Effect of Adding Probiotic Yeast and Fermentation Time on Probiotic Tempeh Characteristics

Mari'ah Qibty, Wisnu Adi Yulianto*, Dwiyati Pujimulyani

Agricultural Product Technology, Faculty of Agroindustry, Mercu Buana Yogyakarta University
Jl Wates km 10, Argomulyo, Sedayu, Bantul Yogyakarta 55753, Indonesia
*Correspondence author, E-mail: wisnuadi@mercubuana-yogya.ac.id

Submitted: 06 July 2023; Revised: 10-9-2023 ; Accepted: 15-10-2023; Published: 15-11-2023

ABSTRACT

Tempeh is one of the foods made from soybean seeds or other ingredients that are processed through fermentation. Tempeh currently circulating in the community does not contain probiotic yeast, the addition of probiotic yeast is intended to provide probiotic properties to tempeh. From previous studies, yeast isolates from radishes and tomatoes have been identified as potential probiotic yeasts. This study aimed to determine the effect of adding the type of probiotic yeast isolate and fermentation time on the number of fungi, the number of yeasts, pH, antioxidant activity, and the level of preference by panelists for the resulting probiotic tempeh. This study used a completely randomized design with a factorial pattern. The first factor was the type of probiotic inoculum (*Saccharomyces boulardii*, yeast isolate LBK K, yeast isolate TMT 2), and the second factor was incubation time (0 hours, 24 hours, and 48 hours). The analysis included the number of fungus and yeast (plate count agar), antioxidant activity (radical scavenging activity (%), and preference level for probiotic tempeh. The data obtained were analyzed for variance (ANOVA) at a 95% confidence level. If there was a significant difference between each treatment was continued with Duncan's multiple range test (DMRT). The results showed that adding probiotic yeast type and a long incubation time affected the microbiological properties, antioxidant activity, and panelist preference. The most preferred tempeh was tempeh with the addition of LBK K isolate with an incubation time of 48 hours with a pH of 5.46, the total number of fungi was 6.62 log CFU/g, the total amount of yeast was 8,63 log CFU/g, the antioxidant activity was 18.58% RSA. Tempeh, with the addition of LBK K yeast isolate, has the potential as a probiotic food.

Keywords: *Probiotic yeast; Saccharomyces boulardi; Tempeh*

INTRODUCTION

Functional food is food or drink with a healthy nutritional content for the human body when used in a predetermined amount. Functional food is expected to prevent disease, slow aging, restore health, and increase endurance. According to Salminen et al (2004), one form of functional food is probiotic food. Probiotics are additional foods that are easily digested by the body and contain non-pathogenic live microbes that are beneficial to the health of living things that consume them. This makes processed food included in functional food because it has a positive effect on health. Probiotics that are often used in food applications such as yogurt, tapai, and tempeh are lactic acid bacteria (LAB) and probiotic yeast. Several criteria must

How to cite: M, Qibty., Yulianto, Wisnu., & Pujimulyani, Dwiyati. (2023). Effect of Adding Probiotic Yeast and Fermentation Time on Probiotic Tempeh Characteristics. *Journal of Food and Agricultural Technology. Vol 1 (*No 1). doi: https://doi.org/10.26486/jfat.v1i1.3390

be met by a microorganism to be categorized as a probiotic, namely having resistance to stomach acid and bile salts, the ability to inhibit pathogenic bacteria, the ability to attach to intestinal epithelial cells, and the ability to inhibit attachment of pathogenic bacteria to epithelial cells (Nuraida et al, 2018).

One product that uses microorganisms is tempeh. Tempeh is a highly nutritious, easy to digest, and delicious product, meeting the increasing demand from consumers looking for high-quality meat substitutes. Tempe is a traditional Indonesian fermented food made from soybeans and is known as a result of mold fermentation. Probiotic bacteria have many benefits, especially in suppressing the growth of pathogenic bacteria in the digestive tract. Based on research, it is known that tempeh has anti-diarrhea properties. The results of Setiawati's research (2015) stated that the majority of the frequency of defecation (BAB) in children before being given the tempeh porridge diet was between 5-10 times/day, which was 46.67%, after being given the tempeh porridge diet the majority of the frequency of diarrhea in children became 1-4 times/day. Hartiningrum's research (2010) concluded that tempeh formula can be used as a substitute for reliever formulas in children with acute diarrhea. The average duration of diarrheal disease when giving the reliever formula was 4.95 days, and giving tempeh porridge was 4.21 daysThe potential of probiotics in tempeh can be increased by adding probiotic yeast. According to Marteau et al. (2001), probiotic yeast is often used as a human drug supplement to reduce diarrhea symptoms and shorten acute gastroenteritis, so it needs to be further developed for its use in the food sector. One of the potential yeasts for probiotics is *Saccharomyces boulardii* which is believed to be a deterrent to *Clostridium difficile* which causes diarrhea due to antibiotics (Stefani, 2019). The results of previous research from Kustyawati (2009) stated that tempeh with the addition of yeast was able to grow together with tempe mold in an amount of 10^7 CFU/g. In this study, the probiotic yeast *Saccharomyces boulardii* and probiotic yeast from fruits and vegetables were used. The results of Yulianto and Pujimulyani's research (2022) stated that there is potential for probiotic yeast from fruits and vegetables, namely, yeast isolates LBK K (from radishes) and TMT 2 (from tomatoes). The LBK K and TMT-2 yeast isolates grow at low pH, are resistant to bile salts, can inhibit pathogenic bacteria, and have autoaggregation, coaggregation, and hydrophobicity properties. This research is expected to know the effect of adding the type of probiotic yeast inoculum added to tempeh. The addition of probiotic yeast inoculum types added to probiotic tempeh is expected to be synergistic in the addition of fungus and yeast so that it can increase health benefits.

The introduction states background of the research supported mainly by the relevant references and ended with the objectives of the research.

MATERIALS AND METHODS

Materials

The main ingredients used in the manufacture of probiotic tempeh are imported soybeans obtained from Godean Market, RAPRIMA brand tempeh yeast obtained from Tempe Bumbung e-commerce/web, Rose Brand rice flour from AR shop, probiotic yeast isolate (*Saccharomyces boulardii* culture, yeast LBK K

How to cite: M, Qibty., Yulianto, Wisnu., & Pujimulyani, Dwiyati. (2023). Effect of Adding Probiotic Yeast and Fermentation Time on Probiotic Tempeh Characteristics. *Journal of Food and Agricultural Technology. Vol 1 (*No 1). doi: https://doi.org/10.26486/jfat.v1i1.3390

and yeast isolate TMT 2) from the Microbiology Laboratory, Faculty of Agro-industry, UMBY. Materials used for microbiological analysis (Potato Dextrose Agar=PDA) as a medium for calculating the number of fungus and yeast. The material for the analysis of antioxidant activity was methanol, 0.1 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution.

Equiptment

The tools for making probiotic tempeh are scales, basins, stoves, pans, filters, cloth covers, and plastic. Equipment for microbiological analysis includes Petri dishes, a laminar airflow chamber, an incubator, a refrigerator, a test tube, a micropipette, a blue pipette, and an autoclave. Equipment for measuring pH and antioxidant activity is a pH meter, measuring pipette, pro pipette, UV-vis spectrophotometer (Shimadzu UV mini 1240), test tubes, Erlenmeyer glass, beaker glass, stirring rod, vortex (Type 37600 mixer).

Methods

This research was divided into several stages, namely preparation of probiotic yeast inoculums, including preparation of yeast and probiotic inoculums, preparation of probiotic tempeh, and analysis of probiotic tempeh.

Preparation of Probiotic Yeast Inoculum

Yeast Tempe (RAPRIMA)

Raprima is weighed as much as 2 grams for 1 kg of soybeans in making tempeh. Then add 1.5 grams of rice flour which have been roasted for 2-3 minutes. The correlation for every 1 gram of yeast has a cell number of 1 x 106 CFU/g. The concentration that has been obtained is then inoculated on tempeh.

Probiotic Cells

Saccharomyces boulardii culture, LBK K yeast isolate, and TMT 2 yeast isolate were taken as much as 2 oses and put into PDA slanting media, then incubated for 24 hours at room temperature. The regenerated isolates were used to make inoculum stock. The correlation for every 1 mL of yeast has a cell count of 1 x 108 CFU/g with an initial optical density (OD) of 0.8 for 250 g of cooked soybeans. The concentration that has been incubated is then inoculated on tempeh.

Making Probiotic Tempeh

Probiotic tempeh is made based on the procedure for making tempeh in general. Dry soybeans are cleaned to remove foreign objects that are mixed with the soybean seeds. Soybeans are washed with water until clean, and boiled with water until 30 minutes. Furthermore, the soybeans are skinned and then soaked for 36 hours. After that, the soybeans are drained thoroughly, then steamed for 1 hour. Soybeans were inoculated with 0.5 g of tempeh yeast and 1 mL of probiotic yeast for 250 grams of soybeans. Furthermore, curing was carried out for 0 hours, 24 hours, and 48 hours. Soybeans are put in plastic which is given air circulation then closed and ripened at room temperature and dark.

How to cite: M, Qibty., Yulianto, Wisnu., & Pujimulyani, Dwiyati. (2023). Effect of Adding Probiotic Yeast and Fermentation Time on Probiotic Tempeh Characteristics. *Journal of Food and Agricultural Technology. Vol 1 (*No 1). doi: https://doi.org/10.26486/jfat.v1i1.3390

Analysis of Probiotic Tempeh

The analysis carried out on probiotic tempeh included microbiological analysis, namely the number of fungi and yeast using the pour plate method (Fardiaz, 1993), pH using a pH meter (Muchtadi dan Ayustaningwarno, 2010), antioxidant activity using the DPPH method (Subagio et al, 2001), and test the level of preference using the hedonic test method (Rahayu, 1998). This preference level test involved 25 panelists to finding out which probiotic tempeh product they liked the most (aroma, taste, texture, color, and overall) using six hedonic scales, namely: 1 (very much dislike), 2 (did not like), 3 (rather dislike), 4 (rather like), and 5 (like), 6 (like very much).

Statistic analysis

The experimental design used was a completely randomized design (CRD) factorial pattern using two factors, namely the type of inoculum (inoculum of tempeh fungi, Saccharomyces boulardii, yeast isolates of LBK K and TMT 2) and incubation time (0 hours, 24 hours, and 48 hours).The data obtained was calculated statistically using ANOVA with a 95% confidence level, and if there was a significant difference between the treatments, then it was continued with the DMRT test.

RESULT AND DISCUSSION

Number of fungi

The number of fungi was determined at incubation times of 0 hours, 24 hours, and 48 hours for all types of probiotic yeast inoculums. The results of the analysis of the number of fungi in probiotic tempeh with the addition of probiotic yeast inoculum types and variations in incubation time are presented in Table 1.

Table 1. Number of fungi (log CFU/g) in probiotic tempeh with the addition of yeast during 48 hours of fermentation

Inoculum type	Fungi Count Log		
	0 hours-Incubation	24 Hour-Incubation	48 hour-incubation
Tempeh Fungi	3.02± 0.02^{a}	4.79 ± 0.07^{c}	6.39±0.12^{d}
Tempeh Fungi + *Saccharomyces boulardii*	2.97±0.03^{a}	4.90 ± 0.00^{c}	6.74± 0.05^{f}
Tempe Fungi + Yeast Isolate LBK-K	2.92±0.03^{a}	4.65 ± 0.07^{b}	6.62±0.11^{ef}
Tempeh Fungi + Yeast Isolate TMT-2	2.90±0.00^{a}	4.81±0.04^{c}	6.57 ± 0.04^{e}

*Numbers followed by different letter notations show significantly different based on Duncan's test a 95% confidence level

Based on the results of factorial ANOVA statistical analysis ($p < 0.05$) there was an interaction between the type of inoculum and the length of incubation that was significantly different. The longer the incubation time, the higher the number of fungi. The addition of inoculum to tempeh can increase the number of fungi by 2 log cycles per 24 hours. The number of fungi incubated at 0 hours, 24 hours and 48 hours was significantly different. Tempeh added *Saccharomyces boulardii* culture, LBK K isolate, and TMT 2 isolate had a higher value than tempeh fungi inoculum at 48 hours of incubation. In the fermentation phase of 30-50 hours optimal growth of tempeh occurs (Fardiaz, 1989). High fungal growth in tempeh with

How to cite: M, Qibty., Yulianto, Wisnu., & Pujimulyani, Dwiyati. (2023). Effect of Adding Probiotic Yeast and Fermentation Time on Probiotic Tempeh Characteristics. *Journal of Food and Agricultural Technology. Vol 1 (*No 1). doi: https://doi.org/10.26486/jfat.v1i1.3390

the addition of *Saccharomyces boulardii* is thought to be due to the high presence of yeast which encourages mold growth in tempeh. The tempeh RAPRIMA fungi contains a single fungus, namely *Rhizopus oligosporus*. The research results in it was found that the fungi can grow simultaneously with the yeast *Saccharomyces boulardii*, LBK K isolate, and TMT 2 isolate. The addition of yeast can significantly increase the growth of the fungus.

Number of Yeast

The number of yeasts was determined at incubation times of 0 hours, 24 hours, and 48 hours for all types of probiotic yeast inoculums. The results of the analysis of the number of yeasts in probiotic tempeh with the addition of probiotic yeast inoculum types and variations in incubation time are presented in Table 2. Based on the results of factorial ANOVA statistical analysis ($p < 0.05$) there was an interaction between the type of inoculum and the length of incubation that was significantly different. This means there is a significantly different effect on adding inoculum types and incubation time on the resulting probiotic tempeh.

Table 2. Number of yeast (log CFU/g) in probiotic tempeh with the addition of yeast during 48 hours of fermentation

Type of Inoculum	Yeast Count Logs		
	0 Hours-Incubation	24 Hour-Incubation	48 Hour-Incubation
Tempeh Fungi	4.06± 0,02[a]	5.41±0.00[d]	6.52± 0.05[e]
Tempeh Fungi + *Saccharomyces boulardii*	4.12 ± 0.00[b]	6.68 ± 0.00[e]	8.85±0.00[g]
Tempe Fungi + Yeast Isolate LBK-K	4.17 ± 0.01[c]	6.56 ± 0.00[e]	8.63±0.00[f]
Tempeh Fungi + Yeast Isolate TMT-2	4.08±0.00[a]	6.56 ± 0.00[e]	8.59±0.00[f]

*Numbers followed by different letter notations show significantly different based on Duncan's test a 95% confidence level

The longer the incubation time, the number of yeast increases. The addition of inoculum to tempeh can increase the number of yeast by 2 log cycles per 24 hours. The number of yeast in tempeh with the addition of *Saccharomyces boulardii* culture, LBK K yeast isolate, and TMT 2 yeast isolate was higher than in tempeh with the addition of tempeh fungi inoculum. *Saccharomyces boulardii* has followed the growth of *Rhizopus oligosporus* mold which is likely to have a mutually beneficial symbiosis regarding nutrient availability between the two (Kustyawati, 2009). In general, yeasts contribute to interactions between microorganisms, texture changes, and biosynthesis of flavor components (Buckle et al, 1987). In this study, it was found that yeast *Saccharomyces boulardii*, LBK K isolate, and TMT 2 isolate can grow together with *Rhizopus oligosporus* or tempeh fungi. Its growth can encourage mold growth on tempeh and change the appearance and flavor of tempeh.

How to cite: M, Qibty., Yulianto, Wisnu., & Pujimulyani, Dwiyati. (2023). Effect of Adding Probiotic Yeast and Fermentation Time on Probiotic Tempeh Characteristics. *Journal of Food and Agricultural Technology. Vol 1 (*No 1). doi: https://doi.org/10.26486/jfat.v1i1.3390

Degree of Acidity (pH)

The degree of acidity (pH) was determined at incubation times of 0 hours, 24 hours, and 48 hours for all types of probiotic yeast inoculums. The results of determining the pH of probiotic tempeh with the addition of probiotic yeast inoculum types and variations in incubation time are presented in Table 3. The results of factorial ANOVA statistical analysis ($p < 0.05$) found that there was a significant interaction between the type of inoculum and the length of incubation. This means that the interaction treatment of inoculum types and incubation time for probiotic tempeh has a significant effect on pH determination.

Table 3. The pH value of probiotic tempeh with the addition of yeast during 48 hours of fermentation

Type of Inoculum	pH value		
	0 Hours-Incubation	24 Hour-Incubation	48 Hour-Incubation
Tempeh Fungi	4.66±0.03[a]	5.05±0.01[b]	5.36±0.08[c]
Tempeh Fungi + *Saccharomyces boulardii*	4.66± 0.00[a]	5.04±0.03[b]	5.47±0.01[d]
Tempe Fungi + Yeast Isolate LBK-K	4.66± 0.01[a]	5.63 ± 0.02[e]	5.46±0.04[s]
Tempeh Fungi + Yeast Isolate TMT-2	4.66± 0.02[a]	5.63 ± 0.04[e]	5.42±0.01[cd]

*Numbers followed by different letter notations show significantly different based on Duncan's test a 95% confidence level

The longer the incubation time, the higher the pH of the probiotic tempeh produced. At 24 hours of incubation, tempeh with the addition of LBK K and TMT 2 isolates was higher than tempeh fungus and *Saccharomyces boulardii*, even though at 48 hours it experienced a decrease in pH. Based on research conducted by Kiers et al (1997) increase in pH value during fermentation occurs due to the increased protein degradation into free amino acids. Following Nurdiani et al (2015) 's increase in pH during the tempeh fermentation process is caused by the presence of *Rhizopus oligosporus* mold which has a high proteolytic activity which can break down protein compounds into amino acids and ammonia. The presence of alkaline ammonia compounds causes the pH of tempeh to increase gradually. Tempeh with inoculum types LBK K and TMT 2 experienced a decrease in pH with an incubation time of 48 hours, so this type of tempeh can increase the age of tempe longer, this decrease is thought to be due to the fermentation of organic acids such as lactic acid and acetic acid.

Antioxidant Activity

Antioxidant activity analysis was carried out at 0 hours, 24 hours, and 48 hours of incubation for all types of probiotic yeast inoculums. The results of the analysis of antioxidant activity with the addition of probiotic yeast inoculum types and variations in incubation time are presented in Table 4. The results of factorial ANOVA statistical analysis ($p < 0.05$) found that there was a significant interaction between the type of inoculum and the length of incubation. This means that the interaction treatment of inoculum types and incubation time for probiotic tempeh significantly affect the results of the analysis of antioxidant activity. The longer the incubation time, the higher the value of antioxidant activity in the resulting probiotic tempeh, and the antioxidant activity increased significantly. The antioxidant activity of probiotic tempeh was

How to cite: M, Qibty., Yulianto, Wisnu., & Pujimulyani, Dwiyati. (2023). Effect of Adding Probiotic Yeast and Fermentation Time on Probiotic Tempeh Characteristics. *Journal of Food and Agricultural Technology. Vol 1 (*No 1). doi: https://doi.org/10.26486/jfat.v1i1.3390

significantly different, except for tempeh with the addition of LBK K yeast isolates, which did not differ significantly at 24 hours and 48 hours of fermentation time.

Table 4. Antioxidant activity (% RSA) on probiotic tempeh with the addition of yeast during 48 hours of fermentation

Type of Inoculum	Antioxidant Activity (% RSA)		
	0 Hours-Incubation	24 Hour-Incubation	48 Hour-Incubation
Tempeh Fungi	11.33 ± 0.55^{a}	21.30 ± 1.82^{s}	24.70 ± 0.42^{e}
Tempeh Fungi + *Saccharomyces boulardii*	11.72±0.00^{a}	16.45 ± 0.45^{b}	25.68 ± 0.00^{e}
Tempe Fungi + Yeast Isolate LBK-K	10.36±0.00^{a}	18.40±1.34^{bc}	18.58±0.48^{bc}
Tempeh Fungi + Yeast Isolate TMT-2	10.94±1.92^{a}	18.70±0.91^{bc}	19.59±0.95^{cd}

*Numbers followed by different letter notations show significantly different based on Duncan's test a 95% confidence level.

Tempeh with the addition of LBK K and TMT 2 yeast isolates had low antioxidant activity, presumably due to the tempeh's low content of phenolic and flavonoid compounds. Tempeh fermented with tempeh fungus had higher antioxidant activity than tempeh added with yeast isolate during fermentation, except for the addition of *Saccharomyces boulardii* at 0 hours and 48 hours of fermentation which was higher. Fungi tempeh and yeast Saccharomyces boulardi produce stronger antioxidant compounds. Tempeh with the addition of *Saccharomyces boulardii* has high antioxidant activity presumably because it contains polyphenolic metabolites. Reported by Datta et al. (2017) in Fahrizal (2022) that the extracellular fraction of Saccharomyces cerevisiae var. boulardi cultures were found to be rich in polyphenolic metabolites viz. *vanillic acid, cinnamic acid, phenyl ethyl alcohol (rose oil), erythromycin, amphetamine,* and vitamin B6 which have antioxidant activity potential 6-10 times greater (assessed by the DPPH test). The stronger the antioxidant activity, the greater the decrease in purple color intensity. Apart from lactic acid, there is an increase in antioxidant activity caused by the activity of probiotic bacteria which will produce compounds that act as antioxidants.

Levels of preference

The preference level test was carried out at 48 hours of incubation on all probiotic yeast inoculums. Test the level of preference for probiotic tempeh through testing of 25 panelists. The quality attributes that were assessed were the aroma, taste, texture, color, and overall attributes of the probiotic tempeh produced. The results of the preference level test for probiotic tempeh with the addition of inoculum type and 48-hour incubation time are presented in Table 5. Based on these data the aroma, taste, texture, and overall attributes of tempeh with the addition of *Saccharomyces boulardii* are significantly different from other types of tempeh. However, the aroma attribute was not significantly different from TMT 2 inoculum type tempeh. Overall tempeh with probiotic yeast was significantly different, except with the addition of TMT 2 yeast isolate. The most preferred tempeh by the panelists was tempeh with the addition of LBK K isolate with aroma, taste, texture, and color values, namely a score of 4 more or somewhat preferred.

How to cite: M, Qibty., Yulianto, Wisnu., & Pujimulyani, Dwiyati. (2023). Effect of Adding Probiotic Yeast and Fermentation Time on Probiotic Tempeh Characteristics. *Journal of Food and Agricultural Technology. Vol 1 (*No 1). doi: https://doi.org/10.26486/jfat.v1i1.3390

Table 5. The level of preference for probiotic tempeh with the addition of yeast during 48 hours of fermentation

Inoculum type	Test Parameters				
	Aroma	Flavor	Texture	Color	Totality
Tempeh Fungi	4.32±1.03[b]	4.52 ± 0.92[b]	4.32 ± 0.99[b]	4.40±1.04[a]	4.32 ± 0.85[b]
Tempeh Fungi + *Saccharomyces boulardii*	3.72 ± 1.17[a]	3.40±1.04[a]	3.84±1.03[a]	4.40±0.96[a]	3.80±1.00[a]
Tempe Fungi + Yeast Isolate LBK-K	4.44 ± 0.92[b]	4.48 ± 0.92[b]	4.40 ± 1.12[b]	4.48±0.77[a]	4.76 ± 0.78[c]
Tempeh Fungi + Yeast Isolate TMT-2	4.16±0.99[ab]	4.16 ± 0.99[b]	4.36 ± 0.70[b]	4.60 ± 0.76[a]	4.28 ± 0.68[b]

*Numbers followed by different letter notations show significantly different based on the Tukey test at the 95% level of confidence
**The higher the attribute value, the more like it is (1=dislike very much 2=dislike 3=somewhat dislike 4=somewhat like 5=like 6=like very much).

Aroma

Aroma is one of the essential parameters in the assessment of food products that determine the level of consumer preference. Aroma is a parameter that can be assessed by the sense of smell (nose). The brain generally recognizes the smell as a mixture of four primary odors, fragrant, sour, rancid, and charred (Winarno, 2007). At 48 hours of incubation, tempeh has a strong tempeh aroma. Based on table 8, the results of the ANOVA statistical analysis ($p < 0.05$) showed an interaction between the type of inoculum and the incubation time of probiotic tempeh. Yeast type and incubation period significantly affect the organoleptic properties of the aroma of probiotic tempeh. The aroma attribute is in the range of 3.72 to 4.44 with a slightly disliked to slightly liked level. Fermentation of soybeans with *Rhizopus oligosporus* and *Saccharomyces boulardii* produces tempeh with a sweet-smelling aroma that covers the aroma of soybeans in general because the yeast has very high proteolytic and lipolytic activity so that it can hydrolyze proteins and fats to produce amino acids, esters, fatty acids, ethanol, acetaldehyde, ethyl acetate, and ethyl butyrate which are components of flavor and aroma (Villijoen and Greyling, 1995).

Flavor

Flavor is one of the important parameters in the assessment of food products that determine the level of consumer preference. Taste is a parameter that can be assessed by the sense of taste (tongue). Taste can be distinguished between sweet, sour, salty, and fresh which is influenced by the ingredients used. At 48 hours of incubation, tempeh has a strong tempeh flavor. Based on table 8, the results of the ANOVA statistical analysis ($p < 0.05$) showed an interaction between the type of inoculum and the incubation time of probiotic tempeh. This means that the interaction treatment of inoculum type and incubation period significantly affect on the organoleptic properties of probiotic tempeh flavor. The taste attribute has a value in the range of 3.40 to 4.52 with a slightly disliked to slightly liked level. In this case, the panelists preferred to control tempeh over tempeh with the addition of probiotic yeast inoculums, this could be because the panelists were used to consuming tempe obtained in the market. In addition, the taste of tempeh with *Saccharomyces boulardii* is sweeter than other tempeh with a strong tempeh flavor. The process of soaking

How to cite: M, Qibty., Yulianto, Wisnu., & Pujimulyani, Dwiyati. (2023). Effect of Adding Probiotic Yeast and Fermentation Time on Probiotic Tempeh Characteristics. *Journal of Food and Agricultural Technology. Vol 1 (*No 1). doi: https://doi.org/10.26486/jfat.v1i1.3390

soybeans causes fermentation by bacteria causing a sour taste and aroma due to the formation of lactic acid (Nurrahman et al, 2015).

Texture

The texture is one of the essential parameters in the assessment of food products that determine the level of consumer preference. The texture is a parameter that the sense of touch can asses. At 48 hours of incubation, tempeh has a compact texture because the entire cotyledon mass is well overgrown by the fungal mycelium. Based on the results of statistical analysis ($p < 0.05$), there was no significant difference between the addition of inoculum types. Tempeh without the addition of probiotics was not significantly different from tempeh added with probiotic yeast inoculums, except for *Saccharomyces boulardii*. The texture attribute has a value in the range of 3.84 to 4.40 with a slightly disliked to slightly liked level. In tempeh with the addition of *Saccharomyces boulardii*, it has a low value, this is because the mycelium in tempeh does not grow evenly covering the material so that the resulting texture is relatively soft. The soft texture is because the number of inoculum cells used will increase organic acids where the compound is in liquid form (Weny et al, 2015). White threads bind the soybeans in fungal fermentation, forming a solid and unified texture (Nurrahman et al, 2015).

Color

Color is one of the essential parameters in the assessment of food products that determine the level of consumer preference. Color is a parameter that the sense of sight can asses. At 48 hours of incubation, the tempeh has a characteristic white color. Based on the statistical analysis results ($p < 0.05$), there was no significant difference between the addition of inoculum types, meaning that all types of tempeh were not significantly different. The color attribute has a value in the range of 4.40 to 4.60 with a slightly like level. The color change is due to the yeast being carried to the surface during fermentation so that it is fermented by carbon dioxide bubbles (Barus, 2011). The longer the incubation time, the white color of the fungal hyphae turns black because sporangiospores begin to form (Nurrahman et al, 2015).

Totality

The overall test parameters are assessed to see the overall acceptability of the product produced. Based on the results of ANOVA statistics ($p < 0.05$), yeast types and incubation time were significantly different, meaning that the interaction between inoculum types significantly affect on the organoleptic properties of the probiotic tempeh produced. The overall attribute has a value in the range of 3.80 to 4.76 with a slightly disliked to slightly liked level. Panelists most liked tempeh with the addition of yeast isolate LBK K. In general, panelists liked tempeh with a non-sweet aroma and taste, white tempeh, and tempeh with a compact or solid texture. In addition, the panelists also liked tempeh with high microbiological properties so that it is safer and healthier when consumed.

How to cite: M, Qibty., Yulianto, Wisnu., & Pujimulyani, Dwiyati. (2023). Effect of Adding Probiotic Yeast and Fermentation Time on Probiotic Tempeh Characteristics. *Journal of Food and Agricultural Technology. Vol 1 (*No 1). doi: https://doi.org/10.26486/jfat.v1i1.3390

CONCLUSION

The addition of probiotic yeast species and long incubation times affected the microbiological properties, pH, antioxidant activity, and the level of panelists' preferences. The tempeh probiotic most preferred by the panelists was tempeh with the addition of LBK-K yeast isolate with an incubation time of 48 hours which had a pH of 5.46, a fungal count of 6.62 log CFU/g, a yeast count of 8.63 log CFU/g, the antioxidant activity was 18.58% RSA. Tempeh with the addition of LBK-K yeast isolate has the potential to be a probiotic food.

REFERENCES

Barus, T., & Lydia, Natalia W. (2010). Dominant Microbiota and Its Role in the Taste of Cassava Tape. *Biota 16*(2), 354-361

Buckle, KA., Edwards, Fleet, & Wootton. (1987). *Food Science*. UI-Press, Jakarta.

Espinosa-Martos, I., & Rupérez, P. (2006). Soybean oligosaccharides. Potential as new ingredients in functional food. *Nutricion hospitalaria*, *21*(1), 92–96.

Fahrizal, AG. (2022). Characteristics and Level of Likeness of Glutinous Tapai Ice Cream and Probiotic Cassava*. Jurnal Teknologi dan Industri Pangan Unisri* 7(2), 153-165

Fardiaz, S. (1989). Food Microbiology. Ministry of Education and Culture. The Directorate of General of Higher Education. *PAU Food And Nutrition*. IPB, Bogor, Indonesia.

Gibson, G R., & M B Roberfroid. (2008). *Prebiotics: Concept, Definition, Criteria, Methodologies, and Products*. Handbook of Prebiotics. New York: CRC Press

Hartiningrum, S Y. (2010). The Effect of Giving Preda and Tempe Formulas on the Old Age of Acute Diarrhea in Children Aged 6 – 24 Months Study at RSU RA. Jepara Regency Kartini. *Thesis.* Semarang: Diponegoro University.

Hayati, E N. (2010). Study of antioxidant activity and dietary fiber content in soybean/rice-based tempeh with the addition of Angkak and variations in fermentation time. *Thesis*. Surakarta : Universitas Negeri Surakarta.

Kiers, J. L., Schreuder, J., Nout, M. J. R & Rombousts, F. (1997). Tempe fermentation, using different substrates and pure culture states, and the effect of substrate modification on in vitro digestibility.

Kustyawati, M. E. (2009). Study of the Role of Yeast in Making Tempe. *Agritech Journal 29*(2)

Marteau, P. R., de Vrese, M., Cellier, C. J., & Schrezeenmeier, J. (2001). Protection from gastrointestinal diseases with the use of probiotics. Am. *J. ClinNutr* 73, 430-436.

Muchtadi, T & F, Ayustaningwarno (2010). *Teknologi Proses Pengolahan Pangan*. Bandung: Alfabeta. Bandung.

Nuraida, L., et al. (2018). Selection of Lactic Acid Bacteria Isolates from Tempe and Tape as Probiotic Candidates. *Journal of Food Technology and Industry* 29(2), 175-184.

Nurdini, A. L., Lilis, N., Suwanto, A., & Suliantari. (2015). Microbial growth dynamics during tempe

How to cite: M, Qibty., Yulianto, Wisnu., & Pujimulyani, Dwiyati. (2023). Effect of Adding Probiotic Yeast and Fermentation Time on Probiotic Tempeh Characteristics. *Journal of Food and Agricultural Technology. Vol 1 (*No 1). doi: https://doi.org/10.26486/jfat.v1i1.3390

fermentation in two different home industries. *International Food Research Journal* 22(4), 1668–1674

Nurrahman, Suparmo, et al. (2012). Fungus Growth, Organoleptic Properties, and Antioxidant Activity of Black Soybean Tempe Produced with Various Types of Inoculums. *Journal of Agritech* 32(1).

Pangastuti, HP & Triwibowo, S. (1996). The process of making soybean tempeh. *Mirror of the World of Medicine* 190, 54-56.

Rahayu, W.P. (1998). *Penuntun Praktikum Penelitian Organoleptik*. Bogor: IPB

Salminen, S., & von Wright, A. (Eds.). (2004). Lactic Acid Bacteria: Microbiological and Functional Aspects, Third Edition (3rd ed.). CRC Press. https://doi.org/10.1201/9780824752033

Setiawati H. (2015), The Effect of Giving Tempe Porridge Diet on the Frequency of Defecation in Children with Diarrhea in the Mina Room of PKU Muhammadiyah Surakarta Hospital.

Stefani, A. (2019). Probiotic *Saccharomyces boulardii* As A Prevention Of Clostridium Difficile Infection Due To The Use Of Antibiotics. *Anatomical Medical Journal*, 2614-5219.

Subagio A, Shigemura Y & Morita (2001). Color stability and lipid oxidation of a dried food model to which carotenoids have been added. *Food Sci. Technol. Res.*(7), 231-234

Vilijoen, B C., & Greyling, T. (1995). Yeast is associated with cheddar and gouda making. *International Journal of Food Microbiology* 28, 79-88

Villijoen, B, -C., & Greyling, -T., 1995. Yeast associated with cheddar and gouda making. *International Journal of Food Microbiology* 28, 79-88. https://doi. org/10.1016/0168-1605(94)00114-L

Weny, A., Darmawati, Sri W. (2015). Old Fermentation Study of the Alcohol Content of Black Glutinous Tape (Oryza Sativa Glutinosa) as the Development of Student Worksheets on Conventional Biotechnology Concepts. Class XII SMA. Riau. Riau University.

Winarno, F. G. (2004). *Kimia Pangan dan Gizi*. Jakarta: Main Library Gramedia

Yulianto, W. & Dwiyati P. (2022). Isolation and Characterization of Yeast Probiotic Potency from Various Sources. *Umbrella Research Report*. Yogyakarta: Mercu Buana University, Yogyakarta.

How to cite: M, Qibty., Yulianto, Wisnu., & Pujimulyani, Dwiyati. (2023). Effect of Adding Probiotic Yeast and Fermentation Time on Probiotic Tempeh Characteristics. *Journal of Food and Agricultural Technology. Vol 1 (*No 1). doi: https://doi.org/10.26486/jfat.v1i1.3390