

# Formulation of nutritious biscuits fortified with minced catfish and *Ulva* sp for malnourished children

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## Abstract

Stunting is a critical problem that deserves attention and has become a global problem. The approach to reducing stunting is to provide nutritious supplementary food in the form of biscuits for children, especially those who are malnourished. The purpose of this study is to formulate nutritious biscuits enriched with minced catfish as a source of amino acids and *Ulva* sp as a source of dietary fiber. Design expert software (DX13) ® was used to analyze sensory data gathered from a hedonic scale assessment with 25 trained panelists to determine the best formulation for the fortified biscuit product. Taste and texture were among the sensory qualities that were assessed. In addition, physical aspects such as hardness and color (L, a, and b) are analyzed to determine the quality of the biscuit formula. The best formulas based on sensory evaluation and physical analysis undergo further analysis of their nutritional composition, including proximate analysis and amino acid profile. The best quality products found are biscuits fortified with a formula consisting of 33% wheat flour, 14% chopped catfish, and 1% *Ulva* flour. The state of the art of this research is the development of functional biscuits fortified with a combination of animal protein (catfish) and dietary fiber as well as antioxidants from *Ulva* sp., using an RSM-based formulation optimization approach to produce a highly nutritious product that is acceptable to children.

**Keywords:** *dietary, sensory, mixture design, seaweed*

## Introduction

Malnutrition is a serious problem that deserves attention. Various forms of malnutrition have become global concerns. As many as 49.5 million children under five suffer from wasting (Golden *et al.*, 2021; UNICEF *et al.*, 2021). Acute malnutrition is a significant global public health issue that affects 49.5 million children yearly, over half of whom die as a result of it (Daures *et al.*, 2020; UNICEF/WHO/World Bank, 2021). Stunting, caused by malnutrition, is indicated by a low height-for-age (HAZ) level. In contrast, wasting is marked by a low weight-for-height (WHZ) level. A low height-for-age (HAZ) level is indicative of stunting, which is brought on by malnutrition. On the other hand, a low weight-for-height (WHZ) level indicates wasting. The World Health Organization (WHO) and UNICEF define severe acute malnutrition (SAM) as meeting at least one of the following criteria: less than 115 mm in the mid-upper arm circumference, a low WHZ, or clinical indications of bilateral oedema (WHO, 2009). According to the World Health Organization's (WHO) child growth criteria, a child is considered stunted if

their height for their age is fewer than two standard deviations below the median. Stunting occurs in children under five years old, which is defined as inadequate linear growth during a critical developmental phase (WHO, 2006). The effects of child stunting are both short-term and long-lasting, encompassing higher rates of illness and death, impaired development and learning ability, greater susceptibility to infections and noncommunicable diseases in adulthood, and diminished productivity and economic potential. The Global Nutrition Targets for 2025 have six targets, the main one being the decrease in stunting among children (WHO, 2012).

In Indonesia, the prevalence of stunting decreased from 27.67% in 2019 to 24.4% in 2021 (Rahmi *et al.*, 2022). In Indonesia, various factors contribute to stunting, including immediate causes like maternal nutrition, breastfeeding habits, complementary feeding practices, and infection exposure. Furthermore, underlying factors including healthcare, food systems, education, and the infrastructure and services related to water and sanitation all play important roles. (Beal *et al.*, 2018). To improve nutritional status during

adolescence and pregnancy, support appropriate gestational growth, and prevent child stunting, efforts should be made before conception and continue at least until the kid is 24 months old. (Dewey *et al.*, 2021; Harper, 1981). Dietary guidelines implemented in some countries promote milk and other dairy foods, as well as foods that are high in protein and calcium for toddler growth and development to reduce the prevalence of stunting. (Ciudadanos *et al.*, 2018; Plaza-Díaz *et al.*, 2020; Slavin, 2012). A wide variety of healthy foods with different tastes, flavors, and textures as complementary foods should be gradually introduced to toddlers (Harris & Pomeranz, 2020).

Popular foods serve as effective carriers for nutrient incorporation, making them a focus for the growing and increasingly demanding market aimed at managing health disorders and preventing malnutrition. (Goubgou *et al.*, 2021). As one of the most popular ready-to-eat foods, biscuits have significant nutritional potential and can be easily modified. Biscuits are also a favored snack among children (Cayres *et al.*, 2021; Giuberti *et al.*, 2021; Urganci & Isik, 2021). Biscuits offer a variety of ways to treat human nutrition-related illnesses. They are frequently consumed as appetizers or as a side dish. They are very well-liked because of their variety of forms, delicious tastes, extended shelf life, and convenience. (Agama-Acevedo *et al.*, 2012; Goubgou *et al.*, 2021; Manley, 2011). Studies on the nutritional composition of commercial biscuits highlight the importance of balancing macronutrients and minimizing unhealthy components like trans fats (Norhayati *et al.*, 2015; Silveira *et al.*, 2023).

Modified biscuits, by adding ingredients rich in amino acids and other nutrients, can help reduce malnutrition and stunting in children. Catfish (*Clarias* sp) which is rich in protein and contains all essential amino acids, includes 16 different types of amino acids (AA), divided into 2 semi-essential (SEAA) types, 7 non-essential (NEAA) types, and 7 essential (EAA) types. Six of these amino acids generate flavors (FAA). The most glycine was present at 14.15%, followed by proline, alanine, and phenylalanine (Zhang *et al.*, 2022). Catfish meat contains 71.30% moisture, 19.03% protein, 8.10% lipids, and 1.5% ash. It is rich in calcium (304.82 mg/100 g), phosphorus (279.45 mg/100 g), iron (17.03 mg/100 g), and essential amino acids, particularly oleic acid (Abdel- Mobdy *et al.*, 2021a). Other research was reported that Catfish extract contains 4.97% water, 8.91% ash, 29.87% fat, 48.85% protein, 7.40% carbohydrates, and 0.12% total polyphenols, with an antioxidant activity measured by an IC<sub>50</sub> value of 4.9724%, highlighting its nutritional potential for improving stunted toddlers' diets (Sihombing *et al.*, 2024).

The average production of catfish increased by 15.84% between 2015 - 2019. In the 2019 saw Indonesia produce 1.2 million tons of catfish, compared to a target of 1.7 tons.

It is anticipated that catfish production will rise by 100% by 2030 (Diatin *et al.*, 2021; Henriksson *et al.*, 2019). Another ingredient that could be added to the biscuits is *Ulva* sp., which contains a high nutrient composition, especially fiber and natural antioxidants. *Ulvans* from *Ulva lactuca* proved potent in antioxidant activity in vitro (Guidara *et al.*, 2021). The dietary fiber content of *Ulva* is advantageous to children. The American Academy of Pediatrics recommends two formulas to determine the bare minimum of fiber consumed each day for children: one depending on the child's weight (0.5 grams of fiber each kilogram, up to 35 grams per day) and the other based on their age (age in years + 5 grams) (Salvatore *et al.*, 2023). Unlike commercial biscuits that are generally low in protein and dietary fiber, and unlike previous studies on fish-based biscuits which focused on other fish species or single nutrient enhancement, this study introduces a novel formulation by combining catfish, as a rich source of essential amino acids, with *Ulva* sp., as a source of dietary fiber and antioxidants. This unique combination has not been previously explored, highlighting the gap in existing research and the potential of such a formulation to provide a more balanced and functional food product for improving children's nutrition.

The objective of this study is to develop a nutritious biscuit intended to prevent malnutrition in children by fortifying it with minced catfish and *Ulva* sp.

## Material and Method

### Material

Catfish (*Clarias* sp) is purchased from a local market, in West Java, Indonesia which is stored in a cooler box and transported to a laboratory. *Ulva* flour is obtained from suppliers in West Nusa Tenggara, Indonesia. The *Ulva* flour used was ready-to-use *Ulva* flour. The ingredients are wheat flour (Kunci Biru, Indonesia), cornstarch (Mama Suka, Indonesia), mung bean flour, milk powder (Dancow, Indonesia), eggs, refined sugar (Claris, Indonesia), coconut oil (Barco, Indonesia), margarine (Blue band, Indonesia), refined salt (Refina, Indonesia), maltodextrin, and sodium bicarbonate, and commercial biscuits as a comparison.

To optimize biscuit formulation, the experiment was set up to model and examine the impacts of four independent variables on response. With 18 sets and 3 independent elements, a mixed design technique is used to accomplish this. This is accomplished using the Stat-

Ease Design-Expert Software (Version 103 13.1.0.1, Stat-Ease, Inc., USA). Three separate ingredients affect the finished biscuit product: Ulva flour, minced catfish, and wheat flour.

### Minced catfish processing

Catfish were cleaned, degutted beheaded, and then filleted. The fillets were washed twice using 0.3% (w/w) salt in cold water (at a temperature of 5-8 °C) for 15 min and stirred. The ratio of water and fillets was 4:1. Then it was pressed at 1300 rpm for 5 minutes, then crushed with a meat cleaver (Panasonic MK-MG 1300, Japan). The minced meat was packaged and frozen.

### Biscuit formulation

Sugar 15%, egg yolk 15%, and ammonium bicarbonate (0.5%) were mixed at high speed until the dough rises. Salt (0.2%), maltodextrin (0.3%), cornstarch (5%), mung bean flour (5%), milk (5%), butter (5%), coconut oil (2%), minced catfish (14%), Ulva flour (1%), and wheat flour (33%) were then added. The dough was mixed until homogen rolled to 5 mm thickness, cut into biscuits, and baked at 140 °C for 25 minutes. The biscuits were stored in plastic jars at room temperature for further analysis.

### Proximate analysis

The proximate analysis followed Indonesian National Standard (SNI) guidelines for moisture content. (BSN, 2006c), crude protein (BSN, 2006b), crude fat (BSN, 2006a), and ash content (BSN, 2010). Moisture content was measured by drying at 105 °C. Crude protein content was determined using the macro-Kjeldahl method.

### Textural analysis

The texture of the samples was analyzed using a TA.XT Plus texture analyzer (Stable Micro Systems, Surrey, UK). The product sheets were divided into 30 mm by 30 mm squares. The spherical probe was attached to the load cell using a probe adaptor (AD/100) on the Heavy-Duty Platform (HDP/90) with a stainless steel spherical probe (P/5S). A 5-kilogram load cell and a 5 mm diameter cylindrical flat-faced probe 129 were utilized. Measurements were performed at a crosshead speed of 1 mm/s, with the probe positioned 20 mm from the surface, following. (Arimi *et al.*, 2010).

### Sensory analysis

A panel of 25 trained assessors conducted the sensory evaluation with a nine-point hedonic rating

system (5 being neither like nor disliked, 6 being somewhat liked, 7 being highly liked, 8 being very much liked, and 9 being extremely liked) (Charutigon *et al.*, 2008; Lawless & Heymann, 2010; Meilgaard, M., Civille, G.V., Carr, 2007; Sinurat *et al.*, 2023) Before being placed on a tray and distributed to the panelists, each sample was given a random three-digit code and scrambled.

### Colour analysis

A HunterLab LabScan XE Spectrophotometer (Hunter Associates Laboratory, Inc., USA) equipped with a D65 light source was used to measure the L\*, a\*, and b\* values to assess the color of the samples. The sample preparation and measurement processes were carried out in compliance with the guidelines provided (del Olmo *et al.*, 2018).

### Amino Acid Analysis

Amino acid composition in the biscuit was analyzed using High-Performance Liquid Chromatography (HPLC) as outlined by (Wang *et al.*, 2011). Amino acids were produced by hydrolyzing proteins, and HPLC was used to measure and identify the resulting compounds..

### Statistical analysis

This research employed Response Surface Methodology (RSM) using Mixture Design - Design Expert 13 (MD-DX13) ®. A hedonic test with 25 trained panelists assessed product liking, with data analyzed using SPSS Statistic V22. All experimental data are presented as the mean values from three repetition along with their respective standard deviations.

## Result and Discussion

### Proximate composition of raw materials

The proximate composition and crude fiber content of the minced catfish and *Ulva* flour are tabulated in Table 1.

The minced catfish showed a high protein content (17.6±0.4%), in line with the reported results Ajibare *et al.* (2023) 17.45±1.7%, Shadyeva *et al.* (2019) 17.03±0.37. Slightly different results were reported by Sulieman *et al.* (2019) and Abdel-Mobdy *et al.* (2021a) the protein content of minced catfish of 18.18±0.20% and 19.03%, respectively. Catfish is categorized as a protein fish, containing 15-20% protein (Shadyeva *et al.*, 2019). The fat content of catfish is low (3.64±0.06%) and It is indicated that catfish is classified as a medium-fat fish (fat content 2-6%) (Shadyeva *et al.*, 2019).

The proximate composition of seaweed varies by species, environmental conditions, season, and geographical location. (Rohani-Ghadikolaei *et al.*, 2012). In this study, the moisture content of *Ulva* was  $12.45 \pm 1.27\%$ , consistent with previous research of  $0.95\% - 14.57\%$  (Khairy & El-Shafay, 2013). *Ulva*'s ash content was  $36.32 \pm 0.62\%$ , higher than reported in another study. (Abdel-Khaliq *et al.*, 2014; Khairy & El-Shafay, 2013; Rasyid, 2017; Rohani-Ghadikolaei *et al.*, 2012). The high ash content is linked to mineral absorption through the thallus surface (Santoso *et al.*, 2024).

The protein content of *Ulva* in this study was  $5.18 \pm 0.05\%$ . This result was lower than the protein content reported by Abirami & Kowsalya (2011),  $12.9\%$ , (Tabarsa *et al.*, 2012);  $10.89\%$ , (Khairy & El-Shafay, 2013)  $16.78 - 17.88\%$ , (Abdel-Khaliq *et al.*, 2014)  $17.6\%$  and (Rasyid, 2017)  $13.6\%$ . The fat content studied in this study was  $0.25 \pm 0.04\%$ . This result is similar to that reported by (Rasyid, 2017), which is  $0.19\%$ , but lower than (Abirami & Kowsalya, 2011)  $1.2\%$ , and Abdel-Khaliq *et al.* (2014)  $0.7\%$ .

Table 1 The proximate composition and crude fiber of minced catfish and *Ulva* flour

No	Parameters	Minced catfish	<i>Ulva</i> flour
1	Water (%)	$71.64 \pm 3.77$	$12.45 \pm 1.27$
2	Ash (%)	$0.74 \pm 0.39$	$36.32 \pm 0.62$
3	Protein (%)	$17.60 \pm 0.00$	$5.13 \pm 0.07$
4	Fat (%)	$3.64 \pm 1.42$	$1.27 \pm 1.44$
5	Carbohydrate (%) (by different)	$8.26 \pm 3.90$	$44.84 \pm 3.25$
6	Crude fiber (%)	$3.71 \pm 0.44$	$25.80 \pm 1.13$

The content of *Ulva*'s crude fiber in this study was  $26.6 \pm 0.41\%$ , this result was similar to (Rasyid, 2017) which was  $28.4\%$ . The high crude fiber content in *U. lactuca* suggests that it may serve as a promising alternative source of dietary fiber.

#### Formulation of fortified biscuits and response analysis

With the use of the DX13® program, the formula determination from the experimental design outcomes of Mixture Design-Design Expert (MD-DX) produced 18 formulas (run orders). Table 2. presents 18 biscuit formulations generated from the Mixture Design-Design Expert, with varying proportions of wheat flour, minced catfish, and *Ulva* sp. flour. The observed responses include sensory attributes (texture and taste), hardness, and color parameters (L, a, b\*), which were analyzed to determine the optimal fortified biscuit formulation.

#### Sensory Properties of Nutritious Biscuits with Minced Catfish

Hedonistic sensory testing of the nutritious biscuits' texture and taste quality characteristics with minced meat refers to SNI 2346:2016 (BSN, 2015) for guidelines on using a 1-9 scale for sensory testing. Sensory reports with scores of three or higher were accepted, whereas those with values lower than three were discarded. Fifteen trained panelists conducted the sensory test.

#### a. Sensory test response for texture and taste

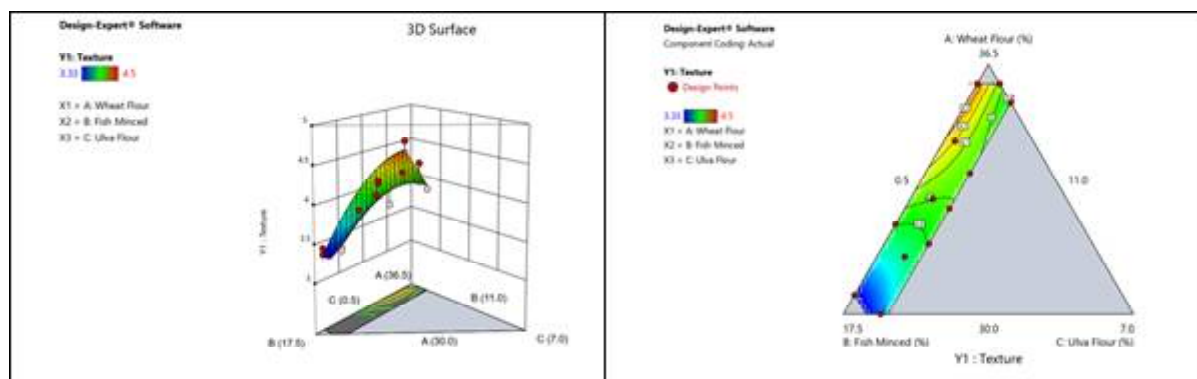
The polynomial equation for the sensory response of texture in fortified biscuits is the square model, with a p-value ( $p < 0.0001$ ) with a coefficient of determination ( $R^2 = 0.8843$ ). Compared to the error of the value of p-value =  $p > 0.3056$  (insignificant).

The texture received a rating from panelists ranging from 3.7 to 4.5, which indicates that it is neutral to highly favored. Biscuits made with 36% wheat flour, 11.5% minced catfish, and 0.5% *Ulva* flour received the highest rating. Biscuits with formula 5 (Table 2) show the best value. Panelists found that this recipe produces biscuits with a texture that is easy to bite into and chew, resulting in the desired crunch. The amount of amylose in wheat flour has an impact on the taste of the food. The ratio of amylose and amylopectin affects the texture of biscuits (Harzau & Estiasih, 2013) In addition, the structure of starch gel, namely the content of amylose and amylopectin, affects the texture of the product including hardness, adhesion, durability, cohesion, and annoyance (Irani *et al.*, 2019). According to Istinganah *et al.* (2017) the moisture content will also affect how soft or hard the texture of the biscuit product is. Figure 1 can give the conclusion that wheat flour tends to respond and contribute more to the texture of the biscuits.

A cubic model with a determination coefficient ( $R^2 = 0.9799$ ) and a p-value ( $p < 0.0001$ ) is the polynomial equation for sensory responses. The sensory reaction to the Lack of Fit was p-value =  $p > 0.1759$ , indicating insignificance.

Table 2. Formulation Runs and Response Results of Minced Catfish and *Ulva* sp. Fortified Biscuits Based on Mixture Design

Run	Wheat flour	Minced Catfish	<i>Ulva</i> flour	Texture	Taste	Hardness	Color		
							L*	a*	b*
1	30.5	17.0	0.5	3.41	4.03	5622.47	65.79	7.39	20.31
2	36.0	11.5	0.5	4.33	3.83	3853.66	53.71	8.54	18.19
3	30.5	17.0	0.5	3.33	4.08	5605.04	65.04	8.90	19.58
4	32.3	15.2	0.5	3.72	4.00	5160.43	58.12	8.28	18.68
5	36.0	11.5	0.5	4.50	3.79	3812.72	53.07	5.65	17.12
6	34.5	12.8	0.8	4.13	4.00	3867.79	61.10	5.65	18.52
7	36.0	11.0	1.0	3.90	4.08	3736.16	56.47	5.06	20.31
8	33.0	14.0	1.0	4.12	4.10	4391.32	59.96	4.21	19.74
9	33.0	14.0	1.0	4.08	4.11	4205.97	56.52	5.87	18.35
10	36.0	11.0	1.0	4.18	4.04	3800.17	56.56	4.99	18.14
11	33.0	14.0	1.0	3.90	4.10	4032.52	54.33	6.41	18.31
12	31.5	15.4	1.1	3.83	4.05	4274.53	62.00	6.40	18.81
13	30.0	16.7	1.3	3.42	4.12	5566.63	63.78	6.78	18.44
14	30.0	16.7	1.3	3.47	4.15	5541.57	59.61	5.78	18.04
15	35.5	11.0	1.5	3.83	3.75	3493.06	57.07	4.14	19.56
16	31.8	14.7	1.5	4.00	3.80	4228.31	58.33	5.21	18.13
17	32.8	13.8	1.5	3.81	3.88	4229.25	58.41	5.53	17.97
18	33.7	12.8	1.5	4.19	3.84	4220.10	59.85	5.83	17.01

Figure 1. 3D graphic and contour of the textural response of biscuits fortified with minced catfish and *Ulva* flour.

The addition of wheat flour and *Ulva* flour will reduce the sweetness of the biscuits, thus affecting the panelists' assessment. According to Gracia *et al.* (2009), Adding water to minced fish will cause sugar to dissolve and decrease the amount of sweetness in the finished cake.

The taste was rated the best at 4.15 (very preferable) and the lowest rate at 3.75 (neutral) Panelists liked the biscuits which are mostly made from wheat flour and contain less *Ulva* flour and minced catfish. The highest sensory taste optimization points with a scale value of

4.15 (like) is indicated by the red area which is greatly influenced by the wheat flour variable. The presence of protein contained in wheat flour can cause a Maillard reaction in a food (Istinganah *et al.*, 2017). The Maillard reaction is a crucial chemical process in baking that occurs between proteins, particularly gluten, and reducing sugars, leading to the development of the characteristic flavor of baked goods like biscuits. The Maillard reaction is responsible for the desirable flavors and aromas in baked items, enhancing their overall appeal (Kerler *et al.*, 2010). Using minced catfish increased the protein content in the biscuits; however,

during baking, the higher protein content promoted Maillard reactions, resulting in a more pronounced bitter taste and off-flavor due to peptide-derived compounds (Liu *et al.*, 2022). Fish rich in free amino acids and nucleotides, such as inosine 52 -monophosphate (IMP) and guanosine 52 -monophosphate (GMP) are known to enhance the umami taste, a savory flavor that is one of the five basic tastes. These compounds are crucial

in creating the umami flavor profile, which is often associated with a pleasant and savory taste experience. The presence of these compounds in fish and other seafood can significantly influence their taste and culinary appeal (Lindemann *et al.*, 2002). Figure 2 can give the conclusion that wheat flour tends to respond and contribute more to the taste of biscuits. The addition of fish meat also contributes to the flavor, even though it is small.

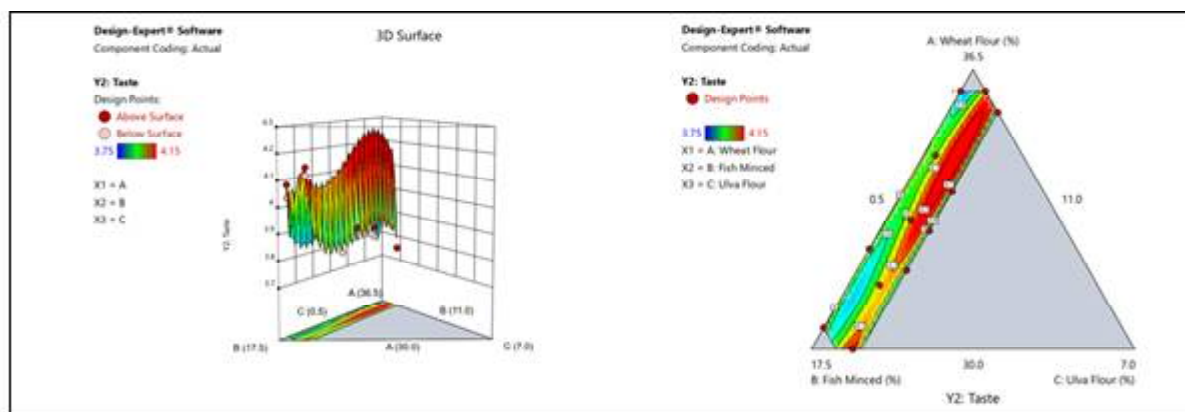


Figure 2. 3D graphic and *contour* biscuit taste response plots fortified with minced catfish and *Ulva* flour.

### Physical Properties Hardness

The polynomial equation of hardness response is a cubic model with a p-value ( $p < 0.0001$ ). The value of the coefficient of determination ( $R^2 = 0.9923$ ) shows that the Cubic model can explain the diversity of hardness of nutritious biscuits by 99.23%. The result of Lack of Fit obtained taste response is p value =  $p > 0.9448$  (not significant) which shows that Lack of Fit biscuit hardness is relatively insignificant to pure error.

Figure 3 illustrates how interactions between wheat flour, minced fish, and *Ulva* flour affect biscuit

hardness. Wheat flour and minced fish significantly influence hardness, which results from gluten-fiber interactions. Dietary fiber increases water absorption and disrupts gluten development. (Najjar *et al.*, 2022). Thus, wheat flour and minced catfish are key contributors to biscuit hardness. The incorporation of *Ulva* flour in biscuit formulations increased product hardness. This effect is mainly attributed to the high dietary fiber and mineral content of *Ulva* flour, which interferes with gluten network formation and results in a denser and firmer texture after baking (Sadkey, 2023).

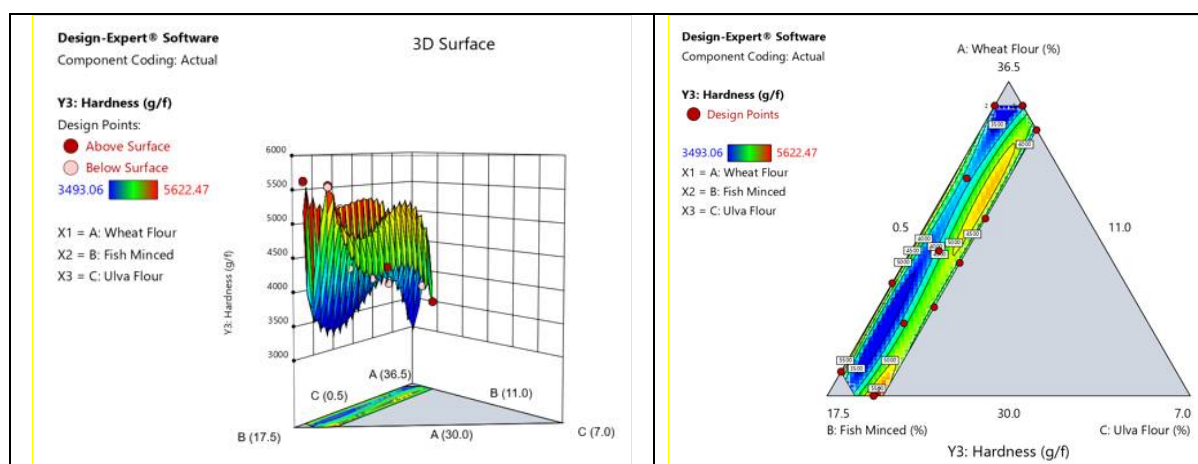


Figure 3. 3D graphic and *contour* of hardness response *plots* of biscuits fortified with minced catfish and *Ulva* flour.



### Sensory test response for color

The color polynomial equation  $L^*$  is a linear model with a determination coefficient value of  $R^2 = 0.5656$  and a p-value of  $p < 0.0019$ . The result of the Lack of Fit obtained by the color response of  $L^*$  is the value of  $p = p > 0.2130$  (insignificant).

The movement of  $L^*$  color from the formulation interaction between wheat flour, minced catfish, and Ulva flour can be seen in Figure 4. The highest optimization points of  $L^*$  color with a value of 65.79 is indicated by the red area which is greatly affected by the variable of chopped catfish. According to (Astiana *et al.*, 2023) the appearance of the color of biscuits turning brown with the addition of surimi is due to the protein content in surimi which makes the biscuits quickly brown or brown on the surface of the biscuits.

The color polynomial equation  $a^*$  is a p-valued linear model ( $p < 0.0011$ ) with a determination coefficient value ( $R^2 = 0.5967$ ). The Lack of Fit result obtained by the color response\* is a value of  $p = p > 0.9175$  (insignificant).

The interaction of color movement formulation between wheat flour, minced catfish, and Ulva flour can be seen in Figure 5. The conclusion is that the highest optimization point of color  $a^*$  with a value of 8.9 is indicated by the red area that is heavily influenced by the minced catfish.

The color polynomial equation  $b^*$  is a square model with a p-value ( $p < 0.3397$ ) with a coefficient of determination value ( $R^2 = 0.5833$ ). The Lack of Fit result obtained by the  $L^*$  color response is the value  $p = p > 0.8652$  (insignificant).

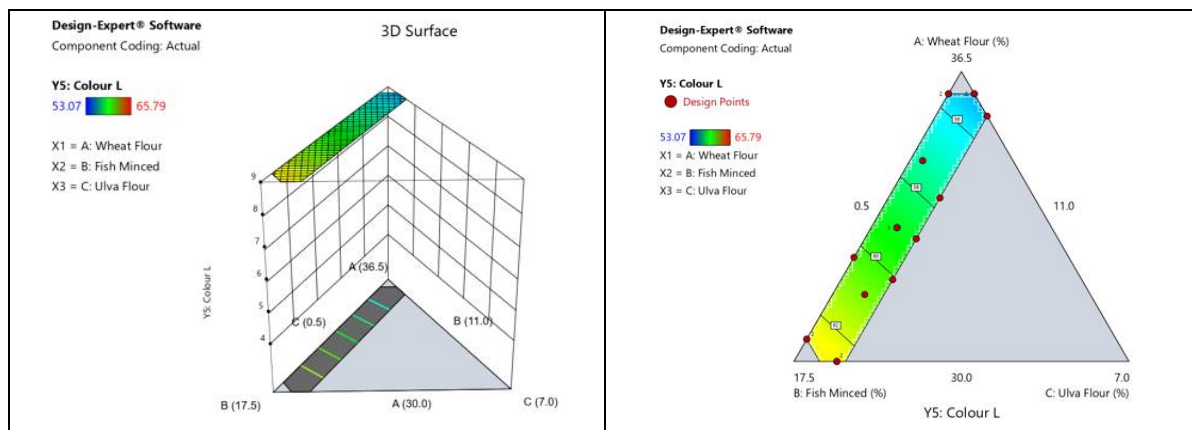


Figure 4. 3D graphic and contour response plot of Color  $L^*$  of biscuits fortified with minced catfish and *Ulva* flour.

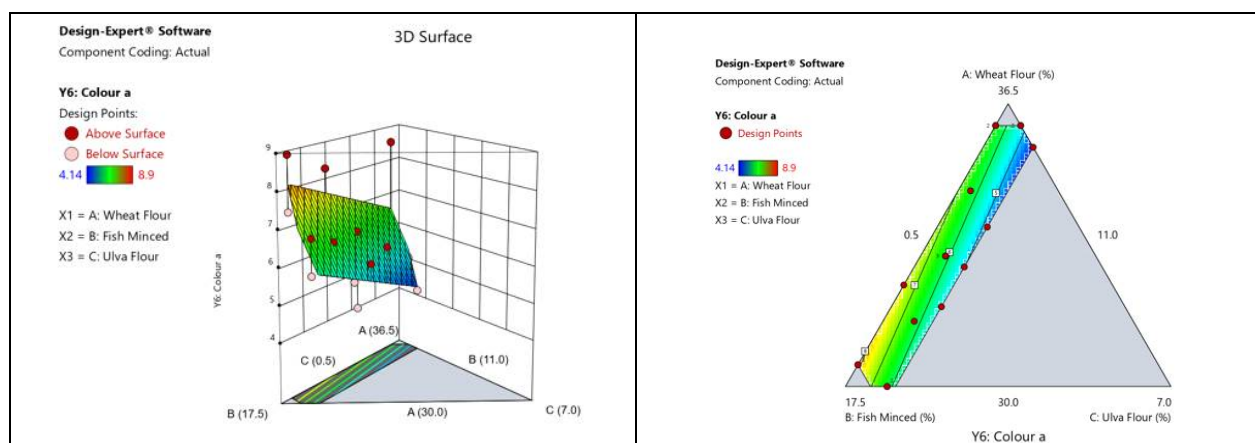


Figure 5. 3D graphic and contour of response plot color  $a^*$  of biscuits fortified with minced catfish and *Ulva* flour.

The interaction of the  $b^*$  color movement formulation between wheat flour, minced catfish, and *Ulva* flour can be seen in Figure 6. The conclusion is that the highest optimization points of color  $b^*$  with a value of 20.11 are indicated by the red area that is greatly influenced by the minced catfish.

### Comparison of Contribution to Nutrition Adequacy

The daily values for biscuits are presented in Table 3. Based on the Recommended Dietary Allowance (RDA) for children aged 4-6 years, the percent daily value is calculated (Indonesian Ministry of Health, 2019).

Table 3. Contribution to nutrition adequacy

Product		Energy (Kcal)	Protein (g)	Fat (g)	Carbohydrate rate (g)	Dietary fiber (g)	Ca (mg)	Mg (mg)	P (mg)	Fe (mg)	Zinc (mg)	K (mg)
Fortified biscuits	Products contain	231.86	6.59	5.75	35.24	0.56	88.4	44.4	99.7	4.2	1.6	185.5
	RDA	1400	25	50	220	20	1000	95	500	10	5	2700
	% RDA	16.6	26.4	11.5	16.0	2.8	8.84	46.8	19.9	42	32	6.9
Commercial biscuits	Products contain	115.93	4.41	8.67	35.64	0.52	103.6	48.7	35.6	4.3	1.6	184.4
	RDA	1400	25	50	220	20	1000	95	500	10	5	2700
	% RDA	8.3	17.6	17.3	16.2	2.6	10.36	51.3	7.1	43	32	6.8
FAO/WHO		1400	25	50	220	20	1000	95	500	10	5	2700

**Note:** Serving size: 50 g

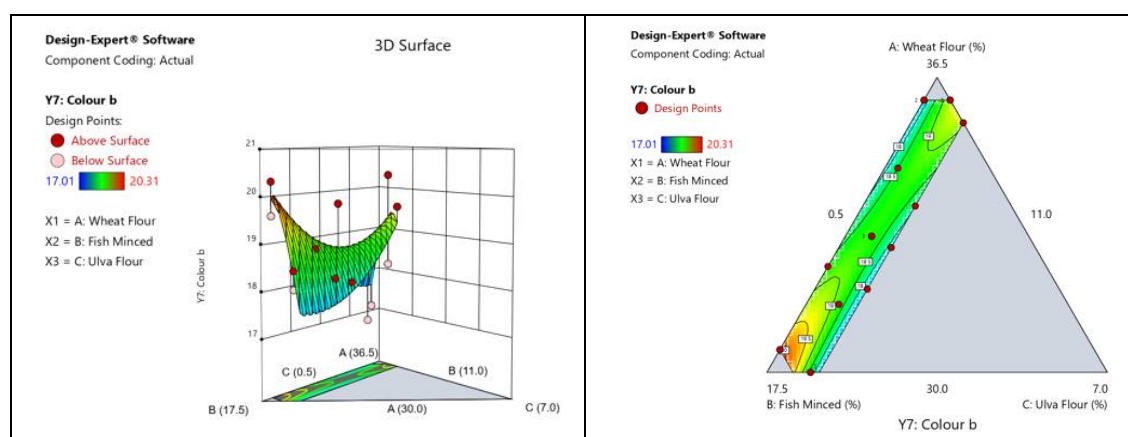


Figure 6. 3D graphic and contour of response plot color  $b^*$  of biscuits fortified with minced catfish and *Ulva* flour.

The daily value for the biscuits is presented in Table 3. Percent daily values are based on the Recommended Dietary Allowance/ RDA of children aged 4-6 years (Indonesian Ministry of Health, 2019). Fortified biscuits provided higher protein, phosphorus, iron, and zinc contents due to the addition of minced catfish and *Ulva* flour, whereas commercial biscuits contained higher fat levels, largely from wheat flour, sugar, and margarine. These nutritional differences highlight the potential role of fortified biscuits in addressing children's nutritional problems, particularly protein, iron, and zinc deficiencies that are closely related to growth and development. The contribution per serving of biscuits was assessed by analyzing their energy and nutrient content and comparing these results to the RDA (Dewi *et al.*, 2020). The recommended daily allowance for energy (16.6%) and protein (26.4%) was greater in the fortified biscuit compared to commercial biscuits (energy 8.3% and protein 17.6%). On the other

hand, the percentages of the RDA for carbohydrate and dietary fiber were similar in both fortified (carbohydrate 16.0% and dietary fiber 2.8%) and commercial biscuits (carbohydrate 16.2% and dietary fiber 2.6%). The commercial biscuits had a higher percentage of the %RDA only for fat (17.6%) compared to the fortified biscuits (11.5%).

The % RDA for protein of fortified biscuits was higher than commercial biscuits. Fortifying biscuits with catfish significantly increased their protein content. The lower %RDA for the fat of fortified biscuits than that of commercial biscuits. According to the results reported by Klerks *et al.* (2023), the fat content in biscuits varied significantly ( $P < 0.05$ ) due to different recipes used by the manufacturers, which affected the amount of fat included in the biscuit formulations. Rutkowska *et al.* (2012) reported that the fat content of various biscuit products ranged from 2.2% to 22.8%. The % RDA for dietary fiber of both



fortified and commercial biscuits was no different. The result was due to the addition of *Ulva* sp. in the formula (1%) being too little to increase the dietary fiber content in the fortified biscuits. According to Klerks *et al.* (2023), the dietary fiber content in biscuits, including those for babies, children, and adults, tends to be low, in the range of 3.0-3.1%.

In terms of mineral adequacy, the fortified biscuits have a higher % RDA of phosphorus (19.9%) compared to commercial biscuits (7.1%), but a lower % RDA of magnesium, with 46.8% compared to 51.3%. The %

RDA of calcium, iron, zinc, and potassium in fortified and commercial biscuits was not different. According to the % RDA, both types of biscuits have a high content of magnesium, zinc, and iron.

### Amino acids profiles

The quality of protein content in biscuits can be evaluated by their amino acid composition. The amino acid profiles of fortified and commercial biscuits are shown in Table 4.

Table 4. Amino acids profile of fortified and commercial biscuits (mg/g DW)

No	Constituents	Fortified biscuits	Commercial biscuits	FAO/WHO
<b>Essential amino acids (EAAs)</b>				
1	Histidine	51	22	18
2	Leucine	79	60	66
3	Lysine	28	48	57
4	Methionine	10	15	25
5	Phenylalanine	61	33	63
6	Threonine	34	31	34
7	Valine	50	37	35
8	Isoleucine	41	34	28
9	Tryptophan	12	11	11
	<b>Total</b>	<b>366</b>	<b>295</b>	<b>337</b>
<b>Non-essential amino acids (NEAAs)</b>				
1	Cystine	15	6	25
2	Arginine	143	48	-
3	Aspartic acid	42	63	-
4	Glutamic acid	418	149	-
5	Serine	54	34	-
6	Glycine	40	28	-
7	Tyrosine	27	21	63
8	Alanine	35	36	-
9	Proline	12.7	36	-
	<b>Total</b>	<b>786</b>	<b>421</b>	<b>88</b>

Amino acid profiles showed that both fortified biscuits and commercial biscuits contain nine categories of amino acids: nine essential and nine non-essential. Compared to commercial biscuits, fortified biscuits contain higher levels of both essential and non-essential amino acids. Compared to the necessary amino acids in commercial biscuits, which are 295 mg g<sup>-1</sup>, the amount of essential amino acids in the fortified biscuits is higher at 366 mg g<sup>-1</sup>. Non-essential amino acid content in fortified biscuits was also higher at 786 mg g<sup>-1</sup> compared to that in commercial biscuits (421 mg

g<sup>-1</sup>). The amount of essential and non-essential amino acids in the biscuits rose dramatically with the addition of minced catfish, which added 24% and 87% of amino acids, respectively. All essential amino acids in fortified biscuits were higher than those in commercial biscuits, except for lysine and methionine. Similar results were observed in the non-essential amino acid content, where most were higher in fortified biscuits, except for aspartic acid and proline.

Table 4 shows that in fortified biscuits, non-essential amino acids are dominated by leucine (79

mg/g), while essential amino acids are dominated by glutamic acid at 418 mg/g. According to Dang *et al.* (2019) and Zhao *et al.* (2003) contributes to umami flavor, affecting the savory taste in the resulting fish biscuits. The components of the sweet taste include glycine, alanine, serine, and threonine, while the components of the bitter taste include phenylalanine, histidine, arginine, leucine, valine, methionine, and isoleucine (Ma *et al.*, 2017). Fortified biscuits showed higher levels of essential amino acids such as histidine (51 mg/g, more than double that of commercial biscuits at 22 mg/g and exceeding the FAO/WHO requirement of 18 mg/g), leucine (79 mg/g, higher than 60 mg/g in commercial biscuits and meeting the FAO/WHO

standard of 66 mg/g), and valine (50 mg/g, compared to 37 mg/g in commercial biscuits and above the FAO/WHO standard of 35 mg/g), with glutamic acid (418 mg/g vs. 149 mg/g in commercial biscuits) being the most dominant non-essential amino acid, indicating a better amino acid profile although lysine (28 mg/g, lower than 48 mg/g in commercial biscuits and below the FAO/WHO requirement of 57 mg/g) remained inadequate.

### Fatty acids profile

The fatty acid profile of fortified and commercial biscuits is shown in Table 5.

Table 5. Fatty acids profile of fortified and commercial biscuits (mg/g DW)

Constituents	Fortified Biscuits	Commercial Biscuits	Standar FAO/WHO
Caprylic acid C8:0	0.50	1.29	
Capric acid C10:0	0.80	0.99	
Lauric acid C12:0	3.00	9.41	
Myristic acid C14:0	8.00	3.97	
Palmitic acid C16:0	22.83	14.54	
Stearic acid C18:0	0.80	2.39	
Arachidic acid C20:0	25.00	0.09	
<b>Saturated Fatty Acids (SFAs)</b>	<b>60.93</b>	<b>32.68</b>	<b>&lt;10%</b>
Palmitoleic acid C16:1	0.80	0.41	
Oleic acid C18: 1	23.58	15.51	
<b>Monounsaturated Fatty Acids (MUFAs)</b>	<b>24.38</b>	<b>15.92</b>	<b>15-20%</b>
Linoleic acid C18: 2	7.48	5.79	
Linolenic acid C18: 3	1.80	0.18	
<b>Polyunsaturated Fatty Acids (PUFAs)</b>	<b>9.28</b>	<b>5.97</b>	<b>6-11%</b>
<b>Total Fatty Acids</b>	<b>94.59</b>	<b>54.57</b>	

Fortified biscuits had a higher content both saturated (SFA) and unsaturated fatty acids (UFA), compared to commercial biscuits. The SFA content of fortified biscuits was dominated by palmitic and arachidic acid. The high content of arachidic acid and palmitic acid in the fortified biscuits is due to the addition of egg (12.5%) and minced catfish (14%) in the formulation. Many studies reported that catfish contain high levels of palmitic acid, with concentrations ranging from 20.62 to 30.74% (Abdel- Mobdy *et al.*, 2021b). Palmitic oil is a vital element in membrane structure, secretory, and transport of lipids. It plays a key role in protein palmitoylation and the function of signaling molecules in children (Innis, 2016). Early nutrition is a crucial factor in determining the composition of the gut microbiota, which can impact metabolic and immune

system development both in the short term and long term. Increased *Lactobacillus* and *Bifidobacterium* colonization of the infant's intestines may be facilitated by palmitic acid (Yaron *et al.*, 2013). A diet rich in 16:0 fatty acids promotes enhanced cell proliferation and decreases apoptosis, which improves the growth and adaptability of the ileum and jejunum in bowel syndrome (Sukhotnik *et al.*, 2011).

Fortified biscuits also have higher levels of unsaturated fatty acids (UFA), including oleic acid (23.58%) and linoleic acid (7.48%), compared to commercial biscuits. The high content of both fatty acids is due to the addition of minced catfish in the fortified biscuits. According to Abdel- Mobdy *et al.* (2021a), the composition of oleic acid and linoleic acid

in catfish is 36.35% and 19.76%, respectively. Linoleic acid is crucial for maintaining “metabolic integrity.” It serves as a precursor to arachidonic acid (ARA), which is vital for the normal growth and development of the brain. In term infants, insufficient levels of linoleic acid have been linked to poor growth and potential skin health problems. (González *et al.*, 2020). Oleic acid has been shown to produce positive anti-inflammatory properties in autoimmune disorders, provide advantages in preventing breast cancer, and strengthen immune system performance. (Sales-Campos *et al.*, 2013).

The higher and more complete total fatty acid content and profile of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids in the fortified biscuits, particularly marked by a tenfold higher level of linolenic acid (Omega-3), is directly influenced by the use of fish meat which is rich in complex lipids in the product's formulation (Garg *et al.*, 2006).

## Conclusion

This study successfully achieved its objective to develop a nutritious biscuit for preventing malnutrition in children through fortification with minced catfish and *Ulva* sp. The optimal formulation, containing 33% wheat flour, 14% minced catfish, and 1% *Ulva* sp. flour, yielded a product with significantly enhanced nutritional profile compared to commercial biscuits. The fortified biscuits contained 15.06% protein—more than double the commercial variant (7.14%)—and exhibited substantially higher phosphorus content (296.48 mg/100 g). The essential amino acid profile was notably improved, with levels of leucine, valine, and isoleucine exceeding the FAO/WHO reference pattern, although lysine content remained slightly below the standard. Furthermore, the total fatty acid content was higher (94.59 mg/g), dominated by beneficial unsaturated fats like oleic acid and linoleic acid. Critically, this nutritional enhancement was achieved without compromising sensory acceptability, as the product received favorable scores for taste, color, texture, and hardness in evaluations. Therefore, the developed fortified biscuit demonstrates strong potential as an effective functional food to address protein and micronutrient deficiencies, thereby supporting efforts to combat malnutrition and stunting among Indonesian children.

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