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Research Article

Quality and Shelf Life Assessment of Modified Pekasam Ale-ale (*Meretrix meretrix*)

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Abstract

Pekasam ale-ale is spontaneously fermented *ale-ale* (*Meretrix meretrix*) flesh with fine salt and carbohydrates, such as sugar, rice porridge or *angkak* (red fermented rice) followed by incubation for 3-7 days. This product has a slightly fishy odour based on the communities' opinion at Ketapang and its unknown shelf life. The original recipe added granulated sugar and garlic powder might be able to remove the fishy odour. The effects of the addition of both ingredients are still unknown for the quality, consumer acceptability and shelf life. The objective of the study was to evaluate the quality and shelf life of *pekasam ale-ale* added granulated sugar and garlic powder based on sensory, physicochemical and microbiological profiles. Three recipes for this study were A (1 kg of fresh *ale-ale* flesh, 400 g of fine salt), B (A recipe, 200 g of granulated sugar, 55 g of garlic powder), and C (A recipe, 200 g of granulated sugar, 125 g of garlic powder) then tested physicochemical, microbiological and sensory properties. The water content, pH, and free fatty acids of A, B, and C differ significantly except for the ash content. All recipes were safe for consumption based on their physicochemical and microbiological properties. The best taste and aroma were awarded to C and B, respectively. The best acceptance score was awarded for C, with days 40 and 60 for the best maturity and shelf life, respectively. In conclusion, garlic powder successfully enhances the taste and aroma and reduces the fishy aroma of *pekasam ale-ale*.

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1. Introduction

Fermented fish products, such as cincalok, buduk, and petis, are common in Southeast Asia. These products are made by mixing raw materials with salt in a 3:1 ratio, followed by spontaneous fermentation that lasts from six days to several months (Liao *et al*., 2019). To promote the growth of lactic acid bacteria (LAB), some producers add carbohydrates like sugar, angkak (red fermented rice), or boiled rice. Salt serves to limit the growth of spoilage and pathogenic microorganisms, but high salt concentrations can also inhibit LAB growth (Lee *et al*., 2018; Nofiani *et al*., 2022). Microorganisms, including LABs, can degrade protein into peptides and amino acids that contribute to developing aroma and taste during food fermentation (Solms, 1969; Zhao *et al*., 2016). For example, cheese product and fish sauce from the fermentation of tilapia fish head hydrolysate (Smit *et al*., 2005; Gao *et al*., 2020). The lactic acid produced will sharpen and strengthen the flavour so that it becomes delicious and can cause a sour taste and a decrease in pH which causes protein coagulation (Nursyam, 2011). The protein degradation in this fermentation probably contributes to unpleasant flavours for consumers.

Fermented fish products are often enhanced with various food ingredients to improve their overall quality. One such ingredient is garlic, which not only imparts aroma and taste but also acts as a deodorizing agent for fish and helps inhibit the growth of spoilage or pathogenic bacteria (McFeeters, 2004; Tao *et al*., 2022). Salt can obtain specific conditions to restrict certain microorganism growth and improve taste (Lee *et al*., 2018; Persulessy *et al*., 2020). Garlic is a seasoning used in most cooking spices that give aroma and taste to food and fermented products. The garlic contains several bioactive molecules, including diallyl trisulfide, diallyl thio-sulfonate (allicin), S-allyl-cysteine sulfoxide (alliin), diallyl disulfide, diallyl sulfide, and allyl mercaptan (Li *et al*., 2022). Garlic could act as fish deodorizing, inhibit spoilage or pathogenic bacteria, stimulate or inhibit particular LABs, antioxidant, immune-stimulating, antimicrobial, antiarthritic, antithrombotic, antitumor, anti-inflammatory, hypoglycemic, and hypolipidemic activities (Li *et al*., 2016; Mondal *et al*., 2022). Fructan from garlic is similar to inulin and can play a role as a prebiotic to increase the LAB total in fermented *som-fak* (Paludan-Müller *et al*., 2002). *Cirsium setidens* Nakai blends fermented with garlic can produce a healthy beverage with strong antioxidant activity and immune-stimulating abilities (Daliri *et al*., 2019). Microorganism starters such as LABs can reduce undesirable flavours and improve the aroma profile by some alcohol and ester productions (Wieczorek and Drabińska, 2022; Tao *et al*., 2022). Microbial flora of various food systems contributes to producing specific flavours in food fermentation by various metabolic functions (Zhang *et al*., 2023).

Ale-ale is widespread in Asia countries and contains 9-45% protein and 0.18% fat (Sawant and Mohite, 2013; Chowdhury *et al*., 2019; Wen *et al*., 2020). Furthermore, its fermented form, known as *pekasam ale-ale* is a popular fish fermentation from Ketapang District, Province of Kalimantan Barat, Indonesia. This product is usually produced from the fermentation of fresh *ale-ale* flesh with salt and rice porridge, or *angkak*, followed by incubation for seven days at room temperature (Nofiani *et al*., 2010). The original *pekasam ale-ale* is still a fishy odour based on the community's opinion at Ketapang, and its shelf life has been unknown. This study enriched the original *pekasam ale-ale* recipe with sugar and garlic powder that probably reduced off-flavour. However, the effects of the addition of both ingredients into *pekasam ale-ale* are still unknown for the quality, consumer acceptability and shelf life. Therefore, this study aimed to evaluate the quality, consumer acceptability and shelf life of *pekasam ale-ale* supplemented with sugar and garlic powder (modified *pekasam ale-ale*) based on physicochemical, microbiological and sensory profiles.

2. Materials and Methods

2.1 Materials

All chemicals and microbiological reagents used in this research were analytical/microbiological grade. The chemicals reagents were formaldehyde (Merck), NaOH (Merck), phenolphthalein (Merck), K_2CrO_4 (Merck), $AgNO_3$ (Merck), NaCl (Merck), KI (Merck), H_2SO_4 (Merck), $Na_2S_2O_3$ (Merck), HCl (Merck), $K_2Cr_2O_7$ (Merck), and Starch (Difco), The microbiological reagents used in this research were Plate Count Agar/PCA (Difco), Oxytetracycline Glucose-Yeast Extract Agar/OGYE (Oxoid), de Man, Rogosa and Sharpe agar/MRSA (Scharlau 01-135), Mannitol Egg Yolk Polymyxin agar/MYPA (Oxoid), Tryptose Sulfite Cycloserine Agar/TSCA (Himedia), Violet Red Bile Glucose Agar/VRBGA (Scharlau 01-295), Mannitol Salt Phenol Red Agar/MSPRA (Mercks 1.05404.0500), and McBride Listeria Agar Base/MLAB (Fluka 62355). The equipments used in this research were pH-Meter (Contech, India), balance (Ohaus, USA), Laminar flow (Sterimac, India), and oven (Vinci, France).

2.1.1 Ethical approval

This study does not require ethical approval because it does not use experimental animals.

2.2 Methods

All experiments were conducted from May to September 2012 in Chemistry and Biotechnology Laboratories, Faculty of Mathematics and Natural Sci-

ences, Universitas Tanjungpura.

2.2.1 Preparation of various Pekasam Ale-Ale recipes

Pekasam ale-ale was prepared using three recipes, namely A (1 kg of fresh *ale-ale* flesh, 400 g of fine salt), B (A recipe, 200 g of granulated sugar, 55 g of garlic powder), and C (A recipe, 200 g of granulated sugar, 125 g of garlic powder). Before production, fresh *ale-ale* flesh was washed with tap water to remove impurities such as sand. The samples were soaked in what for approximately 10 hours until they became white and fluffy. Subsequently, they were drained and mixed evenly with fine salt and sugar or garlic powder based on the recipes.

2.2.2 Sensory analysis

The sensory analysis was conducted using the hedonic rating test and measured consumer acceptability based on four criteria: taste (sweet, salt, sour), aroma, texture, and product appearance using a nine-point hedonic rating scale (1-dislike extremely; 2-dislike very much; 3-dislike moderately; 4-dislike slightly; 5-neither like nor dislike; 6-like slightly; 7-like moderately; 8-like very much; 9-like extremely). The twenty selected consumers were *pekasam ale-ale* producers. Before the sensory test, the consumers were trained toward the sensory attributes, particularly for ability and sensitivity to discriminate tastes, aromas, appearances and textures. The tastes were divided into savoury, sweet, salty, and sour. They also were instructed to cleanse their palate using drinking water before and after the test. Each consumer received the sample in a small container with a spoon and drinking water and then asked to answer each questionnaire. Data from the questionnaire were analyzed using the analytical hierarchy process (AHP) technique. The consistency level of the consumers was calculated by consistency ratio (CR) as follows (Alonso and Lamata, 2006):

$$CR = \frac{CI}{RI}$$

$$CI = \frac{\lambda_{max} - n}{n - 2}$$

$$RI = \frac{\bar{\lambda}_{max} - n}{\lambda_{max} - n}$$

Where:

CI = consistency index

RI = the average value of CI

λ_{max} = A consistency index (eigenvalue maximum)

RI = the average value of CI for random matrices using the Saaty scale

2.2.3 Shelf life determination

Shelf life was calculated from the overall acceptability score, TVBN, FFA, TMABs, and selected pathogenic bacterial data. All data were predicted shelf life using the regression method described in terms of zero-order (Eq. 1), first-order (Eq. 2), and second-order kinetic reactions (Eq. 3).

Zero-order reaction: $k = (A-Ao)/t$ ..... Eq. 1

First-order reaction: $k = (\ln A - \ln Ao) / t$ ..... Eq. 2

Second-order reaction: $k = (1/A - 1 / Ao) / t$ ..... Eq. 3

Where:

Ao is the initial value
A is the freshness at any storage time t (d)
k is the rate constant

The shelf life model was validated using the coefficient of determination (R^2) (Eq. 4) and non-linear Chi-square test (X^2) (Eq. 5). The error encountered in the experiment was calculated using five different methods, i.e. the sum square of errors (ERRSQ) (Eq. 6), hybrid fractional error function (HYBRID) (Eq. 7, average relative error (ARE) (Eq. 8), Marquardt's percent standard deviation (MPSD) (Eq. 9), and the sum of absolute errors (EABS) (Eq. 10)

$$R^2 = \frac{\Sigma(Q_{e,cal} - Q_{e,mexp})^2}{\Sigma(Q_{e,calc} - Q_{e,mexp})^2 - (Q_{e,cal} - Q_{e,mexp})^2} \quad \text{Eq. 4}$$

$$X^2 = \sum_{i-1}^{n} \frac{(Q_{e,cal} - Q_{e,mexp})^2}{Q_{e,meas}} \quad \text{Eq. 5}$$

$$RSQ = \sum_{i=1}^{n} (Q_{e,i,calc} - Q_{e,i,exp})^2 \quad \text{Eq. 6}$$

$$HYBRID = \frac{100}{n-p} \sum_{i=1}^{n} \left[\frac{(Q_{e,i,exp} - Q_{e,i,calc})^2}{Q_{e,i,exp}} \right] \quad \text{Eq. 7}$$

$$ARE = \frac{100}{n} \sum_{i=1}^{n} \left[\frac{Q_{e,i,calc} - Q_{e,i,exp}}{Q_{e,i,exp}} \right] \quad \text{Eq. 8}$$

$$MPSD = \sqrt{\frac{1}{n-1} \sum_{i=1}^{n} \left(\frac{(Q_{e,i,exp} - Q_{e,calc})}{Q_{e,exp}} \right)^2} \quad \text{Eq. 9}$$

$$EABS = \sum_{i=1}^{p} [Q_{e,exp} - Q_{e,calc}] \quad \text{Eq. 10}$$

2.2.4 Physicochemical analysis

Each *pekasam ale-ale* recipe (500 g) was homogenized using a sterilized blender to obtain a homogenate sample for physicochemical analysis (ash content, water content, pH, lactic acid content, free fatty acids (FFA) and total volatile base nitro-

gen (TVBN)). The ash content was carried out by the gravimetric method based on Indonesian National Standard 01-2891-1992 (SNI, 1992). The water content was determined using the oven method based on Indonesian National Standard 01-2891-1992 (1992).

2.2.4.1 pH

Two g of the homogenated sample was diluted with 18 mL of distilled water. Then, the diluted sample was measured pH using pH meter digital.

2.2.4.2 Free fatty acid (FFA)

FFA was determined using the acid-base titration method following modified Bernadez et al. (2005) (Bernadez *et al*., 2005). A 5 g of the homogenated sample was added to 50 mL of neutralized ethanol 95% and then boiled with stirring. After cooling down, the homogenated sample was added 2 drops of phenolphthalein indicator, then titrated with NaOH 0.1 N until light pink formed that stable until 30 min. The FFA was calculated as follows:

$$\%\ FFA = \frac{\text{mL NaOH x N NaOH x 282}}{\text{The sample weight x 1000}}\ x100$$

2.2.4.3 Titratable acidity (TA)

The TA was analyzed using by acid-base titration method based on the AOAC official procedure 942.15 (AOAC, 2000). The fermented ale-ale homogenate (1 g) was centrifuged at 3,200 x g for 2 hours then the supernatant was collected. The supernatant was added distilled water until 20 mL, then boiled for 1 min. After cooling down, the sample was added 5 drops of phenolptalein indicator and titrated with NaOH 0.1 N. The titration was stopped when pink colour formed in the sample. The TA was calculated as follows:

$$TA\ (^{mM}/_{mL}) = \frac{\text{M NaOH x V NaOH x 0.9}}{\text{The sample volume}}$$

2.2.4.4 Total volatile base nitrogen (TVBN)

The TVBN was determined using a modified Conway's microdiffusion method (Conway, 1933). Before the test, each edge of the inner and outer rings from Conway's unit was applied with a sealing agent (vaseline). A two g of the homogenate samples (W) was ground carefully with 8 mL of trichloroacetic acid (TCA) 4%, using a mortar for 30 min, and then filtrated using a paper filter (Whatman No.41) to obtain a filtrate. One mL of the filtrate was poured into the outer ring then it was slanted to add 1 mL of saturated K_2CO_3 solution. The saturated K_2CO_3 solution was prepared by mixing 60 g of potassium carbonate and 50 ml of distilled water, boiling gently for 10 min, and letting it cool down before filtration. The inner ring was put 1 mL of H_3BO_3 1% and added 1-2 drops of a mixed indicator solution (dissolved bromocresol green (0.01 g) and methyl red (0.02 g) in 10 ml of ethanol). Finally, Conway's unit closed, and the sample was mixed gently before incubating at 37ºC for 60 min. After 1 hour, the sample in the inner ring was titrated with HCl 0.02 N until a green-turned-pink colour formed and the volume used was recorded (s mL). A blank test was also carried out using 1 mL of 1% TCA, instead of the sample. The TVBN was calculated as follows:

$$TVBN\ (\frac{mg}{100}gr = (V_s - V_a) \times (N_{HCl} \times A_n) \frac{\left[\left(W_s \times \frac{M}{100}\right) + V_E\right]}{W_s} x100$$

Where:

V_S = Titration volume of 0.02N HCI for sample extract (ml)

Vb = Titration volume for blank (ml)

N_{HCl} = Normality of HCI (=0.02N x f, factor of HCI)

A_N = Atomic weight of Nitrogen (x 14.00)

W_S = Weight of muscle sample (g)

M = percentage moisture of muscle sample.

V_E = Volume of 4% TCA used in the extraction

2.2.5 Microbiological analysis

Each *pekasam ale-ale* recipe for microbiological analysis was prepared with 5 g of *pekasam ale-ale* and suspended in 45 mL of saline buffer (NaCl 0.9%). The microbiological analysis was carried out, namely total mesophilic aerobic bacteria (TMABs), lactic acid bacteria (LABs), and pathogenic bacteria (*B. cereus, C. perfringens, Enterobacteriaceae, L. monocytogenes, S. aureus*).

The LAB total, the suspension sample was inoculated on MRS media added with 1% of $CaCO_3$ and anaerobically incubated (Monika *et al*., 2017) After 3-4 days, a clear zone around colonies was counted as the number of LABs.

The fungal total was determined by inoculating 50 µL of the suspension sample onto Potatoes Dextrose Agar (PDA) supplemented with NaCl 15 g/L and incubated at 37ºC. After five days, appeared colony was counted.

The suspension sample was inoculated on *Mannitol Egg Yolk Polymyxin* (MYP) supplemented with 5 % NaCl and incubated on days 2-3 to determine

B. cereus (Han *et al*., 2001). The pink colonies surrounded by zone precipitation were counted and followed further identification based on Bergey's manual determinative bacteriology (Holt *et al*., 1994).

Tryptose Sulfite Cycloserin Agar (TSCA) supplemented with NaCl 5 % was used to count the total of *C. perfringens* (Han *et al*., 2001; Tirillini *et al*., 2019). After anaerobic incubation at 37°C for 18-24 h, black colonies were counted and followed by confirmation using fermentation of lactose (+), gelatin liquefaction (+), motility (A) and nitrate reduction (A) as criteria after anaerobic incubation for 24 h at 37°C.

Enterobacteriaceae was evaluated following a procedure described by Han *et al.* (2001) using a selective medium, Violet Red Bile Glucose Agar (VRBG) supplemented with NaCl 5 % (Han *et al*., 2001). After three days of incubation at 37ºC, the large colonies with purple haloes on the media surface and were recorded as Enterobacteriaceae.

L. monocytogenes was determined by inoculating 50 µL of the suspension sample onto McBride *Listeria agar* base (MLAB) medium containing NaCl 15 g/L and incubated at 37ºC for a day (Nofiani *et al*., 2019). Presumptive yellow colonies on the media surface were confirmed based on Bergey's manual determinative bacteriology (Holt *et al*., 1994).

Mannitol Salt Agar (MSA) supplemented with 15 % NaCl was used to inoculate *S. aureus* (Nofiani *et al*., 2021). After three days of incubation at 37ºC, the yellow colonies with yellow zones were tested for coagulase, then the positive coagulase test was counted as log CFU of *S. aureus*.

2.2.6 Statistical analysis

The physicochemical analysis of this experiment was conducted using a completely randomized design and repeated three times. The variation among the treatments was analyzed through a one-way analysis of variance (ANOVA) using Tukey's test as the *post hoc* comparison test ($p < 0.05$).

3. Results and Discussion

3.1 Results

3.1.1 Consumers' acceptability of Pekasam Ale-Ale added sugar and garlic powder

The sensory analysis was carried out in using the hedonic rating method to evaluate the effect of added garlic powder on consumer acceptability of A, B, and C recipes. The result showed that B recipe showed the best aroma value, followed by C and A recipes, while C recipe obtained the best taste value, followed by B and A recipes (Table 1). The best acceptance among recipes (C recipe) was re-tested by the sensory to determine the shelf life or duration of consumer acceptance of the modified *pekasam ale-ale* in Part 3.1.4.

3.1.2 Physicochemical analysis

Physicochemical properties (water content, ash content, pH, TA, FFA, and TVBN) were used to assess the quality of the modified pekasam ale-ale recipes. Overall, all recipes still fulfilled the standard quality for consumption based on the physicochemical properties (Table 2). The water content in all recipes had a value range of 66.04 – 79.60%, which was significantly different ($p < 0.05$) among the recipes for each observation time. The ash content of each recipe in different incubation times generally showed a significant difference ($p < 0.05$) except C recipe. However, all recipes for all incubation times showed insignificant differences ($p < 0.05$). The pH value in different incubation times in and among the recipes showed a fluctuating trend. B recipe generally showed lower pH and higher TA than A and C recipes. The FFA of A recipe was significantly lower ($p < 0.05$) than that of A and B recipes, but no significant difference between B and C recipes (Table 2). Sugar and garlic added to B and C recipes could seem to increase hydrolysis of the ale-ale fat. The FFA value of fermented ale-ale for all recipes ranged from 1.88 to 11.63% (Table 2). TVBN values among recipes or incubation times in one recipe fluctuated from 5 to 47 mg/100 g (Table 2).

3.1.3 Microbiological analysis

The TMAB, the total of LABs and fungi were determined for all *pekasam ale-ale* recipes (Table 3). Most TMAB values for all recipes were approximately 10^{-3} CFU/g. The TMABs were detected for all observation times, and the value tended to fluctuate. The TMAB values of B and C recipes are lower than those of A, which means that garlic powder and FFA content probably decreased the TMABs. The sugar added to B and C recipes did not seem to increase the LAB total (Table 3), even though the LABs of B recipe were slightly higher than those of C recipe (Table 3). A recipe did not identify the fungi, but B recipe exhibited fungal presence on day 30, while C recipe detected fungi on days 30 and 40 (Table 3). All colonies on B and C recipes showed white mycelia and had similar phenotypes. Furthermore, one of them was microscopically identified its morphological characteristics using a light microscope with 40 x magnification. Some hyphae might have swollen and were slightly larger than the normal hyphae (Figure 1). The spores and mycelia were similar to *Phytium* (Watanabe, 2010). Therefore, the fungus belongs to *Phytium* sp. *Phytium* sp. is an aquatic fungus that causes disease in plants and fish (Al-Sheikh and Abdelzaher, 2012). All recipes also did not contain pathogenic bacteria.

Table 1. Consumers' acceptability of *pekasam ale-ale* added with sugar and garlic powder.

Recipe	1 kg of fresh flesh added ale-ale			Criteria score of				Overall acceptability
	Fine salt, g	Sugar, g	Garlic powder, g	Appearance	Texture	Aroma	Taste	
A	400	0	0	0.4177	0.2544	0.1141	0.3959	0.3600
B	400	200	55	0.3021	0.4375	0.5757	0.0738	0.2342
C	400	200	125	0.2802	0.3081	0.3102	0.5303	0.4058
Dominant Criteria				0.4778	0.1201	0.0912	0.4778	

Table 2. Physicochemical properties of various *pekasam ale-ale* recipes.

Incubation time, day	Water content (%)			Ash content,%		
	A	B	C	A	B	C
15	77.44 ± 0.57^{aA}	74.19 ± 0.05^{aB}	66.04 ± 0.51^{aC}	19.49 ± 0.07^{aA}	14.38 ± 0.05^{aA}	17.02 ± 0.06^{aA}
30	79.60 ± 0.05^{bA}	78.22 ± 0.09^{bB}	67.48 ± 0.44^{bC}	20.20 ± 0.11^{aA}	16.85 ± 4.14^{aA}	16.77 ± 0.06^{aA}
40	78.84 ± 0.06^{bcA}	77.02 ± 0.24^{cB}	71.50 ± 0.05^{cC}	17.19 ± 0.09^{bA}	13.41 ± 0.09^{aA}	14.56 ± 0.05^{aA}
50	78.67 ± 0.02^{bcA}	71.84 ± 0.15^{dB}	67.17 ± 0.16^{abC}	16.98 ± 0.00^{bA}	16.54 ± 0.10^{aA}	17.40 ± 0.00^{aA}
60	78.41 ± 0.08^{cA}	71.31 ± 0.14^{dB}	68.14 ± 0.21^{bC}	16.51 ± 0.01^{bA}	16.43 ± 0.54^{aA}	16.48 ± 0.13^{aA}
70	78.05 ± 0.06^{acA}	75.03 ± 0.00^{eB}	72.44 ± 0.25^{cC}	15.20 ± 0.01^{cA}	17.20 ± 0.00^{aA}	24.86 ± 4.34^{bA}
Incubation time, day	**pH**			**TA, mM/mL**		
	A	B	C	A	B	C
15	4.4 ± 0.00^{aA}	3.89 ± 0.11^{aB}	4.31 ± 0.00^{aC}	0.36 ± 0.02^{aA}	0.91 ± 0.08^{aB}	0.73 ± 0.03^{aB}
30	4.7 ± 0.00^{bA}	4.23 ± 0.02^{bB}	4.32 ± 0.04^{aC}	$0.73 \pm 0.01b^{cA}$	0.55 ± 0.03^{bB}	1.00 ± 0.07^{bB}
40	5.4 ± 0.00^{cA}	3.93 ± 0.01^{aB}	4.28 ± 0.01^{aC}	0.91 ± 0.02^{cdA}	0.64 ± 0.07^{bB}	1.09 ± 0.01^{cB}
50	4.7 ± 0.00^{bA}	3.85 ± 0.00^{aB}	3.93 ± 0.02^{aC}	1.09 ± 0.13^{dA}	1.55 ± 0.06^{cB}	0.91 ± 0.03^{dB}
60	4.4 ± 0.00^{aA}	3.96 ± 0.01^{aB}	4.39 ± 0.01^{bC}	0.55 ± 0.25^{abA}	1.46 ± 0.04^{cB}	1.18 ± 0.07^{eB}
70	4.47 ± 0.06^{aA}	4.00 ± 0.01^{aB}	4.4 ± 0.00^{bC}	0.36 ± 0.06^{aA}	0.55 ± 0.06^{bB}	1.09 ± 0.01^{cB}
Incubation time, day	**FFA, %**			**TVBN, mg N/100 g**		
	A	B	C	A	B	C
15	1.88 ± 0.08^{aA}	10.36 ± 0.13^{cB}	7.59 ± 0.08^{aB}	6.68 ± 0.01^{aAB}	12.33 ± 1.60^{abA}	5.22 ± 0.02^{aB}
30	3.54 ± 0.01^{bcA}	6.10 ± 0.08^{aB}	8.72 ± 0.18^{bB}	9.40 ± 1.90^{aAB}	12.04 ± 1.55^{abA}	11.12 ± 0.92^{bB}
40	3.02 ± 0.24^{bA}	8.79 ± 0.08^{cB}	7.70 ± 0.16^{aB}	$13.41 \pm 0.02b^{AB}$	5.34 ± 0.02^{cA}	19.80 ± 1.87^{cB}
50	3.42 ± 0.16^{bcA}	11.63 ± 0.16^{dB}	9.76 ± 0.08^{cB}	$16.75 \pm 0.95b^{AB}$	10.57 ± 0.61^{aA}	30.08 ± 1.85^{dB}
60	5.08 ± 0.08^{dA}	11.52 ± 0.17^{dB}	9.81 ± 0.08^{cB}	24.11 ± 0.02^{cAB}	15.83 ± 0.09^{bA}	47.15 ± 0.01^{cB}
70	3.77 ± 0.16^{cA}	7.83 ± 0.05^{bB}	7.62 ± 0.13^{aB}	29.45 ± 0.01^{dAB}	22.61 ± 1.88^{dA}	101.83 ± 1.85^{fB}

The value is mean ± standard deviation. The lowercase in a column indicated a significantly different value ($p < 0.05$). The uppercase in a row indicated a significant difference ($p < 0.05$). A recipe: 1 kg of fresh ale-ale flesh, 400 g of fine salt. B recipe: A recipe added 200 g of granulated sugar and 55 g of garlic powder. C recipe: A recipe added 200 g of granulated sugar and 125 g of garlic powder.

3.1.4 Determination of maturity and shelf life of c recipe

C recipe was awarded the highest acceptability score based on the total score of all criteria (taste, appearance, texture and aroma) among recipes based on sensory analysis. Therefore, C recipe was re-tested the sensory to assess the best maturity and shelf life for 90 days, namely on days 15, 30, 40, 50, 60, 70, 80 and 90. The result was that all consumers did not want to consume C recipe on day 70. During the observation, the best acceptance fell on day 30 based on the total score of all criteria, with 2 of all criteria showing the highest, namely taste and texture (Table 4). Therefore, the 30th day could be determined as the best maturity time for C recipe, and its shelf life was proposed as days 60 (Table 4).

Table 3. Microbiological profiles of various *pekasam ale-ale* recipes.

Incubation time, day	TMABs (CFU/g)			LABs (CFU/g)			Fungi (CFU/g)		
	A	B	C	A	B	C	A	B	C
15	5.95×10^3	$<10^3$	$<10^3$	2.38×10^5	2.00×10^4	$<10^3$	-	-	-
30	0.40×10^3	$<10^3$	1.05×10^3	$<10^3$	12.56×10^5	$<10^3$	-	2.00×10^3	2.00×10^3
40	0.01×10^3	0.01×10^3	$<10^3$	$<10^3$	$<10^3$	$<10^3$	-	-	2.00×10^3
50	0.34×10^3	0.05×10^3	0.91×10^3	4.98×10^5	$<10^3$	$<10^3$	-	-	-
60	0.10×10^3	$<10^3$	$<10^3$	$<10^3$	1.00×10^5	$<10^3$	-	-	-
70	0.52×10^3	12.55×10^3	$<10^3$	$<10^3$	4.40×10^4	$<10^3$	-	-	-

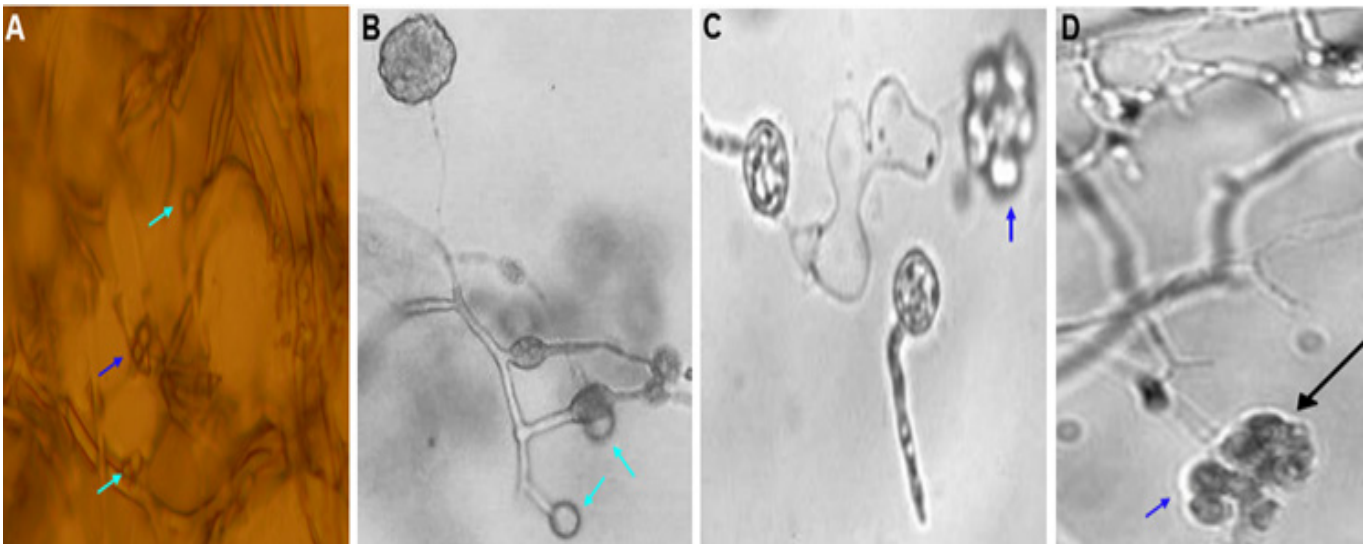

Figure 1. Morpholagical characteristics of *Phytium* sp. A. *Phytium* sp. in *pekasam ale-ale*; B. *Phytium elongatum* (Watanabe, 2010); C. *Phytium dissotocum* (Watanabe, 2010); D. *Phytium diclinum* (Al-Sheikh and Abdelzaher, 2012); Blue arrow. A vesicle contains differentiated zoospores; Tosca arrow. Lobate sporangia with vesicle formation.

3.1.5 Shelf life prediction model of c recipe

The acceptability score and TVBN data were used to determine the shelf life of the C recipe. The shelf life model prediction based on the highest R^2 model was fit with the second-order kinetic and the first-order, respectively, while the FFA showed no fit for all orders (Table 5). If the overall acceptability of 5.5, the shelf life model prediction fell on days 63 following the second order reaction and on days 70 based on the sensory test. Meanwhile, with the TVBN score of 100 mgN/100 g, the shelf life fell on day 67 based on the zero-order reaction (Table 5).

3.2 Discussion

Modified *pekasam ale-ale* in this study was prepared by adding sugar and garlic powder with a certain ratio to the original *pekasam ale-ale* recipe. To evaluate these effects, the modified *pekasam ale-ale* was characterized by the sensory test, shelf-life, and physicochemical and microbiological properties. The sensory results, physicochemical and microbiological properties were used as parameters to determine the quality, safety and shelf-life of the modified *pekasam ale-ale.*

The sensory test for the modified *pekasam ale-ale* was conducted in two steps. The first step was used to determine the best-modified *pekasam ale-ale* recipe. The second step was conducted as one of the parameters to determine the shelf-life of the best-modified *pekasam ale-ale* recipe. The first sensory test showed that adding sugar and garlic powder successfully removes the fishy aroma and improves the taste of B and C recipes. Li *et al.* (2016) reported that garlic could play a role in fish deodorizing, suppressing spoilage or pathogenic bacteria and stim-

ulating or inhibiting particular LAB growth (Li *et al.*, 2016; Qiu *et al.*, 2020). The garlic powder was added to B and C recipes, causing them to turn brown due to the browning reactions. Browning reactions are affected by the chemical environment of food, including water activity, pH, chemical compositions of the food system, and temperature (Corzo-Martinez *et al.*, 2012). As a result, the appearance criteria for the B and C recipes were lower than those for the A recipe (Table 1). The greater the garlic powder added, the darker the colour of the product produced. However, the C recipe was awarded the highest score for the overall priority value, followed by B and A recipes.

The physicochemical properties involved ash content and water content, pH, TA, FFA, and TVBN. Ash content can be used to predict the total amount of minerals in foodstuffs. Adding granulated sugar and garlic powder could not increase the ash content. The ash content of all recipes ranged from 13-24%, which is still in the commercial *pekasam ale-ale* range, namely 14.68% for white *pekasam ale-ale* and 20.16% for red pekasam ale-ale (Nofiani *et al.*, 2010).

The high water content in food can increase the growth of spoilage microorganisms. The water content for all recipes in this study was higher than that

Table 4. Consumer's acceptability of C recipe based on storage time at room temperature.

Recipe	K, g	Criteria score of				Overall acceptability
		Appearance	Texture	Aroma	Taste	
C15	15	0.2319	0.2342	0.2463	0.2460	80.233
C30	30	0.2870	0.3156	0.2856	0.2727	91.609
C40	40	0.1773	0.1756	0.2046	0.1835	66.288
C50	50	0.1616	0.1445	0.1379	0.1599	55.794
C60	60	0.1423	0.1300	0.1257	0.1380	51.499
C70	70	0.1336	0.1269	0.1208	0.1344	50.485
C80	80	0.1279	0.1249	0.1156	0.1272	49.126
C90	90	0.1149	0.1072	0.1037	0.1206	46.259
Dominant Criteria		0.2272	0.1833	0.2748	0.3146	

Table 5. Shelf life prediction of C recipe based on order reactions.

Quality index, error and validity analysis	Acceptability score			TVBN		
	Zero order	First order	Second order	Zero order	First order	Second order
Regression equation	Y = -0.057X + 9.2379	Y = -0.011X + 0.0909	Y = 0.0014X + 0.0937	Y = 1.9582X – 47.252	Y = 0.042X + 0.1343	Y = -0.0022X + 0.1687
K	-0.0570	-0.0011	0.0014	1.9582	0.042	-0.0022
R^2_{model}	0.7725	0.7911	0.8769	0.8984	0.9701	0.7625
ERRSQ	4.3674	0.2213	0.0014	1.982.8370	1.6332	0.0036
ARE	0.8824	-26.1049	-0.2646	-365,344	25.4145	-51.9075
HYBRID	8.6351	13.0446	0.1469	1.959.8443	15.9012	3.7890
MPSD	0.1137	10.731	0.1080	1.7167	0.3814	2.0784
EABS	0.0210	0.8187	0.0173	0.0059	-3.1553	-0.0366
Nonlinear Chi-Square Test ($\chi 2$)	0.6045	0.9131	0.0103	137.1891	1.1131	0.2652
ρ korelasi	-0.8790	-0.8984	0.9365	0.9478	0.9825	-0.8732
Shelf life for overall acceptability score of 5.5, days	65.5772	54.6450	62.9416	-	-	-
Shelf life for TVBN value of 100 mg N/100 g, days	-	-	-	75.1976	67.0927	72.1364

of commercial *pekasam ale-ale,* 60.16% (Nofiani *et al.,* 2010). The inhomogeneous or drained samples with different levels in preparation of *ale-ale* flesh for fermentation might also contribute to this issue. The water content of the C recipe was significantly lower ($p < 0.05$) than that of the A and B recipes, probably caused by the low number TMABs in the C recipe (Table 2). The low number of microorganisms in the degradation of smoked hilsa fish is also reported to reduce the water content (Hossain *et al.,* 2012).

pH is determined to measure proton concentration in food and is important to differentiate microorganisms in fermentation. A lower pH than five can inhibit most microorganism contaminants, such as toxin-producing microorganisms, spoilage and pathogenic bacteria. Most pH of the modified pekasam ale-ale in this study was lower than 5, which probably limited most microorganism contaminants (Table 2). These pH were lower than the commercial pekasam ale-ale, ranging from 5.06 to 5.19 (Nofiani *et al.,* 2010).

The TA is used to determine the total organic acid concentration. Some organic acids in TA positively correlated with pH. In fermentation, organic acid production (such as lactic acid, tartaric acid, malic acid, and citrate acid) by microorganism activities can decrease the pH, notably LAB, which is the main contributor to acetic acid and lactic acid production. Besides salt, the low pH and high TA are pivotal to restricting the type and growth of microorganisms involved in fermentation (Moschopoulou *et al.,* 2019). Most bacteria can grow optimally on pH optimum 5-5.75.

Triglycerides or fat can be hydrolyzed to FAA (i.e. diglyceride or monoglyceride) by enzymes (i.e. lipase or phospholipase) or microorganism activities during food fermentation. This lipid hydrolysis also co-occurs with lipid oxidation (Xu *et al.,* 2019). Lipid hydrolysis and oxidation of unsaturated fatty acids can improve the flavour, but the excessive oxidation of polyunsaturated fatty acids causes low nutritional quality and deteriorates the products (Mariutti and Bragagnolo, 2017). The FFA of B and C recipes ranged from 1.88 to 11.63 % (Table 2) and was higher than cincalok ranging from 0.57 to 1.05% (Nofiani *et al.,* 2021) and *buduk*, ranging from 17.96 to 21.71% (Nofiani *et al.,* 2019). The sugar and garlic added to B and C recipes could seem to increase the hydrolysis of the modified *pekasam ale-ale.*

TVBN is a method to quantify the presence of nitrogenous compounds (ammonia, methylated amines, isobutylamine, putrescine, and cadaverine) as biochemical and microorganism activities to degrade protein or other nitrogen (N)-containing compounds (Bekhit *et al.,* 2021). Most TVBN values were used to assess the freshness of fish and meat products or as an index for spoilage and indicator for accumulation of trimethylamine (TMA)(Castro *et al.,* 2006; Liao *et al.,* 2019). The TMA is one of the compounds responsible for the fishy smell in fish (Xu *et al.,* 2010). High TVBN values can increase the pH of food products. A fluctuating TVBN values trend is also reported in the fermented sardine fish sauce (Kilinc *et al.,* 2006). However, the TVBN for all recipes was lower than the maximum limit of 200 mg N/100 g for fermented fish (Kilinc *et al.,* 2006), 30 mg N/100 g for fresh fish (The Decision of The Head of Fish Quarantine, Quality Control, and Safety of Fishery Products Number 37/Kep-Bkipm/2017and http://dkp.jatimprov.go.id/index.php/2019/09/19/apa-itu-tvb-dan-tma/). Therefore, all recipes in this study were categorized as safe products for consumption. In this study, the FFA value from each recipe was higher than *cincalok* ranging from 0.57 to 1.05% (Nofiani *et al.,* 2021) and buduk, ranging from 17.96 to 21.71% (Nofiani *et al.,* 2019).

Microorganisms in a nonstarter food fermentation play a role in decomposing foodstuffs into simple compounds that can increase or decrease the food quality, improve nutrition, extend the shelf life of food health benefits, or develop specific senses. Some microorganisms in fermentation are also harmful to human health, particularly fish fermentation without a starter, which can cause uncontrolled physicochemical and microbiological changes.

Microbiological parameters such as TMAB value can also be used to assess shelf life. TMAB value can used as an organism indicator to describe how hygienic and safe the products are produced. The TMAB values of the B and C recipes were lower than those of the A recipe, which means that garlic powder and FFA content probably decreased the TMABs (Table 2). Garlic was reported to have antimicrobial and antifungal activities such as *S. aureus, E. coli, C. albicans, Staphylococcus, Escherichia, Salmonella, Streptococcus, Klebsiella, Proteus, Bacillus, and Clostridium* (Hughes and Lawson, 1991; Lim *et al.,* 2015). *Somfak* fermented with adding garlic shows a decrease in the TMABs (Paludan-Müller *et al.,* 1999). FFA also can inhibit or not inhibit particular bacterial growth (Bao *et al.,* 2016; Nair *et al.,* 2005). A product containing TMABs is more than 5 x 10^5 CFU/g indicating that the product is unsafe for consumption based on the Decision of The Head of Fish Quarantine, Quality Control, and Safety of Fishery Products Number

37/Kep-Bkipm/2017. However, the TMABs for all formulas are less than 5 x10^5 CFU/g indicating safe products and hygienic processing based on microbiological profiles.

LABs are beneficial in improving food product quality and health. These bacteria play to convert carbohydrates such as glucose to lactic acid. As a result, the pH in fermented food products decreases. Most LABs also showed antimicrobial activities against foodborne pathogenic bacteria such as *B. cereus, E. coli, S. aureus,* and *Shigella dysenteriae* (Monika *et al.,* 2017). Sugar or carbohydrate added to fermentation plays a role in stimulating LAB growth.

A higher concentration of garlic powder added to C than B recipes seemed to inhibit the LAB total. Some LAB genera in the recipes were probably sensitive to allicin from the garlic powder. *Enterococcus, Pediococcus* and *Streptococcus* were dominant LABs in this study (unpublished data). Only a few LABs were identified as *Leuconostoc* sp. Aa8 (Sari *et al.,* 2012) and *Lactobacillus* (unpublished data). Most commercial *pekasam ale-ale* LABs contained *Pediococcus* and *Enterococcus* (Nofiani *et al.,* 2022). Particular LAB genera sensitive to allicin from garlic is *Bifidobacteria* (Kim *et al.,* 2009). Some LAB genera resist allicin from garlic, such as *Lactobacillus, Leuconostoc,* and W*eissella* (Kim *et al.,* 2009; Lim *et al.,* 2015). Besides allicin, more than 2% salt added to food fermentation can inhibit LAB growth (Kim *et al.,* 2021). All modified *pekasam ale-ale* in this study contained high salt concentrations, which probably caused a low LAB total.

Pathogenic bacteria that probably contaminate seafood or fish fermentation are *B. cereus, S. aureus, C. perfringens, Enterobacteriaceae* and *L. monocytogenes.* This study detected no pathogenic bacteria in all *pekasam ale-ale* recipes (Table 3), indicating that the raw material was probably not contaminated with pathogenic bacteria or contaminated. The other reason, the chemical compositions, such as lactic acid, salt, LABs, and garlic can probably inhibit or kill pathogenic bacterial growth. Therefore, all recipes were safe for consumption.

The shelf life of food is the period of food that is safe for consumption (Rasane *et al.*, 2015). It can be assessed by combining sensory, physicochemical and microbiological parameters, even though there are no standard rules for determining shelf life (Guo *et al.,* 2023). The sensory parameter calculated from the overall acceptability score can describe the deterioration of different criteria as a result of physical, chemical and microbiological changes during food storage (Gram *et al.,* 2002; Young and O'Sullivan, 2011). Some physicochemical parameters, such as TVBN, can also be chosen based on harm to health. Chinese mitten crab (*Eriocheir sinensis*) combines sensory tests (particularly fishy aroma), histamine content, TVBN content, and total viable count to assess shelf life. The fishy aroma plays a role as the sensitive index for shelf life due to the close correlation with histamine content and toxic substances (Guo *et al.,* 2023). The shelf life prediction model of C recipe was calculated using data consumers' acceptability and TVBN value by a kinetic equation. The FFA, TMABs and the selected pathogenic bacterial data were not used to determine the shelf life. The FFA and TMAB data did not follow all reaction order predictions, while the pathogenic bacteria were undetected.

4. Conclusion

Garlic powder added to *pekasam ale-ale* recipes affected its quality and safety based on the physicochemical, microbiological, and sensory properties of each recipe. Physicochemical properties (the water content, pH, FFA) among A, B, and C differ significantly except for the ash content. For the TA, TVBN, and FFA, between B and C showed insignificant differences ($p > 0.05$) but significant differences ($p < 0.05$) with A. Garlic powder decreased the TMAB of recipes and increased B LABs but no LABs in C. The best taste and aroma were awarded C and B, respectively, showing that the added garlic powder improved the taste and aroma. The most preferred recipe based on the overall criteria score from the sensory test was C. The best maturity time and the predicted shelf life for C fell on days 40 and 60, respectively.

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Authors' Contributions

RN has contributed to the research design, processing, interpretation of the experimental data, and writing the manuscript. N performed the experiments. P supervised N in doing the experiments and revised the manuscript.

Conflict of Interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Declaration of Artificial Intelligence (AI)

The author(s) affirm that no artificial intelligence (AI) tools, services, or technologies were employed in the creation, editing, or refinement of this manuscript. All content presented is the result of the independent intellectual efforts of the author(s), ensuring originality and integrity.

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