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Research Article

Optimum Processing Conditions for Bakasang Using the Response Surface Methodology with Central Composite Design (CCD)

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Abstract

Background and Objective: Bakasang is a typical food of North Sulawesi and its surroundings, made from fermented fish viscera. This food is made by fermentation so that it is rich in amino acids and polypeptides. This study aimed to determine the optimum processing conditions for Bakasang with Peroxide Value (PV) parameters based on Response Surface Methodology (RSM) with Central Composite Design (CCD). **Materials and Methods:** Viscera from fresh Skipjack tuna consisting of intestines, liver, heart and eggs were mixed with salt and left in a fermenter for varying times and temperatures to obtain Bakasang. The fermentation temperature, salt content, and fermentation time, determined based on the Central Composite Design (CCD), were variables in this study. Thus, 20 experiments consisted of eight quadratic points, six centre points and six axial points. **Results:** The results showed that the correlation test between temperature, salinity and fermentation time variables on PV were 0.521, 0.305 and 0.591. The regression test resulted in an R^2 value of 0.988. The model equation obtained was $y = 4.35194 - 0.11363x_1 - 0.07459x_2 - 0.25300x_3 + 0.00157x_1^2 + 0.00310x_2^2 + 0.01571x_3^2 - 0.00064x_1x_2 + 0.00172x_1x_3 + 0.00340x_2x_3$. The contour plot graph obtained from the experiment using CCD showed that the optimum processing conditions for Bakasang were at a temperature of 32.9324°C, salinity 10.2631% and fermentation time of 4.7793 days, which will produce Bakasang with an optimum PV of 1.51256 meq kg⁻¹ sample. **Conclusion:** According to the findings of this study, the optimum temperature, salinity and fermentation time for producing Bakasang with a PV of 1.51256 meq kg⁻¹ sample were 32.932°C, 10.263% and 4.779 days, respectively.

Key words: Bakasang, Skipjack tuna, response surface methodology, peroxide value, salinity, fermentation

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Skipjack tuna (Cakalang fish), a leading commodity in North Sulawesi province, is a Pelagic fish group member. The fish is widely processed by the community by utilizing its meat, while its waste (viscera) has not been fully utilized. One of the uses of Cakalang fish viscera in North Sulawesi is processing it by fermentation to make a fish sauce, called by the local community as Bakasang. It is commonly used as a spice in cooking^{1,2}.

Fermented fish sauce products are also available in various other countries with different names. These fish sauces are made with different fermentation techniques, such as fish-water proportion, salinity, use of yeast/enzyme, spices, storage time and temperature. Fukui *et al.*³ examined the quality of fermented fish sauce made using a 3:1 proportion of fish-water, with a salt content of 15%, storage at room temperature and 16% of *Aspergillus oryzae*. Anto *et al.*⁴ studied the effect of fermentation time on the chemical and microbiology properties of Bakasang from Oci fish (*Rastrelliger* sp). On the other hand, the processing of fermented Caspian Kilka fish sauce was evaluated by Sabour and Moini⁵. According to their findings, the quality of the fish sauce produced is affected by the type of fish used and the conditions under which it is processed. Purwaningsih *et al.*⁶ studied the physicochemical, microbiological and histamine changes of Bakasang from tuna Skipjack during fermentation and storage. They discovered that fermentation and storage times have an impact on the physicochemical quality of Bakasang.

Fish oil is known as an essential source of essential fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic (DHA)⁷. Simat⁸ stated that tuna waste refined oil contained polyunsaturated fatty acid (PUFA) above 40% consisting of 30% EPA and DHA. Like other fish-based products, Bakasang, made from fish viscera contains quite a large amount of oil (1.11%)². The oil in Bakasang has a high degree of unsaturation, so it is susceptible to oxidation reactions, which result in off-flavour⁹. According to Mohamed¹⁰, the total lipid content in raw, salted and fermented fish products was 3.85, 4.04 and 3.76%, respectively. Altogether, the most abundant fatty acids were 14:0 (myristic acid), 16:0 (palmitic acid), 16: 1n-7 (palmitoleic acid), 18:1n-9 (oleic acid), 20:5n-3 (eicosapentaenoic acid) and 22:6n-3 (docosahexaenoic acid, DHA). The major Fatty Acid (FA) group was polyunsaturated. Among unsaturated FA, n-3 forms were the most dominant. Raw fish had the greatest concentrations of n-3 fatty acids, followed by fermented and salted fish. Based on this, the oxidation parameter is very critical in producing quality Bakasang products.

Bakasang is produced through a spontaneous fermentation process involving Lactic Acid Bacteria (LAB) that is naturally present in fish viscera. Lactic acid bacteria play a significant role in the fermentation process of available carbohydrates, causing a pH decrease. The combination of low pH and organic acids (mostly lactic acid) is the main preservative factor in fermented fish products¹¹.

The Response Surface Methodology (RSM) is a way to determine the relationship between several explanatory variables and one or more response variables. This method can be used successfully in the determination of optimum conditions in food technology. Can and Ersan¹² used RSM to investigate the effect of cooking carp (*Cyprinus carpio*) fillets at various temperatures and with varying amounts of oil. Motevalzadeh *et al.*¹³ used RSM to investigate the effects of soybean oil and carrot extract on the physicochemical and textural properties of pizza cheese containing carrot extract. According to the preceding explanation, the oxidation parameter is critical in determining the quality of Bakasang products. Hence, the purpose of this study was to optimize the processing of Cakalang (*Katsuwonus pelamis*) with the RSM based on oxidation parameters, namely Peroxide Value (PV).

MATERIALS AND METHODS

Bakasang processing: The research was conducted between April, 2019 and June, 2021. The method for making Bakasang followed the procedure done by Fatimah *et al.*¹ Viscera from fresh Skipjack tuna consisting of intestines, liver, heart and eggs were washed using clean running water and then cut into smaller pieces. After that, the pieces of viscera were drained to remove water and then weighed. Furthermore, the viscera were mixed with salt and left in a fermenter for varying times and temperatures to obtain Bakasang. The fermentation temperature, salt content and fermentation time, determined based on the Central Composite Design (CCD) were variables in this study.

Application of response surface methodology in bakasang processing: Bakasang processing optimization was done using three variables: Fermentation temperature, salt content and fermentation time obtained based on the previous studies¹. Based on that, then the fermentation temperature ranges used in this study were 30-70°C, with a centre point of 50°C. The range of variations in salt concentration was 10-30% with a centre point of 20%. The fermentation time ranged from 5-15 days with a centre point of 10 days. Of the three variables, variable coding was x_1 for fermentation temperature (°C), x_2 for salt content (%) and x_3 fermentation time (day).

Therefore, there were 20 combinations made determined by the RSM program. Based on the CCD scheme using a centre point of 6 repetitions and 6 axial points, the coding results are shown in Table 1. The experimental design is depicted using code variables and the actual variables are presented in Table 2.

Statistical analysis: The relationship between the variables and the response was carried out employing a correlation test, followed by regression analysis and significance test with Analysis of Variance (ANOVA) to obtain the R^2 value using the software SPSS 20. To find out the results of the observations with the predicted results, a graph plot of the mathematical model equations obtained was carried out using the software Minitab 14. Then contour plots and surface response plots were made in 3 dimensions to determine each variable's optimum point for the PV response.

RESULTS AND DISCUSSION

PV is one of the parameters for oxidation reactions in food products containing oil/fat. In this study, testing for PV was carried out to determine hydroperoxide content in the oil in

Bakasang, which is the primary product of the oxidation process. The hydroperoxide content is directly proportional to the damage done to fish oil. The greater the hydroperoxide content, the greater the level of damage to the fish oil¹⁴. According to Tenyang *et al.*¹⁵, the most detrimental effect on lipid oxidation of fish oil was generally found to be associated with processing.

In this study, the optimization of Bakasang processing was carried out using three variables: fermentation temperature, salt content and fermentation time. In experiments using the RSM, correlation, regression and determination of the optimum variables were also carried out. The correlation test for observing the effect of the three independent variables on the PV response is presented in Table 3.

According to Table 3, the variable fermentation time has a correlation coefficient of 0.591, which is greater than the fermentation temperature's correlation coefficient of 0.521 and the salt's correlation coefficient is 0.305. Fermentation time and temperature have the highest correlation compared to salt content, implying that fermentation temperature and duration are more important than salt content in producing Bakasang.

Table 1: Determination of central composite design (CCD) codes with three variables

Parameters	Value the CCD to be converted into parameters of				
	-1.682	-1	0	1	1.682
Fermentation temperature ($^{\circ}\text{C}$) (x_1)	16.36	30	50	70	83.64
Salt content (%) (x_2)	3.18	10	20	30	36.82
Fermentation time (day) (x_3)	1.59	5	10	15	18.40

Table 2: CCD (central composite design) experimental design with three variables

Experiment number	Experiment sequence	x_1	x_2	x_3	Fermentation temperature (x_1) ($^{\circ}\text{C}$)	Salt content (x_2) (%)	Fermentation time (x_3) (days)
1	5	-1	-1	-1	30	10	5
2	19	1	-1	-1	70	10	5
3	13	-1	1	-1	30	30	5
4	12	1	1	-1	70	30	5
5	15	-1	-1	1	30	10	15
6	6	1	-1	1	70	10	15
7	20	-1	1	1	30	30	15
8	14	1	1	1	70	30	15
9	16	-1.68	0	0	16.36	20	10
10	1	1.68	0	0	83.64	20	10
11	4	0	-1.68	0	50	3.18	10
12	18	0	1.68	0	50	36.82	10
13	17	0	0	-1.68	50	20	1.59
14	11	0	0	1.68	50	20	18.40
15	2	0	0	0	50	20	10
16	3	0	0	0	50	20	10
17	7	0	0	0	50	20	10
18	8	0	0	0	50	20	10
19	9	0	0	0	50	20	10
20	10	0	0	0	50	20	10

Table 3: Correlation of variables to the PV (peroxide value) response

Variables (x)	Response (y)	Correlation value (r)
Fermentation temperature (x_1) (°C)	PV	0.521
Salt content (x_2) (%)	PV	0.305
Fermentation time (x_3) (days)	PV	0.591

Greater the correlation value (r), the stronger the correlation between the variables (x) and the response (y)

Table 4: Regression analysis for the determination of the second-order model equation

Term	Coefficient	p-value
Constant	2.277	0.000
x_1	0.944	0.000
x_2	0.562	0.011
x_3	1.081	0.000
x_1^2	0.636	0.004
x_2^2	0.264	0.161
x_3^2	0.402	0.044
$x_1 * x_3$	-0.118	0.625
$x_1 * x_2$	0.181	0.457
$x_2 * x_3$	0.160	0.510

x_1 : Fermentation temperature (°C), x_2 : Salt content (%), x_3 : Fermentation time (day), R-sq: 90.34% and R-sq(adj): 81.64%

Optimum variables were identified to get an overview of the variables to be included in the linear regression equation determination. The optimum variable was determined by performing regression analysis and ANOVA. Variables with a $p > 0.05$ were eliminated because they did not have a significant effect and had a confidence level below 95%. Table 4 summarizes the results of the regression analysis, square and their interaction to determine the p-value in this experiment.

The p-value is useful for determining whether the variables x_1 (fermentation temperature, in degrees Celsius), x_2 (salt content in percent), x_3 (fermentation time in days) and square and its interactions are correlated with the variable y (peroxide value in meq kg⁻¹). The absolute correlation requirement for a variable to be included in the regression formula is if the p-value is greater than the significance or alpha level (in this study $\alpha = 0.05$). In Table 4, the term constant $t = 2.277$ is the slope/coefficient, while the coefficient for each variable, for example, 0.944 is the coefficient for the variable x_1 . Coefficient x_1 of 0.944 indicates that each increase in fermentation temperature of 1 degree Celsius will cause an increase in the response variable y (peroxide number) of 0.944 meq kg⁻¹. Thus, an equation for calculating the predicted y value (peroxide number) (meq kg⁻¹) is obtained as follows:

$$y_2 = 4.35194 - 0.11363x_1 - 0.07459x_2 - 0.25300x_3 + 0.00157x_1^2 + 0.00310x_2^2 + 0.01571x_3^2 - 0.00064x_1x_2 + 0.00172x_1x_3 + 0.00340x_2x_3$$

From the equation, it can be observed that the variables included in the model determination are the fermentation temperature variable (x_1) of 4.35194-0.11363, salt content (x_2)

of -0.07459, fermentation time (x_3) of -0.25300, square of fermentation temperature (x_1^2) of 0.00157, the square of salt content (x_2^2) of 0.00310, the result of multiplication of fermentation temperature and salt content (x_1x_2) produces -0.00064, the multiplication of fermentation temperature and fermentation time (x_1x_3) is 0.00172 and the multiplication of salt content and fermentation time (x_2x_3) is 0.00340.

The function of determining the equation of the linear regression model in this CCD analysis is to determine the ideal response value, namely the peroxide number by using the variables in the model equation. Furthermore, from the equation, the predicted PV (y_2) was obtained based on the second-order equation. Table 5 shows the difference in the peroxide number (meq kg⁻¹) from the experimental results (y_1) and the peroxide number (meq kg⁻¹) predicted using the above equation (y_2). As a result, the fermentation temperature ranges used in this study was 30-70°C, with a centre point of 50°C. The range of variations in salt concentration was 10-30%, with a centre point of 20%. The fermentation time ranged from 5 to 15 days, with a centre point of 10 days.

As shown in Table 5, the peroxide value between the experiment (y_1) and prediction (y_2) is not significantly different for the same fermentation variables, i.e., temperature (°C), salt content (%) and time (days). Additionally as shown in Table 5, the lowest peroxide value (0.9622 meq kg⁻¹) was found in the 13th experiment, where Bakasang was processed at a temperature of 50°C, with a salt content of 20% and a processing period of 1.59 days. Meanwhile, the highest peroxide value (6.2208 meq kg⁻¹), was discovered in the 8th experiment, where Bakasang was processed at a temperature of 70°C, with the salt content of 30% and a 15 days processing duration. Thus, the peroxide value of the experimental findings ranged between 0.9622-6.2208 (meq kg⁻¹) in this study. There is currently no maximum limit for the peroxide value in some food products, including fermented fish sauce products¹⁴.

According to Tenyang *et al.*¹⁵, PV in fish products varies depending on the type of fish and the processing method. The initial PV of raw herring was 23.31 meq kg⁻¹ of oil. The PV of all smoked herring increased significantly during processing, with unbleached smoked herring having a higher PV (30.12 meq O₂/kg of oil) than bleached smoked herring. According to Popa¹⁶, the recommended PV limit for quality fish oil was less than 3 (meq kg⁻¹ dry sample) when the oil leaves the factory, less than 5 (meq kg⁻¹ dry sample) after the bottle was open and less than 10 (meq kg⁻¹ dry sample) when it was used. One of the food products that use PV parameters in quality standards is food oil, including fish oil. The PV standard for fish oil, according to De Boer *et al.*¹⁷ is ≤ 5.00 meq kg⁻¹.

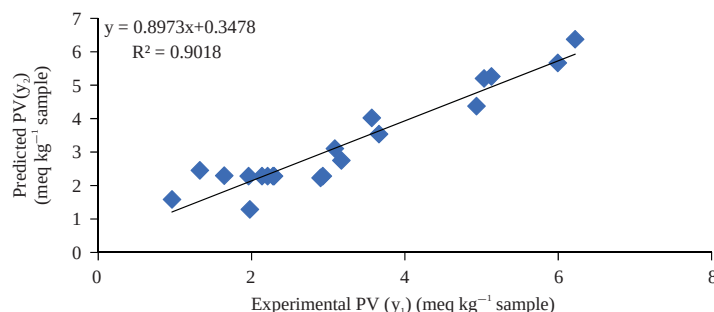


Fig. 1: Relationship between experimental (y_1) and predicted (y_2) peroxide value (PV) of the bakasang processing from cakalang fish

Table 5: Experimental and predicted peroxide value (PV) of the bakasang processing from cakalang fish

Fermentation temperature (x_1) ($^{\circ}\text{C}$)	Salt content (x_2) (%)	Fermentation time (x_3) (days)	Experimental PV (y_1) (meq kg $^{-1}$ sample)	Predicted PV (y_2) (meq kg $^{-1}$ sample)
30	10	5	1.9773	1.2849
70	10	5	3.0855	3.1067
30	30	5	2.8991	2.2281
70	30	5	3.6611	3.5389
30	10	15	3.1705	2.7519
70	10	15	5.1283	5.2627
30	30	15	4.9342	4.3738
70	30	15	6.2208	6.3749
16.36	20	10	1.3247	2.4531
83.64	20	10	5.9938	5.6667
50	3.18	10	1.6432	2.2959
50	36.82	10	3.5674	4.0250
50	20	1.59	0.9622	1.5859
50	20	18.40	5.0329	5.2017
50	20	10	2.2075	2.2836
50	20	10	2.2919	2.2836
50	20	10	2.2799	2.2836
50	20	10	1.9613	2.2836
50	20	10	2.1351	2.2836
50	20	10	2.9292	2.2836

Following up on the previous finding, the Bakasang PV results from this study ranged from 0.9622-6.2207 meq kg $^{-1}$ sample. This value is undoubtedly relatively low compared to the reported PV for other food products. Nevertheless, if we take the benchmark from the standard PV for fish oil quality¹⁷, then some PVs are not included in the fish oil quality standard. According to IFOS, the optimum PV for fish oil is ≤ 5.00 meq kg $^{-1}$ sample. This result shows that the smaller the PV value in the product, the better because the product undergoes a smaller oxidation reaction.

This study showed a correlation between fermentation temperature and PV with a positive correlation of 52.1%. The salt content gave a weak correlation to the observed response, which was 30.5% positively correlated with PV. The fermentation time variable gave a positive correlation of 59.1% to PV. The correlation test shows the influence of other variables that are not observed in the response. The higher the

fermentation temperature, the PV will increase. The addition of salt did not have a significant effect on PV. This can be observed from the weak correlation between salt content and PV. Fermentation temperature and fermentation time significantly affect PV, the longer the fermentation time, the PV will increase. An increase in fermentation temperature will cause the reaction to take place faster. The collisions that occur between particles are faster so that more products will be formed.

Additionally, to determine the relationship between the experimental (y_1) and the predicted (y_2) peroxide number response as provided in Table 5, a graph plot was created in Fig. 1. The difference between the two PVs, experimental and predicted PV, is not too big, meaning that the model used is appropriate. According to Assagaf *et al.*¹⁸, one of the parameters of the suitability of the CCD model is the small difference between experimental and predicted responses.

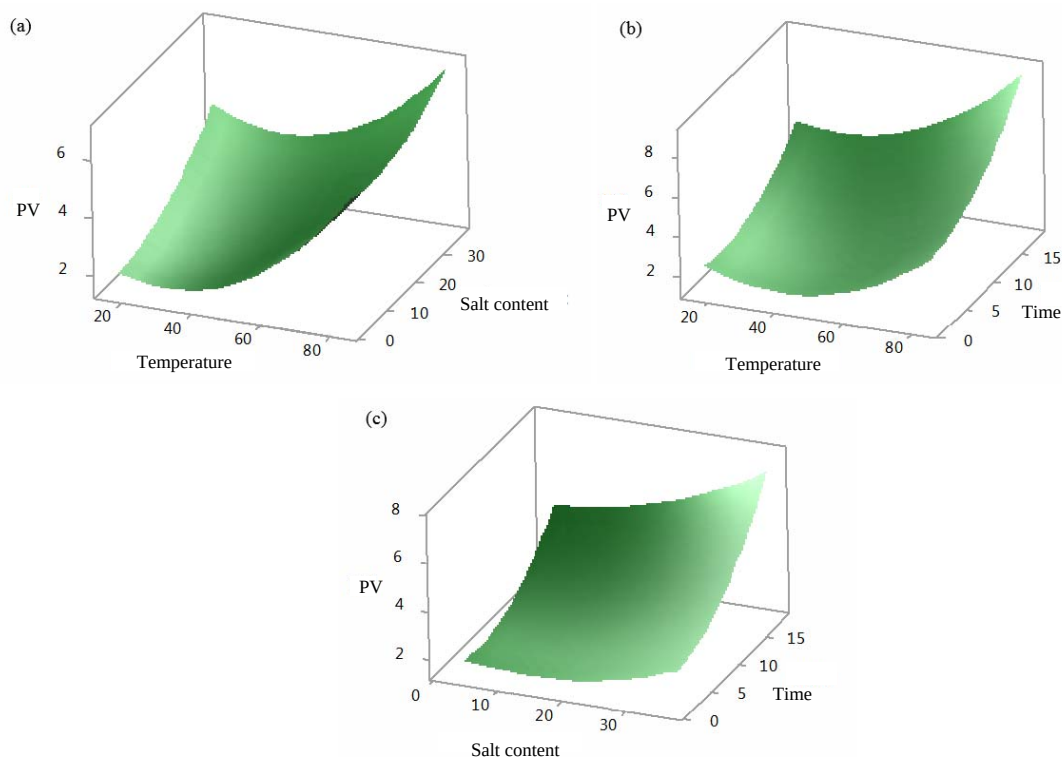


Fig.2(a-c): Three-dimensional curves of surface response, (a) Fermentation temperature and salt content against PV, (b) Fermentation temperature and fermentation time against PV and (c) Salt content and fermentation time against PV

Fermentation temperature ($^{\circ}\text{C}$), salt content (%), fermentation time (day) and peroxide value (PV) (meq kg^{-1} sample)

The coefficient of determination is 0.9018. This shows that the confidence level of the observation data on the prediction data is 90.18%. There is a data error of 9.82%, which was caused by factors that were not taken into account in this study.

Figure 2 showed the three-dimensional surface response and two-dimensional contour plots. The response surface and contour plots are the graphical representation of the regression equation used to visualize the relationship between each factor response and experimental levels¹⁹. The response surface of the three-dimensional fixed-variable of PV plotted with independent variables such as fermentation temperature and salt content (2a), fermentation temperature and fermentation time (2b) salt content and fermentation time (2c) was processed using Minitab software. Figure 2 also shows that the relationship between fermentation temperature, salt content and fermentation time with the PV response is minimal.

According to Bezerra *et al.*²⁰, surface response curves can be classified into five types: Maximum, horizontal, maximum outside the experimental area, minimum and saddle curves.

A saddle-shaped curve is depicted in Fig. 2(a-c). To obtain the minimum surface response curve, one of the variables must be replaced with one that has a more significant effect on PV, namely the variable with the lowest correlation (i.e. salt content).

The increase in fermentation temperature, salt content and fermentation time, the greater the PV, then further analysis is carried out, namely the contour plot in Fig. 3. The variable fermentation temperature and salt content affect PV and reach the optimum under certain conditions. The resulting contour plot consists of various colour variations. Each colour shows the range of the resulting response. The most optimal condition shown in Fig. 3a is light green, where $\text{PV} < 2 \text{ meq kg}^{-1}$ sample. Based on Fig. 3a, the optimum PV response at fermentation temperature and salt content was 30.926°C and 9.226% . Under these conditions, the PV is obtained at the optimum point of $1.579 \text{ meq peroxide kg}^{-1}$ of oil. Figure 3b shows the variable fermentation temperature and fermentation time has an effect on PV and reaches the optimum at fermentation temperature and fermentation time of 34.939°C and 4.868 days. Under these conditions, it was found that the PV was at the optimum point of 1.446 meq

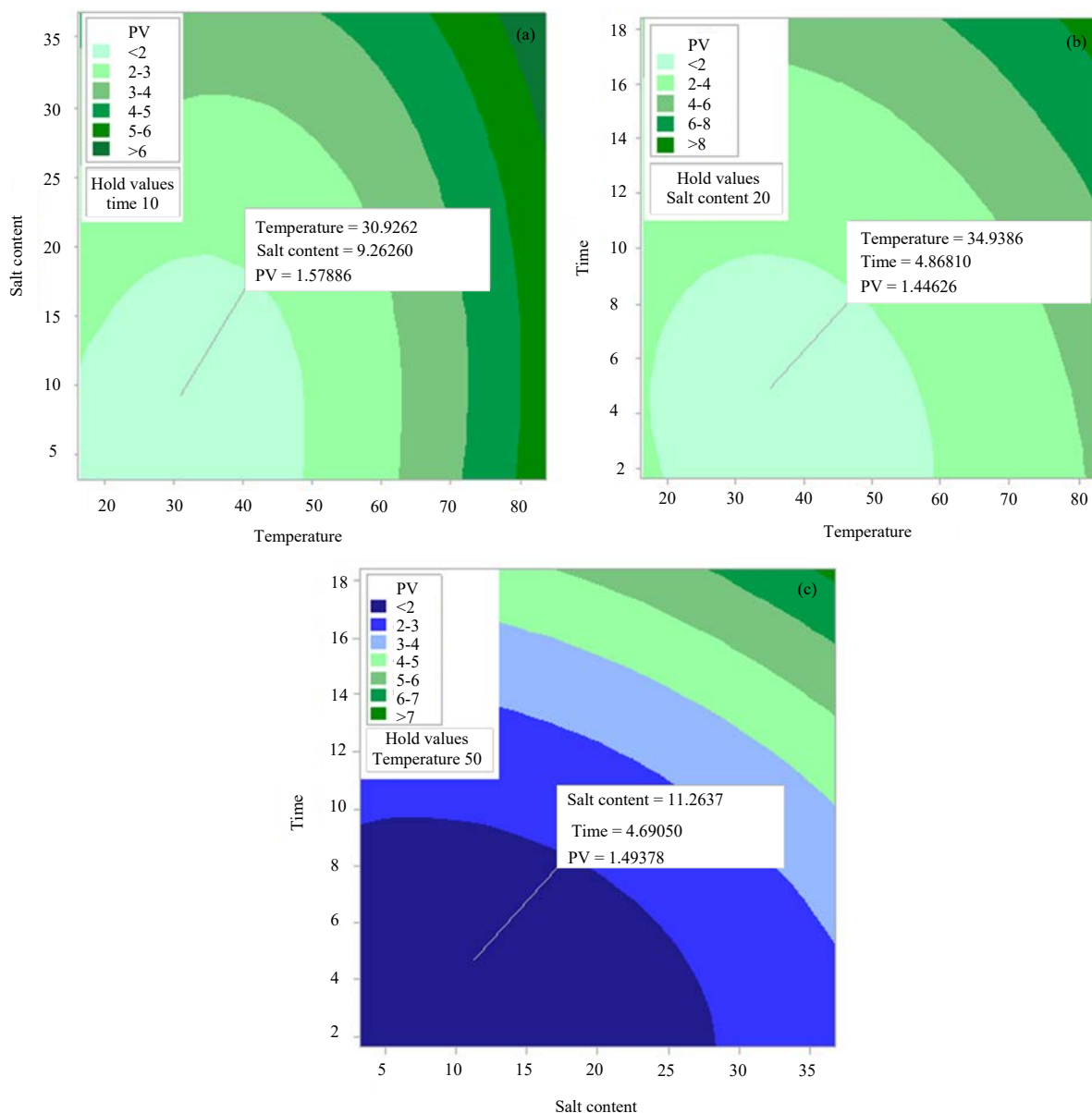


Fig. 3(a-c): Contour Plot of PV, (a) Fermentation temperature and salt content, (b) Fermentation temperature and fermentation time and (c) Fermentation time and salt content
Fermentation temperature (°C), salt content (%), fermentation time (day) and peroxide value (PV) (meq kg⁻¹ sample)

peroxide kg⁻¹ of oil. Figure 3c showed the fermentation time and salt content have an effect on PV and reach the optimum at the salt content and fermentation time of 11.264% and 4.690 days with a PV of 1.494 meq peroxide kg⁻¹ of oil. Based on Fig. 3a-c, the resulting optimum point values are fermentation temperature 30.926-34.939°C, salt content 9.263-11.264% and fermentation time 4.690-4.868 days and with PV Bakasang of 1.446-1.579 meq peroxide kg⁻¹ of oil. Thus, it can be concluded that the optimum processing point for Bakasang is at a temperature of 32.932°C,

fermentation time of 4.779 days and salt content of 10.263%, with a PV of 1.513 meq peroxide kg⁻¹ of oil. The PV is below the fish oil standard according to De Boer *et al.*¹⁷, which is ≤5.00 meq kg⁻¹.

The low PV in Bakasang is thought to be caused by the antioxidant content in Bakasang. Several researchers have reported the presence of antioxidants in fermented fish. Najafian and Babji²¹ show that Loma Fermented Fish (LFF) contains peptides that may have potential as natural functional ingredients in the food and pharmaceutical

industries. Furthermore, it is said that the presence of hydrophobic amino acids (isoleucine (Ile), alanine (Ala) and proline (Pro)) and basic amino acids in the peptide sequence, lysine (Lys), is thought to contribute to the high antioxidant activity of LFF.

In this connection, Choksawangkarn *et al.*²² stated that Fish sauce by-product (FSB) contains low molecular weight FSB fraction PQLLLLLL and LLLLLLL, which are potent antioxidative molecules. Hamzeh *et al.*²³ stated that Thai fish sauces contain peptides that have antioxidant and ACE-inhibitory activities. Ruthu *et al.*²⁴ stated that the protein isolated from fish heads has antioxidant and antimicrobial properties to be used as a functional ingredient in food formulations to provide benefits to human health and/or increase the shelf life of food. Based on this, it is suspected that the peptide content in Bakasang is also potential as an antioxidant.

CONCLUSION

In this study, the optimum conditions for Bakasang processing were determined based on PV parameters according to the Response Surface Methodology (RSM) with the Central Composite Design (CCD). With this CCD, the Peroxide Value (PV) of the Bakasang can be adjusted by searching for the optimum variables involved in the process, namely the fermentation temperature (°C), salt content (%) and fermentation time (day). Based on the correlation coefficients, regression and equation model, the three variables (fermentation temperature (°C), salt content (%) and fermentation time (day)) can increase PV. However, in this CCD, there must be variables that are set constant in the midpoint, so that two other variables can be optimized. Referring to the contour plot of PV versus fermentation temperature and salt content, fermentation temperature and fermentation time and fermentation time and salt content, it can be concluded that to produce Bakasang with a minimum PV of 1.513 meq kg⁻¹ sample, then the processing condition has to be set at a temperature 32.932°C, salt content 10.263% and fermentation time 4.779 days.

SIGNIFICANCE STATEMENT

The new finding that can be generated through this study is information about a model that can be used in the determination of PV in products other than Bakasang, with a recommendation that controlled variables used should be the same.

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