Revista de Ciências Agroveterinárias
Universidade do Estado de Santa Catarina (UDESC)

Research Article

Effectiveness of green betel leaf infusa as a natural preservative of white tofu by maintaining its sensory characteristics

Eficácia da infusa de folha de betel verde como conservante natural de tofu branco, mantendo suas características sensorios

Aminullah Aminullah[*(ORCID 0000-0003-2354-6025)], **Muhammad Muslih** [(ORCID 0009-0007-1214-3147)], **Titi Rohmayanti** [(ORCID 0000-0002-2618-3015)]

Universitas Djuanda, Bogor Indonesia. *Author for correspondence: aminullah@unida.ac.id

Submission: October 02th, 2024 | Acceptance: June 16th, 2025

ABSTRACT

Preserving white tofu with natural ingredients was one solution due to its relatively short shelf life and prevent from the dangerous ingredients. A lack of natural ingredients usually changes the product's sensory quality. This research aimed to utilize green betel leaf infusion as a preservative while maintaining the sensory characteristics of white tofu. The research method consisted of two stages. Preliminary research was conducted to select the concentration of 0.01%, 0.025%, 0.05%, 0.075%, 0.1%, 1%, 2% and 4% betel leaf infusion based on the sensory of taste, aroma, and color. The primary research was two factor research of selected betel leaf infusion concentrations and the storage time of the tofu at room temperature by testing total plate numbers, *E. coli* bacteria, and sensory and hedonic quality. The preliminary research produced the selected concentrations of 0.01%, 0.025%, and 0.05%, which had minimal effects on the taste, aroma, and color sensory. The primary research showed that the higher the concentration of betel leaf infusion, the decreased microbial growth, and the longer the storage time, the more microbes grew. However, the number of microbes in treated tofu was much lower compared to control tofu. In addition, the results showed that the higher the infusion concentration led to the lower the sensory value. However, the concentration level had no significant effect on the texture sensory. The longer storage time tended to reduce white tofu's sensory and hedonic quality, but it was still in a relatively normal condition. The *E. coli* bacteria was negative on white tofu. All infusion concentration treatments inhibited microbial growth, and the sensory value of white tofu can be maintained.

KEYWORDS: *Piper betle* L. Natural preservatives. Total microbes. *Escherichia coli*. Soaking.

RESUMO

Preservar o tofu branco com ingredientes naturais foi uma solução devido ao seu prazo de validade relativamente curto e à prevenção de ingredientes perigosos. A falta de ingredientes naturais costuma alterar a qualidade sensorial do produto. Esta pesquisa teve como objetivo utilizar a infusão de folhas verdes de betel como conservante, mantendo as características sensoriais do tofu branco. O método de pesquisa consistiu em duas etapas. Pesquisa preliminar foi realizada para selecionar a concentração de 0,01%, 0,025%, 0,05%, 0,075%, 0,1%, 1%, 2% e 4% de infusão de folhas de betel com base no sensorial de sabor, aroma e cor. A pesquisa primária foi a pesquisa de dois fatores de concentrações selecionadas de infusão de folhas de betel e o tempo de armazenamento do tofu em temperatura ambiente, testando o número total de placas, bactérias E. coli e qualidade sensorial e hedônica. A pesquisa preliminar produziu as concentrações selecionadas de 0,01%, 0,025% e 0,05%, que tiveram efeitos mínimos no sabor, aroma e sensorial da cor. A pesquisa primária mostrou que quanto maior a concentração da infusão de folhas de betel, menor o crescimento microbiano e quanto maior o tempo de armazenamento, mais micróbios cresceram. No entanto, o número de micróbios no tofu tratado foi muito menor em comparação com o tofu controle. Além disso, os resultados mostraram que quanto maior a concentração da infusão, menor o valor sensorial. Entretanto, o nível de concentração não teve efeito significativo na sensorialidade da textura. O

maior tempo de armazenamento tendeu a reduzir a qualidade sensorial e hedônica do tofu branco, mas ele ainda estava em condições relativamente normais. A bactéria E. coli foi negativa em tofu branco. Todos os tratamentos de concentração de infusão inibiram o crescimento microbiano e o valor sensorial do tofu branco pode ser mantido.
PALAVRAS-CHAVE: *Piper betle* L. Conservantes naturais. Micróbios totais. *Escherichia coli*. Imersão.

INTRODUCTION

Tofu is a processed product made from soybeans as a primary ingredient of high protein. Tofu is one of the products most in demand by the majority of the Indonesian population because the price is still relatively very affordable (FEBRIYOSSA & KOTEN 2022). In standard storage, the average shelf life of tofu is ± 1 to 2 days. If it has passed two days of storage, the taste usually becomes sour, the texture changes quickly, the aroma is unpleasant, and the color becomes slightly dull, so the tofu is no longer suitable. This condition is due to the growth of spoilage microbes in tofu (LEE et al. 2017) (ROSSI et al. 2016). Raw materials, water, storage, production environment, and workers can cause sources of microbial contamination in tofu. (SIMANJUNTAK et al. 2022) and (ANANCHAIPATTANA et al. 2012) stated that, on average, white tofu marketed in South Sulawesi Province of Indonesia and Thailand, was contaminated with coliform bacteria, and some were also contaminated with *Escherichia coli* bacteria.

Some traders carry out illegal preservation practices using the chemical formaldehyde. (SARI et al. 2021) and (BABARINDE et al. 2015) reported the presence of tofu identified as positive for formaldehyde. Based on the Republic of Indonesia Minister of Health Regulation Number 033 of 2012 concerning food additives, the use of the two chemicals, borax and formaldehyde, is prohibited. Therefore, to prevent the use of dangerous chemicals as food preservatives, it is necessary to innovate or develop preservatives that are not dangerous or toxic, do not have long-term effects on the body, and are relatively affordable. This effort refers to using natural ingredients for food preservation, one of which is green betel leaves.

Green betel leaves are a potential spice or natural ingredient that can be used as a preservative. Green betel leaf (*Piper betle* L) is a plant that has several antibacterial components that can be used as natural preservatives, such as essential oils, phenols, flavonoids, saponins, and tannins (PATEL & JASRAI 2013). Several previous studies, such as (HAMZAH et al. 2021), tested the in vitro antibacterial activity of green betel liquid extract in methanol solvent and prevented the development of E. coli bacteria in all betel leaf infusion treatments tested. Furthermore, (PRATIWI et al. 2019) reported that tofu soaked for 15 minutes in betel leaf-infused water with concentrations of 0%, 3%, 6%, and 9% produced a significant effect on bacterial growth. Where the higher the concentration, the higher the level of inhibition. Regarding the influence on organoleptic tofu, (SEPTIANA 2018) and (DIENY et al. 2023) in preserving tofu soaked with an infusion of green betel leaves and beluntas leaves, respectively, significantly inhibited microbial growth. However, the sensory value of aroma and color in white tofu becomes distorted, where the aroma of the betel leaves was still strong, and had green color. Constraints that still exist in previous research related to the declining sensory value of tofu are the background for further research regarding appropriate

concentrations where microbial growth is inhibited. However, the sensory quality value of white tofu can be maintained under normal conditions. This research aimed to study the effect of betel leaf infusion concentration and storage time at room temperature on white tofu's sensory and microbiological quality.

RESEARCH METHOD

Materials and Equipment

The materials used in this research included betel leaves (Piper betle L.), tofu, water and distilled water. The chemicals used were NaCl (Merck), Tyrptone Glucose Yeast Agar (TGY) media (HI Media GM014), Mac Conkey Agar (MCA) media (Oxoid CM0007b), and 95% alcohol (Merck).

Equipment used in the research included an oven, sterile sample container, sampling box, lighter/match, spray bottle, pH meter (Mettler Toledo), analytical scale (Sartorius CPA224S), thermometer (ASTM S64C), plastic petri dish (Labware CLW-HP1002), micro pipette, 1ml measuring pipette (Iwaki), drop pipette (Iwaki), beaker (Iwaki), water bath (Memmert), hotplate (Thermolyne SP131), bulb, incubator (Memmert IN110), autoclave (Companion ST-G Jeio Tech), sample box, 1000ml measuring cup (Iwaki), 100ml measuring flask (Iwaki).

Research procedure

The research was divided into two stages: the first stage was preliminary research, and the second stage was primary research. In the preliminary research, two stages of activities were carried out, namely making simplicia and betel leaf infusions and determining the concentration of infusion to be used in the primary research by testing taste, aroma, and color parameters separately. The research was qualitative.

1. Preliminary Research

The research began with a crucial step-the selection of green betel leaves for infusion. The betel leaves used were carefully chosen from the Caringin area, Bogor Regency, with a focus on fresh, young leaves from the second segment. This meticulous selection process was a key part of the research. Next, the green betel leaves that will be used were sorted and cleaned with running water, then drained and dried in the oven. Drying the betel leaves was conducted at a temperature of 40-50 ℃ for 4 days, ensuring the leaves were perfectly dry. After the betel leaves were dry, they were then ground using a blender until they form a fine powder (RAHAYU et al. 2019). The yield was calculated using the following formula:

$$\%\text{Yield} = \frac{\text{Final simplicia mass}}{\text{initial simplicia mass}} \times 100\%$$

After simplicia, the preliminary research continued with the experimental process of making betel leaf extract using the infusion method. This experimental process was carried out with various betel leaves concentrations of 0.01%, 0.025%, 0.05%, 0.075%, 0.1%, 1%, 2%, and 4%.

Making betel leaf infusion was done by cooking or boiling green betel leaf simplicia at temperature of 90 ℃ for ±15 minutes; for an example, making an infusion of 0.01% concentration, betel leaf simplicia was weighed 0.1 g and then 1000 ml of water was added and then heated for 15 minutes at temperature of 90 ℃. After that, the solution was filtered and collected in a 1000 ml measuring flask; rinse the remaining

filter with hot water and let it sit until it cools, then measured the volume with water until the flask limits (KUNCARLI & DJUNARKO 2014).

The subsequent step involved a visual assessment of the betel leaf infusion, considering key parameters such as taste, aroma, and color. This evaluation aimed to identify the optimal concentration of betel leaf infusion from a sensory perspective, one that would provide the minimum sensory impact on taste, aroma, and color. From these tests, the selected three concentrations of betel leaf infusion were used in the primary research.

2. Primary Research

The first activity was taking samples of white tofu. White tofu was taken directly from the tofu MSME production site in Cinagara Village, Bogor Regency. Sampling was carried out by taking the tofu directly after the molding and cutting process with a size of 5x5 cm; 6 tofu was immediately put into a sterile container, then added ±450 ml of betel leaf infusion solution until the tofu was submerged in the container. Tofu samples for microbiological testing and sensory quality were stored in a separate container and identified.

After that, the tofu samples were stored at storage room temperature. On the 0th, 2nd, and 4th day of storage, microbiological parameter analysis (total plate count, *Escherichia coli*), sensory parameter quality (color, appearance, aroma, texture), overall hedonic test (SEPTIANA 2018) were carried out.

This research was meticulously designed using a two-factor Completely Randomized Design (CRD). Factor A involved three concentration levels of betel leaf infusion, carefully selected at the preliminary research stage. Factor B was the storage time of tofu in betel leaf infusion water, set at 0, 2, and 4 days. Each treatment was carried out twice, resulting in 18 experimental units. As a control, tofu was soaked in well water, the same water used in production facilities. This systematic approach ensured the validity and reliability of our findings.

Total plate count (TPC) testing (KEMENTERIAN KESEHATAN REPUBLIK INDONESIA 2017)

The TPC test uses Tryptone Glucose Yeast Agar (TGY) media. The tofu sample was first mashed using a sterile spoon. This measurement was done by weighing a 10 g sample of white tofu and putting it in a bottle, then adding 90 ml of sterile physiological NaCl, homogenizing it, and waiting for it to settle. Next, the dilution was carried out aseptically by pipetting 1 ml of the solution into 9 ml of NaCl solution with a concentration of 10^{-6} or until the growing colonies can be counted, then pipetting into the medium, incubating at a temperature of 30-35 ℃ for 3-5 days. Counting the colonies at the end of the incubation period in colonies/ml.

Eschesichia coli testing (KEMENTERIAN KESEHATAN REPUBLIK INDONESIA 2017)

Identification of Eschesichia coli bacteria was carried out by weighing a 10 g sample of tofu that had been mashed using a sterile spoon, put it in a bottle and added 90 ml of sterile physiological NaCl then homogenized and wait until the solution settled. Next, 1 ml was pipetted and grown on Mac Conkey Agar (MAC) media for incubation for 24-28 hours at a temperature of 30-35 ℃. *Escherichia coli* colonies grew red when compared with positive controls, namely standard Eschesichia coli bacteria, tofu

without treatment, and negative controls in the form of blanks.

Organoleptic Testing (SETYANINGSIH et al. 2014)

The organoleptic tests carried out were sensory quality and overall hedonic tests by 30 semi-trained panelists. This test used a line scale (0-10 cm). The test was carried out using a description of 0 was undesirable, and 10 was desired. For the color sensory, 0 was yellowish white, and 10 was typical of white tofu. For the appearance test, 0 was slimy and moldy and 10 was typical of not slimy and moldy tofu. In the aroma test, 0 was a distinctive aroma of betel leaves, and 10 was the typical aroma of white tofu. For texture testing, 0 was is less chewy texture, and 10 was chewy. The overall hedonic test was labeled with a description of intensity on a scale of 0 was dislikes and 10 was likes.

Data analysis

Data analysis in this study used the SPSS program with a statistical test of analysis of variance (ANOVA), to determine whether the treatment carried out in the study had a significant effect or not; if $p<0.05$ then the treatment has a significant effect, then followed by Duncan's post hoc test at a confidence interval of 95% (α level = 0.05) to find out which treatments were significantly different.

RESULTS AND DISCUSSION

Yield and water content of green betel leaves

Yield compares the product's dry weight with the raw material's weight. A high yield value indicates the amount of a bioactive component contained in the plant due to the large number of active compounds in the material (SENDUK et al. 2020). Based on the analysis results, the initial weight of betel leaves before drying is 223.21 g, and the final weight after drying is 59.24 g. Based on calculations, the yield of green betel leaves is 26.54%. Based on previous research of (KUNCARLI & DJUNARKO 2014), the yield from betel leaves was 23.018%.

According to (DAUD et al. 2019), water content was a chemical laboratory test method that is very important in the food industry to determine the quality and resistance of food to damage that may occur. The higher the water content of a raw material, the greater the possibility of damage either due to internal biological activity (metabolism) or the entry of destructive microbes. Reducing the water content in raw materials can reduce the availability of water that can be used for the life of microorganisms and the occurrence of physicochemical reactions, thus inhibiting the growth of microorganisms and physicochemical reactions or both; food ingredients can survive longer against damage.

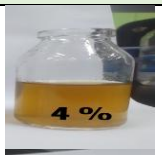
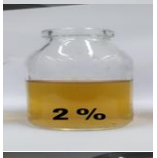
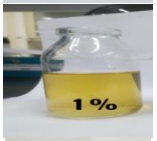
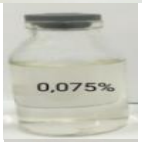
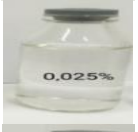
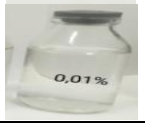
Determining water content is one of the most essential things in food technology. Based on the analysis results, it is found that the water content of green betel leaves is 3.22%. This result was included in the (KEMENTERIAN KESEHATAN REPUBLIK INDONESIA 2017) standard for the water content requirements of simplicia leaves, namely ≤10%.

Observation of green betel leaf infusion solution

Preliminary research is carried out to determine the desired concentration of betel leaf infusion, namely the infusion solution concentration that provides the minimum sensory effect of color, aroma, and taste. These conditions allow the sensory

characteristics of tofu to be maintained in standard conditions during storage. The initial concentration chosen was taken based on previous research by (SEPTIANA 2018) at a concentration of 2.5% betel leaf infusion solution affected the aroma of tofu, and the minor concentration of 0.05% was able to restrain the growth rate of bacteria compared to the bay leaf and lemongrass leaf infusion solution. For this reason, the researchers tried to get the best concentration of betel leaf infusion. The the sensory test of the betel leaf extract solution are presented in Table 1.

Table 1. Results of qualitative sensory observations of betel leaf extract solution.

Concentration (%)	Taste	Aroma	Color	Appearance
4	Very bitter	typical betel leaf (+ + + + +)	Dark Brown	
2	Bitter	typical betel leaf (+ + + +)	Brown	
1	A bit bitter	typical betel leaf (+ +)	Yellowish-brown	
0.1	A bit bitter	typical betel leaf (+ +)	Brownish yellow	
0.075	A bit bitter	typical betel leaf (+ +)	Clear, slightly yellow	
0.05	A bit bitter	typical betel leaf (+ +)	Clear, slightly yellow	
0.025	Not Bitter	typical betel leaf (+)	Clear	
0.01	Not Bitter	typical betel leaf (+)	Clear	

Description: Very Strong (+ + + + +); Strong (+ + + +); Medium (++); Weak (+)

Table 1 shows that concentrations of 4%, 2%, and 1% have a solid sensory color ranging from brown to yellowish brown, a strong betel leaf aroma, and a strong bitter taste. From all concentrations, there were two concentrations, namely 0.01% and 0.025%, which had sensory properties close to water's sensory properties, such as a tasteless taste, a weak betel odor, and a clear color. Based on these results, the concentrations chosen were 0.01%, 0.025%, and 0.05% to reduce the sensory influence on stored tofu. The impact of color and taste on betel leaf infusion is due to

the presence of alkaloids, tannins, glycosides, reducing sugars, and saponins (KAVETI et al. 2011), which cause the color of the solution to be more brownish-yellow.

In contrast, the aroma is affected by the essential oils of estragole, linalool, α-copaene, anethole, and caryophyllene α-terpinene, p-cymene, 1,8-cineole, β-caryophyllene, α-humulene, allyl pyrocatechol, allylcatechol, methyl eugenol, estragol (methyl chavicol), chavibetol, chavibetol acetate, safrol, 4-allyl-2-methoxy-phenolacetate, and 3-allyl-6-methoxypheno (MADHUMITA et al. 2019, PRAKASH et al. 2010, SALEHI et al. 2019).

Primary Research

TPC (Total Plate Count) test for white tofu

TPC testing on tofu infused with betel leaf extract is crucial for evaluating the safety, antimicrobial effectiveness, and product quality during storage. The core principle involves counting the number of microorganisms capable of growing on culture media, which reflects the level of microbial contamination in the product. The TPC test results for tofu immersed in betel leaf infusion (colony/ml) are shown in Table 2.

Table 2 shows that the higher the concentration of green betel leaf infusion, the higher the inhibitory power of microbial growth. These results align with (ULYA 2019), who stated that the higher concentration of betel leaf infusion was followed by the higher tannin and flavonoid compounds in betel leaves, resulting in higher inhibition of bacterial growth. Green betel leaves themselves contain essential oils, which are derivatives of phenol compounds, and most of them are chavicol. This chavicol has a distinctive smell and is five times the bacteria-killing power of ordinary phenol. The mechanism of phenol as an antibacterial is that it is a toxin in the protoplasm that damages and penetrates the walls. Cells and precipitates denature bacterial cell proteins to disrupt the formation of cell walls in bacteria (SYAHRINASTITI et al. 2015). Apart from that, (WIDIYANTI et al. 2017) explained that the effectiveness of antimicrobials was influenced by concentration and storage time, and (DINOS et al. 2016) stated that antibacterials could work as bactericidal and can also work as bacteriostatic by affects protein synthesis.

Table 2. TPC test results of tofu soaked with betel leaf infusion (colony/ml).

Concentration of Betel Leaf Infusion (%)	Storage time in infusion water (days)			Average
	B1 (0)	B2 (2)	B3 (4)	
A1 (0.01)	$8.94\times10^{5\ a}$	$7.32\times10^{6\ c}$	$1.99\times10^{7\ e}$	$9.37\times10^{6\ r}$
A2 (0.025)	$7.1\times10^{5\ a}$	$6.55\times10^{6\ c}$	$1.71\times10^{7\ d}$	$8.12\times10^{6\ q}$
A3 (0.05)	$7.19\times10^{5\ a}$	$4.91\times10^{6\ b}$	$7.04\times10^{6\ c}$	$4.22\times10^{6\ p}$
Average	$7.74\times10^{5\ x}$	$6.26\times10^{6\ y}$	$1.47\times10^{7\ z}$	
Control (0% infusion)	7.625×10^{5}	7.14×10^{9}	2.045×10^{10}	

Note: Different superscript letter indicate significant differences at the confidence level α = 0.05.

Table 2 also shows that the longer the storage time, the higher the number of microbes. This result was in line with research by (ULYA 2019), where during storage,

the number of microbes and water content of tofu would increase, and a decrease in protein content, which was caused by the use of protein in bacterial growth. When compared with the control tofu shown in Table 2, the number of microbes that grew on the treated tofu are much lower and succeeded in inhibiting around ± 1,805,166,667 or 99.2% of microbes even though it exceeded the standard of 1.0 x 106. This phenomenon can be categorized as still quite good because tofu has advanced processing stages before consumption, for example, frying, which can cause the microbes contained in tofu to decrease due to the heating process.

Table 2 also shows that there is an interaction between the concentration of betel leaf infusion and storage time, which has a significant effect on microbial growth. (DIENY et al. 2023) reported an interaction between the two treatments on the number of bacteria and sensory properties in white tofu.

Although adding betel leaf infusion to TGY medium significantly reduces microbial counts, certain microorganisms continue to grow. This condition is because betel leaf extract exhibits an antimicrobial effect, but its inhibitory efficacy varies among microbial species. Gram-positive bacteria, especially Staphylococcus aureus, tend to be more susceptible to betel leaf-derived compounds. However, growth suppression is not absolute; some S. aureus strains may still proliferate at lower extract concentrations. Notably, one study determined the MIC for red betel leaf extract against S. aureus at approximately 6.25%, with the MBC (minimum bactericidal concentration) at 12.5%, indicating that concentrations below these thresholds may allow bacterial survival and growth (ZULKARNAIN et al. 2023)

Escherichia coli bacteria test results.

Table 3 shows that no growth of *Escherichia coli* bacteria is identified in white tofu soaked with betel leaf infusion. For the positive control, standard *Escherichia coli* bacteria are used, and white tofu without treatment soaked in well water is grown on Mac Conkey Agar (MCA) media with the same concentration and treatment. The negative control use Mac Conkey Agar (MCA) media (blank without the addition of any substances); the observation results can be seen in Figure 1.

Table 3. Test results for *Escherichia coli* bacteria (colony/ml).

Concentration of Betel Leaf Infusion (%)	Storage time in infusion water (days)		
	B1 (0)	B2 (2)	B3 (4)
A1 (0.01)	Negative	Negative	Negative
A2 (0.025)	Negative	Negative	Negative
A3 (0.05)	Negative	Negative	Negative
Control (0% infusion)	Negative	Negative	Negative

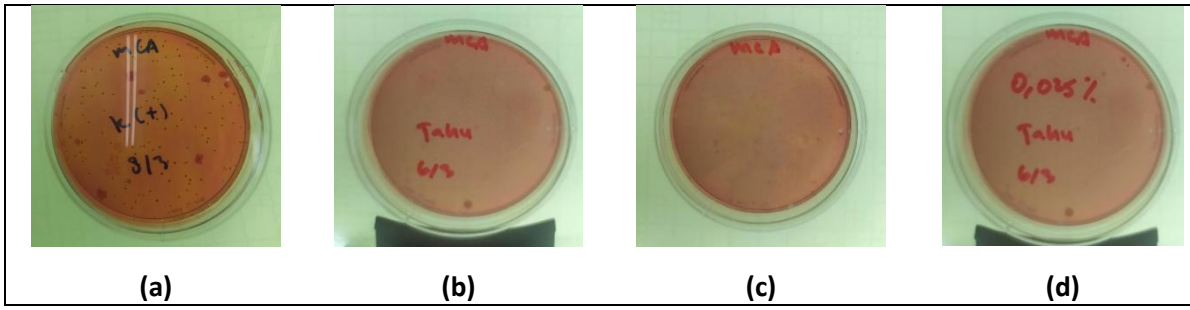


Figure 1. Data from observations of *Escherichia coli* bacteria in (a) positive control, (b) blank, (c) untreated white tofu, and (d) treated white tofu.

This result is significant as it suggests that the well water used to make white tofu is not polluted. However, the location of well water can influence the presence of dangerous microorganisms such as Escherichia coli. Water sources close to waste disposal or septic tanks were often a source of E. coli bacterial contamination (BANSEKA & TUME 2024). This highlights the importance of water source management in food production.

Sensory quality testing results

Table 4 shows the results of testing the sensory quality of tofu soaked in a green betel leaf infusion solution.

Table 4. Sensory quality of white tofu soaked in green betel leaf infusion for several periods of storage at room temperature.

Treatment	Sensory parameter				
	Color	Appearance	Aroma	Texture (elasticity)	Overall hedonic
Effect of green betel leaf infusion concentration					
0.01%	7.3^{r}	7.2^{r}	7.2^{r}	8.4^{x}	7.2^{r}
0.025%	7.1^{q}	7.4^{q}	7.0^{q}	8.4^{x}	7.0^{q}
0.05%	6.7^{p}	7.9^{p}	6.0^{p}	8.3^{x}	6.4^{p}
Effect of storage time					
0 days	9.0^{z}	9.2^{z}	9.0^{z}	9.3^{p}	9.0^{z}
2 days	5.9^{y}	7.4^{y}	5.2^{y}	8.5^{q}	5.8^{y}
4 days	6.2^{x}	5.9^{x}	5.9^{x}	7.3^{r}	6.5^{x}
Interaction of green betel leaf infusion concentration and storage time					
0.01% and 0 days	9.2^{f}	9.3^{g}	9.1^{d}	9.3^{c}	9.1^{d}
0.025% and 0 days	9.1^{f}	9.2^{g}	9.0^{d}	9.4^{c}	9.0^{d}
0.05% and 0 days	8.7^{e}	9.2^{g}	8.9^{d}	9.3^{c}	9.0^{d}
0.01% and 2 days	6.2^{cd}	7.0^{d}	5.7^{b}	8.5^{b}	6.3^{b}
0.025% and 2 days	6.0^{bc}	7.2^{e}	5.6^{b}	8.4^{b}	5.9^{b}
0.05% and 2 days	5.6^{a}	8.0^{f}	4.4^{a}	8.4^{b}	5.2^{a}
0.01% and 4 days	6.5^{d}	5.4^{a}	6.4^{c}	7.2^{a}	6.9^{c}
0.025% and 4 days	6.3^{d}	6.8^{b}	6.3^{c}	7.3^{a}	6.8^{c}
0.05% and 4 days	5.8^{b}	6.4^{c}	4.8^{a}	7.3^{b}	5.8^{b}

Note: different letters in a column indicate significantly different results at α=0.05.

Color sensory quality

According to (SPENCE 2015), color is a measurement factor that can influence the quality of ingredients before other characteristics, such as taste, aroma, texture, etc. Based on Table 4, the concentration of betel leaf infusion significantly affects the color sensory of white tofu. The higher the concentration of betel leaf infusion, the white color sensory value tends to decrease but still tends to be white. These results are in line with research by (HAFIZAH et al. 2021), where the concentration level of betel leaf infusion influenced the color of yellowish white. This infusion contained tannin and flavonoid compounds, which play a role in giving the betel leaf infusion a yellowish green color.

Also Table 4 explains that the storage time significantly affects the color sensory value, where the longer the storage time, the more likely it becomes yellowish-white. This was explained by (DIENY et al. 2023), who stated that the length of soaking affected the color sensory, where beluntas leaf extract changes the color of the tofu to green. The smallest sensory value is obtained on the second day, namely 5.55, indicating that the color of the tofu was still at the typical white tofu level even though

there was a slight change. Visual changes in the color of white tofu can be seen in Figure 2.

The results are not significantly different for the interaction between infusion concentration and storage time. This finding aligns with the research conducted by (SEPTIANA 2018), which similarly reported that the interaction between the concentration of betel leaves used and the length of storage time did not have a significant effect on the color sensory quality test. This research by (SEPTIANA 2018) served as a crucial reference point, providing a solid foundation for our current study. The water contained in tofu during storage will come out along with a decrease in protein levels, which function as a water binder, so the water affects the concentration of the betel leaf infusion.

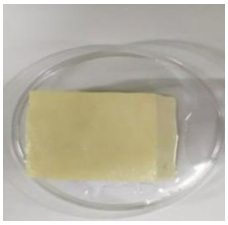
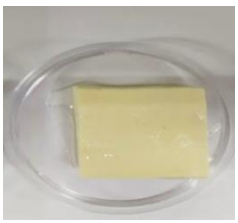
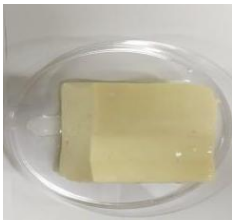
	Concentration of green betel leaf infusion (%)			
Storage time (day)	**0 (control)**	**0.01**	**0.025**	**0.05**
0				
2				
4				

Figure 2. Visual observation of white tofu on days 0, 2, and 4 days in blank samples and soaking treatment in green betel leaf infusion.

Appearance sensory quality

Appearance can influence consumer acceptance of a product. The uniformity and integrity of a product will make panelists more interested in and like it. This is because, in general, consumers would choose food forms with an attractive appearance (JANJIĆ et al. 2023).

Table 4 demonstrates that the concentration of betel leaf infusion significantly affects the appearance sensory of white tofu. Specifically, a higher concentration of betel leaf infusion prolongs the appearance of the tofu under normal conditions. These findings have practical implications for the food industry. The ability of betel leaf infusion to inhibit the appearance of mucus and mold on tofu due to the presence of

anti-microbial active substances of essential oils, phenols and flavonoids (SARMA et al. 2018), can be leveraged to enhance the shelf life and visual appeal of white tofu products.

Table 4 also shows that the length of storage time significantly affects the appearance sensory quality, where the longer tofu is stored, the appearance value tends to decrease. This is in line with (ARANDINI et al. 2022), namely that the longer the storage time, the surface of the tofu becomes slimy and moldy. This is related to the results of the total plate count test, which shows an increase in the total number of microbes in tofu as they are stored longer. Even though the results of the appearance of tofu are decreasing daily, the average assessment of the appearance of tofu is still in normal tofu conditions typical of white tofu. The interaction between the concentration of betel leaf infusion and the storage time shows that the results significantly affect the appearance sensory quality.

Aroma sensory quality

Aroma is a volatile compound contained in a material; a processing process can also influence the appearance of an aroma. Table 4 shows that the higher the concentration of betel leaf infusion, the lower the aroma sensory value of white tofu. These results are similar with (KIRVE 2024), who reported that the higher the concentration of betel leaves used, the more intense the distinctive aroma of betel leaves that emerged from the essential oil compounds. The aroma is influenced by the content of essential oils and phenolic compounds, chavicol, eugenol, and allyprocathecin (SINGH et al. 2023). However, the white tofu soaked in betel leaf infusion solution still has the typical aroma of white tofu.

Apart from that, the storage time for white tofu soaked in betel leaf infusion significantly affects the aroma sensory value, where the longer the storage time, the aroma of betel leaves tends to weaken or decrease. This condition is due to the nature of the aromatic compounds in betel leaves, which evaporate quickly so that the aroma of betel leaves gradually decreases over time of storage (INDRIASTUTI et al. 2012).

The average storage time for the aroma sensory quality test, namely day 0, day 2, decreased, and on day 4, there is a slight increase. This is in line with the results of the color sensory test, where the longer the storage time, the water content in the tofu will come out and mix. This condition resulting in the water in the solution increasing and making the concentration of the betel leaf infusion decrease, so that the aroma of the betel leaf tending to weaken or decrease. The interaction between the addition of concentration and the length of storage time showed that the results significantly affects the aroma sensory quality test.

Elasticity texture sensory quality

Texture parameter is an essential part of consumer acceptance of a product. Based on Table 4, the infusion concentration has no significant effect on the sensory value of white tofu texture. (INDRIASTUTI et al. 2012) reported that the concentration of betel leaf infusion did not affect the sensory texture of food. Table 4 also shows that storage time significantly affects the texture sensory value. The longer the storage time, the softer the texture sensory value, even though the average of all treatments is in normal conditions.

These results align with (INDRIASTUTI et al. 2012), where the longer the storage

time, the more efficiently the texture of food tends to be destroyed. According to (ARANDINI et al. 2022), the binding capacity of protein and water becomes weaker due to damage to the protein in tofu during storage, so the texture of tofu becomes not chewy or easy to break. The interaction between the concentration of betel leaf infusion and the length of storage time has no significant effect. In general, according to the results of observations from the panelists, the sensory value is still in the high score towards the average, namely 7.2, still chewy, typical of white tofu.

Overall hedonic

The principle of this hedonic test is that panelists are asked for their responses regarding their likes or dislikes on a hedonic scale. This is necessary to determine whether further improvements are needed in the product (TARWENDAH 2017).

Table 4 reveals the crucial finding that the concentration of betel leaf infusion has a significant effect on the hedonic value of tofu. The higher the concentration, the lower the hedonic value. This aligns with (SEPTIANA 2018), which found that the higher the concentration of betel leaf infusion, the lower the panelists' receptivity. The average panelist assessment of the tofu hedonic test is in the range of 5.25-9.12, underscoring the importance of these findings.

Furthermore, Table 4 also indicates that storage time significantly affects the hedonic effect of white tofu, where the longer the storage time, the lower the preference. This is because the condition of the tofu is increasingly changing, as noted in (SEPTIANA 2018). This explains that the longer it is stored, the color, appearance, and aroma change towards signs of damage marked by the appearance of mucus and mold. This highlights the dynamic nature of the product.

The interaction between the concentration of betel leaf infusion and the length of storage time has a significant effect on the hedonic value. In general, the panelists' preference for white tofu soaked in betel leaf infusion is still within the normal value typical of white tofu. The lowest value is obtained by treatment (A3) concentration on the second day, 5.25 of 10.

CONCLUSION

The research results showed that the higher the concentration of betel leaf infusion, the lower the microbial growth. However, it tended to reduce the sensory qualities of white tofu's color, aroma, appearance, and overall hedonics. Meanwhile, on texture sensory, the infusion concentration had no significant effect. This research also showed that the longer the storage time, the more microbes grew, but the number of microbes in treated tofu was much lower compared to control tofu. Storage time also significantly affected the sensory qualities of color, aroma, texture, appearance, and overall hedonics.

On average, tofu stored for four days experienced sensory loss but was still in average condition. The interaction between concentration and storage time significantly affected the number of microbes and the sensory qualities of aroma, appearance, and overall hedonic of white tofu but had no significant effect on the color and texture sensory of white tofu. All concentrations of betel leaf infusion generally had good effectiveness in inhibiting bacterial growth and could maintain the sensory characteristics of white tofu.

AUTHOR CONTRIBUTIONS

Conceptualization, methodology, and formal analysis**, A and MM**; software and validation**, A, MM, and TR;** investigation**, MM**; resources and data curation**, MM**; writing-original draft preparation**, A**; writing-review and editing**, A, and TR**; visualization**, A, and MM**; supervision, **A and TR**; project administration, **MM**; funding acquisition, **A and MM**. All authors have read and agreed to the published version of the manuscript.

FUNDING

This work was not supported by any funding agency.

INSTITUTIONAL REVIEW BOARD STATEMENT

Not applicable for studies not involving humans or animals.

INFORMED CONSENT STATEMENT

Not applicable because this study did not involve humans.

DATA AVAILABILITY STATEMENT

The data can be made available under request.

CONFLICTS OF INTEREST

The authors declare no competing interests

REFERENCES

ANANCHAIPATTANA C et al. 2012. Bacterial contamination of soybean curd (tofu) sold in Thailand. Food Science and Technology Research 18: 843–848.

ARANDINI NPCA et al. 2022. Preservation of tofu with liquid smoke from coconut shell. SEAS. Sustainable Environment Agricultural Science 6: 102–111.

BABARINDE GO et al. 2015. Formaldehyde, a food additive as preservatives: Its applications and health implications-A review. World Journal of Engineering Research and Technology 9: 126–150.

BANSEKA YJ & TUME SJP. 2024. Coliform bacteria contamination of water resources and implications on public health in Fako Division, South West Region, Cameroon. Advances in Environmental and Engineering Research 05: 1–13.

DAUD A et al. 2019. Kajian penerapan faktor yang mempengaruhi akurasi penentuan kadar air metode thermogravimetri. Lutjanus 24: 11–16.

DIENY A et al. 2023. The effectiveness of beluntas (Pluchea indica) leaf extract as antimicrobial in white tofu. Biota: Jurnal Ilmiah Ilmu-Ilmu Hayati 8: 31–40.

DINOS GP et al. 2016. Chloramphenicol derivatives as antibacterial and anticancer agents: Historic problems and current solutions. Antibiotics 5: 20.

FEBRIYOSSA A & KOTEN MI. 2022. Analysis of the contamination of coliform bacteria in tofu soaking water at the tofu factory in the Kalideres Region, West Jakarta. Jurnal Kesehatan Saelmakers PERDANA 5: 217–222.

HAFIZAH I et al. 2021. Effect of betel type (Piper sp) and concentration of betel leaf extract on quality and antibacterial activities of glycerine bar soap. IOP Conference Series: Earth and Environmental Science: 012016.

HAMZAH H et al. 2021. Uji aktivitas antibakteri infusa daun sirih hijau (Piper betle L.) terhadap bakteri Escherichia coli. Jurnal Penelitian Farmasi Indonesia 10: 35–41.

INDRIASTUTI ATD et al. 2012. The effect of betle leaf juice (Piper betle Linn) as precuring agent and storage time on chemical composition and peroxide value of chicken layer dendeng. Buletin Peternakan 35: 182–187.

JANJIĆ L et al. 2023. The impact of food visual aesthetics on consumer perception of a healthy and tasty meal in the hospitality and tourism industry. Economics of Agriculture 70: 689–710.

KAVETI B et al. 2011. Antibacterial activity of Piper betel leaves. International Journal of Pharmacy Teaching & Practices 2: 129–132.

KEMENTERIAN KESEHATAN REPUBLIK INDONESIA. 2017. Farmakope Herbal Indonesia. In: Panjaitan R et al. Eds. 2.Ed. Kementerian Kesehatan Republik Indonesia.

KIRVE DC. 2024. An overview of betel leaf: Green gold of India. Journal of Pharmacognosy and Phytochemistry 13: 240–248.

KUNCARLI I & DJUNARKO I. 2014. Uji toksisitas subkronis infusa daun sirih merah (Piper crocatum Ruiz & Pav) pada tikus: Studi terhadap gambaran mikroskopis jantung dan kadar sgot darah. Jurnal Farmasi Sains Dan Komunitas 11: 86–95.

LEE DY et al. 2017. Microbial contamination of tofu in Korea and growth characteristics of Bacillus cereus isolates in Tofu. LWT 78: 63–69.

MADHUMITA M et al. 2019. Extraction of betel leaves (Piper betle L.) essential oil and its bio-actives identification: Process optimization, GC-MS analysis and anti-microbial activity. Industrial Crops and Products 138: 111578.

PATEL RM & JASRAI YT. 2013. Evaluation of fungitoxic potency of Piper betel l. (mysore variety) leaf extracts against eleven phyto-pathogenic fungal strains. Cibtech Journal of Bio-Protocols 2: 21–28.

PRAKASH B et al. 2010. Efficacy of chemically characterized Piper betle L. essential oil against fungal and aflatoxin contamination of some edible commodities and its antioxidant activity. International Journal of Food Microbiology 142: 114–119.

PRATIWI P et al. 2019. Extraction of betel leaves (Piper betle L) and salam leaves (Syzygium polyanthum) as alternative tofu preservatives. Jurnal Pendidikan Teknologi Pertanian 5: 97.

RAHAYU CW et al. 2019. Aktivitas kombinasi infusa daun sirih merah dan infusa daun sirih dengan klorheksidin terhadap pertumbuhan Porphyromonas gingivalis. Majalah Farmasetika 4: 17–21.

ROSSI F et al. 2016. Microbiological characteristics of fresh tofu produced in small industrial scale and identification of specific spoiling microorganisms (SSO). LWT 70: 280–285.

SALEHI B et al. 2019. Piper species: A comprehensive review on their phytochemistry, biological activities and applications. Molecules 24: 1364.

SARI AN et al. 2021. Identifikasi kadar formalin pada tahu mentah yang dijual di pasar tradisional kota banjarmasin. Journal of Pharmaceutical Care and Sciences 2: 5–14.
SARMA C et al. 2018. Antioxidant and antimicrobial potential of selected varieties of Piper betle L. (Betel leaf). Anais da Academia Brasileira de Ciências 90: 3871–3878.
SENDUK TW et al. 2020. The rendement of boiled water extract of mature leaves of mangrove Sonneratia alba. Jurnal Perikanan Dan Kelautan Tropis 11: 9–15.
SEPTIANA W. 2018. Pengaruh penggunaan ekstrak daun salam, daun sirih, dan serai sebagai pengawet alami tahu terhadap sifat organoleptik. Universitas Lampung.
SETYANINGSIH D et al. 2014. Analisis sensori untuk industri pangan dan agro. IPB Press.
SIMANJUNTAK ADR et al. 2022. Analysis of contamination of Escherichia coli bacteria in white tofu sold all over the market of Lhokseumawe City. Jurnal Ilmiah Manusia Dan Kesehatan 5: 414–425.
SINGH T et al. 2023. A literature review on bioactive properties of betel leaf (Piper betel L.) and its applications in food industry. Food Chemistry Advances 3: 100536.
SPENCE C. 2015. On the psychological impact of food colour. Flavour 4: 21.
SYAHRINASTITI TA et al. 2015. Perbedaan daya hambat ekstrak daun sirih hijau (Piper betle L.) dan daun sirih merah (Piper crocatum Ruiz & Pav) terhadap pertumbuhan Escherichia coli. Jurnal Kesehatan Andalas 4: 421–424.
TARWENDAH IP. 2017. Comparative study of sensory attributes and brand awareness in food product: A review. Jurnal Pangan Dan Agroindustri 5: 66–73.
ULYA N. 2019. Pengaruh konsentrasi dan lama perendaman larutan infusa daun salam (Syzygium polyanthum) terhadap total bakteri Staphylococcus aureus, Escherichia coli, Salmonella sp., dan kadar protein pada daging ayam. Universitas Islam Negeri Maulana Malik Ibrahim.
WIDIYANTI NLPM et al. 2017. Analisis ekstrak tumbuhan rempah sebagai preservatives makanan tahu diuji secara in vitro. Jurnal Sains Dan Teknologi 5: 833–848.
ZULKARNAIN AASD et al. 2023. The antibacterial activity of red betel (*Piper crocatum*) leaf extract toward *Staphylococcus aureus*. Indonesian Journal of Dental Medicine 6(2): 70-73.