during moromi fermentation. During the koji stage (0–72 h), the moisture content of C1 sample decreased to 20.6 ± 1.30% ($p<0.05$), while the pH dropped to 5.08 ± 0.00. The α-amylase activity increased significantly to 66.56 U/mL ($p < 0.05$). During the 32-month moromi fermentation, salt concentration decreased from 18% to 12.75 ± 0.35%, whereas reducing sugars increased to 2.93 ± 0.05 g/100 mL and total acidity declined to 2.31 ± 0.00. The lower amino nitrogen content (0.66 ± 0.03 g/100 mL) in C1 was attributed to microbial metabolism, oxidation, and Maillard reactions. For microbiology quality of soy sauce, there was no presence of yeasts and molds (TYMC) and total plate count (TPC) among all 4 conditions. The sensory evaluation revealed acceptable consumer preference for all samples, with mean scores showing only a slight difference where 6.00 ± 0.79 for C1, 6.38 ± for C3 and 6.82 ± 0.98 for C4 compared to the commercial Kbal Kor soy sauce (6.39 ± 0.87; $p>0.05$). These findings indicate that the experimental soy sauce possessed comparable quality to the commercial product, suggesting its potential acceptability in the consumer market.*

Keywords: Koji fermentation; Moromi fermentation; Different molds; Physicochemical quality; Microbiological quality.

1. INTRODUCTION

Soy sauce is the most popular among traditional soy-based foods used in Asian cuisine worldwide, including fermented products such as miso, tempeh, and natto, as well as tofu and soymilk [1]. It is commonly characterized by a light brown to black coloration and a distinctly salty, umami-rich taste [2].

The first stage of soy sauce process is koji-making, which is fermented under 30°C with *Aspergillus oryzae* or *Aspergillus. sojae*. The resulting culture mold (koji) is then mixed with a brine solution containing approximately 16-22% salt and the resulting (moromi) is traditionally fermented for 4-12 months. Finally, the refining stage includes pressing, pasteurization, and packaging [3]. Furthermore, researchers have discovered that soy sauce contains bioactive compounds that have antibacterial, anticarcinogenic, antioxidant, and antiplatelet properties [4]. As is well known, there are three major groups of microorganisms involved in soy sauce fermentation. They have included fungi such as *A. oryzae* and *A. sojae* involved in koji production; halotolerant lactobacillus; and yeast strains responsible for moromi fermentation [5]. *A. oryzae* and *A. sojae* play an important role by producing various enzymes in fermenting and producing their different flavors [5]. In soy sauce brewing, enzymes from koji molds break down proteins and starches into amino acids and sugars, which contribute to flavor and serve as substrates for lactic acid bacteria and yeasts to produce organic acids and aromatic compounds. The color develops through reactions between amino acids and sugars. Thus, koji mold is vital for producing the enzymes that drive fermentation and flavor formation in soy sauce [6].

* Corresponding author: Reasmey Tan
E-mail: rtan@itc.edu.kh;

This study investigated the effects of different koji mold strains on soy sauce fermentation. The physicochemical characteristics of koji and enzymatic activities of molds were analyzed during koji fermentation. And the soy sauce quality was assessed through sensory evaluation, microbiological analysis, and physicochemical measurements during the final stage of processing.

2. METHODOLOGY

2.1 Koji mold preparation

The four commercial mold strains available in the stocks were evaluated to assess their effects on fermentation performance and final product quality. PDA (Potato dextrose agar) was prepared for incubating the koji mold used in soy sauce experiment. A total of 39 g of PDA was weighted and put into a beaker by dissolving with 1L distilled water (DW). It was then autoclaved at 121 ℃ for 15 minutes. Koji mold strains stored at –80 °C were revived by transferring a loopful of each strain onto PDA plates. The plates were then incubated at 30 °C for 5–7 days. After incubation, the active mold cultures were used to inoculate steamed rice, which was then incubated for approximately 7 days.

2.2 Mold spore counting

Koji mold spores were harvested by mixing the rice medium with wheat flour, followed by using 100 mesh size for sieving the mixture. A dilution solution was prepared by pipetting 25 µL of Tween® 80 into a 250 mL volumetric flask and filling it to the mark with distilled water. To quantify the spores, 1 g of the mold–flour mixture was weighed and thoroughly mixed with the Tween® 80 solution. Serial dilutions (10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}) were prepared by duplicate. A hemocytometer (Millicell_2ch, Millipore) and a light microscope (OPTIKA, Italy) were used to count the spores. The number of spores was calculated using Eq. 1.

$$\text{Number of spores} = \frac{A \times 1000}{0.1} \times df \quad \text{(Eq. 1)}$$

where A is the average number of spores in one large square, and 0.1 is the volume of the square in the hemocytometer.

2.2 Koji fermentation

Koji fermentation is a process cleaned and left to soak in water for four hours. Then soybeans were steam-cooked for 30 minutes at 121 °C using autoclave equipment [6,7]. Next, 2kg of whole wheat was cleaned and roasted for 30 min at 140 ℃. The roasted wheat was crushed into 4–5 pieces per grain. Furthermore, roasted wheat and steamed soybeans were mixed at a ratio of 1:1 (w/w). Koji spores, at an approximate concentration of 10^5 spores per gram of substrate, were added to the mixture. The inoculated substrate was placed in a stainless-steel tray and incubated at ambient temperature for 72 hours.

Four different conditions were used in the experiments. The koji fermentation using different molds from different sources were named as C1 (*A. oryzae*), C2 (*A. oryzae*), C3 (*A. oryzae*) and C4 (*A. oryzae* with *A. sojae*), respectively.

2.3 Moromi fermentation

This step, 18% of brine solution was allowed to mix with 1:1.5 (w/v) of initial koji fermentation. *Zygosaccharomyces. rouxii* was added to moromi fermentation after the moromi took 4 weeks with a pH mash drop down to below 5.2. Moromi fermentation will become to black soy sauce with contain the good nutrition after 32 weeks of fermentation. During the fermentation period, the tanks were located in an oven (98 × 82 × 133 cm) to controlled temperatures (30±0.5 ℃). The mash was stirred for 3 min once every day.

2.4 *Physicochemical analysis*

2.4.1 Alpha-amylase activities

The analysis for amylase activity in koji was conducted using a quantitative starch-iodine essay with minimal modifications [8]. This procedure involved preheating 3 mL of a 0.2% starch solution for 10 minutes at 60°C. 3 mL of a diluted koji enzyme was added, and the mixture was incubated for ten more minutes. 1.5 mL of 1 M HCl was added to the reaction in order to stop the enzyme activity. After transferring 3 mL of the reaction mixture to a fresh tube, it was combined with 3 mL of distilled water and 0.1 mL of iodine solution to form a blue color by binding with the starch. A UV-Vis spectrophotometer was then used to measure the absorption at a wavelength 580 nm. Alpha-amylase activity was determined using Eq. 2 below:

$$\alpha\text{-amylase activity (U. ml}^{-1}) = \frac{mass_{control} - mass_{assay}}{Time \times v} \times df \text{(Eq. 2)}$$

where $m_{control}$ is the mass of starch without a sample (mg), $massay$ is the mass of starch with sample (mg), time is hydrolysis duration (min), V is a volume of sample used for assay (mL), and df is total dilution applied to the commercial solution of the sample.

2.4.3 Moisture content

The moisture content of koji fermentation was determined by using oven drying following the standard method of AOAC (2012). The empty metal cup was first dehydrated for three hours at 105 °C in the oven before being placed in a desiccator to cool (M_S). Weigh the aluminum cup after placed in a desiccator to chill (M1). Next, measure out 3 grams of the koji sample, then spread it well in aluminum cups. Next, put the aluminum cup with the

koji sample inside the oven (Memmet UNE400, Germany) and let it dry for 24 hours at 105 °C. The dried koji sample was placed in a desiccator to chill after drying and then weighed the remaining mass (M0). The moisture content in koji fermentation was considered by calculation through this formula in Eq. 3.

$$\text{Moisture content (\%)} = \frac{M_S-(M_0-M_1)}{M_S} \times 100 \quad \text{(Eq. 3.)}$$

where M_S is the Mass of the sample (g), M1 is the Mass of the aluminum cup (g), and M0 is Mass of the dried sample with the aluminum cup (g).

2.4.4 pH value

pH meter was calibrated with different buffers (pH = 7 as the neutral point, pH = 4 as the acidity, and pH = 10 as alkalinity), with all calibration performed at 25°C. Since pH will change depend on temperature. pH was measured in the first fermentation and second fermentation. The pH values of koji samples at 0, 24 , 48, and 72 h, and measured in moromi fermentation from week 0 until week 32. Lastly, approximately 5 ml of sample allow to analyzed the pH value, e.g.

2.4.5 Amino acid nitrogen

To determine total acidity as lactic acid, 25 ml of properly final diluted sample was titrated with 0.05 N NaOH to pH 8.2 [9]. Firstly, 20 ml of moromi of sample was mixed with 60 ml of distilled water in a beaker. After that, the mixture was stirred using a magnetic stirrer, and then 0.05 mol/l of NaOH solution was added dropwise until pH reached 8.2. However, we used a V blank 20 mL as a V sample to prepare the blank test. To analyze lactic acid, TA was analyzed by following the formula in Eq. 4.

$$\text{Total acidity} = \frac{N_{NaOH} \times V_{NaOH} \times 0.09}{V_{sample}} \times 100 \quad \text{(Eq. 4)}$$

where N_{NaOH} is the concentration of NaOH standard titration solution (N), V_{NaOH} is the volume of NaOH standard titration solution used for titration (ml), V sample is the volume of sample used (ml), and 0.09 is the conversion factor of lactic acid.

Later, 10 ml of formaldehyde solution was added to the mixture until the pH reached 8.2, followed by titration with 0.05 mol/l sodium hydroxide until the pH reached 9.2. To calculate AAN, this Eq. 5 below represents the formulation used:

$$\text{Amino acid nitrogen (g/100ml)} = \frac{(V1-V2) \times C \times 0.014}{5 \times V3/100} \times 100 \quad \text{(Eq. 5)}$$

where V1 is the volume of NaOH standard titration solution after adding formaldehyde in the sample, V2 is the volume of NaOH standard titration solution after adding formaldehyde in the blank test, C is the concentration of NaOH standard titration solution, and V3 is the volume of diluted sample.

2.4.6 Salinity

The evaluate the salt content of moromi, salt was measured from week 0 until week 32 by using a salt meter (model ES-421; ATAGO®). To analyze salt content, sample was dropped on salt meter, and then click the button start to present the result.

2.4.7 Reducing sugar

Reducing sugar (RS) content in the moromi sample 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. 1 ml 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, 5ml of distilled water was added and the OD_{540nm} was measured by using distilled water as a blank control. The concentration of glucose solutions in range of 1.0 mg/ml was used to make a calibrated curve for quantitative analysis.

2.5. Microbiological analysis

1ml sample was diluted with 9 ml of saline solution, solution became 10^{-1}. Serial dilutions prepared from 10^{-1} to 10^{-4} of the brine fermentation were prepared in 9ml volumes of sterile distilled peptone water. Using a sterile pipette tip, 1ml aliquots of each dilution were transferred into sterile Petri dishes having a label corresponding to the dilution. To each dilution in the sterile petri dish, was added 15ml of PDA. For the aerobic plate count, use Plate Count Agar (PCA, BIOTEC Laboratories Ltd., United Kingdom); for yeast and fungi, use Potato Dextrose Agar (PDA; 250g potato, 9g agar, and 5g glucose to form 500mls). Cover the base of the petri dishes with this agar [11]. After allowing the petri dishes to set, they were twirled both clockwise and counterclockwise to guarantee even mixing. PCA and PDA plates were incubated for 24 to 48 h at 37 °C.

After 24 hours of incubation, colonies on plates showing growth were counted using the Colony Counter (STUART Scientific Colony Counter, UK). Representative colonies on the corresponding media were kept for further identification. The yeast and microbial counts were conducted using the following equation (Eq. 6).

$$\text{Halophile yeast and Total plate count (CFU/ml)} = \frac{\sum c}{n \times d \times v} \quad \text{(Eq. 6)}$$

where $\sum c$ is the sum of counted colonies, n is the number of plates, d is the dilution factor, v is the amount of inoculating volume.

2.6. Sensory evaluation

Soy sauce products were evaluated based on the sensory evaluation with preference test method with parameters such as appearance, color, odor, flavor, taste, and texture, using a 9-point hedonic scale. The panelist untrained with the range of age 20–30-year-old. The test was performed in individual booths under controlled of lighting. The codes of samples were randomly presented. Panelists selected their preferred sample based on overall liking, with water provided as a palate cleanser between samples. Soy sauce samples were prepared in cup refer to different conditions such as C1, C2, C3, and C4 as the name refer to different condition.

2.7. Statistical analysis

The variance (ANOVA) was employed to find the significant differences in means the results of the physiochemical characteristics, enzymatic activities and sensory evaluation during fermentation time. Using SPSS software, Turkey's new multiple range test was used to evaluate whether there was a significant difference between the means of the samples and controls within a 95% confidence interval.

3. RESULTS AND DISCUSSION

3.1 Physicochemical analyze during koji

3.1.1 Moisture content

Fig. 1 the moisture content at the interval process (0, 24, 48, and 72h) of koji was represented were significantly different *($p<0.05$)*. To start fermentation, the moisture increased with a high material moisture content of 45–50%. At the end of koji fermentation, moisture dramatically drop down until below 30%. Based on additional research [12], the lowering the moisture, after the initial day (24h) permits the fungus to infiltrate the grain in search of oxygen and water, allowing it to flourish independently of outside moisture sources. Furthermore, moisture steady state failed down after the first day. Based on [13] during koji mold grows, it consumes the materials' carbohydrates and produces heat. This is the reason that moisture reduce to low amount. According to the experimental results, the first condition (C1) of the koji mold showed a dramatic decrease compared to the other conditions. Compared with the other conditions, C1 exhibited a substantial decrease in moisture, likely due to the higher growth rate of the mold strain. In other study, slight moisture reduction may help prevent contamination by unwanted microbes and keep koji fermentation in quality condition [12].

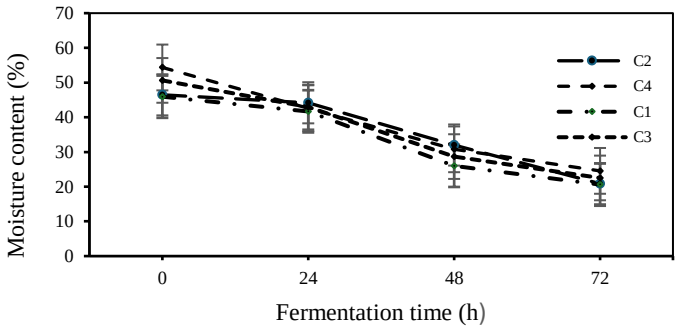

Fig. 1. Moisture content during koji fermentation

3.1.2 Alpha amylase activity

The alpha-amylase enzyme result was observed at the initial of fermentation (0h), and at 24h with 3.50 ± 0.480, 3.53 ± 0.299, and 3.86 ± 0.665 (U/g) there is not significant difference between *($p>0.05$)* for C1, C2, C3 and C4 respectively. However, this result still increased gradually from the first day until the final day of fermentation (72h) with not significantly different *($p>0.05$)* for C1 and C2 66.92 ± 16.563, 46.09 ± 6.737 (U/g). Based on [14] substrate ratio was more favorable to *A. oryzae* which produces more amylase. Furthermore, the enzymatic activity is influenced by the koji depends on the qualities of the raw material, the type of mold, the period of fermentation, and the storage condition. Ambient temperature is a condition for the synthesis period of koji fermentation [14].

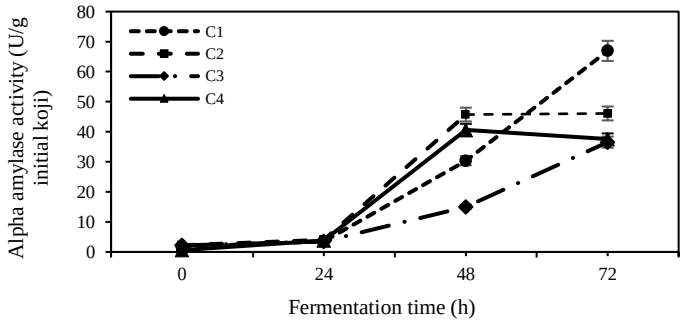

Fig. 2. Alpha-amylase activity during koji fermentation

3.1.3 pH value

The significant different were observed all samples with p-values ($p<0.05$) from 0 week to 2 week of moromi fermentation. On the final day of moromi fermentation (week 32), the pH values for C1, C2, C3, and C4 were recorded at 4.46±0.005, 4.51±0.00, 4.58±0.00, and 4.40±0.035, respectively. These final values were found to be significantly different ($p<0.05$) among the treatment conditions. Based on the Institute of Standard of Cambodia, the pH value in soy sauce should be

around 4.2 to 4.6 [15]. Low pH also helps to preserve the soy sauce quality. Since most bacterial spores cannot develop in high-acid food, which has a pH of less than 4.6, pasteurization is sufficient to destroy the spoiling microbe and preserve soy sauce [16]. Otherwise, the pH value of all conditions illustrated significantly different (P<0.05) while the highest pH value was noticed for C3. During fermentation, the first lactic acid bacteria took place and it resulted in a decrease in the pH from 7 to below 5. **Fig. 3**. below represent about the value of pH contain in soy sauce.

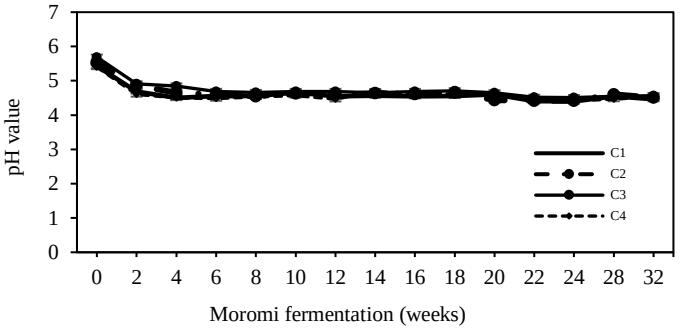

Fig. 3. pH value during koji fermentation

3.1.4 Reducing sugar

Reducing sugar is one of the very critical quality indicator a favorable sensory profile. Based on Figure 4 represent a signification different between week 0 and week 2, the value of C1, C2, C3, and C4 were shown 6.6813 ± 0.98, 6.0093 ± 0.15, 6.3427 ± 0.08, and 5.4360 ± 0.27, respectively. According to **One-way ANOVA** statistics, the p-value (p<0.05) and there is significantly different at the 0 and 2 of weeks. C4 exhibited the lowest reducing sugar content (5.4360%), indicating a higher degree of sugar utilization by fermentative microorganisms. In this case, *A. oryzae* is the main factor break down the raw material such as protein, carbohydrates, and lipids [17]. One month after adding *Z. roxii* yest, reducing sugar start to decrease dramatically with not significantly different with the p-value (p>0.05). In this case pH value drop down due to carbohydrate amino acid released during fermentation accumulation by microbial cells of yeast [18]. Reducing sugar continue to decline until week 32 after inoculation of yeast due to the type of sugar was hydrolyzed during the presence of yeast such as maltose and glucose which were synthesized by enzymic action [19,20]. Based on the experiment, the last condition of moromi fermentation has the highest concentration compared to other condition. As illustration in **Fig. 4**. the factors increase of reducing sugar are koji mold species that produced strong amylase, substrate composition of raw material, and include the condition of fermentation [14] Reducing sugar value demonstrate to the **Fig. 4**

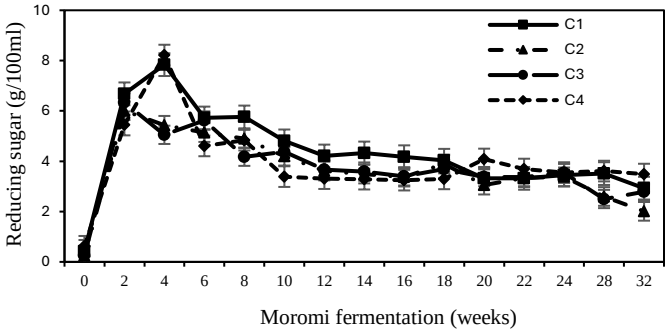

Fig. 4. Reducing sugar during moromi fermentation

3.1.5 Total acidity

Total acidity is an essential parameter used to determine the quality of soy sauce. Result of total acidity was significantly different if comparing week 0 to week 2 with (p<0.05). Compared to after 2 weeks of moromi fermentation, the results were changed from a low level up to a slightly high level among 0.95 ± 0.00, 0.95 ± 0.00, 0.90 ± 0.00, and 1.17 ± 0.00 g/100ml with the (p<0.05) was significantly different for C1, C2, C3, and C4. C4 show the strongest fermentation effect. Based on the previous study expressed that, the increase in total acidity caused by the decline in pH during fermentation may be attributed to the autolysis of microbial cells, accumulation of free fatty acid, amino acid, and peptides containing carboxylic side chain as the result of hydrolysis of material in soy sauce, as well as the microbial fermentation of carbohydrate [21]. Otherwise, the total acidity from week 4 end the nearly final fermentation (week 24) dramatically decreases with significantly different (p<0.05). According to a previous study, yeast still grows depending on the presence of air during fermentation as well as the pH contained in the mash which is a good range for yeast growth [22].

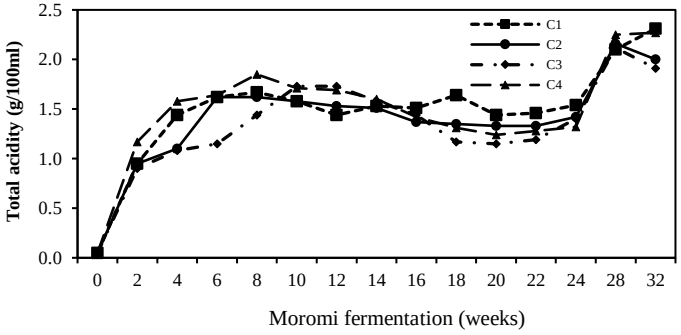

Fig. 5. Total acidity during moromi fermentation

3.1.6 Amino acid nitrogen

Amino Acid Nitrogen (ANN) is one of the main organic compounds that are produced while koji incubation and moromi fermentation, *A. oryzae*, and lactic acid bacteria produce enzyme or create acid environment that can help denature protein convert to amino acid compound [23,24]. According to **Fig. 6** at the initial week of moromi fermentation, Amino Acid Nitrogen (ANN) presented with significantly different (p<0.05) at the final week fermentation 0.66 ± 0.01, 0.73 ± 0.08, 0.80 ± 0.00, and 0.76 ± 0.00 g/100ml for C1, C2, C3, and C4 respectively. For exactly, Amino Acid Nitrogen (ANN) is starting to increase from the second with the variation value from one condition to each condition, due to the hydrolyzation of the raw material in Koji by the enzyme from the mold. Protease enzymes break down protein, leading to an increase in the formal nitrogen, measured as the Amino Acid Nitrogen (ANN) [25]. Based on this study, the concentration of amino acids in the third condition shows the highest nutritional quality. As demonstrated in **Fig. 6**, at the final week of fermentation, C3 contained 0.80 g/100 ml. According to Chinese National Standard GB/T2717-2003, Amino Acid Nitrogen (ANN) better contain in soy sauce is not less than 0.4g/100ml. However, compare to this research soy sauce at week 32, the quality is under standard of great nutrition soy sauce. Amino Acid Nitrogen (ANN) content show more detail in **Fig. 6** below.

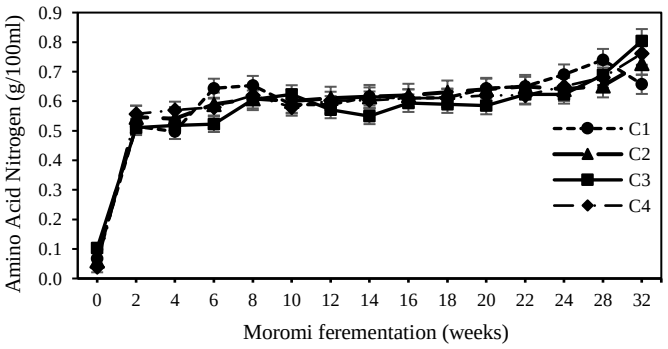

Figure 6. Amino acid nitrogen during moromi fermentation

3.1.7 Salinity

Salt is an essential ingredient used in soy sauce fermentation products. The great soy sauce product should contain a range from 18%-20% [26]. Based One-way ANOVA statistic was analysis that 16.25 ± 0.35, 16.00 ± 0.00, 17.00 ± 0.00, and 16.50 ± 0.00 respectively for C1, C2, C3, C4 significantly different by comparing to the first week and second week. C2 dramatically fall down than other condition. Salinity progresses to decreases after the second week until the final week of fermentation. Salinity consistent downward trend due to the presence of precipitates of raw materials such as soybean, wheat, and mold growth on raw material as well. These phenomena affect brine solution and salt content in moromi fermentation exactly fall. For significantly different with reason decrease in salt concentration step might be due to the enzyme's breakdown of the nutrition value. The increased protein rate breaks down, regardless of the decrease in salt concentration during fermentation [27]. Salinity was indicated in **Fig. 7** below.

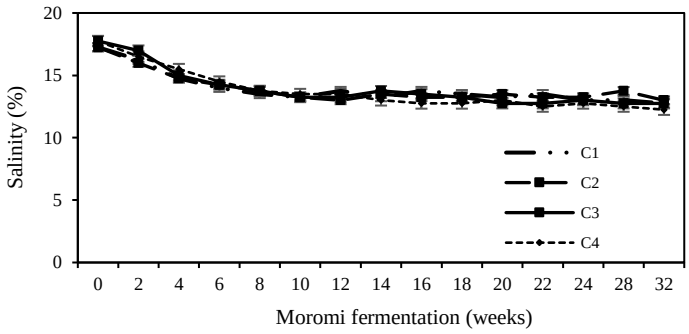

Fig. 7. Variation of salinity during moromi fermentation

3.1.8 Microbiological quality of soy sauce

Total plate count and halophile yeast and mold were also analyzed as indicators of the microbiological quality of soy sauce. Halophile yeast is a type of microbial tolerant with high salt concentration and grows well with a low pH level [28,4]. The microbiological quality in soy sauce at the final week of moromi fermentation after pasteurizing is indicated in Table 2.

Table 2. Total plate count and halophile yeast and mold in soy sauce

Microbial parameters	C1	C2	C3	C4	Standard
TPC (CFU/ml)	ND	ND	ND	ND	<5000 (China)
TYMC (CFU/ml)	ND	ND	ND	ND	ND (Cambodia)

Based on **Table 2**, microbiological in soy sauce indicator quality safe for consumption. As shown in the table TYMC and TPC in each condition found has ND that presence below <10 FCU/ml in the samples C1, C2, C3, and C4 due to good hygienic practice while processing, fermentation, pasteurization, to activate micro and alter the flavor, aroma, and composition. According to [29], the refinement is used to clarify soy sauce and a significant process to remove microorganisms to stop the fermentation in soy sauce. According to the Institute of Standard of Cambodia, yeast must not be present in soy sauce products. Thus, each condition's TYMC levels were complaint with regulatory standards. On the other hand, TPC is not detected (ND) after the pasteurization process as indicated in **Table 2**. was ND for C1, C2, C3, and C4 respectively. According to [30], the food product spoils when the

number of microorganisms is around 10 $\times 10^7$ CFU/ml. Therefore, the microbial number in this study is an acceptable limit at the final product. However, based on the National Standard of the People's Republic of China, bacteria should be less than 5000 CFU/ml [31]. Thus, the microbial standard in each food product is not the same according to countries. The variation in microbial numbers is also due to the right pH and moisture content that did not support for microbial growth [32]. Normally, decrease in spoilage microorganisms due to production containing alcohol and acid as the acid condition is unavailable for spoilage micro survive [33]. Further [33], stated that the high salt concentration is around 18%, effectively the growth rate of desirable osmophile microorganisms. Based on this research, at the end of fermentation pH of soy sauce stays in low acid with a range from 4.5 to 4.8. As indicated in the report of [18], most microorganisms can be prevented or inactivity by adjusting the initial microbial number and temperature storage, using preventives, and lowering pH. In addition, to prevent or develop microbial growth sodium benzoate has been applied to soy sauce products regardless of their effect on human health [34].

3.1.9 Sensory evaluation

Sensory analysis is a scientific discipline used to evoke, measure, interpret, and analyze the reaction to the characteristics of food and materials as perceived by the sense of sight, taste, hearing, and touching applied to development, grading, quality control, and ensuring, marketing, measuring, and predicting the shelf-life of the product [35]. Following to statistics analyzed by One-way ANOVA, results indicate p-value (p>0.05) is not significantly different for color, sourness, bitterness, and other points as proven in figures above such as odor, texture, aroma, sweetness was significantly different (p<0.05) by observation between fermented sample and control sample. According to the rating results, C3 achieved the highest score compared to the other conditions. However, the rating scale control sample got higher score than ferment product. Due to, many commercial soy sauces are acid-hydrolyzed vegetable protein (HVP) or blended products rather than fully fermented, which can result in a simpler or harsher flavor profile in product and more often panelists have consumed the soy sauce from commercials than fermented products. **Figure 8** indicates the rating scale in each condition of soy sauce.

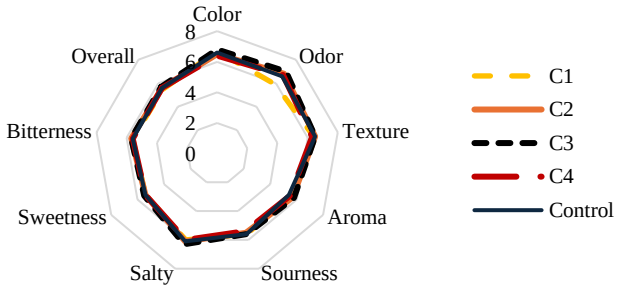

Fig. 8. Sensory evaluation of soy sauce with different conditions

4. CONCLUSION

In this study, *Aspergillus oryzae* and *Aspergillus sojae* were applied in koji fermentation. During the koji stage, moisture content and α-amylase activity showed no significant differences among the koji fermentation. Throughout 32 weeks of moromi fermentation, the values of amino acid nitrogen (AAN), total acidity, reducing sugars, and pH remained within the acceptable ranges for high nutritional quality according to Cambodian standards and the National Food Safety Standards of the People's Republic of China. Overall, all fermentation conditions (C1–C4) produced soy sauce of acceptable quality; however, C3 (*Aspergillus oryzae)* exhibited the most favorable physicochemical characteristics, indicating superior fermentation performance and product quality.

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