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EFFECT OF SALT CONCENTRATION AND FERMENTATION TIME IN THE DEVELOPMENT OF ANCHOVY (*Stolephorus sp*) BEKASAM AS TEMPURA RAW MATERIAL

Ririn Margiati, Ilham Marvie*, Syahrizal Nasution

Food Technology Study Programe, Faculty of Industrial Technology, Institut Teknologi of Sumatera, Jl. Terusan Ryacudu, Way Huwi, Jati Agung, Lampung Selatan, Lampung 35365, Indonesia

*Email: ilham.marvie@tp.itera.ac.id

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ABSTRACT

Bekasam is a spontaneously fermented fish product popular among the people of South Sumatra. The raw material in making bekasam is fish, with the addition of salt around 10%-20% of the weight of the fish and rice as a nutrient source. The study aimed to analyze the effect of salt concentration and fermentation time on the chemical characteristics and lactic acid bacteria of anchovy bekasam and the sensory of anchovy bekasam as tempura raw material. Tests were carried out in the form of water content (thermogravimetry), pH (pH meter), fat content (soxhlet), bacterial count (TPC), and sensory (hedonic). The randomized design used was a Randomized Complete Block Design (RCBD) with two factors, namely 15% and 20% salt concentration and fermentation time of 4 days and 7 days. The results showed that salt concentration and fermentation time affected the water content, pH, and total lactic acid bacteria. In contrast, the fat range of the two factors did not have a significant effect but decreased. Differences in salt concentration and fermentation time affect the panelist's liking of color, aroma, taste, texture, and overall parameters. The overall level of liking resulted in the highest mean value in sample K1L2 (15% salt concentration and 7 days fermentation time).

Keywords : Bekasam; Fish; Fermentation; Salt; Tempura

INTRODUCTION

Anchovy (*Stolephorus sp*) is a fishery commodity with high selling value and complete nutritional content (Aryati & Dharmayanti, 2014). According to the Central Bureau of Statistics of Bandar Lampung City in 2020, anchovy is a fish that was widely produced in the Bandar Lampung waters, 25,7.36 tons in 2020 and is ranked third after mackerel and Simba fish (BPS kota Bandar Lampung, 2020). Fresh anchovy in 100 g contains 77 kcal of energy, 16 g of protein, 500 mg of calcium, 1 g of fat, 500 mg of phosphorus, 1 mg of iron, 0.1 mg of vitamin B, and 47 mg of vitamin A (Aryati & Dharmayanti, 2014). The complete nutritional content of anchovies allows for rapid bacterial growth, which can cause damage to the fish if not appropriately handled. One of the efforts that can be used to extend the shelf life of fish is fermentation (Widayanti et al., 2015).

Fermentation is a method of preserving and decomposing compounds with the help of enzymes from microorganisms (Rinto et al., 2019). The benefits of food fermentation are that it can provide taste or flavor to food products, extend product shelf life, and form a new texture from the original product (Putri et al., 2014). One of the fermented fish products is *bekasam*. *Bekasam* is a product of the spontaneous fermentation of freshwater fish with the addition of salt and rice, which is well known among the people of South Sumatra (Huda, 2012). The raw material used to make *bekasam* is fish with salt around 15% to 20% and rice as a nutrient source (Mumtiah et al., 2014).

The fermentation process in '*bekasam*' is influenced by many factors, including the concentration of salt and the fermentation time used (Alyani et al., 2016). During fermentation, salt serves as a texture shaper, enhances flavor, extends shelf life, controls the growth of desirable bacteria, and inhibits unwanted pathogenic bacteria (Majid et al., 2014). The fermentation time affects the characteristics of the resulting *bekasam*, such as softer meat, aroma, sour taste, decreasing water content, decreasing pH, and increasing lactic acid bacteria (Huda, 2012). The difference in salt concentration and fermentation time in making *bekasam* is expected to affect the moisture content, acidity (pH), fat content, organoleptic value, and total lactic acid bacteria (LAB) produced.

The advantages of processing *bekasam* are inexpensive costs, processed products that are easily digested, and increased nutritional value, such as an increase in the amount of protein and a decrease in carbohydrates and fats (Rinto et al., 2019). Organoleptic characteristics include taste, color, aroma, and texture that are distinctive and safe for consumption because the bacteria that grow are lactic acid bacteria, which play a role in helping the digestive process (Marvie et al., 2022). *Bekasam* has a distinctive taste, combining salty and sour flavors that can increase appetite. However, *bekasam* is rarely known to the general public (Widayanti et al., 2015). The dull and unattractive color and shape of *bekasam* make *bekasam* not in demand by the community (Rinto et al., 2022). Based on this phenomenon, it is necessary to innovate the processing of *bekasam* into products that are more attractive to the public, such as tempura. The purpose of this study is to analyze the effect of salt concentration and fermentation time in the development of anchovy (*Stolephorus sp*) *bekasam* as tempura raw material.

RESEARCH METHODS

Material

The ingredients included anchovies from Way Kandis Market, Bandar Lampung, salt (Segitiga), and rice (Bunga). Supporting ingredients included medium protein wheat flour (Segitiga Biru), tapioca flour (Pak Tani Gunung), chicken eggs, kilo panir flour, and cooking oil (Tawon). Materials used for analysis in the form of mineral water (Tripanca), hexane (Himedia), equates, media de Man Rogosa Sharpe Agar (Merck), dan NaCl fisiologis (Merck).

Tools

The tools used include glass jars, digital scales SF-400, pH meter (Biobase 920), oven (Mettler), desiccator, Soxhlet (Buchi 815), analytical scales (Mettler Toled ME204), hotplate (maspion S-301), saucer, clamp, filter paper, cotton, mortar, autoclave (Tommy SX500), incubator (Mettler IN110), laminar airflow (Thermo Scientific Heraguard ECO 0.9), bunsen, Petri dish, Erlenmeyer 100 mL, 250 mL dan 500 mL, beaker glass 50 mL, 100 mL and 250 mL, test tube, threaded tube, test tube racks, micropipettes, spatula, tip, rubber, spray bottle, destruk, vortex (Ohaus VXMNFS), magnetic stirrer, volume pipette, aluminum foil, heat-resistant plastic, plastic wrap, label paper, pens, and score sheet.

Time and Place

The research was conducted from February to May 2023 at the Food Chemistry and Nutrition Laboratory, Microbiology Laboratory, Food Sensory Evaluation Laboratory, Food Processing Process Engineering Laboratory, Food Technology Study Program, Institut Teknologi Sumatera.

Methods

The randomized design used in this research is a Complete Randomized Group Design (RCBD) using two factors. The first factor was 15% and 20% salt concentration, the second factor was 4 days and 7 days fermentation time, with four treatments namely K1L1 (15% salt concentration with 4 days fermentation time), K1L2 (15% salt concentration with 7 days fermentation time), K2L1 (20% salt concentration with 4 days fermentation time) and K2L2 (20% salt concentration with 7 days fermentation time). The study was conducted with two repetitions in Duplo.

Anchovy Bekasam Making

Making bekasam begins with weeding and washing anchovies until clean, mixing 100 g anchovies with 15% and 20% salt concentration and 50 g rice. After that, it is put into a jar to ferment for 4 and 7 days at room temperature.

Bekasam Tempura Making

It made tempura from bekasam anchovies by preparing 100 g of ground bekasam and mixing it with 20 g of wheat flour, 10 g of tapioca flour, and chicken eggs. It is then molded and dipped in egg white (battering) and smeared with panir flour (breading) until the tempura surface is evenly covered. Next, the frying stage is carried out for approximately one minute at 170°C.

Analysis of anchovy scum

Analysis of anchovy bekasam included water content, fat content, pH, lactic acid bacteria, and sensory. Water content analysis using the thermogravimetry method at 105°C is then weighed until the weight is constant (Kiwak et al., 2018). Fat content analysis by extracting fat using the Soxhlet method (Kalor et al., 2022). Analysis of pH using a pH meter by dipping the electrode into a sample dissolved in distilled water (Bawinto et al., 2015). Lactic acid bacteria with total plate count (TPC) method using de Man Rogosa Sharpe Agar (MRSA) media. The number of lactic acid bacteria (30-300 colonies) expressed in log CFU/mL was counted (Arfianty et al., 2017). The sensory test used a hedonic test (favorability test) with a total of 80 untrained panelists. This test was conducted to determine the level of liking for tempura samples from anchovy bekasam with different salt concentrations and fermentation times used.

Data Analysis

The test data were analyzed using Two-way Analysis of Variance (ANOVA) in a Randomized Complete Group Design (RCBD) and further tested with the Duncan Multiple Range Test (DMRT). The applications used to process data were IBM SPSS Statistics 25 and Microsoft Excel.

RESULTS AND DISCUSSION

Moisture Content

Moisture content shows the amount of water contained in food ingredients, where higher moisture content causes food ingredients to deteriorate quickly (Marvie & Sunarti, 2021). This is why water is often removed in food processing by heating or evaporation (Mumtiah et al., 2014). Water content in bekasam is tested before and after fermentation to determine the changes that occur during the fermentation process (Majid et al., 2014). Based on the analysis of the water content of anchovy bekasam with ANOVA, it shows a significant difference between the treatment of salt concentration and fermentation time ($\alpha < 0.05$), so a further test of DMRT 5% is conducted. The results of the water content test on the anchovy

bekasam are presented in Figure 1.

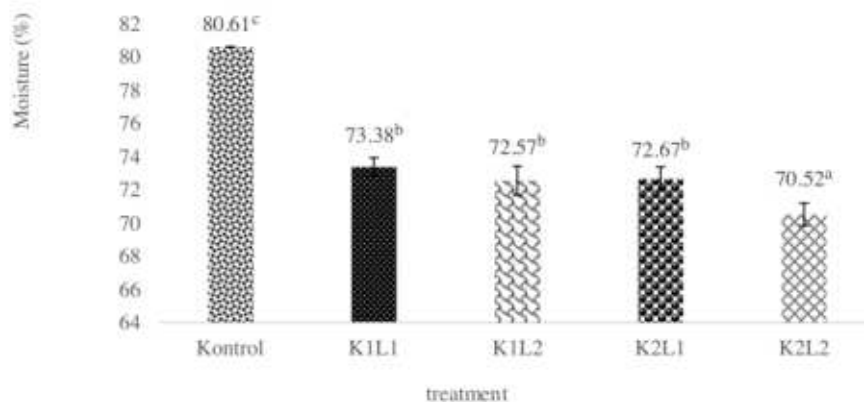


Figure 1. Moisture content of anchovy paste

Description: control (0% salt concentration and 0 days fermentation time), K1L1 (15% salt concentration and 4 days fermentation time), K1L2 (15% salt concentration and 7 days fermentation time), K2L1 (20% salt concentration and 4 days fermentation time), K2L2 (20% salt concentration and 7 days fermentation time).

Figure 1 shows that the water content value of fresh anchovy (control) is 80.61%; after being fermented into bekasam, the water content value ranges from 70.52%-73.38%. The higher the salt concentration with the longer fermentation time, the lower the water content (Alyani et al., 2016). Bekasam, with a higher salt concentration, produces a lower water content; this happens because a high salt concentration will result in a more excellent absorption of the water content in the food material (Majid et al., 2014). Likewise, the longer the fermentation time, the more salt seeps into the fish meat, and the more water is bound by salt so that the water content will decrease (Majid et al., 2014).

The results of this study agree with the research on the water content of catfish powder with a salt concentration of 20%, which produces a lower water content than bekasam with a salt concentration of 10%, which is 4.36% (Puspita et al., 2019). The decrease in water content occurs due to salting during fermentation (Mumtiah et al., 2014). This happens because of the hygroscopic nature of salt, the difference in osmotic pressure between salt and the liquid contained in the fish body, so that water is bound to come out of the food (Puspita et al., 2019).

Fat Content

Fat content in anchovy paste can be known by extracting fat. Based on the ANOVA test results, it was found that the control sample was not significantly different from samples K1L1 (15% salt concentration and 4 days fermentation time), K1L2 (15% salt concentration and 7 days fermentation time), K2L1 (20% salt concentration and 4 days fermentation time) and K2L2 (20% salt concentration and 7 days fermentation time). The test results for the fat content of anchovy paste are presented in Figure 2.

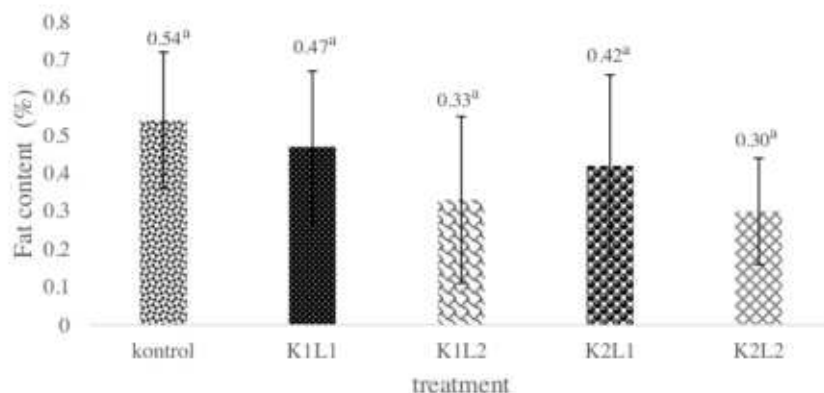


Figure 1. Fat content of anchovy paste

Description: control (0% salt concentration and 0 days fermentation time), K1L1 (15% salt concentration and 4 days fermentation time), K1L2 (15% salt concentration and 7 days fermentation time), K2L1 (20% salt concentration and 4 days fermentation time), K2L2 (20% salt concentration and 7 days fermentation time).

Based on the study's results in Figure 2, the fat content of anchovies before fermentation was 0.54%; after fermentation into bekasam, the resulting fat content was around 0.30%-0.47%. Bekasam, after being fermented for 4 days and 7 days with 15% salt concentration (K1L1 and K1L2), produced a higher fat content of 0.47% ± 0.20 and 0.33% ± 0.22. Bekasam with 20% salt concentration (K2L1 and K2L2) produces lower fat content than Bekasam with 15% salt concentration, which is 0.42% ± 0.24 and 0.30% ± 0.14. This is because the fat breaks down into free fatty acids during fermentation. Similar research shows that the fat content at 20 days of rusip fermentation with an initial fat content of 2% decreased by 0.5% (Putri et al., 2014). The decrease in fat content during fermentation of bekasam is caused by the degradation of fat into fatty acids and lipase enzyme activity (Putri et al., 2014).

Degree of Acidity (pH)

pH analysis was conducted to measure the concentration of hydrogen ions in the sample of anchovy bekasam that had been dissolved with distilled water. Based on the ANOVA test, there was a significant difference between salt concentration and fermentation time. Therefore, a DMRT 5% further test was conducted, and the test results are presented in Figure 3.

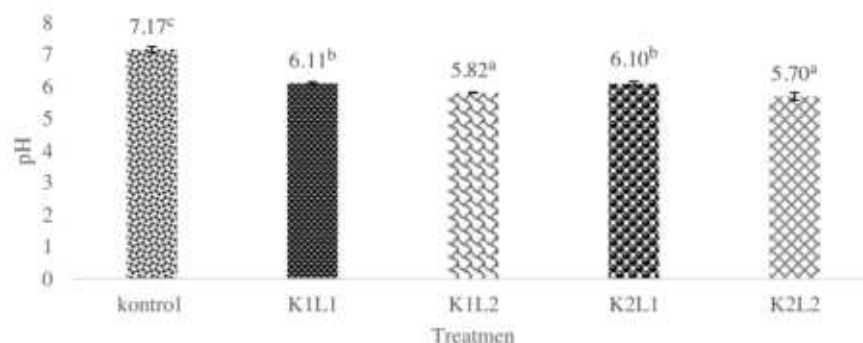


Figure 2. pH of anchovy bekasam

Description: kontrol (0% salt concentration and 0 days fermentation time), K1L1 (15% salt concentration and 4 days fermentation time), K1L2 (15% salt concentration and 7 days fermentation time), K2L1 (20% salt concentration and 4 days fermentation time), K2L2 (20% salt concentration and 7 days fermentation time).

The results showed that the pH value of anchovies before fermentation was 7.17; after fermentation into bekasam, the resulting pH ranged from 6.11 to 5.70. bekasam, after fermentation for 4 days and 7 days with a salt concentration of 15% (K1L1 and K1L2), produced a higher acidity (pH) of 6.11 ± 0.04 and 5.82 ± 0.01 . Bekasam with 20% salt concentration (K2L1 and K2L2) produces a lower pH than bekasam with 15% salt concentration, which is 6.10 ± 0.07 and 5.70 ± 0.12 .

The higher the salt concentration with the longer fermentation time, the more acidic the pH obtained will decrease or become (Arfianty et al., 2017). Salt concentration treatment affects the decrease in acidity due to the presence of Na^+ and Cl^- ions that will bind to free water in the sample, which causes the environmental atmosphere to become acidic due to the formation of HCl compounds (Arfianty et al., 2017). The treatment of fermentation time affects the degree of acidity; the longer the fermentation time, the lower the pH value produced; this is due to the growth of lactic acid bacteria microorganisms that can produce lactic acid so that it can reduce the pH value (Kurnianto & Munarko, 2022).

Lactic Acid Bacteria

Lactic acid bacteria can be classified as homofermentative because the metabolic process can produce large amounts of lactic acid, cells, and few by-products through the Embden-Meyerhof pathway (Huda, 2012). The metabolic pathway of homofermentative lactic acid bacteria can be seen in Figure 4.

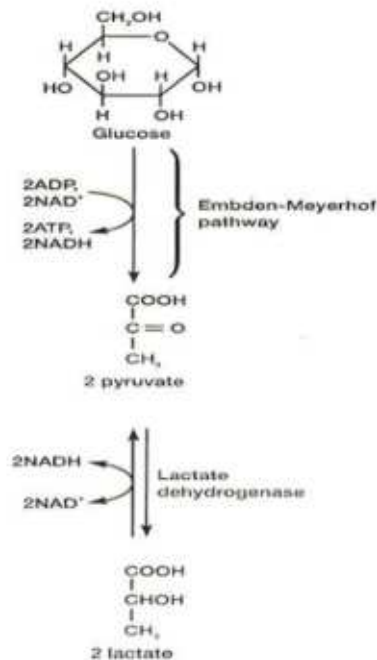


Figure 3. Metabolic pathway homofermentative lactic acid (Huda, 2012)

Based on the results of the ANOVA test, it was found that there was a significant difference between the treatments of salt concentration and fermentation time ($\alpha < 0.05$), so the DMRT 5% test was conducted. The results of the LAB test can be seen in Figure 5.

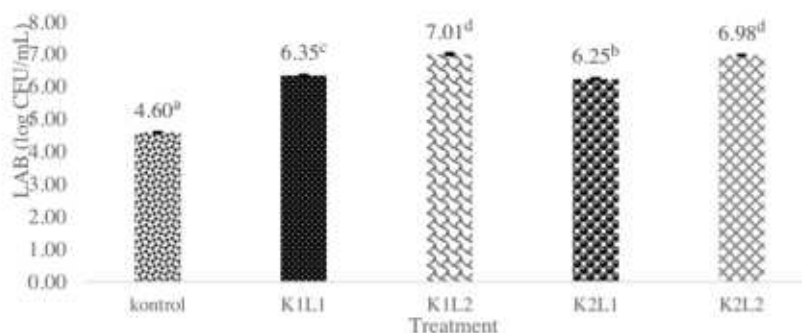


Figure 4. LAB bekasam anchovy

Description: kontrol (0% salt concentration and 0 days fermentation time), K1L1 (15% salt concentration and 4 days fermentation time), K1L2 (15% salt concentration and 7 days fermentation time), K2L1 (20% salt concentration and 4 days fermentation time), K2L2 (20% salt concentration and 7 days fermentation time).

Figure 5. shows the average results of lactic acid bacteria in anchovy paste. Lactic acid bacteria (LAB) ranged from 4.60 log CFU/mL to 7.01 log CFU/mL. In contrast, in the fresh anchovy sample (kontrol), the lactic acid bacteria produced was 4.60 log CFU/mL. Fermentation of bekasam for 4 days and 7 days with 20% salt concentration (K2L1 and K2L2) produced lower lactic acid bacteria of 6.25 log CFU/mL and 6.98 log CFU/mL. At the same time, the fermentation of bekasam with 15% salt concentration produced a higher lactic acid bacteria of 6.35 log CFU/mL and 7.01 log CFU/mL. Figure 6 shows the colonies of lactic acid bacteria (LAB) in bekasam samples with four treatments that grew on MRS agar media after incubating 48 hours at 37°C.

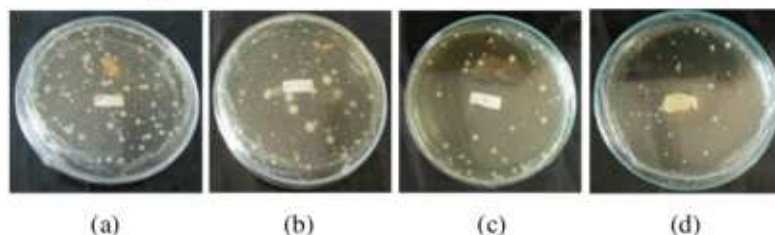


Figure 1. Sample K1L1(a), Sample K1L2 (b), Sample K2L1 (c), Sample K2L2 (d)

Description: kontrol (0% salt concentration and 0 days fermentation time), K1L1 (15% salt concentration and 4 days fermentation time), K1L2 (15% salt concentration and 7 days fermentation time), K2L1 (20% salt concentration and 4 days fermentation time), K2L2 (20% salt concentration and 7 days fermentation time).

Lactic acid bacteria increase with the length of fermentation time (Marvie et al., 2022). The increase in lactic acid bacteria occurs due to adding rice as a carbohydrate source, significantly affecting the number of lactic acid bacteria that grow (Rinto et al., 2019). The optimal amount of rice is the primary substrate that encourages the growth of increasing lactic acid bacteria (Rinto et al., 2019). In addition, the increase in lactic acid bacteria occurs due to salting in the fermentation process, which can stimulate the growth of lactic acid bacteria (Arfianty et al., 2017). Lactic acid bacteria will be inhibited and decreased with the higher concentration of salt added in the process of making bekasam, so the ability to produce lactic acid becomes not optimal (Rinto et al., 2022). In addition to these two things, the growth rate of lactic acid bacteria (LAB) can also be caused by intrinsic and extrinsic factors (Indah et al., 2014). Intrinsic factors include nutrient availability, water activity (A_w), and pH, while extrinsic factors are temperature and inhibiting substances. Bacteria can grow and develop if nutrients are available properly, one of which is the availability of nutrients (Indah et al., 2014).

Based on previous research, fermentation of bekasam with 5% salt concentration produces a total of 8.05 log CFU/mL lactic acid bacteria, 7.5% salt concentration as much as 7.73 log CFU/mL, while 10% salt concentration with a total of 7.13 log CFU/ml lactic acid bacteria (Waty et al., 2019). Indicates that the total lactic acid bacteria produced will decrease the higher the salt concentration used (Waty et al., 2019). This proves that the length of fermentation time and salt concentration affect the increase in total lactic acid bacteria, which is increasing (Puspita et al., 2019). The longer fermentation will also affect the lower pH value with increasing lactic acid concentration (Waty et al., 2019). However, when the performance of lactic acid bacteria has reached its maximum, the changes will not be significant even though the fermentation time is added (Huda, 2012).

Sensory Analysis of anchovy Bekasam

Sensory analysis of anchovy bekasam was conducted using the hedonic method. The hedonic method was conducted by 80 untrained panelists with color, aroma, taste, texture, and overall parameters. Regarding panelists' liking for the color parameter, sample K1L1 produces the lowest value of 2.76. In contrast, sample K2L1 produces the highest value of 2.96, which is in the criteria somewhat like. In the aroma parameter, sample K1L2 produces the highest value of 3.16, which is in the liking criteria. In contrast, sample K2L1 produces the lowest value of 2.95 in the mild liking criteria. The flavor parameter, sample K1L2, produced the highest value of 3.13, which was in the liking criteria. In contrast, sample K2L1 produced the lowest value of 2.65 in the mild liking criteria.

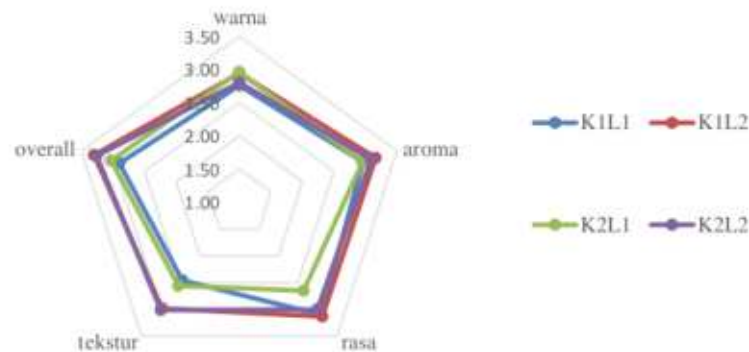


Figure 2. Hedonic Test Results

Description: control (0% salt concentration and 0 days fermentation time), K1L1 (15% salt concentration and 4 days fermentation time), K1L2 (15% salt concentration and 7 days fermentation time), K2L1 (20% salt concentration and 4 days fermentation time), K2L2 (20% salt concentration and 7 days fermentation time) The scale of liking ranges from 0-5, namely (0) dislike, (1) neutral, (2) somewhat like, (3) like, (4) very like, (5) very very like

The lowest tempura texture parameter was in sample K1L1, which amounted to 0.46, somewhat similar to the criteria. The highest texture value in the K2L2 sample is 3.01, which is in the criteria like, which shows that the higher the salt concentration and the longer the fermentation time, the panelists like the more tempura texture (Arfianty et al., 2017). The overall liking level of tempura resulted in the highest average in sample K1L2, which amounted to 3.30, which was in the liking criteria. The lowest average result in sample K1L1 is 2.90, somewhat similar to the criteria. This is because the 15% salt concentration with a fermentation time of 7 days produces a taste and aroma that is not too salty and sour, causing the panelists to overall like the K1L2 sample (15% salt concentration and 7 days fermentation time).

CONCLUSIONS

Salt concentration and fermentation time affected water content, pH, and total lactic acid bacteria. Meanwhile, the fat content of the two factors did not have a significant effect but decreased. Differences in salt concentration and fermentation time affect the level of panelist preference on the parameters of color, aroma, taste, texture, and overall. The overall level of liking resulted in the highest mean value in sample K1L2 (15% salt concentration and 7 days fermentation time).

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