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Yusmarini Yusuf
Universitas Riau


Usman Pato
Universitas Riau


Shanti Fitriani
Universitas Riau


Evy Rossi
Universitas Riau

**Research Article**

# Evaluation of the Quality of Fishballs Using Several Types of Preservatives During Early Frozen Storage

**Yusmarini Yusuf*, Usman Pato*, Shanti Fitriani, Emma Riftyan, Evy Rossi, Diky Arma Fauzi, Ghina Ismadiah, Miftahul Hidayah, Windy Sabiliani**

*Agricultural Technology Department, Faculty of Agriculture, Universitas Riau, Pekanbaru 28293 Indonesia.*

**Abstract** | Fishballs are processed food products with high water content and are easily damaged. The addition of several types of preservatives can be used as an effort to extend the shelf life of fishballs. This study aimed to study the quality of fishballs using several preservatives following fish standards during early frozen storage. A completely randomized design with four treatments and four replications was used in this experiment to create 16 experimental units. The treatments in this study were the addition of several types of preservatives with different concentrations, namely without the addition of preservatives (P1), the addition of 0.3% sodium nitrite (P2), 2.5% chitosan (P3), and 0.6% bacteriocin (P4). All samples were stored at freezing temperatures for 30 days. Data were analyzed statistically using analysis of variance, and differences between treatments were analyzed using DNMRT at the 5% level. The results showed that the application of various preservatives might impact the quality of fishballs during the first 30 days of frozen storage. Even though the chemical and sensory parameters of fish meatballs without preservatives that were kept at freezing temperatures showed the count of microorganisms that had exceeded the quality standard on the early 30$^{th}$ day, they were still comparable to those preserved with nitrite, chitosan, and bacteriocin preservatives. Based on sensory evaluation, the fishballs for all treatments had a smooth surface, were slightly hollow and bright, tasted delicious and had somewhat product-specific flavor, and had a dense, compact, and slightly chewy texture.

**Keywords** | Fishballs, Bacteriocin, Chitosan, Sodium nitrite, Freezing temperature

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***Correspondence** | Usman Pato and Yusmarini Yusuf, Agricultural Technology Department, Faculty of Agriculture, Universitas Riau, Pekanbaru 28293 Indonesia; Email: usmanpato@yahoo.com and Email: marini_thp@yahoo.co.id
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## INTRODUCTION

Meatballs are made by mashing processed meat products, adding flour, spices, and other ingredients, forming small balls, and then boiling them in hot water. Wodi et al. (2019) stated that meatballs are one of the popular foods and are also liked by all groups because meatballs can be served in various ingredients and forms on the market to attract consumer interest in meatballs. Generally, the meat used as raw material in the manufacture of meatballs is beef, chicken, and fish. Fishballs are processed fishery products that use minced fish meat or a minimum of 40% surimi mixed with flour and spices, which undergo formation and cooking (National Standardization Agency, 2017). Fish used to make meatballs include mackerel, tilapia, snapper, grouper, and milkfish. This statement follows Zamili et al. (2020), so far, the community has made meatballs from mackerel (*Scomber scombrus*) and tilapia (*Tilapia mariae*), which are relatively expensive.

Catfish is one of the fish that might be exploited as a source of primary materials for making meatballs and is widely cultivated, especially in Riau Province (Indonesia). Catfish production in 2019 in Indonesia reached 384,883 tons, and in Riau Province, 27,335 tons (Central Bureau of Statistics, 2020). Catfish are known as freshwater fisheries commodities that have the potential to become local superior processed food products taste like meat and are high in unsaturated fatty acids, and are low in cholesterol. Almost all fish parts can be processed (Simanjuntak, 2019). Several studies regarding processed catfish products, namely the manufacture of fish nuggets by Ayu et al. (2020), fish sausages by Rifa'i et al. (2021), and fishballs by Sinaga et al. (2017).

Fish balls have a very short shelf-life (24h) at room temperature. This statement follows Rohimadilwa et al. (2021), which stated that meatballs based on fish meat have high levels of protein and water, and the pH is close to neutral, 6–6.5. As a result, the maximum shelf life when stored at room temperature is just one day. One way to get meatballs with a longer shelf life is to use preservatives. According to the National Food and Drug Supervisory Agency (2013), preservatives are substances added to foods to stop or slow down microbial processes such as fermentation, acidification, breakdown, and other food-damaging processes. Preservatives used can be natural preservatives and chemical preservatives. A natural preservative produced by lactic acid bacteria is called bacteriocin. Bacteriocins can be used as natural agents to inhibit Gram-positive and Gram-negative bacteria (Skariyachan dan Govindarajan, 2019). Pato et al. (2020) stated that the bacteriocins produced by lactic acid bacteria isolated from dadih, mainly *Pediococcus pentosaceus* strain 2397, still exhibited antimicrobial activity at 121°C with an inhibition zone of 5 mm, while at pH 3.0 and 9.0 with inhibition zones respectively are 5.77 and 6.77 mm (Arief et al., 2012). Bacteriocin from *Lactobacillus plantarum* 2C12 had antimicrobial activity against spoilage microbes for beef meatballs (Arief et al., 2012).

In addition to natural preservatives produced by lactic acid bacteria, there are also natural preservatives that are derivatives of chitin (usually found in crustaceans), namely chitosan. Chitosan's ability to prevent pathogenic and putrefactive bacteria gives it antimicrobial capabilities, such as *Salmonella enterica*, the pathogenic bacteria that cause typhus (Pratiwi, 2014). Erlina (2021) reported that adding 1.5% chitosan showed a lower number of bacteria growing, namely 9 x $10^4$ CFU/g, compared to adding 1% chitosan with the number of bacteria which grows at about 14 x $10^4$ CFU/g. Several chemical preservatives still allowed as food preservatives are sodium nitrite, sodium benzoate, sodium nitrate, and sorbic acid. These preservatives can be used as long as they are within limits set by the National Food and Drug Supervisory Agency (2013). One of the preservatives that can be used as a preservative for fishballs is sodium nitrite. According to the National Food and Drug Supervisory Agency (2013), the maximum limit for nitrite preservatives in processed meat, poultry, and game meat products is not more than 30 mg/kg. The use of sodium nitrite as a preservative has been carried out by Siregar (2013), who stated that sodium nitrite has antimicrobial activity, which can prevent the growth of spoilage and toxic bacteria in meat sausage from extending the product's shelf life. Several types of preservatives are expected to have an antimicrobial activity that can inhibit the growth of microorganisms, thereby extending the shelf life of a product. The product's shelf life can be extended through storage at proper temperatures, namely frozen storage. Food is stored to maintain product temperature at -18°C or lower with minimal temperature fluctuations (National Food and Drug Supervisory Agency, 2013). Storage at freezing temperatures has various advantages because it can retain the nutritional content, taste, color, and aroma for ± 4 weeks (Asiah et al., 2020). However, frozen processed food derived from poultry, fish, and meat is a food that has a high risk. So, it requires proper temperature control during handling, processing, distribution, and serving at retail (National Food and Drug Supervisory Agency, 2013). The purpose of this study was to assess fishballs' quality using several preservatives and to obtain the selected treatment according to SNI 01-7266-2017 during early frozen storage.

## MATERIALS AND METHODS

### Raw materials, media, chemicals

The culture used in this study was *Pediococcus pentosaceus* strain 2397, isolated from dadih, traditional fermented milk. Raw fishball materials were fresh catfish (*Pangasianodon hypophthalmus)* (Figure 1), tapioca, pepper, table salt, garlic, and ice. The chemical composition of catfish is presented in Table 1.

**Figure 1:** Catfish (*Pangasianodon hypophthalmus*).

### Bacterial activation and propagation

Bacterial activation is conducted according to Pato et al. (2017). *Pediococcus pentosaceus* strain 2397 isolate was taken as much as 1 ml, inoculated into a test tube containing

5 ml MRS broth (MRSB) medium, and incubated in an incubator at 37°C for 24 hours. The active culture obtained was marked by a change in the medium to become cloudy. The active bacterial suspension was inoculated into a test tube containing MRSB medium. *Pediococcus pentosaceus* strain 2397 was incubated at 37°C for 24 hours.

**Tabel 1:** The chemical composition of catfish.

| Compounds | Number (%) |
|---|---|
| Moisture content | 82.20 |
| Ash | 0.74 |
| Protein | 14.54 |
| Fat | 1.09 |
| Carbohydrate | 1.43 |

**Source:** Sumarto and Pareng (2014).

### Preparation of cell-free supernatant

The preparation of cell-free supernatant was conducted according to Afriani et al. (2017). A 5% culture of *Pediococcus pentosaceus* strain 2397 was inoculated into an Erlenmeyer containing 300 ml of MRSB medium, which had been added with 2% yeast extract and incubated at 37°C for 48 hours in an incubator. The incubated media was poured into a 15 ml centrifuge tube and centrifuged at 4500 rpm for 10 minutes. The resulting liquid is called cell-free supernatant, which contains metabolite compounds formed during fermentation.

### Bacteriocin preparation

Bacteriocin preparation is conducted according to Pato et al. (2021). The supernatant separated from the cells was added with 70% ammonium sulfate. The resulting cell-free supernatant was ±300 ml, and then 143.32 g of ammonium sulfate was added and stirred until dissolved. This mixture was then stored in the refrigerator at 4°C for 24 hours to precipitate the protein. This mixture was centrifuged at 4500 rpm for 10 minutes to obtain pellets. The pellets obtained were then dissolved in 10% of the initial supernatant volume in phosphate buffer (0.1 M, pH 7.0). This mixture was left for 24 hours in the refrigerator at 4°C to obtain a concentrated bacteriocin.

### Preparation of fishballs

Formula and preparation of fishballs were conducted according to Arief et al. (2012). 200 g of catfish meat is mixed with spices (pepper, garlic, sugar, and salt) and mashed using a chopper. Tapioca of as much as 50 g is mixed with the mashed meat little by little and stirred until a homogeneous dough is obtained. The homogeneous dough is molded into meatball balls and ready to be boiled. The meatball balls are boiled in boiling water for 10–15 minutes until the meatballs float. Control treatment referred to Sinaga et al. (2017) without preservatives. The treatment used 0.6% bacteriocin and sodium nitrite, referred to Arief et al. (2012). A 1.2 g of bacteriocin was added before the catfish meat and spices were mashed using a chopper. As much as 0.6 g of sodium nitrite was added before the catfish meat and spices were mashed using a chopper. The use of chitosan referred to Purba et al. (2014); as much as 5 g of chitosan was added to 200 ml of meatball cooking water.

### Chemical and microbiological analysis

Chemical components, including ash and moisture contents, were evaluated according to National Standardization Agency (2017). *Escherichia coli* was quantified using the Most Probable Number (MPN) method following ISO 7251, and the total plate count (TPC) and amount of *Staphylococcus aureus* were quantified using the surface dish method per AOAC (2005).

### Sensory analysis

Sensory testing was carried out according to Setyaningsih et al. (2010). The sensory test carried out is a descriptive test. The descriptive test aimed to determine the characteristics of fishballs resulting from the treatment tested for appearance, aroma, taste, and texture. The panellists who tested were semi-trained panellists from students of the Agricultural product technology study program, Faculty of Agriculture, the University of Riau, who had taken and passed the Sensory Evaluation course, consisting of 15 people. Samples are presented randomly and coded. The sensory assessment was carried out by presenting a sample of fish meatballs, as much as one round of meatballs (± 5 g) on a clean small plate given a random code. Panellists were asked to assess appearance, aroma, taste, and texture for the quality attributes of the descriptive test on the questionnaire sheet provided.

### Experimental design and data analysis

The investigation was conducted experimentally, utilizing a completely randomized design with four treatments and four replications to produce 16 experimental units. All treatments were stored at a freezing temperature of around -18°C and observed on days 0, 10, 20, and 30. The data obtained from the observations were analyzed statistically using analysis of variance. If F count ≥ F table, continue with the duncan multiple range test at the 5% level. Data were analyzed using SPSS software version 23.

## RESULTS AND DISCUSSION

Moisture content is the percentage of water contained in a material. Moisture content can affect the quality of a food product related to its shelf life. The results showed that several preservatives significantly affected fishballs moisture, ash, and protein contents (Table 2).

The P4 treatment had a lower water content value than the other treatments. This finding is because the amount of bacteriocin used is tiny, so it does not affect the water content produced. This statement is in line with Shefira (2019), who states that adding bacteriocin in the form of a small solution does not affect the moisture content of the sausages. Because, during the cooking process, the moisture content of the bacteriocins expands. The influence of other factors, such as freezing temperatures, freezing techniques, and imperfect thawing processes, results in significant differences in the moisture content obtained (Beltran dan Belles, 2018). The moisture content of fishballs without preservatives had a higher value than sodium nitrite and bacteriocin on days 0, 10, 20, and 30. This finding was due to adding more ice water in the production of fishballs in the control treatment than in the other treatments. According to Paulus (2009), giving ice water can affect the increase in water content in meatballs. The treatment using chitosan was not significantly different from the control treatment. Chitosan is thought to have a higher ability to bind water than sodium nitrite and bacteriocin and can prevent the evaporation of water in the product. This finding is in line with Cahyono (2018), which states that chitosan contains polar hydroxyl groups (H+), which can bind water molecules through the bonds between chitosan and water in fishballs; the presence of free water is getting less. Chitosan has hygroscopic properties, resulting in chitosan samples having the ability to absorb water during storage (Walke et al., 2014). The use of sodium nitrite had a moisture content that was not significantly different from bacteriocin on days 10, 20, and 30. This finding was thought to be due to sodium nitrite and bacteriocin compounds which can bind with water. Porto et al. (2017) stated that bacteriocin consists of hydrophilic amino acids.

Ambarwati (2012) stated that sodium nitrite is a white crystalline compound that can dissolve and bind with water. Sausages using sodium nitrite had the highest water content value (61.00%) compared to controls (58.55%) and bacteriocins (58.54%). Sausage dough added with sodium nitrite undergone a cooking process so that the free water in the dough will bind to sodium nitrite and is challenging to evaporate (Shefira, 2019). According to Arief et al. (2012), the moisture content in products is influenced by several factors, including raw materials, additives, processing, packaging, and storage. The results of the moisture content of the fishballs in the P1 and P3 treatments on day 0 to day 30 did not meet the quality standard for fishballs, namely a maximum of 70% (National Standardization Agency, 2017). The moisture content of fishballs treated P2 and P4 on days 10, 20, and 30 met the quality standards of fishballs. The moisture content in a material varies depending on the food ingredients used, both vegetable and animal, environmental conditions, processing, and storage temperature (Sundari et al., 2015).

**Table 2:** The chemical composition of fishballs use of different types of preservatives and stored at frozen temperature.

| Type of preservative | 0 | 10 | 20 | 30 |
|---|---|---|---|---|
| **Moisture content (%) on the day** | | | | |
| Control | 72.26$^{c}$ | 71.17$^{b}$ | 71.83$^{b}$ | 73.33$^{b}$ |
| Nitrite 0.3% | 70.73$^{b}$ | 68.69$^{a}$ | 68.94$^{a}$ | 69.67$^{a}$ |
| Chitosan 2.5% | 71.14$^{b}$ | 70.86$^{b}$ | 72.27$^{b}$ | 73.64$^{b}$ |
| Bacteriocin 0.6% | 67.35$^{a}$ | 68.67$^{a}$ | 68.87$^{a}$ | 69.90$^{a}$ |
| **Ash (%) at day** | | | | |
| Control | 1.22$^{a}$ | 1.15$^{a}$ | 1.08$^{a}$ | 1.03$^{a}$ |
| Nitrite 0.3% | 1.38$^{c}$ | 1.32$^{c}$ | 1.27$^{b}$ | 1.04$^{a}$ |
| Chitosan 2.5% | 1.26$^{ab}$ | 1.22$^{ab}$ | 1.16$^{ab}$ | 0.95$^{a}$ |
| Bacteriocin 0.6% | 1.32$^{bc}$ | 1.29$^{bc}$ | 1.17$^{ab}$ | 0.96$^{a}$ |
| **Concentration of bacteriocin** | **Protein (%) at day** | | | |
| Control | 8.86$^{ab}$ | 10.47$^{c}$ | 9.54$^{b}$ | 8.96$^{c}$ |
| Nitrite 0.3% | 8.52$^{a}$ | 9.04$^{b}$ | 8.65$^{a}$ | 8.61$^{b}$ |
| Chitosan 2.5% | 9.22$^{bc}$ | 8.40$^{a}$ | 8.37$^{a}$ | 8.25$^{a}$ |
| Bacteriocin 0.6% | 9.71$^{c}$ | 8.37$^{b}$ | 9.25$^{b}$ | 8.60$^{b}$ |

Means followed by the lowercase letters indicate a significant difference ($P < 0.05$).

Ash is an inorganic material left over from the combustion of organic material at high temperatures. The results showed that using several types of preservatives significantly affected the ash content of fishballs during frozen storage (Table 2). The treatment of several preservatives did not significantly affect the ash content of fishballs on the 30$^{th}$ day. It is suspected that there was a decrease in the antimicrobial activity of each preservative, so the ash content values are not significantly different. The highest ash content was on the 20$^{th}$ day, namely treatment P2, which was not significantly different from treatments P3 and P4, but significantly different from P1. This finding is due to the mineral content contained in each preservative, namely sodium nitrite, chitosan, and bacteriocin. Sodium nitrite is a salt group compound with a high mineral content and turns into ash when the ashing process is carried out (Shefira, 2019). Shefira (2019) stated that the ash content value of sausages using sodium nitrite is higher (2.91%) than the control treatment (2.85%) but lower than using bacteriocins (2.98%). Chitosan contains mineral elements such as calcium carbonate and a little calcium phosphate which are insoluble in water. These minerals can increase the ash content of food products (Cahyono et al., 2018).

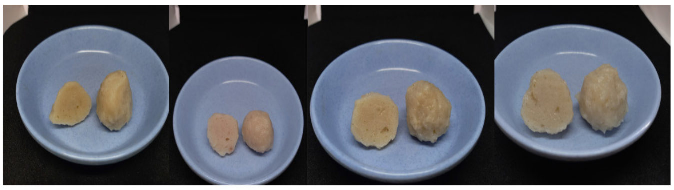
Descriptively, the ash content of fishballs decreased during storage. This finding is due to the longer storage time and more spoilage microbes that grow in fishballs. Based on the total plate count analysis, the data obtained up to the 30th day exceeded the quality standard, namely $10^7$ CFU/g (Table 3). According to Nirmala et al. (2016), spoilage microbes will use the essential elements contained in the product, such as phosphorus, magnesium, iron, and others, so that the ash content decreases. The ash content of fishballs on days 0, 10, and 20, treatment P4 descriptively had a higher value than P1 and P3. This phenomenon was because fishballs treated with P4 in the study used bacteriocins obtained by partial purification by adding ammonium sulfate to the cell-free supernatant to precipitate protein. Bacteriocins are in the partial purification stage during the fractionation process using ammonium sulfate to produce proteins with high salt content containing inorganic compounds. So bacteriocins must be purified through dialysis (Suwayvia, 2017). The ash content results of fishballs for each treatment met the quality standard for fishballs, namely a maximum of 2.5% (National Standardization Agency, 2017).

**Table 3:** Microbial counts of fishballs use of different types of preservatives and stored at frozen temperature.

| Type of preservative | 0 | 10 | 20 | 30 |
|---|---|---|---|---|
| **Total plate count (CFU/g) a day** | | | | |
| Control | $1.09x10^4$ | $1.41x10^5$ | $2.72x10^6$ | $3.32x10^7$ |
| Nitrite 0.3% | $6.15x10^3$ | $9.80x10^4$ | $1.80x10^5$ | $2.72x10^5$ |
| Chitosan 2.5% | $9.59x10^3$ | $1.17x10^4$ | $1.94x10^5$ | $3.43x10^5$ |
| Bacteriocin 0.6% | $5.83x10^3$ | $1.22x10^4$ | $2.17x10^5$ | $2.84x10^5$ |
| **Staphylococcus aureus (CFU/g) a day** | | | | |
| Control | 0 | 0 | 0 | 0 |
| Nitrite 0.3% | 0 | 0 | 0 | 0 |
| Chitosan 2.5% | 0 | 0 | 0 | 0 |
| Bacteriocin 0.6% | 0 | 0 | 0 | 0 |
| ***E. coli*** **(MPN/g) a day** | | | | |
| Control | 14.7 | 0 | 0 | 0 |
| Nitrite 0.3% | 0 | 0 | 0 | 0 |
| Chitosan 2.5% | 0 | 0 | 0 | 0 |
| Bacteriocin 0.6% | 0 | 0 | 0 | 0 |

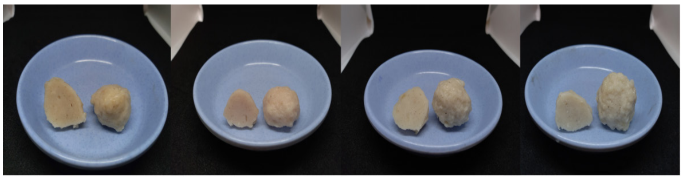
Protein is a substance that functions as a builder, regulator, and source of energy in the body. The variance analysis showed that using several preservatives significantly affected the protein content of fishballs (Table 2). The protein content value of P1-treated fishballs decreased and increased during storage. This finding is presumably due to the constraints that existed at the time of the study, such as a ding power outage that caused the refrigerator current to turn off, affecting the fishballs during frozen storage. Fishball protein content on days 10 and 30 of treatment P2 was not significantly different from treatment P4. The protein content of fishballs with sodium nitrite decreased descriptively from the 10th to the 30th day. It is suspected that there is a link between protein content and water content in the fishballs produced. Protein content has an inverse relationship with water content. Protein content value can be affected by the amount of water content lost from the ingredients, the decrease in water content that occurs results in an increase in the protein content contained in the food (Pratama et al., 2014). Shefira (2019) stated that sausages using sodium nitrite have the highest moisture content, 61.00%, resulting in a low protein content of 11.70%. All bacteriocins are made of protein, so when added to processed food products, it can increase the protein value of the product (Fuziawan, 2012). The addition of various bacteriocin concentrations from *Lactobacillus plantarum* MXG 68 to chicken meat sausage increased protein levels (Ahmad, 2019). This finding follows Fuziawan (2012), who stated that bacteriocin produced from *Lactobacillus plantarum* could increase protein levels in meatballs because it consists of complex peptides. Fishball protein content in the P1 treatment study was higher than P4, in line with Ambarwati et al. (2012) stated that the protein content of boiled cob sardines without the use of preservatives had a higher value (24.36%) compared to the use of sodium nitrite (19.20%) and chitosan with concentrations of 1.5% and 3% (20.00%). Research by Badai et al. (2017) stated that the protein content of processed meat with chitosan stored at freezing temperatures decreased. The results of fishball protein content for each treatment met the quality requirements of fishballs with a minimum of 7% (National Standardization Agency, 2017).

One of the criteria for fishball quality is the quantity of pathogenic and spoilage bacteria. The number of pathogenic and spoilage bacteria in fishballs during cold storage for 30 days is presented in Table 3. The longer the frozen storage time, the TPC value increased in all treatments. However, the TPC value in control was higher than the other treatments from day 0 to day 30. On day 0, the TPC value was relatively high, ranging from $10^3$–$10^4$ CFU/g. This finding probably due to the boiling process was being carried out until the meatballs floated. Thus, it is estimated that heat-resistant bacteria can still live in this process. The TPC value of fishballs in this study was almost the same as the TPC value of beef meatballs using the preservatives nitrite and bacteriocin (Arief et al., 2012) and chitosan (Purba et al., 2014). The TPC value on the 10th day still met the fishball quality standards. However, on the 20th day, the control TPC value exceeded the quality standard, while the nitrite and bacteriocin chitosan treatment still met the quality standard until the 30th day. In general, meatballs have a maximum shelf life of 3 months (Herawati, 2008). The appropriate sanitation and hygiene standards used during the processing likely prevented *S. aureus* growth in all treatments of the fishball samples. Table 3 also showed that on day 0, nitrite, chitosan, and bacteriocin inhibited the growth of *E. coli*. Our previous research supports this finding (Pato et al., 2021; Arief et al., 2012). From day 10 to day 30, no further growth of *E. coli* was found in all treatments. This finding is probably due to the bacteria not growing and dying in cold temperatures. *E. coli* grows well at moderate temperatures, around 36–40°C (Jang et al., 2017).



P1 P2 P3 P4

**a. appearance of fish balls on day 0**



P1 P2 P3 P4

**b. The Appearance of fish balls on the 30$^{th}$ day**

**Figure 2:** The appearance of fishballs stored at freezing temperatures.

**Table 4:** Sensory evaluation of fishballs use of different types of preservatives and stored at frozen temperature.

| Type of preservative | 0 | 10 | 20 | 30 |
|---|---|---|---|---|
| **Appearance at day** | | | | |
| Control | 7.9 | 7.8 | 7.6 | 7.0 |
| Nitrite 0.3% | 7.8 | 7.1 | 7.1 | 7.1 |
| Chitosan 2.5% | 7.8 | 7.4 | 7.4 | 7.4 |
| Bacteriocin 0.6% | 7.9 | 7.8 | 7.6 | 7.6 |
| **Odor at day** | | | | |
| Control | 7.8 | 7.8 | 7.5 | 7.4 |
| Nitrite 0.3% | 7.6 | 7.5 | 7.5 | 7.5 |
| Chitosan 2.5% | 7.6 | 7.6 | 7.6 | 7.6 |
| Bacteriocin 0.6% | 8.2 | 8.2 | 7.8 | 7.8 |
| **Taste at day** | | | | |
| Control | 7.8 | 7.5 | 7.2 | 6.7$^{a}$ |
| Nitrite 0.3% | 7.2 | 7.1 | 7.0 | 7.0$^{ab}$ |
| Chitosan 2.5% | 7.4 | 7.4 | 7.2 | 7.6$^{b}$ |
| Bacteriocin 0.6% | 8.2 | 8.0 | 7.6 | 7.6$^{ab}$ |
| **Texture at day** | | | | |
| Control | 8.3 | 7.8 | 7.6 | 6.3$^{a}$ |
| Nitrite 0.3% | 7.8 | 7.5 | 7.5 | 7.2$^{b}$ |
| Chitosan 2.5% | 7.4 | 7.9 | 7.2 | 7.1$^{b}$ |
| Bacteriocin 0.6% | 8.3 | 8.0 | 7.8 | 7.8$^{b}$ |

Means followed by the lowercase letters indicate a significant difference ($P < 0.05$)

Appearance is an initial characteristic used to determine the quality value and level of product acceptance by consumers using the five senses of sight. The results showed that using several preservatives did not significantly affect the appearance of fishballs (Table 4). The sensory assessment scores for the appearance of fishballs for all treatments from day 0 to day 30, ranging from 7.0–7.9 (the surface is relatively smooth, slightly hollow, and slightly bright). Observations from day 0 to day 30 showed that the use of several preservatives did not significantly affect the appearance of the fishballs. This finding is presumably due to the preservative's ability to maintain the resulting fishballs' appearance. This fact was in line with research by Herzegovina et al. (2022), who stated that the use of sodium nitrite did not affect the appearance of sliced kebab meat. Research conducted by Pitayati et al. (2021) stated that the application of chitosan in the form of an edible coating did not affect the appearance of pempek. Fuziawan (2012) stated that adding bacteriocins did not cause changes in meatballs' physical and sensory characteristics. The appearance of fishballs can be seen in Figure 2. The resulting fishballs have a relatively smooth, slightly hollow, and slightly bright surface appearance. This phenomenon is due to the use of catfish and tapioca meat which can affect the appearance of the meatballs. According to Primadini et al. (2021), tapioca at a concentration of 15% resulted in the appearance of hollow fishballs. According to Husain et al. (2021), tapioca will form a brighter amylopectin paste. Setyadi et al. (2020) stated that adding white catfish meat produced bright meatballs. In line with Fuziawan (2012), which stated that the myoglobin content of the meat used strongly influences the appearance of meatballs. The sensory test results for the appearance of fishballs for each treatment met the quality standards with a minimum score of 7; namely, the surface is rather smooth, slightly hollow, and relatively bright (National Standardization Agency, 2017).

Aroma is one of the sensory attributes that can be tested by the sense of smell and can be used as an indicator of product damage. The results showed that using several preservatives did not significantly affect the aroma of fishballs (Table 4). The sensory assessment score for the aroma of catfish meatballs for all treatments from day 0 to day 30 ranged from 7.4–8.2 (rather a product specific). Observations from day 0 to day 30 produced the aroma of fishballs which were not significantly different for each treatment in frozen storage. This finding is because the components contained in the preservative cannot affect the aroma of the fishballs. According to Sholihatunnisa et al. (2015), chitosan can be added to food and is not harmful to humans. Besides that, the use of chitosan does not affect color and aroma. Cahyono et al. (2018) stated that sodium nitrite, as a food additive, functions as a stabilizer for the red color of meat and slows down the occurrence of rancidity. Bacteriocins do not affect the aroma of fishballs because they did not give rise to an aroma in the product (Patrovsky et al., 2016). The aroma of the fish meatballs produced is somewhat product-specific. The aroma of fishballs is influenced by the aroma of catfish meat used. The use of catfish produces a distinctive fishy odor from fish caused by the protein content in the fish Setyadi et al. (2020). The aroma of meatballs in fishballs arises due to the evaporation of volatile compounds in fish meat during processing or cooking, such as mercaptans and skatole (Harmain et al., 2017). Several factors that can affect the aroma of meatballs are the aroma of meat, the aroma of flour as a filler, spices, and other additional ingredients added Fuziawan (2012). The cooking process can also affect the resulting meatballs color, aroma, taste, texture, and elasticity. The results of the sensory aroma test for each treatment met the fish meatball quality standard with a minimum score of 7, namely the product's somewhat specific aroma (National Standardization Agency, 2017).

Taste is one of the essential characteristics to determine a product's quality and consumer evaluation. The results showed that using several preservatives did not significantly affect days 0, 10, and 20. However, on the 30$^{th}$ day, it significantly affected the taste of the fishballs. The sensory assessment scores for the taste of fishballs for all treatments from day 0 to day 30 ranged from 7.0–8.2 (rather product specific). Using several preservatives in fishballs does not affect the taste of fishballs during frozen storage. This finding is presumably due to the preservative ability of chitosan, sodium nitrite, and bacteriocin to maintain the taste of fishballs. Observations on the 30th day of treatment P1 had the lowest score, significantly different from treatment P3. This finding is because the P1 treatment was fishballs without preservatives, so it is suspected that there was still the activity of spoilage microorganisms which caused the taste of the fishballs to start to change on the 30$^{th}$ day. The resulting taste is caused by the fish used and the addition of spices such as pepper and garlic. Adding spices such as garlic will affect the taste produced because it has several bioactive components, namely sulfide compounds with the highest amount Ardianti et al. (2014). This finding is in line with Sujianti et al. (2020), who state that chemical compounds, concentration, temperature, and the interaction of food ingredients with other taste components can influence taste. Preservatives did not affect the taste of the mackerel meatballs produced. The savory taste produced in the meatballs comes from the fish and the spices used when making the meatballs Syafira (2022). The results of the sensory taste test for each treatment met the fish meatball quality standard with a minimum score of 7, which is a rather specific product taste (National Standardization Agency, 2017).

The texture is one of the organoleptic test parameters that can be felt through the skin or by the senses of taste. The results showed that using several types of preservatives did not significantly affect the texture of the fishballs on days 0, 10, and 20, while on days 30, it had a significant effect (Table 3). The score of sensory assessment of fishballs texture for all treatments from day 0 to day 30 ranged from 6.3–8.3 (dense, compact, and slightly chewy). Observations on days 0, 10, and 20 showed that the texture of the fishballs produced was not significantly different for each treatment. This fact indicated that the preservative used could maintain the texture of the resulting fishballs. Observations on the 30$^{th}$ day of treatment P1 had the lowest score and significantly differed from treatments P2, P3, and P4. Treatment P1 was fishballs without preservatives, so there is a possibility that the activity of spoilage microorganisms could begin to affect the texture of the fishballs. The textural sensory assessment scores decreased descriptively until the 30th day. Yulianti dan Cakrawati (2017) stated that the decrease in the texture level of the meatballs was due to the high total microbes, which caused softening of the meatballs during storage. A decrease in the protein content also caused the texture quality of the meatballs, so the texture assessment also decreased Wirawan et al. (2016).

The higher the protein, the better the water-holding capacity and resulting texture. Protein has hydrophilic properties, so the presence of carboxyl groups in the protein in the product will affect the resulting texture (Lestari et al., 2018). Treatment using sodium nitrite and chitosan can maintain the texture of fishballs on days 0, 10, and 20. This finding is because sodium nitrite is a chemical preservative with a long shelf life. According to Siregar (2013), nitrites added to food can increase its shelf life 10 times longer before it is heated. The addition of chitosan did not affect the texture of keumamah, a typical Aceh food made from tuna and skipjack, because chitosan prevents damage by decomposing microorganisms, which will cause changes in protein and carbohydrates during metabolism, then producing water. Moreover, it causes the texture to become soft (Fauziyani et al., 2019). The texture sensory assessment scores for treatment P4 were higher than those for treatments P1, P2, and P3. This data shows that the panellists prefer the P4 treatment. Panellists preferred the texture of the fishballs with the addition of bacteriocins compared to the control treatment and the addition of nitrites. This data is due to the addition of bacteriocin to the dough in liquid form so that the resulting texture is smoother and more compact (Fuziawan, 2012). The texture of fishballs can be affected by starch, the meat used, salt, water absorption, and other food additives (Fuziawan, 2012). The results of the sensory texture test for each treatment met the fish meatball quality standard with a minimum score of 7, namely a dense, relatively compact, and chewy texture (National Standardization Agency, 2017).

## CONCLUSIONS AND RECOMMENDATIONS

The use of several types of preservatives can affect the quality of fishballs during frozen storage for the initial 30 days. Fishballs without preservatives stored at freezing temperatures showed the number of microbes that had exceeded the quality standard on the 30th day. However, the chemical and sensory parameters were still close to those treated with nitrite, chitosan, and bacteriocin preservatives. Descriptive sensory assessment of fishballs for all treatments had a smooth surface, slightly hollow and slightly bright, flavorful and a somewhat product-specific taste, and dense, compact, and slightly chewy texture.

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## NOVELTY STATEMENT

The novelty of the paper is the utilization of bacteriocin from indigenous Indonesian bacteria as a source of natural preservatives.

## AUTHORS CONTRIBUTION

The research was started by UP, who also oversaw various project phases. UP participated in the experiment's design and oversaw several work phases. The main contributors to the article's research and writing were UP, YY, SF, and ER. The experiment was planned by UP, EmR, DAF, GI, MH, and SY, who also proofread the article. The article's writing and editing were done in collaboration with UP and ER.

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### Ethical approval

The Research Ethics Committee of the Faculty of Nursing, Universitas Riau, Pekanbaru, Indonesia, approved this experimental investigation on sensory analysis (Reference No. 156/UN.19.5.1.8/KEPK.FKp/2021).

### Conflict of interest

The authors have declared no conflict of interest.

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