Vol. 14, No. 6 (2025): 2229 - 2238 http://dx.doi.org/10.23960/jtepl.v14i6.2229-2238

JURNAL TEKNIK PERTANIAN LAMPUNG

ISSN 2302-559X (print) / 2549-0818 (online)
Journal homepage : https://jurnal.fp.unila.ac.id/index.php/JTP

Effect of Packaging on Protein Content, pH, and Sensory of Probiotic Tempeh Juice During Cold Storage

Nur Aini Sahara[1], Attika Nur Aisah[1], Maria Erna Kustyawati[1,✉], Sussi Astuti[1], Wisnu Satyajaya[1]

[1] Department of Agricultural Product Technology, Faculty of Agriculture, Universitas Lampung, Bandar Lampung, INDONESIA.

Article History:

Received : 02 July 2025
Revised : 01 August 2025
Accepted : 29 August 2025

Keywords:

Cold storage,
Packaging,
Protein content,
Shelf life,
Tempeh juice.

Corresponding Author:
✉ maria.erna@fp.unila.ac.id
(Maria Erna Kustyawati)

ABSTRACT

Tempeh juice with probiotics is considered as functional probiotic foods with health benefits. This study aimed to evaluate the effect of packaging type (glass bottle and aluminum foil), and cold storage duration on chemical, microbial and sensory properties of probiotic tempeh juice. The research used a two-factorial Randomized Complete Block Design with three replications. ANOVA was used to analyze the data and it was followed by DMRT test at 5% significance level. The results showed that storage duration had no significant effect on viscosity and other chemical analysis, but was significant on sensory attributes of color, aroma, taste, and overall acceptability. Protein decreased on the 8th day probably due to protein hydrolysis by microbial activity, but increased on the 12th day of storage due to the accumulation of microorganism cell walls during storage. However, sensory changes included a paler color, a stronger fermentation aroma, an acidic alcoholic taste, and overall acceptance was acceptable. It was concluded that glass bottle packaging was more effective in maintaining the stability of protein content, pH, and sensory of probiotic tempeh juice beverage compared to the aluminum foil standing pouch with a spout, with the estimated shelf life was 8 days in cold storage.

1. INTRODUCTION

Probiotic beverages are functional beverage enriched with live microorganisms or probiotics (Dhahana *et al*., 2021). They are considered functional foods if they contain at least 10^6 cells per g/ml of probiotics after consumption. Probiotic beverage has benefits in increasing endurance and digestive health. Probiotics in food can improve health by optimizing mucosal performance, balancing nutrient absorption and gut microbiota, thereby enhancing the host's immune system (Labiba *et al*., 2020). The benefits of probiotics come not only from live microorganisms, but also from parabiotics, inactive cells or their fractions, and posbiotics, secreted metabolites (Teame *et al*., 2020). Selected microorganisms classified as probiotics include lactic acid bacteria (LAB), Bifidobacteria, *Bacillus subtilis*, and *Saccharomyces cerevisiae* and *Saccharomyces boulardii* (Kustyawati *et al*., 2022). Probiotic beverage are generally fermented milk products; however, tempeh also has the potential to be processed into probiotic beverage by adding probiotic microbes during fermentation. Based on Purry & Rafiony (2018), the principle of making tempeh juice beverage is the extraction of tempeh with water to obtain a solution with a soluble solid component. Tempeh juice beverage contain protein, carbohydrates, fat, vitamins and isoflavones (Bintari *et al*., 2022), but *Mosaccha* tempeh, tempeh fermented with the addition of *Saccharomyces cerevisiae* to *Rhizopus oligosporus* starter, disguise beany aroma and has β-glucan offered an antioxidant activity (Rizal & Kustyawati, 2019).

The quality of food products can easily change during storage, thus requiring special attention to packaging. Packaging is a material that wraps or containers food products (Budiningrum *et al*., 2022) that protect products from physical, chemical and biological damage, maintain temperature, humidity and light, increase shelf life and facilitate distribution. Glass packaging materials have been used for some time to package beverage products and have proven to be superior in terms of extending the shelf life of certain products compared to PE and PP plastic packaging (Rosmawati *et al*., 2021). Glass bottles have *inert* properties and are not permeable to gas (Nilakrisna *et al*., 2024). The aluminum foil standing pouch with a spout also has good barrier properties against water vapor and oxygen, although with potential small molecule permeability. The differences in permeability characteristics and interactions of each packaging type have the potential to affect product quality. Fermentation of tempeh juice with the addition of inulin, *Saccharomyces cerevisiae* and *Lactobacillus casei* produces probiotic juice with a fragrant aroma, fizzy taste and as a source of protein that is likely to change during storage. Therefore, it is very important to reveal the influence of the type of packaging material used to store the product during storage. This study was to evaluate the effectiveness of glass bottle and aluminum foil standing pouch with a spout packaging in maintaining the stability of protein, pH, and sensory (color, aroma, taste, overall acceptance) of probiotic tempeh juice beverage during cold storage.

2. RESEARCH MATERIALS AND METHODS

2.1. Material

This study used *Mosaccha* tempeh as the main ingredient, and additional ingredients included sugar, inulin, *Saccharomyces cerevisiae* culture, *Lactobacillus casei* culture, deMan Rogosa Sharpie agar (MRSA), Malt Extract Agar (MEA) and Nutrient Agar (NA) media, yeast extract agar, peptone, and KH_2PO_4. Chemical analysis used buffer solution, H_2SO_4, Na_2SO_4, HgO, NaOH, Na_2SO_3, Na_2SO_3, H_3BO_3, methylene red/blue indicator, 0.02 N HCl, and distilled water.

The equipment used in this experiment was a measuring cup, beaker, pH meter, Kjeldahl apparatus, burette, volume pipette, and sensory test equipment as well as a tempeh juice maker and filters (200 mesh). Culture preparation involved test tubes, racks, vortex, Erlenmeyer, petri dish, glass beaker, stirring rod, drop pipette, ose needle, Bunsen, laminar air flow, autoclave, and incubator. Glass bottles and aluminum foil standing pouch with a spout 500 ml, and refrigerator were prepared for storage study.

2.2. Methods

This study used a 2-factorial Randomized Complete Block Design (RCBD) with 3 replicates to analyze the effect of two factors: type of packaging (glass bottle and aluminum foil standing pouch with a spout) and length of storage of probiotic tempeh juice beverage in refrigerator at temperature (5°C) in 4 levels of 0 days (P0), 4 days (P4), 8 days (P8), and 12 days (P12) on protein content, pH, and sensory (color, aroma, taste, overall acceptance of probiotic tempeh juice. Data were tested for Bartlett's homogeneity, then analyzed using ANOVA 5% to see significant effect of the treatments, and followed by DMRT 5% further test.

2.3. Research Implementation

2.3.1. Preparation of *Saccharomyces cerevisiae*

1 g of dry yeast containing *S. cerevisiae* was taken, then homogenized with 9 ml of sterile distilled water in a test tube. One ose was taken to be inoculated into a Petri dish containing MEA media (18 ml), then incubated at 25°C for 24 hours. After incubation, an ose of pure culture colony *of S. cerevisiae* was taken to be cultured into a test tube containing NA slanted medium (7 ml), then incubated at 25°C for 24 hours.

2.3.2. Preparation of *Lactobacillus casei* Culture

One ose of *L. casei* culture was taken, then put into a petri dish containing MRSA media (18 ml). Next, it was incubated at 37°C for 24 hours. After incubation, 1 ose of pure culture colony of *L. casei* was taken to be cultured into a test tube containing NA slant medium (7 ml), then incubated at 37°C for 24 hours.

2.3.3. Preparation of Working Culture or Starter Culture

The working culture of *S. cerevisiae* and *Lactobacillus casei* as fermentation culture in tempeh juice beverage was pure tempeh juice beverage without other ingredients. Exactly 100 g of *Mosaccha* tempeh was reduced in size, then blanched in boiling water (100°C) for 5 min, and drained. Tempeh was pulverized using a blender for 45 s, adding 500 mL of distilled water (tempeh : water = 1 : 5). Tempeh juice was boiled for 10 min, then filtered and put into a 100 mL bottle to add several colonies of *S.cerevisiae* or *L.casei* cultures that have been dissolved in 5 mL of 0.85% physiological solution, 0.32 g yeast extract agar, 0.53 g peptone, and 0.11 g KH_2PO_4. The working culture was homogenized, then put into glass bottles and incubated for 24 h at 30°C.

2.3.4. Preparation of Probiotic Tempeh Juice Beverage

Tempeh as much as 200 g was blanched at boiling water temperature for 5 minutes to remove the unpleasant odor of soybeans through inactivation of the lipoxygenase enzyme. Next was smoothing with a blender accompanied by the addition of water with a composition of tempeh: water = 1 : 5, for 45 seconds and continued with heating for 10 min and followed by the addition of granulated sugar as much as 5% of the total volume weight and when it was cold a 0.25% inulin (w/v) was added. Furthermore, filtration was carried out to separate the tempeh dregs and tempeh juice water and continued with inoculation of 1% (v/v) *L. casei* and 0.5% (v/v) *S. cerevisiae*. Fermentation was in an incubator at a 30°C for 24 hours. The addition of sugar can balance acidity, maintain pH and improve sensory quality, while inulin can maintain protein stability, support probiotic growth, and improve viscosity (Fikriyah *et al*., 2024).

2.3.5. Storage Study of Probiotic Tempeh Juice

Storage of probiotic tempeh juice was carried out after the fermented tempeh juice was produced and analyzed for its characteristic. It then was packed in glass bottles (K1) and aluminum foil standing pouch with a spout (K2) of 400 ml each. Samples were stored in a refrigerator at 5°C. Storage was carried out for 12 days with observation intervals carried out according to the treatment, they were 0 days (P0), 4 days (P4), 8 days (P8), and 12 days (P12). During storage, changes in protein content, pH, and sensory parameters (color, aroma, taste, and overall acceptance) were monitored.

2.4. Observation

2.4.1. Chemical and physical analysis

Chemical and physical analysis included protein, pH, and viscosity, respectively. The protein content of probiotic tempeh juice was analyzed using the Indophenol blue method to determine total N and the conversion factor from N content to protein content based on Sudarmadji *et al*. (1997). The protein content of probiotic tempeh juice beverage was carried out by the Kjeldahl semimicro method. The principle of this method involves the process of protein destruction into ammonia, then ammonia is converted into ammonium sulfate, followed by the release of ammonia through distillation with NaOH. The released ammonia is captured by boric acid to form an ammonium borate complex, which is then titrated with HCl to determine the amount of total nitrogen. A 10 ml sample was put into a 100 ml volumetric flask and diluted with distilled water to the mark. The solution was taken 10 ml, put into a 500 ml Kjeldahl flask and added 10 ml of H_2SO_4. Catalyst mixture of Na_2SO_4-HgO (20 : 1) was added as much as 5 g, and the solution was boiled until clear and continued boiling for 30 minutes. After cooling, the wall of the Kjeldahl flask was washed with distilled water and boiled again for 30 minutes. After cooling, 140 ml of distilled water was added and as well 35 ml of NaOH-Na_2SO_3solution, then homogenized until dissolved. The distillate was collected to 100 ml in an Erlenmeyer containing 25 ml of saturated boric acid solution (H_3BO_3) and added a few drops of methylene red/blue indicator, then titrated with 0.02 N HCl.

The pH meter was calibrated with a buffer solution of pH 4 and 7. Then, the pH meter was dipped in the sample of probiotic drink of tempeh juice. The number on the pH meter is waited until stable, the results are recorded, then the electrode is rinsed with distilled water. Measurement of the viscosity of probiotic tempeh juice drink was carried out using an Ostwald U viscometer. Viscosity is the measure of a fluid's resistance to flow in Pascal-second (Pa·s) or Poise (P), with 1 Pa·s being equal to 10 Poise.

2.4.2. Total microbial count

The total microbial count was calculated by taking 1 mL of sample and putting it into a solvent (0.85% NaCl), homogenized, then diluted to 10^{-7}. A total of 1 mL of sample dilutions 10^{-5}, 10^{-6}, and 10^{-7} were sprinkled on petri dishes containing MEA and MRSA to calculate the growth of *S.cerevisiae* and *L. casei*. Then, the petri dishes were incubated for 24-48 hours at 25°C and 37°C.

2.4.3. Sensory Test

The sensory test of probiotic tempeh juice beverage was conducted through hedonic test based on the level of panelists' preference based on SNI 01-2346-2006 (BSN, 2006). Untrained panelists consisting of 40 students majoring in Agricultural Product Technology, University of Lampung who had taken sensory courses were involved in the testing. The same panelists conducted each observation time (day 0, 4, 8, and 12). The observed parameters included color, aroma, taste, and overall acceptance of the product with a scale of 7 = very like, 6 = like, 5 = somewhat like, 4 = neutral, 3 = somewhat dislike, 2 = dislike, and 1 = very dislike.

3. RESULTS AND DISCUSSION

The parameters observed in this study were protein and pH levels, which were significantly affected by the storage time factor. The further test of the DMRT 5% of protein content and pH of probiotic tempeh juice beverages are presented in Table 1. It shows that the storage time significantly affected on the changes in protein content and pH of probiotic tempeh juice.

Table 1. The data of protein, pH, microbial load and viscosity of the probiotic tempeh juice in various types of packaging and storage time and its DMRT analysis.

Treatment	Protein (% wb)±Sd	pH±Sd	Microbial load (cfu/mL)±Sd	Viscosity (cPas)±Sd
K1P0 (glass bottle. 0 days storage)	3.66±0.09[a]	4.58±0.20[b]	7.21±0.59[a]	0.31±0.12[a]
K1P4 (glass bottle. 4 days storage)	4.23±0.10[a]	4.49±0.05[ab]	7.23±0.74[a]	0.36±0.1[b]
K1P8 (glass bottle. 8 days storage)	3.87±0.52[a]	4.46±0.01[ab]	7.19±0.83[a]	0.33±0.18[a]
K1P12 (glass bottle. 12 days storage)	4.88±0.90[b]	4.38±0.17[a]	7.15±0.51[a]	0.41±0.12[b]
K2P0 (aluminium foil pouch. 0 day storage)	3.63±0.05[a]	4.57±0.21[b]	7.55 ±0.41 [a]	0.50± 0.21 [b]
K2P4 (aluminium foil pouch. 4 days storage)	3.93±0.18[a]	4.35±0.04[a]	7.61± 0.21 [a]	0.47± 0.12 [b]
K2P8 (aluminium foil pouch. 8 days storage)	3.81±0.75[a]	4.33±0.03[a]	7.90 ±0.12 [a]	0.44 ±0.32 [a]
K2P12 (aluminium foil pouch. 12 days storage)	4.95±0.54[b]	4.37±0.06[a]	7.83± 0.16 [a]	0.39± 0.32 [ab]

Note: Numbers followed by the same letter in same column indicate no difference in the 5% DMRT test.

3.1. Protein, microbial load and viscosity

The results of the analysis of variance (p-value <0.05) showed that the storage time had a very significant effect on the protein content of probiotic tempeh juice beverages, while the packaging type factor and the interaction of the two factors did not have a significant influence. Based on DMRT test $\alpha = 5\%$ (Table 1), the protein content at P0 was different from P4 and P12. Protein levels increased on days 4 and 12, while it decreased on day 8. Protein changes during cold storage can be caused by several factors, including growth of microorganisms and chemical reactions influenced by temperature, time, and packaging. The increased protein in the probiotic tempeh juice could be some reasons including the use of *Mosaccha* tempeh which contain high protein, 17.4% (Rizal & Kustyawati, 2019); and accumulation of the cell wall of microorganisms involved during fermentation. Darmawan *et al*. (2023) found that bacterial cell lysis contributes to the increase in protein. While Walker & Stewart (2016) suggested that *S. cerevisiae* plays a role in increasing protein through anabolic pathways due to the availability of amino acids, Lozančić *et al*. (2021) reported that about 50 - 60% of protein is contained in the cell wall of *Saccharomyces cerevisiae*. Pradana *et al*. (2018) in their research on yogurt tempeh, found that addition of tempeh in yogurt making increased the protein content (19.3 g/100 g), and clarified that during the fermentation process lactic acid bacteria (LAB) might not break down protein but instead utilized fat. On the other hand, the decrease of protein can be caused by microbial activity during fermentation, including production of proteolytic enzyme which degrades protein into amino acids, peptides and NH_3, thus causing protein solubility. Muchtar & Hastian (2020) found that the longer the storage at low temperatures, the lower the protein content of tofu, which is likely caused by the decomposition of the protein bond chain and as a result the protein becomes soluble in water. Rizqiati *et al*. (2021) explained that protein hydrolysis into amino acids may facilitate metabolism and release nitrogen which in turn lesser nitrogen during titration in analyzing protein. Protein denaturation may unlikely to occur due to acidity (pH 4) which has little influence on the denaturation process, and also cold storage condition.

The addition of inulin into the probiotic drink of tempeh juice in this research may help enhance the growth of *S.cerevisiae*, since inulin served as prebiotic supports the growth of probiotic *S.cerevisiae*. the increase growth of microorganism may improve the protein through the cell wall lysis.

Viscosity of the probiotic tempeh juice was a value of particles 'resistance to flow, how thick or thin it is. Protein concentration and pH of the solution correlate to viscosity, of which as protein concentration was higher, the viscosity increases due protein-protein interaction such as hydrophobic interaction, van der Walls forces, hydrogen bonding leading to a more resistant flow and create a more viscous solution (Aranda-González *et al*., 2016). The pH affects viscosity by altering the protein's charge and conformation leading to increase viscosity. Probiotic tempeh juice stored at 12 days has high viscosity but it was low at the beginning of storage (day 0) (Table 1). During storage, microorganisms break down carbohydrates, protein, fats into simple charged molecules that can interact with each other, resulting in a more viscous solution. Some organic acids may also produce which can lower the pH of the solution causing an increase in viscosity through the protein conformation.

3.2. pH

The results of the analysis of variance (p-value < 0.05) showed that the storage time had a significant effect on the pH while the packaging factor and the interaction of the two factors did not have a significant influence. The results of the DMRT α = 5% (Table 1), the pH at 0 days storage was different from 4, 8, and 12 days of storage. The decrease pH in this experiment can be as a result of microbial activity of *S. cerevisiae and L. casei*. During cold storage, psychrophilic microorganisms were able to grow and produce acid resulting in reducing pH. *Lactobacillus casei* produces amylase, lactase which break down carbohydrate to produce organic acids. *S.cerevisiae* produces zymase to break down alcohol into organic acids. Lactic acid bacteria continued to grow although it slowed down during cold storage causing an increase in acid levels (Archadiya *et al*., 2021). Tefa *et al*. (2023), microbial activity degraded sugar from the fruit and produce organic acid, such as acetic and pyruvic acid which affected the pH of the must in wine fermentation. LAB, Gram-positive bacteria ferment carbohydrates into lactic acid (Sukmaningrum *et al*., 2021), and thus increase in organic acids indicated the breakdown of carbohydrates by microorganisms, resulting in a decrease in pH. Sabil *et al*. (2023) stated that the high addition of *Lactobacillus casei* correlated with an increase in total lactic acid, hence increasing acidity and lowering pH. Nilakrisna *et al*. (2024) explained that acid can be formed from the spontaneous reaction between CO_2 and H_2O, where CO_2 is produced from the decomposition of sucrose by microbes. According to Rosmawati *et al*. (2021), *S.cerevisiae* plays a role in fermentation by producing the enzymes amylase (breaks down starch), zymase (converts sugar into ethanol). Moreover, it was likely that the maximum utilization of sugar by microbes during fermentation of probiotic tempeh juice could result in lowering pH. According to Khazalina *et al*. (2020), invertase of *S. cerevisiae* breaks down glycosidic bond in sucrose and produce glucose and fructose, then converts glucose into organic acids to reduce pH in robusta coffee fermentation. According to Pangestu *et al*. (2021), sucrose in yogurt is utilized by *Lactobacillus casei* as nutrients so that sucrose was broken down into glucose and fructose before being fermented into lactic acid.

3.3. Sensory Analysis

Sensory analysis of probiotic tempeh juice during cold storage was conducted by hedonic test with the attribute of color, aroma, taste, and overall acceptance on a scale of 1 - 7 (strongly disliked - strongly liked). Table 2 showed the interaction between [packaging type and storage time on probiotic tempeh juice.

3.3.1. Color

Color is an important initial impression for product acceptance (Tursina *et al*., 2019). Analysis of variance (p-value < 0.05) showed that between treatments had a significant effect on the color assessment of probiotic tempeh juice beverage. Panelists' assessment of color was greatly influenced by the type of packaging and storage period (DMRT test on α = 5% in Table 2). The color assessment of products in glass bottle packaging was generally better (rather like = 5) compared to aluminum foil standing pouch (neutral = 4). However, the assessment of color based on storage period and type of packaging showed no significant differences somewhat in the range of neutral (4) and somewhat like (5) from out of the highest value of very like (7). The product color was yellowish at the beginning of storage until the 4th day

Table 2. Effect of packaging and storage time on sensory analysis of probiotic tempeh juice (DMRT test at 5%)

Treatment	Attributes			
	Color	Aroma	Taste	Overall Acceptance
K1P0	5.10 ± 1.13^{c}	3.68 ± 1.67^{bc}	4.28 ± 1.45^{d}	4.28 ± 1.55^{d}
K2P0	4.56 ± 1.38^{ab}	2.55 ± 1.39^{a}	2.93 ± 1.26^{a}	3.08 ± 1.22^{a}
K1P4	5.05 ± 1.06^{bc}	4.20 ± 1.28^{cd}	4.23 ± 1.34^{d}	4.58 ± 1.23^{d}
K2P4	4.75 ± 1.10^{abc}	3.70 ± 1.10^{bc}	3.50 ± 1.06^{bc}	4.28 ± 1.17^{bc}
K1P8	4.78 ± 1.23^{abc}	4.00 ± 1.46^{cd}	4.18 ± 1.43^{d}	4.50 ± 1.34^{d}
K2P8	4.38 ± 1.21^{a}	3.28 ± 1.37^{b}	3.18 ± 1.41^{ab}	3.58 ± 1.25^{ab}
K1P12	4.78 ± 1.27^{abc}	4.45 ± 1.13^{d}	4.35 ± 1.44^{d}	4.83 ± 1.19^{d}
K2P12	4.53 ± 1.22^{a}	3.65 ± 1.31^{bc}	3.95 ± 1.58^{cd}	4.10 ± 1.31^{cd}

DMRT 5%: 2.772; 2.918; 3.017; 3.089; 3.146; 3.193; 3.232

Note: Number of followed by the same letter indicate no significant difference in the DMRT test at the 5% level. K1 (Glass bottle, 0 days), K2 (aluminum foil standing pouch with a spout) with storage levels of P0, P4, P8 and P12 were at 0, 4, 8, and 12 days, respectively. Hedonic scale of 7 is very like, 6 is like, 5 is somewhat like, 4 is neutral, 3 is somewhat dislike, 2 =is dislike, and 1 is strongly dislike.

and gradually changed to pale yellow and cloudy on the 8th to 12th day (Figure 1). The yellowish color of the fermented tempeh juice drink at the beginning of storage may be caused by the isoflavone found in tempeh. During storage, isoflavones degrade and dissolve in water due to microbial activity and turn pale yellow in tempeh juice solution (Abdullah & Asriati, 2016).

The changes of color can be influenced by the microbial activity and the pH in the beverage product. *S.cerevisiae* and *L.casei* degrade macromolecular components such as proteins, carbohydrates and fats into basic components such as amino acids, peptides, saccharides, thus causing turbidity due to ionization of basic compounds at low pH (4) in the probiotic tempeh juice. Basic compounds will ionize into positive ions/cations and easily dissolve in acidic solutions, whereas in high pH solutions, basic compounds will not ionize and are less soluble (Santoso *et al*., 2022). Mohammed (2015), that at acidic pH, particles in solution are more easily dissolved and cause turbidity, while at alkaline pH (6 - 8) precipitation occurs. This phenomenon occurs in probiotic tempeh juice drinks in glass bottles and stored for 12 days with a stable bright yellow color. This is likely the role of inulin as a prebiotic that supports the growth of probiotics that convert inulin into organic acids lowering the pH of the prebiotic tempeh juice. Therefore, it can be said that glass bottles packaging was better as it was able to maintain color stability.

Figure 1. Probiotic sari tempeh juice after storage showing a little color change.

3.3.2. Aroma

Analysis of variance (p-value < 0.05) showed that between treatments had a very significant effect on the aroma assessment of probiotic tempeh juice beverages. The results of the DMRT further test α = 5% (Table 2), showed that aroma assessment of K1P0 was difference from K2P0, K2P0 from K2P8 and K1P12. Overall, the panelists' assessment of the aroma of the product in the glass bottle was better than that of the aluminum foil standing bag. The product with a fermented aroma was preferred compared to the musty and sharp alcoholic aroma arising from the product stored in the aluminum foil standing bag. The research was in line with Nirmalasari *et al*. (2023), found that panelists liked the aroma of probiotic jackfruit seed milk which was not too pungent or sharp. The aroma produced by the probiotic tempeh juice likely comes from metabolite products resulting from the growth activity of *S.cerevisiae* and *L.casei*, including organic acid, carbon dioxide, and some alcohol. According to Ayun *et al*. (2023), storage time was positively correlated with an increase in acid and alcohol content. Tursina *et al*. (2019) stated that the higher the acid, the stronger the distinctive aroma of fermented beverages and proportional to the increase in the number of microbes during storage. In line with Nurman *et al*. (2020), the formation of the distinctive aroma of yogurt is influenced by lactic acid and carbonyl compounds such as acetaldehyde. Udin *et al*. (2020) *Saccharomyces cerevisiae* plays a role in alcohol production through the breakdown of carbohydrates in the presence of the enzyme zymase. Lestari *et al*. (2021), LAB and yeast in corn milk kefir produce compounds such as lactic acid, ethanol, and acetaldehyde, as well as carbon dioxide, which contribute to the distinctive aroma and flavor of kefir.

3.3.3. Taste

Analysis of variance (p-value <0.05) showed that between treatments had a very significant effect on the taste of probiotic tempeh juice. Further DMRT test ($\alpha = 5\%$, Table 2), there were differences in the taste of probiotic beverages stored for 0, 4, and 8 days between glass bottle packaging and aluminum foil standing pouch with a spout, but the difference is not significant after 12 days of storage. The taste of probiotic tempeh juice on day 0 was still predominantly sweet, but changed to sour and slightly alcoholic along with the length of storage due to the presence of hydrolysis products of *S.cerevisiae* and *L.casei* such as amino acids, esters, and alcohol. According to Rizal & Kustyawati (2019), *Saccharomyces cerevisiae* in *Mosaccha* tempeh break down protein and fat to produce compounds such as amino acids, esters, and alcohols, covering the sour taste and create a distinctive aroma of fermentation. Beany taste in soy fermentation which may be caused the hydrolysis production of by lipoxygenase did not arise due to *blanching* or short heating serving to inactivate the enzyme (Purry & Rafiony, 2018).

The research was in line with Lailia *et al*. (2023) and Musoffin *et al*. (2024) found that LAB broke down glucose into lactic acid in yoghurt. Tefa *et al*. (2023) mentioned that the high glucose in fermented materials encourages yeasts (such as *Saccharomyces cerevisiae*) to produce high concentrations of alcohol, such as in *wine* fermentation. The strong sour taste in fermented products such as kombucha comes from the metabolism of sucrose into organic acids by bacteria and yeasts (Nurhayati *et al*., 2020). In addition, *Saccharomyces cerevisiae* can stimulate the growth of lactic acid bacteria (LAB) by breaking down sugars, then lowering the pH of products such as kefir (Rossi *et al*., 2016). Overall, prebiotic tempeh juice in the bottle glass with slightly sour and alcoholic taste (K1P12) was somewhat like by the panelist.

3.3.4. Overall Acceptance

Analysis of variance (p-value <0.05) showed that between treatments had a very significant effect on the overall acceptance of probiotic tempeh juice beverage. DMRT test at α 5% (Table 2) showed that there were differences in the overall acceptance of probiotic tempeh juice stored for 0, 4, and 8 days between glass bottle packaging and aluminum foil standing pouch, but the difference was not significant after 12 days of storage. The hedonic value of overall acceptance of the tempeh juice probiotic drink ranged from 3.08 (slightly dislike) to 4.83 (neutral) with a tendency to increase in favorability with the longer storage time. The highest overall acceptance value was in the beverage stored for 12 days in glass bottles, indicating very distinctive fermentation characteristics and good acceptance by panelists.

3.4. Determination of the Shelf Life of Probiotic Tempeh Juice Beverage

Probiotic tempeh juice beverage is thought to be a functional food because it contains *Saccharomyces cerevisiae*, *Lactobacillus casei*, and proteins with physiological functions. Its health benefit was by increasing endurance and digestive health, and has immunomodulatory and antimicrobial functions from bioactive peptides (Isnaeni *et al*., 2016; Nabilah *et al.,* 2022). Therefore, maintaining its nutritional quality during storage is very important. One of which was through the extended storage studies (ESS) method. ESS conventionally determines product shelf life by monitoring sensory and nutritional quality until unfit for consumption (Tritisari *et al*., 2024). Using ESS method, it showed that protein content and pH was influenced by the storage time regardless type of packaging either glass bottle or alu foil standing pouch. However, based on sensory evaluation probiotic tempeh juice in the glass bottle had better panellist assessment on color, aroma, taste and overall acceptance after stored at 12 days. In addition, pale yellow, cloudy and sour-musty taste were the given sensory assessment by the panellists on the probiotic tempeh juice in the alu foil standing pouch stored at 12 days. According to Usman *et al*., (2023), sensory assessment was subjective and reflects individual preferences. However, referring to sensory analysis, the products were acceptable by the panellist assessment (score of 4.83 and 4.10, Table 2); chemical analysis showed that protein content, pH, microbial load were stable during storage. There is only a slight change in viscosity on day 12. Therefore, a shelf life of 8 days was recommended to maintain optimal sensory quality. In line with Abdullah & Asriati (2016) found that tempeh juice beverage with the addition of vanilla flavor can maintain quality for up to 7 days at 4 °C in glass bottles.

3.5. Determination of the Best Packaging for ProbioticTempeh Juice Beverage

Determination of the best packaging for probiotic tempeh juice beverages during cold storage can be based on changes in protein content, pH, and sensory. The changes that occur are due to the activity of microorganisms, so quantitative monitoring of LAB and yeast is important to validate the probiotic effect and understand chemical and sensory changes during storage. According to Nilakrisna *et al*. (2024), the decline in the quality of functional beverages can be minimized by proper packaging. In this study, the chemical and sensory stability of tempeh juice probiotic drink was proven to be better in glass bottle (K1) than aluminium foil standing pouch with a spout (K2). The inert nature and low permeability of glass bottles are thought to maintain protein structure and function by preventing protein denaturation. According to Nilakrisna *et al*. (2024) glass bottle packaging is inert. Mustofa *et al*. (2014) explained that the inert nature of packaging means that the packaging does not react to chemical substances and its ability to prevent contamination of the packaged product, including the migration of substances from the packaging into the product.

The low permeability of the glass bottle packaging controls the entry of oxygen, maintaining conditions for the activity of lactic acid bacteria (LAB) in a stable pH reduction. According to Devianti & Arifiyan (2022), glass bottles as solid and non-porous packaging, effectively block external factors such as water vapor and oxygen. This is because oxygen can mediate the entry of microbes into food (Nilakrisna *et al*., 2024). The inert nature and low permeability of glass bottles can inhibit microbial contamination and support the desired viability of probiotics. By maintaining protein stability, pH, and limiting unwanted microbial activity, glass packaging can minimize changes in taste, aroma, color, and overall acceptability of probiotic tempeh juice beverage. Rosmawati *et al*. (2021), glass packaging has low permeability so that it can withstand moisture and oxygen from outside and from inside so that the fermentation process runs smoothly and the resulting ethanol content is low.

4. CONCLUSION

Storage time significantly affected the protein content and pH of the probiotic tempeh juice drink, while packaging type (bottle or standing pouch alu foil) had no significant effect. Furthermore, there was no interaction between the treatments (storage time and packaging type). However, storage time and packaging type have a significant influence on the sensory properties of the product. It was concluded that the probiotic tempeh juice beverage in glass bottle was better in maintaining the quality of protein content, pH, and sensory during cold storage than aluminium foil standing pouch with a spout due to the characteristics of inert glass bottle packaging and low permeability with an optimal shelf life of 8 days.

ACKNOWLEDGMENT

The authors would like to express their deepest gratitude to the Faculty of Agriculture, University of Lampung for the research funding provided on behalf of Prof. Dr. Dra. Maria Erna Kustyawati, M.Sc.

REFERENCES

Abdullah, K., & Asriati, D.W. (2016). Karakteristik minuman sari tempe dengan penambahan rasa vanila. *Journal of Agro-based Industry*, ***33***(1), 1-8.

Aranda-González, I., Perera-Pacheco, M., Barbosa-Martín, E., & Betancur, D. (2016). Replacing sugar with *S. rebaudiana* extracts on the physicochemical and sensory properties of strawberry ice cream. *Ciência Rural*, ***46***(4), 604–609. https://doi.org/10.1590/0103-8478cr20150505

Archadiya, M., Radiati, L.E., & Sawitri, M.E. (2021). Pengaruh waktu penyimpanan seasoning whey kefir terhadap kualitas fisik, kimia dan mikrobiologis. *Jurnal Agripet*, ***21***(1), 35–39. https://doi.org/10.17969/agripet.v21i1.19406

Ayun, Q., Muthiáh, S. N., & Sukmalara, D. (2023). Potensi bakteri asam laktat (BAL) dari jus tempe sebagai kandidat probiotik. *Jurnal Al-Azhar Indonesia*, ***8***(2), 171–177. https://doi.org/10.36722/sst.v8i2.1673

BSN (Badan Standarisasi Nasional). (2006). SNI 01-2346-2006 – Petunjuk pengujian organoleptik dan atau sensori. Jakarta: Badan Standarisasi Nasional.

Bintari, S.H., Purnama, D.F.E., Saputro, D.D., Sunyoto, S., Dewi, P., & Mubarok, I. (2022). Microbiological and biochemical tests on tempe production using tempe mold innovation. *Journal of Biology & Biology Education*, ***14***(2), 245-253. https://doi.org/10.15294/biosaintifika.v14i2.37700

Budiningrum, E.W., Wahyudiyono, W., & Murdapa, P.A. (2022). Kemasan untuk meningkatkan kualitas dan penjualan produk. *Hikmayo: Jurnal Pengabdian Masyarakat Amayo*, ***1***(2), 58–67.

Darmawan, A., Suliasih, S., & Malianti, L. (2023). Pengaruh sari buah naga merah (*Hylocereus polyrhizus* L.) terhadap kandungan protein, karbohidrat, dan pH pada yoghurt drink dengan starter komersil. *Jurnal Inspirasi Peternakan*, ***3***(2), 64–72.

Devianti, V.A., & Arifiyana, D. (2022). Pengaruh bahan kemasan penyimpanan minuman infused water jeruk nipis terhadap kadar vitamin C. *Journal Pharmasci (Journal of Pharmacy and Science)*, ***7***(2), 91–94. https://doi.org/10.53342/pharmasci.v7i2.293

Dhahana, K.A.P., Nocianitri, K.A., & Duniaji, A.S. (2021). Pengaruh lama fermentasi terhadap karakteristik soyghurt drink dengan penambahan *Lactobacillus rhamnosus* SKG 34. *Jurnal Ilmu dan Teknologi Pangan (ITEPA)*, ***10***(4), 646-656. https://doi.org/10.24843/itepa.2021.v10.i04.p10

Fikriyah, H., Habibi, N., Ismanilda, I., Darningsih, S., Zulkifli, Z., Andrafikar, A., & Edmon, E. (2024). Pengaruh penambahan inulin terhadap mutu sensorik, kandungan serat, dan daya terima yoghurt. *Jurnal Sehat Mandiri*, ***19***(1), 369–379. https://doi.org/10.33761/jsm.v19i1.1389

Khazalina, T. (2020). *Saccharomyces cerevisiae* in making halal products based on conventional biotechnology and genetic engineering. *Journal of Halal Product and Research (JHPR)*, ***3***(2), 88-94. https://doi.org/10.20473/jhpr.vol.3-issue.2.88-94

Kustyawati, M.E., Nurlita, M.E., Fadhallah, E.G., & Rizal, S. (2022). Prebiotic activity of Lactobacillus casei grown on medium containing hylocereus undatus extract and its use in the fermentation of goat's milk kefir. *Biodiversity*, ***23***(12), 6513-6519. https://doi.org/10.13057/biodiv/d231249

Isnaeni, Sugiyartono, Kusumawati, I., dan Rijal, M.A.S. (2016). Efek imunomodulator kombinasi susu probiotik dan ekstrak daun jambu biji. *Jurnal Farmasi Indonesia*, 8(1), 277–282.

Labiba, N.M., Marjan, A.Q., & Nasrullah, N. (2020). Pengembangan soyghurt (yoghurt susu kacang kedelai) sebagai minuman probiotik tinggi isoflavon. *Amerta Nutrition*, ***4***(3), 244–249. https://doi.org/10.20473/amnt.v4i3.2020.244-249

Lailia, R.P., Kentjonowaty, I., & Dinasari, I.R. (2023). Pengaruh lama simpan yoghurt susu kambing dengan penambahan sari jambu biji merah (*Psidium guajava* L.) terhadap total mikroba dan uji organoleptik. *Dinamika Rekasatwa: Jurnal Ilmiah (e-Journal)*, ***6***(1), 177–184.

Lestari, Y., Amin, N., Ananda, D., & Rengganis, N.A. (2021). Identifikasi karakteristik kimiawi dan daya simpan kefir susu jagung (Zea mays L. Saccharata). *Jurnal Gizi*, ***10***(2), 20–32. https://doi.org/10.26714/jg.10.2.2021.20-32

Lozančić, M., Žunar, B., Hrestak, D., Lopandić, K., Teparić, R., & Mrša, V. (2021). Systematic comparison of cell wall-related proteins of different yeasts. *Journal of Fungi*, ***7***(2), 128. https://doi.org/10.3390/jof7020128

Mohammed, S.S. (2015) Effect of pH on the turbidity removal of wastewater. *Open Access Library Journal*, 2: e2283. http://dx.doi.org/10.4236/oalib.1102283

Muchtar, F., & Hastian, L.P. (2020). Pengaruh penyimpanan pada suhu rendah terhadap kandungan protein dan sifat organoleptik tahu. *Jurnal Sains dan Teknologi Pangan*, ***5***(5), 3256–3264.

Musoffin, A., Kentjonowaty, I., & Puspitarini, O.R. (2024). Pengaruh berbagai jenis gula terhadap nilai pH, sineresis dan kualitas organoleptik yoghurt. *Jurnal Dinamika Rekasatwa*, ***7***(1), 118–126.

Mustofa, Rohmaniyati, A., Gani, I.A., Sampurno, B., Ardi, B.N., Limbu, Y.S., Fatima, F., dan Hasanah, H. (2014). *Pedoman Pemilihan Jenis Kemasan Pangan*. Jakarta Pusat: Badan POM RI.

Nabilah, F.V., Listiyowati, S., dan Astuti, R.I. (2022). Diversitas pangan fermentasi berbasis susu di Indonesia dan kandungan gizinya. *Jurnal Ilmu Pertanian Indonesia*, 27(4), 552–561.

Nilakrisna, N., Patang, & Fadilah, R. (2024). Pengaruh jenis kemasan dan lama penyimpanan terhadap kualitas minuman pangan fungsional berbahan dasar jahe (*Zingiber officinale*). *Jurnal Review Pendidikan dan Pengajaran*, ***7***(3), 6607–6616.

Nirmalasari, R., Norrahmah, M., & Septina, N. (2023). Pengaruh suhu penyimpanan terhadap kualitas organoleptik minuman probiotik susu biji nangka (*Artocarpus heterophyllus*). *Jurnal Ilmiah Biosaintropis (Bioscience-Tropic)*, ***9***(1), 70–80. https://doi.org/10.33474/e-jbst.v9i1.539

Nurhayati, N., Yuwanti, S., & Urbahillah, A. (2020). Karakteristik fisikokimia dan sensori kombucha cascara (kulit kopi ranum). *Jurnal Teknologi dan Industri Pangan*, ***31***(1), 38–49. https://doi.org/10.6066/jtip.2020.31.1.38

Nurman, M.N., Azhar, M., Amelia, F., & Oktavia, B. (2020). Pengaruh penambahan ekstrak inulin umbi dahlia terhadap karakteristik organoleptik sinbiotik set yoghurt. *Periodic: Chemistry Journal Universitas Negeri Padang*, ***9***(2), 55–59.

Pangestu, A.D., Kurniawan, dan Supriyadi. (2021). Pengaruh variasi suhu dan lama penyimpanan terhadap viabilitas bakteri asam laktat (BAL) dan nilai pH yoghurt. *Borneo Journal of Medical Laboratory Technology (BJMLT)*, 3(2), 231–236.

Pradana, A.S., Srijuliani, E., & Risnantoko, W. (2018). Karakteristik kimia dan organoleptik yoghurt tempe dengan penambahan ekstrak buah bit (*Beta vulgaris*). *Prosiding Seminar Nasional 5th FP "Pemanfaatan Sumber Daya Lokal Menuju Kemandirian Pangan Nasional*", 1–8. Fakultas Pertanian, Universitas Veteran Bangun Nusantara.

Purry, A.P.K., & Rafiony, A. (2018). Pembuatan minuman sari tempe dengan ekstrak jeruk siam (*Citrus nobilis*) ditinjau dari mutu organoleptik kadar vitamin C dan kadar aktivitas antioksidan isoflavon. *Prosiding Nasional Jurnal*, ***1***(2), 60–65. https://doi.org/10.30602/pnj.v1i2.289

Rizal, S., & Kustyawati, M.E. (2019). Karakteristik organoleptik dan kandungan beta-glukan tempe kedelai dengan penambahan *Saccharomyces cerevisiae*. *Jurnal Teknologi Pertanian*, ***20***(2), 127–138.

Rizqiati, H., Susanti, S., Nurwantoro, Albaarri, A.N., & Slamet, Y.B. (2021). Pengaruh waktu fermentasi terhadap sifat fisiko kimia kefir whey dari susu kambing. *Warta IHP*, ***38***(1), 54–60.

Rosmawati, R., Syam, H., & Sukainah, A. (2021). Pengaruh jenis kemasan dan lama penyimpanan terhadap kualitas minuman khas Sinjai (Ires). *Jurnal Pendidikan Teknologi Pertanian*, ***7***(1), 79–92. https://doi.org/10.26858/jptp.v6i2.13107

Rossi, E., Hamzah, F., & Febriyani. (2016). Perbandingan susu kambing dan susu kedelai dalam pembuatan kefir. *Jurnal Peternakan Indonesia*, ***18***(1), 13–20. https://doi.org/10.25077/jpi.18.1.13-20.2016

Sabil, S., Amin, M., Maruddin, F., Husnaeni, H., & Taggo, S. (2023). Sifat fisiko-kimia dan fungsional susu kurma dengan penambahan bakteri asam laktat pada level berbeda. *Jurnal Ilmu dan Industri Peternakan*, ***9***(2), 203–213. https://doi.org/10.24252/jiip.v9i2.41824

Santoso, T.L.A., Wicaksono, D.A., & Gunawan, P.N. (2022). Effects of carbonated soft drink on saliva pH in the occurrence of dental caries. *E-GiGi*, ***10***(1), 66–74. https://doi.org/10.35790/eg.v10i1.37606

Sudarmadji, S., Haryono, B., Suhardi. (1997). *Prosedur Analisa Untuk Bahan Makanan dan Pertanian*. Yogyakarta : Liberty.

Sukmaningrum, H., Darmayanti, T., & Puspawati, G.A.K.D. (2021). The changes of characteristic fermented milk during room temperature storage. *Jurnal Ilmu dan Teknologi Pangan (ITEPA)*, ***10***(1), 119–130. https://doi.org/10.24843/itepa.2021.v10.i01.p11

Teame, T., Wang, A., Xie, M., Zhang, Z., Yang, Y., Ding, Q., Gao, C., Olsen, R.E., Ran, C., & Zhou, Z. (2020). Paraprobiotics and postbiotics of probiotic Lactobacilli, their positive effects on the host and action mechanisms: A review. *Frontiers in Nutrition*, ***7***, 570344. https://doi.org/10.3389/fnut.2020.570344

Tefa, P., Ledo, M.E.S., & Nitsae, M. (2023). Variasi konsentrasi Saccharomyces cerevisiae dalam pembuatan wine buah dilak (*Limonia acidissima*). *SCISCITATIO*, ***4***(1), 32–38. https://doi.org/10.21460/sciscitatio.2023.41.112

Tritisari, A., Lianto, A., & Suhana, S. (2024). Pengaruh variasi konsentrasi natrium benzoat terhadap kualitas dan daya simpan sirop nanas. *Jurnal Teknologi Pangan dan Industri Perkebunan (LIPIDA)*, ***4***(2), 41–50. https://doi.org/10.58466/lipida.v4i2.1678

Tursina, T., Irfan, I., & Anwar, S.H. (2019). Tingkat penerimaan panelis terhadap yoghurt dengan perlakuan lama fermentasi, jenis susu, dan lama penyimpanan yang berbeda. *Jurnal Ilmiah Mahasiswa Pertanian*, ***4***(3), 65–74. https://doi.org/10.17969/jimfp.v4i3.11637

Udin, J., Nurlaelah, I., & Priyanto, A. (2020). Pengaruh kadar konsentrasi *Saccharomyces cerevisiae* terhadap sifat organoleptik dan sifat kimia (alkohol dan gula) pada brem cair *Ipomea batatas* L. *EDUBIOLOGICA: Jurnal Penelitian Ilmu dan Pendidikan Biologi*, ***8***(1), 25–34.

Usman, M., Tarigan, B.Y., Aprilia, M., Zalvi, A.P., Sari, F.I., Romauli, N.D.M., & Sinaga, H. (2023). Pengujian daya terima (uji hedonik) pada empat merek produk yoghurt yang dijual pada pasar modern (supermarket) di Kecamatan Medan Kota. *Jurnal Agroindustri Pangan*, ***2***(2), 1–16. https://doi.org/10.47767/agroindustri.v2i2.523

Walker, G.M., & Stewart, G.G. (2016). *Saccharomyces cerevisiae* in the production of fermented beverages. *Beverages*, ***2***(4), 30. https://doi.org/10.3390/beverages2040030