EFFECT OF STARTER CONCENTRATION AND STIRRING FREQUENCY ON THE PHYSICOCHEMICAL CHARACTERISTICS OF COCONUT AMINOS

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Abstract. Coconut aminos is a soy sauce alternative produced from coconut sap through moromi fermentation, which is traditionally spontaneous and time-consuming. The addition of a starter can accelerate the fermentation time. Stirring during the fermentation facilitate the process by evenly distributing the substrate and microbial cells. This study aimed to investigate the effect of starter concentration and stirring frequency on the physicochemical characteristic of coconut aminos. This research employed a Randomized Complete Block Design with 12 treatment combinations (4 starter concentrations: 0%, 5%, 10%, 15%; and 3 stirring frequencies: no stirring, daily stirring, and stirring every 3 days). The results showed that starter concentration had no significant effect on any of the physicochemical parameters. In contrast, stirring frequency significantly affected reducing sugar and total sugar contents, while moisture content and dissolved protein were not significantly influenced by either factor. The highest reducing sugar and total sugar contents were observed in samples that were not stirred. Increasing stirring frequency resulted in lower reducing sugar levels, and treatments without stirring differed significantly from daily and every-three-day stirring.

Keywords: Coconut aminos, coconut sap, moromi fermentation, starter concentration, stirring frequency

1. Introduction

Soy sauce is defined as a liquid product obtained from the fermentation or hydrolysis of soybeans (*Glycine max L.*), with or without the addition of other food ingredients and permitted food additives (1). Soy sauce can be classified into two types based on taste, flavor, and viscosity, namely salty soy sauce and sweet soy sauce. The distinction between the two lies in the addition of sugar to sweet soy sauce, which yields a different viscosity and flavor profile than salty soy sauce (2). The process of soy sauce production involves two stages of fermentation: solid-state fermentation using a starter culture for 2–4 days, followed by moromi fermentation, in which the koji product is soaked in a salt solution of a certain concentration for several months, as this fermentation occurs spontaneously (3,4).

Soybeans are classified as food ingredients that can trigger allergic reactions due to the presence of water-soluble glycoproteins contained in soybeans (5). Symptoms of soy allergy may include itching, watery eyes and nose, swelling, and even anaphylaxis, which can be life-threatening (6). This highlights the need for innovation to develop non-soy-based soy sauce alternatives that are free of allergens, including sap-based soy sauce, commonly known as coconut aminos. Coconut aminos is a salty soy sauce substitute made from fermented coconut sap using the moromi fermentation method (7). Beyond serving as a solution for individuals with soy allergies, coconut aminos also represents the utilization of local natural resources available in the Banyumas region and its surroundings.

Coconut sap has characteristics similar to koji fermentation products, with a nutritional composition of 15–18% sugars, most of which are sucrose (8,9). This underlies the production of coconut aminos via moromi fermentation alone, without prior koji fermentation, with the expectation that moromi fermentation of coconut sap can serve as an alternative to soybean-based salty soy sauce. Moromi fermentation is generally carried out spontaneously, without the addition of starter microorganisms, which makes the process relatively long (10). The slow progress of spontaneous fermentation is influenced by the growth of undesired microbial groups such as *Lactobacillaceae, Streptococcus, Aerococcus,* and *Corynebacterium*, which are difficult to control and may cause contamination. The addition of inoculum and starter cultures can accelerate fermentation (3). The starter's concentration indicates the number of microorganisms present. The starter used contains halophilic microorganisms that are tolerant of high salt concentrations. The activity of these microorganisms optimizes the conversion of amino acids and glucose into aroma-forming compounds, including alcohols, esters, phenols, aldehydes, and ketones (11).

The moromi fermentation process is generally stirred, which enhances contact between cells and substrates, prevents microbial sedimentation at the bottom, and homogenizes the temperature throughout the medium. Stirring can break down colonial cell structures, thereby preventing microbial cells from forming flocs or aggregates that may disrupt cell proliferation. Flocs or aggregates can hinder cells from obtaining sufficient nutrients from the substrate (12). Stirring also ensures uniform salt concentration, provides adequate aeration, and prevents the growth of undesirable anaerobic microorganisms (13). The stirring process during moromi fermentation can be applied at varying frequencies. One objective of this study is to identify the optimal stirring frequency for producing coconut aminos with desirable physicochemical properties. Based on the background described, both the addition of starter and the stirring frequency are expected to influence the quality of the resulting coconut aminos. This study aims to examine the effect of starter concentration and stirring frequency on the physicochemical characteristics of coconut aminos.

2. Methods

2.1 Materials

The materials used in this study included coconut sap obtained from Banyumas regency, starter moromi, distilled water, sea salt, pH buffer solution, Nelson reagent A, nelson reagent B, arsenomolybdate reagent, lowry reagent A, lowry reagent B, HCl, NaOH, Bovine Serum Albumin (BSA) solution, H2SO4 and other analytical reagents.

2.2 Experimental design

This research used a Randomized Complete Block Design with 12 treatment combinations: 4 starter concentrations (0%, 5%, 10%, 15%) and 3 stirring frequencies (no stirring, every 1 day, and every 3 days). Moromi fermentation was carried out using clay jar containers with 20% sea salt. The fermentation was conducted for 30 days in room temperature. The filtrate was heated to obtain the Total Soluble Solids (TSS) at 50 °Brix.

2.3 Determination of Moisture Content (14)

Moisture content was determined gravimetrically by weighing. Moisture content represents the amount of unbound water molecules (free water) present in a material. The principle of this method is that water molecules are removed by heating, either in a vacuum or a non-vacuum oven, or in another moisture-determination instrument operating at 80–110 °C, until a constant dry weight is obtained. The water content is then calculated gravimetrically from the difference in sample weight before and after drying.

2.4 Determination of Reducing Sugar (15)

The measurement of reducing sugar was initiated by preparing a standard curve. A standard glucose solution was prepared at a concentration of 10 mg anhydrous glucose per 100 mL. Serial dilutions of the glucose solution were then made to obtain concentrations ranging from 0.02 to 0.08 mg/mL. A total of 1 mL of each glucose solution was placed in a test tube and mixed with 1 mL of Nelson reagent. The test tubes were heated in a water bath for 20 minutes, cooled, and subsequently added with 1 mL of arsenomolybdate reagent. The mixture was shaken until the Cu_2O precipitate was fully dissolved; 7 mL of distilled water was then added, and the mixture was homogenized. The Optical Density (OD) was measured at 540 nm. The standard curve was constructed by plotting glucose concentration against OD values. The determination of reducing sugar in the samples was carried out by preparing sample solutions containing approximately 2–8 mg of reducing sugar per 100 mL. Clarification was performed using lead acetate. Then, 1 mL of the clarified sample solution was transferred into a test tube, mixed with 1 mL of Nelson reagent, and treated as in the preparation of the standard curve.

2.5 Determination of Total Sugar (15)

Total sugar content was determined by preparing 25 mL of the sample filtrate in an Erlenmeyer flask, then adding 15 mL of distilled water and 5 mL of HCl. The mixture was heated in a water bath at 70 °C and then rapidly cooled to 20 °C. The solution was neutralized with 45% NaOH and diluted to a final volume of 100 mL, resulting in a solution containing 2–8 mg/mL of reducing sugar. The total sugar content was determined from the Optical Density (OD) of the sample solution, using a standard glucose calibration curve.

2.6 Determination of Soluble Protein (16)

The determination of soluble protein content was carried out using the Lowry Method. A standard solution of Bovine Serum Albumin (BSA) was prepared with various concentrations ranging from 30 to 300 µg/mL. From each concentration, 1 mL was taken and placed into a test tube. g. Subsequently, 8 mL of Lowry Reagent B was added and allowed to stand for 10 minutes at room temperature. Then, 1 mL of Lowry Reagent A was added, mixed, and left for 20 minutes. The absorbance was measured at a wavelength of 600 nm using a spectrophotometer. A standard curve was prepared on graph paper, plotting concentration (µg/mL) against absorbance. For protein measurement, 2 g of the sample was weighed into a volumetric flask, and distilled water was added to the calibration mark. The solution was transferred to an Erlenmeyer flask and shaken at 157 rpm for 15 minutes. The liquid was then transferred into a centrifuge tube and centrifuged for 15 minutes at 3000 rpm. From the filtrate, 1 mL was taken and subjected to serial dilutions of 0, 5, and 10. From each dilution, 1 mL was taken, then 8 mL of Lowry Reagent B was added, the mixture was vortexed, and the mixture was incubated for 10 minutes. Then, 1 mL of Lowry Reagent A was added, the mixture was vortexed, and it was allowed to stand for 20 minutes. Absorbance was measured at a wavelength of 600 nm.

2.7 Data analysis

The data obtained from the study were analysed using Analysis of Variance (ANOVA) at 95% confidence level with IBM SPSS Statistics version 27. The statistical test applied was Two-Way ANOVA for normally distributed data followed by Duncan's Multiple Range Test as a post-hoc analysis.

3. Results and Discussion

3.1 Moisture Content

The analysis of variance showed that starter concentration, stirring frequency, and their interaction had no significant effect on the moisture content of Coconut aminos. The values of moisture content range from 47,81% to 56,36%. The highest moisture content was observed in the sample with a 10% starter concentration and daily stirring, whereas the lowest was observed in the sample without starter addition and without stirring during fermentation. There are no significant differences that might be attributed to the fact that the moisture content in soy sauce-like products subjected to heating is more strongly affected by the heating process than by fermentation. In this study, the moromi liquid was cooked for the same duration across treatments, with no significant differences among samples. Moisture content in soy sauce after heating is primarily affected by heating duration; longer cooking times result in greater water evaporation, thereby reducing moisture content. Soy sauce with low moisture content tends to exhibit higher viscosity because heating increases particle collisions and reduces the spacing between particles (17).

3.2 Reducing Sugar

The analysis of variance showed that stirring frequency significantly affected the reducing sugar content of coconut aminos. The average reducing sugar content was 40.98% (range, 18.08-70.57%). The lowest reducing sugar content was observed in the sample with 15% starter concentration and stirring every three days, while the highest was found in the sample with 5% starter concentration without stirring. Stirring frequency significantly affected the reducing sugar content of coconut aminos. Samples without stirring exhibited the highest reducing sugar levels, whereas increasing stirring frequency reduced them. This trend may be attributed to uneven oxygen distribution in non-stirred conditions, which limits microbial activity and reduces enzymatic sugar utilization (18). Consequently, reducing sugars were less metabolized, resulting in higher levels than in stirred treatments.

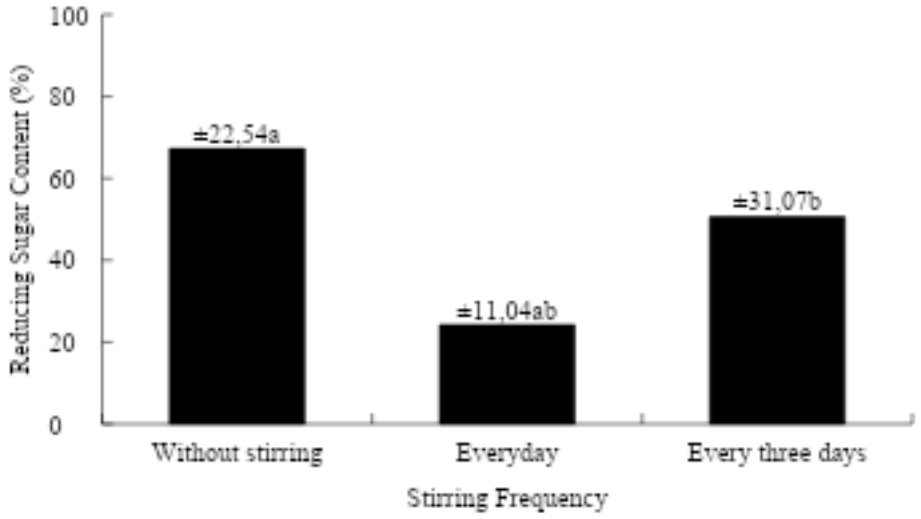

Figure 1. Effect of stirring frequency on reducing sugar content of coconut aminos

Starter concentration did not significantly affect the reducing sugar content of coconut aminos. This result can be explained by the fact that all samples were subjected to the same heating temperature and duration during processing. Heating is a major factor influencing sugar reduction, as thermal treatment may alter the chemical structure of the fermented liquid, thereby affecting the effects of starter concentration.

3.3 Total Sugar

The analysis of variance showed that stirring frequency had a significant effect on the total sugar content of coconut aminos, while the starter concentration and the interaction between the two factors were not significant. The average total sugar content of the product was 53.96% (range, 29.43-85.08%). The lowest total sugar content was observed in the sample with 0% starter concentration and stirring every 3 days, whereas the highest total sugar content was found in the sample with 5% starter concentration without stirring.

The total sugar content of coconut aminos was significantly affected by stirring frequency. The highest total sugar content was observed in samples that were not stirred. The no-stirring treatment also showed a significant difference compared to the treatments with daily stirring and stirring every three days. This is because, in the absence of stirring, oxygen distribution within the fermentation medium is more limited, resulting in lower microbial activity compared to stirred samples. Consequently, the sugar content in the medium was not extensively degraded into other compounds. In contrast, stirring improved medium homogeneity and oxygen distribution, thereby enhancing microbial metabolism. This condition accelerated sugar utilization for microbial growth and enzymatic activity, thereby reducing total sugar content in stirred treatments. Stirring functions to maintain nutrient uniformity, prevent sedimentation, and accelerate microbial metabolic activity (17).

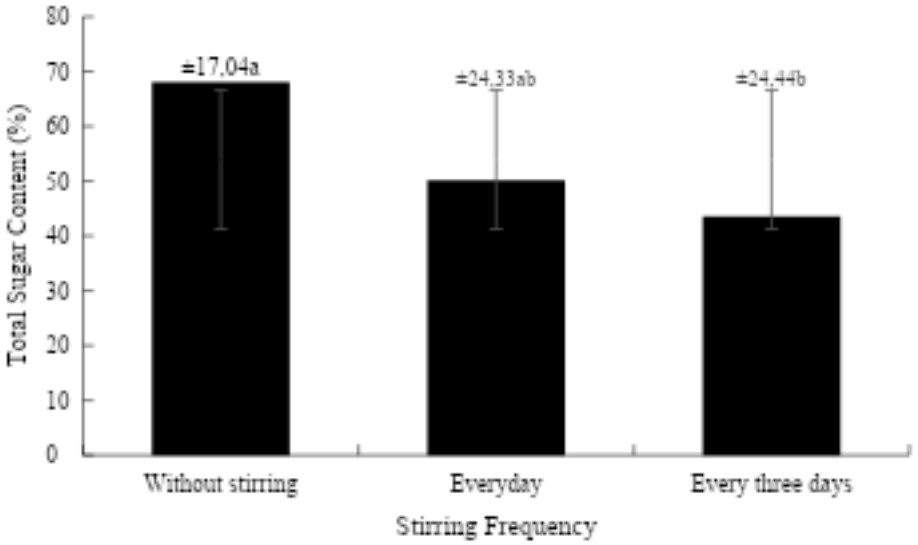

Figure 2. Effect of stirring frequency on total sugar content of coconut aminos

Starter concentration did not significantly affect the total sugar content of coconut aminos. This is likely because all samples were heated at the same temperature for the same duration. Heating plays a more dominant role in influencing total sugar content than starter concentration during fermentation. Although starter amounts varied, total sugar content tended to remain similar across treatments because the heating process had a stronger effect than microbial starter influence during fermentation.

3.4 Soluble Protein

The analysis of variance showed that starter concentration, stirring frequency, and the interaction between these two factors did not have a significant effect on the soluble protein content of coconut aminos. The average soluble protein content of the product was 0.84%. The lowest soluble protein content was 0.70%, while the highest was 1.27%. The lowest soluble protein content was found in the sample treated with 15% starter concentration and stirring every three days, whereas the highest soluble protein content was observed in the sample with 15% starter concentration without stirring. There were no significant differences in soluble protein content because all samples were heated at the same temperature. Heating

can cause protein denaturation, which involves the disruption of secondary and tertiary protein structures. This process changes the protein structure from folded into an unfolded form, thereby affecting its solubility (19).

The highest soluble protein content was obtained with the 15% starter concentration without stirring, whereas the lowest was obtained with the 15% starter concentration and stirring every three days. Stirring frequency is closely related to aeration and the distribution of substrate and cells during fermentation. Samples without stirring were more stable because the absence of agitation prevented significant movement of the substrate and microbial cells. In contrast, samples stirred every three days exhibited less uniform substrate and cell distribution due to infrequent agitation, resulting in less efficient protein hydrolysis (20).

4. Conclusion

Stirring frequency significantly affected the reducing sugar and total sugar content of coconut aminos. On the other hand, starter concentration had no significant effect on the characteristics of coconut aminos. The treatment without stirring produced the highest reducing sugar content (67.26%) and the highest total sugar content (68.09%).

5. Acknowledgement

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