Revisions made

| No | | RESPON |
|---|---|---|
| 1 | TITLE | no changes |
| 2 | ABSTRAK | STTP abbreviation extension |
| 3 | Keyword | No Changes |
| 4 | Introduction | grammar and Typo fix |
| 5 | Material and Methods | Perbaikan grammers |
| 6 | Results and Discutions | Added anova Placket Burman Table and improved numbering order, as well as discution, grammer and typo fix |
| 7 | Tabels | Table 3 made 3 layers for proximate, fish freshness and surimi characteristics |
| 8 | Figure | Adding Font enlargement |
| 9 | Conclution | No Chanes |
| 10 | Reference | Fixed Reference writing and typos |
| 11 | Other Command | General typo and grammatical error fixes |

Reviewer: 1

Comments to the Author
The authors have not improved the quality of the manuscript. The data do not have analysis of variance and means were not separated. This is very important and should be done. The manuscript contains old references, authors should cite recent publications 2020-2022. 2000 to 2009 citations are old.

Reviewer: 2

Comments to the Author
Abstract needs to contain some data (as in real numbers). Not just an explanation of results.
Table 1. Data with different superscripts in a row….not "raw"
Table 2. Data with different superscripts in a row….not "raw"
Table 3. Data with different superscripts in a row….not "raw"
Table 4. Data with different superscripts in a row….not "raw"
Table 4. Scale for sensory should be included
Table 5. Data with different superscripts in a row….not "raw"
Table 6. Data with different superscripts in a row….not "raw"
Discussion does a good job of referring to previous reported results in the literature.
Conclusion should at least acknowledge a potential negative of using vegetable oils… decreases DHA which could be perceived as a negative for nutritional value of the fillet.
WAFP-2022-0121.R1
Selamat pagi bu Rakhmawati. Terima ksh untuk atensinya. Paper yg sdh dimasukkan hampir 1 th yg lalu baru di.kembalikan lagi dng bnyk mssukkan. Bu Rakhma bisa menjawab line 6, 9. 39 dan 155 ya bu.
Line 6. For this project…not aim
Line 9 physicochemical parameters… not all one word
Line 39 …and good
Line 155 why is salt-soluble protein in bold? Don't start a sentence with a number. It is not clear that the next sentence is a description of the salt-soluble protein. Presenting you methods in a list like this is not typical. Please look at other papers in this journal to see how methods are normally presented.
Suggest you create separate paragraphs
Ex:
Proximate composition Moisture content was analyzed by drying samples in the oven at 105°C for 18 hours. The ash content was determined by burning samples in the furnace at 550 ± 5 oC overnight. Protein content was determined based on analysis of total nitrogen content in the samples, using Kjeldahl method taking 6.25 as the conversion factor value. Fat content was analyzed by solvent extraction methods using Soxhlet.
pH pH analysis was measured with a digital pH meter (Thermo Fisher Scientific Orion). Was sample diluted? If so, explain how.
Total Volatile Bases – Provide a brief description of the method and the reference.
Hardness Surimi, 5 g, was mixed with 50 ml of 5% NaCl solution and homogenized for 2-3 minutes in a waring blender at a low temperature (Balange and Benjakul 2009). The mixture was subsequently centrifuged at 3400 x G for 30 minutes at 10 oC and filtered using Whatman filter paper No. 1. The filtrate was collected in the erlenmeyer and kept at 4 oC. Approximately 25 ml of filtrate was determined for protein content using the Kjeldahl semi-micro method.
Color
Water holding capacity
Salt Soluble Proteins

Line 342 Improve the
Line 352 Check the document to make sure you have gotten rid of all comma in numbers…ex 2687.50 NOT 2687,50
Line 377 Do you mean 1,279 and 1,913.58…. carefully check document to make sure the comma's and decimals are in the appropriate places.

Editor's Comments to Author: As seen above, the first reviewer is still not pleased with the revised manuscript, and the 2nd reviewer has quite some more comments to you paper.

Receiving Editor: 1
Comments to the Author:
(There are no comments.)

Receiving Editor: 2
Comments to the Author:
(There are no comments.)

# Application of Placket Burman Design Analysis for Optimization of the Physicochemical Properties of Multispecies Surimi

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## Abstract

The implementation of strict fishing regulations in Indonesia and Thailand caused scarcity of tropical fish in the Southeast Asia. One strategy for overcoming raw material scarcity is to use multi-species fish. This study aims to screen processing parameters affecting the physicochemical attributes of multispecies surimi using the Plackett - Burman design. There were eleven (11) processing variables evaluated in this study, i.e. fish species, cryoprotectant components (4 variables), gelling agents (2 variables), and stirring periods (1 variable). Surimi was observed for the physicochemical parameters including moisture, salt soluble protein, pH, hardness, whiteness degree, and water holding capacity. The results showed, based on the Plackett - Burman design analysis, that the physicochemical properties that positively influenced the moisture content of multi-species surimi were sodium lactate and *Laticce monocle* bream. The screening process using the Plackett - Burman design concluded that four (4) of the eleven (11) selected variables had a positive effect on the main attributes of the phychochemical properties of surimi, namely Sodium tripoli phospat (STPP), carrageenan, Calsium lactate, and the stirring process. These four variables demonstrated the effects of different magnitudes according to the resulting coefficient. In addition, tilapia and sorbitol have more negative effects on the physicochemical properties of surimi multi-species.

**Key Words**: Surimi, Muti spesies, Chemical properties, Physical properties

## 1. Introduction

Surimi is a myofibril protein concentrate made from minced fish, washed in cold water to remove blood, fat, and sarcoplasmic protein. Fish mince after being washed was mixed with cryoprotectant, and frozen (Walayat et al., 2020). Basically, surimi is an intermediate product and has good gel-forming ability that can be processed into various value-added products such as chikuwa, kamaboko, sausages, kani crab, etc. (Cando et al., 2017). In general, the fish used for surimi production are less economical or underutilized fish, white meat, low-fat fish, abundantly available,and good gelling ability (Watabe et al., 2020). In Southeast Asia (Thailand, Vietnam, Malaysia, and Indonesia), the raw materials use for surimi processing industries are tropical demersal fish species considered before 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 hypopthalmus*), and yellow tail snapper (*Caeso* sp) (Yingchutrakul et al., 2022). However, with the implementation of strict fishing regulations in Indonesia and Thailand, fish catches have decreased drastically, limiting the supply of surimi raw materials ( Djunarsjah et al., 2021). The significantly reduced raw material supply led to the closure of several surimi processing plants in Indonesia and decreased surimi production in China, Vietnam, Thailand, and Malaysia (Guenneugues & Park, 2020). Surimi production of Thailand and China fell from 75.000 to 55.000MT and 225.000 – 160.000 MT during the period 2014 – 2018 respectively. While surimi production in Vietnam and Malaysia both fell by 5.000 MT in 2019 (Guenneugues & Park, 2020). The reduction of raw material availability can be overcome by employing multi-species technology, based on surimi processing through the composition method. That surimi processing method has been explored by Cornellia *et al.,* (2008), Djazuli et al., (2009), and Santoso et al., (2011). The findings from those experiments were that surimi processed using multi-species increased the gel strength and functional properties. Due to the limited availability of fish catches, the raw materials of surimi can also be substituted for cultivated fish whose production increases rapidly more than 18.5 million tons of Asian carp were produced globally ( Yingchutrakul 2022). Multi-species surimi has been implemented in China using silver carp, sea bream, and mixed with ribbonfish (Guenneugues & Park, 2020). While in Vietnam, surimi multi-species using thread bream, red snapper, and other fish with a lower gel such as goatfish, croaker, and bar tail has been commercially produced (Anon, 2017)

The processing of multi-species surimi involves many factors besides the fish itself. 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 gel characteristics of surimi and storage the surimi (Nopiati et al., 2011, Fahrizal et al, 2018). A blend of sorbitol and sucrose resulted in a stronger cryoprptective effect of myofibrillar protein than did sorbitol or sucrose alone.(Yoo, 2014). 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). Other cryoprotectants, such as sodium lactate with a concentration of 8%, are also effective in preventing the denaturation of tilapia surimi protein during frozen storage (Zhou et al., 2006). The addition of 0,3% STTP improved physical properties and had a significant effect on the increasing value of texture, sensory, and microstructure profiles of gel surimi (Etemadian et al., 2012; Julavittayanukul et al., 2006; Laksono et al., 2019). The gelling agent of konjac at a level of 0.5%-2% improves the physicochemical properties of myofibrillar protein and surimi gel. While that agent can inhibit protein denaturation and reduce the decrease in gel strength. (Santana et al., 2013; Liang et al., 2017). The use of 2% refined carrageenan in surimi can improve water holding capacity and gel strength, as well as 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;). Surimi gel was also affected by the stirring treatment, the different stirring durations produce surimi with different characteristics, in which a prolonged stirring period induced changes in the functional properties of protein such as gelling ability (Ducept et al., 2012).

From the above explanation, it is clear that many processing factors are indicated to have an effect on surimi. Plackett–Burman design was particularly helpful in the study to determine those factors (Abdel-Fattah et al., 2005). This method statistically reduces the number of experiments tremendously, thus saving time, glassware, chemicals, and manpower (Quinlan & Lin, 2015). This study was aimed at determining the processing factors that really affect the quality of the surimi by employing a screening process using the Plackett-Bruman design method. Even though this method does not accurately explain the effect of variables on parameters, it can provide important information about the level of significance of each variable on the analysis parameters with just a few experiments (Syamdidi & Suryaningrum, 2015). This approach is popular because it is quite simple. It is a useful tool for screening and searching for variables demonstrating significant effect rapidly in a multivariable system. The method does not require many trials and, most importantly, is statistically reliable (Nguyen et al., 2021. Abdel-Fattah et al., (2005) stated that Plackett - Burman design can identify significant factors quickly and effectively among many variables so that it will save time and clearly reveal all the information from the attributes. Therefore, in this study Plackett - Burman's experimental design was employed to determine the fish species, cryoprotectant types, gelling agents, and stirring times affecting the physicochemical properties of the surimi produced.

## 2. Materials and Methods

### 2.1. Materials

Marine and freshwater fish were both used for surimi processing. Marine fish including threadfin bream (*Nemipterus* sp), croaker (*Argyrosomus japonicas),* and lattice monocle bream (*Scolopsis taeniopterus)* were purchased from the fish landing place of Belanakan, West Java, Indonesia. Freshwater fish of tilapia (*Oreochromis mossambicus)* were obtained from a fresh water fish landing place in Subang, West Java, Indonesia. While, cryoprotectants, namely sucrose, sorbitol, sodium tripolyphosphate (STPP), and sodium lactate were supplied by CV Setia Makmur, Jakarta, Indonesia. The hydrocolloids employed as gelling agents were *k-carrageenan* and konjac, bought from Setia Guna Chemical Shop in Bogor, Indonesia.

### 2.2. Methods

#### 2.2.1. Preparation of Surimi.

Fish used as raw material *for* surimi was head-cut and eviscerated, then passed through a meat bone separator machine to obtain minced fish. The minced was then washed three times in 15 minutes with 5oC cold water in a 1:4 fish-to-water ratio. Approximately 0.5% (w/v) of NaCl was added in the last wash. Water was removed by placing minced fish into a dehydrator machine to reduce the moisture content. The surimi was then ready for further study.

#### 2.2.2. Plackett - Burman Design

A Plackett – Burman *design* was employed to select variables using minimum and maximum values, which were based on the assumption that the value range adopted for each variable still produced surimi. Eleven factors at two levels (minimum and maximum values) were applied for the preliminary screening of the main effects of eleven variables can be seen in Table1.

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

| Independece varaibles | Minimum Value | Maximum Value | Unit |
|---|---|---|---|
| Threadfin Bream | 300 | 600 | g |
| Croaker | 300 | 600 | g |
| Lattice monocle bream | 300 | 600 | g |
| Tilapia | 300 | 600 | g |
| Sorbitol | 6 | 36 | g |
| Sucrose | 6 | 36 | g |
| Sodium Tripoliphosphat | 0.3 | 3 | g |
| *k*-carrageenan | 3 | 30 | g |
| Sodium lactate | 18 | 60 | g |
| Konjac | 3 | 30 | g |
| Stirring period | 5 | 15 | Min |

The minimum and maximum values of variables were selected for the basis of previous experimental and literature reviews. Composition of surimi by mixing based on the type of fish, the type of cryoprotectant, the addition of hydrocolloids, and the length of stirring resulted in 14

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formulations as shown in Table 2.

**Table 2.** The formulation of surimi using various variables based on the Placket-Burman Design method

| STAD ORDER | TDF (g) | CF (g) | LMBF (g) | TF (g) | SORB (g) | SUC (g) | STTP (g) | CARR (g) | SOD LACT (g) | KONJ (g) | STIR TIME (min) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 600 | 300 | 600 | 300 | 6 | 6 | 3 | 30 | 60 | 30 | 15 |
| 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 | 16.5 | 10 |
| 14 | 450 | 450 | 450 | 450 | 21 | 21 | 1.85 | 16.5 | 39 | 16.5 | 10 |

***Note*** : TBF = Threadfin Bream, CF = Croaker, LMBF = Lattice monocle bream, TF = Tilapia, SORB = Sorbitol, SUC= Sucrose, STTP = Sodim Tripolifosfat, CARR = *k*-carrageenan, SOD LACT = Sodium Lactat, KONJ = Konjac ,

The main effect was calculated basically as a difference between the average measurements of each variable made at a high level (+1) and a low level (−1). This design screened variables based on a first-order model: $Y \mid X = A_o + A_1 X_1 + A_2 X_2 + A_3 X_3 + A_4 X_4 + \ldots A_n X_n$ (1), where Y is the response to surimi quality, $A_o$ is the constant, $A_1$, $A_2$, $A_3$, A4,... An is the response coefficient, and $X_1$, $X_2$, $X_3$, $X_4$……$X_n$ denotes the effect of a variable with a value between -1 and +1 (Kuchekar and Pawar, 2014; Sahu & Jain 2017).

### 2.3. Observations

Observations were made on the raw materials and surimi produced. Observations of the raw material were carried out to the proximate composition, fish freshness pH and total volatile bases nitrogen (TVBN) content determined using the standard reference methods of the AOAC (2005). (1). Moisture content was analyzed by drying samples in the oven at 105°C for 18 hours. (2) The ash content was determined by burning samples in the furnace at 550 ± 5$^o$C overnight (3). Protein content was determined based on analysis of the total nitrogen content in the samples, using the Kjeldahl Method taking 6.25 as the conversion factor value (4) Fat

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content was analyzed by solvent extraction methods using Soxhlet, (5) pH analysis was measured with a digital pH meter (*Thermo Fisher Scientific Orion*) and (6) TVBN content was analyzed using Conway methods. The quality of the surimi was determined chemically and physically. The chemical properties are observed on moisture content, pH, and salt soluble protein. The physical properties analyzed were hardness, whiteness degree., and water holding capacity (WHC) (7) **Salt-soluble protein** (Weng and Sheng, 2015). 5 grams of surimi were mixed with 50 ml of 5% NaCl solution and homogenized for 2-3 minutes in a waring blender at a low temperature. The mixture was subsequently centrifuged at 3400 x G for 30 minutes 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. (8) **Hardness** (Balange & Benjakul, 2009). Surimi was blended thoroughly with 30% cold water(5°C) and 3% salt (NaCl), then stirred using a food processor at below 10°C for 10 minutes. 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 minutes, followed by 20 minutes at 90°C. The gel formed was allowed to cool and left in the refrigerator overnight. Hardness was measured using a Texture Analyzer$TAXT_1$ equipped with a spherical plunger (5 mm diameter, 60 mm/min depression speed). (9) **Water Holding Capacity *(WHC)*** (Xiong et al., 2009): The surimi gel was sliced to a 0.5 cm thickness and then weighted (X grams). 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 two minutes. The pressed gel was weighted (Z grams). The WHC was calculated by the difference in weight of surimi before and after being pressed, divided by the weight of surimi gel after being pressed. The result was expressed in percent. (10) Whiteness degree (Pathare et al., 2013). The color of samples was measured using the Color Flex EZ Hunter Lab. The result of color was expressed by the value of the L* value, indicating the brightness or darkness has a value from 0 black to 100 white. . +a * for a reddish color, -a * for a greenish color, +b * for a yellowish color, and -b * for a greenish color.

### 2.4. 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 a p-value <0.05 will be used in further research or optimization. Variables that are declared significant may have more than one test attribute (Karlapudi et al., 2018).

## 3. Results and Discussions

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### 3.1. Proximate Analysis, Characteristics of fresh fish and surimi

The characteristics of fish and the surimi used in this study was shown in Table 3 . The proximate composition of threadfin bream, croaker, lattice monocle bream, and tilapia used for preparing surimi was insignificantly different. 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.

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

| Parameters | Threadfin Bream/ *Nemipterus* sp | Croaker/ *Argyrosomus japonicas* | Lattice monocle bream/ *Scolopsis taeniopterus* | Tilapia/ *Oreochromis mossambicus* |
|---|---|---|---|---|
| Proximate composition | | | | |
| Moisture content (%) | 77.48 ± 0.38 | 79.4 ± 0.10 | 78.00 ± 0.61 | 79.95 ± 0.08 |
| Ash content (%) | 1.37 ± 0.09 | 1.50 ± 0.00 | 1. 95 ± 0.68 | 1.17 ± 0.02 |
| Protein content (%) | 18.88 ± 0.10 | 18.77 ± 0.42 | 18.75 ± 1.13 | 17.72 ± 0.65 |
| Fat content (%) | 0.77 ± 0.19 | 0.66 ± 0.17 | 0.45 ± 0.16 | 0. 81 ± 0. 01 |
| fish freshness | | | | |
| TVBN (mgN/100g) | 20.53 ± 0.38 | 4. 65 ± 1.89 | 16.89 ± 0.75 | 12. 72 ± 1. 00 |
| pH | 6.60 ± 0.21 | 6. 98 ± 0. 04 | 6. 65 ± 0.13 | 6. 60 ± 0. 09 |
| Characteristics of surimi | | | | |
| Yield of surimi (%) | 33.11 ± 1.72 | 34.54 ± 0.76 | 33. 66 ± 0. 24 | 26. 51 ± 1. 40 |
| Moisture content (%) | 80.88 ± 0.98 | 81.21 ± 0.21 | 81.96 ± 0.19 | 81.98 ± 0.50 |
| Hardness (g/cm$^2$) | 1279.77 ± 0.44 | 2060.61 ± 0.74 | 1933.84 ± 061 | 1913 .58 ± 0.52 |
| Whitness degree (%) | 60.18 ± 1.55 | 57.00 ± 1.59 | 62.17 ± 2.00 | 55.95 ± 0.43 |

The protein content of the fish was quite high, i.e., (17,72 ± 0,65%) – (18,88 ± 0,10%), and thus the fish would produce a good gel structure (Bhattacharya & Prajapati, 2016). All fish were classified as lean fish with a fat content of less than 5% (Tasbozan & 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 (Jiao et al., 2019; Lin et al., 2020). Based on TVBN content, croaker fish was considered very fresh (prime quality) with TVBN levels ≤ 10 mgN/100g. Tilapia and Lattice monocle bream were categorized as fresh with TVBN contents of 12.72 mgN/100g and 16.89 mgN/100g, respectively. While the thread bream was fairly fresh with a TVBN content of 20.53 mgN/100g, it was still accepted for consumption. This fish is still at the borderline of freshness and can still be consumed with TVBN 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, that moisture content was slightly higher than the moisture content of commercial surimi according to Indonesian Nasional Standard (SNI) 01-2694.1-2013, i.e., maximum 80%.

Croaker surimi had the highest hardness (2060.61 g/cm$^2$), while the threadfin bream surimi showed the lowest hardness (1279.77 g/cm$^2$). In fresh condition threadfin bream fish make a high-quality surimi with good gel strength, with the average gel strength is 2.424.5 + 22.61 g/cm$^2$ (Nopiati et al, 2011). Surimi produced from this study had a whiteness degree range of (55.95 ± 0.43%) – (62.17 ± 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.

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

To find out the effect of an independent factors on the chemical properties of surimi, 14 experiments were employed to determine by using the formulation of various variables based on the Plackett - Burman design method based in Table 2. The chemical properties of surimi can be seen in Table 4.

**Table 4**: Effect of independent factors on the chemical properties of surimi

| STAD ORDER | 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 |

Surimi had an average moisture content ranging from 78.26–80.90%, with some surimi slightly exceeding the maximum moisture content required for commercial surimi according to Indonesian Nasional Standard i.e. maximum is 80% (SNI 2694, 1. 2013). Accordig Park and Lin (2005) the range of moisture content of commercial surimi were 72-77%, The average moisture contents of surimi prepared in this study were higher than commercial surimi were suggedted by Park and Lin 2005.

According to the Pareto Chart, the moisture content of surimi was significantly affected by sodium lactate, sorbitol, tilapia, and lattice monocle bream, whereas salt soluble protein and pH were not significantly (Fig. 1). The results of the ANOVA analysis showed that the 4 dependent factors had a significant effect with $P < 0.05$ and that there was a linear correlation between water content and the dependent factors with a confidence level of $R = 0.9997$ (Table 5).

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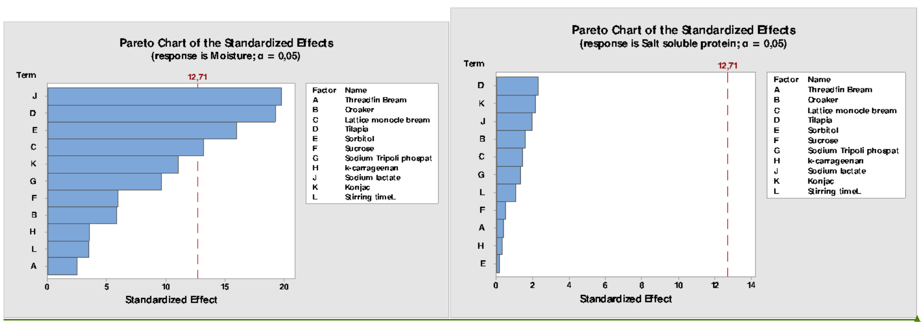
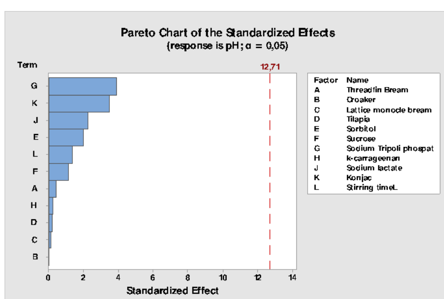



(a) (b)

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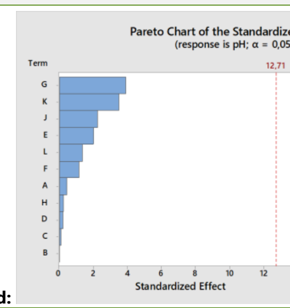

**Figure 1**: Standardized Pareto Chart for (a) Moisture content, (b) Salt Soluble Protein (c ) pH of surimi.

Table 5: Anova Placket Burman Screening Regression Parameters of Dependent Variables on Moisture Content, Salt Soluble Protein, and pH

| | Moisture Content R2= 0,9997 | | Salt Soluble Protein R2 = 0,9619 | | pH R 2 = 0.9839 | |
|---|---|---|---|---|---|---|
| Factor | F Value | P-Value | F Value | P-Value | F Value | P-Value |
| TB | 6.23 | 0.243 | 0.18 | 0.747 | 0.19 | 0.739 |
| C | 34.13 | 0.108 | 2.48 | 0.36 | 0 | 0.984 |
| LMB | 173.9* | 0.048 | 2.06 | 0.387 | 0.02 | 0.919 |
| TLPF | 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 |

***Note*** : TBF = Threadfin Bream, CF = Croaker, LMBF = Lattice monocle bream, TF = Tilapia, SORB = Sorbitol, SUC= Sucrose, STTP = Sodim Tripolifosfat, CARR *k*-carrageenan, Sod LACT = Sodium Lactat, KONJ = Konjac , ,

Based on the normal standard effect, Sodium lactate and lattice monocle bream demonstrated a positive effect on a significant reduction in moisture content. While the addition of sorbitol and tilapia had a significant negative effect on the moisture content of surimi (Fig 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 can increase the water binding and affect the water availability and water activity of meat products. It also maintains color stability while increasing cooking yield due to its humectant properties (Quilo et al., 2009). Sorbitol, on the other hand, is a glucose derivative that can bind water and protein, improve texture, and act as an anti-denaturant (Klinmalai, 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. The addition of tilapia surimi, which has a higher moisture content exhibited a negative effect on increasing the water content of surimi produced.

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) (Fig 1b & 1c). Salt soluble protein is a myofibril protein consisting of actin and myosin that are responsible for gel formation. Based on the obtained stad order, the salt soluble protein content ranged from (1,27 ± .018%) to (1,96 ±0.06%). Although the fish used had different salt soluble

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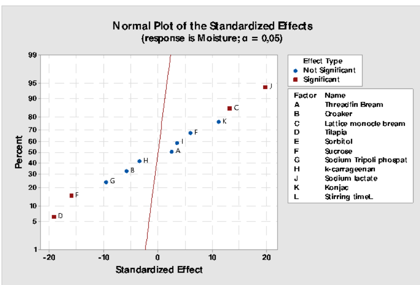


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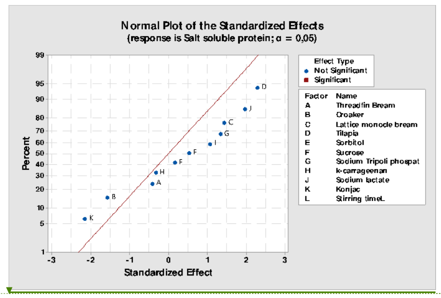


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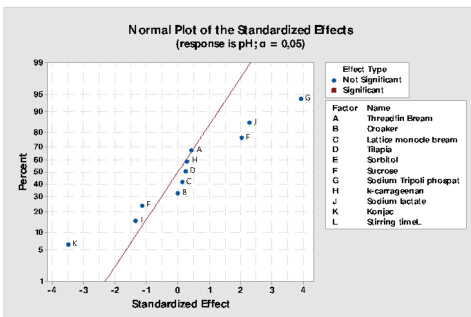


( c )

Figure 2 : Effect of dependent variables on (a) Moisture content (b) Salt Soluble protein and (c) pH

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 were threadfin bream fish, croaker, lattice monocle 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 are obtained. (Gultom et al., 2015).

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 the fermentation of sugar, to produce lactic acid,

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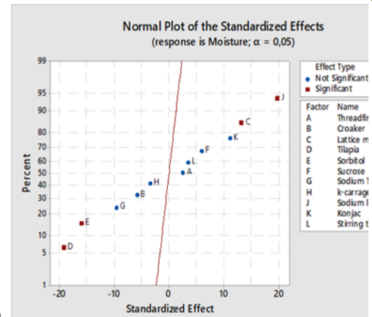


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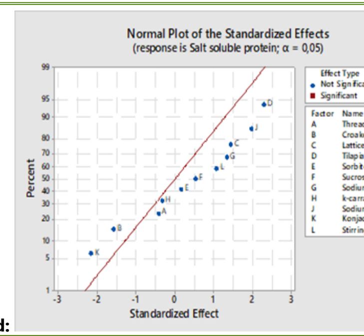


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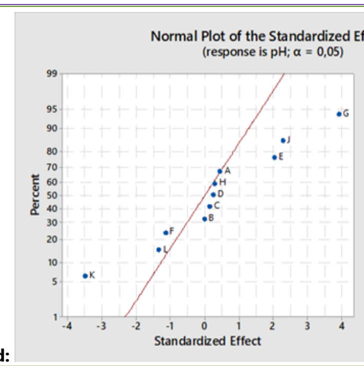

and then neutralized using NaOH to obtain sodium lactate which can cause an acidic taste (Choi et al., 2014). The low degree of acidity can affect the functional properties of surimi. The optimum pH range to produce elastic gel is 6.0-8.0, while the best condition is at a pH of 6.5–7.0. Surimi with a pH of less than 6 will produce a brittle or breakable gel. The gel formed by surimi with a pH of more than 8.0 is not compact, and a pH of less than 6 causes instability of salt-soluble protein or myofibrillar protein in fish meat, indicating a decrease in gelling ability (Sun & Holley, 2010).

### 3.3. Effect of dependent factors on the physical properties of surimi

#### 3.3.1. Hardness

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

**Table 6** : Effect of independent factors on the physical properties of surimi

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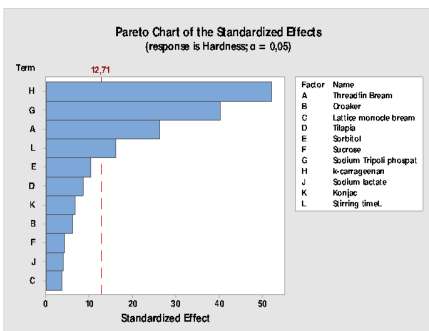
| STAD ORDER | Hardness (g/cm$^2$) | Whitness (%) | WHC (%) |
|---|---|---|---|
| SO 1 | 2296.95 + 1.55 | 70.25 ± 0.01 | 29.90 ± 0.45 |
| SO 2 | 1410.40 ± 1.97 | 62.15 ± 0.07 | 31.24 ± 0.20 |
| SO 3 | 751.62 ± 1.52 | 79.12 ± 0.01 | 16.57 ± 0.73 |
| SO 4 | 897.80 ± 1.22 | 72.50 ± 0.02 | 16.01 ± 0.78 |
| SO 5 | 1230.96 ± 1.15 | 66.08 ± 0.10 | 23.32 ± 1.48 |
| SO 6 | 980.52 ± 1.11 | 65.36 ± 0.08 | 20.44 ± 0.42 |
| SO 7 | 1580.50 ± 1.41 | 68.09 ± 0.08 | 16.72 ± 1.19 |
| SO 8 | 2687.50 ± 1.92 | 58.91 ± 0.01 | 29.68 ± 0.56 |
| SO 9 | 2136.99 ± 1.62 | 69.89 ±0.12 | 24.74 ± 0.78 |
| SO 10 | 996.92 ± 1.44 | 78.95 ± 0.01 | 20.03 ± 1.39 |
| SO 11 | 3088.25 ± 1.10 | 58.83 ± 0.01 | 40.72 ± 0.93 |
| SO 12 | 778. 04 ± 1.87 | 69.37 ± 0.04 | 23.72 ± 0.66 |
| SO 13 | 1536.66 ± 1.25 | 70.99 ± 0.04 | 28.44 ± 0.80 |
| SO 14 | 1586.52 ± 1.37 | 70.40 ±0.08 | 28.02 ± 1.50 |

Based on the 14 experimental formulations, it was seen that the hardness of the surimi gel ranged between (751.62 ± 1.52 g/cm$^2$) – (3088.25 ± 1.10 g/cm$^2$), the whiteness was (58.83 ± 0.01%) – (78.95 ± 0.01%), and the WHC (16.01 ± 0.78%) – (29.90 ± 0.45%). The use of *k*-carrageenan with a maximum value produced surimi with a better gel strength than minimum value such as SO1, S02, SO8, SO9 and SO11 (Table 4). This same results as obtained by Yu et al., (2021), where *k*- carrageenan can function as an adhesive to strengthen this matrix to make it more compact and firmer, however, excessive use of *k*-carrageenan (>2% w/w) seems to cause a decrease in gel strength.

While the Pareto Chart shows (Figure 3 a), only 4 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 4 dependent factors had a significant linear correlation between that dependent factor and hardness with a confidence level of $R^2$ = 0.9997.

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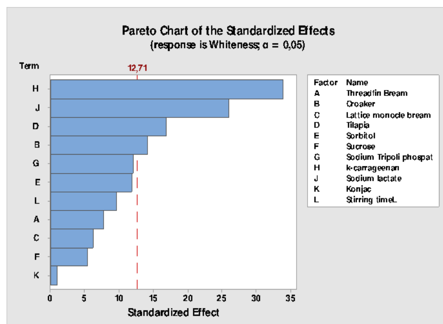


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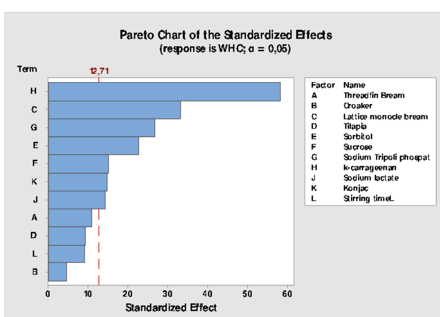


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**Figure 3** : Pareto Chart of (a) Hardness, ( b ) Whiteness and (c) Water Holding Capacity of surimi formulated using Plackett - Burman design



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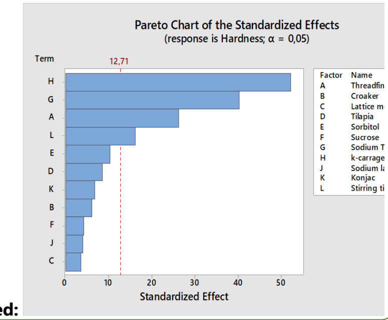


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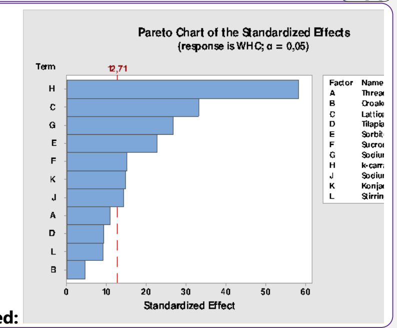


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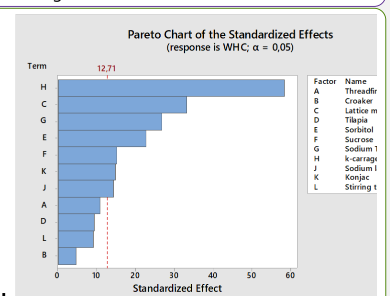


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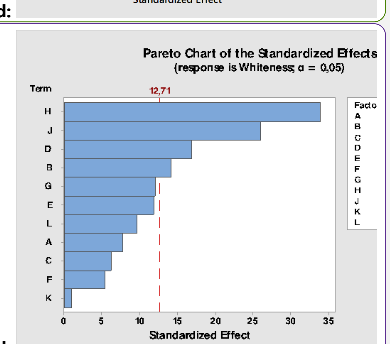


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Table 7: Anova Placket Burman Screening Regression parameters of Dependent Variables on Hardness, Water Holding Capacity (WHC), and Whiteness

| | Hardness $R^2$ = 0,9996 | | WHC $R^2$= 0,9999 | | Whiteness $R^2$ = 0,9995 | |
|---|---|---|---|---|---|---|
| Factor | 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 | 1626.64* | 0.016 | 711.11* | 0.024 | 144.65 | 0.053 |
| CARR | 2715.94* | 0.012 | 3385.63* | 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 |

Based on the normal standard effect, *k-carrageenan*, Sodium Tripolifospat, and stirring times demonstrated a significant positive effect on the hardness of surimi. While the use of Threadfin bream had a significant negative effect on the hardness of surimi (Fig. 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 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 3-dimensional structure network into a colloidal form which causes the surimi gel to become stronger (Ramirez,et al., 2011, Yu et al., 2021). 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 is able to 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 & Guo, 2019).

The stirring times and the addition of carrageenan also increased the gel strength of surimi. Similar result was reported by Liang et al., (2017) that adding *k*-carrageenan and high pressure processing can be a potential method to improve the gel quality of surimi. Tabilo and



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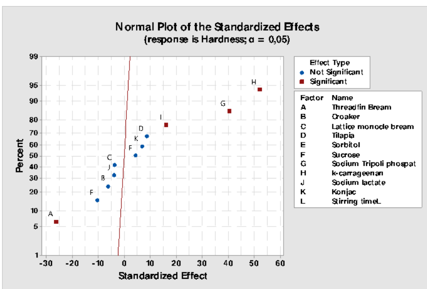
Canova (2004) also noted that the use of ultra high pressure on imitation seafood analogues can improvethe texture properties such as gel strength and elasticity.

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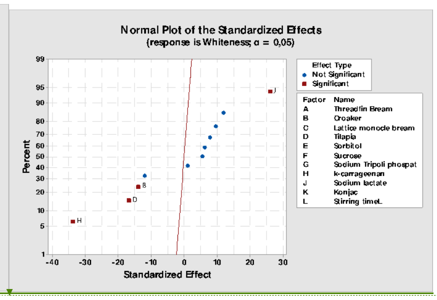


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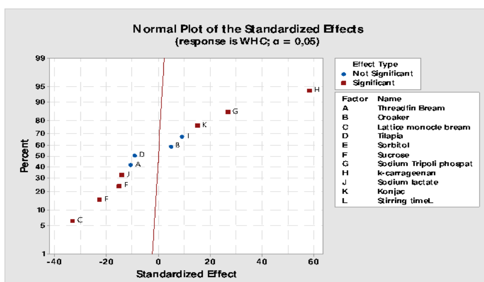


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**Figure 4 :** Effect of dependent variables on (a) Hardness (b) Whitness and (c) Water Holding Capacity (WHC).

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 (2296.95 + 1.55 g/cm$^2$), SO8 (2687,50 + 1.92 g/cm$^2$), and SO11 (3088.25 + 1.10 g/cm$^2$) (Table 4.). 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 et al., 2017). Nopianti et al. (2011) 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 increasing the gel



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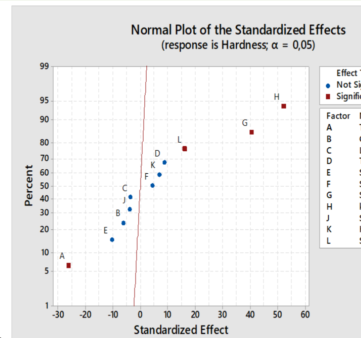

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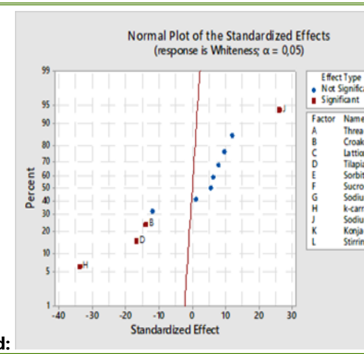

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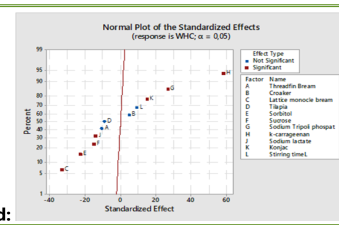

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strength, fracturability, chewiness, gumminess, bite, and folding properties as well as the smooth and solid surfaces of surimi (Laksono et al., 2019). A similar indication is provided by Julavittayanukul et al. (2006) that the addition of polyphosphates combined with CaCI2 can increase the gel strength and water binding capacity of surimi, making it better compared to the one without the addition of polyphosphates. 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 °C which is usually mixed with sorbitol and sucrose as a cryoprotectant (Nopiati et al, 2011).

Based on the normal standard effect (Fig 4 a) the use of thread bream fish had a negative effect on the hardness of the surimi produced. This can be related to the freshness of the thread bream being used has decreased. 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 are released. Denaturation results in the loss of protein functionality, including the loss of gel-forming ability (Julavittayanukul et al., 2006). The hardness of surimi from thread bream was 1. 279 + 0.44 g/cm$^2$ below the hardness of other surimi, 1. 913.58 ± 0.51 – 2060.61 + 0.74 g/cm$^2$ (Table 3). The Threadfin bream used in the experiment were obtained from fishermen who may have kept the fish on ice for more than two days. Thread bream has an indigenous proteolytic enzyme that causes myofibril protein degradation, which causes the gel strength of surimi to decrease (Bashir et al., 2017). Fish kept on ice for more than two days brings about the breakdown of myofibril protein by proteolytic enzymes, thereby reducing the ability to form gels in the surimi (Julavittayanukul et al., 2006). 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 its 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 at the beginning of the freshly lifted common carp from the water had a gel strength of 668 2 g/cm$^2$ decreased to 318.70 g/cm$^2$ at the end of the 15 days ice storage.

Stirring was done to facilitate physically extracting 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 being produced. 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

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This study revealed that 15 minutes of stirring resulted in a better surimi gel than 5 minutes of stirring. 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 strength of the surimi produced as shown 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.

### 3.3.2. Whiteness degree

The highest whiteness degree of surimi was found in SO3 (79.12 + 0.01%) and the lowest was shown by surimi SO11 (58.83 + 0.01 % ) (Table 4.). The whitness degree values of Vietnamese commercial surimi processed from a mixture of tread bream fish, red snapper, and others a mixture of tread break fish, red snapper, and others have 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 Anova and the Pareto Chart (Fig 4 b), the independent factors influencing the whiteness of surimi were *k-carrageenan*, , sodium lactate, tilapia, and croaker fish. The results of the ANOVA analysis showed that the 4 dependent factors had a significant linear correlation between that dependent factor and hardness with a confidence level of $R^2$ = 0.9995. 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 (Fig 4 b). The addition of sodium lactate was in line with a study on catfish surimi conducted by Suryaningrum et al. (2009) demonstrating that the addition of 0.05% lactic acid produced surimi with a better whiteness degree compared to the one without lactic acid addition. 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 an decrease in the whiteness value of surimi gels. While Chen et al. (2020) revealed that the addition of κ-carrageenan remarkably decreased the whiteness of surimi gel, and the whiteness decreased when the addition level of κ-carrageenan reached 0. 5% (b/b). Based on the Stad Order obtained, the k–carrageenan used in this study was approximately 0,25 -2,5% (b/b). The color of commercial carrageenan in the market were used in this study a yellowish-white,. Djaeni et al., (2012) revealed that temperature and drying time of carrageenan produces an unpleasant colour, so that the resulting carrageenan is



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Formatted: Normal, Justified, Indent: Left: 0 cm, First line: 0 cm, Right: 0 cm, Tab stops: 7,34 cm, Left yellowish white colour . Therefore, the addition of *k-carrageenan* at a higher concentration will bring about a decrease in 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 + 1.59% and 55.95 + 0.43 % (Table 3), 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 correspondingly. Abdelwahab (2020) noted that the color indices of Tilapia flesh are L * = 58.08 + 2.26 %, + a* = 6.31 1.07 %, and + b* = 16.42 0.21, indicating that the tilapia flesh is slightly yellowish.

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#### 3.3.3. Water Holding Capacity (WHC)

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 + 0.93%, while the lowest was encountered in the surimi SO10, i.e. 20.03 + 1.39%. According to Anova (Table 7) and the Pareto Chart, (Fig 3 c) the dependent factors that affected the WHC were *k*-carrageenan, lattice monocle bream, STTP, sorbitol, sucrose, konjac, and sodium lactate. The results of the ANOVA analysis showed that the 7 independent factors had a significant linear correlation between that dependent factor and WHC with a confidence level of $R^2$ = 0.9999. 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 (Fig 4 c) . Kim et al, (2018) found that k-carrageenan forms strong complexes with myofibril proteins, which can increase the water-holding capacity, gel strength, and cohesiveness of meat products. Konjac has a high molecular weight (200–2000 kDa) consisting of mannose and glucose. Konjac is not only widely recognized for its strong water-binding ability but is also a synergistic ingredient in protein gelation, water binding, and textural properties of meat products (Chin et al., 2009).

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Gonçalves (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 is able to open the protein structure, which facilitates to hold of more water (Nopiati et al, 2011). 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 at the minimum limit tended to reduce the WHC of the surimi. The addition levels of sucrose and sorbitol ranged from 0.3 to 3%, but 4% sucrose and sorbitol is the most commonly used cryoprotectant in the surimi industry (Bashir et al., 2017). Therefore, the addition of sucrose and sorbitol with a minimum limit had a negative effect on the WHC of surimi.

## 4. Conclusion

Plackett – Burman's design analysis revealed that moisture content of the multi-species surimi was positively influenced by sodium lactate and *Laticce 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 tripolofosfat, and Laticce 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.

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# Application of Placket Burman Design Analysis for Optimization of the Physicochemical Properties of Multispecies Surimi

**Abstract**

The application of Placket - Burman design in investigating the effect of physicochemical attributes of multispecies surimi was performed. For this aim, surimi was made from 5 species of marine and freshwater fish, added with 4 types of cryoprotectant and 2 different gelling agents, thus constructing eleven (11) processing variables. Surimi was observed for the physicochemical parameters including moisture, salt soluble protein, pH, hardness, whiteness degree, and water holding capacity. Based on the Plackett - Burman design analysis, that the physicochemical properties that positively influenced the moisture content of multi-species surimi were sodium lactate and *Laticce monocle* bream. The screening process using the Plackett - Burman design concluded that four (4) of the eleven (11) selected variables had a positive effect on the main attributes of the physicochemical properties of surimi, namely (STPP) Sodium tripolyphosphate, carrageenan, sodium lactate, and the stirring process. Based on the physical and chemical properties of surimi, the S01 formulation is considered the best formulation, producing surimi with a gel strength of 2.296.95 ± 1.55 g/cm2, a whiteness degree of 70.29 ± 0. 01 %, WHC 29.90 ± 0. 45 %, water content of 80.80 ± 0.27 %, salt-soluble protein of 1.87 ± 0.00 %, and a pH of 5.90 ± 0.04 .

**Key Words**: Surimi, multispecies, physico-chemical properties, Placket Burman design.

## 1. Introduction

Surimi is an intermediate product made from minced fish that possess 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 manufacture, the material used for surimi is generally from less economical or underutilized fish, which typically has a white meat, low-fat content, abundance source; and good gelling ability (Watabe et al. 2020).

In Southeast Asia (Thailand, Vietnam, Malaysia, and Indonesia), the raw materials use 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 hypopthalmus*), 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 fish catches drastically, limiting the supply of surimi raw materials (Djunarsjah et al. 2021). The significant reduction of raw material supply leads to the closure of several surimi processing plants in Indonesia and also the decrease of surimi production in several countries including China, Vietnam, Thailand, and Malaysia (Guenneugues and Park 2020). It is reported that surimi production in Thailand and China fell from 75.000 to 55.000MT and 225.000 – 160.000 MT during the period 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 overcomed by varying the composition of surimi, regarded as multi-species surimi technology. It is reported that surimi processed using multi-species possesses high gel strength and functional properties (Santoso et al. 2011). Beside from fish catches, the composition of multi-species surimi can also be substituted from cultivated fish, utilising the increase 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 bar tail (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 (Nopiati et al. 2011; Fahrizal et al. 2018). A mixture of sorbitol and sucrose resulted in a stronger cryoprotective effect of myofibrillar protein than did sorbitol or sucrose alone (Yoo 2014). Other cryoprotectants, were added to improve the gelling properties of surimi is Calsium lactate can increase the protein–protein interactions via the formation of a salt-bridge between negatively charged myofibrillar proteins. Addition of 1.5%, Calsium lactate significantly increased gel strength and whiteness, while cooking loss decreased (Sang et al. 2022).

The addition of 0,3% STTP improved physical properties and had a significant effect on the increasing value of texture, sensory, and microstructure profiles of surimi gel (Etemadian et al. 2012;; and Laksono et al. 2019). The gelling agent of konjac at a level of 0.5%-2% improves the physicochemical properties of myofibrillar protein and surimi gel. While that agent can inhibit protein denaturation and reduce the decrease in gel strength. (Santana et al. 2013; Liang et al. 2017). The use of 2% refined carrageenan in surimi can improve water holding capacity and gel strength, as well as 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 durations 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).

From the above explanation, it is clear that many processing factors are indicated to have an effect on surimi. Plackett–Burman experimental design is used to identify the most important factors early in the experimentation phase when complete knowledge about the system is usually unavailable. (Anand et al, 2018). This method statistically reduces the number of experiments tremendously, thus saving time, glassware, chemicals, and manpower (Quinlan and Lin 2015). This study was aimed at determining the processing factors that really affect the quality of the surimi by employing a screening process using the Plackett-Bruman design method. Even though this method does not accurately explain the effect of variables on parameters, it can provide important information about the level of significance of each variable on the analysis parameters with just a few experiments (Syamdidi and Suryaningrum 2015). This approach is popular because it is quite simple. It is a useful tool for screening and searching for variables demonstrating significant effect rapidly in a multivariable system. The method does not require many trials and, most importantly, is statistically reliable (Nguyen et al. 2020) stated that Plackett - Burman design can identify significant factors quickly and effectively among many variables so that it will save time and clearly reveal all the information from the attributes. Therefore, in this study Plackett - Burman's experimental design was employed to determine the fish species, cryoprotectant types, gelling agents, and stirring times affecting the physicochemical properties of the multi-species surimi.

## 2. Materials and Methods

### *2.1. Materials*

Marine and freshwater fish were both used for surimi processing in this study. Marine fish including threadfin bream (*Nemipterus* sp), croaker (*Argyrosomus japonicas),* and lattice monocle bream (*Scolopsis taeniopterus)* were purchased from the fish landing place of Belanakan, West Java, Indonesia. Freshwater fish of tilapia (*Oreochromis mossambicus)* were obtained from a freshwater fish landing place in Subang, West Java, Indonesia. Cryoprotectants, namely sucrose, sorbitol, sodium tripolyphosphate (STPP), and sodium lactate were supplied by CV Setia Makmur, Jakarta, Indonesia. The hydrocolloids (*k-carrageenan* and konjac) employed as gelling agents, were bought from Setia Guna Chemical Shop in Bogor, Indonesia.

### *2.2. Methods*

#### *2.2.1. Preparation of Surimi.*

Fish used as raw material for surimi was head cut and eviscerated, then passed through a meat bone separator machine to obtain minced fish. The minced fish was then washed three times with 5°C cold water at a ratio of fish : water = 1 : 4. Approximately 0.5% (w/v) of NaCl was added in the final wash. Water was removed using a dehydrator to reduce the moisture content and the surimi was kept in cold storage until further study.

#### *2.2.2. Plackett - Burman Design*

A Plackett – Burman *design* was employed to select variables using minimum and maximum values, which are based on the assumption that the value range adopted for each variable is still produced good quality of surimi. Eleven factors at two levels (minimum and maximum values) were applied for the preliminary screening of the main effects of eleven variables can be seen in Table1.

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

| Independece varaibles | Minimum Value | Maximum Value | Unit |
|---|---|---|---|
| Threadfin Bream | 300 | 600 | g |
| Croaker | 300 | 600 | g |
| Lattice monocle 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 |
| *k*-carrageenan | 3 | 30 | g |
| Sodium lactate | 18 | 60 | g |
| Stirring period | 5 | 15 | Min |

The minimum and maximum values of variables were selected based on previous experimental and literatures. Combination of surimi based on type of fish, type of cryoprotectant, presence of hydrocolloids, and length of stirring resulted in 14 formulations as shown in Table 2.

**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 | 30 | 15 |
| 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 |

***Note*** : TBF = Threadfin Bream, CF = Croaker, LMBF = Lattice monocle bream, TF = Tilapia, SORB = Sorbitol, SUC= Sucrose, STTP = Sodium Tripolyphosphate, CARR = *k*-carrageenan, SOD LACT =

Sodium Lactate, KONJ = Konjac

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: $Y \mid X = A_o + A_1 X_1 + A_2 X_2 + A_3 X_3 + A_4 X_4 + \ldots. \quad A_n X_n$ (1), where Y is the response to surimi quality, $A_o$ is the constant, $A_1$, $A_2$, $A_3$, A4,... $A_n$ is the response coefficient, and $X_1$, $X_2$, $X_3$, $X_4$……$X_n$ denotes the effect of a variable with a value between -1 and +1 (Kuchekar and Pawar 2014; Sahu and Jain 2017).

## 2.3. *Observations*

Observations were conducted on the raw materials and surimi. Observations on the raw material were carried out to the proximate composition, pH and total volatile bases (TVB) content determined using the standard reference methods of the AOAC (2005).

Moisture content was conducted by drying the dish at 105 ºC for 2 hours and cooled in desiccator for 30 minutes. The dish was then weighed until reach the constant weight. 2 grams of samples were placed inside the dish, dried in the oven at 105 ºC for 18 hours, and cooled in desiccator for 30 minutes and weighed. Moisture content (%)

= (B−C) (1)

B−A) $x$

Note: A : The initial weight of empty dish (g)

B : The weight of dish and sample (g)

C : The weight of dish and sample after dried (g).

The ash content was determined by burning samples from the moiture content. The dish was then put in the furnace and burned at 550ºC for 8 hours and weighed after to get ash content.

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

Note:

A: The initial weight of empty dish (g) B: The weight of dish and sample after burned (g)

B: The weight of dish and sample after burned (g)

. (3) Protein content was measured by weighing 2 g of samples, placed inside the destruction flask, added with 2 pieces of boiling rock, 15 mL concentrated H2SO4 (95% - 97%), 3 mL H2O2, destructed at 410ºC for ± 2 hours until the solution clearer, and cooled at room temperature. 25 mL H3BO3 4% solution was prepared, the flask contained the solution resulted from destruction was mounted on a steam distillation apparatus. 50 – 70 mL Natrium hydroxide – thiosulfate (Na2S2O3) 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 (%).,

$$\text{Protein content } (\%0 = \frac{(Va - Vb)\ \text{HCl x N HCl x } 14.007 \text{ x } 6.25}{W} \ x\ 100\% \qquad 3$$

Note:

Va : mL HCl for sample titration (ml)

Vb : mL HCl for blank titration (ml)

N : Normality of HCl standard being used

4,007 : Weight of nitrogen atom

6,25 : Protein conversion factor for fish

W : Weight of sample (g

Fat content was measured by weighing 2 g of samples and extracted with 150 mL chloroform in the soxhlet fat extractor at 60ºC for 8 hours. The mixture of fat and chloroform was evaporated in the flask and dried in the oven at 105ºC for ± 2 hours to remove the residual chloroform and water vapor. The flask was then cooled in the desiccator for 30 minutes and weighed until reached the constant weight.

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

Note: A: The initial weight of empty flask (g)

B: The weight of sample (g)

C: The weight of flask contained fat after extraction (g)

pH analysis was measured with a digital pH meter (*Thermo Fisher Scientific Orion*). Measurement of pH of surimi was performed by dissolving 10 g in 90 mL sterile distilled water. Sample was homogenized and the then the pH was measured using a pH meter. Determination of pH is done after pH meter is calibrated first. After that, the electrodes are rinsed with distilled water and dried. The electrode is dipped in the sample solution, and the pH measurements can be set. The electrode is left immersed for a while until a stable reading is obtained, then the sample pH can be recorded .(Purnomo et al, 2017)

7 **TVB content** was analyzed using Conway methods. 25 g of surimi was added to 75 mL of perchloric acid solution 7% (PCA) filtered with filter paper. Configure the Conway cup, fill the inner chamber of the Conway cup with 1 ml of boric acid, and on the left and right sides each with 1 ml of sample and 1 ml of K2 C03 Shake the cup for 1 minute, then cover the Conway cup which has been smeared with Vaseline. Incubation at 35°C for 2 hours Titration of boric acid in the Inner Chamber with 0.02 N HCl. The titration process is carried out until the boric acid turns pink.

$$\text{TVB-N (mg N/100 g)} = \frac{(Vc-Vb) \times N\ HCl \times 14{,}007 \times 2 \times 100}{W} \qquad (5)$$

Note : Vc = volume HCl solution in sample titration

Vb = volume HCl solution in blank titration

N = Normalitet HCl solution

W = Weight sample (g)

14,007 = Weight nitrogen atom

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

Salt-soluble protein (Weng and Zheng 2015). 5 grams of surimi were mixed with 50 ml of 5% NaCl solution and homogenized for 2-3 minutes in a waring blenderat a low temperature. The mixture was subsequently centrifuged at 3400 x G for 30 minutes at $10^{o}C$ and filtered using Whatman filter paper No. 1. The filtrate was collected in the erlenmeyer and kept at $4^{o}C$. Approximately 25 ml of filtrate was determined for protein content using the Kjeldahl semi-micro method.

(8) **Hardness** (Zeng et al, 2022). Surimi was mixed thoroughly with 30% cold water($5^{o}C$) and 3% salt (NaCl), then stirred using a food processor at below $10^{o}C$ for 10 minutes. 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–$50^{o}C$ for 40 minutes, followed by 20 minutes at $90^{o}C$. 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 –1; test speed = 1 mm·s –1; compression deformation, 75% were set.

(9) **Water Holding Capacity *(WHC)*** (Xiong et al. 2009): The surimi gel was sliced to a 0.5 cm thickness and then weighted x 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 two minutes. The pressed gel was weighted z grams (M2)

. The WHC (100%) $= \frac{M1-M2}{M2} \times 100\%$ (6)

Note : M1 = weight of surimi before pressed

M2 = the weight of surimi gel after being pressed.

(10) Whiteness degree (Duan et al, 2022). The color of 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

$$W = [\,100 - (100 - L^{*})^{2} + a^{+2} + b^{+2}]^{½} \quad \ldots\ldots 7$$

### *2.4. 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 a p-value <0.05 will be used in further research or optimization. Variables that are declared significant may have more than one test attribute (Karlapudi et al. 2018).

## 3. Results and Discussions

### *3.1. Characteristics of raw material and surimi product*

The characteristics of fish as raw material used in this study and the resulting surimi were shown in Table 3. The proximate compositions of threadfin bream, croaker, lattice monocle bream, and tilapia were insignificantly different ($p>0.05$). 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 ± 0.65%) – (18.88 ± 0.10%), and thus the fish would produce a good gel structure (Bhattacharya and Prajapati 2016). All fish were classified as lean fish with a fat content of less than 5% (Tasbozan 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 (Jiao et al. 2019; Lin et al. 2020). Based on TVBN content, croaker fish was considered very fresh (prime quality) with TVB levels ≤ 10 mgN/100g. Tilapia and Lattice monocle bream were categorized as fresh with TVBN contents of 12.72 mg N/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.

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

| Parameters | Threadfin Bream/ *Nemipterus* sp | Croaker/ *Argyrosomusjaponicas* | Lattice monocle bream/ *Scolopsis taeniopterus* | Tilapia/ *Oreochromis mossambicus* |
|---|---|---|---|---|
| Proximate composition | | | | |
| Moisture content (%) | 77.48 ± 0.38 | 79.4 ± 0.10 | 78.00 ± 0.61 | 79.95 ± 0.08 |
| Ash content (%) | 1.37 ± 0.09 | 1.50 ± 0.00 | 1.95 ± 0.68 | 1.17 ± 0.02 |
| Protein content (%) | 18.88 ± 0.10 | 18.77 ± 0.42 | 18.75 ± 1.13 | 17.72 ± 0.65 |
| Fat content (%) | 0.77 ± 0.19 | 0.66 ± 0.17 | 0.45 ± 0.16 | 0.81 ± 0.01 |
| Fish freshness | | | | |
| TVBN (mgN/100g) | 20.53 ± 0.38 | 4.65 ± 1.89 | 16.89 ± 0.75 | 12.72 ± 1.00 |
| pH | 6.60 ± 0.21 | 6.98 ± 0.04 | 6.65 ± 0.13 | 6.60 ± 0.09 |
| Characteristics of surimi | | | | |
| Yield of surimi (%) | 33.11 ±1.72 | 34.54 ± 0.76 | 33. 66 ±0.24 | 26. 51 ± 1.40 |
| Moisture content (%) | 80.88 ±0.98 | 81.21 ±0.21 | 81.96 ± 0.19 | 81.98 ± 0.50 |
| Hardness (g/cm$^2$) | 1279.77 ±0.44 | 2060.61 ±0.74 | 1933.84 ±0.61 | 1913 .58 ±0.52 |

| | | | | |
|---|---|---|---|---|
| Whitness degree (%) | 60.18 ±1.55 | 57.00 ±1.59 | 62.17 ±2.00 | 55.95 ± 0.43 |

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, that moisture content was slightly higher than the moisture content of commercial surimi according to Indonesian Nasional Standard (SNI) 01-2694.1-2013, (BSN, 2013) i.e., maximum 80%.

Croaker surimi had the highest hardness (2.060.61 g/cm2), while the threadfin bream surimi showed the lowest hardness (1.279.77 g/cm2). In fresh condition threadfin bream fish make a high-quality surimi with good gel strength, with an average gel strength is 2.424.5 + 22.61 g/cm2 (Nopiati et al. 2011). Surimi produced from this study had a whiteness degree range of (55.95 ± 0.43%) – (62.17 ± 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.

### *3.2. Effect of independent factors on the chemical properties of surimi*

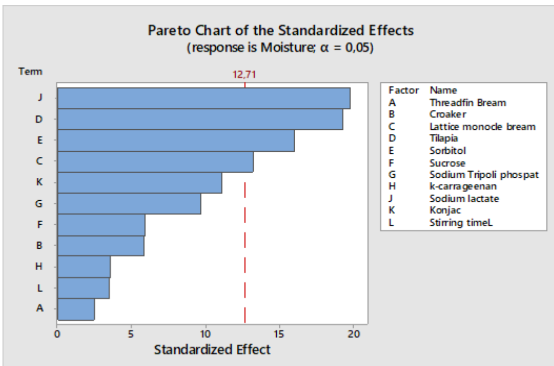
To find out the effect of an independent factor on the chemical properties of surimi, 14 experiments were employed using the formulation of various variables applied in the Plackett - Burman design method (Table 2). The chemical properties of surimi are presented in Table 4.

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 78.26–80.90%, where several treatments produced moisture content slightly exceeding the maximum moisture content required for commercial surimi according to Indonesian Nasional Standard (≤ 80%) (SNI 2694, 1. 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% (Riyanti, 2017)

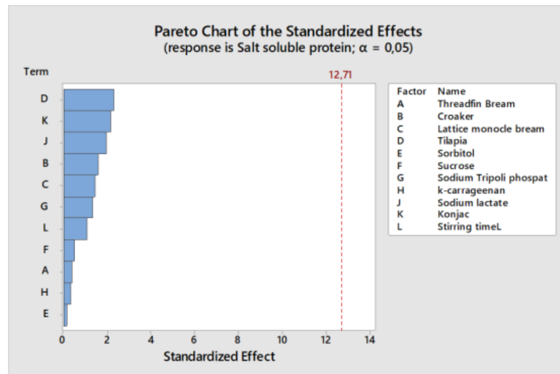
**Table 4**: Effect of independent factors on the chemical properties of multi-species surimi

| STAD ORDER | 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 |

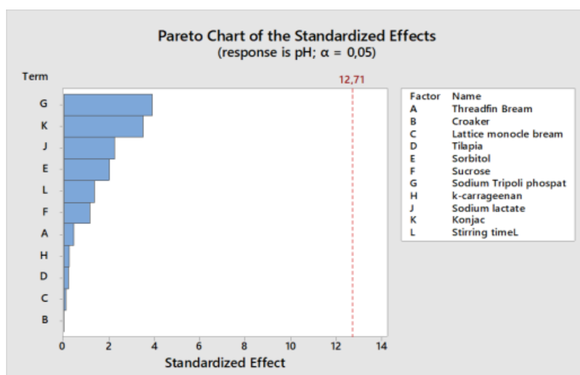
According to the Pareto Chart, the moisture content of the multi-species surimi was significantly affected by sodium lactate, sorbitol, tilapia, and lattice monocle bream, whereas salt soluble protein and pH were not significantly influenced (Fig. 1). The results of the ANOVA analysis that the 4 dependent factors had a significant effect with P <0.05 and that there was a linear correlation between moisture content and the dependent factors with a confidence level of R = 0.9997 (Table 5).




(a)




(b)




(c)

**Figure 1**: Standardized Pareto Chart for (a) Moisture content, (b) Salt Soluble Protein (c ) pH of surimi

Table 5: Anova Placket Burman screening regression parameters of dependent variables on moisture content, salt soluble protein, and pH

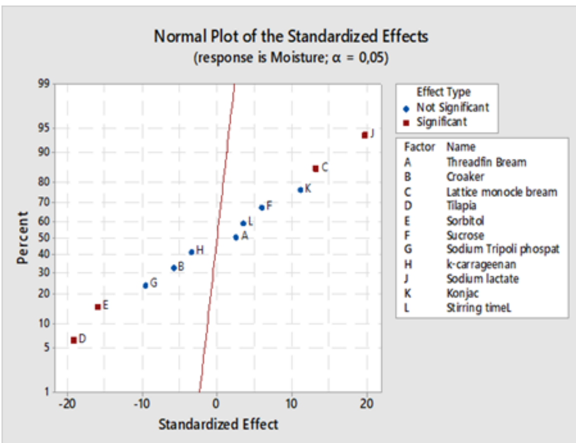
| Moisture content | Salt soluble protein | pH |
|---|---|---|
| R2= 0.9997 | R2 = 0.9619 | R 2 = 0.9839 |

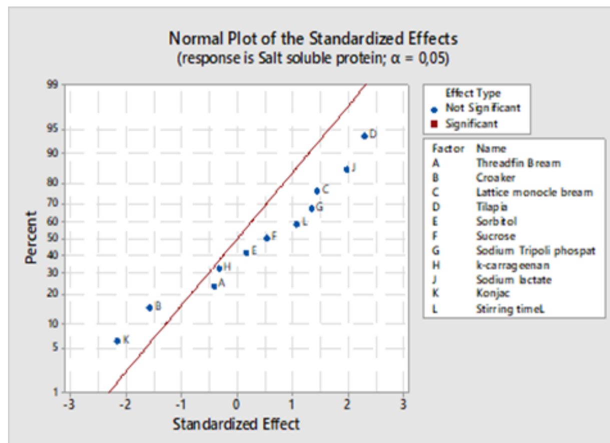
| Factor | F Value | P-Value | F Value | P-Value | F Value | P-Value |
|---|---|---|---|---|---|---|
| TBF | 6.23 | 0.243 | 0.18 | 0.747 | 0.19 | 0.739 |
| CF | 34.13 | 0.108 | 2.48 | 0.36 | 0 | 0.984 |
| LMBF | 173.9* | 0.048 | 2.06 | 0.387 | 0.02 | 0.919 |
| TF | 371.2* | 0.033 | 5.21 | 0.263 | 0.05 | 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 |

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

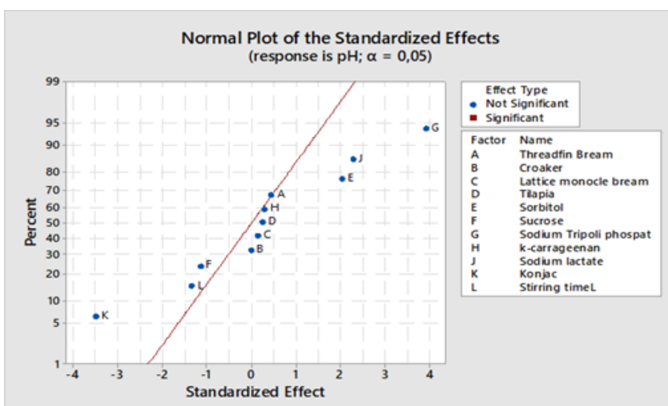
Based on the normal standard effect, sodium lactate and lattice monocle bream demonstrated a positive effect on the significant reduction of the moisture content of surimi, while the addition of sorbitol and tilapia produced an opposite effect (Fig 2a). Sodium lactate is a food additive used as an antimicrobial agent for meat products. Sodium lactate is effective at inhibiting most spoilage and pathogenic bacteria (Choi et al. 2014). The use of sodium lactate can bind water molecules, thereby reducing the moisture content of the resulting surimi, 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 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 ($p<0.5$). In this research, the use of tilapia fish has an impact on increasing the water content of the resulting surimi. This is because surimi from tilapia has the highest water content (79.95 ± 0.08 %) compared to 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 )




(a) (b)




( c )

Figure 2 : Effect of dependent variables on (a) Moisture content (b) Salt soluble protein and (c) pH

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) (Fig. 1b & 1c). 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 ± 0.18%) to (1.96 ±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 monocle 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).

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 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 surimi-based product, especially its physical properties such as hardness, water binding capacity, emulsion properties, and protein rheology (Gao et al, 2018)

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

#### *3.3.1.Hardness*

Observations of the physical properties of surimi from various formulations based on

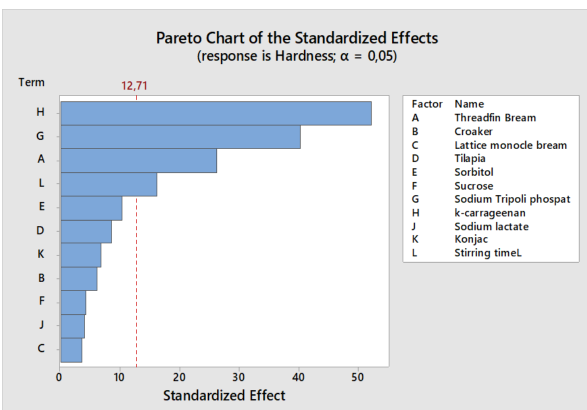
Plackett - Burman design, which was observed on hardness, whiteness, and water holding capacity, can be seen in Table 6.

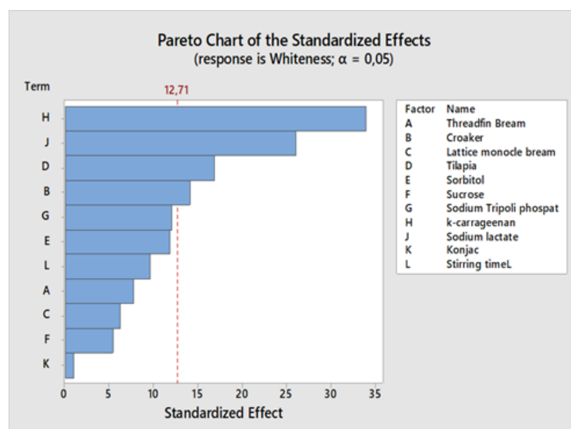
**Table 6**: Effect of independent factors on the physical properties of surimi

| STAD ORDER | Hardness ($g/cm^2$) | Whitness (%) | WHC (%) |
|---|---|---|---|
| SO 1 | 2.296.95 + 1.55 | 70.25 ± 0.01 | 29.90 ± 0.45 |
| SO 2 | 1.410.40 ± 1.97 | 62.15 ± 0.07 | 31.24 ± 0.20 |
| SO 3 | 751.62 ± 1.52 | 79.12 ± 0.01 | 16.57 ± 0.73 |
| SO 4 | 897.80 ± 1.22 | 72.50 ± 0.02 | 16.01 ± 0.78 |
| SO 5 | 1.230.96 ± 1.15 | 66.08 ± 0.10 | 23.32 ± 1.48 |
| SO 6 | 980.52 ± 1.11 | 65.36 ± 0.08 | 20.44 ± 0.42 |
| SO 7 | 1.580.50 ± 1.41 | 68.09 ± 0.08 | 16.72 ± 1.19 |
| SO 8 | 2.687.50 ± 1.92 | 58.91 ± 0.01 | 29.68 ± 0.56 |
| SO 9 | 2.136.99 ± 1.62 | 69.89 ±0.12 | 24.74 ± 0.78 |
| SO 10 | 996.92 ± 1.44 | 78.95 ± 0.01 | 20.03 ± 1.39 |
| SO 11 | 3.088.25 ± 1.10 | 58.83 ± 0.01 | 40.72 ± 0.93 |
| SO 12 | 778. 04 ± 1.87 | 69.37 ± 0.04 | 23.72 ± 0.66 |
| SO 13 | 1.536.66 ± 1.25 | 70.99 ± 0.04 | 28.44 ± 0.80 |
| SO 14 | 1.586.52 ± 1.37 | 70.40 ±0.08 | 28.02 ± 1.50 |

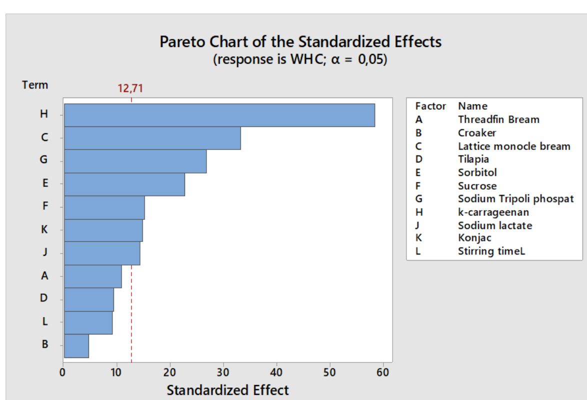
Based on the 14 experimental formulations, it was seen that the hardness of the surimi gel ranged between (751.62 ± 1.52 $g/cm^2$) – (3.088.25 ± 1.10 $g/cm^2$), the whiteness was (58.83 ± 0.01%) – (78.95 ± 0.01%), and the WHC (16.01 ± 0.78%) – (29.90 ± 0.45%). The use of *k*-carrageenan with a maximum value produced surimi with a better gel strength than other treatments ( SO1, S02, 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% w/w) seems to cause a decrease in gel strength.

While the Pareto Chart shows (Figure 3 a), only 4 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 4 dependent factors had a significant linear correlation between that dependent factor and hardness with a confidence level of $R^2 = 0.9997$ (Table 7).




(a) (b)



(c)

**Figure 3** : Pareto Chart of (a) Hardness, ( b ) Whiteness and (c) Water Holding Capacity of surimi formulated using Plackett - Burman design.

Table 7: Anova Placket Burman Screening regression parameters of dependent variables on hardness, Water Holding Capacity (WHC), and whiteness

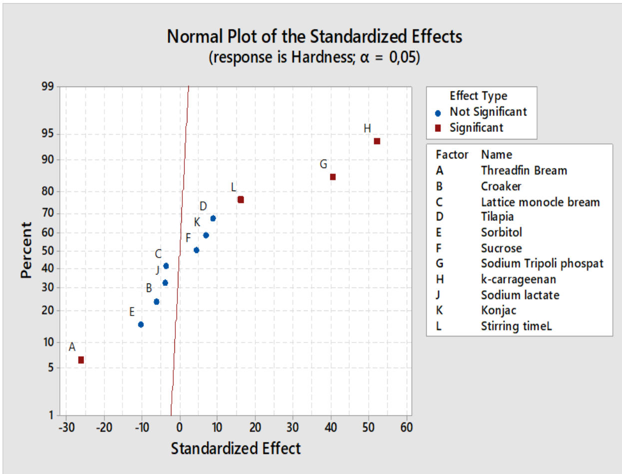
| | Hardness $R^2$ = 0.9996 | | WHC $R^2$= 0.9999 | | Whiteness $R^2$ = 0.9995 | |
|---|---|---|---|---|---|---|
| Factor | F Value | P Value | F Value | P Value | F Value | P Value |
| TBF | 690.54* | 0.024 | 116.53 | 0.059 | 59.48 | 0.082 |
| CF | 37.91 | 0.103 | 22.55 | 0.132 | 198.57* | 0.045 |
| 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 |

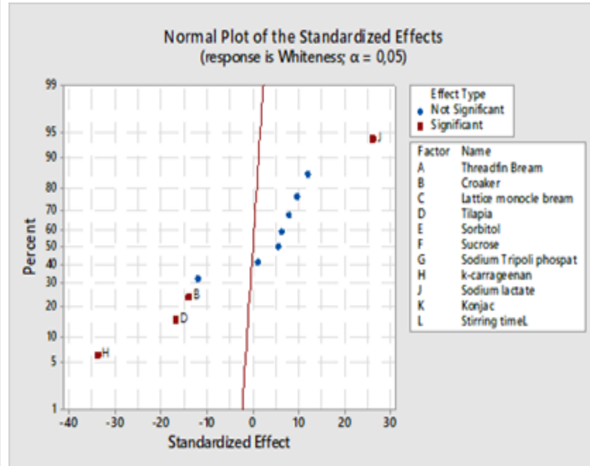
***Note*** : TBF = Threadfin Bream, CF = Croaker, LMBF = Lattice monocle bream, TF = Tilapia, SORB = Sorbitol, SUC= Sucrose, STTP = Sodium Tripolyphosphate, CARR *k*-carrageenan, SOD LACT = Sodium Lactate, KONJ = Konjac.

Based on the normal standard effect, *k-carrageenan*, Sodium tripolyphospate, 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 (Fig. 4a).

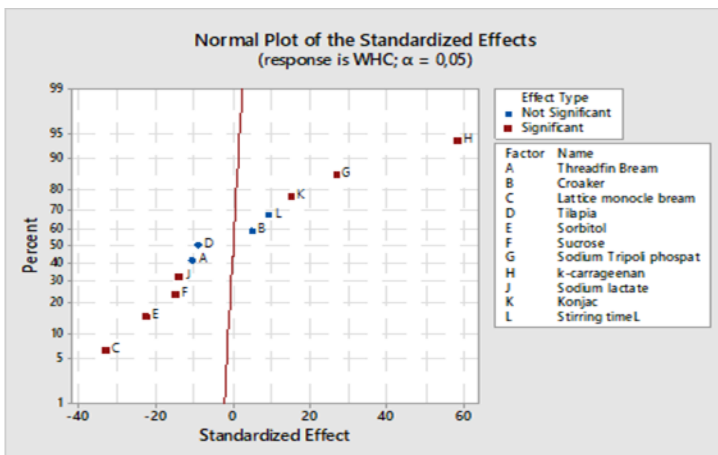
Carrageenan is widely used in the food industry for its unique texture and stability. In this study, *k*- carrageenan was shown as a gelling agent to have an important role in improving the texture of surimi processed from various types of fish. Due to its hydrophilic properties, *k*-carrageenan was able to absorb water in the product and convert it into hydrocolloid form. The addition of *k*- carrageenan will 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 is trapped in the 3-dimensional structure network into a colloidal form which causes the surimi gel to become stronger (Ramirez,et al. 2011; 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 Liang et al. (2017) that adding *k*-carrageenan and high-pressure processing can be a potential method to improve the gel quality of surimi. Meanwhile, Lu et al. (2020) reported in their study that high hydrostatic pressure treatments on *Orechromis niloticus* surimi gels resulted in increased water-holding capacity, color, gel strength, microstructure, texture, and proteins of the surimi gels produced.




(a) (b)




(c )

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

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 g/cm$^2$), SO8 (2.687,50 + 1.92 g/cm$^2$), and SO11 (3.088.25 + 1.10 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 et al. 2017). Nopianti et al. (2011) 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 of 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) reporting that the use of 0.8% phosphate significantly affects the texture parameters of the produced surimi from pike conger fish (*Muraenesox cinerus)*. . 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 $^{o}$C which is usually mixed with sorbitol and sucrose as a cryoprotectant (Nopiati et al. 2011).

Based on the normal standard effect (Fig 4a), the use of thread bream fish had a negative effect on the hardness of the surimi produced. This can be related to the decreased of 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 are released. Denaturation results in the loss of protein functionality, including the loss of gel-forming ability (Moosoud et al,. 2015) The hardness of surimi from thread bream was 1. 279 + 0.44 g/cm$^2$ below the hardness of other surimi, 1. 913.58 ± 0.51 – 2.060.61 + 0.74 g/cm$^2$ (Table 3). The Threadfin bream used in the experiment were obtained from fishermen who may have kept the fish on ice for more than two days. Thread bream has an indigenous proteolytic enzyme that causes myofibril protein degradation, which causes the decrease of 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 strenght decreased from 668. 2 $g/cm^2$ of the freshly lifted common carp to 318.70 $g/cm^2$ at the end of the 15 days ice storage.

Stirring was done to facilitate physically extracting 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 being produced. 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 minutes resulted in a better surimi gel compared to those 5 minutes. 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.

### 3.3.2. Whiteness degree

The highest whiteness degree of surimi was shown by SO3 (79.12 ± 0.01%) and the lowest was shown by SO11 (58.83 ± 0.01 %) (Table 4.). The whiteness degree values of Vietnamese commercial surimi processed from a mixture of tread bream fish, red snapper, and others a mixture of tread break fish, red snapper, and others have 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 (Fig 4 b), 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 (Fig 4 b). Whiteness is an important parameter to determine the quality of surimi, with values greater than 75 generally considered acceptable (Priyadashini 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) increasing calcium lactate contents in surimi, the results showed rising values of whiteness. Therefore, adding 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 the decrease in the whiteness value of surimi gels. Chen et al. (2020) revealed that the addition of κ-carrageenan remarkably decreased the whiteness of surimi gel, and the whiteness decreased when the addition level of κ-carrageenan reached 0. 5% (b/b). Based on the Stad Order obtained, the k–carrageenan used in this study was approximately 0,25 -2,5% (b/b). The color of 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 ±1.59% and 55.95 ± 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 correspondingly. Abdelwahab et al. (2020) noted that the color indices of Tilapia flesh are L * = 58.08 ± 2.26 %, + a* = 6.31 1.07 %, and + b* = 16.42 0.21, indicating that the tilapia flesh is slightly yellowish.

### *3.3.3. Water Holding Capacity (WHC)*

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 ± 0.93%, while the lowest was encountered in the surimi SO10, i.e. 20.03 ± 1.39%. According to the Pareto Chart (Fig 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 (Fig 4c). Kim et al. (2018), found that k-carrageenan forms strong complexes with myofibril proteins, which can increase the water-holding capacity, 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 (Yang et al., 2017).

Gonçalves (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 is able to open the protein structure, which facilitates keeping more water (Nopiati et al. 2011). 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 at 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 commonly 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**

## 4. Conclusion

Plackett – Burman's design analysis revealed that moisture content of the multi-species surimi was positively influenced by sodium lactate and *Laticce 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 *Laticce 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.

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