

Process optimization of salt-fermented green mussel (*Perna viridis* Linnaeus, 1758) using response surface methodology

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Article history:

Received: 28 September 2022

Received in revised form: 19 February 2023

Accepted: 9 April 2024

Available Online: 6

December 2024

Keywords:

Bivalves,
Fermentation,
Quality,
Safety,
Acceptability

DOI:

[https://doi.org/10.26656/fr.2017.8\(6\).490](https://doi.org/10.26656/fr.2017.8(6).490)

Abstract

Green mussels are bivalve molluscs that can be preserved through salt fermentation to enhance their quality. This study aimed to optimize the fermentation process of green mussels using response surface methodology (RSM) and employing the D-optimal design. Variables include salt concentration (5-30%) and fermentation period (1-4 weeks). A total of 16 combination conditions of salt concentration and fermentation period were produced by the RSM. Responses include pH, total plate count (TPC), and overall acceptability. Based on the result, the optimized condition for fermented green mussel was 15.05% salt concentration and 2.6 weeks fermentation period. The desirability value was 0.733. The optimum condition has 4.71 pH, 3.63 log CFU/g total plate count, and 8.99 overall acceptability score. Overall, the result of this study could be applied in the process standardization of producing high-quality salt-fermented green mussels. Further studies on the identification of bacteria in the fermented product and longer fermentation period are recommended.

1. Introduction

The green mussel, (*Perna viridis* Linnaeus, 1758) is a bivalve mollusk that thrives in almost tropical waters in the Indo-Pacific region of Asia (Siddall, 1980). They usually settle in mid-littoral and sub-littoral areas in densities of up to 35,200 individuals/m² (Lee, 1985). Locally known as “tahong”, it is considered a cheap source of protein for people living in coastal areas (Masniyom and Benjama, 2007), as it contains 36.15% of protein (Saritha *et al.*, 2015). Samar province is one of the major producers of green mussels in the Philippines where production reached 100,738.69 metric tons in 2018 (PSA, 2019). This bivalve is mostly cultured in the municipality of Jiabong, being the top producer in the whole region.

Bivalves are highly perishable due to their neutral pH, and high initial bacterial load due to their filter-feeding characteristics (Batista and Mendes, 2012). Mussels are processed in order to extend their shelf-life and produce more innovative food products. In the Philippines, value-added products from green mussel were developed, such as mussel crackers and “adobong tahong” produced in the Municipality of Jiabong, Samar. Doncillo and Combras (2017) developed mussel meatloaf, sausage, and dried mussel.

Fermentation is one of the oldest preservation methods that enhance the flavor and nutritional value of food (Visessanguan *et al.*, 2004). Some of the evaluations used to determine the quality of fermented goods include sensory evaluation, pH level, and total plate count. During fermentation, fish sensory attributes change over time which can be favorable or undesirable such as off flavor and odor flavor, and therefore fermentation parameters should be altered to prevent these (Giyatmi and Irianto, 2017). The pH levels typically decline slightly during fermentation and range between 6.47 and 6.58 most probably due to the formation of lactic acid by LAB (Bao *et al.*, 2018).

Fermentation is a widely acceptable preservation method due to its simplicity, efficiency, and lower cost (Battcock and Azam-Ali, 1998). Several aquatic species are utilized for fermentation such as sardines, shrimps, squids, fish eggs, and shellfish (Steinkraus, 1983). However, little information is available on the fermentation of green mussels. There are few vendors in Samar that sell fermented mussel, but no data on the process standardization of the product was recorded nor reported. Optimization of the process is necessary in order to produce high-quality food products. Hence, this study aimed to optimize the process of making salt-fermented green mussels specifically on the salt

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concentration, and fermentation period.

2. Materials and methods

2.1 Sample preparation

One sack of fresh green mussel was procured from a mussel farmer in Brgy. Bahay, Tarangnan, Samar, Philippines. Samples were transported immediately to the fish processing laboratory of Samar State University, Mercedes Campus. Upon arrival, green mussels were cleaned with clean running water to remove dirt and unwanted particles adhering to their shells, and they were blanched in hot water (80°C) for 2 mins (Arcales and Nacional, 2019).

2.2 Salting process

Blanched mussels were placed in a clean container and mixed with table salt. The variable factor for salt concentration was 5-30% by total weight. After mixing, salted mussels were packed in sterile bottles. They were stored for 1-4 weeks at room temperature until product analyses.

2.3 pH determination

Approximately 10 g of the sample was homogenized in 10 mL of distilled water. The pH of the homogenate was measured using a digital pH meter (AOAC, 2005).

2.4 Total plate count determination

Approximately 10 g of sample was homogenized in 90 mL peptone water. One ml from the stock solution was dispensed in 9 mL tubes with peptone water for serial dilution (up to 10⁶). Samples (0.1 mL) from the dilution tubes were dispensed and spread into plat count agar medium. Inoculated plates were incubated at 37°C for 24 hrs. Colonies were counted and recorded as log CFU/mL (AOAC, 2005).

2.5 Sensory evaluation

Acceptability of the samples was determined by 10 semi-trained panelists using a 9-point hedonic scale scorecard (1=dislike extremely, 9=like extremely). Semi-trained panelists refer to panelists who have no formal training but are knowledgeable in conducting the sensory evaluation. These include faculty, students, and laboratory technicians. Prior to the start of the experiment, panelists were oriented on their tasks and about the sample.

2.6 Statistical analysis

Data were analyzed and processed using Design Expert software. In response surface methodology (RSM), D-optimal design was used to determine the

effect of the independent variables, i.e. the salt concentration (5-30%), and the fermentation period (1-4 weeks), on the dependent variables i.e. pH, total plate count, and overall acceptability. ANOVA was used to determine significant differences ($p < 0.05$) among treatments that were done in triplicate.

3. Results and discussion

This study investigated the effects of independent process variables i.e. fermentation time and salt concentration on the fermentation of green mussel. The parameters determined were pH, total plate count, and overall acceptability and responses are revealed in Table 1.

Table 2 shows the model summary statistics of the different parameters that are cubic and linear. Significance models are all < 0.05 , which indicates that all math models were significant. All lack of fit values were revealed to be not significant which was good and preferable. Adequate precision that measures the signal to ratio was all greater than 4 which is desirable.

Figure 1 shows that the pH of fermented green mussels decreases with a longer fermentation period, and decreasing salt concentration. The decrease in pH with decreasing salt concentration was because salt inhibits the growth of microorganisms including lactic acid bacteria. The same results were obtained by Duman *et al.* (2007), El Hag *et al.* (2012a), and El Hag *et al.* (2012b) where a slight decrease in pH values was observed in their fermented fish samples during fermentation. Petrus *et al.* (2013) also reported that salt has a slight impact on the pH value of fermented fish. As shown in Table 1, all samples in all experiments had pH values below 6.5, thus suitable for human consumption. Fermented fish products with a pH level of 6.5 or higher are considered bad quality (Anihouvi *et al.*, 2012).

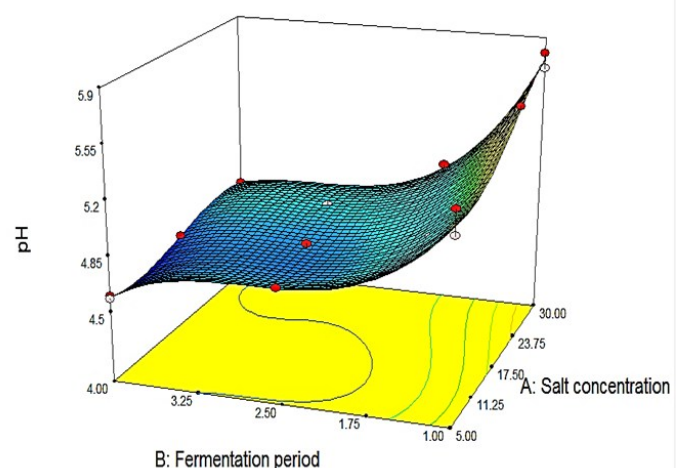


Figure 1. A 3D model of the relationship between (A) salt concentration and (B) fermentation relative to the pH of the fermented green mussel.

Table 1. The pH, total plate count and overall acceptability responses for salt-fermented green mussel as the result of RSM optimization.

Experiment no.	Salt concentration (%)	Fermentation period (weeks)	pH value	Total plate count (log CFU/g)	Overall acceptability
1	22.53	1.0	5.60	2.8	6
2	24.79	2.8	4.76	3.2	7
3	15.89	2.7	4.74	3.4	9
4	17.50	4.0	4.65	3.8	8
5	30.00	4.0	4.71	3.6	6
6	30.00	1.9	4.98	3.1	6
7	5.00	1.0	5.22	3.5	6
8	5.00	4.0	4.60	4.5	7
9	5.00	1.0	5.38	3.7	6
10	5.00	4.0	4.58	4.6	7
11	5.00	2.5	4.78	4.0	6
12	14.38	1.5	4.92	3.2	8
13	30.00	1.0	5.70	2.5	5
14	30.00	4.0	4.71	3.4	5
15	5.00	2.5	4.78	4.1	6
16	30.00	1.00	5.80	2.4	5

Table 2. Model summary statistics of the different parameters.

Parameter	Math Model	Significance model	Lack of fit value	Adj. R ² model	Pred. R ² model	Adequate precision
pH	Cubic	<0.0001	0.86	0.9792	0.6053	24.877
Total plate count (TPC)	Linear	<0.0001	2.58	0.9459	0.9320	31.054
Overall acceptability	Cubic	0.0004	0.063	0.9365	0.8054	17.225

The model chosen by the program for the pH response was cubic with an R² value of 0.9792. The pH response model has an F value of 79.50 with a p-value (Prob> F) of <0.0001 indicating the model was significant at $\alpha < 0.05$. ANOVA results showed that the salt concentration and fermentation period affected the pH response with not significant lack of fit value of more than 0.05 (0.3954).

Figure 2 shows that the total plate count (TPC) of fermented green mussels increases with increasing fermentation period but decreases with increasing salt concentration. This only showed that salt being a preservative agent, inhibits the growth of microorganisms. High salt concentration in fermentation removes water from flesh and is replaced by salt molecules, thus inhibiting the growth of pathogenic and non-pathogenic bacteria (Karyantina *et al.*, 2021). Similar results were obtained by Petrus *et al.* (2013) where a decreasing number of microorganisms was observed in wadi betok a traditional fermented fish added with 100% salt. Desniar *et al.* (2009) also reported a decrease in the number of microorganisms in peda samples added with 50% salt.

The model chosen by the program for the TPC response was linear with an R² value of 0.9459. The TPC response model has an F value of 132.12 with a p-value (Prob>F) of <0.0001 indicating the model was significant at $\alpha < 0.05$. ANOVA results showed that the

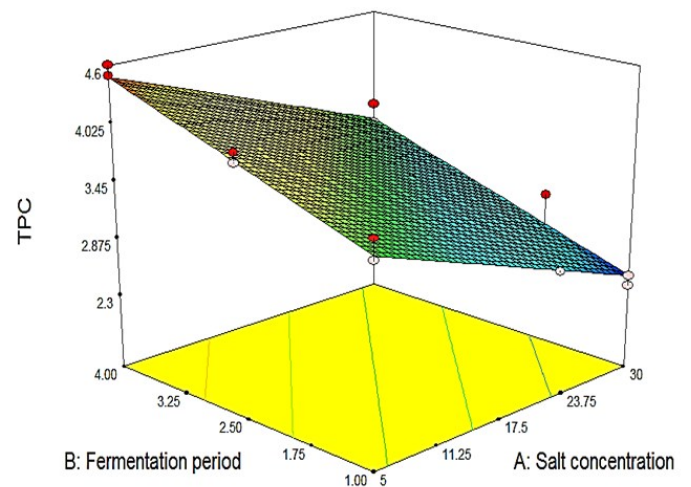


Figure 2. A 3D model of the relationship between (A) salt concentration and (B) fermentation relative to the total plate count (TPC) of the fermented green mussel.

salt concentration and fermentation period affected the TPC response with not significant lack of fit value of more than 0.05 (0.1555).

Figure 3 shows the overall acceptability of fermented green mussels. Results revealed that experiment no. 3 with variables of 15.89% salt and 2.7-week fermentation period obtained the highest sensory score of 9 with an adjectival rating of like extremely showing no significant difference with experiments no. 4 and 12. It can be observed that the panelists preferred those samples that had a medium amount of salt. Noted comments include

that the samples with 30% salt were too salty, and those samples with 5% salt had slight off-odor. Petrus *et al.* (2013) also reported the highest sensory score in fermented fish that utilized a 15% salt concentration.

The model chosen by the program for the overall acceptability response was cubic with an R^2 value of 0.9365. The overall acceptability response model has an F value of 25.59 with a p-value (Prob> F) of 0.0004 indicating the model was significant at $\alpha < 0.05$. ANOVA results show that the salt concentration and fermentation period affected the overall acceptability response with not significant lack of fit value of more than 0.05 (0.8121).

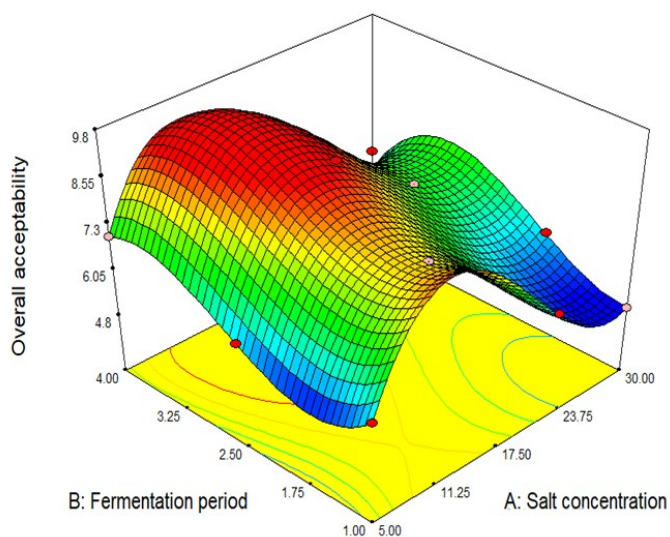


Figure 3. A 3D model of the relationship between (A) salt concentration and (B) fermentation relative to the overall acceptability of the fermented green mussel.

The optimization and desirability of the response variable were obtained by specifying the goal. In this study, the goal chosen for salt concentration and fermentation period was within range. For the pH and total plate count (TPC) the target was to minimize, and the overall acceptability was set to maximize. Numerical optimization was revealed to have a desirability value of 0.733. The desirability value is a parameter that is used to determine the best-optimized condition ranging from 0 -1. A value closer to 1 is more acceptable. The condition chosen by the program for fermented green mussels is 15.05% salt concentration and 2.6 weeks fermentation period.

4. Conclusion

The D-optimal design and response surface methodology (RSM) were effectively used to determine optimized conditions for fermented green mussels. The pH level, total plate count (TPC), and overall acceptability responses were measured to optimize 16 conditions. The optimized condition for fermented green

mussel was 15.05% salt concentration and 2.6 weeks fermentation period, with an optimum formula of 4.71 pH, 3.63 log CFU/g total plate count, and 8.99 overall acceptability score. Further studies on the identification of bacteria in the fermented product and longer fermentation period are recommended.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

This study was funded by the Center of Fisheries and Aquatic Resources Research and Development (CFARRD RD Proj. 021-13) of Samar State University with special order no. 44 s. 2021.

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