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SHORT PAPER

Optimising the Brining-Steaming Process of Pearl Oyster *Pinctada radiata* (Leach, 1814) Meat

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Abstract

This study aimed to optimise the brine's salt content (BSC) and steaming temperature (ST) for the brined-steamed pearl oyster (BSPO) process using Response Surface Methodology. The experimental design employed a two-level Central Composite Design, comprising thirteen combinations, and a polynomial regression model was developed to assess the effects of BSC and ST on yield and water activity (αw). ST linearly influenced the yield and αw. The BSC did not significantly affect yield but had a linear, negative effect on αw. The optimal combination of BSC and ST was 20% (w/v) at 105°C; the yield and αw were 64.645% and 0.945.

Introduction

Marine bivalves are regarded as a rich source of protein and contain high levels of polyunsaturated fatty acids, essential vitamins, and minerals; therefore, they are consumed worldwide (Ackman, 1990; Rey et al., 2012; Zgouridou et al., 2022). *Pinctada radiata*, commonly known as the pearl oyster (PO), is a non-native benthic species with a notable presence in the Mediterranean Sea (Theodorou et al., 2023). In Greece, this PO has been available in local markets since the 1960s, and recent studies have confirmed its ongoing expansion (Theodorou et al., 2019). Its high meat yield (over 20%) and consistent year-round high protein content (approximately 60%) make the PO a viable alternative to seasonal shortages of key commercial species (Theodorou et al., 2021, 2023). Furthermore, this suggests its potential for further processing to develop new value-added seafood products (Moutopoulos et al., 2022; Theodorou et al., 2019, 2021). Oysters are often preferred as raw food by humans, making safety a critical factor for human consumption (Froelich & Noble, 2016; Moutopoulos et al., 2022; Nenciu et al., 2022). Consequently, the risk of food poisoning outbreaks is notably high due to the perishability of their meat. This emphasises the need for rapid, simple, and cost-effective methods for processing seafood. Oyster processing aims to significantly extend their shelf life, facilitate transportation, and ensure preservation. Typically, oysters are preserved through high-pressure treatments with or without heat application, high hydrostatic pressure, ultraviolet sterilisation, and other modern or traditional techniques (Cruz-Romero et al., 2007; Jeong et al., 2021; Liu et al., 2022; López-Caballero et al., 2000; Ma et al., 2021). Conventional methods, such as drying, extensive

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heating, and salting, are also employed, though they have different impacts on the organoleptic properties of the products.

Salting is a traditional method of processing seafood. Salt acts as a flavour enhancer, improves texture, and exerts a remarkable preservative effect on salted food products. Salt reduces the αw of foods, slowing or stopping spoilage processes (Whittle & Howgate, 2002). However, salting alone is regarded as an inadequate method for preserving ready-to-eat foods and should be combined with other preservation techniques (e.g., drying, osmotic dehydration) (Albarracín et al., 2011). The salting methods commonly used in the fish processing industry are as follows: a) dry salting, b) dry-wet salting (pickling), c) wet salting (brining), and d) injection salting. In dry salting, fish is placed in layers separated by layers of dry salt. The brine formed through the osmotic process is removed via holes in the bottom of the containers (FAO, 1989). Pickling serves as an alternative to the previous method. The resulting brine is not removed but stays in the salting tank, establishing a dry-wet salting condition in the fish (FAO, 1989). In brining, the fish is soaked in low-temperature (3-5°C) brine solutions, usually of medium strength (15-20%), to minimise bacterial growth in the product. The time interval depends on the size and chemical composition (fat content) of the fish (Gallart-Jornet et al., 2007). Injection salting involves pouring a brine solution into the product. This method is quick and cost-effective, ensuring an even distribution of sodium chloride, but it results in products with a shorter shelf life (FAO, 1989). Salting is utilised in a wide range of fish products, including salted cod (Barat et al., 2002; Thorarinsdottir et al., 2004), sea bream (Goulas & Kontominas, 2007) and chub mackerel (Goulas & Kontominas, 2005).

Response surface methodology (RSM) is a statistical technique used to develop, improve, and optimise processes involving multiple factors that affect the response of interest. RSM aims to generate a mathematical model that accurately represents the entire process (Myers et al*.,* 2016). It has been applied to optimise processes across various types of seafood, including sausages made from minced mullet (Daley & Deng, 1978), surimi from mechanically recovered fish muscle (Fogaça et al., 2013), smoked mussels (Petridis et al., 2013), and sea bream fillets smoked with liquid smoke (Makri et al., 2016).

Although the effects of various traditional processing methods on the quality parameters of different oyster species have been examined, there is no reference to using salting and steaming to produce brined-steamed pearl oyster (BSPO) in the literature. Therefore, this study aimed to develop a method for producing BSPO and to optimise the brining-steaming process using RSM.

Materials and Methods

Sample Collection

Whole-shelled pearl oysters (WSPO) were hand-harvested from the Saronic Gulf region. Immediately after harvest, the WSPO were packed and stored in insulated polystyrene cold boxes filled with flaked ice, then transported to the laboratory. The day after harvesting, the WSPO were shucked, and the pearl oyster meat (POM) was processed according to the following experimental design (ED).

Experimental Design

Statistical analyses, response surface generation, and optimisation were carried out using Expert Design version 9 (Stat-Ease Inc., Minneapolis, USA).

The ED employed was a two-level Central Composite Design (CCD) with thirteen runs. Equations were formulated to estimate the effects of the two factors, A and B (Chew et al., 2024; FAO, 2021; Makri et al., 2016) on the response variable Y (Table 1), and a numerical optimisation method was used to identify the factor values that optimise the production process, utilising first- or second-degree polynomial regression models as appropriate.

$$Y = b_0 + b_1 \times A + b_2 \times B + b_{11} \times A^2 + b_{22} \times B^2 + b_{12} \times A \times B$$
(Equation 1)

Where Y represents the response variable (either yield or αw); A and B are the coded factors — A being the brine's salt content (BSC) and B the steaming temperature (ST) — and b_1 and b_2 are the linear coefficients, with b_{12} as the interaction coefficient. At the same time, b_{11} and b_{22} are the quadratic coefficients of the model. The significance of all coefficients in the equations was evaluated statistically at a probability (*P*) of 0.05. Model adequacy was assessed using adjusted R-squared (R^2), prediction error sum of squares (PRESS), and adequacy precision (Barat et al., 2002). A prediction R^2, comparable to the adjusted R^2, along with a low PRESS value and an adequacy precision greater than four, indicates that the model is suitable for predictions.

Initially, the linear model was evaluated for its ability to fit the data. When this proved inadequate, modifications to the equation involved either adding squared terms or interaction terms to the linear model.

Table 1. Factors and their corresponding levels affecting the production yield and products' αw of brine-steamed pearl oysters

Notation	Factor	Unit	Low level (-1)	Middle level (0)	High level (+1)
A	Brine salt content (%)	w/v	0	10	20
B	Steaming temperature	ºC	105	113	120

If the fit remained insufficient, the complete quadratic model, which included all these modifications, was used. The fitted model identified the trend, whether linear or curvilinear (Makri et al*.,* 2016).

Processing of the Pearl Oyster Meat

Fifty-two POM samples of Pinctada radiata (Leach, 1814) were used to prepare BSPO according to the ED detailed in Table 2. The POMs were divided into 13 groups, each corresponding to one of the study's 13 ED runs. To remove excess water, the POMs were placed on filter paper No. 41 for 2 minutes, then weighed. Subsequently, the POMs were placed in brine at salt concentrations ranging from 0% to 20% (w/v) for 5 minutes. The ratio of POMs to brine was 1:3, and during the brining process, the containers were shaken in a suitable water bath. The brine was kept at 4°C to prevent microbial spoilage. The brined POMs were placed on a rack for 2 minutes to drain, then weighed again. After draining, the POMs were steam-cooked in an autoclave (Sanyo MLS-3020) at STs of 105°C, 113°C, and 121°C for 6 minutes. They were then allowed to cool at room temperature for 60 minutes. Afterwards, the POMs were wrapped in aluminium foil and sealed inside food-grade plastic bags (INPULSE SANI-SEALER ME-2010HC). At this stage, the process was considered complete, and the POMs were stored in the laboratory refrigerator at 2°C until the following day, when they were weighed to determine the yield of the final product and the αw.

Physicochemical analysis

Yield

The yield percentage was calculated by comparing the raw pearl oyster meat weight (POMW) to the processed POMW, using the following formula.

$$\text{Yield (\%)} = \frac{\text{processed POMW}}{\text{raw POMW}} \times 100$$

Water Activity (αw)

αw measurements were performed using the LabMaster-αw (Novasina AG, Switzerland). 2 g of POM from each group (4 raw POs) were milled and dispersed within the measuring container. All measurements were taken at room temperature (25°C). Each measurement was carried out in triplicate, and the average was used as the αw of the sample.

Results and Discussions

The data and results of processing POMs at different BSCS and STs are shown in Figure 1 (yield percentage) and Figure 2 (αw).

The data of the production yield (Figure 1) were fitted to the following model equation:

Yield= 60.813 - 3.833 X B

The data for the αw (Figure 2) were fitted to the following model equation:

αw= 0.951 - 0.004 X A + 0.0016 X B

ST had a negative and linear effect ($P<0.001$) on the yield, but BSC showed no significant effect (Equation 1, Figure 1). Yield ranged from 55.70% to 65.43% and peaked at the lowest ST. High STs cause protein denaturation, which reduces the water-holding capacity of tissue proteins (Alcicek, 2014). This may lead to greater oyster liquid loss at higher STs and, consequently, lower processing yields.

Meat products are traditionally soaked in concentrated solutions containing sodium chloride and other ingredients. Pieces of meat or fish are processed by immersing them in aqueous solutions of sodium chloride as the main dissolved substance. These processes help the tissues absorb sodium chloride while preventing dehydration, especially at concentrations up to 250 g/L. Therefore, this salting process (soaking in aqueous sodium chloride solutions) is usually followed by a dehydration step to stabilise the product (Collignan

Table 2. Design matrix for brining and steaming pearl oysters

Runs	Brine salt content (%)	Steaming temperature (°C)
1	10	113
2	0	113
3	20	105
4	20	113
5	10	121
6	10	113
7	10	105
8	10	113
9	10	113
10	0	105
11	20	121
12	0	121
13	20	113

et al., 2001). Therefore, given that in the present study the salt concentration in brines ranged from 0 to 20% (w/v), the salt concentration would have a limited effect on oyster dehydration and, by extension, on the final product yield. The levels of b1 and b2 values (Equation 1) indicate an adverse linear effect of BSC (factor A) and a positive linear effect of ST (factor B) on the αw of BSPO, with factor A exerting a much greater influence on αw than factor B (Figure 2). These effects resulted in a slight reduction of αw from 0.956 (0% salt

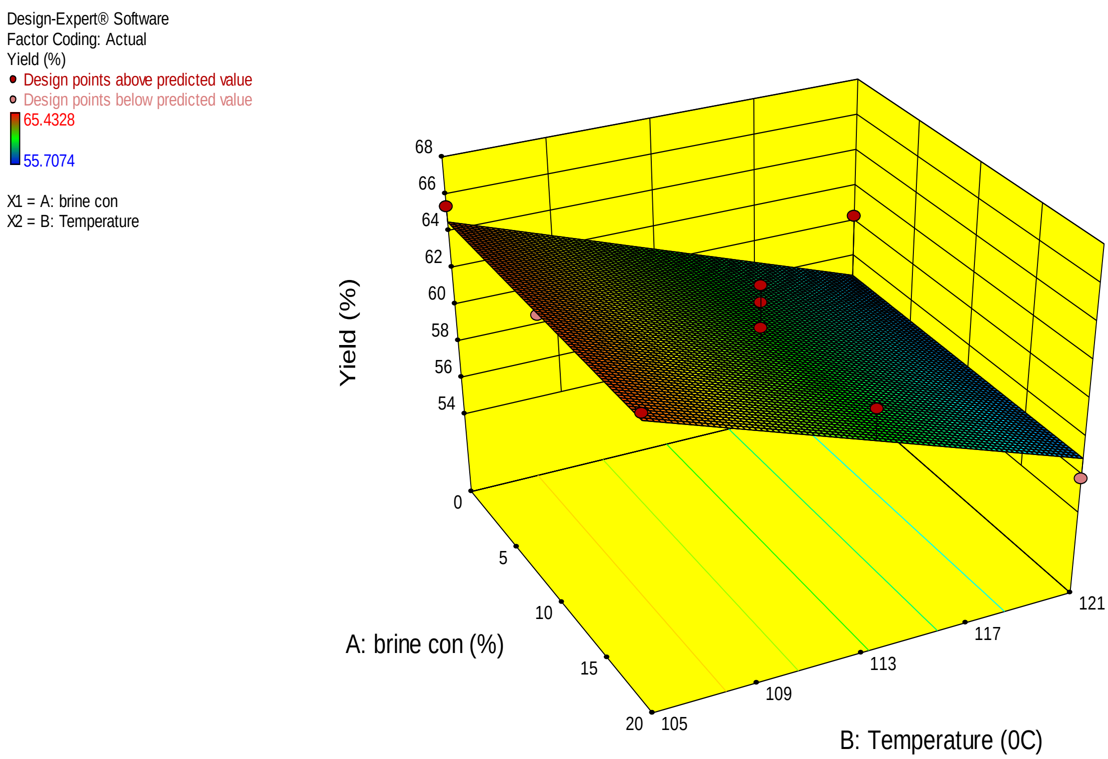

Figure 1. Response surface of the yield of the brining-steam-cooking process.

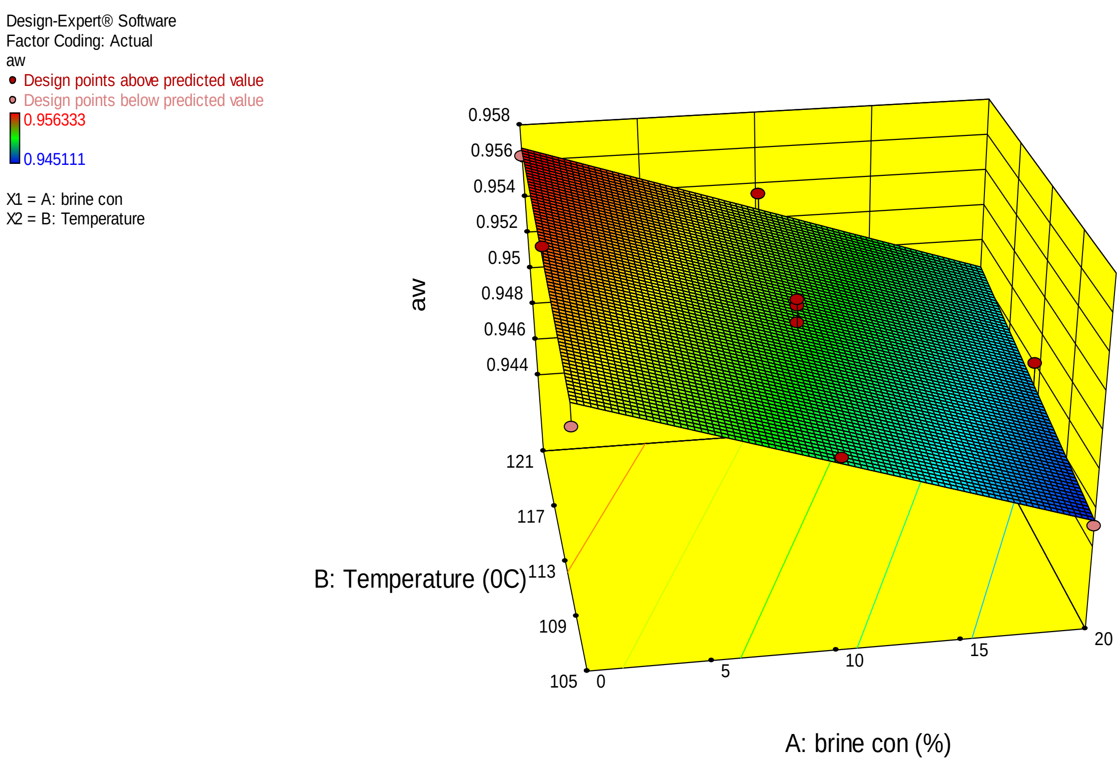

Figure 2. Response surface of water activity (αw) of brining–steam cooking process.

concentration, T= 121°C) to 0.945 (20% salt concentration, T= 105°C) in the samples. The αw of a food product represents the amount of unbound water available for microbial growth and chemical reactions. Salt's ability to decrease αw is believed to stem from the association of sodium and chloride ions with water molecules incre. Therefore, increased salt levels in brines could have caused increased concentration of salt ions in the oyster's meat. This could have reduced the unbound water by associating with salt ions, thereby decreasing the αw of the samples (Damodaran & Kirk L. Parkin, 2017). On the contrary, ST could have disrupted the hydrogen bonds of the side groups of the oyster's proteins with water molecules. This could have induced an increase in free water molecules in the oysters' meat and, in consequence, an increase in αw of the samples (Pérez-Reyes et al., 2021).

The process was optimised using the numerical method for each factor and response, as shown in Table 2. The optimal combination of BSC and ST was 20% (w/v) and 105°C, respectively, with an overall desirability of D= 0.946 (Table 3). Under this optimal response combination, the yield and αw of BSPO were 64.645% and 0.945, respectively.

Conclusions

A polynomial regression model for the yield and αw parameters of BSPOs was developed as functions of BSC and ST. Within the experimental range, response surface methodology and numerical optimisation proved useful in identifying the optimal BSC and ST for BSPO production. The optimal conditions were 20% (w/v) for BSC and 105°C for ST. Under these conditions, processing yield and αw reached 64.645% and 0.945, respectively.

Ethical Statement

Not applicable.

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Author Contribution

First Author: Conceptualization, Formal Analysis, Investigation, Methodology Visualization and Writing - original draft; Second Author: Data Curation, Visualization and Writing -original draft; Third Author: Data Curation, Writing -review and editing; and Fourth Author: Supervision, Funding Acquisition, Project Administration, Resources, Writing - review and editing.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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Table 3. Output for the numerical optimisation of the brine-steamed pearl oyster process

Combinations	Salt content (%)	Temperature (ºC)	Yield (%)	αw	Desirability
1	20.000	105	64.645	0.945	0.946
2	18.500	105	64.645	0.946	0.919
3	17.163	105	64.645	0.947	0.894
4	8.500	105	64.645	0.950	0.715

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