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CHEMICAL AND MICROBIOLOGICAL PROPERTIES OF SYMBIOTIC YOGURT ICE CREAM WITH THE ADDITION OF WHITE OYSTER MUSHROOM JUICE (*Pleurotus ostreatus*)

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Abstract

This study aims to analyze the effect of symbiotic yogurt with the addition of white oyster mushroom juice on the chemical and microbiological properties of symbiotic yogurt ice cream. This study used a complete randomized design (RAL) with white oyster mushroom juice concentration treatment consisting of 0%, 2%, 4%, 6% and each treatment was repeated 4 times then yogurt as much as 60% of each treatment was added to ice cream. Based on the results of the study, protein levels and fat and crude fiber levels, there was a very real difference (P≤0.01) to the average protein levels between the treatment of symbiotic yogurt ice cream with the addition of white oyster mushroom juice (Pleurotus ostreatus). The results of the study of total lactic acid bacteria and the viability of lactic acid bacteria showed that there was a very noticeable difference (P≤0.01). The conclusion of this study is that symbiotic yogurt ice cream with the addition of white oyster mushroom juice (Pleurotus ostreatus) chemical and microbiological properties is a highly nutritious drink.

Key words*: protein, fat, crude fiber, total BAL, viability.*

INTRODUCTION

Semi-solid food made from a mixture of milk, animal or vegetable fat, sugar with or without other food ingredients and other permitted food ingredients is called ice cream (NSA., 2009). The high nutritional content and people's preference for ice cream has given rise to various innovations and variations of ice cream. The innovation that is developing today is to create ice cream by improving its quality and benefits, so that the ice cream consumed has the effect of improving health or is often referred to as a functional food (Putri et al., 2020).

The development of ice cream products is widely carried out to increase consumer acceptance, for example the development of symbiotic yogurt ice cream. This is because yogurt has a sour taste when mixed in the manufacture of yogurt ice cream, it will increase consumer acceptance because it will produce a sweet and fresh taste, in addition to having good probiotic viability. Symbiotic yogurt ice cream is an ice cream that uses a mixture of symbiotic yogurt in its manufacture (Tamime & Robinson, 2021; Widyaningsih et al., 2021; Rosida et al., 2022).

Beside adding probiotic lactic acid bacteria as a starter, ingredients containing prebiotics are also added to make yogurt, so it is called symbiotic yogurt. Prebiotics are food ingredients that are useful in increasing and influencing probiotic bacteria activity and growth. The role of prebiotics is to stimulate the growth of probiotic bacteria by providing a substrate that can be digested by the bacteria so that the population increases. An increasing population of probiotic bacteria in the gastrointestinal tract can reduce the growth of harmful pathogenic bacteria, increase gastrointestinal endurance, prevent constipation and help better absorption (Putri et al., 2020).

The process of making ice cream includes mixing of the ingredients, pasteurization, homogenization, cooling, aging, freezing, hardening and storage (Arbuckle et al., 2018). Symbiotic yogurt ice cream is a product obtained by modification of ice cream recipe by using symbiotic yogurt.

Symbiotic yogurt contains lactic acid bacteria *Lactobacillus bulgaricus*, *Streptococcus thermophilus* and *Lactobacillus acidophilus* as probiotic also have immunomodulatory functions, antioxidant properties and hypocholesterolemic effects, as well as contribute to the balance of microbes in the host's digestive tract when consumed in sufficient quantities as well as dietary fiber for the probiotic bacteria food in yogurt (Kusumaningtyas et al., 2019; Tari et al., 2020). The types of prebiotics known so far are fructo - oligosaccharides, galactose, oligosaccharides and inulin.

White oyster mushroom (*Pleurotus ostreatus*) is a food commodity that has high nutritional value, containing β-glucans. The β-glucan fiber found in the juice of white oyster mushrooms can act as a prebiotic so that it can increase the viability of lactic acid bacteria in the probiotic group (Anissa & Radiati, 2018).

This study aims to analyze the quality of symbiotic yogurt ice cream with the addition of white oyster mushroom juice based on chemical properties and microbiology to produce functional drinks.

MATERIALS AND METHODS

This research was carried out in May 2024 at the Animal Husbandry Product Technology laboratory, Faculty of Animal Husbandry, Sam Ratulangi University, Manado, starting from sample preparation, and the process of making symbiotic yogurt ice cream with the addition of white oyster mushroom juice (*Pleurotus ostreatus*).

Testing of crude fiber, total protein, total fat, total lactic acid bacteria and the viability of lactic acid bacteria was carried out in the Applied Chemistry Laboratory of the Department of Medical Laboratory Technology, Manado Polytechnic.

The ingredients used in this study are white oyster mushroom juice from the traditional market in Manado city, milk, whippy cream, eggs, skimmed milk, gelatin, sugar, starter culture consisting of *Streptococcus thermophilus, Lactobacillus bulgaricus, Lactobacillus acidophilus* bacteria as much as 3% from the microbiology laboratory of the medical faculty of Brawijaya University, Malang Indonesia.

Starter preparation: *Lactobacillus bulgaricus* from the microbiology laboratory of the medical faculty of Brawijaya University, Malang Indonesia 1mL of was grown in MRS broth media up to 10mL, then incubated for 24 hours at 37°C. Skimmed milk, 16 g and sugar 2 g and pure water 200 mL were sterilized at 121°C for 15 minutes and *Lactobacillus bulgaricus* were inoculated into a sterile solution and incubated for 18 hours at 37°C, as well as in the manufacture of *Streptococcus thermophilus* and *Lactobacillus acidophilus* starters (Tamin et al., 2001).

Crude fiber determination: 2 to 3 g of sample was weighted, introduced into a reflux flask or fiber extraction device, then hydrolysed with 200 mL H_2SO_4 1.25%, heated to boiling, maintained for 30 minutes while stirring slowly; after that, the strain was filtered through a filter paper, then the residue was washed with hot water until neutral. The residue was dried in the oven at 105°C for 2 to 4 hours until it reached a constant weight after which the dry residue was weighed. The residue was calcinated at 550-600^{0}C for 2 to 3 hours to remove the organic substances and cooled in a desiccant and weighed the remaining residue again (to measure the content of cellulose, lignin, and a small portion of hemicellulose).

The total protein determination by Kjelhadl method (measuring the total nitrogen content, then multiply it by a conversion factor (usually 6.25) to obtain the crude protein content): 2-5 mL of sample, introduced in a Kjeldahl flask, added concentrated sulphuric acid (H_2SO_4) and catalyst; heated until the solution becomes clear (all organic matter is oxidized). After cooling, NaOH solution is added to make the solution alkaline. The resulted NH_3 was distilled into a flask containing boric acid solution (H_3BO_3). Then the ammonium borate solution is titrated with standard acid (usually HCl or H_2SO_4). Using methyl red or bromocresol blue indicator to see the end point of the titration. The volume of acid used is calculated to determine the nitrogen content (AOAC., 2005).

Fat determination: 8mL sample was mixed with 8 g of sand (that has been heated until smooth); the mix was put in a thimble and placed into a Soxhlet tube. Next, cooling flow is carried out through the condenser. The extraction tube is installed on the Soxhlet distiller and inserts enough petroleum ether to be extracted for 4 hours. Residue stirring in the extraction tube is carried out. After extraction, the solvent evaporated from the boiling flask, then the remaining fat was dried to residue at 105°C to remove traces of solvent, cooled in a desiccator and weighed to constant mass (AOAC., 2005).

Testing the viability of lactic acid bacteria: the measurement of the growth curve of lactic acid bacteria can be carried out on two types of medium namely MRS broth (Man Rogosa Sharpe Broth) media and 100 mL milk media. Growth measurements in both mediums were carried out based on colony calculations using the pouring cup method using MRS media so that the turbidity measured λ=600 nm for MRS broth media. Measurements are taken every two hours for 24 hours. The bacterial culture used was conditioned on high viability, so that the population size after powder form was obtained at least 107CFU/g. The growth curve in MRS broth was carried out by inoculating 1 mL of lactic acid bacterial broodstock into 10 mL of MRS broth and incubating at 370C, agitation of 200 rpm. Its turbidity was measured at 600 nm. The growth curve in the 100 mL milk medium is carried out by, as much as 1 mL of lactic acid bacterial broodstock resulting from incubation in MRS broth is differentiated centrifugated at 8000 rpm for 10 minutes at 4°C, then washed once using 0.85% NaCl by re-centrifugation at 8000 rpm for 10 minutes at 4°C to obtain bacterial pellets. The bacterial pellets obtained were then inoculated in a 100 mL milk medium, and incubated at 37°C with agitation of 200 rpm, then measured the pH value and viability of the bacteria with a pouring cup at a dilution of 10-6-10-8 using MRS agar media. Sampling was carried out at a time range of every 2 to 24 hours and the viability of bacteria was calculated with a pouring cup (Arbuckle et al., 2009).

Making the white oyster mushroom juice: white oyster mushrooms were cleaned and washed, then weighed 500 g, after which were cut and extracted with 500 mL pure water homogenized for 10 minutes using a blender and pasteurized until it reaches 75oC and maintained for 30 minutes, after which it was cooled to 45°C and filtered using a filter cloth (AOAC, 2005).

Obtaining the symbiotic yogurt: 500 ml milk supplemented with white oyster mushroom juice (with 0%, 2%, 4%, 6% volume ratio) according to the treatment, pasteurized for 15 minutes at 85oC, cooled to 45°C; 3% of a starter was added and then incubated for 18 hours at 27°C.

The pros of making symbiotic yogurt ice cream with the addition of white oyster mushroom extract consists of homogenization of all the ingredients (500 mL of milk, 125 g of sugar, 100 g of powdered milk and 100 g of whipped cream, until evenly mixed; then, the other ingredients (300 mL of water, 3 g of gelatine and 2 egg yolks) are beaten and cooked. The two mixtures were combined, then pasteurized at 80°C, maintained for 10 minutes, with continuous stirring during pasteurization. The final pasteurized mixture was cooled at 45°C, added with 60% symbiotic yogurt from each treatment of white oyster mushroom juice while stirring. The symbiotic mix was aged at 10°C for 24 hours, aerated 30 minutes and feezed at -10°C for 24 hours. Symbiotic yogurt ice cream is ready to be analyzed.

RESULTS AND DISCUSSIONS

The results on total average value of crude fiber, protein, fat, of symbiotic yogurt ice cream with white oyster mushroom juice *(Pleurotus ostreatus)* can be seen in Table 1.

Table 1. Average total value of crude fiber, protein, fat, of symbiotic yogurt ice cream

Treatment (%)	Crude Fiber (%)	Total Protein (%)	Fat Total (%)
0	1.813±0.010[a]	4.483±0.480[a]	4.464±0.011[a]
2	1.955±0.054[b]	5.633±0.925[b]	3.975±0.035[b]
4	2.020±0.024[c]	8.667±0.185[c]	3.704±0.001[c]
6	2.941±0.009[d]	9.646±0.105[d]	3.341±0.170[d]

The research results from Sakul et al. (2020) about synbiotic yogurt provide results for protein value of 2.77-3.26%, crude fiber value of 2.90-3.15%, and fat value of 2.96-3.49%, and these findings are incorporated into the production of ice cream to enhance the quality of ice cream into a functional beverage. Based on the results of the study, it was shown that the addition of symbiotic yogurt to the ice cream formulation provided a very real difference ($P \leq 0.01$) to the total value of crude fiber of symbiotic yogurt ice cream. The average value of total crude fiber varies from 1.813 to 2.941%. The average value of the total crude fiber produced increases further depending on how much symbiotic yogurt is added in the ice cream formulation. The juice of white oyster mushrooms contains polysaccharides so that they cannot be enzymatically degraded into subunits that can be absorbed in the stomach and intestines. White oyster mushroom juice has dietary fiber which is very important for digestive health (Rochmayani et al., 2019).

Crude fiber is part of dietary fiber that cannot be digested by human digestive enzymes, but it has an important role in digestive health. Crude fiber is generally composed of lignin, cellulose, and hemicellulose that are resistant to hydrolysis by digestive enzymes. In fermented dairy products such as symbiotic yogurt, the fiber content can come from additives such as prebiotics, fruits, or other plant-based ingredients. Crude fiber in symbiotic yogurt ice cream provides benefits, including maintaining digestive health, being food for probiotic bacteria so that it increases the benefits of symbiotics, can increase the viscosity in symbiotic yogurt ice cream, increasing the crude fiber in symbiotic yogurt ice cream provides functional benefits and improves quality (Widyaningsih et al., 2021).

Based on the results of the study, the addition of symbiotic yogurt to the ice cream formulation made a very significant difference ($P \leq 0.01$) to the total protein value of symbiotic yogurt ice cream. The average protein value varied from 4.483 to 9.646%. This protein content has met the ice cream quality local standard set in SNI 01- 3713-1995 which is at least 2.7%. The protein content of symbiotic yogurt ice cream can be affected by the type of raw materials, the proportion of ingredients, and the type of prebiotics used in the formulation. The average value of the resulting protein depends on how much symbiotic yogurt is added in the ice cream formulation. The higher the concentration of white oyster mushroom extract the higher is the increase in protein value. Proteins are complex organic compounds of high molecular weight that are polymers of amino acid monomers that are linked to each other by peptide bonds. It was stated that the more lactic acid bacteria present in yogurt ice cream (Mukhoiyaroh et al., 2022). The main composition that plays a role in protein content is milk. The more dissolved protein, the higher protein levels. This can happen because prebiotics are a source of energy that lactic acid bacteria use for metabolism that produces lactic acid. Lactic acid is the result of the breakdown of various organic substances (Putri et al., 2020). During the fermentation process, several essential amino acids are also formed that make up proteins from the bacterial cell mass of lactic acid. Protein in ice cream making serves to stabilize fat emulsions after homogenization, add flavor, help foaming, increase and stabilize water binding power which affects the viscosity and texture of soft ice cream (Puspa et al., 2022).

Based on the results of the study, it was shown that the addition of symbiotic yogurt to the ice cream formulation provided a very real difference ($P \leq 0.01$) to the fat value of symbiotic yogurt ice cream. The average value of fat content varied from 4.464 to 3.341%. The average value of the resulting fat content decreases depending on how much symbiotic yogurt is added in the ice cream formulation. This fat content has met the ice cream quality local standard set in SNI 01-3713-1995, which is 5%. Fat is a very important source of nutrients, improving texture and taste, as well as a source of vitamins A, D, E, K. The fat content of symbiotic yogurt ice cream can play a role in protecting probiotic bacteria during the manufacturing and storage process, especially against temperature changes, in addition to providing health benefits, namely maintaining the intestinal microbe (Sitompul et al., 2022). Low fat levels can be affected by the lipolytic activity of lactic acid bacteria. that can cause a decreasing of fat content and an increasing of lactic acid levels (Ngatini et al., 2018). The fat content of symbiotic yogurt ice cream is determined by the concentration of symbiotic yogurt added in the ice cream mix because during the yoghurt fermentation the fat is hydrolyzed into simpler compounds like fatty acids and glycerol, with a distinctive aroma in yogurt and ice cream (Widyaningsih et al., 2021).

Table 2. Total average value and viability of lactic acid bacteria in the symbiotic yogurt ice cream

Treatment (%)	Total Lactic Acid Bacteria (CFU/mL)	Viable Lactic Acid Bacteria (CFU/mL)
0	6,822±0.018a	1,125.05±10.10a
2	6,952±0.002a	1,296.35±15.88b
4	7,137±0.005b	1,477.63±19.74c
6	8,243±0.001c	1,566.67±14.31d

The results of the total statistical analysis of lactic acid bacteria showed that there was a very significant difference ($P \leq 0.01$) in the average total value of lactic acid bacteria between the treatment of symbiotic yogurt ice cream with the addition of white oyster mushroom juice (Pleurotus ostreatus). The increase in the total value of lactic acid bacteria is influenced by the increasing quantity of white oyster mushroom juices in the fermentation yogurt used by lactic acid bacteria to grow and develop. The total lactic acid bacteria in this study ranged from 1,125.05-1,566.67 CFU/mL. The polysaccharides found in the juice of white oyster mushrooms can function as prebiotics. Prebiotics function to support the growth and development of lactic acid bacteria. This total lactic acid bacteria occurs because symbiotic yogurt ice cream in the manufacturing process must go through a freezing and hardening process, causing bacteria to experience "cold" and difficult to develop (Puspa et al., 2022). Lactic acid bacteria utilize lactose and prebiotics found in symbiotic yogurt as a source of energy during the fermentation of milk into yogurt. The results of statistical analysis of the viability of lactic acid bacteria showed that there was a very significant difference ($P \leq 0.01$) in the average viability value of lactic acid bacteria between the treatment of symbiotic yogurt ice cream with the addition of white oyster mushroom juice *(Pleurotus ostreatus)*. The viability value of lactic acid bacteria ranged from 6,822-8,243 CFU/mL. According to the National Standardization Agency (2009), the quality requirement for the number of lactic acid bacteria starter bacteria in yogurt is at least 107 CFU/mL or 7 log CFU/mL, so that the symbiotic yogurt ice cream products produced from this study are in accordance with and meet SNI standards for yoghurt quality requirements and the consumption of symbiotic yogurt products can provide health effects for the body (Nadisyah et al., 2023). Along with the addition of white oyster mushroom juice, the viability of lactic acid bacteria in symbiotic yogurt ice cream increases. This is suspected to be due to the influence of polysaccharide carbohydrates found in the juice of white oyster mushrooms that function as prebiotics for lactic acid bacteria so as to increase their growth (Rosida et al., 2022). The viability of lactic acid bacteria in symbiotic yogurt ice cream is influenced by a variety of factors, including the addition of prebiotic ingredients and the length of storage. The main component of the lactic acid bacterial fermentation substrate in yogurt is milk containing lactose and protein, as well as the influence of white oyster mushroom juice (Puspa et al., 2022).

CONCLUSIONS

Based on the research results, it can be concluded that the addition of symbiotic yogurt with 6% white oyster mushroom juice to the ice cream mixture produces ice cream that is highly nutritious and beneficial for health in terms of crude fiber, total protein, total fat, total lactic acid bacteria and the viability of lactic acid bacteria.

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