Asian Journal of Applied Research for Community Development and Empowerment. Vol 8 (2024), No.3

AJARCDE
ASIAN JOURNAL OF APPLIED RESEARCH FOR COMMUNITY DEVELOPMENT AND EMPOWERMENT

Journal home page: http://ajarcde-safe-network.org ISSN 2581-0405

Physicochemical and Organoleptic Characteristics of Sourdough Bread with Kefir Grain Added to the Starter

Nayli Husni, Novelina, and Purnama Dini Hari*

Food Technology and Agricultural Product Dept. Faculty of Agricultural Technology, Andalas University, Padang, West Sumatra. Indonesia

ARTICLE INFO

Article History:
Received: 2 October 2024
Final Revision: 28 November 2024
Accepted: 01 December 2024
Online Publication: 03 December 2024

KEYWORDS

fermentation, kefir grains, lactic acid, natural yeast, sourdough bread

CORRESPONDING AUTHOR

*E-mail: *novelina@ae.unand.ac.id*

ABSTRACT

Kefir grains function as a multifaceted starter comprising a variety of microbiota, such as lactic acid bacteria, yeast, and acetic acid bacteria, encapsulated within a polysaccharide matrix. This study investigates the efficacy of kefir grains in creating sourdough starters to assess sourdough bread's physical and chemical properties and identify the ideal formulation based on sensory acceptance. A Completely Randomized Design (CRD) comprised five treatments and three replications. Data were subjected to statistical analysis by Analysis of Variance (ANOVA) followed by Duncan's New Multiple Range Test (DNMRT) at a 5% significant threshold. The results indicated that including kefir grains in the sourdough starter markedly affected dough development, bread volume, acidity (pH), moisture content, ash content, protein content, carbohydrate content, and sensory characteristics, including texture and flavor. Treatment C, utilizing a 10% starting concentration, had the most advantageous outcomes, attaining enhanced sensory and compositional quality.

1. INTRODUCTION

1.1. Research Background

Sourdough bread is one of the oldest biotechnology processes in bread making. Sourdough bread is processed by a fermentation process that utilizes natural microbes from the ingredients. Regular bread uses instant yeast in fermentation, while sourdough bread uses yeast and lactic acid bacteria in the sourdough starter. Sourdough bread is generally made from rye flour and high-protein wheat flour. The taste of sourdough bread is slightly sour with a softer, more moist texture and has a more fragrant aroma [1]. Starter sourdough is made from the fermentation of flour and water. Making a sourdough starter with flour and water alone takes a long time to make the starter stable and active [2]. To accelerate the fermentation process, yeast or fermented ingredients can be incorporated. One such ingredient is kefir grain, a rich source of lactic acid bacteria suitable for enhancing sourdough starter formulations. Kefir grain consists of a symbiotic community of bacteria and yeast embedded within a matrix of proteins and complex carbohydrates [3].

The use of kefir grain in making sourdough starters in bread making has been carried out. Still, the physicochemical and organoleptic characteristics of the sourdough bread produced are not yet known. Using 20% sourdough with the addition of kefir grain can maintain the shelf life of bread up to 15 days [4]. The results of HPLC analysis show that the concentration of lactic acid in sourdough bread using kefir grain is higher than that of ordinary sourdough, where lactic acid plays an important role in the antimicrobial activity of sourdough bread. Other researchers also say that kefir seeds exhibit antimicrobial properties in various food systems [5]. According to Ref.[6], Kefir has been applied to making sourdough bread, which can improve the sensory characteristics, shelf life, and degradation rate compared to bread with commercial yeast

1.2. Research Objective

This study was conducted to determine the effect of adding kefir grain to starters on the characteristics of the sourdough bread produced and to determine the best formulation based on organoleptic acceptance.

CC BY SA

This work is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License
Published under licence by SAFE-Network

2. MATERIALS AND METHODS

2.1 Material and Tools

The ingredients used in this study are kefir grain, high-protein wheat flour, rye flour, sugar, boiled water, and salt. Meanwhile, the chemicals used for the analysis are aquades, NaOH 30% solution, HCl 0.02N, red methyl indicator (mm); blue methyl (mb), H_2SO_4, K_2CrO_4, $AgNO_3$ 0.1 N, selenium catalyst, non-polar organic solvent, alcohol, filter paper.

The equipment used in this study is glass jars, containers, knives, spatula, analytical scales, baking ovens, and baking pans, and the analytical tools used are ovens, aluminum cups, gauges, scales, greying cups, hot plates, kilns, Kjeldahl flasks, distillation flasks, desiccants, fume cabinets, pH meters, measuring flasks 100 ml, erlemeyer, burettes 50 ml, fat flasks, goblet glasses, droppers, filter paper, test tubes, spatula, ultrasonic bath, stirring rods, calipers and apparatus for organoleptic testing.

2.2 Research Design

The research design used was a Complete Random Design (RAL) with five levels of treatment and three replicates. The observation data was analyzed using Analysis of Variance (ANOVA) and continued with Duncan's New Multiple Range Test (DNMRT) test at 5%. The treatment used is a lot of starter concentrations used in making sourdough bread: A = 0% (Starter sourdough without the addition of kefir grain); B = 5%; C = 10%; D = 15%; and E = 20%

2.3 Research Implementation

2.3.1 Determination of Formulation

The formulation used in this study is based on preliminary research—the stages of making sourdough bread start with the development of the sourdough starter. The recipe for making sourdough starters can be seen in Table 1.

Table 1. Formulation of making sourdough starter

No.	Treatment	Composition		
		Kefir Grain (g)	Flour (g)	Water (ml)
1.	Starter Sourdough I (Control)	0	20	20
2.	Starter Sourdough II	10	20	20

Next, the sourdough starter will be added to the sourdough bread dough. The sourdough bread recipe can be seen in Table 2.

Table 2. The formulation for Making Sourdough Bread

Composition	Treatment				
	A	B	C	D	E
Wheat Flour (g)	225	225	225	225	225
Rye flour (g)	25	25	25	25	25
Water (ml)	200	200	200	200	200
Starter sourdough (g)	12.5*	12.5	25	37.5	50
Sugar	10	10	10	10	10
Salt (g)	6	6	6	6	6

Note: Starter concentration is calculated based on total flour (wheat flour and rye flour) and (*) sourdough starter without adding kefir grain.

2.3.2 Making Sourdough Starter

Making a sourdough starter starts with making starter dough by dissolving kefir grain with boiled water, then mixing it with high-protein wheat flour and stirring until well combined. After that, the starter dough is stored in a sealed glass jar for 12 hours at room temperature. After 12 hours, the starter dough is weighed, taken in part, and then given the addition of high protein wheat flour and water in a ratio of 1: 2: 2 after being mixed well, stored until the next 12 hours, and repeated until the starter dough expands double or triple. The control sourdough starter was manufactured for treatment A using the same procedure, namely mixing high-protein wheat flour and boiled water without adding grain kefir.

2,3,3 Making Sourdough Bread

First, carry out the autolyze process, mixing water, high-protein wheat flour, and rye flour until smooth to form a dough, then let it sit for 30-120 minutes at room temperature. Then the mixing process is carried out by adding a sourdough starter to the existing dough by slowly kneading it by hand and then letting it sit for 1 hour at room temperature, here the first fermentation process begins. After an hour, add natural salt and let it sit again for 1 hour until the dough expands 2 times. The dough is pulled and folded (stretched and folded) by hand slowly, then let sit again for 3 hours, and every 30 minutes, the dough is pulled and folded. The dough is pre-shaped to remove excess gas. Shaping is done by forming a dough and placing it on a container covered with cloth, then letting it sit at room temperature ±2 hours; here, the second fermentation process occurs. Next, the dough is transferred to a baking sheet lined with baking paper and baked at a temperature of 235°C for 35 minutes or until the color is browned.

2.4 Observations

Observations of raw materials carried out on sourdough starters are total lactic acid bacteria (BAL), mold/yeast number (AKK), and acidity degree (pH). Meanwhile, the observation of sourdough bread is a physical analysis consisting of the degree of dough development, the degree of bread baking, and the porosity of the bread, and chemical analysis consisting of pH, moisture content, ash content, NaCl content, protein content, fat content, and carbohydrate content, microbiological analysis, namely mold contamination, and organoleptic analysis of color, aroma, taste, and texture.

3 RESULT AND DISCUSSION

3.1 *Raw Material Analysis*

Analysis of raw materials in sourdough starters made with high-protein wheat flour and water and sourdough starters made with grain kefir. The analysis carried out was the degree of acidity (pH), The mold and yeast count (MYC), and the total lactic acid bacteria (BAL). The results of the analysis of raw materials can be seen in Table 3.

The acidity degree (pH) of a sourdough starter without adding kefir grain is 3.65, while the pH of a sourdough starter that adds kefir grain is 3.48. The pH results of sourdough starter I are by; namely, the sourdough starter ranges from 3.5 to 4.3 [7]. Meanwhile, according to Lau, sourdough starters have a low pH range from 4.0 to 4.8 [8]. The pH yield of sourdough starter II is lower, which can occur due to the variable concentration of nutrients during the fermentation process. The treatment of adding kefir grain to the sourdough II starter is the cause of the low pH of the starter. pH starter sekitar 4,0 bahkan dapat lebih rendah yang disebabkan oleh bakteri asam laktat [9]. The same thing was obtained by [4] pH of the control sourdough starter, along with the addition of kefir grain, which was below 4.0.

The average total values of the control sourdough starter's yeast mold and lactic acid bacteria were 4.2 x 107 and 3.7 x 109, respectively. The results obtained in the sourdough starter I are in line with the research of [10]; the sourdough starter that is stable and active has an MYC content of 1.0 x 106 – 5.0 x 107 and BAL 1.0 x 109 – 3 x 109. Meanwhile, sourdough II starter with kefir grain addition treatment had a higher average total AKK and BAL, namely 2.4 x 108 and 2.8 x 1010. This is because the content of kefir grain has lactic acid bacteria as the main population and is accompanied by acetic acid bacteria and yeast. Complex microbiota in kefir grain, which has a symbiotic community where BAL 108 – 109 CFU/g and yeast 107 – 108 CFU/g are energy sources and microbial growth factors during the fermentation process of starter-making [11].

Table 3. Raw Material Analysis Results

Analysis	Raw Material	
	Starter Sourdough I (Control))	Starter Sourdough II
pH (Rata-rata ± SD)	3.65 ± 0.91	3.48 ± 0.73
MYC (CFU/g)	4.2×10^7	2.4×10^8
LAB (CFU/g)	3.7×10^9	2.8×10^{10}

Note: LAB (Lactic Acid Bacteria), MYC (Mold Yeast Count).

3.2 *Physical Characteristics of Sourdough Bread*

3.2.1 Degree of Development of Sourdough Bread Dough

The results of the variance fingerprint analysis showed that the degree of development of sourdough bread dough with the addition of kefir grain in the starter was significantly different at the level of α = 5%. Table 4 shows the results of the degree of development analysis.

In Table 4, it can be seen that the higher the rate of addition of kefir grain, the higher the rate of development of bread dough. This is due to the content of lactic acid bacteria in the sourdough starter. In the fermentation of sourdough bread, there are two types of lactic acid bacteria: homofermentative and heterofermentative. Heterofermentative lactic acid bacteria will ferment glucose into lactic acid, acetic acid, and CO2. The resulting CO_2 gas will be trapped in the gluten, so the bread dough will rise [12]. Therefore, the higher the concentration of kefir used, the more lactic acid bacteria will be contained in the dough. So, the higher the CO2 produced during bread fermentation, the more the development ability of bread dough increases.

Table 4. The average degree of dough development

Treatment	Degree of Dough Development (%) (Mean ± SD)
A (0%)*	22.42 ± 4.87^a
B (5%)	26.80 ± 7.34^b
C (10%)	37.75 ± 5.86^b
D (15%)	40.27 ± 4.20^b
E (20%)	46.00 ± 3.38^b

Note: Numbers in the same column followed by the same lowercase letter differing intangibly at the 5% level of Duncan's New Multiple Range Test (DNMRT)

3.2.2 Degrees of Sourdough Bread Development

The degree of development of sourdough bread ranges from 41.08 – 60.88%. The fingerprint results of various degrees of sourdough bread development with adding kefir grain to the starter significantly differed at the level of α = 5%.%. Table 5 shows the results of the degree of bread development analysis.

Table 5. The average degree of bread development

Treatment	Degree of Bread Development (%) (Mean ± SD)
A (0%)*	41.08 ± 6.46^a
B (5%)	48.35 ± 3.78^b
C (10%)	54.39 ± 5.11^b
D (15%)	55.39 ± 2.59^b
E (20%)	60.88 ± 2.66^b

Note: Numbers in the same column followed by the same lowercase letter differing intangibly at the 5% level of Duncan's New Multiple Range Test (DNMRT)

Based on Table 5, the higher the concentration of kefir grain used, the higher the value of the degree of development of the sourdough bread produced. During the bread dough fermentation, the greater the concentration of kefir, the more CO2 gas will be formed. During the baking process, the gases trapped in the gluten will expand and push the walls of the dough to cause the bread to rise [13]. The baking process on bread can cause some of the water to be lost, gelatinization of the starch, yeast to die, and proteins to clump, which gives the bread a stable shape and makes the dough more fluffy [14].

3.2.2 Bread Porosity

Observing bread porosity was carried out to determine the pores of sourdough bread produced using starters added to kefir grain. Porosity is a cavity found in bread. The porosity of bread is affected by the CO2 gas produced during fermentation [15]. Thus, the more CO2 gas trapped by gluten during the fermentation process, the more fluffy the dough will be. The image of the pores of sourdough bread is enlarged by 80% of the image of a whole bread slice with a photo ratio of 3 4. The picture shows that the

https://doi.org/10.29165/ajarcde.v8i3.538

pores of treatment A bread look small and tend to be uneven, and there are some parts where the pores are not visible.

In contrast to treatments B, C, D, and E, which have bread pores, they tend to be more significant and clearly visible. The image of the appearance of the sourdough bread pores is in line with the results of the bread development degree obtained, where the higher the concentration of the sourdough starter used, the higher the average value of the bread development degree. The pores of the sourdough bread produced by each treatment can be seen in Figure 1.

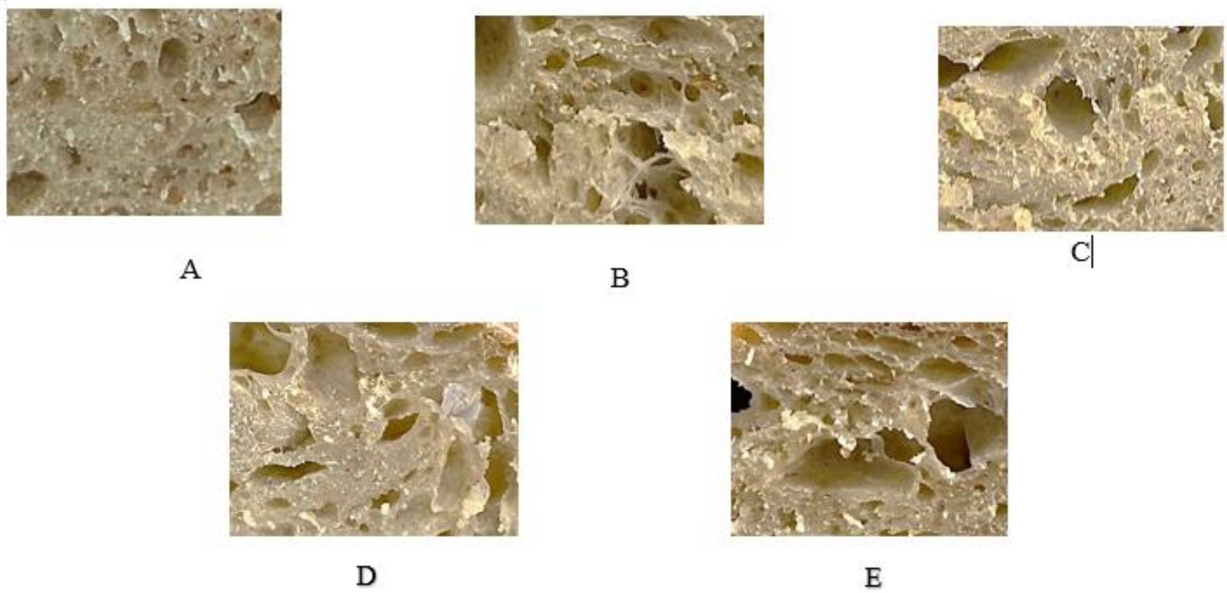

Fig 1. Porosity of sourdough bread per treatment

3.3 Chemical and Microbiological Characteristics of Sourdough Bread

The chemical and microbiological characteristics of sourdough bread observed are moisture content, ash, unsalted ash, NaCl content, protein, fat, carbohydrates, acidity degree (pH), and mold contamination. The results of the analysis can be seen in Table 5.

Table 6. Results of analysis of sourdough bread characteristics

Chemical characteristics	Sourdough starter with the addition of kefir grains				
	A (Control)	**B (5%)**	**C (10%)**	**D (15%)**	**E (20%)**
Water content	32.85±1.04[a]	33.84±1.71[a]	34.49±2.02 [a]	35.03±0.57 [a]	38.33±1.16[b]
Ash content	1.63±0.04[a]	1.73±0.18[a]	1.83±0.21[ab]	2.08±0.17[bc]	2.19±0.17[c]
Ash without salt	0.42±0.12	0.44±0.20	0.49±0.23	0.68±0.13	0.71±0.06
NaCl levels	1.21±0.08	1.29 ± 0.20	1.34 ± 0.11	1.40 ± 0.21	1.48±0.12
Proteins	6.97±0.29[a]	7.35±0.50[a]	7.56±0.27[ab]	8.15±0.43[b]	8.32±0.51[b]
Fat	2.48±0.14	2.64±0.13	2.70±0.11	2.72±0.17	2.77±0.16
Carbohydrate	56.06±0.92[a]	54.45±1.08[b]	53.42±1.83[bc]	52.02±0.80[cd]	48.39±0.63[d]
pH	4.76±0.07[a]	4.68±0.04[ab]	4.65±0.04[ab]	4.63±0.05[bc]	4.57±0.05[c]
Mold	8.0 x 10^2	3.0 x 10^2	2.0 x 10^2	3.0 x 10^2	6.0 x 10^2

Based on the results of the analysis of the water content of sourdough bread in Table 6 with the addition of kefir grain to the starter, it shows that the more concentration of starter is used, the starter sourdough is the result of fermentation of lactic acid bacteria which tend to contain more water than commercial yeast. Kefir grains contain active bacterial and yeast cultures. Using kefir grain to manufacture sourdough starters increases the activity of enzymes in the flour, thereby breaking down more starch in the flour into simple sugars that can absorb more water. According to Jitrakbumrung & Therdthai, sourdough starter can cause partial hydrolysis of starch resulting in a by-product that can bind water [16]. Therefore, the more concentration of starter used, the more moisture content in the sourdough bread produced. The moisture content of bread obtained from the use of sourdough starter with the addition of kefir grain meets the quality requirements (SNI 01-3840-1995) regarding the quality requirements of bread, which is a maximum of 40% [17].

3.3.1 Ash Content

The analysis of ash content from sourdough bread (table 6) with the addition of kefir grain to the starter shows that the more concentration of starter used, the more ash content yield increases. Kefir grains used to manufacture starters contain minerals such as calcium, magnesium, and sodium [18]. The results of the moisture content analysis in sourdough bread can be seen in Table 6.

Turker's research proved that the mineral content of cow's milk kefir is higher than that of cow's milk [19]. Concentrations of Ca, P, Mg, Cu, and Fe increased significantly in cow's milk kefir compared to regular cow's milk. This proves that kefir grain contains minerals and influences the increase in ash content as the concentration of sourdough starter used in bread making increases. In addition, the value of ash content in sourdough bread is due to the mineral content contained in other bread-making ingredients, such as flour. Ash content is a mixture of inorganic components or minerals found in a food. The higher the mineral content in a product, the greater the value of ash content obtained in the product [20].

The ash content without salt was analyzed to determine the minerals in sourdough bread without NaCl. The value of the unsalted ash content is calculated based on the difference between the ash content and the naCl content obtained. The results meet (SNI 01-3840-1995) a quality requirement for bread where the ash content, excluding salt, is a maximum of 1% [17]. Based on Table 8, the highest value is found in treatment E at 0.71%, and the lowest value is found in treatment A at 0.42%. The results are in line with the ash content value, where the higher the starter concentration, the higher the value. The statistical analysis results showed that the ash content excluding salt did not significantly affect the ash content excluding salt of sourdough bread.

3.3.2 NaCl Levels

Sodium chloride (NaCl) in bakery products has an important role in the bread-making process. NaCl influences the development of gluten structure, dough fermentation process, and bakery products' water activity. NaCl can make bread dough stronger and more stable. The more stable structure of the dough contributes to the formation and retention of gas cells during the fermentation process. Bread products without the addition of NaCl will be flat with much larger pores because the dough is sticky and cannot withstand the gases formed due to the weak structure of the dough [21]. The results of the obtained NaCl level follow SNI 01-3840-1995 concerning bread quality requirements, where the maximum NaCl level is 2.5%. Based on the statistical analysis, the difference in the concentration of the sourdough starter used was not real on the NaCl level in the sourdough bread produced.

3.3.3 Protein Content

Based on Table 5, the results of the fingerprint of the protein content of sourdough bread with the addition of kefir grain in the starter used were significantly different at the level of α = 5%. The higher the concentration of starter used, the higher the protein level obtained. Kefir grain contains high protein ranging from 40-60% [22]. The protein content is also sourced from the flour used, namely high-protein wheat flour, which has a 12-14% protein content. Higher protein content in the bread, which is in the range of 6.57 – 18.22% [23]. The high protein levels obtained by Putri are due to the flour formulation with an increase in the number of starter concentrations used [23].

3.3.4 Fat Content

Based on Table 5 above, the results of the fat content analysis show that the higher the concentration of starters used, the higher the fat content value obtained. Fat is one of the body's nutrients because it provides 9 calories/gram of energy. In this study, the difference in the concentration of starter added to kefir grain in making sourdough bread was not real on the fat content of bread. This is in line with the research of Limbad who used kefir grain with coconut water as a sourdough starter, obtaining the fat content in the resulting bread had no real effect between treatments [24].

3.3.5 Carbohydrate Content

Based on the analysis of the carbohydrate content of sourdough bread, the higher the concentration of starter used, the lower the carbohydrate content. The analysis of the variance fingerprint showed that the carbohydrate content of sourdough bread with the addition of kefir grain in the starter used was significantly different at the level of α = 5%. The fermentation process in making sourdough bread using natural yeast can convert the compounds in the bread into simpler compounds. The lactic acid bacteria contained in the sourdough starter produce exopolysaccharide compounds when fermented [25]. The carbohydrate content in sourdough bread is calculated by difference. The results of the obtained carbohydrate content are influenced by other nutritional content such as water, ash, fat, and protein content [26]. So, the higher the content of other nutrients (water, ash, protein, and fat), the lower the carbohydrate content obtained.

3.3.6 Degree of Acidity (pH)

Based on Table 4 above, the results of the pH analysis show that the higher the concentration of starter used, the lower the pH value of the sourdough bread. The results of the various fingerprints showed that the value of the degree of acidity (pH) of sourdough bread with the addition of kefir grain in the starter used was significantly different at the level of α = 5%. The average pH value of the sourdough bread produced ranged from 4.76 - 4.57%. This pH value follows research on the quality of sweet bread with a sourdough starter concentration treatment of 3.5 – 5% [27]. These results stated that the higher the concentration of kefir in the sourdough starter, the more acidic the bread produced. The low pH value is caused by lactic acid production by lactic acid bacteria during the fermentation process of sourdough bread. Microorganisms found in sourdough bread ferment carbohydrates, fats, and proteins that can produce acids [28].

3.3.7 Mold Contamination

Based on Table 5. above, the average value of mold contamination in sourdough bread is < 10 CFU/g. Analysis of mold contamination determines the quality and safety of sourdough bread. According to the bread quality requirements, contamination of bread is a maximum of 1 x 10^4. The results of the mold contamination testing that was carried out met the Indonesian National Standard (SNI) for bread products. The acidification level and antimicrobial properties effectively delay bread spoilage [4]. Other researchers have also said that kefir

https://doi.org/10.29165/ajarcde.v8i3.538

grain exhibits antimicrobial properties in various food systems [4]. pH of sourdough bread made with a mixture of Kluyveromyces marxianus yeast culture 4.3 – 4.6 was able to maintain the shelf life of bread up to 12 days. Meanwhile, regular sourdough bread lasts only 8 days and has a pH value of 5.2 [29].

3.4 Organoleptic

Organoleptic test of sourdough bread with the addition of kefir grain to the starter using the hedonic test method. The observations carried out included aroma, color, taste, and texture with 25 semi-trained panellists. The results of the Organoleptic Test of Sourdough Bread can be seen in Table 7.

Table 7. Average Organoleptic Value of Sourdough Bread

Addition of kefir grains	Aroma	Color	Flavor	Texture
A (0 %)*	3.36 ± 0.64	3.60 ± 0.65	3.00 ± 0.71^{b}	3.20 ± 0.76^{b}
B (5%)	3.72 ± 0.54	3.68 ± 0.75	3.44 ± 0.87^{ab}	3.36 ± 0.70^{ab}
C (10%)	3.76 ± 0.83	3.72 ± 0.84	3.80 ± 0.87^{a}	3.80 ± 0.82^{a}
D (15%)	3.68 ± 0.85	3.64 ± 0.91	3.68 ± 0.85^{a}	3.76 ± 0.93^{a}
E (20%)	3.44 ± 0.65	3.64 ± 0.70	3.64 ± 0.64^{a}	$3.72\ 0.74^{a}$

3.4.1 Color

The color organoleptic test aims to determine the color preference of the panellists for the sourdough bread produced. Based on Table 7 above, the results of the analysis of the various fingerprints of the organoleptic test on sourdough bread with the addition of kefir grain in different starters were not real at the level of α = 5% of the level of preference. The color of the sourdough bread produced is brownish-yellow. The maillard reaction during the baking process causes the formation of color in bread. The maillard reaction is a non-enzymatic browning reaction in carbohydrates, specifically reducing sugars with primary amine groups [30]. The maillard reaction occurs due to the condensation of reducing sugars such as fructose and glucose-containing carbonyl groups with free amine group [31]. The main ingredients for making sourdough bread are high-protein wheat flour and rye flour (rye flour).

3.4.2 Taste

The average organoleptic value on the taste of sourdough bread ranged from 3.00 to 3.80. The results of various fingerprints of organoleptic tests on the taste of sourdough bread were significantly different at the level of α = 5%. The lactic acid bacteria found in starters are responsible for the formation of certain flavor compounds in sourdough bread [32]. The lactic acid bacterial fermentation produces acetic acid and lactic acid causes a sour taste in sourdough bread. The BAL fermentation process also allows protein degradation to produce amino acids that can engulf the umami flavor of sourdough bread [9]. Based on the results of the statistical analysis, sourdough bread without the addition of kefir grain in the starter got the lowest value and was significantly different from the treatment B, C, D, and E where using kefir grain in the starter was preferred by the panellists.

3.4.3 Texture

The results of the various organoleptic tests on the texture of sourdough bread with the addition of kefir grain in the starter used were significantly different at the level of α = 5%. The sourdough bread has a unique texture, the outside of the sourdough bread has a firm texture, while the inside of the bread feels soft and chewy. This is one of the reasons why panellists like sourdough bread: it has a different texture from bread in general. The fermentation of heterofermentative lactic acid bacteria will produce CO2 gas, which will then be trapped in the bread dough and make it fluffy. Lactic acid bacteria will also inhibit starch retrogradation and tighten the texture of bread [33]. The high moisture content of sourdough bread maintains a moist inner bread texture during the baking process. In contrast, the outer texture of the bread is firmer and crispier due to the high baking temperature of the bread. Based on the statistical analysis results, sourdough bread without the addition of kefir grain in the starter got the lowest value and was significantly different from treatments B, C, D, and E where using kefir grain in the starter was preferred by the panellists.

The products preferred by the panellists can be identified through the organoleptic test radar. The average organoleptic radar of the panellists' reception of the color, aroma, taste, and texture of sourdough bread with the addition of kefir grain to the starter can be seen in Figure 2.

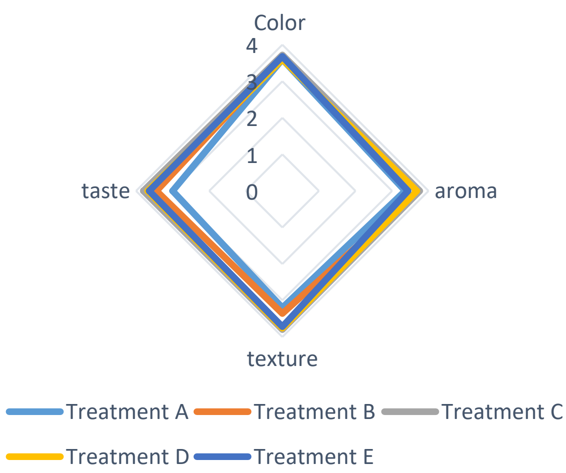

Fig 2. Organoleptic analysis radar

Based on the organoleptic radar graph, it can be concluded that the best treatment is in treatment C (10%) with an average value of 3.76 (likes), 3.72 (likes), 3.80 (likes), and 3.80 (likes) of taste. Overall, the organoleptic value of sourdough bread added by kefir grain in the making of starters was acceptable to the panellists both in terms of aroma, color, taste, and texture of the sourdough bread produced at all levels of starter concentration.

4. CONCLUSION

This study on the production of sourdough bread using kefir grain-enhanced starters yielded the following conclusions: (1) The concentration of sourdough starter with kefir grain significantly influenced dough development, bread expansion, acidity (pH), moisture content, ash content, protein content, carbohydrate content, and sensory attributes such as texture and taste at a 5% significance level; (2) The optimal formulation for sourdough bread was achieved with Treatment C (10% starter concentration). This formulation demonstrated an average dough development of 37.75%, bread expansion of 54.39%, acidity (pH) of 4.65, moisture content of 34.49%, ash content of 1.83%, protein content of 7.56%, fat content of 2.70%, and carbohydrate content of 53.42%, with no mold contamination; (3) Organoleptic evaluations for Treatment C indicated favorable results, with aroma scoring 3.76 (like), color 3.72 (like), taste 3.80 (like), and texture 3.80 (like), highlighting its acceptability and potential for high-quality sourdough bread production. Based on the research that has been carried out in making sourdough bread with the addition of kefir grain to the starter on the characteristics of sourdough bread, it is recommended for the next researcher: (1) Research further regarding the functional properties of sourdough bread[(2) Further research on the physical properties of the sourdough bread produced using different methods to analyze the degree of development and porosity of the bread.

References

[1] S. Ayse, E. Osman, and K. Sinem, "Identification of lactic acid bacteria and yeasts from traditional sourdoughs and sourdough production by enrichment," *Czech J. Food Sci.*, vol. 39, no. 4, pp. 312–318, 2021, doi: 10.17221/56/2021-CJFS.

[2] C. M. Mandey, “Yoghurt Sebagai Starter Sourdough Dan Aplikasinya Dalam Pembuatan Roti Tawar Dengan Substitusi Tepung Kedelai,” Unika Soegijapranata Semarang, 2015.

[3] N. Mohammad Kresna Bayu, Heni Rizqiati, “Analisis Total Padatan Terlarut , Keasaman , Kadar Lemak , dan Tingkat Viskositas pada Kefir Optima dengan Lama Fermentasi yang Berbeda,” vol. 1, no. April, pp. 33–38, 2017.

[4] I. Mantzourani, S. Plessas, G. Saxami, A. Alexopoulos, A. Galanis, and E. Bezirtzoglou, "Study of kefir grains application in sourdough bread regarding rope spoilage caused by Bacillus spp .," *Food Chem.*, vol. 143, pp. 17–21, 2014, doi: 10.1016/j.foodchem.2013.07.098.

[5] A. Santos, M. S. Mauro, A. Sanchez, J. M. Torres, and D. Marquina, "The Antimicrobial Properties of Different Strains of Lactobacillus spp . Isolated from Kefir," vol. 437, no. 2003, pp. 434–437, 2020.

[6] S. Plessas, A. Alexopoulos, A. Bekatorou, I. Mantzourani, A. A. Koutinas, and E. Bezirtzoglou, "Examination of freshness degradation of sourdough bread made with kefir through monitoring the aroma volatile composition during storage," *Food Chem.*, vol. 124, no. 2, pp. 627–633, 2011, doi: 10.1016/j.foodchem.2010.06.086.

[7] C. Thiele, M. G. Gänzle, and R. F. Vogel, "Contribution of Sourdough Lactobacilli, Yeast , and Cereal Enzymes to the Generation of Amino Acids in Dough Relevant for Bread Flavor," vol. 79, no. 1, pp. 45–51, 2002.

[8] S. W. Lau, A. Q. Chong, N. L. Chin, R. A. Talib, and R. K. Basha, "Sourdough microbiome comparison and benefits," *Microorganisms*, vol. 9, no. 7, 2021, doi: 10.3390/microorganisms9071355.

[9] L. De Vuyst, A. Comasio, and S. Van Kerrebroeck, "Sourdough production: fermentation strategies, microbial ecology, and use of non-flour ingredients," *Crit. Rev. Food Sci. Nutr.*, vol. 63, no. 15, pp. 2447–2479, 2023, doi: 10.1080/10408398.2021.1976100.

[10] W. P. Hammes, M. J. Brandt, K. L. Francis, J. Rosenheim, M. F. H. Seitter, and S. A. Vogelmann, "Microbial ecology of cereal fermentations," *Trends Food Sci. Technol.*, vol. 16, no. 1–3, pp. 4–11, 2005, doi: 10.1016/j.tifs.2004.02.010.

[11] J. Dong, B. Liu, T. Jiang, Y. Liu, and L. Chen, "The biofilm hypothesis: The formation mechanism of Tibetan kefir grains," *Int. J. Dairy Technol.*, vol. 71, no. March, pp. 44–50, 2018, doi: 10.1111/1471-0307.12473.

[12] T. Sopandi and Wardah, *Mikrobiologi Pangan (Teori dan Pratikum)*. Yogyakarta: CV. Andi Offset., 2014.

[13] A. G. Gumelar, “Formulasi Sourdough dengan Sari Buah Belimbing Wuluh (Averrhoa Bilimbi L.) Sebagai Asidulan Formulation of Sourdough With Wuluh Starfruit (Averrhoa Bilimbi L.) as Acidulant.," 2019.

[14] L. Makarim, “Pengaruh Perbandingan Buah Mentimun (Cucumis sativus L.) dan Air dalam Pengembangan Natural Yeast Refreshed Starter dalam Bentuk Roti Sourdough,” Universitas Andalas, 2023.

[15] D. F. Muthoharoh and A. Sutrisno, "The Making of Gluten-Free Bread of Arrowroot Flour, Rice Flour, and Corn Flour (Study of Glucomannan Concentration and Proofing Time)," *J. Pangan dan Agroindustri*, vol. 5, no. 2, pp. 34–44, 2017.

[16] S. Jitrakbumrung and N. Therdthai, "Effect of addition of sourdough on physicochemical characteristics of wheat and rice flour bread," *Kasetsart J. - Nat. Sci.*, vol. 48, no. 6, pp. 964–969, 2014.

[17] SNI, “Standar Nasional Indonesia Roti Tawar (SNI 01-3840-1995),” in *SNI*, Badan Standar Nasional-BSN : Jakarta.

[18] G.-S. Z. Gokırmaklı C, Yuceer Y, "Chemical, microbial, and volatile changes of water kefir during fermentation with economic substrates," *Eur. Food Res. Technol.*, vol. 249, no. 7, pp. 1717–1728, 2023.

[19] G. Turker, B. Kizilkaya, and N. Cevik, "The mineral composition of kefir produced from goat and cow milk," *J. Food, Agric. Environ.*, vol. 11, no. 2, pp. 62–65, 2013.

[20] N. Richana, A. Budiyanto, and I. Mulyawati, “Pembuatan Tepung Jagung Termodifikasi dan Pemanfaatannya untuk Roti,” *Pros. Pekan Serealia Nas.*, vol. 1, no. 1, pp. 446–454, 2010, [Online]. Available: balitsereal.litbang.pertanian.go.id/wp-content/uploads/2016/12/p57.pdf

[21] C. Silow, C. Axel, E. Zannini, and E. K. Arendt, "Current status of salt reduction in bread and bakery products – A review," *J. Cereal Sci.*, vol. 72, pp. 135–145, 2016, doi: 10.1016/j.jcs.2016.10.010.

[22] B. Bahar, *Kefir. Minuman Susu Fermentasi dengan Segudang Khasiat Untuk Kesehatan.* P.T. Gramedia Pustaka Utama, Jakarta., 2008.

[23] D. N. Putri, N. Harini, L. N. Azizah, and H. A. Manshur, “Karakteristik Kimia Roti Manis Sourdough yang Menggunakan Ragi Alami dari Apel Manalagi (Malus sylvestris),” *agriTECH*, vol. 42, no. 4, p. 380, 2022, doi: 10.22146/agritech.61100.

[24] M. Limbad, N. G. Maddox, N. Hamid, and K. Kantono, "Sensory and physicochemical characterization of sourdough bread prepared with a coconut water kefir starter," *Foods*, vol. 9, no. 9, 2020, doi: 10.3390/foods9091165.

[25] P. S. Rimada and A. G. Abraham, "Kefiran improves rheological properties of glucono-δ-lactone induced skim milk gels," *Int. Dairy J.*, vol. 16, no. 1, pp. 33–39, 2006, doi: 10.1016/j.idairyj.2005.02.002.

[26] R. Intan Pratama, I. Rostini, and D. E. Liviawaty, "Rusky Intan Pratama : Karakteristik Biskuit dengan Penambahan Tepung Tulang Ikan Jangilus … Karakteristik Biskuit dengan Penambahan Tepung Tulang Ikan Jangilus (Istiophorus Sp.) Characteristics of Biscuit with Jangilus (Istiophorus sp.) Fish Bone Flour Su," *Akuatika ISSN*, vol. 2, no. 31470–1483, 2014.

[27] R. K. Putra, "Pengaruh konsentrasi starter sourdough terhadap mutu roti manis," no. 1, pp. 430–439, 2018.

[28] M. Corsetti, A., Gobbeti, Marco., Ganzle, "Technology of Sourdough Fermentation and Sourdough Applications," in *Handbook on Sourdough Biotechnology.*, New York : Springer Heidelberg Dorderecht, 2013.

[29] S. Plessas, A. Fisher, K. Koureta, C. Psarianos, P. Nigam, and A. A. Koutinas, "Application of Kluyveromyces marxianus, Lactobacillus delbrueckii ssp. bulgaricus and L. helveticus for sourdough bread making," *Food Chem.*, vol. 106, no. 3, pp. 985–990, 2008, doi: 10.1016/j.foodchem.2007.07.012.

[30] F. Kusnandar, *Kimia Pangan Komponen Makro*. Bumi Aksara, Jakarta, 2019.

[31] T. E. Catrien, Yusi Stephanie Surya, "Reaksi Maillard pada Produk Pangan," in *Program Kreativitas Mahasiswa Penulisan Ilmiah*, 2013.

[32] M. G. Gänzle, "Enzymatic and bacterial conversions during sourdough fermentation," *Food Microbiol.*, vol. 37, pp. 2–10, 2014, doi: 10.1016/j.fm.2013.04.007.

[33] E. K. Arendt, L. A. M. Ryan, and F. Dal Bello, "Impact of sourdough on the texture of bread," *Food Microbiol.*, vol. 24, no. 2, pp. 165–174, 2007, doi: 10.1016/j.fm.2006.07.011.