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Physicochemical Quality and Antioxidant Enhancement of Jack Bean Sauce with Pineapple Extract Addition and Different Fermentation Time

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Abstract

Jack bean (*Canavalia ensiformis* L.) is one of the underutilized local Indonesian commodities that has the potential to be an alternative source to substitute soybean, which has been widely used as a source of protein. High nutritional value, such as essential amino acids, kaempferol glycoside, and higher protein content, is found in this legume. There are two prominent steps in soy sauce production that promote enzymatic reactions and enhance nutritional value. The first step is fermentation involving mold such as *Rhizopus* sp. called koji, and the second one is fermentation in the brine solution called moromi. However, the fermentation process entails a prolonged duration. The addition of another enzyme, such as bromelain from pineapple extract, is needed to accelerate the rate of enzymatic reactions. This study aims to determine the effect of bromelain addition from pineapple extract on the physicochemical and antioxidant quality of jack bean sauce. Bromelain was extracted from pineapple, which was added in the jack bean sauce fermentation process. Jack bean was inoculated with tempeh culture (*Rhizopus oryzae*), incubated for 3-4 days, and added with various bromelain concentrations. Significant differences in protein content, glutamic acid content, and antioxidant activity of jack bean sauce were reported, affected by bromelain concentrations and fermentation time. Moreover, a significant difference in pH was affected by bromelain concentrations. Total soluble solids were found to be similar and not significantly different. Fermentation time for 60 days and 5% addition of bromelain was the optimum condition for jack bean sauce, which exhibited the highest content of glutamic acid, protein content, and antioxidant activity.

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Introduction

Jack bean (*Canavalia ensiformis* L.) is widely grown in Indonesia and has become a local resource alternative in Indonesia instead of soybean due to its high level of protein. The protein content of jack bean is 33.12 – 33.97%, slightly higher than soybean (31.47 – 32.58%) (containing essential amino acids such as arginine, threonine, methionine, phenylalanine, isoleucine, leucine, valine, thryptophan, and histidine) (Yudiono, 2020; Agustia et al., 2023). Bioactive compound which is called kaempferol glycoside can be discovered in jack bean and has benefit to inhibit alpha glucosidase activity in a way has anti-diabetic potential (Sutedja et al., 2020). Jack bean has high in nutritional value such as high in carbohydrate (58.4%), protein (25.2%), but low in fat content (5.21%) (Okomoda et al., 2016). However, this legume has not been utilized and becomes one of 48 species of underutilized legumes in tropical country (Tiamiyu et al., 2016). Furthermore, there was an increase in soybean import from 2023 to 2024 (6,041,094 to 6,051,178 tons) (Pusat Data dan Sistem Informasi Pertanian,2024). Due to its excellence and high import of soybean, jack bean can be used as soy alternative in soy-based products such as soy sauce.

Fermentation for making soy sauce requires two steps which are called koji (dry condition) and moromi (wet condition, in brine). Koji process will hydrolyze several nutrients due to its enzymatic activity such as

protease, amylase, and other enzymes which contribute to final flavor of the products. Simple sugars which were produced during koji process will be hydrolyzed to form acetic acid and lactic acid in moromi process, resulting in pH decrease. Protease and amylase enzyme will convert protein into peptides and amino acids to bring distinct umami taste in soy sauce, one of them is glutamic acid (Widiantara, 2019; Setiani et al., 2024). The conversion of protein into peptides during soy sauce fermentation also induced the formation of bioactive peptides which have several functional benefits such as antioxidant (Daliri et al., 2017). The increase of antioxidant activity during soy sauce fermentation was reported by several studies (Gao et al., 2019a; Ham et al., 2019). Soy sauce production requires the long process of fermentation. However, the addition of protease enzyme can enhance its nutritional value, hydrolysis, functionality, and reducing fermentation time. The addition of protease enzyme during fermentation accelerates the breakdown of proteins into peptides and amino acids. This acceleration is crucial because traditional soy sauce fermentation is a slow process, often requiring extended periods to achieve desirable levels of protein hydrolysis, flavor development, and nutritional enhancement (Yao et al., 2023; Elhalis et al., 2024; Y. Liu et al., 2025). Protease enzyme such as bromelain which is can be obtained from pineapple is more affordable than other proteases (Putriana et al., 2020). In addition, bromelain which is plant-based enzyme is recognized as *halal* (*halal* means permissible and lawful for Moslem) compared to other general proteases such as pepsin, pancreatin, and trypsin which are derived from animal (Utami et al., 2019). Similar as other proteases, bromelain accelerates protein breakdown and soluble protein content. Aside having proteolytic enzyme, pineapple has sugar, vitamin B, vitamin A, citric acid and malic acid. The use of bromelain enzyme from pineapple extract for fermentation had been used in several study for fish sauce and sesbania seeds (Prasetyo et al., 2012; Asngad et al., 2015). In addition, bromelain had also been used for soybean-based peptone production and soybean-based milk replacer for lamb which result in higher protein digestibility, higher degree of hydrolysis, and higher soluble nitrogen (Utami et al., 2019; Putriana et al., 2020).

The utilization of jack bean had been conducted in several studies such as for protein hydrolysate using various proteases enzyme and tempeh (Andriati et al., 2018; Wijatniko and Murdiati, 2019; Zainol et al., 2020; Purwandari et al., 2024). The studies about jack bean sauce had been also conducted using combination of tofu waste and various salt concentration in which salt concentration of 20-25% is the optimal condition for fermentation (Garnida and Taufik, 2014; Widiantara, 2019). However, the prior studies was exploring about the optimal salt concentration and combination with other protein sources while the use of bromelain enzyme from pineapple extract for jack bean sauce fermentation and the study about its antioxidant activity has not been explored. Thus, this study aimed to investigate the effects of various pineapple extract concentration and different fermentation time on the physicochemical properties and antioxidant activity of jack bean sauce and determine the optimum condition of jack bean fermentation using pineapple extract.

Materials and Methods

Materials

Jack bean (*Canavalia ensiformis* L) seeds were collected from Wonosari, Gunung Kidul, Yogyakarta. Commercial tempeh culture (*Rhizopus oligosporus*) and unripe pineapple were purchased from Pasar Niten, Bantul, Yogyakarta. All chemicals used in this study are for analytical purposes such as DPPH (Sigma Aldrich) and ninhydrin (WARCHEM).

Research Methods

Preparation of Pineapple Extract

The preparation of Pineapple Extract was conducted using the method of Pang et al. (2020). The whole unripe pineapple was washed, and all unwanted parts were removed until the flesh was obtained. Approximately 100 g of pineapple flesh was cut into pieces and blended for 2 min with 100 mL of water. The extract was then filtered using a clean muslin cloth and stored at 5°C for further use.

Preparation of Jack Bean

Jack bean was prepared according to Puspitojati et al. (2019) with slight modifications in boiling time and the addition of sodium bicarbonate soaking. Jack bean seeds were soaked in 2% sodium bicarbonate solution (1:3) for 10 hours, washed thoroughly with water, and then soaked in water for 24 hours. The seeds were then boiled for 90 min, peeled, and soaked again for 48 hours. The soaked seeds were boiled again for 15 min and boiled Jack Bean seeds were obtained.

Preparation of Jack Bean Sauce

Jack bean sauce was prepared according to Muangthai et al. (2009) and Zahidah and Lo (2022) with two-phased fermentation (koji and moromi). Boiled Jack Bean seeds were inoculated with commercial tempeh culture (*Rhizopus oligosporus*) (2 g in 1000 g w/w) and incubated at room temperature for 3-4 days for koji fermentation. Approximately 125 g of fermented Jack Bean seed was then placed in a jar and fermented again with brine solution (20%) and pineapple extract concentrations of 0% (1000 mL of 20% brine solution), 5% (50 mL of pineapple extract and 950 mL of 20% brine solution), and 10% (100 mL of pineapple extract and 900 mL of 20% brine solution) for moromi fermentation. Moromi fermentation was conducted for 30 and 60 days. Afterward, the solution was filtered and cooked with 700 g of palm sugar. Jack Bean sauce was then stored at room temperature for further analysis.

Protein Content Analysis

The protein content of jack bean sauce was calculated as total nitrogen with Kjeldahl method according to AOAC (1995).

Glutamic Acid Content Analysis

Glutamic acid content was determined according to the method of Khokhani et al., 2011). Approximately 1 g of sample was diluted in 100 mL of distilled water. The mixture was then filtered and centrifuged until the sample extract was obtained. The sample extract (1 mL) was placed in the reaction tube

and 2 mL of ninhydrin solution was added. The mixture was then heated in the water bath for 30 min. The mixture was cooled, and 96% ethanol solution was added until a volume of 8 mL was obtained. The mixture was incubated for 15 min until the purple color was formed and the absorbance was measured with a wavelength of 520 nm. Ethanol solution (96%) was used instead of sample as a blank. The concentration of glutamic acid was calculated based on the standard curve of glutamic acid and glutamic acid content can be calculated using the equation as follow:

$$\text{Glutamic acid content (\%)} = \frac{\text{Concentration of sample x dilution factor}}{\text{Sample weight (mg)}} \times 100\%$$

Antioxidant Activity Analysis

Antioxidant activity was determined based on the method of DPPH Radical Scavenging Activity (Tang et al., 2023). Approximately 1 g of the sample was diluted in ethanol, and 1 mL of the diluted sample was then mixed with 200 µM DPPH. The mixture was incubated for 30 min in the dark room and was diluted again until a volume of 5 mL was obtained. The absorbance was measured with a wavelength of 517 nm. The mixture of DPPH (1 mL) and methanol (4 mL) was used as a blank. The antioxidant activity was calculated using the equation as follow:

$$\text{Antioxidant activity (\%)} = \frac{\text{OD blank} - \text{OD sample}}{\text{OD blank}} \times 100\%$$

pH Analysis

Approximately 10 mL of sample was placed into the beaker glass and the pH was measured using digital pH meter that had been calibrated using buffer 4.0 and 7.0 (Andriati et al., 2018).

Total Soluble Solid Analysis

The Total Soluble Solid (TSS) was measured using hand refractometer and expressed as °Brix (Chen et al., 2015).

Statistical Analysis

The data from different pineapple extract concentration were analysed using analysis of variance (ANOVA) with Duncan Multiple Range Test (DMRT) for further test. The data from different fermentation time were analysed using Independent T-Test. Data analysis was conducted using SPSS (22.0).

Results and Discussion

Protein Content

The protein content of the jack bean sauce can be seen in Figure 1. There were significant differences in protein content with different fermentation time and different pineapple extract concentration at fermentation time of 30 days and there was an interaction between different fermentation time and pineapple extract concentration ($p<0.05$). Protein content of jack bean increased as pineapple extract concentration rose up to 5%. Similar result was found in the study conducted by (Yarlina et al., 2024) in which protease activity can enhance the protein content based on total nitrogen content. Pineapple extract contains bromelain which is one of protease enzymes with a proteolytic activity and are able to breakdown protein into smaller pieces such

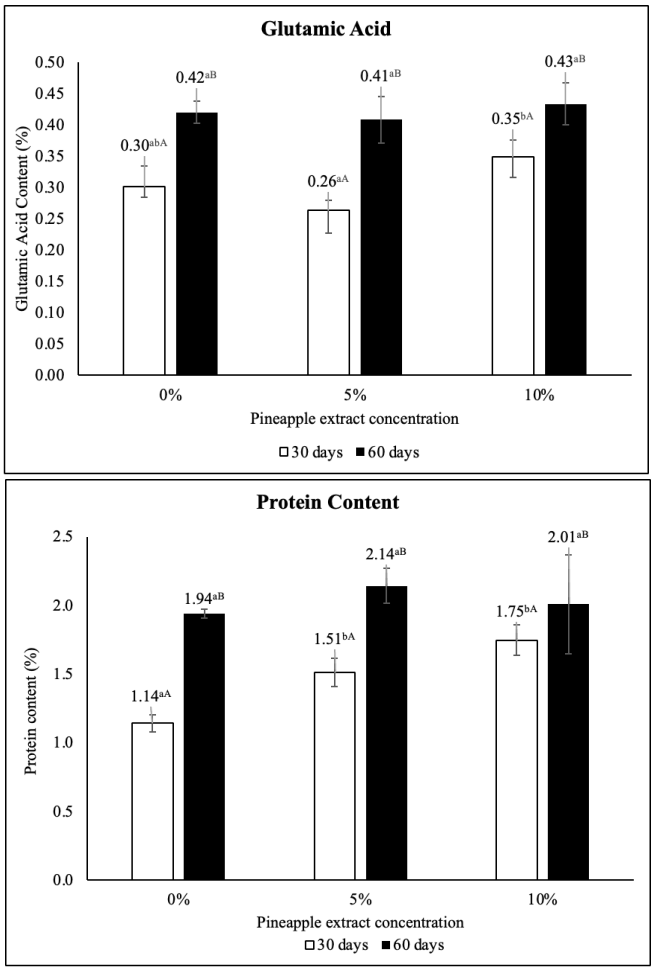

Figure 1. Protein Content and Glutamic Acid Content of Jack Bean Sauce in Different Pineapple Extract Concentration and Fermentation Time. Different letters in the label exhibit significant difference between groups (a,b for different pineapple extract concentration; A,B for different fermentation time).

as peptides and amino acids (Susanti et al., 2022). The rise of protein content can be related to the protein breakdown into peptides and amino acids facilitated by bromelain. In addition, bromelain can denaturate protein leading to the exposure of more sites for enzymatic cleavage resulting in higher degree of hydrolysis (Aziz et al., 2025; Zhu et al., 2025). The Kjeldahl method quantification is based on the total nitrogen content. When protein undergo the degradation into smaller pieces during fermentation process, the total nitrogen content increased (Yarlina et al., 2024). However, there is no significant difference in the protein content from the pineapple extract concentration 5% to 10%. This suggests that a 5% concentration was already sufficient to maximize the rate of protein hydrolysis, as the available protein substrate in the moromi was limited. Once this saturation point was reached, increasing the enzyme concentration further did not enhance protein breakdown, consistent with findings that higher enzyme levels accelerate hydrolysis only up to the point where substrate becomes the limiting factor (Hafizh et al., 2022; Setiani et al., 2024). This decrease also can be due to the denaturation of proteases in a high salt concentration leading to the reduction of its biological activity (Setiani et al., 2024).

Greater protein content was found in the

fermentation time of 60 days than 30 days. Study by (Gao et al., 2019b) revealed that there was an elevation on Free Amino Acids (FAA) and peptides during moromi fermentation in soy sauce with the most abundant molecular weight peptides of 1-3 kDa. This is relatable to the study conducted by (Tang et al., 2023) that fermentation process during soy sauce making increase the FAA due to the activity of proteases. FAA and peptides contributes to the total nitrogen content in a product (Kavian et al., 2022). Protein contents of jack bean sauce yielded in this study were ranged from 1.14 – 2.14%. This range met the Indonesian National Standard's (SNI 3543.1:2013) requirements in which minimal protein content for sweet soy sauce is 1% (Badan Standarisasi Nasional (BSN), 2013).

Glutamic Acid Content

The Glutamic Acid is one of non-essential amino acids which is contributed to the umami taste and can be formed during fermentation process. Glutamic acid contents of jack bean were ranged from 0.26 – 0.43% (Figure 1). There were significant differences in glutamic content with different fermentation time and different pineapple extract concentration at fermentation time of 30 days. However, there was no interaction between different fermentation time and pineapple extract concentration ($p<0.05$). Glutamic acid content of jack bean sauce increased from the addition of pineapple extract 5% to 10%. This is relatable with the study conducted by Listyaningrum et al. (2022) in which glutamic acid increases as pineapple extract concentration increased up to 15%. Glutamic acid was reported to be the most abundant amino acid produced during soy sauce fermentation (Tang et al., 2023). In addition, it is the most abundant amino acid in jack bean protein (15.8 g per 100 g proteins) which is most likely to be freely released during fermentation (Anuntagool and Soonthonsun, 2023). Protease activity of bromelain can breakdown protein into peptides and FAA resulting in enhancement of specific amino acids content such as glutamic acid (Susanti et al., 2022). Bromelain exhibit a wider activity and can hydrolyze various variety of protein leading to the increase of amino acid formation including glutamic acid (Orynbekov et al., 2024)

There was also an escalation in glutamic acid content of jack bean sauce with the increase of fermentation time. Glutamic acid content with the fermentation time of 60 days was greater than 30 days. Similar result was found on the study conducted by Wang et al. (2024) in which prolonged fermentation time significantly improve the glutamic acid content and its umami taste. The increase in glutamic acid content in prolonged fermentation is primarily attributed to the ongoing hydrolysis of existing proteins by the enzymes present during fermentation. As fermentation progresses, these enzymes continuously break down protein, releasing more glutamic acid into the substrate. This enzymatic activity is a key factor driving the accumulation of glutamic acid throughout the fermentation process. Protease bacterial produce enzyme which can hydrolyzed proteins into amino acids, peptides and small molecular weight proteins resulting in higher free amino acid content (X. Liu, 2019; Tang et al., 2023).

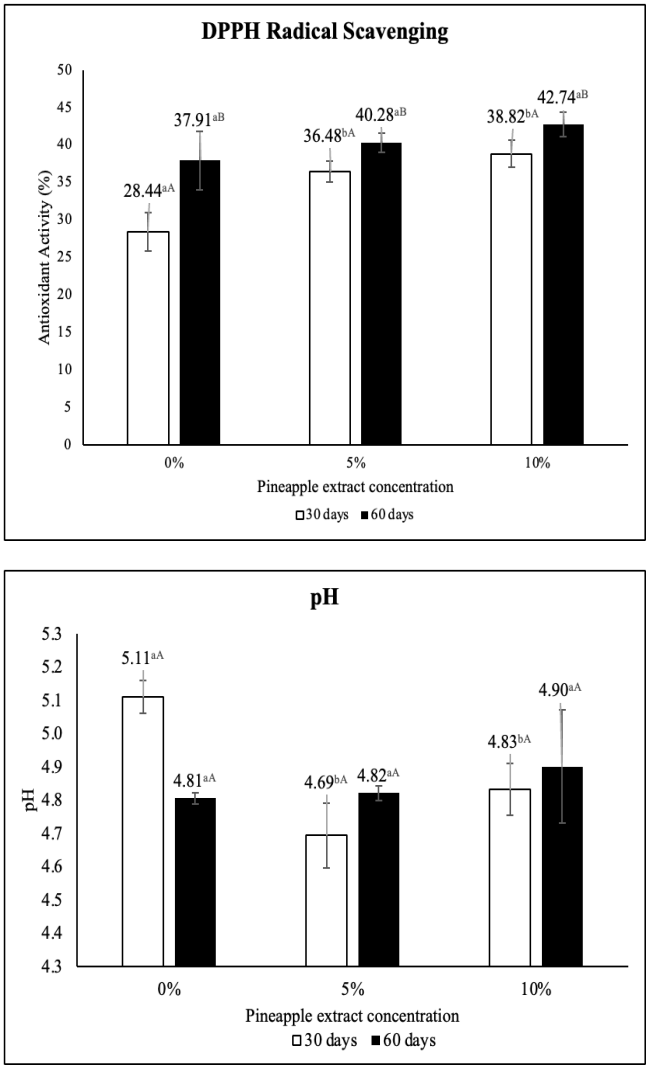

Figure 2. Antioxidant Activity and pH of Jack Bean Sauce in Different Pineapple Extract Concentration and Fermentation Time. Different letters in the label exhibit significant difference between groups (a,b for different pineapple extract concentration; A,B for different fermentation time).

Antioxidant Activity

The antioxidant activity of jack bean sauce samples was investigated using DPPH method. There were significant differences during fermentation and the addition of pineapple extract. However, there was no interaction between different fermentation time and pineapple extract concentration ($p<0.05$). Results on antioxidant activity are shown in Figure 2. Fermented samples for 60 days had higher antioxidant activity than samples which were fermented for 30 days. Samples with different concentrations of pineapple extract (0%, 5%, and 10%) were compared and showed higher concentrations resulted in higher antioxidant activity. Antioxidant activity increased during fermentation, notably with a longer fermentation time of 60 days. The addition of pineapple with different concentrations also influenced antioxidant activity. Bromelain can hydrolyze protein into smaller fraction caused the formation of bioactive peptides during fermentation (Daliri et al., 2017). Bromelain is endopeptidase enzyme which hydrolyze protein within its peptide chain and cleavage

Table 1. The total soluble solids (TSS) of jack bean sauce in different concentration of pineapple extract and fermentation time

Treatment	Total Soluble Solid (°Brix)
0% Pineapple extract	
30 days	76.83±2.25^{aA}
60 days	77.5±1.64 ^{aA}
5% Pineapple extract	
30 days	79.33±2.08 ^{aA}
60 days	75.67±2.08 ^{aA}
10% Pineapple extract	
30 days	76.67±2.47 ^{aA}
60 days	77.33±2.89 ^{aA}

Different letters in the label exhibit significant difference between groups (a,b for different pineapple extract concentration; A,B for different fermentation time

peptide bonds between aromatic, basic, and hydrophobic amino acids leading to the formation of antioxidant peptides (Johny et al., 2022; Venetikidou et al., 2025). This study similar with the results from (Gao et al., 2019a; Ham et al., 2019) in which antioxidant activity increased during moromi fermentation and can be applied to prevent lipid oxidation. Other results also exhibited by the study of (Carlos et al., 2017; Wijatniko and Murdiati, 2019) in which antioxidant activity increased due to the hydrolysis process.

In addition, same result was reported that fermentation in jack bean tempeh was responsible for the increased in antioxidant activity due to some antioxidant compounds from jack bean which then was changed functionally during fermentation, such as isoflavones and phenolic compounds (Tsalissavrina et al., 2023). Fermentation promotes the hydrolysis of glycosides, resulting in the release of substituted hydroxyl groups, which enhances the ability to capture free radicals. Isoflavones, which contain phenolic groups, can bind free radicals and donate a hydrogen atom (a proton and an electron). During fermentation, isoflavone glycosides and isoflavone aglycones were released from sugar due to the hydrolysis reaction of o-glycosidic bonds. Another specific isoflavone compound called factor-2 (6,7,4-trihydroxy isoflavone) was formed, which has strongest antioxidant properties than other forms of isoflavones. Flavonoids can be found in jack beans which are trapped in a matrix but then released during fermentation. Some studies also reported that antioxidant activity released along with the fermentation period due to the releasing of flavonoid compounds (Y. Chen et al., 2020; Lo et al., 2022; Tsalissavrina et al., 2023).

pH

The pH of jack bean sauce samples ranged from 4.69 – 5.11 (Figure 2). This range meets the Indonesian National Standard's (SNI 3543.1:2013) requirements inwhich pH range required for sweet soy sauce is 3.5 – 6.0 (Badan Standarisasi Nasional (BSN), 2013). The pH values for different pineapple extract concentration at fermentation time of 30 days were significantly different while pH with different fermentation time were not significantly different. However, there was an interaction between different fermentation time and pineapple extract concentration ($p<0.05$). The pH value of jack bean sauce decreased from the pineapple concentration extract of 0% (5.11) to 5% (4.69). Similar result was found in the study by Setiani et al. (2024) in which there is a decrease of pH during soy sauce fermentation. The decrease is relatable to the natural organic acids contained in pineapple extract as the concentration increased (Hafizh et al., 2022). In addition, during the moromi stage fermentation, the pH falling can be due to the activity of halophilic lactic acid bacteria which generate organic acids from the simple sugars (Setiani et al., 2024). Lactic acid bacteria fermentation during moromi stage was dominated by *Tetragenococcus halophilus* which produces lactic acids and acetic acids (Zhou et al., 2022). Protelytic activity of bromelain from pineapple extracts contributed to the breakdown of protein into free amino acids which can facilitate the growth of lactic acid bacteria to produce lactic acids and other organic acids (Sebastián-Nicolas et al., 2021).

Total Soluble Solids

The Total Soluble Solids (TSS) of jack bean sauce ranged from 75.67 – 79.33 (Table 1). Total soluble solids pointed to the total concentration of dissolved solid in a liquid system and is important to soy sauce quality. It contains bacteria and organic compounds (Lubis, 2019; Setiani et al., 2024). The improvement of TSS also can be related to the increase of free amino acids content (Setiani et al., 2024). The Total Soluble Solids of Jack Bean Sauce with different fermentation time and pineapple extract concentration were not significantly different and there was no interaction between different fermentation time and pineapple extract concentration ($p<0.05$).. This result is quietly similar as the study conducted by Setiani et al. (2024) in which there is no increase of total soluble solid in the final stage of fermentation. However, it is not consistent with the increase of the glutamic content in this study. However, this may be due to the other factors which can play a role in the rise of TSS. Based on the study by Tan et al. (2022), bacteria population had significant role in the increase of TSS.

Conclusion

This study demonstrated that both pineapple extract concentration and fermentation time significantly influenced the protein content, glutamic acid content, and antioxidant activity of jack bean sauce, with the combination of 5% pineapple extract and 60 days fermentation yielding the highest values protein content (2.14%), glutamic acid content (0.41%), and antioxidant

activity (40.28%). In contrast, total soluble solids remained unchanged across treatments, and only pineapple extract concentration significantly affected pH. These results suggest that incorporating bromelain-rich pineapple extract and optimizing fermentation duration can enhance the nutritional and functional qualities of jack bean sauce, making it a promising plant-based seasoning with potential applications in the development of high-protein, umami-rich, and antioxidant-enriched food products. However, the study was limited by the scope of pineapple extract concentrations and fermentation times tested, as well as the lack of sensory evaluation and shelf-life analysis. Future research should explore a broader range of process variables and assess consumer acceptance and product stability to fully realize the commercial potential of jack bean sauce.

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