Application of High-Frequency Defrosting, Superheated Steam, and Quick-Freezing Treatments to Improve the Quality of Seafood Home Meal Replacement Products Consisting of the Adductor Muscle of Pen Shells and Common Squid Meat

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Abstract: We developed a new seafood home meal replacement (HMR) product containing the adductor muscle of the pen shell (AMPS) and common squid meat (CSM) via high-frequency defrosting (HFD), superheated steam, and quick freezing. Test HMR products were produced by mixing defrosted and roasted AMPS, CSM, and sauce in ratios of 27.5, 27.5, and 45.0% (w/w), respectively, followed by quick freezing at 35 °C in a polypropylene plastic bowl covered with a plastic film. The chemical characteristics, nutritional quality, microbial and sensory properties, and shelf life of the product were examined. The response surface methodology identified the optimal temperature and heating time of the superheated steam for AMPS (220 °C, 1 min) and CSM (300 °C, 1.5 min). Chemical characteristics showed low levels of volatile basic nitrogen (9.45 mg%) and thiobarbituric acid-reactive substances (1.13 mg Malondialdehyde [MDA]/kg). No significant changes (p < 0.05) were observed in microbial, color, flavor, taste, texture, and overall acceptance at −23 °C for 90 days. After reheating, the sensory scores varied from “like moderately” to “like very much.” The shelf life of the HMR product was estimated to be 24 months. In conclusion, HFD, superheated steam, and quick freezing successfully improved product quality, with little loss of nutrition and texture.

Keywords: HMR; pen shell; squid meat; superheated steam; high-frequency defrosting

1. Introduction

In recent decades, people have dramatically changed the way they cook food because of the changes in attitude towards food. In the last few years, with increasing lifestyles, the number of people choosing to embrace these convenient food products at the expense of taste and health has steadily increased. Consequently, the popularity of packaged food and instant meals has revolutionized the packaged food industry, with manufacturers seeking new methods to offer fresh and delicious food without inconvenience. Nowadays, home meal replacement (HMR) products that are simple meals to prepare and consume have become increasingly popular in the food and beverage industry. HMR products have the advantage of long shelf life while maintaining the nutrient content of the food. Globally, the demand for HMR products has increased due to increasing single-person households, women’s social activities, and the population of elderly people.
Increases in HMR products have encouraged the development of new high-quality seafood HMR products with a long shelf life; these contain the seasoned adductor muscle of the pen shell (AMPS) and common squid meat (CSM). Pen shell (Atrina pectinata), a bivalve and common squid (Todarodes pacificus), a cephalopod, are two popular and commercially important species. Globally, these organisms are distributed from southeast Africa to Malaysia and New Zealand, the Indo-western Pacific region, Japan, and Korea [1,2]. However, South Korea and Japan are the largest markets for raw and processed products of these two species [3]. Khi-Jo-Gae and O-Jing-Eo are popular names for A. pectinate and T. pacificus in Korea. Due to their high nutritional value, these species are often found on the seafood menu. For instance, the AMPS and CSM, the protein-rich, highly nutritional products of pen shell and common squid, have high consumption demand.

As the quality of seafood HMR products continues to improve, it is believed that applying advanced technologies to handle raw material, cook, and freeze during the manufacturing process is likely to result in excellent quality products [4]. To this end, several technologies, including high-frequency defrosting (HFD), superheated steam cooking, and quick freezing techniques, are believed to enhance product quality. HFD, in which the amount of heat generated inside the product and the defrosting time are accurately controlled, can reduce thawing time, inhibit microbial activities, reduce drip loss, and maintain the quality of raw materials [5,6]. Furthermore, oxidation is minimized in superheated steaming due to the lack of oxygen during heating and roasting [7], rendering superheated steam cooking an effective method in the food industry. In addition, superheated steam roasting reduces energy consumption during the roasting process [8] and has been proven to reduce lipid oxidation and preserve food nutrient substances, color, and texture better than traditional cooking methods [7,9–11]. Quick freezing is a key technology for maintaining the quality and prolonging the shelf life of frozen food. This technology successfully inhibits changes in flavor, color, and texture due to oxidative, enzymatic, and microbial changes [12–14].

Though these techniques have been explored in several food products, their applications in seafood-based HMR products are limited. Therefore, we hypothesized that the development of new high-quality fresh product-equivalent seafood HMR products, especially those containing the seasoned AMPS and CSM, could be achieved by employing advanced techniques. Moreover, the shelf life of packaged seafood products is an important factor determining the consumer acceptability and saleability of the product. Therefore, it is essential to decipher the ideal conditions that could prolong the shelf life of HMR products while improving their quality.

In this study, we aimed to elucidate the best method to produce good quality, highly nutritious HMR products with an improved shelf life prepared by mixing roasted AMPS and roasted CSM. Here, we produced the seasoning-mixed AMPS and CSM as test HMR products using a high-frequency defroster, superheated steam, and quick freezing techniques and evaluated its quality characteristics of test HMR products. The findings could unravel the potential of improved technologies, which could be used in the seafood industry to produce high-quality HMR products with little loss of nutrition and texture.

2. Materials and Methods
2.1. Materials

All chemicals used in this study, including potassium carbonate, sulfuric acid, boric acid, sodium hydroxide, trichloroacetic acid, phosphoric acid, N-tert-butylidimethylsilyl-N-methyltrifluoroacetamide with 1% Butyldimethylchlorosilane (MTBSTFA with 1% t-BDMCS), methyl red solution, methylene blue solution, acetonitrile, 2-thiobarbituric acid, ethanol, and sodium bicarbonate, were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA). The Difco plate count agar and EC medium used for microbiological analysis were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA), whereas Sanita-Kun plates were obtained from JNC Corp. (Tokyo, Japan).
2.2. Experimental Sample

Frozen AMPS and CSM were obtained from EBADA Fishery Co. Ltd. (Busan, Korea). Sample weights were 46–60 g (average 53.7 g) for AMPS and 188–266 g (average 221.8 g) for CSM.

2.3. Drip Loss Analysis

Frozen AMPS and CSM were thawed under three different conditions: room temperature, running water, and HFD. For running water and room temperature thawing, samples were placed in plastic bags. For HFD thawing, the controller of the HFD machine (CHRFT-100, Chamco Co. Ltd., Busan, Korea) was set at 27 MHz for 10 min (with an input power of 11 kW). Drip loss was analyzed using the filter-paper wetness (FPW) method following Kauffman et al. [15]. Quantitative filter paper No. 2 (55 mm; Advantech, Tokyo, Japan) was weighed \(y\), placed on the samples during thawing, and then filter paper with absorbed fluid was weighed again \(x\). The weight difference of filter paper was expressed as the weight of the absorbed exudate. Drip loss was quantified as a percentage following this formula:

\[
\text{Drip loss (\%)} = \frac{x - y}{x} \times 100
\]

2.4. Roasting Treatment

Thawed AMPS and CSM were cut into approximately 3.5 × 0.5 cm and 1 × 5 cm pieces, respectively. The inedible part of the CSM was removed, and the samples were then washed with tap water. Next, all samples were roasted using a superheated steam in an Aero Steam Oven (DFC-560A-2R/L, Naomoto Co., Osaka, Japan) at different temperatures and roasting times. For AMPS, the temperatures used were 192, 200, 220, 240, and 248 °C for 0.53, 0.67, 1.00, 1.33, and 1.47 min, respectively, while for CSM, the temperatures used were 272, 280, 300, 320, and 328 °C for 0.79, 1.00, 1.50, 2.00, and 2.21 min, respectively.

2.5. Sample HMR Product Preparation

First, the stock of sauce was prepared by mixing the ingredients shown in Table 1. A test seafood HMR product was then produced by mixing roasted AMPS, roasted CSM, and sauce at ratios (w/w) of 27.5, 27.5, and 45.0%, respectively. Afterward, 180 g of product was packaged in a polypropylene plastic bowl (New Ecopack Co. Ltd., Jeonju, Korea) and sealed with a plastic film using a tray sealing machine (TPS-TS3T, TPS Co. Ltd., Kyungki-do, Korea) at 180 °C for 5 s. Next, packaged products were frozen using a quick freezer (QF-700, Alpha Tech Co. Ltd., Incheon, Korea) at −35 °C for 10 min, and then stored at −13, −18, and −23 °C in a deep freezer (DF35035, IlShin BioBase Co. Ltd., Dongducheon, Korea) for shelf life estimation.

Table 1. Ingredients used to prepare the sauce (stock), a key ingredient of the new home meal replacement (HMR) product.

| Ingredients                                      | (%)   |
|--------------------------------------------------|-------|
| Mixed soy sauce                                  | 24.69 |
| Food additive (D-sorbitol liquid)                | 49.19 |
| Fructose                                         | 9.27  |
| Caramel food coloring                            | Trace |
| Beverage base (lemon powder)                    | Trace |
| Fruit whine                                      | Trace |
| Starch                                           | Trace |
| Sodium L-glutamate                               | Trace |
| Chilli powder                                    | Trace |
| Food additive (ethyl p-hydroxybenzoate)         | Trace |
| Purified water                                   | Adequate amount (~16%) |
2.6. pH Measurement

Two grams of product were added to 3rd-distilled water at a ratio of 1:9 (w/v), and then homogenized using a homogenizer (SHG-15D, SciLab Co. Ltd., Seoul, Korea). Next, the pH of each sample was measured using a pH meter (ST 3100, Ohaus Co., Parsippany, NJ, USA).

2.7. Measurement of Volatile Basic Nitrogen (VBN)

VBN was performed using the Conway micro diffusion method. Five grams of product were diluted with 25 mL of 3rd-distilled water and homogenized using a vortex for 5 min. Filter paper No. 2 (55 mm; Advantech, Tokyo, Japan) was used to filter the mixture sample. Following filtration, potassium carbonate was added to the filtrate solution at a ratio of 1:1 (v/v) in the outer chamber, while 1 mL of 0.01 M H\textsubscript{2}SO\textsubscript{4} was added to the inner chamber of the Conway unit. Conway cells were incubated at 37 °C for 90 min. Brunswick reagent (2–3 drops) was added to the inner chamber and titrated with 0.01 N NaOH.

2.8. Measurement of Thiobarbituric Acid-Reactive Substances (TBARS)

TBARS were measured following the method by Peiretti et al. [16] with modifications. Briefly, 5 g of each sample was homogenized (SHG-15D, SciLab Co. Ltd., Seoul, Korea) in 12.5 mL of TCA solution containing 20% trichloroacetic acid in 2 M phosphoric acid and adjusted to 25 mL with 3rd-distilled water. Next, homogenate samples were centrifuged at 1500 rpm for 10 min, and the upper layer was collected and filtered using filter paper No. 2 (55 mm; Advantech, Tokyo, Japan). The supernatant was then mixed with a 0.005 M thiobarbituric acid solution at a ratio of 1:1 (v/v) and incubated at 95 °C for 30 min in a water bath (JSWB-22TL, JS Research Inc., Gongju City, Korea). The samples were then left to cool to room temperature. Samples (200 µL) and the blank group (3rd-distilled water) were placed on a 96-well plate and measured at 530 nm using a SPECTROstar Nano Microplate Reader (S/N:601-0618, BMG Labtech Ltd., Ortenberg, Germany). Malondialdehyde (MDA) bis (dimethyl acetal) was used as the standard.

2.9. Proximate Analysis

Proximate analysis was performed using AOAC standard methods. Moisture was measured following the methods AOAC 952.08 [17] using an oven at 105 °C for 24 h. Ash was determined following the methods AOAC 938.08 method [18] in a furnace at 550 °C. Sodium was measured following AOAC 971.27 method [19]. Crude protein was determined following AOAC 960.48 method [20]. N content from the test product was then multiplied with 6.26 to obtain the value of crude protein. Calories, carbohydrates, sugars, dietary fiber, crude fat content, cholesterol, vitamin D, potassium iron, and calcium were measured following AOAC 971.10 [21], AOAC 998.18 [22], AOAC 985.29 [23], AOAC 948.15 [24], AOAC 994.10 [25], AOAC 936.14 [26], AOAC 2011.11 [27], AOAC 990.05 [28], and AOAC 984.27 [29], respectively.

2.10. Amino Acid Analysis

Prior to hydrolysis, the protein was extracted following the Kjeldahl method, and the amino acid analysis was performed using the AOAC 994.12b method [30]. Briefly, a 50 µL aliquot of a solution containing a mixture of 91 µg/mL L-amino acids in 0.1 N HCl was dried. Subsequently, 100 µL of neat MTBSTFA, followed by 100 µL of acetonitrile, were added. The mixture was then heated to 100 °C for 4 h. Next, the sample was neutralized with sodium bicarbonate and subjected to gas chromatography-mass spectrometry (GC-MS) analysis using the GCMS-QP2020 system (Shimadzu Corp., Kyoto, Japan). An injection volume of 0.5 µL/min was used for the separations performed on an SLB™ 5ms Capillary GC Column (20 m × 0.18 mm I.D., 0.18 µm; Sigma-Aldrich, Inc., St. Louis, MO, USA) using helium as the carrier gas. During the separation, the oven temperature was programmed as follows—initially, it was set at 100 °C for 1 min, then increased to 290 °C at 35 °C/min and held for 3 min, and finally, it was raised to 360 °C at a rate of 40 °C/min and held for
2 min. The temperature for the inlet was set at 250 °C, while the temperature for the mass storage device (MSD) interface was set at 325 °C.

2.11. Fatty Acid Composition Analysis

The fatty acid composition analysis was performed following a hydrolytic method described by Sutikno et al. [11]. Briefly, ether was used to extract fatty acids and methylate them into fatty acid methyl esters (FAMEs). FAMEs were then analyzed using gas chromatography (GCMS-QP2020) fitted with a DB-wax capillary column (30 m × 0.25 mm i.d., 0.25 mm film thickness, Agilent). Helium at a constant linear velocity of 30 cm/s was used as the carrier gas. The split ratio was set at 1/10. During the separation, the column oven was programmed as follows: initial column oven temperature was set at 100 °C; held for 1 min, and increased to at 25 °C/min to 100 °C and after 1 min, and finally, it was raised to 240 °C at 5 °C/min; held for 2 min. The temperature for the inlet was set at 250 °C, while the temperature for Flame Ionization Detector (FID) was set at 270 °C. FAME standard mixture (EN 14078, Paragon Scientific Ltd., Wirral, UK) was used to identify the peak and calculate the response factor. The results were expressed as g/100 g of dry matter.

2.12. Total Bacterial Count (TBC) and Total Coliform Count

TBC was determined according to the instructions of Chen et al. [31]. The sample was mixed with sterile saline at a ratio of 1:9 (w/v) in sterilized bags, and homogenized using a Stomacher 400 Circulator (Seward Ltd., West Sussex, UK) for 3 min. Three serial dilutions of an aliquot of the homogenate were plated onto a specific medium. For TBC, Difco plate count agar (BD Co., Franklin Lakes, NJ, USA) was used and incubated at 37 ± 1 °C for 48 h. For the coliform count, EC medium (BD Co., Franklin Lakes, NJ, USA) was used and incubated at 37 ± 1 °C for 24 h; if no gas was observed, results were recorded as negative (−). For Salmonella sp. and Staphylococcus sp. counts, Sanita-Kun plates (JNC Corp., Tokyo, Japan) were used and incubated at 35 ± 1 °C for 48 h.

2.13. Texture Analysis

A texture analyzer (CT3 4500, Brookfield Engineering Laboratories Inc., Middleboro, MA, USA) operated by TexturePRO CT software (Middleboro, MA, USA) was used to measure hardness. The texture analysis was performed by compressing the sample to 50% of its height using a cylinder glass probe (12.7 mm in diameter and 35 mm in length). The test speed was 0.5 mm/s. The textural analysis was performed at room temperature with triplicate measurements of each sample.

2.14. Sensory Evaluation

Sensory analysis, including color, flavor, aroma, texture, and overall acceptability, were evaluated. Test seafood HMR was reheated using a microwave (RE-M50, Samsung Electronics Co. Ltd., Seoul, Korea) for 1 min 30 s (700 W). Twenty-one trained and certified sensory evaluation panelists (aged 25–40 years) were employed in this test—all of them were researchers at the Industry Academic Cooperation Foundation, Silla University (Busan, Korea). The sensory test was approved by the Silla University Institutional Review Board (Approval No. 1041449-202006-HR-007; 4 October 2020). Each panelist was asked to evaluate and numerically rate all samples. A hedonic scale of 1 to 9 points was used—1 indicating ‘remarkably dislike’ and 9 indicating ‘extreme like.’ Five was considered the threshold value; any sample less than a score of 5 was considered unacceptable [32].

2.15. Shelf Life Estimation

The expiration date was established following the Ministry of Food and Drug Safety guidelines, the Republic of Korea. The accelerated experiment to define the expiration date and evaluate the shelf life qualities of the test-HMR products was conducted for 90 days. Test HMRs were stored at −18 °C (distribution temperature) as a control, −13 °C, and −23 °C. Samples were collected seven times over 90 days, and microbiological and physical
tests were performed. The number of common bacteria, such as *E. coli*, *Staphylococcus aureus*, and *Salmonella* spp., and the sensory evaluation scores were used as indicators of quality shelf life. Program simulation (https://www.foodsafetykorea.go.kr, accessed on 17 December 2020) was used to estimate the product’s shelf life.

2.16. Statistical Analysis

All experiments were performed in triplicate (n = 3); the data are displayed as mean ± standard deviation (SD). Drip loss, TBC, and sensory properties were analyzed via a one-way analysis of variance (ANOVA) at a 95% level of probability (p < 0.05) using IBM SPSS v 23.0 (IBM, Corp., Armonk, NY, USA) software. Response surface methodology (RSM) was analyzed using Minitab v 14.0 (Minitab Inc., Birmingham, UK). Temperature and heating time were set as independent variables, while overall acceptance and hardness were set as dependent variables.

3. Results and Discussion

3.1. Drip Loss

Drip loss was analyzed via the exudate absorbed into the quantitative filter paper during the thawing process under three different conditions: room temperature, running water, and HFD. Table 2 shows the drip loss results of frozen AMPS and CSM. HFD resulted in the lowest drip loss value of both AMPS and CSM. The drip loss values of AMPS from the high-frequency defroster were significantly different from those thawed using the conventional methods (p < 0.05). The highest drip loss value was observed by thawing at room temperature, followed by running water.

Table 2. Drip loss analysis of frozen adductor muscle of the pen shell (AMPS) and common squid meat (CSM) under different conditions.

| Raw Materials | Drip Loss (%) |
|---------------|---------------|
|               | Room Temperature | Running Water | High-Frequency Defroster |
| AMPS          | 8.15 ± 0.07 a   | 7.32 ± 0.14 ab | 5.74 ± 0.39 c |
| CSM           | 4.65 ± 0.70 a   | 2.80 ± 0.60 ab | 2.20 ± 0.80 b |

Data are presented as mean ± SD. Different superscript letters (a–c) show significantly different values according to Duncan’s test (p < 0.05).

The thawing process for frozen fish and fishery products should be performed as quickly as possible. Water changing from its original position during the thawing process leads to drip loss, resulting in a dry, stringy, and less tasty fish. Nutrient losses, such as proteins, vitamins, and minerals, can occur with drip loss [33], with high drip loss often linked to protein denaturation. In addition, high drip loss also decreases attractiveness, nutritional value, texture, and appearance [34].

Applying technology during the thawing process may improve the quality of fishery products, also likely decreasing thawing time [35]. Furthermore, rapid thawing can maintain fish quality [36] and reduce mechanical damage to cell membranes by decreasing recrystallization [37]. Recrystallization, which can be problematic during both thawing and freezing, leads to cellular damage, increasing drip production [38].

Therefore, HFD is considered an effective option to decrease drip loss and maintain the quality of raw materials in the test HMR product. The results of the present study confirm the effectiveness of HDF compared to conventional methods.

3.2. Optimum Conditions for Roasting

The optimum conditions for roasting were analyzed using RSM. Temperature (X₁) and heating time (X₂) were set as independent variables, while overall acceptance (Y₁) and hardness (Y₂) were set as dependent variables. The model in this study was build using the actual value of independent variables. The model obtained for overall acceptance and
hardness of AMPS was $Y_1 = 7.8097 + 0.9016X_2 - 0.9525X_1^2 - 1.4762X_2^2$ with 92.1% of $R^2$, and $Y_2 = 471.5 + 63.588X_1 + 28.777X_2 + 79.759X_1^2 + 46.134X_2^2$ with 95% of $R^2$, respectively (Table 3). Three-dimensional response surface plots of AMPS showed an increase in the score of overall acceptance and decrease in hardness value with an increase in time and temperature until an optimum condition was attained (Figure 1a,b). The overall acceptance score started to decline beyond 217.8 °C and 1.08 min (Figure 1a), whereas the hardness value tended to increase by increasing the temperature and time beyond 217.8 °C and 1.08 min (Figure 1b). Therefore, the optimum temperature and time for the superheated steam roasting of AMPS were set at 217.8 °C for 1.08 min (Table 4).

Table 3. Response surface model equations of AMPS and CSM.

| Raw Materials | Responses          | Quadratic Polynomial Model Equations | $R^2$ |
|---------------|--------------------|-------------------------------------|-------|
| AMPS          | $Y_1$: Overall acceptance (score) | $7.8097 + 0.9016X_2 - 0.9525X_1^2 - 1.4762X_2^2$ | 0.921 |
|               | $Y_2$: Hardness (g)    | $471.5 + 63.588X_1 + 28.777X_2 + 79.759X_1^2 + 46.134X_2^2$ | 0.950 |
| CSM           | $Y_1$: Overall acceptance (score) | $8.07933 - 0.54590X_2 - 0.76879X_1^2 - 1.54254X_2^2$ | 0.916 |
|               | $Y_2$: Hardness (g)    | $508.67 + 63.44X_1 + 121.91X_2 - 49.13X_1^2 + 47.90X_2^2$ | 0.965 |

$Y_1$: overall acceptance, and $Y_2$: hardness are the dependent variables; $X_1$: Temperature (°C); $X_2$: heating time (min) are the independent variables.

Table 4. Optimal cooking conditions of AMPS and CSM using response surface methodology (RSM)-analyzed superheated steam roasting.

| Raw Material | $X_1$ Temp (°C) | $X_2$ Time (min) |
|--------------|----------------|-----------------|
| AMPS         |                |                 |
| Coded value  | $-0.1102$      | 0.2435          |
| Actual value | 217.80         | 1.08            |
| Predicted values | $Y_1$: 7.9086, $Y_2$: 421.1653 |       |
| Experimental values | $Y_1$: 7.83 ± 0.55, $Y_2$: 393.33 ± 87.32 |    |
| CSM          |                |                 |
| Coded value  | $-0.1964$      | 0.0514          |
| Actual value | 296.07         | 1.53            |
| Predicted values | $Y_1$: 8.00, $Y_2$: 500.00 |   |
| Experimental values | $Y_1$: 8.07 ± 0.15, $Y_2$: 465.33 ± 49.47 |    |

The CSM response surface model is displayed in Table 3. The model obtained for overall acceptance and hardness was $Y_1 = 8.07933 - 0.54590X_2 - 0.76879X_1^2 - 1.54254X_2^2$ with 91.6% of $R^2$, and $Y_2 = 508.67 + 63.44X_1 + 121.91X_2 - 49.13X_1^2 + 47.90X_2^2$ with 96.5% $R^2$, respectively. Three-dimensional response surface plots of CSM for overall score and hardness (Figure 1c,d) showed the same pattern as those for AMPS. The overall acceptance score increased with the increasing time and temperature until the optimum condition was attained, while a reverse trend was observed for hardness. Increasing the temperature and time beyond 296.07 °C and 1.53 min decreased the overall acceptance scores (Figure 1c), while a temperature and time beyond 296.07 °C and 1.53 min increased the hardness (Figure 1d). The optimum temperature and time for CSM superheated steam roasting was 296.07 °C for 1.53 min (Table 4).
The optimum temperature and time of AMPS and CSM were 217.8 °C for 1.08 min and 296.1 °C for 1.53 min, respectively. These values were in the range −0.1964 to 0.2435 (Table 4). Moreover, central composite design results (Table 5) showed that the optimum practical temperature and time values for superheated steam roasting was 220 °C for 1 min for AMPS and 300 °C for 1.5 min for CSM. In this study, the roasting time for AMPS and CSM was faster than that reported by Mohibullah et al. [10] and Sutikno et al. [11]. According to Mohibullah et al. [10], the superheated steam roasting time of AMPS was 4 min at 270 °C to achieve a good panelist-determined sensory evaluation score, which included color, odor, texture, flavor, and overall enjoyment. Moreover, Sutikno et al. [11] proposed the optimum superheated steam roasting time for squid meat was 10 min at 240 °C, as evaluated by panelists based on sensory characteristics.

Table 5. Symbol, experimental range and values of the independent variables in the central composite design.

| Raw Materials | Independent | Symbol | Range Level |
|---------------|-------------|--------|-------------|
| AMPS          | Temperature (°C) | X₁     | 192 200 220 240 248 |
|               | Time (min)    | X₂     | 0.53 0.67 1.00 1.33 1.47 |
| CSM           | Temperature (°C) | X₁     | 272 280 300 320 328 |
|               | Time (min)    | X₂     | 0.79 1.00 1.50 2.00 2.21 |
The optimum conditions for roasting both AMPS and CSM indicated the best combination of independent and dependent variables. Furthermore, RSM has been effectively used to improve food products [39,40]. Here, we showed RSM successfully optimized superheated steam conditions for efficient production of AMPS and CSM. Moreover, applying RSM to AMPS and CSM roasting resulted in high sensory evaluation values. Taken together, these results indicate the potential of RSM to optimize or choose operating conditions to achieve a particular set of objectives.

3.3. Chemical Characteristics

A test HMR product was prepared by thawing frozen AMPS and CSM using HFD, followed by superheated steam roasting at a particular value for both AMPS and CSM. The test product consisted of 27.5% AMPS, 27.5% CSM, and 45% sauce. Chemical properties, such as pH, VBN, and TBARS, were used to evaluate the quality of the product (Table 6). The pH, VBN, and TBARS of the test HMR were 6.30 ± 0.13, 9.45 ± 1.09 mg%, and 1.13 ± 0.27 mg MDA/kg, respectively. Consistent with the results of Sutikno et al. [11] and Mohibullah et al. [10], who reported near-neutral pH in seafood products (squid meat and AMPS) following superheated steam roasting, the pH of the test HMR was close to neutral (7). Moreover, for fresh fish and fishery products, an ideal pH level is almost neutral. However, post-mortem period-associated nitrogenous compounds increase the pH, leading to decreased quality [41].

Table 6. Chemical characteristics of test seafood HMR products consisting of a mixture of AMPS, CSM, and sauce.

| Sample          | pH     | VBN (mg%) | TBARS (mg MDA/kg) |
|-----------------|--------|-----------|-------------------|
| HMR Products    | 6.30 ± 0.13 | 9.45 ± 1.09 | 1.13 ± 0.27       |

Data are presented as mean ± SD.

A product’s VBN value indicates the amount of nitrogen derived from proteolytic bacteria and endogenous enzymes [29], with the VBN test examining the level of protein breakdown and the presence of non-protein nitrogenous compounds. Furthermore, unpleasant smells generally correlate with high VBN values. Superheated steam roasting has been observed to lead to low VBN values. Similar results were reported by Sutikno et al. [11], who found that the VBN of roasted squid meat was lower when using superheated steam roasting compared to other methods. Furthermore, Mohibullah et al. [10] also reported a lower VBN for superheated steam roasted AMPS.

The low TBARS value of a product labels it as a perfect product according to Yildiz [41], who categorized seafood products into the following three TBARS value-based groups: perfect product (less than 3 mg MDA/kg), good product (3–5 mg MDA/kg), and consumable limit (7–8 mg MDA/kg). Using superheated steam roasting resulted in a low TBARS in squid meat [11] and AMPS [10]. This could be attributed to the reduced oxygen availability during this process resulting in higher inhibition of lipid oxidation and decreased product peroxide (POV) and TBARS values [42].

Collectively, the results of chemical characteristics of the test HMR indicated the quality of the product. According to VBN and TBARS values, test seafood HMR products can be classified as very good.

3.4. Nutritional Quality

Nutritional quality results are shown in Table 7. Proximate composition results, including moisture, crude protein, and ash, were 57.21, 11.6, and 1.62 g/100 g, respectively. Furthermore, mineral contents, such as sodium, calcium, and potassium, were 1.04, 0.19, and 0.001 g/100 g, respectively. Other parameters of nutritional quality, including calories, salt, carbohydrates, sugars, and crude fat, were 167.2 kcal/100 g, 1.03 g/100 g, 28.4 g/100 g, 14.9 g/100 g, and 0.8 g/100 g, respectively.
Table 7. Nutritional values of test seafood HMR products consisting of a mixture of AMPS, CSM, and sauce.

| Chemical Composition | Unit             | Contain |
|----------------------|------------------|---------|
| Ash                  | g/100 g          | 1.62    |
| Calcium              | g/100 g          | 0.19    |
| Calories             | kcal/100 g       | 167.2   |
| Carbohydrate         | g/100 g          | 28.4    |
| Cholesterol          | g/100 g          | 0.63    |
| Crude fat            | g/100 g          | 0.8     |
| Crude protein        | g/100 g          | 11.60   |
| Moisture             | g/100 g          | 57.21   |
| Potassium            | g/100 g          | 0.001   |
| Salt                 | g/100 g          | 1.03    |
| Saturated fat        | g/100 g          | 0.3     |
| Sodium               | g/100 g          | 1.04    |
| Sugar                | g/100 g          | 14.9    |
| Trans fat            | g/100 g          | 0.0     |
| Vitamin D            | µg/100 g         | 0.0     |

The nutritional quality of test HMR products indicated that they contained essential nutrition for humans. Wholesome food consists of macronutrients, such as protein, carbohydrates, and fat, thereby offering calories to fuel the body and energy for specific health-maintaining roles. The human body requires nutrition from food, including proteins, carbohydrates, minerals, and crude fat, to participate in daily activities and maintain good health [43]. This study suggests that the product has the essential nutrients required for healthy human body functioning, also contributing to daily nutrition needs.

3.5. Amino Acid Composition

The amino acid composition of the HMR product, consisting of mixed AMPS, CSM, and sauce, is presented in Table 8. The total amino acid content was 7.93 g/100 g, with glutamine as the predominant amino acid. Glutamine and glutamate are abundant amino acids in fish and fishery products [44,45]. In the human body, glutamine is an essential source of energy for the immune system [46].

Non-essential amino acids were dominant (53.84%) compared with essential amino acids (46.16%). Glutamine, aspartate, and alanine were the most abundant non-essential amino acids found, whereas lysine and arginine were the predominant essential amino acids (both at 8.20%). Both essential and non-essential amino acids benefit human health, such as lowering the risk of cardiovascular disease and enhancing the immune system [47]. Furthermore, following digestion, amino acids provide various benefits to human health, including repairing tissue, supporting growth, breaking down food, and providing energy [48].

This study indicates that test seafood HMR products contain amino acids essential for the human body. In addition, the product’s amino acid content contributes to the overall daily amino acid requirement.
Table 8. Amino acid analysis of test seafood HMR products consisting of a mixture of AMPS, CSM, and sauce.

| Amino Acid | g/100 g | %  |
|------------|---------|----|
| Alanine    | 0.52    | 6.56 |
| Aspartate  | 0.81    | 10.21 |
| Cysteine   | 0.11    | 1.39 |
| Glutamine  | 1.47    | 18.54 |
| Glycine    | 0.45    | 5.67 |
| Proline    | 0.33    | 4.16 |
| Serine     | 0.38    | 4.79 |
| Tyrosine   | 0.20    | 2.52 |
| Total NE   | 4.27    | 53.84 |
| Arginine   | 0.65    | 8.20 |
| Histidine  | 0.15    | 1.89 |
| Isoleucine | 0.31    | 3.91 |
| Leucine    | 0.63    | 7.94 |
| Lysine     | 0.65    | 8.20 |
| Methionine | 0.19    | 2.40 |
| Phenylalanine | 0.32  | 4.04 |
| Threonine  | 0.36    | 4.54 |
| Tryptophan | 0.07    | 0.88 |
| Valine     | 0.33    | 4.16 |
| Total E    | 3.66    | 46.16 |
| Total amino acid | 7.93 | 100 |

NE: Non-essential; E: Essential.

3.6. Fatty Acid Composition

Fatty acid composition results are presented in Table 9. Total saturated fatty acids (SFAs) showed the highest value (0.71 g/100 g), followed by polyunsaturated fatty acids (PUFAs) (0.26 g/100 g), and total monounsaturated fatty acids (MUFAs) (0.18 g/100 g). The three predominant fatty acids found in the sample were palmitic acid, docosahexaenoic acid (DHA), and oleic acid. Due to their nutritional value, fatty acids have important health benefits. Palmitic acid, the most common fatty acid in meat, fish, and fishery products, accounted for approximately 50–60% of total fats [49]. Furthermore, DHA is a known agent in treating primary and secondary heart disease and neurological and neuropsychiatric disorders [50]. The Food and Agriculture Organization (FAO) of the United Nations and the World Health Organization (WHO) have given special consideration to fatty acids in food as essential nutrients, and their impact on early growth, development, and nutrition-related chronic diseases in humans.

In processed seafood products, amino acids contribute to the taste of the product [51]. A sweet taste is caused by glycine, alanine, and trimethyl, whereas a bitter taste results from arginine [52]. The fatty acid content in the test seafood HMR product indicated its good nutritional value, thereby suggesting its potential to contribute to daily fatty acid requirements.
Table 9. Fatty acid contents of test seafood HMR products consisting of a mixture of AMPS, CSM, and sauce.

| Fatty Acids               | C   | (g/100 g) |
|---------------------------|-----|-----------|
| Lauric acid C12: 0        |     | 0.03      |
| Myristic acid C14: 0      |     | 0.05      |
| Pentadecanoic acid C15: 0|     | 0.01      |
| Palmitic acid C16: 0      |     | 0.52      |
| Palmitoleic acid C16: 1   |     | 0.00      |
| Heptadecanoic acid C17: 0|     | 0.01      |
| Stearic acid C18: 0       |     | 0.09      |
| Tricosanoic acid C23: 0   |     | 0.00      |
| **Σ SFA**                 |     | 0.71      |
| Oleic acid C18: 1 n−9c    |     | 0.10      |
| cis-11-Eicosenoic acid C20: 1 n−9 | | 0.03 |
| Erucic acid C22: 1 n−9     |     | 0.01      |
| Nervonic acid C24: 1 n−9   |     | 0.04      |
| **ΣMUFA**                 |     | 0.18      |
| Linolelaidic acid C18: 2 n−6t | | 0.00 |
| Linoleic acid C18: 2 n−6c  |     | 0.08      |
| cis-11,14-Eicosadienoic acid C20: 2 n−6 | | 0.00 |
| Arachidonic acid C20: 4 n−6 | | 0.01 |
| **Σ n−6**                 |     | 0.09      |
| cis-11,14,17-Eicosatrienoic acid C20: 3 n−3 | | 0.00 |
| Eicosapentaenoic acid C20: 5 n−3 | | 0.03 |
| Docosahexaenoic acid C22: 6 n−3 | | 0.14 |
| **Σ n−3**                 |     | 0.17      |
| **ΣPUFA**                 |     | 0.26      |
| n−3/n−6                   |     | 1.89      |

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

3.7. Effect of Storage Duration on Microbiological Changes

Microbiological changes in food can indicate product safety. Table 10 shows the microbial change during storage duration. The score of TBC increased in conjunction with the storage time. At −13 °C, bacterial growth increased significantly \((p < 0.05)\) on day 90 \((3.20 \pm 0.00)\), while at −18 °C, bacterial growth increased significantly \((p < 0.05)\) after days 75 and 90 compared to day 0. However, at −23 °C, bacterial growth was maintained throughout the 90-day storage period. The optimum temperature in which to store products based on the TBC was less than −23 °C. In this study, the TBC of the product was less than 5 log CFU/g, which is acceptable for consumption (International Commission of Microbiological Specification for Food [ICMSF]) \([53]\). Overall, the findings suggest that low TBC values are influenced by the storage temperature—low temperatures, especially freezing, can decrease microbial activity.

The results of this study align with those of Alizadeh et al. \([37]\), who reported that microbial and enzymatic activities successfully decrease in salmon fillets stored at freezing conditions, therefore maintaining a better nutritional content than those in chilled storage. Furthermore, Pohoro and Ranghoo-Sannukhiya \([54]\) reported that the TBC of frozen fish sold in Mauritius was lower during storage. Similar results were reported by Gornik et al. \([55]\), who reported that low temperatures during storage result in low TBC values in lobster (Nephrops norvegicus).
Table 10. TBC and microbiology of test seafood HMR products, consisting of a mixture of AMPS, CSM, and sauce, stored at different temperatures.

| Temperature | Day | TBC (Log CFU/g) | S. aureus | Salmonella spp. | E. coli |
|-------------|-----|----------------|----------|-----------------|--------|
| −13 °C      | 0   | 2.94 ± 0.10 ab | -        | -               | -      |
|             | 15  | 2.85 ± 0.12 a  | -        | -               | -      |
|             | 30  | 2.94 ± 0.02 ab | -        | -               | -      |
|             | 45  | 2.99 ± 0.21 ab | -        | -               | -      |
|             | 60  | 2.98 ± 0.03 ab | -        | -               | -      |
|             | 75  | 3.11 ± 0.04 bc | -        | -               | -      |
|             | 90  | 3.20 ± 0.00 c  | -        | -               | -      |
| −18 °C      | 0   | 2.94 ± 0.10 ab | -        | -               | -      |
|             | 15  | 2.86 ± 0.05 a  | -        | -               | -      |
|             | 30  | 2.94 ± 0.04 ab | -        | -               | -      |
|             | 45  | 2.98 ± 0.03 b  | -        | -               | -      |
|             | 60  | 2.93 ± 0.03 ab | -        | -               | -      |
|             | 75  | 3.07 ± 0.04 c  | -        | -               | -      |
|             | 90  | 3.11 ± 0.04 c  | -        | -               | -      |
| −23 °C      | 0   | 2.94 ± 0.10 a  | -        | -               | -      |
|             | 15  | 2.90 ± 0.03 a  | -        | -               | -      |
|             | 30  | 2.95 ± 0.03 a  | -        | -               | -      |
|             | 45  | 2.94 ± 0.05 a  | -        | -               | -      |
|             | 60  | 2.93 ± 0.07 a  | -        | -               | -      |
|             | 75  | 2.96 ± 0.04 a  | -        | -               | -      |
|             | 90  | 2.97 ± 0.03 a  | -        | -               | -      |

TBC: total bacteria count; Data are presented as mean ± SD. Means in each column at each temperature group with different letters (a–c) differed significantly according to Duncan’s test (p < 0.05).

In this study, pathogenic bacteria, such as S. aureus, Salmonella spp., and E. coli, were not detected during storage at all temperatures. These results suggest that temperature plays an important role in the growth of pathogenic bacteria in fishery products. At low temperatures, microbial activity is suppressed, and most bacteria cannot grow [56,57]; hence, the growth of many spoilage-causing bacteria is prevented via temperature reduction.

3.8. Sensory Evaluation of the Product

Five sensory parameters, including color, flavor, odor, texture, and overall acceptance, were used to evaluate changes in sensory properties following storage and reheating using a microwave. On day 0, sensory evaluation scores of the test HMR products ranged from 8.24 to 8.38, with the highest value for flavor and odor. However, during storage, the scores were decreased. When stored at −13 °C and −18 °C, the sensory evaluation score started to decrease significantly (p < 0.05) on day 45 and day 90, respectively, compared to day 0. In contrast, storage at −23 °C did not significantly alter the sensory evaluation scores throughout the 90-day storage period. Sensory evaluation scores ranged from 7.14 to 8.62 (Table 11). According to the hedonic scale, the test HMR was scored by a panelist from “like moderately” to “like very much.” These results indicate that these products maintained favorable sensory properties following storage and reheating, likely due to rapid freezing. The quality deterioration of frozen food may be promoted by three factors: ice crystal formation, dehydration of protein molecules, and an increase in solid concentration during the freezing process [58].
Table 11. Sensory evaluation of test seafood HMR products, consisting of a mixture of AMPS, CSM, and sauce, stored at different temperatures.

| Temperature | Day | Color         | Flavor       | Odor          | Texture       | Overall Acceptance |
|-------------|-----|---------------|--------------|---------------|---------------|--------------------|
| −13 °C      | 0   | 8.33 ± 0.47 a | 8.38 ± 0.49 a| 8.38 ± 0.49 a | 8.24 ± 0.43 a | 8.33 ± 0.47 a      |
|             | 15  | 7.86 ± 0.71 b | 7.95 ± 0.72 b| 8.14 ± 0.56 b | 8.05 ± 0.65 ab | 7.89 ± 0.77 b      |
|             | 30  | 8.00 ± 0.69 ab| 7.81 ± 0.66 b| 7.95 ± 0.65 b | 7.86 ± 0.71 b | 7.88 ± 0.69 b      |
|             | 45  | 7.48 ± 0.66 c | 7.14 ± 0.47 c| 7.48 ± 0.59 c | 7.43 ± 0.58 c | 7.33 ± 0.56 c      |
|             | 60  | 7.38 ± 0.49 c | 7.29 ± 0.43 c| 7.52 ± 0.50 c | 7.24 ± 0.43 c | 7.33 ± 0.45 c      |
|             | 75  | 7.29 ± 0.45 c | 7.05 ± 0.21 c| 7.19 ± 0.39 c | 7.24 ± 0.43 c | 7.19 ± 0.33 c      |
|             | 90  | 7.14 ± 0.35 c | 7.21 ± 0.50 c| 7.33 ± 0.47 c | 7.19 ± 0.39 c | 7.18 ± 0.32 c      |
| −18 °C      | 0   | 8.33 ± 0.47 a | 8.38 ± 0.49 a| 8.38 ± 0.49 a | 8.24 ± 0.43 a | 8.33 ± 0.47 a      |
|             | 15  | 8.10 ± 0.29 ab| 8.38 ± 0.49 a| 8.29 ± 0.45 a | 7.95 ± 0.21 ab | 8.19 ± 0.29 ab     |
|             | 30  | 8.29 ± 0.82 ab| 8.14 ± 0.77 ab| 8.38 ± 0.72 a | 8.24 ± 0.81 a | 8.26 ± 0.81 a      |
|             | 45  | 7.90 ± 0.68 bc| 7.76 ± 0.61 bc| 8.00 ± 0.76 a | 7.81 ± 0.66 b | 7.86 ± 0.71 bc     |
|             | 60  | 8.10 ± 0.29 abc| 7.90 ± 0.29 bc| 8.17 ± 0.32 a | 8.00 ± 0.00 ab | 8.07 ± 0.14 abc    |
|             | 75  | 8.26 ± 0.61 ab| 8.12 ± 0.53 ab| 8.36 ± 0.64 a | 8.07 ± 0.49 ab | 8.19 ± 0.45 ab     |
|             | 90  | 7.76 ± 0.61 c | 7.57 ± 0.79 c | 7.95 ± 0.79 a | 7.76 ± 0.68 b | 7.76 ± 0.63 c      |
| −23 °C      | 0   | 8.33 ± 0.47 a | 8.38 ± 0.49 ab| 8.38 ± 0.49 a | 8.24 ± 0.43 a | 8.33 ± 0.47 a      |
|             | 15  | 8.48 ± 0.50 ab| 8.48 ± 0.50 a | 8.62 ± 0.49 a | 8.48 ± 0.50 a | 8.57 ± 0.47 a      |
|             | 30  | 8.38 ± 0.84 a | 8.00 ± 0.87 b | 8.24 ± 0.97 a | 8.19 ± 1.05 a | 8.33 ± 0.99 a      |
|             | 45  | 8.48 ± 0.66 a | 8.38 ± 0.65 ab| 8.62 ± 0.65 a | 8.24 ± 0.53 a | 8.48 ± 0.61 a      |
|             | 60  | 8.40 ± 0.48 a | 8.26 ± 0.43 ab| 8.45 ± 0.49 a | 8.38 ± 0.49 a | 8.43 ± 0.47 a      |
|             | 75  | 8.24 ± 0.53 a | 8.19 ± 0.59 ab| 8.43 ± 0.58 a | 8.19 ± 0.59 a | 8.31 ± 0.47 a      |
|             | 90  | 8.19 ± 0.39 a | 8.00 ± 0.44 b | 8.38 ± 0.49 a | 8.10 ± 0.29 a | 8.17 ± 0.24 a      |

Data are presented as mean ± SD. Means in each column at each temperature group with different letters (a–c) differed significantly according to Duncan’s test (p < 0.05).

Ice crystal formation during freezing alters the physical properties of muscle tissues, thus distorting the structure of the meat [12,59–61]. Furthermore, ice crystal formation in frozen seafood reportedly decreases the sensory properties of the products [62,63]. Interestingly, the size of ice crystals is dependent on the freezing rate. Quick freezing leads to a greater number of fine ice crystal formations within muscle cells, whereas slow freezing leads to large ice crystal formations outside of muscle cells [12,37,64]. As such, the sensory properties of the products were largely preserved after reheating. Therefore, applying quick freezing to test seafood HMR products successfully maintained their sensory properties during storage or after reheating using microwaves.

3.9. Shelf Life Estimation

Changes in sensory and microbial parameters indicate product quality, potentially affecting the shelf life of fish products [65]. On day 0, the sensory characteristics score was above 8.24 for all parameters. During storage at −13 and −20 °C, the product began to lose its sensory characteristics; however, panelists still scored products with decreased sensory characteristics above 7. The product stored at −23 °C showed no significant (p < 0.05) differences throughout the 90-day storage period. At −23 °C, test seafood HMR products maintained their sensory characteristics, obtaining a score above 8 from the panelists.

The physicochemical properties of fish and fishery products correlate with their sensory properties and storage time [66]. Moreover, the microbial activity gives flavors and odors to products during the storage period [67], and the decreases in sensory properties could be caused by changes in microbial parameters (Table 10). The TBC of the HMR product at −18 and −13 °C significantly (p < 0.05) increased during storage time; however, at −23 °C, there was no significant change (p < 0.05). These results suggest that lower temperatures decrease microbial activity in the product, thereby increasing shelf life.
Moreover, the microbial quality of seafood can be classified into the following three TBC value-based groups: satisfactory (TBC < 5 log CFU/g), acceptable (≥5 TBC < 6 log CFU/g), and unsatisfactory (TBC ≥ 6 log CFU/g) [68]. Therefore, based on the TBC results of this study, the test seafood HMR product could be classified as satisfactory.

The test seafood HMR product’s expiry date and overall acceptance values were established using TBC and the simulator program. It identified the shelf life of test seafood HMR products was 29.62 months when the sensory evaluation, as the main criteria, had the highest statistical value. However, the final shelf life was set to 24 months by multiplying it with the safety factor (0.82), which considers temperature changes during production, purchase, storage, and consumption by the consumer; hence, the quality of HMR products could be maintained for 24 months. The shelf life of fishery products is influenced by temperature and limited by biochemical and microbiological changes. Low temperatures may reduce microbial activity, enzymatic autolysis, and oxidation in fishery products, thereby prolonging shelf life [69–72]. Furthermore, although frozen storage shelf life has constraints, it may vary from a few weeks to years [73].

4. Conclusions

This study revealed that the technological improvements produced high-quality test HMR products by mixing AMPS and CSM. HFD maintained the nutritional quality of the raw materials with little drip loss. Optimization of superheated steam roasting using RSM successfully roasted AMPS and CSM at the optimum temperature and time, resulting in good overall acceptance and hardness. Moreover, quick freezing prevented nutrition and texture loss during storage and after reheating. Overall, the application of HFD, superheated steam, and quick freezing successfully produced test HMR products with high nutrition, good texture, and long shelf life, suggesting the potential of the methods reported here for use in the seafood industry to produce new HMR products.

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