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# Application of Plackett–Burman Design Analysis for Optimization of the Physico-chemical Properties of Multispecies Surimi

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## ABSTRACT

The application of Plackett–Burman design in investigating the effect of physico-chemical attributes of multispecies surimi was performed. The surimi was made from five species, added with four types of cryoprotectant and two different gelling agents, thus constructing 11 processing variables. Surimi was observed for the physico-chemical parameters including moisture, salt-soluble protein, pH, hardness, whiteness degree, and water-holding capacity and sensory evaluation. The best formulation was obtained from consisting of threadfin bream, croaker, lattice monocle bream, tilapia, sorbitol, sucrose, sodium tripolyphosphate, k-carrageenan, sodium lactate, and konjac with 15 min stirring period.

## KEYWORDS

Surimi; multispecies; physico-chemical properties; Plackett–Burman design

## Introduction

Surimi is an intermediate product made from minced fish that possesses good gel-forming ability. As a transitional product, surimi can be processed into various value-added products such as chikuwa, kamaboko, sausages, crabmeat imitation, etc. (Cando et al. 2017). Basically, surimi is a myofibril protein concentrate of the minced fish which is processed by washing the minced fish in cold water for several times to remove blood, fat, and sarcoplasmic protein; mixing the washed meat with cryoprotectant; and keeping the preparation in frozen product (Walayat et al. 2020). In manufacturing, the source material used for producing surimi is generally from less economical or underutilized fish, which is typically not only available in abundance but also has a white meat, low-fat content, and good gelling ability (Watabe et al. 2020).

In Southeast Asia (Thailand, Vietnam, Malaysia, and Indonesia), the raw materials used for surimi processing industries are typically from tropical demersal fish species which is considered as by-catch, such as threadfin bream (*Nemipterus* sp) (68%), big eye snapper (*Priacanthus tayenus*), croaker (*Pseudociena amoyensis*), and lizardfish (Sauridia spp), goatfish (*Openeus tragula*), as well as other fish species such as conger (*Congresox talaban*), catfish (*Pangasius hypophthalmus*), and yellow tail snapper (*Caeso* sp) (Yingchutrakul et al. 2022). However, the implementation of strict fishing regulations in Indonesia and Thailand nowadays has decreased the number of fish catches drastically, limiting the supply of surimi raw materials (Djunarsjah et al. 2021). The significant reduction of raw material supply not only leads to the closure of several surimi processing plants in Indonesia but also the decrease in surimi production in several countries including China, Vietnam, Thailand, and Malaysia. It is reported that surimi production in Thailand and China fell from 75.000 to 55.000 MT and 225.000–160.000 MT during the period of 2014–2018, respectively, while surimi production in Vietnam and Malaysia fell by 5.000 MT in 2019 (Guenneugues and Park 2020). The reduction of raw

material availability can be overcome by varying the composition of surimi, regarded as multi-species surimi technology. It is reported that surimi processed using multi-species fish possesses higher gel strength and functional properties (Santoso et al. 2011). Besides fish catches, the composition of multi-species surimi can also be substituted from cultivated fish, utilising the increased production of global Asian carp for more than 18.5 million tons (Yingchutrakul et al. 2022). In China, multi-species surimi has been processed from silver carp, sea bream, and mixed with ribbonfish (Guenneugues and Park 2020), while in Vietnam, it is produced from thread bream, red snapper, and other fish with a lower gel such as goatfish, croaker, and hairtail (Anon 2017).

The presence of cryoprotectant in the processing of multi-species surimi is essential. Cryoprotectants have been found to be effective in protecting the physical, functional, and structural properties of myofibrillar proteins and preserving the gel-forming property during frozen storage of surimi (Walayat et al. 2020). Cryoprotectant from low molecular weight carbohydrates such as sucrose, sorbitol, polydextrose, lactitol, maltodextrin, litesse, sodium lactate, trehalose, and phosphates are among the most studied cryoprotectants used to enhance the gelling characteristics and the storage of the surimi (Fahrizal et al. 2018). A mixture of sorbitol and sucrose results in a stronger cryoprotective effect of myofibrillar protein than does sorbitol or sucrose alone (Yoo 2014). Another cryoprotectant widely used to improve the gelling properties of surimi is calcium lactate. It can increase the protein–protein interactions via the formation of a salt-bridge between negatively charged myofibrillar proteins. Addition of 1.5% calcium lactate significantly increased gel strength and whiteness of surimi, while its cooking loss decreased (Sang et al. 2022).

The addition of 0.3% sodium tripolyphosphate (STTP) improved the physical properties of surimi, where a significant increase in texture, sensory, and microstructure profiles of surimi gel were observed (Laksono et al. 2019). Other gelling agent, i.e konjac, when added at a level of 0.5%–2%, can improve the physicochemical properties of myofibrillar protein and surimi gel, inhibit protein denaturation, and reduce the decrease in gel strength (Liang et al. 2017; Santana et al. 2013). The use of 2% refined carrageenan in surimi can improve surimi's water-holding capacity and gel strength but decrease the whiteness degree of surimi. In addition, the carrageenan gives a finer and denser network structure (Astutiek et al. 2020; Chen et al. 2020). Different stirring duration also affects the characteristics of surimi, in which prolonged stirring period induces changes in the functional properties of protein such as gelling ability (Ducept et al. 2012).

As discussed above, it is clear that many processing factors are indicated to influence the physicochemical properties of surimi. To identify the most important factors early in the experimentation phase when complete knowledge about the system is usually unavailable, the Plackett–Burman experimental design is widely applied (Anand et al. 2018). The method can provide important information about the level of significance of each variable on the analysis parameters through a small number of experiments, thus saving time, equipment, chemicals, and manpower (Quinlan and Lin 2015; Syamdidi and Suryaningrum 2015). This approach is popular since it is not only a simple, rapid, and useful tool for screening and searching for variables that had a significant effect in a multivariable system but also statistically reliable (Nguyen et al. 2020). This study aims to determine the factors that affect the quality of the multispecies surimi by employing a screening process using the Plackett–Burman design method. The variables used in the experiment were fish species, cryoprotectant types, gelling agents, and stirring times that determine the physicochemical properties of the multi-species surimi.

## Materials and methods

### Materials

Surimi was made from marine and freshwater fish. The marine fish were threadfin bream (*Nemipterus* sp.), croaker (*Argyrosomus japonicus*), and lattice monocle – bream (*Scolopsis taeniopterus*), which were purchased from a fish landing site in Belanakan, West Java, Indonesia. Meanwhile, the main source of freshwater fish was tilapia (*Oreochromis*

*mossambicus*), which was obtained from a freshwater fish landing place in Subang, West Java, Indonesia. As cryoprotectants, sucrose, sorbitol, sodium tripolyphosphate (STPP), and sodium lactate were supplied by CV Setia Makmur, Jakarta, Indonesia. While *k-carrageenan* and konjac were employed as gelling agents, purchased from Setia Guna Chemicals, Bogor, Indonesia.

## Methods

### Preparation of surimi

To produce surimi, fish was firstly head cut and eviscerated, then passed through a meat bone separator machine to obtain minced fish. The minced fish was washed three times with 5°C cold water at a ratio (fish:water = 1:4). NaCl was added at approximately 0.5% (w/v) in the final washing and then water was removed using a dehydrator. Surimi was then molded and kept in cold storage until further analysis.

### Screening using Plackett–Burman design

A Plackett–Burman *design* was employed to select variables using minimum and maximum values, which assumes that the value range adopted for each variable still produces good quality of surimi. Eleven factors at two levels (minimum and maximum values) were applied for the preliminary screening of the main effects, as shown in Table 1.

The minimum and maximum values of each variable were selected based on previous experiments and literature. Combination of surimi based on type of fish, type of cryoprotectant, presence of hydrocolloids, and time of stirring resulted in 14 formulations as shown in Table 2.

The main effect was calculated as the difference between the average measurements of each variable at high level (+1) and at low level (–1). This design-screened variables based on a first-order model:  $Y | X = A_0 + A_1 X_1 + A_2 X_2 + A_3 X_3 + A_4 X_4 + \dots + A_n X_n$  (1), where  $Y$  is the response to surimi quality,  $A_0$  is the constant,  $A_1, A_2, A_3, A_4, \dots, A_n$  is the response coefficient, and  $X_1, X_2, X_3, X_4, \dots, X_n$  denotes the effect of a variable with a value between –1 and +1 (Kuchekar and Pawar 2014; Sahu and Jain 2017).

## Experimental parameters

Analysis of the experimental parameters was conducted on the raw materials and surimi. The raw material was analyzed for its proximate composition, pH, and total volatile base (TVB) content (AOAC 2005).

**Table 1.** The minimum and maximum limits of surimi processing variables used in the Plackett–Burman design method.

| Independent variables   | Minimum value | Maximum value | Unit |
|-------------------------|---------------|---------------|------|
| Threadfin Bream         | 300           | 600           | g    |
| Croaker                 | 300           | 600           | g    |
| Lattice monacle bream   | 300           | 600           | g    |
| Tilapia                 | 300           | 600           | g    |
| Sorbitol                | 6             | 36            | g    |
| Sucrose                 | 6             | 36            | g    |
| Sodium tripolyphosphate | 0.3           | 3             | g    |
| Konjac                  | 3             | 30            | g    |
| <i>k-carrageenan</i>    | 3             | 30            | g    |
| Sodium lactate          | 18            | 60            | g    |
| Stirring period         | 5             | 15            | Min  |

**Table 2.** The formulation of surimi using various variables based on the Plackett–Burman design method.

| STAD ORDER | TDF (g) | CRF (g) | LMBF (g) | TLPF (g) | SORB (g) | SUCC (g) | STTP (g) | CARR (g) | SOD LACT (g) | KONJ (g) | STIR TIME (min) |
|------------|---------|---------|----------|----------|----------|----------|----------|----------|--------------|----------|-----------------|
| 1          | 600     | 300     | 600      | 300      | 6        | 6        | 3        | 30       | 60           | 3015     |                 |
| 2          | 600     | 600     | 300      | 600      | 6        | 6        | 0.3      | 30       | 60           | 30       | 5               |
| 3          | 300     | 600     | 600      | 300      | 6        | 6        | 0.3      | 3        | 60           | 3        | 15              |
| 4          | 600     | 300     | 600      | 600      | 36       | 36       | 0.3      | 3        | 18           | 3        | 15              |
| 5          | 600     | 600     | 300      | 600      | 6        | 6        | 3        | 3        | 18           | 3        | 15              |
| 6          | 600     | 600     | 600      | 300      | 36       | 36       | 0.3      | 30       | 18           | 30       | 5               |
| 7          | 300     | 600     | 600      | 600      | 36       | 36       | 3        | 3        | 60           | 3        | 5               |
| 8          | 300     | 300     | 600      | 600      | 6        | 6        | 3        | 30       | 18           | 30       | 5               |
| 9          | 300     | 300     | 300      | 600      | 36       | 36       | 0.3      | 30       | 60           | 30       | 15              |
| 10         | 600     | 300     | 300      | 300      | 36       | 36       | 3        | 3        | 60           | 3        | 5               |
| 11         | 300     | 600     | 300      | 300      | 36       | 36       | 3        | 30       | 18           | 30       | 15              |
| 12         | 300     | 300     | 300      | 300      | 6        | 6        | 0.3      | 3        | 18           | 3        | 5               |
| 13         | 450     | 450     | 450      | 450      | 21       | 21       | 1.65     | 16.5     | 39           | 165      | 10              |
| 14         | 450     | 450     | 450      | 450      | 21       | 21       | 1.85     | 16.5     | 39           | 16.5     | 10              |

TBF = Threadfin Bream, CF = Croaker, LMBF = Lattice monacle bream, TF = Tilapia, SORB = Sorbitol, SUC = Sucrose, STTP = Sodium tripolyphosphate, CARR = *k*-carrageenan, SOD LACT = Sodium Lactate, KONJ = Konjac

### Moisture content

Moisture content was conducted by drying the dish at 105°C for 2 h and cooled in a desiccator for 30 min. The dish was then weighed until a constant weight was reached. Two grams of samples were placed inside the dish, dried in the oven at 105°C for 18 h, and cooled in a desiccator for 30 min and weighed. This was calculated using the following equation:

$$\text{Moisture content}(\%) = \frac{(B - C)}{(B - A)x} \quad (1)$$

where A is the initial weight of an empty dish (g), B is the weight of the dish and sample (g), and C is the weight of the dish and sample after being dried (g).

### Ash content

The ash content was determined by burning samples in the dish used in the moisture content analysis. The dish was then put in the furnace and burned at 550°C for 8 h and weighed after to get ash content. This was calculated using the following equation:

$$\text{Ash content}(\%) = \frac{(B - A)}{\text{initial weight of dish with sample}} \times 100\% \quad (2)$$

where, A is the initial weight of an empty dish (g), and B is the weight of the dish and sample after burning (g).

### Protein content

Protein content was measured by weighing 2 g of samples, placed inside the destruction flask, added with two pieces of boiling rock, 15 mL concentrated H<sub>2</sub>SO<sub>4</sub> (95%–97%), 3 mL H<sub>2</sub>O<sub>2</sub>, destructed at 410°C for ±2 h until the solution was clearer, and cooled at room temperature. Twenty-five milliliter H<sub>3</sub>BO<sub>3</sub> 4% solution was prepared, the flask containing the solution resulting from the destruction was mounted on a steam distillation apparatus 50 – 70 mL natrium hydroxide–thiosulfate (Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>) solution was added, and distillation process was run until 150 mL distillate was obtained in the Erlenmeyer flask. The distillate was then titrated with HCl 0,2 N until the color of the solution was changed from green to natural grey. The protein content was stated in g/100 g unit sample (%):

$$\text{Protein content}(\%) = \frac{(V_a - V_b)\text{HCl} \times N \text{HCl} \times 14.007 \times 6.25}{W} \times 100\% \quad (3)$$

Where  $V_a$  is mL HCl for sample titration (ml),  $V_b$  is mL HCl for blank titration (ml),  $N$  is normality of HCl standard being used, 14.007 is weight of nitrogen atom, 6.25 is protein conversion factor for fish,  $W$  is weight of sample (g).

#### **Fat content**

The fat content was measured by weighing 2 g of samples and extracted with 150 mL chloroform in the Soxhlet fat extractor at 60°C for 8 h. The mixture of fat and chloroform was evaporated in the flask and dried in the oven at 105°C for  $\pm 2$  h to remove the residual chloroform and water vapor. The flask was then cooled in the desiccator for 30 min and weighed until a constant weight was reached.

$$\text{Fat content}(\%) = \frac{(C - A)}{B} \times 100\% \quad (4)$$

where  $A$  is the initial weight of an empty flask (g),  $B$  is the weight of the sample (g), and  $C$  is the weight of the flask containing fat after extraction (g).

#### **pH analysis**

The pH measurement was carried out with a digital pH meter (*Thermo Fisher Scientific Orion*). This was performed by dissolving 10 g in 90 mL sterile distilled water. Sample surimi was homogenized, and then pH was measured. A calibration of the pH meter was conducted prior to each measurement. This was performed by immersing the electrode in the sample solution until a stable reading was obtained, and then the pH value of the samples is recorded.

#### **TVBN content**

The TVB N was analyzed using Conway methods. Twenty-five g of surimi was added to 75 mL of perchloric acid solution 7% (PCA) filtered with filter paper. The Conway cup was organized and filled the inner chamber of the Conway cup with 1 ml of Boric acid, 1 ml of sample, and 1 ml of  $K_2CO_3$  on the left and right sides each. The cup is shaken for 1 min, and then cover the Conway cup which has been smeared with Vaseline. Incubation was performed at 35°C for 2 h and then boric acid was titrated with 0.02 N HCl. The titration process is carried out until the boric acid turns pink.

$$\text{TVB} - \text{N}(\text{mgN}/100\text{g}) = \frac{(V_a - V_b)\text{HCl} \times N \text{ HCl} \times 14.007 \times 2}{W} \times 100\% \quad (5)$$

where  $V_a$  is the volume of HCl solution in sample titration,  $V_b$  is the volume of HCl solution in blank titration,  $N$  is the normality of HCl solution,  $W$  is the weight of sample (g), and 14,007 is the weight of nitrogen atom.

The quality of the surimi was determined chemically and physically. The chemical properties of surimi were measured on moisture content, pH, and salt-soluble protein, while the physical properties were hardness, whiteness degree, and water-holding capacity (WHC).

#### **Salt-soluble protein (Weng and Zheng 2015)**

Five grams of surimi was mixed with 50 ml of 5% NaCl solution and homogenized for 2–3 min in a waring blender at a low temperature. The mixture was subsequently centrifuged at  $3400 \times g$  for 30 min at 10°C and filtered using Whatman filter paper No. 1. The filtrate was collected in the Erlenmeyer and kept at 4°C. Approximately 25 ml of filtrate was determined for protein content using the Kjeldahl semi-micro method.

#### **Hardness (Zheng et al. 2022)**

The surimi was mixed thoroughly with 30% cold water (5°C) and 3% salt (NaCl), then stirred using a food processor at below 10°C for 10 min. The dough was inserted into a pipe with a 2.5 cm diameter and a 5 cm height. The dough was gradually heated at 40–50°C for 40 min, followed by 20 min at 90°C. The gel formed was allowed to cool and left in the refrigerator

overnight. Hardness was measured using a TAXT plus texture analyzer (Stable Micro Systems, Vienna, UK) equipped with a probe = P/0.5 s, trigger force = -5 g; pre-test speed = 5 mm·s<sup>-1</sup>; test speed = 1 mm·s<sup>-1</sup>; and compression deformation was set at 75%.

#### **Water-holding capacity (Xiong et al. 2009)**

The surimi gel was sliced to a 0.5 cm thickness and then weighed  $\times$  grams (M1). After two-layer filter papers were placed on the top of the slice and three-layer filter papers at the bottom, a 5 kg load was applied for 2 min. The pressed gel weighed z grams (M2).

$$\text{Water Holding Capacity}(\%) = \frac{M1 - M2}{M2} \times 100\% \quad (6)$$

where M1 is the weight of surimi before being pressed, and M2 is the weight of surimi gel after being pressed.

#### **Whiteness degree (Duan et al. 2023)**

The color of the samples was measured using the Color Flex EZ Hunter Lab. To analyze L\* (lightness), a\* (redness-greenness), and b\* (yellowness-blueness). The whiteness (W) was computed in the following equation:

$$\text{Whitness Degree} = [100 - (100 - L^*)^2 + a^{+2} + b^{+2}]^{1/2} \quad (7)$$

#### **Sensory evaluation of surimi**

A total of 15 semi-trained sensory panelists who are familiar with and knowledgeable about the sensory attributes of surimi gels from the Research Center for Product Processing and Biotechnology were involved in sensory evaluation. Surimi gels were prepared by mixing 100 g surimi samples with 3% (w/w) NaCl at 4°C until homogenous samples were obtained. Homogenous samples were then placed into the mold and the gel was set by incubation in a water bath at 40°C for 30 min, followed by heating at 90°C for 20 min. Surimi gels were then cooled and stored at 10°C overnight. The folding is performed by cutting the kamaboko sample with a thickness of 0.3–0.5 cm. The fold test is conducted by holding the sample between the thumb and index finger. The surimi gel sample is folded into a semicircle. If no cracks are found, the sample is further folded into quarters. The grading and assessment for the fold test are as follows: grade AA (no cracks when folded twice, score 9), grade A (no cracks when folded once, score 7), grade B (slightly cracked when folded once, score 5), grade C (cracked when folded once, score 3), and grade D (crumbles when pressed with a finger, score 1).

The bite test was performed by biting a piece of kamaboko sample with a thickness of 0.3–0.5 cm between the incisors continuously or at several points on the sample. The sample is bitten slowly to assess the firmness of the kamaboko gel. The grading standards for the bite test are as follows: 9 (extremely firm), 8 (very firm), 7 (firm), 6 (moderately firm), 5 (normal), 4 (somewhat soft), 3 (very soft), 2 (extremely soft), and 1 (crumbles). The appearance and texture of the folded sample are observed. Meanwhile, for the appearance test, a frozen surimi was measured and compared to the grading standards based on SNI 01.2694:2013.9 (BSN 2013).

#### **Statistical analysis**

All measurements were repeated three times. The variables were screened using MINITAB 18.0 software for statistical analysis and graph plotting. Plackett–Burman based on the value of the effect coefficient and the significance variable with p-value < .05 will be used for further research or optimization. Variables that are declared significant may have more than one test attribute (Karlapudi et al. 2018).

## Results and discussions

### Characteristics of raw material and surimi product

The characteristics of fish as raw material used in this study and the resulting surimi are shown in Table 3. The proximate compositions of threadfin bream, croaker, lattice monocle bream, and tilapia were insignificantly different ( $p > .05$ ). Those fish had a proximate composition of 77.48–79.95% moisture, 17.72–18.88% protein, 0.45–0.81% fat, and 1.17–1.95% ash.

The protein content of the fish was quite high, i.e., ( $17.72 \pm 0.65\%$ ) – ( $18.88 \pm 0.10\%$ ); thus, the fish meat would produce a good gel structure (Bhattacharya and Prajapati 2016). All the fish were classified as lean fish with a fat content of less than 5% (Taşbozan and Gokce 2017). The fat content of fish is less than 2%, therefore they will not interfere with the formation of gel, destroy the protein matrix, and reduce the gel strength (Lin et al. 2020). Based on the TVBN content, croaker fish was considered very fresh (prime quality) with TVB levels  $\leq 10$  mgN/100g. Tilapia and lattice monocle bream were categorized as fresh with TVBN contents of 12.72 mgN/100g and 16.89 mg N/100g, respectively. While the thread bream was fairly fresh with a TVBN content of 20.53 mg N/100g, it was still accepted for consumption. This fish is still at the borderline of freshness and can still be consumed with TVB levels of 20–30 mgN/100 (Bekhit et al. 2021). The pH of the fish was in the range of 6.5–7.0, indicating that all the fish were still fresh.

The yield of surimi obtained from freshwater fish of tilapia (25.51%) was lower than that of demersal fish (33.11–34.54%). The yield is closely related to the value of its economic feasibility. The surimi processing industry informed that it would be profitable if the yield was more than 20% (Guenneugues and Park 2020). The moisture content of the surimi obtained was in the range of 80.88–81.98%. Thus, the moisture content was slightly higher than the moisture content of commercial surimi according to Indonesian National Standard (SNI) 01-2694.1-2013 (BSN 2013). i.e, maximum 80%.

Croaker surimi had the highest hardness ( $2060.61 \pm 0.74$  N), while the threadfin bream surimi showed the lowest hardness ( $1279.77 \pm 0.44$  N). In fresh condition, threadfin bream fish make a high-quality surimi with good gel strength, with an average gel strength of  $2452.10 \text{ g/cm}^2$  (Lestari et al. 2016). Surimi produced from this study had a whiteness degree in the range of ( $55.95 \pm 0.43\%$ )–( $62.17 \pm 2.00\%$ ). Surimi with the highest whiteness degree was obtained from lattice monocle bream, and the lowest was from tilapia. Surimi products processed from those fish were used for further experiments.

### Effect of independent factors on the chemical properties of surimi

To find out the effect of an independent factor on the chemical properties of surimi, 14 experiments were employed using the formulation of various variables applied in the Plackett–Burman design method (Table 2). The chemical properties of the surimi are presented in Table 4.

**Table 3.** Proximate analysis, fish freshness, and characteristics of surimi.

| Parameters                | Threadfin Bream/<br>Nemipterus sp. | Croaker/Argyrosomus<br>japonicus | Lattice monocle bream/<br>Scolopsis taeniopterus | Tilapia/Oreochromis<br>mossambicus |
|---------------------------|------------------------------------|----------------------------------|--|------------------------------------|
| Proximate composition     |                                    |                                  |  |                                    |
| Moisture content (%)      | 77.48 $\pm$ 0.38                   | 79.4 $\pm$ 0.10                  | 78.00 $\pm$ 0.61                                 | 79.95 $\pm$ 0.08                   |
| Ash content (%)           | 1.37 $\pm$ 0.09                    | 1.50 $\pm$ 0.00                  | 1.95 $\pm$ 0.68                                  | 1.17 $\pm$ 0.02                    |
| Protein content (%)       | 18.88 $\pm$ 0.10                   | 18.77 $\pm$ 0.42                 | 18.75 $\pm$ 1.13                                 | 17.72 $\pm$ 0.65                   |
| Fat content (%)           | 0.77 $\pm$ 0.19                    | 0.66 $\pm$ 0.17                  | 0.45 $\pm$ 0.16                                  | 0.81 $\pm$ 0.01                    |
| Fish freshness            |                                    |                                  |  |                                    |
| TVBN (mgN/100 g)          | 20.53 $\pm$ 0.38                   | 4.65 $\pm$ 1.89                  | 16.89 $\pm$ 0.75                                 | 12.72 $\pm$ 1.00                   |
| pH                        | 6.60 $\pm$ 0.21                    | 6.98 $\pm$ 0.04                  | 6.65 $\pm$ 0.13                                  | 6.60 $\pm$ 0.09                    |
| Characteristics of surimi |                                    |                                  |  |                                    |
| Yield of surimi (%)       | 33.11 $\pm$ 1.72                   | 34.54 $\pm$ 0.76                 | 33.66 $\pm$ 0.24                                 | 26.51 $\pm$ 1.40                   |
| Moisture content (%)      | 80.88 $\pm$ 0.98                   | 81.21 $\pm$ 0.21                 | 81.96 $\pm$ 0.19                                 | 81.98 $\pm$ 0.50                   |
| Hardness (N)              | 1.279.77 $\pm$ 0.44                | 2.060.61 $\pm$ 0.74              | 1.933.84 $\pm$ 0.61                              | 1.913.58 $\pm$ 0.52                |
| Whitiness degree (%)      | 60.18 $\pm$ 1.55                   | 57.00 $\pm$ 1.59                 | 62.17 $\pm$ 2.00                                 | 55.95 $\pm$ 0.43                   |



**Table 4.** Effect of dependent factors on the chemical properties of multi-species surimi using variables based on Plackett–Burman Design.

| STAD ORDER (SO) | Moisture Content (%) | Salt-Soluble Protein (%) | pH          |
|-----------------|----------------------|--------------------------|-------------|
| SO 1            | 80.90 ± 0.27         | 1.87 ± 0.00              | 5.90 ± 0.04 |
| SO 2            | 80.11 ± 0.03         | 1.36 ± 0.11              | 5.70 ± 0.01 |
| SO 3            | 80.89 ± 0.24         | 1.47 ± 0.01              | 5.70 ± 0.13 |
| SO 4            | 80.56 ± 0.12         | 1.65 ± 0.30              | 5.53 ± 0.32 |
| SO 5            | 78.26 ± 0.56         | 1.66 ± 0.14              | 5.88 ± 0.04 |
| SO 6            | 79.79 ± 0.26         | 1.36 ± 0.02              | 5.77 ± 0.06 |
| SO 7            | 80.09 ± 0.23         | 1.96 ± 0.06              | 5.89 ± 0.12 |
| SO 8            | 79.03 ± 0.32         | 1.65 ± 0.01              | 5.81 ± 0.04 |
| SO 9            | 79.57 ± 0.12         | 1.94 ± 0.06              | 5.79 ± 0.13 |
| SO 10           | 80.44 ± 0.09         | 1.52 ± 0.21              | 5.84 ± 0.02 |
| SO 11           | 79.99 ± 0.16         | 1.27 ± 0.18              | 5.65 ± 0.18 |
| SO 12           | 79.93 ± 0.34         | 1.38 ± 0.18              | 5.71 ± 0.07 |
| SO 13           | 77.99 ± 1.98         | 1.59 ± 0.05              | 5.61 ± 0.19 |
| SO 14           | 77.90 ± 0.04         | 1.35 ± 0.06              | 5.54 ± 0.21 |

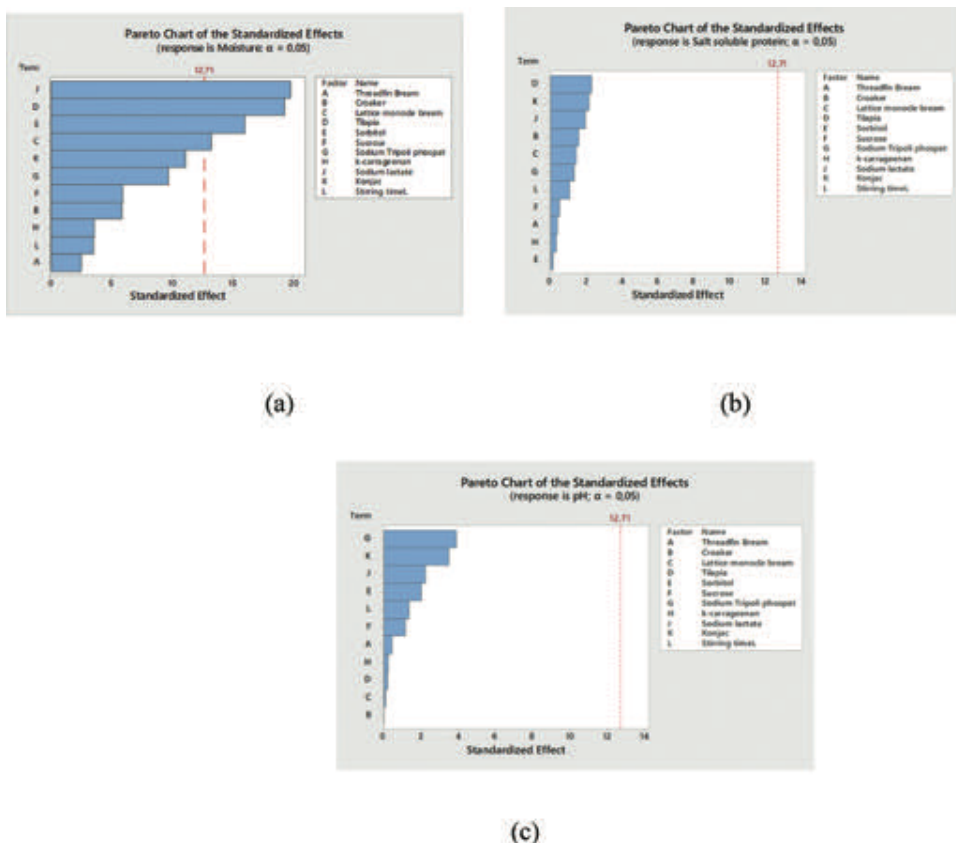
Water content is an important component in surimi because water can affect the appearance, texture, and taste of food. Surimi had an average moisture content ranging from 78.26% to 80.90%, where several treatments produced moisture content slightly exceeding the maximum moisture content required for commercial surimi according to Indonesian National Standard ( $\leq 80\%$ ) (SNI 2694, 1. BSN 2013). The moisture content of this research is above the exportable quality standard set by PT Bintang Karya Laut, a surimi processing company in Rembang, which is 74–75% (Asrianti 2017).

According to the Pareto chart, the moisture content of the multi-species surimi was significantly affected by sodium lactate, sorbitol, tilapia, and lattice monacle bream (Figure 1). The results of the ANOVA analysis showed that the four dependent factors had a significant effect with  $p < .05$  and that there was a linear correlation between moisture content and the dependent factors with a confidence level of  $R = 0.9997$  (Table 5).

Based on the normal standard effect, sodium lactate and lattice monacle bream demonstrated a positive effect on the significant reduction of the moisture content of surimi, while the addition of sorbitol and tilapia produced an opposite effect (Figure 2a). Sodium lactate is a food additive used as an antimicrobial agent for meat products. Sodium lactate is effective at inhibiting most spoilage and pathogenic bacteria (Choi et al. 2014). The use of sodium lactate in surimi can bind water molecules, thereby reducing the moisture content of the product, because sodium lactate can increase the water holding capacity (Walayat et al. 2020).

Sorbitol, on the other hand, is a glucose derivative that can bind water and protein, improve texture, and act as an anti-denaturant (Klinmalai et al. 2021). Sorbitol is used as a humectant or moisturizer in various products to resist water loss. However, the addition of sorbitol at the upper limit showed an effect on increasing moisture content ( $p < .5$ ). In this research, the use of tilapia fish has an impact on increasing the water content of the resulting surimi. This is because the surimi from tilapia has the highest water content ( $79.95 \pm 0.08\%$ ) compared to the surimi from other fish. Tilapia is a freshwater fish that lives in freshwater environments and has a higher osmotic pressure (hyperosmotic) compared to the osmotic pressure of its surroundings. Therefore, water tends to diffuse into the fish's body through the semi-permeable body surface (Gultom et al. 2015).

Based on the Pareto chart effect, coefficient analysis indicated that 11 variables used in this study insignificantly affected salt-soluble protein content and the degree of acidity (pH) (Figure 1b,c). Salt soluble protein is a myofibril protein consisting of actin and myosin that are responsible for gel formation. The salt-soluble protein content obtained in the study ranged from ( $1.27 \pm 0.18\%$ ) to ( $1.96 \pm 0.06\%$ ). Although the fish used had different salt-soluble protein content, they had no impact on salt-soluble protein surimi formulated with Plackett–Burman design. The study conducted by Suryaningrum et al. (2018) revealed that the salt-soluble protein contents of threadfin bream fish, croaker, lattice monacle bream, and tilapia were 5.33%, 6.49%, 3.81%, and 2.6%, respectively. The salt-soluble protein content of fish is influenced by the type of fish, where the more salt-soluble protein content, the better functional properties of the fish gel (Gultom et al. 2015).



**Figure 1.** Standardized Pareto chart effect of dependent variables on (a) moisture content, (b) salt soluble protein, and (c) pH of surimi.

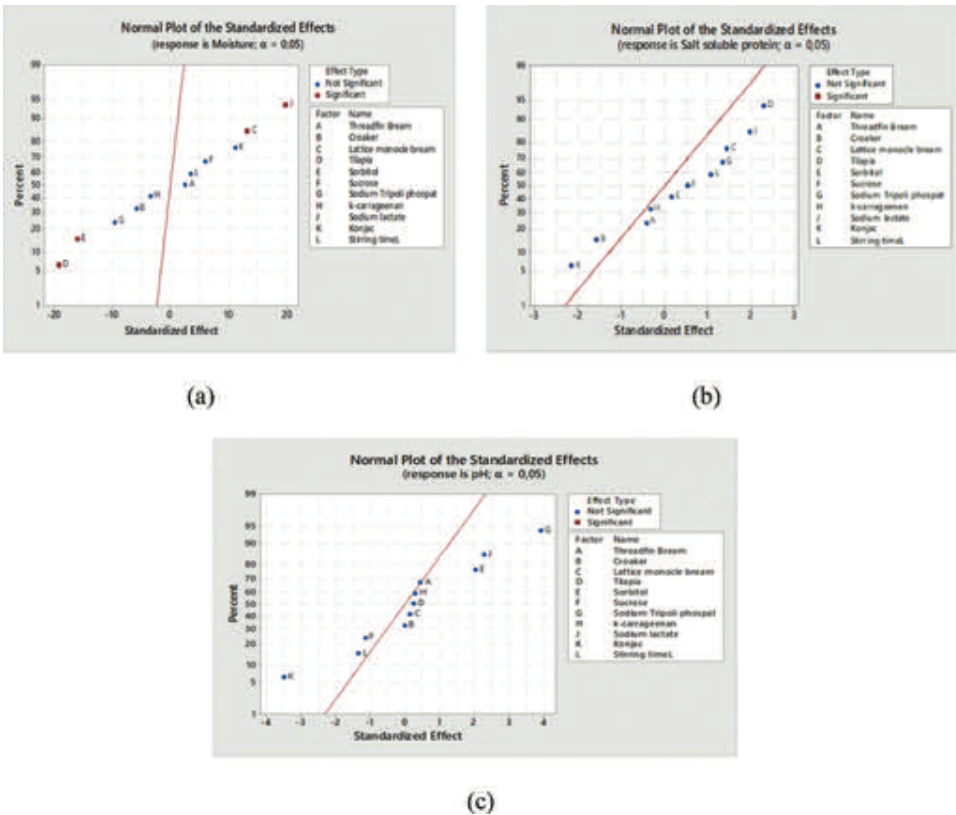
**Table 5.** Analysis of variance of Plackett–Burman screening regression parameters of dependent variables on moisture content, salt-soluble protein, and pH.

| Factor      | Moisture content $R^2 = 0.9997$ |         | Salt-soluble protein $R^2 = 0.9619$ |         | pH $R^2 = 0.9839$ |         |
|-------------|---------------------------------|---------|-------------------------------------|---------|-------------------|---------|
|             | F Value                         | P-Value | F Value                             | P-Value | F Value           | P-Value |
| TBF         | 6.23                            | 0.243   | 0.18                                | 0.747   | 0.19              | 0.739   |
| CF          | 34.13                           | 0.108   | 2.48                                | 0.36    | 0                 | 0.984   |
| LMBF        | 173.9*                          | 0.048   | 2.06                                | 0.387   | 0.02              | 0.919   |
| TF          | 371.2*                          | 0.033   | 5.21                                | 0.263   | 0.05              | 0.856   |
| SORB        | 255.68*                         | 0.04    | 0.03                                | 0.899   | 4.06              | 0.293   |
| SUC         | 34.96                           | 0.107   | 0.28                                | 0.691   | 1.32              | 0.456   |
| STTP        | 93.55                           | 0.066   | 1.79                                | 0.409   | 15.24             | 0.16    |
| CARR        | 12.32                           | 0.177   | 0.11                                | 0.795   | 0.08              | 0.826   |
| SOD LACT    | 391.38*                         | 0.032   | 3.86                                | 0.3     | 5.16              | 0.264   |
| KONJ        | 122.6                           | 0.057   | 4.65                                | 0.276   | 12.22             | 0.177   |
| Stirr Times | 12                              | 0.179   | 1.14                                | 0.48    | 1.83              | 0.405   |

TBF = Threadfin Bream, CF = Croaker, LMBF = Lattice monacle bream, TF = Tilapia, SORB = Sorbitol, SUC = *k*-carrageenan, SOD LACT = Sodium Lactate, KONJ = Konjac.

**Note:** The values in the same column with various superscript letters reflect the significant difference at  $p < 0.05$

The degree of acidity (pH) of various surimi formulations ranged from 5.53 to 5.90, with surimi SO1 having the highest pH (5.90) and surimi SO4 having the lowest pH (5.53) (Table 4). Those pH values of surimi were quite low, i.e. below 6, which was probably due to sodium lactate addition. Sodium lactate is the salt form of lactic acid, which is well recognized as a powerful antimicrobial. Sodium lactate is made through



**Figure 2.** Normal plot of standardized effect of dependent variables on (a) moisture content, (b) salt-soluble protein, and (c) pH.

the fermentation of sugar, to produce lactic acid, and then neutralized using NaOH to obtain sodium lactate which can cause an acidic taste (Choi et al. 2014). The low degree of acidity can affect the functional properties of surimi. The optimum pH range to produce elastic gel is 6.0–8.0, while the best condition is at a pH of 6.5–7.0. Surimi with a pH of less than 6 will produce a brittle or breakable gel. The pH value will affect the physical properties of surimi-based product, such as hardness, WHC, emulsion properties, and protein rheology (Gao et al. 2018).

### **Effect of dependent factors on the physical properties of surimi**

#### **Hardness**

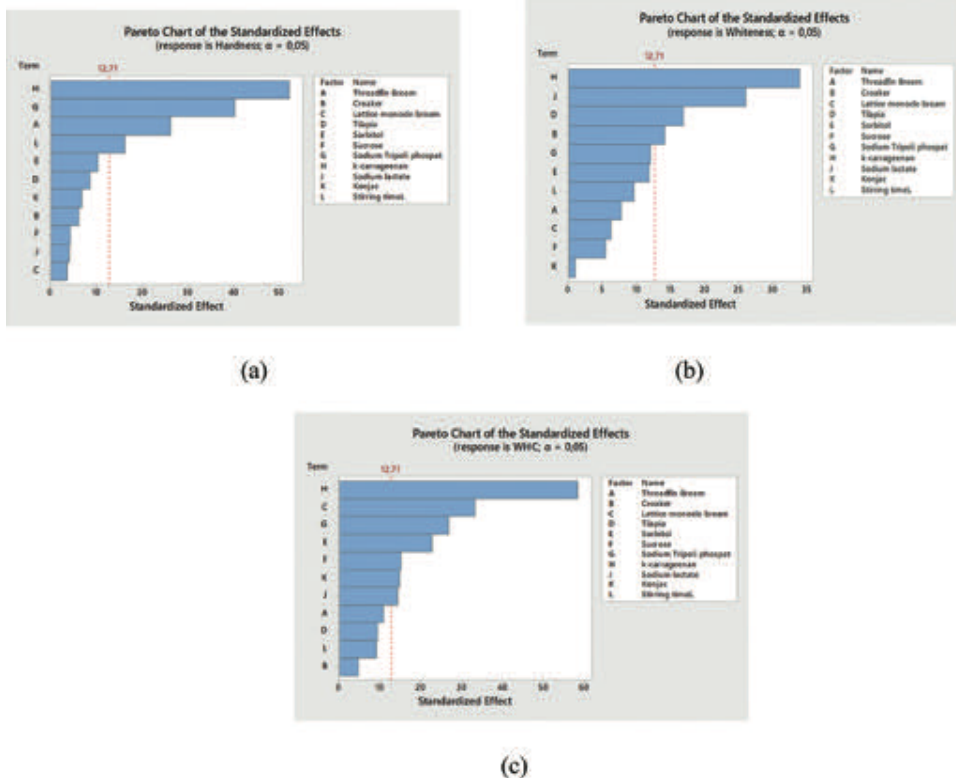
Observations of the physical properties of surimi from various formulations based on Plackett–Burman design, which was observed in hardness, whiteness, and WHC, can be seen in Table 6.

Based on the 14 experimental formulations, it was seen that the hardness of the surimi gel ranged between  $(751.62 \pm 1.52 \text{ N})$  and  $(3.088.25 \pm 1.10 \text{ N})$ , the whiteness was  $(58.83 \pm 0.01\%)$ – $(78.95 \pm 0.01\%)$ , and the WHC  $(16.01 \pm 0.78\%)$ – $(29.90 \pm 0.45\%)$ . The use of *k*-carrageenan with a maximum value produced surimi with a better gel strength than other treatments (SO1, SO2, SO8, SO9, and SO11) (Table 4). This result is similar to outcome obtained by Yu et al. (2022), where *k*-carrageenan has function as an adhesive in strengthening this matrix to make it more compact and firmer; however, excessive use of *k*-carrageenan ( $>2\% \text{ w/w}$ ) seems to cause a decrease in gel strength.

As the Pareto chart shows (Figure 3a), only four factors dependent had a significant effect on the hardness of the resulting surimi gel, namely *k*-carrageenan, sodium tripolyphosphate, threadfin bream fish, and stirring time. The results of the ANOVA analysis showed that the four dependent factors had a significant linear correlation between that dependent factor and hardness with a confidence level of  $R^2 = 0.9997$  (Table 7).

**Table 6.** Effect of dependent factors on the physical properties of multi-species surimi using variables based on Plackett–Burman design.

| STAD ORDER | Hardness (N)    | Whiteness (%) | Water-Holding Capacity (%) |
|------------|-----------------|---------------|----------------------------|
| SO 1       | 2.296.95 + 1.55 | 70.25 ± 0.01  | 70.25 ± 0.01               |
| SO 2       | 1.410.40 ± 1.97 | 62.15 ± 0.07  | 62.15 ± 0.07               |
| SO 3       | 751.62 ± 1.52   | 79.12 ± 0.01  | 79.12 ± 0.01               |
| SO 4       | 897.80 ± 1.22   | 72.50 ± 0.02  | 72.50 ± 0.02               |
| SO 5       | 1.230.96 ± 1.15 | 66.08 ± 0.10  | 66.08 ± 0.10               |
| SO 6       | 980.52 ± 1.11   | 65.36 ± 0.08  | 65.36 ± 0.08               |
| SO 7       | 1.580.50 ± 1.41 | 68.09 ± 0.08  | 68.09 ± 0.08               |
| SO 8       | 2.687.50 ± 1.92 | 58.91 ± 0.01  | 58.91 ± 0.01               |
| SO 9       | 2.136.99 ± 1.62 | 69.89 ± 0.12  | 69.89 ± 0.12               |
| SO 10      | 996.92 ± 1.44   | 78.95 ± 0.01  | 78.95 ± 0.01               |
| SO 11      | 3.088.25 ± 1.10 | 58.83 ± 0.01  | 58.83 ± 0.01               |
| SO 12      | 778. 04 ± 1.87  | 69.37 ± 0.04  | 69.37 ± 0.04               |
| SO 13      | 1.536.66 ± 1.25 | 70.99 ± 0.04  | 70.99 ± 0.04               |
| SO 14      | 1.586.52 ± 1.37 | 70.40 ± 0.08  | 70.40 ± 0.08               |



**Figure 3.** Standardized Pareto chart effect of dependent variables on (a) Hardness, (b) Whiteness, and (c) Water-Holding Capacity of surimi.

Based on the normal standard effect, *k-carrageenan*, sodium tripolyphosphate, and stirring times demonstrated a significant positive effect on the hardness of surimi. On the contrary, the use of Threadfin bream had a significant negative effect on the hardness of surimi (Figure 4a).

Carrageenan is widely used in the food industry for its unique texture and stability. In this study, *k-carrageenan* was shown as a gelling agent to have an important role in improving the texture of surimi processed from various types of fish. Due to its hydrophilic properties, *k-carrageenan* was able to absorb water in the product and convert it into hydrocolloid form. The addition of *k-carrageenan* will

**Table 7.** Analysis of variance of Plackett–Burman screening regression parameters of dependent variables on Hardness, Water Holding Capacity (WHC), and Whiteness.

| Factor      | Hardness R <sup>2</sup> = 0.9996 |         | WHC R <sup>2</sup> = 0.9999 |         | Whiteness R <sup>2</sup> = 0.9995 |         |
|-------------|----------------------------------|---------|-----------------------------|---------|-----------------------------------|---------|
|             | F Value                          | P Value | F Value                     | P Value | F Value                           | P Value |
| TBF         | 690.54*                          | 0.024   | 116.53                      | 0.059   | 59.48                             | 0.082   |
| CF          | 37.91                            | 0.103   | 22.55                       | 0.132   | 198.57*                           | 0.045   |
| LMBF        | 13.38                            | 0.17    | 1101.07*                    | 0.019   | 38.9                              | 0.101   |
| TF          | 74.18                            | 0.074   | 86.91                       | 0.068   | 285.39*                           | 0.038   |
| SORB        | 107.7                            | 0.061   | 514.96*                     | 0.028   | 141.65                            | 0.053   |
| SUC         | 18.52                            | 0.145   | 230.88*                     | 0.042   | 28.99                             | 0.117   |
| STTP        | 1.626.64*                        | 0.016   | 711.11*                     | 0.024   | 144.65                            | 0.053   |
| CARR        | 2.715.94*                        | 0.012   | 3.385.63*                   | 0.011   | 1149.27*                          | 0.019   |
| SOD LACT    | 16.08                            | 0.156   | 200.53*                     | 0.045   | 676.98*                           | 0.024   |
| KONJ        | 46.02                            | 0.093   | 220.37*                     | 0.043   | 0.99                              | 0.502   |
| Stirr Times | 259.84*                          | 0.039   | 82.4                        | 0.07    | 92.8                              | 0.066   |

TBF = Threadfin Bream, CF = Croaker, LMBF = Lattice monacle bream, TF = Tilapia, SORB = Sorbitol, SUC = Sucrose, STTP = Sodium Tripolyphosphate, CARR, *k*-carrageenan, SOD LACT = Sodium Lactate, KONJ = Konjac.

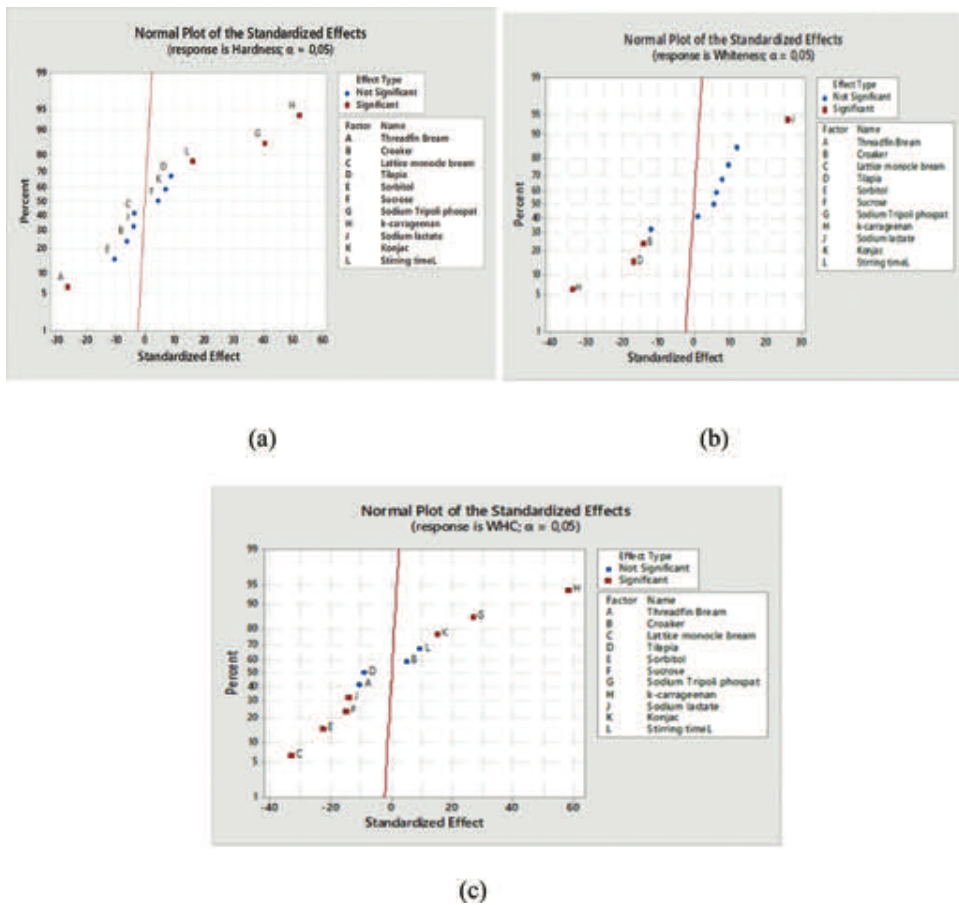
**Note:** The values in the same column with various superscript letters reflect the significant difference at  $p < 0.05$

encourage the formation of a three-dimensional network structure, through hydrogen bonding in the hydroxyl groups of the carrageenan polymer. This will cause the water to be trapped in the three-dimensional structure network into a colloidal form which causes the surimi gel to become stronger (Yu et al. 2022). Carrageenan can interact with myosin and form hydrogen bonds through the carboxyl group, leading to the stability of myofibril protein and WHC during storage (Chen et al. 2020). Carrageenan can interact with negatively charged macromolecules of protein, which causes an increase in the affinity of moles for water and increases interactions between molecules, thereby increasing viscosity, gel formation, deposition, and stability of protein (Goff and Guo 2019).

The stirring times and the addition of carrageenan also increased the gel strength of the surimi. Similar result was reported by Liu et al. (2021) that adding *k*-carrageenan and high-pressure processing can be a potential method to improve the gel quality of surimi and resulted in increased WHC, color, gel strength, microstructure, texture, and proteins of the surimi gels produced.

The use of STTP at the maximum limit resulted in surimi that tended to have high gelling properties. The highest hardness was found in surimi SO1 ( $2.296.95 + 1.55 \text{ g/cm}^2$ ), SO8 ( $2.687.50 + 1.92 \text{ g/cm}^2$ ), and SO11 ( $3.088.25 + 1.10 \text{ g/cm}^2$ ) (Table 6). STTP has been found to be effective at enzyme hydration, which can affect the redox potential of substances such as ferrous ions, ascorbate, and cysteine, potentially leading to the inactivation of the enzyme trimethylamine oxide demethylase (TMAOase). TMAOase is an endogenous enzyme that plays a role in the breakdown of TMAO into formaldehyde and dimethylamine, which will interfere with gel formation (Lee 2016). Walayat et al. (2020) revealed that the use of phosphate in surimi increases gel strength, cohesiveness, and other texture parameters. According to microscopy structure (SEM), the addition of STPP has a significant effect on the increase in the gel strength, fracturability, chewiness, gumminess, bite, and folding properties as well as the smooth and solid surfaces of surimi (Laksono et al. 2019). Azka and Mujiyati (2020) reported that the use of 0.8% phosphate significantly affects the texture parameters of the produced surimi from pike conger fish (*Muraenesox cinereus*). The use of STPP can increase the ability of the gel to capture water and rehydrate it when surimi is thawed, STPP improves the texture of the meat, which causes an increase in meat quality, and can have an impact on pH ionic strength, dissociation of actomyosin complexes, and antibacterial activity (Glorieux et al. 2017). Polyphosphates have an inhibitory effect on protein denaturation of surimi during frozen storage at  $-18^\circ\text{C}$  which is usually mixed with sorbitol and sucrose as a cryoprotectant (Nopianti et al. 2011).

Based on the normal standard effect (Figure 4a), the use of thread bream fish had a negative effect on the hardness of the surimi produced. This can be related to the decrease in the freshness of the thread bream used during experiment. The freshness of the fish plays an important role in the surimi gel quality. As the fish undergoes degradation, some of the denatured myofibril proteins that are responsible for gel formation



**Figure 4.** Effect of independent variables on (a) Hardness, (b) Whiteness, and (c) Water-Holding Capacity (WHC).

are released. Denaturation results in the loss of protein functionality, including the loss of gel-forming ability (Massoud et al. 2015). The hardness of surimi from thread bream was  $1279 \pm 0.44 \text{ g/cm}^2$  below the hardness of other surimi,  $1913.58 \pm 0.51$ – $2060.61 \pm 0.74 \text{ g/cm}^2$  (Table 3). The Threadfin bream used in the experiment was obtained from fishermen who may have kept the fish on ice for more than 2 days. Thread bream has an indigenous proteolytic enzyme that causes myofibril protein degradation, which causes a decrease in surimi gel strength (Bashir et al. 2017). Long duration of fish storage brings about the breakdown of myofibril protein by proteolytic enzymes, thereby reducing the ability to form gels in the surimi (Massoud et al. 2015). The myofibrillar protein content of *Pseudosciaena crocea* stored in crushed ice decreased by 44.48% after 2 days of storage and 88.35% after 6 days of storage (Guan et al. 2021). The gelling properties of surimi were significantly influenced by the freshness of the fish. The results of the research by Tiwo et al. (2018) on common carp (*Cyprinus carpio*) that were stored in ice for 15 days showed that the gel strength decreased from  $668.2 \text{ g/cm}^2$  of the freshly lifted common carp to  $318.70 \text{ g/cm}^2$  at the end of the 15 days ice storage.

Stirring was done to facilitate physical extraction of the myofibrillar protein. Myofibril is a salt soluble protein, but according to Cando et al. (2017), the addition of other additives in combination with high-pressure processing also results in similar physicochemical properties of surimi. Myofibrillar protein is an important functional ingredient and has a significant impact on the gel-forming ability, textural quality, and sensory quality of surimi-based products (Priyadarshini et al. 2018). This study revealed that stirring for 15 min resulted in a better surimi gel compared to those 5 min. Prolonged stirring can induce changes in the functional properties of the protein, such as gelling ability (Ducept et al. 2012). In this

study, stirring was done with a stone mixing and kneading machine, so the surimi was subjected to physical pressure during the mixing. The pressure applied will affect the increase in myofibril proteins so that it can increase the gel strength of the surimi as reported in the study conducted by Liang et al. (2017) on big carp surimi. Maksimenkol et al. (2020) reported that the application of moderate-high hydrostatic pressure has been successfully used to increase the functionality of myofibrillar proteins by modifying the structure due to denaturation, solubilization, aggregation, or gelation.

### Whiteness degree

The highest whiteness degree of surimi was shown by SO3 ( $79.12 \pm 0.01\%$ ) and the lowest was shown by SO11 ( $58.83 \pm 0.01\%$ ) (Table 4). The whiteness degree values of Vietnamese commercial surimi processed from a mixture of tread bream fish, red snapper, and others have an  $L^*$  value of 70–77% (Anon 2017). Formulations that produced surimi with a whiteness degree of more than 70% were SO1, SO3, SO4, SO 10, SO13, and SO14.

According to the Pareto chart (Figure 4b), the dependent factors influencing the whiteness of surimi were *k-carrageenan*, sodium lactate, tilapia, and croaker fish. The effect coefficient analysis showed that sodium lactate had a significant positive effect on the whiteness degree of the surimi. However, the use of croaker, tilapia, and carrageenan induced a significant negative effect on the whiteness degree (Figure 4b). Whiteness is an important parameter to determine the quality of surimi, with values greater than 75 generally considered acceptable (Priyadarshini et al. 2018). The addition of calcium lactate was in line with a study on yellow croaker (*Pseudosciaena crocea*) surimi conducted by Sang et al. (2022), which revealed that increasing calcium lactate contents in surimi increases the values of whiteness. Therefore, the addition of calcium lactate might improve the whiteness of surimi gels. The use of carrageenan at the upper limit encouraged a decrease in the whiteness degree value of the surimi. A similar trend was reported by Eom et al. (2013), in which the addition of *k-carrageenan* caused a decrease in the whiteness value of surimi gels. Chen et al. (2020) revealed that the addition of  $\kappa$ -carrageenan remarkably decreased the whiteness of the surimi gel, and that the whiteness decreased when the addition level of  $\kappa$ -carrageenan reached 0.5% (b/b). Based on the Stad Order obtained, the  $\kappa$ -carrageenan used in this study was approximately 0,25–2,5% (b/b). The color of the commercial carrageenan used in the study was a yellowish white. Djaeni et al. (2012) revealed that this yellowish white colour of carrageenan is caused by higher temperature and longer drying time. Therefore, the addition of *k-carrageenan* at a higher concentration intends to decrease the whiteness of the surimi.

Based on the normal standard effect, the use of croaker and tilapia in surimi processing showed a negative effect that decreased the whiteness degree. The whiteness degree of croaker and tilapia used in this study was  $57.00 \pm 1.59\%$  and  $55.95 \pm 0.43\%$ , respectively, specifying that the color of croaker and tilapia was dark white. The skin and flesh color of tilapia and croaker were black and grayish white, respectively. Abdelwahab et al. (2020) noted that the color indices of Tilapia flesh are  $L^* = 58.08 \pm 2.26\%$ ,  $+ a^* = 6311.07\%$ , and  $+ b^* = 16420.21\%$ , indicating that the tilapia flesh is slightly yellowish.

### Water-holding capacity

WHC is an important factor in gel formation and is closely related to free water released. The highest WHC value was found in the surimi SO11, i.e.  $40.72 \pm 0.93\%$ , while the lowest was encountered in the surimi SO10, i.e.  $20.03 \pm 1.39\%$ . According to the Pareto chart (Figure 3c), the dependent factors that affected the WHC were carrageenan, lattice monocle bream, STTP, sorbitol, sucrose, konjac, and sodium lactate. The effect coefficient analysis revealed that the use of carrageenan, STPP, and konjac had a significant positive effect on WHC. The addition of sucrose, sorbitol, and lattice monocle bream exhibited a significant negative effect on WHC (Figure 4c). Kim et al. (2018) found that *k-carrageenan* forms strong complexes with myofibril proteins, which can increase the WHC, gel strength, and cohesiveness of meat products. Konjac has a high molecular weight (200–2000 kDa) consisting of mannose and glucose. Konjac is well known to strengthen the water-binding ability of meat products but is also a synergistic ingredient in protein gelation and textural properties of meat products (Liu et al. 2019; Yang et al. 2017).

Goncalves (2012) reported that the main functions of phosphate in seafood processing can be used to increase pH and ionic strength, as well as bind myofibril protein and dissociate actomyosin, thereby improving the WHC of fish protein. The addition of phosphate increases the WHC of protein. The addition of phosphate can open the protein structure, which facilitates keeping more water. Therefore, the effect of the three coefficients (k-carrageenan, konjac, and STTP) showed a positive effect on the WHC of the resulting surimi. Sucrose and sorbitol are cryoprotectants that are widely used in the surimi industry to prevent protein denaturation during freezing by inhibiting the hydrophobic interaction of the proteins. Sucrose and sorbitol as cryoprotective agents can increase the water shear surface to protect against the loss of protein molecules. The addition of cryoprotectant can improve the quality and WHC of surimi. In this study, the addition of sucrose and sorbitol in the minimum limit tended to reduce the WHC of the surimi. The addition levels of sucrose and sorbitol ranged from 0.3% to 3%; however, the most common concentration for sucrose and sorbitol used in the surimi industry is 4% (Bashir et al. 2017). Therefore, the addition of sucrose and sorbitol with a minimum limit had a negative effect on the WHC of surimi.

### Sensory evaluation

The sensory evaluation of surimi was carried out on attributes of appearance, folding test, and bite test. The results of sensory evaluation on Run Orders 1 to 14 can be seen in Table 8. According to the effect of dependent variables and the Pareto chart, the sensory component of the multi-species surimi independent factor given has no effect on appearance or folding ability (Figure 5). Only k-carrageenan influences the biting test. According to the analysis of variance, the carrageenan given had an influence

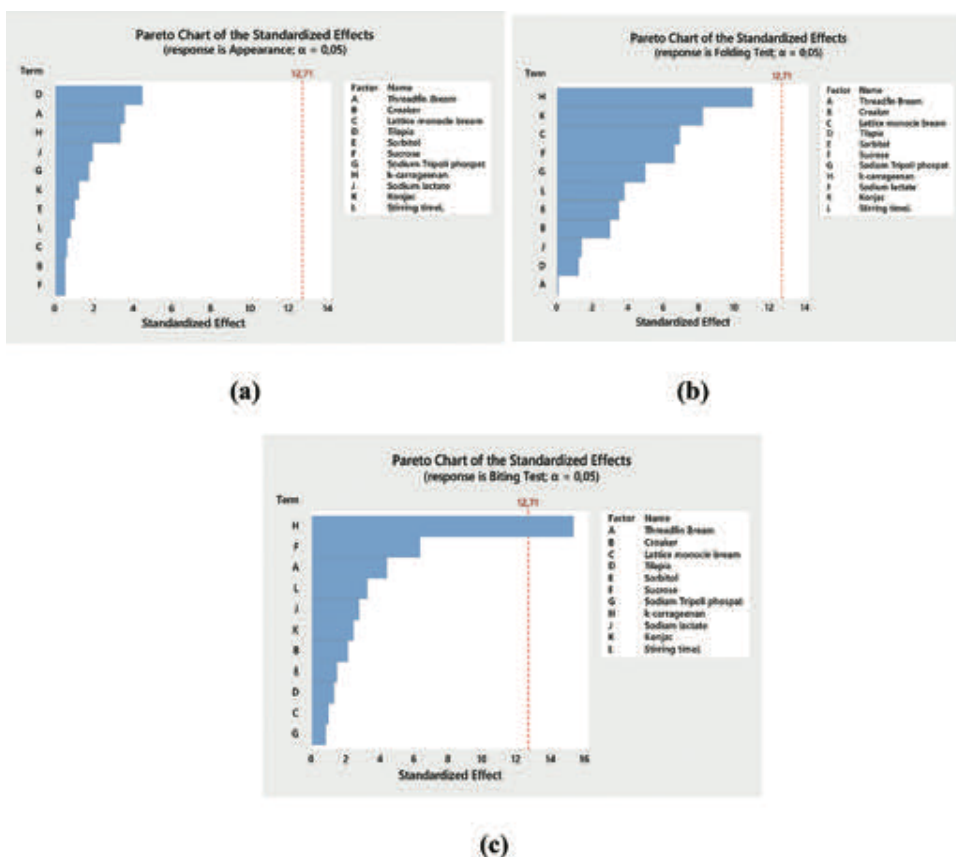
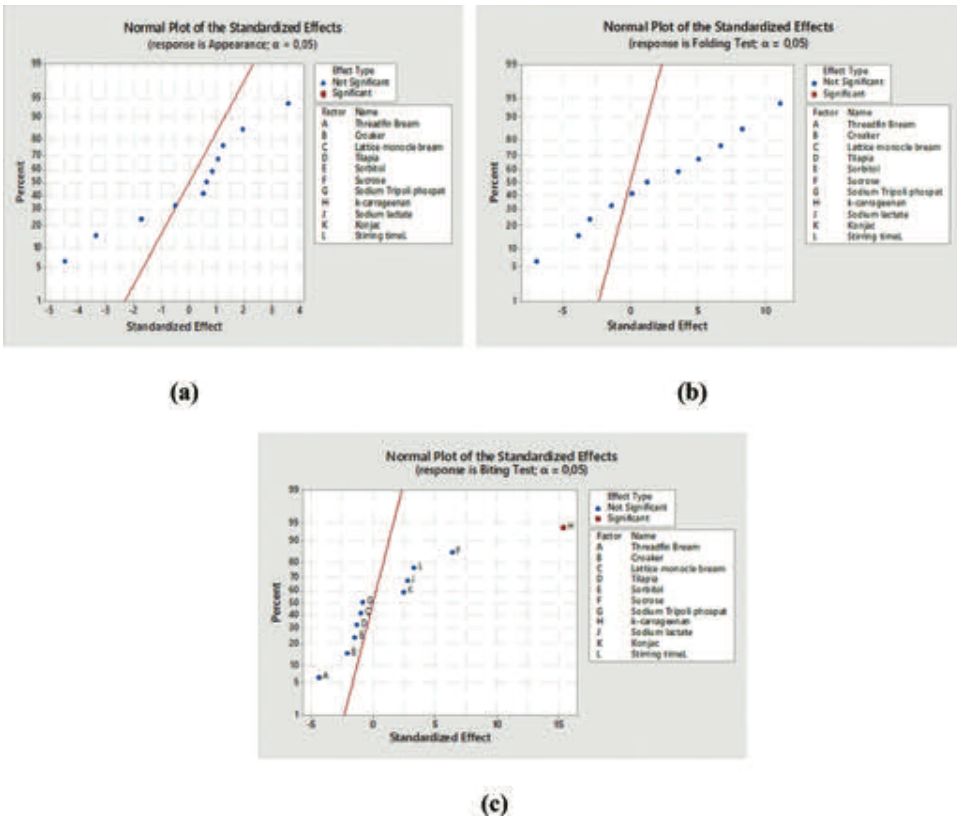


Figure 5. Standardized Pareto chart effect of dependent variables on sensory evaluation: (a) appearance, (b) folding test, and (c) biting test.





**Figure 6.** Normal plot of the standardized effect of dependent factor to sensory evaluation: (a) appearance, (b) folding test, and (c) biting test.

on the bite test given with  $p < .05$ , and there was a linear correlation between the tooth test and the dependent factor with a coefficient of the level of  $R = 99.73\%$  (Table 9).

Panelists gave appearance values ranging from 5.6 to 8.6, and the highest appearance values were obtained from SO3 and SO10 run orders with values of 8.4 and 8.6, indicating that the surimi obtained had pure meat specifications (without bones and scales) containing fiber by 5%. The lowest appearance value is obtained from SO8 with a value of 5.6 with a fiber specification of 10%. Although the appearance was

**Table 8.** Effect of dependent factors on the sensory evaluation of multi-species surimi using variables based on Plackett–Burman design.

| Variable | Appearance | Folding test | Biting test |
|----------|------------|--------------|-------------|
| SO1      | 7,6 ± 0,00 | 6,1 ± 0.12   | 7,5 ± 0.10  |
| SO2      | 7,0 ± 0.20 | 7,9 ± 0.02   | 7,1 ± 0.20  |
| SO3      | 8,6 ± 0.10 | 5,0 ± 0.02   | 5,9 ± 0.00  |
| SO4      | 7,4 ± 0.0  | 6,3 ± 0.20   | 6,1 ± 0.10  |
| SO5      | 7,1 ± 0.20 | 5,9 ± 0.12   | 4,3 ± 0.20  |
| SO6      | 7,6 ± 0.04 | 7,1 ± 0.20   | 7,1 ± 0.20  |
| SO7      | 6,3 ± 0.12 | 5,7 ± 0.24   | 5,9 ± 0.00  |
| SO8      | 5,6 ± 0.04 | 8,7 ± 0.04   | 7,2 ± 0.30  |
| SO9      | 6,1 ± 0.06 | 8,0 ± 0.20   | 8,9 ± 0.10  |
| SO10     | 8,4 ± 0.22 | 8,7 ± 0.00   | 6,0 ± 0.22  |
| SO11     | 6,4 ± 0,20 | 9,0 ± 0.00   | 8,9 ± 0.20  |
| SO12     | 7,1 ± 0.20 | 5,5 ± 0.10   | 5,3 ± 0.02  |
| SO13     | 7,7 ± 0.20 | 7,5 ± 0.12   | 8,1 ± 0.00  |
| SO14     | 7,1 ± 0.24 | 7,9 ± 0.02   | 7,8 ± 0.10  |

**Table 9.** Analysis of variance of Plackett–Burman screening regression parameters of dependent variables on the sensory evaluation (appearance, folding test, and biting test).

| Appearance ( $R^2 = 98.26\%$ ) |         | Folding Test ( $R^2 = 99.72\%$ ) |         | Biting Test ( $R^2 = 99.73\%$ ) |         |
|--------------------------------|---------|----------------------------------|---------|---------------------------------|---------|
| F Value                        | P Value | F Value                          | P Value | F Value                         | P Value |
| 12,76                          | 0,174   | 0,01                             | 0,948   | 19,44                           | 0,142   |
| 0,26                           | 0,7     | 9,13                             | 0,204   | 4,51                            | 0,28    |
| 0,38                           | 0,65    | 48,17                            | 0,091   | 0,96                            | 0,506   |
| 20,17                          | 0,139   | 1,5                              | 0,436   | 1,71                            | 0,416   |
| 1,04                           | 0,494   | 12,33                            | 0,177   | 2,16                            | 0,38    |
| 0,26                           | 0,7     | 43,74                            | 0,096   | 40,56                           | 0,099   |
| 3,01                           | 0,333   | 24,81                            | 0,126   | 0,67                            | 0,564   |
| 11,34                          | 0,184   | 121,5                            | 0,058   | 235,63*                         | 0,041   |
| 3,76                           | 0,303   | 1,93                             | 0,397   | 7,71                            | 0,22    |
| 1,5                            | 0,436   | 68,01                            | 0,077   | 6                               | 0,247   |
| 0,67                           | 0,564   | 14,73                            | 0,162   | 10,67                           | 0,189   |

**Note:** The values in the same column with various superscript letters reflect the significant difference at  $p < 0.05$

not statistically influenced by dependent variables, the panelists tended to evaluate surimi with a higher percentage of tilapia fish and carrageenan a lower value. This is because the surimi produced contains more fiber, thereby reducing the panelist's assessment of the surimi produced. The presence of fiber in the surimi is in accordance with the results of the study by Pamungkas et al. (2022), where the surimi of tilapia has the appearance of meat fiber and the absence of foreign bodies in the resulting tilapia kamaboko product. Besides this, the use of carrageenan and tilapia in higher concentrations tends to decrease the whiteness of surimi, so that the panelist gave lower value to the surimi produced.

The folding test is a way to determine the sensory strength of the kamaboko surimi gel produced by folding the surimi gel into 2 or 4. Statistical analysis showed that the folding test is not affected by the independent variables used (Table 9). Panelists gave the highest score to S011, where surimi can be folded into four without any cracks. This is in line with the texture profile, where surimi formulated with run order S011 has the highest gel strength compared to other treatments. Based on the folding test, surimi was produced with a quality level of grade AA (score 5) with a value of 8.5–9 at runs order S011, S010, and S08. Grade A (score 4) was generated from the run orders S02, S06, S09, S013, and S014, while other run orders produced grade B surimi (BSN 2013).

The bite test is one of the methods used to determine the level of strength of the resulting kamaboko gel. Based on the statistical analysis of the bite test of the resulting kamaboko surimi gel was influenced by the presence of *k*-carrageenan. The addition of *k*-carrageenan will encourage the formation of a 3-dimensional network structure, through hydrogen bonding in the hydroxyl groups of the carrageenan polymer. This will cause the water to be trapped in the three-dimensional structure network in a colloidal form, which causes the surimi gel to become stronger (Ramirez et al. 2011; Yu et al. 2022). Panelists gave a better bite test score (7.7) for surimi processed with the addition of carrageenan at the maximum level compared to the minimum level of (6.4) (shown in Figure 6). Based on a normal plot of the standardized effect of dependent factors on sensory evaluation, only carrageenan influences the biting test (Figure 6). According to the surimi quality assessment system at the Surimi Workshop (Pamungkas et al. 2022), surimi with a bite test value of 10 or 9 is categorized into classes 1 and 2, while surimi with a bite test value of 8 is in class 3. Surimi with a value of 7 was classified into class 4, and surimi with a value of 6 was classified into class 5. Results showed that the surimi formulation with run orders S09 and S011 was classified as grade 2, run orders S01, S013, and S014, were classified as grade 3, and run orders S02, S06, and S08 are classified in grade 4. The other run orders were classified in grade 5 or rejected.

## Conclusion

Plackett–Burman's design analysis revealed that moisture content of the multi-species surimi was positively influenced by sodium lactate and *Lattice monocle* bream. Gel strength was affected by sodium

tripolyphosphate, k-carrageenan, and stirring time, while the whiteness degree was influenced by sodium lactate. WHC was positively impacted by k-carrageenan, sodium tripolyphosphate, and *Lattice monocle* bream. The screening process using the Plackett–Burman design concluded that 4 of the 11 selected variables had a positive effect on the main attributes of the physical–chemical of surimi, particularly STPP, carrageenan, Ca-lactate, and the stirring process. These four variables provided effects of different magnitudes according to the resulting coefficient. While tilapia and sorbitol had more negative effects on the physicochemical properties of multi-species surimi. Based on the physical and chemical properties of surimi, the surimi formulation of SO11 consisting of 300 g threadfin bream, 600 g croaker, 300 lattice monocle bream, 300 g tilapia, 36 g sorbitol, 36 g sucrose, 3 g sodium tripolyphosphate, 30 k-carrageenan, 18 g sodium lactate, and 30 g konjac with 15 min stirring period is considered the best formulation, producing surimi with  $3088.25 \pm 1.10$  N Hardness,  $58.83 \pm 0.01\%$  whiteness degree,  $40.72 \pm 0.93\%$  WHC,  $79.99 \pm 0.10\%$  moisture content,  $1.27 \pm 0.18\%$  salt-soluble protein, pH  $5.65 \pm 0.18$ , appearance of  $6.4 \pm 0.20$ , folding test of  $9.0 \pm 0.00$ , and biting test of  $8.9 \pm 0.20$ .

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No potential conflict of interest was reported by the author(s).

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