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RESEARCH PAPER

Optimisation of nutritious biscuits formulation through fortification with catfish (*Clarias gariepinus*) and *Ulva* sp. flours using response surface methodology

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Abstract

The optimisation of biscuit formulation fortified with catfish (*Clarias gariepinus*) and *Ulva* sp. flours was studied. The Response Surface Methodology (RSM) was employed to determine the best formula using three independent variables, namely wheat, catfish, and *Ulva* sp. flour with upper and lower limits of 33.0–36.5%, 8.0–11.5%, and 0.5–2%, respectively. Analysis was performed on protein content, taste, texture, hardness, and colour of the biscuits and was verified by their nutritional quality, including proximate, dietary fibre, sensory and physical properties, and amino acid profiles minerals, vitamins, as compared to commercial biscuits. The results indicated that the biscuits fortified with catfish and *Ulva* sp. contained 19.38 ± 1.02% of protein, 11.20 ± 0.76% of fat, 63.52 ± 0.89% of carbohydrate, 495 kcal of energy, and 20.83 ± 1.87% of a dietary fibre, which were meeting the Recommended Dietary Allowance (above 20%) for energy, protein, dietary fibre, minerals (magnesium, potassium, iron and zinc), vitamin A, and B6. The biscuits also contained essential amino acids exceeding the Recommended Dietary Allowances (RDA) standards for children established by FAO/WHO, thus it is recommended to prevent malnutrition.

Keywords

Nutritious biscuits, catfish (*Clarias gariepinus*), *Ulva* sp., Response Surface Methodology, formulation

Introduction

Malnutrition in children under the age of 5 has become the major concern worldwide recently. This continuing malnutrition leads to stunting and wasting in children, a disruption in the children's growth and development, which is characterised by inhibited growth and inadequate psychosocial stimulation (Deshpande and Ramachandran 2022). The stunted children receive an average intelligence quotient (IQ) score eleven points below the average IQ score of normal children, affecting academic performance, increasing the risk of obesity, making them more susceptible to non-communicable diseases and raising the risk of degenerative diseases (Zhang et al.

2021). Countries with the highest prevalence of stunting are concentrated in South and Southeast Asia and Africa, including Indonesia, reaching 21.6% in 2023, surpassing the 20% threshold established by the WHO (Vaivada et al. 2020; Hatijar 2023). Therefore, improving the population's nutritional status is an of important means to reduce the prevalence of stunting, which can be achieved by providing high-nutrient supplementary foods, such as biscuits (Astuti and Noor 2023).

Biscuits have a longer shelf life, making them suitable for distribution to remote areas in stunting bags. According to the Indonesian Central Bureau of Statistics (2023), biscuit consumption in Indonesia accounts for 5–8% of the total population, with a per capita consumption of 24.22 ounces (0.1 kg) per year. As supplemental food for children, biscuits must have an energy content of 450 kcal, 14 g of fat, 9 g of protein, 71 g of carbohydrate, vitamins (vitamins A, B1, B2, B3, B6, B12, D, E, K, and folic acid), and minerals (iron, zinc, phosphorus, selenium, and calcium) (Setyawati et al. 2023). However, commercially available biscuits typically have high levels of carbohydrates and fats, while their protein, dietary fiber, and mineral contents are relatively low (Indonesian Standardization Society 2023). Fortification of biscuits with dietary fibre, protein, and antioxidants is aimed at providing better nutrition for children's health. Catfish is a source of high-quality protein (18.9–20.34%) with sufficient essential amino acids as well as minerals such as calcium, phosphorus, iron, and vitamin A in substantial amounts, which is beneficial for children growth (Abdel et al. 2021). Catfish also contains high essential amino acids like lysine, threonine and tryptophan (Adepoju et al. 2022). Fortification of biscuits with catfish can enhance the protein content, especially for the essential amino acids, however, it affects their sensory attributes (Canti et al. 2023).

Another issue faced by children is they often lack vegetable consumption, causing insufficient dietary fibre intake. Dietary fibre plays a role in preventing constipation and maintaining intestinal health, improving intestinal function, and contributing to children's ideal growth and development (Hosjack et al. 2022). The World Health Organisation (WHO) recommends a daily intake of dietary fibre for children aged 2–5, 6–9, and 10 years and older are 15, 21, and 25 g/day, respectively (Salvatore et al. 2023). One of the good sources of dietary fibre is seaweed. *Ulva* sp. seaweed is reported to contain 28.4% of dietary fibre, 13.6% of protein, 0.19% of fat, and is rich in minerals (Rasyid and Timur 2017; Sinurat et al. 2021; Kusumawati et al. 2022).

The purpose of this study is to obtain the optimal formulation of nutritious biscuits using Response Surface Methodology with the Mixture Design-DX 13® software. This method can effectively find the optimum formulation consisting of 2–24 components with varying ranges, with a high level of confidence, by using response data from the respective parameters of the preparations (Damayanti et al. 2018; Sabariman et al. 2021). The use of catfish flour and *Ulva* sp. flour as fortifying agents in the biscuit formulation becomes the novelty in this research since they can provide important substances needed for children's development. The optimal biscuits formulation is expected to contain essential nutrients that can be used as supplementary meals to prevent malnourished children.

Materials and methods

Materials

The materials used in this research were live catfish (*Clarias gariepinus*), purchased from a catfish vendor at Cipayung Market, East Jakarta, Indonesia. *Ulva* sp. flour (80 mesh size, porosity 177 μm) was purchased from processors in Lombok. Other ingredients were wheat flour, corn starch, green bean flour, eggs, powdered milk, fine sugar, butter, coconut oil, salt, ammonium bicarbonate, vanilla L-arome and maltodextrin.

Preparation of catfish flour

Catfish was filleted, and then the fillets were washed with cold water (5–8 °C) with a water-to-catfish ratio of 4:1 for 15 minutes. The washing process was repeated three times and added with 0.3% (v/w) salt at the final wash. The washed catfish meat was drained using a spinner at 1300 rpm for 5 minutes. The fillet meat was then ground and steamed (90–95 °C) for 30 minutes. The steamed catfish meat was pressed, shredded on a tray, and dried in an oven (50–60 °C) for 18 hours. Then, dried catfish meat was ground into flour using a blender and sieved to obtain 80-mesh catfish flour.

Biscuit processing

To prepare 100 g of biscuits, 13.5 g of sugar, 15 g of eggs yolk, and 0.5 g of ammonium bicarbonate were mixed using a high-speed mixer until well incorporated and the dough was expanded. Following this, other ingredients, including 0.2 g of salt, 0.3 g of maltodextrin, 5 g of corn starch, 5 g of green bean flour, 5 g of milk powder, 5 g of butter, and 5 g of coconut oil, as well as wheat flour, seaweed flour and catfish flour at certain amount set by the Response Surface Methodology, were added to form a homogeneous dough. The dough was rolled out to a thickness of 5 mm, shaped, and baked in an oven at 140 °C for 25–30 minutes. The baked biscuits were stored in plastic containers for further analysis.

Experimental design

The optimization formulation of the biscuit was conducted using Response Surface Methodology (RSM) with the Mixture-Design Expert 13 (DX13)® software (USA). The independent variables chosen were wheat flour (A), catfish flour (B), and *Ulva* sp. flour (C). The upper and lower limit values for catfish flour, wheat flour and *Ulva* sp. flour were set based on the preceding work, i.e 33–36.5%; 8–11.5%; and 0.5–2%, respectively (Ikasari et al. 2020; Sinurat et al. 2021). In this study, the ratio between independent and fixed variables was 48:52%. The obtained range was processed by the MD-DX13® software, resulting in 18 formulas or run orders (Table 1). The dependent variables for the formulation of biscuits consist of green been flour (5%), corn starch (5%), milk powder (5%), egg yolk (15%), fine sugar (13.5%), butter (5%), coconut oil (5%), vanilla extract (milk aroma) (0.5%), ammonium bicarbonate (0.5%), and maltodextrin (0.3%).

Table 1. Run order in the optimization research design for the biscuit's formulation.

Run Order	Wheat flour (X1) (%)	Catfish powder (X2) (%)	*Ulva* sp. flour (X3) (%)
1	33.00	10.75	1.25
2	36.50	8.00	0.50
3	34.38	9.38	1.25
4	34.75	9.75	0.50
5	33.67	9.33	2.00
6	33.00	10.75	1.25
7	36.50	8.00	0.50
8	34.38	9.38	1.25
9	35.06	8.69	1.25
10	34.75	9.75	0.50
11	33.69	10.44	0.88
12	33.00	11.50	0.50
13	35.75	8.00	1.25
14	33.00	10.00	2.00
15	34.33	8.67	2.00
16	35.00	8.00	2.00
17	35.75	8.00	1.25
18	34.38	9.38	1.25

Experimental analysis

The experimental analysis was performed on the raw materials and the biscuits product. Total Volatile Base Nitrogen (TVB-N) was performed using Conway methods (Indonesian Standard SNI 2354.8.2009) (BSN 2009), while pH of fresh catfish was analysed using a digital pH meter (Thermo Fisher Scientific Orion) to investigate the freshness of catfish meat. Catfish flour and *Ulva* sp. flour were analysed on proximate values, including (1) moisture content which was analysed by drying samples in the oven at 105 °C for 18 hours, (2) ash content was determined by burning samples in the furnace at 550 ± 5 °C overnight, (3) 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, and (5) fat content was analysed by solvent extraction methods using Soxhlet based on the standard reference methods of the AOAC (2005). The crude fibre was determined gravimetrically after chemical digestion and solubilization of another materials present. The fibre residue weight was then corrected for ash content after ignition (Kaizo and Zibula 2023). The wheat flour was produced by PT Bogasari, which contains 11% of protein (medium protein), 1.5% of fat, and 73% of carbohydrates.

The biscuits samples were evaluated on the responses to nutritional quality i.e the protein content, sensory properties (taste and texture), and physical properties (hardness and objective colour L*a*b*). The sensory properties of the biscuits (appearance, aroma, taste, and texture) were evaluated through a hedonic test on a scale of 1–5 according to Indonesian Standard SNI 2346: 2011 (BSN 2011) by involving 30 well-trained panellists from the National Research and Innovation Agency of Indonesia. Panellist were introduced to the products parameters in the preliminary test, including appearance (color and neatness), aroma (fishy, sweety, undesired odor), taste (fishy, sweety, bitterness), and texture (hardness and crispiness). The sample was prepared using randomized three-digit numbers. Texture profiles of biscuits were assessed on the hardness and fracturability using a Stable Microsystem TAXT Plus (UK), with a 3-Pb-type probe at a test speed of 1.0 mm/s at 3 mm and the results were expressed in compressive force (g) (Istinganah et al. 2017). Colour measurement was performed using a 3nh NH 310 Digital Colorimeter (China), a portable colorimeter that is connected to a personal computer with a measuring aperture Φ8 mm/Φ4 mm. The biscuit samples were mashed into powder and put in the chamber to measure, and the results were presented as L*, a*, and b* values. The L* value indicates lightness, where higher values correspond to lighter colours. Meanwhile, a* represents red/green values, and b* represents blue/yellow values (Pathare et al. 2013). All measurements for both the raw materials and the biscuits product were run in triplicates.

The optimized biscuits by MD-DX 13® software were then observed in terms of nutritional value, sensory and physical properties, amino acid profile, minerals, omega 3, 6, 9 and vitamins compared to commercial biscuits. The commercial biscuit used in the experiment was also fortified with seaweed, which is obtained from Small Medium Enterprise industry. The amino acid and vitamins analysis were analysed using high performance liquid chromatography (HPLC). This method is based on that of Antoine et al. (1999) with several modifications. The sample was prepared by extracting ten grams of biscuit and 40 mL of extracting solvent (75% methanol in distilled deionized water), blended for 2 min, and transferred to a 100 mL volumetric flask, brought up to volume and stored for 60 min (or overnight) at 4 °C. Following this, the samples were transferred to a 50 mL centrifuge tube and centrifuged at 15000 rpm for 40 min at 4 °C using an IEC refrigerated centrifuge model B20A (International Equipment Co., Needham Heights, MA). The supernatant was filtered, diluted as required, and to 100 μL of diluted sample supernatant, 400 μL of OPT (prepared by dissolving 27 mg of o-phthaldialdehyde in 500 μL of absolute alcohol, added with 5 mL of 0.1 M sodium tetraborate ($Na_2B_4O_7$,$10H_2O$) (pH9.5), followed by 50 μL of mercaptoethanol) was added followed by thorough mixing using a vortex for 2 min. The sample was then manually injected onto the column of the HPLC system (Ultrasphere ODS 5 µm particle size, 4.6 mm × 25 cm) (Beckman Instruments, Inc., Fullerton, CA) and the gradient run started. The flow rate was adjusted to 1 ml/min and detection was performed at 330 nm using a Spectroflow 980 programmable fluorescence detector (ABI Analytical, Inc., Kratos Division, Ramsey, NJ). Sodium phosphate buffer was used as a blank, and the concentration of each amino acid of interest was measured using an external calibration curve. SAS (SAS Institute Inc., Raleigh, NC) general linear models' procedure was used to analyse the data obtained.

The micromineral elements (Ca, P, Mg, Na, and K) and micro-minerals (Fe and Zn) were quantified by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) following the method described by Pinto et al. (2022). The samples were firstly extracted using acid digestion by weighing 0.4 g sample and adding 5 mL of 65% nitric acid (HNO3) and 2.5 mL of 37% hydrochloric acid (HCl). The digestion occurred in three-step i.e increasing temperature from room temperature to 90 °C in 30 min, followed by 30 min at this temperature and then 60 min at 105 °C. Next, the sample solutions were cooled and dissolved to 25 mL of deionized water and filtered using a 0.22 µm PTFE syringe filter. A reference material and a blank sample were prepared in the same procedure. Minerals concentration was examined using an iCAP™ Q ICP-MS instrument (Thermo Fisher Scientific, Bremen, Germany). Sample solutions were analyzed in triplicate, and the results were expressed in mg/kg wet weight (ww).

Meanwhile, omega 3, 6 and 9 were determined using a gas chromatography mass spectrometer (GCMS). This was performed by using fatty acid methyl ester (FAME) preparation and chromatographic analysis. The fatty acid methyl ester (FAME) was prepared by a one-step extraction-transesterification method following Majid et al. (2003) with modification. The sample was weighed ± 20 mg and added with 4 mL fresh solution of a mixture of methanol, concentrated sulphuric acid, and chloroform (1.7:0.3:2.0 v/v/v) in a 10 ml bottle. The bottle was closed tightly and placed inside a water bath at 90 °C for 30 min to allow the reaction. Once the reaction was completed, the bottle was cooled down to room temperature, added with1 mL distilled and thoroughly vortexed for 1 min to form two phases. The lower phase containing fatty acid methyl ester (FAME) was transferred to a clean, 10 mL bottle and dried with anhydrous sodium sulphate (Na_2SO_4). The fatty acid compositions of lipid extracts were determined by gas chromatography (Shimadzu QP 5000 M/S and GC-17A equipped with a SHIMADZU capillary column) of the corresponding methyl esters. One microliter of sample was taken and injected into the capillary column CBP-M25-025 (diameter of 0.25 mm and a length of 25 m). The column was set to 43.0 kPa head pressure with 1 mL/ min flow rate using helium as the carrier gas. The initial temperature was set to 60 °C and increased at a rate of 15 °C/min to 230 °C. The injection and detector temperature were set 230 °C. The produced mass spectrum was equated with NBS and NIST Mass Spectra Library, while the retention times was compared to those of the commercial fatty acid methyl ester standards (GC 18-91) to identify the fatty acid methyl esters.

Statistical analysis

All data obtained were statistically analyzed using Analysis of variance (ANOVA) with the level of significance ($p < 0.05$). Regression analysis of the sensory quality attributes of the biscuits was carried out using Design-Expert® version 13.0.0 (Stat-Ease, Inc., USA). The model fitness, indicated by model significance ($p < 0.05$), lack of fit and adjusted coefficient of determination (R^2_{adj}) were defined from the analysis.

Results and discussion

The quality of raw materials

The pH and TVB levels of fresh catfish showed a value of 6.8 ± 0.07 and 12.14 ± 0.78 mgN%, respectively (Table 2), representing a fresh raw material since the TVB content at range between 10–20 mgN% (Bekhit et al. 2021). Processing the catfish into the flour produced the moisture and a protein content of 4.34 ± 1.13% and 58.04 ± 3.83%, respectively, which was meeting the standard established by FAO with maximum moisture content of 10% and minimum protein content of 67.7% (Rieuwpassa et al. 2018).

Table 2. Proximate and crude fibre values of catfish flour and *Ulva* sp. flour.

No	Parameters	Catfish flour	*Ulva* sp. flour
1	Water content (% ww)	4.34 ± 1.13	6.32 ± 0.25
2	Ash content (% ww)	1.79 ± 0.07	28.91 ± 1.21
3	Protein content (% ww)	58.04 ± 3.83	14.36 ± 0.23
4	Fat content (% ww)	11.98 ± 0.11	0.45 ± 0.01
5	Carbohydrate (% ww) (by different)	1.43 ± 0.18	62.06 ± 0.60
6	Crude fibre (% ww)	7.47 ± 0.30	16.60 ± 1.24

Response of biscuit formulation on protein content

The results of the analysis based on the MD-DX® software indicate that three independent factors have a significant impact ($P < 0.05$) (Table 3). There is a linear relationship between the protein content and the dependent factor, with a confidence level of $R = 0.7379$. This suggests that the concentration of wheat flour, catfish flour, and *Ulva* sp. flour influenced the nutritional value of the protein in biscuits. The lack of fit values for each linear protein model was not significant, with a value of 0.0944 (Table 4). The protein content increased when adding the catfish flour and decreasing the amount of wheat flour (Fig. 1). The protein content of biscuits formulated from run orders 1–18 ranged from 11.22% to 14.52%. The high protein content in this study comes from the catfish flour, which has a protein content of 58.04 ± 3.83% (Table 2). The protein content of biscuits from various formulations is partially higher than the protein content of eggs (13%), which was used as a reference for nutritional fulfilment in the community (Nurliyani et al. 2023). This protein content is above the minimum value for biscuits as additional food for toddlers aged 24–59 months, ranging from 6% to 18% (Astuti and Noor 2023). The results of this study are in line with the findings of preceding work, which indicated that biscuits fortified with 10% of catfish flour had a protein content of 11.33% (Arvianto et al. 2016). Similarly, the study by Ikasari et al. (2020) on biscuits fortified with 10% fish protein concentrate demonstrated an increase in protein content to 11.4% and 11.91%.

Response of biscuit formulation on sensory attributes

The analysis results using the software MD-DX 13® for attributes of taste and texture indicated that the recommended predictive model for sensory taste and texture is the Sp Quadratic vs. Quadratic model. The analysis showed that three dependent factors significantly influenced ($P < 0.05$) taste and texture, with a confidence level of $R = 0.8233$ for taste and $R2 = 0.9494$ for texture. A fixed lack of fit value for the Sp quadratic vs. quadratic texture model indicated non-significance with a value of 0.4701 (Table 4). A fixed three-dimensional curve showed the combination of inter-component variables influencing the taste and texture response based on the surface plot graph in Fig. 2.

Based on MD-DX.13® analysis, the highest taste sensory response formulation was obtained at a concentration of 36.5% wheat flour, 8% catfish flour, and 0.5% *Ulva* sp. with a score of 4.1 (like to very like). Panelists gave

Table 3. Response analysis of biscuits on protein content, sensory properties (texture and taste), hardness, and colour obtained from run orders 1–18.

No	Wheat flour	Catfish flour	*Ulva* sp. flour	Protein (%)	Sensory evaluation		Hardness (g)	Colour		
					Texture	Flavour		L*	a*	b*
1	35.1	8.7	1.3	13.77	3.27	3.40	2429.08	60.49	10.03	21.79
2	33.0	10.0	2.0	13.43	3.40	3.30	2225.95	61.31	9.36	21.37
3	33.0	11.5	0.5	14.52	3.59	3.70	2827.55	65.50	9.04	21.64
4	35.0	8.0	2.0	12.06	3.61	3.30	2168.70	60.44	7.89	20.47
5	34.3	8.7	2.0	12.12	3.63	3.50	2341.94	58.68	9.17	20.35
6	34.4	9.4	1.3	12.65	3.81	3.54	2458.04	69.98	4.36	23.16
7	34.4	9.4	1.3	12.40	3.81	3.79	2363.56	65.33	7.68	23.18
8	34.4	9.4	1.3	12.61	3.96	3.60	2380.30	64.16	7.52	23.25
9	33.0	10.8	1.3	13.77	4.00	4.10	2341.00	66.31	7.18	22.92
10	34.8	9.8	0.5	13.20	4.00	4.00	2098.67	71.28	6.51	24.11
11	34.8	9.8	0.5	12.12	4.00	3.75	2049.07	63.93	10.60	23.50
12	36.5	8.0	0.5	11.95	4.09	3.90	2455.02	68.47	7.96	25.48
13	33.7	10.4	0.9	13.76	4.09	3.40	2378.22	67.78	6.30	23.88
14	36.5	8.0	0.5	11.92	4.18	4.09	2488.04	71.62	5.14	24.10
15	33.0	10.8	1.3	13.40	4.27	4.00	2349.06	68.23	4.51	23.28
16	35.8	8.0	1.3	11.39	4.30	4.09	2136.13	64.27	8.26	22.78
17	33.7	9.3	2.0	13.91	4.36	3.60	2001.82	66.96	9.53	24.62
18	35.8	8.0	1.3	11.22	4.43	4.05	2150.93	65.33	6.06	21.78

Table 4. Analysis model of sensory responses and physical quality of biscuit products.

Response	Model/Model	Lack of fit **	Significant *		R-Squared
			Model	Lack of fit	
Protein	Linear.	0.7379	Significant	Not Significant	0.0944
Texture	Sp Quadratic vs Quadratic	0.4701	Significant	Not Significant	0.9494
Taste	Sp Quadratic vs Quadratic	0.0895	Significant	Not Significant	0.8233
Hardness	Sp Quadratic vs Quadratic	0.9652	Significant	Not Significant	0.0537
Lightness (L*)	Linear	0.4609	Significant	Not Significant	0.3930
Redness (a*)	Cubic	0.8251	Significant	Not Significant	0.5378
Yellowness (b*)	Sp Quadratic vs Quadratic	0.1286	Significant	Not Significant	0.8752

Note: *Not significant (The interaction between components did not significantly affect the response) Significant (The interaction between components significantly affected the response).
**Not significant (The response was compatible with the analysis model).
Significant (The response was not compatible with the analysis model).

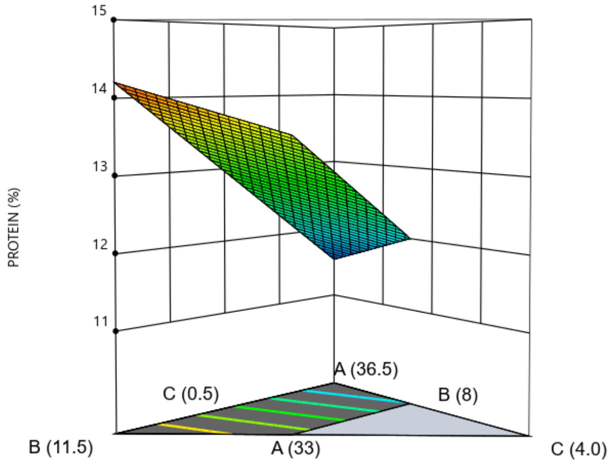

Figure 1. Graphic surface plot (mixture design) response of a protein-nutritional biscuits fortified with catfish flour and *Ulva* sp. flour.

relatively low scores for the taste of biscuits formulated with low wheat flour and high *Ulva* sp. flour. For example, biscuits which were formulated with 33% wheat flour, 10% catfish flour, and 2% *Ulva* sp. flour, have an average taste score of 3.30 (neutral to like). Then, the use of *Ulva* sp. flour at concentrations of 2% in the formulation produced a slightly bitter after taste in the final biscuit's product. *Ulva* sp. is rich in essential amino acids such as lysine, phenylalanine, methionine, leucine, and valine, imparting a bitter taste (Moustafa and Eladel 2015). Meanwhile, increasing the concentration of catfish flour resulted in a less sweet taste due to the caramelization process during roasting and will change the taste characteristics of the biscuit (Widyaningrum et al. 2022). The use of 10% catfish flour were acceptable to panelists, but the panelist's preferences significantly decreased when the catfish flour substitution was increased to 15% (Arvianto et al. 2016).

The MD-DX.13® analysis of the actual components of *Ulva* sp. flour at 1.25% revealed that the texture optimization point achieved the highest value for the wheat flour variable at a concentration of 35.75% and catfish flour at 8%. The optimization value indicated a preference for texture at 4.43 (between like and really like), meaning that biscuits have a crunchy texture, are easy to break, and are chewable. The lowest texture value was obtained in the formulation using wheat flour at a concentration of 35.1%, catfish flour at 8.7% and *Ulva* sp. at 1.3%, with a value of 3.27 (between neutral and like). Fortifying catfish flour at concentrations of 8–10.75% still resulted in an acceptable response of texture from the panelists. Panelists' preferences for texture tend to decrease with increasing catfish flour concentration. This is due to the reduction of the elasticity of gluten at high concentration of catfish flour, resulting in dense and slightly harder biscuits (Arvianto et al. 2016). Both catfish flour and *Ulva* sp. flour addition increased the density of the dough in biscuits. During the baking process, gluten forms air bubbles which are filled with solids produced by catfish and *Ulva* sp. flour, resulting in dense and hard biscuits texture (Guiné 2022).

Response of biscuit formulation on physical properties

The response to the physical properties of nutritious biscuits was observed in terms of hardness and colour (L*a*b*). The recommended prediction model for hardness (MD-DX.13®) is the Sp Quadratic vs. Quadratic model, while for the colour responses are linear, cubic, and Sp Quadratic. vs Quadratic, respectively (Fig. 3). Based on ANOVA analysis, the polynomial model is statistically significant with a p-value <0.05, indicating a relationship between Sp Quadratic vs Quadratic and both dependent and independent factors concerning hardness, with a confidence level of R = 0.9652. This suggests that the concentration of wheat flour, catfish flour, and *Ulva* sp. flour influenced the hardness values of the final biscuit's products ($p < 0.05$) (Table 5). The hardness values of biscuits in the study ranged from 2001.82 to 2827.55 g. Based on MD-DX.13® analysis, the optimization point for hardness was obtained with a concentration of 35.75% wheat

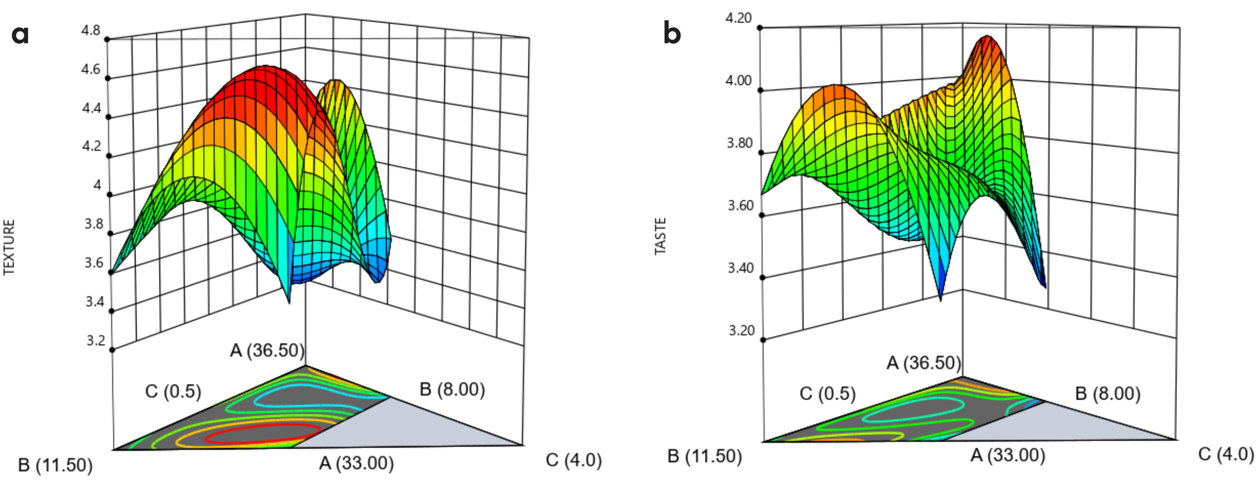

Figure 2. Surface plot graphic of mixture design response for taste (**a**) and texture (**b**) of nutritious biscuits fortified with catfish flour and *Ulva* sp. flour.

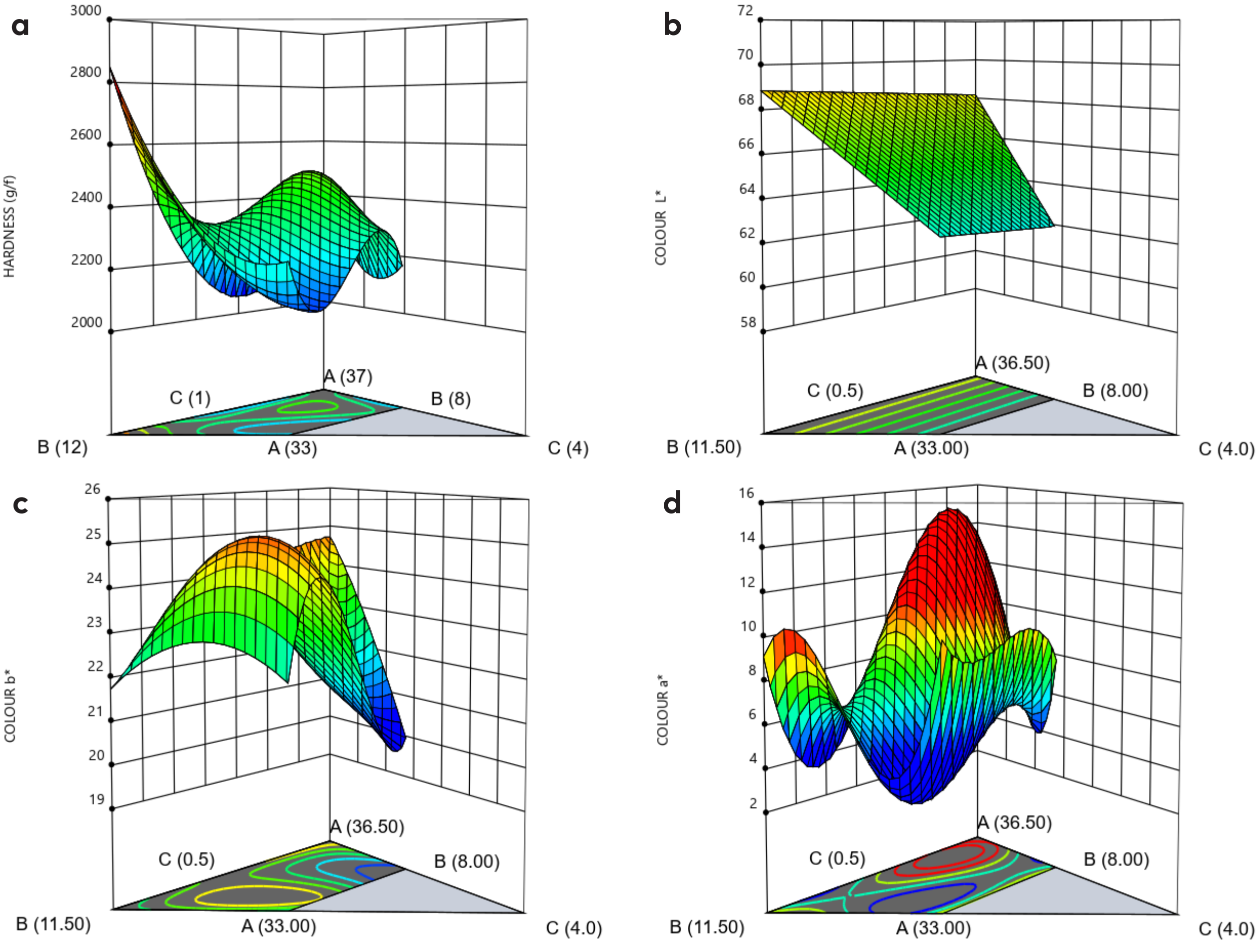

Figure 3. Surface plot graphic of mixture design response for (**a**) hardness (**b**) L* value (**c**) a* value and (**d**) b* value of nutritious biscuits fortified with catfish flour and *Ulva* sp. flour.

flour, 8% catfish flour, and 1.25% *Ulva* sp. flour. At this optimization point, the hardness value for the biscuits was 2136.13 g. This value is above the hardness value for baby biscuits (948–1196 g), representing a soft texture and not easily crumbled (Widyaniputria et al. 2020).

The hardness of biscuits is affected by its protein content, high levels of protein can cause biscuits to become harder and less crunchy (Rauf et al. 2018). Evaporating water during baking will create air cavities, which allows it to be filled with solids materials from catfish and *Ulva* sp., making the product less crunchy. Hardness also depends on the gluten content of the wheat flour, baking temperature, and duration (Guiné 2022). In this study, medium-protein wheat flour (11%) was used, which is easily dispersed and has low water absorption properties, producing dough that is not easily damaged during molding, resulting in non-expanding biscuits with a crunchy texture (Nandiyanto et al. 2022). This result aligns with the findings of previous study on biscuits fortified with catfish flour, where a higher concentration led to harder biscuits (Arvianto et al. 2016).

Colour is one of the physical factors influencing the preference level of panelists. In this study, biscuits fortified with 0.5% *Ulva* sp. flour tend to have a higher brightness compared to biscuits fortified with 2% *Ulva* sp. flour (Fig. 3b). The brightness (L*) level of the resulting biscuits ranges from 58.6 to 71.6. The a* (redness) values range between 4.36 and 10.60, while the b* (yellowness) values range between 20.35 and 25.48. Biscuits with high a* (redness) and low b* (yellowness) produced a brown colour, resulting in a low brightness (L*) level. A lower L* value can be associated with higher levels of protein denaturation and oxidation (Kumoro et al. 2022), and the presence of chlorophyll in the *Ulva* sp. flour. The baking process leads to the degradation of chlorophyll pigments into pheophytins and pheophorbides compounds, causing the green colour to change into brown (Moustafa and El-adel 2015). The brown colour can also be due to non-enzymatic reactions between sugar and proteins in the food ingredients. The higher the protein and sugar content in the ingredients, the higher the browning reactions, leading to lower brightness levels. Fortifying ingredients such as catfish flour and *Ulva* sp. flour contain high levels of the amino acid lysine, which is highly sensitive to heat. Lysine, having a reactive α-amino group, easily binds with reducing sugars, forming melanoidin compounds that are brown in colour. Meanwhile, coconut oil and butter containing high-fat content undergo oxidation during baking, forming radicals that condense to form brown-coloured compounds (Hustiany 2016).

Optimization of biscuits formulation

The optimization of the formula is carried out by establishing criteria based on the importance of response variables (Table 5). The optimization of the components to be achieved (goals) includes the concentration of wheat flour, catfish flour, and *Ulva* sp. flour, as well as response goals for texture, taste, hardness, protein, and colour values L*, a*, and b*. All components input their goals to obtain an optimal formula. Subsequently, the Mixture Design Expert 13® Software will recommend the optimum formula and desired level values. Priority is given with an importance rating of 5 to wheat flour because it is a primary ingredient in biscuit production. Wheat flour plays a role in forming the dough, binding other ingredients, and shaping the biscuit structure during baking (Istinganah et al. 2017). On the other hand, catfish flour addition in the biscuit aims to increase the protein content, amino acids, minerals, and vitamins in the resulting biscuits. Fish protein is composed of complex essential amino acids, including lysine, isoleucine, leucine, and methionine, for growth and muscle formation. Additionally, catfish flour also contains calcium and phosphorus minerals, as well as vitamin B complex, especially vitamin B12 (Abdel et al. 2021). Therefore, the biscuits produced are rich in amino acids, vitamins, and minerals necessary for highly nutritious biscuits.

Ulva sp. flour, with an importance rating of 5, aims to increase the dietary fibre necessary to aid digestion. Texture and taste responses are important sensory properties that ensure the produced biscuits are accepted by consumers (Bekare et al. 2020). Colour and hardness each receive importance ratings of 3 and 4, respectively, as their contributions do not significantly affect the nutritional value of the resulting biscuits. The recommended results obtained are composed of 33.18% wheat flour, 10.57% catfish flour, and 1.25% *Ulva* sp. flour. The MD-DX 13® software recommended an optimal formula solution with a desired desirability level of 0.7835. Furthermore, biscuits with the formulation of 33.18% wheat flour, 10.57% catfish flour, and 1.25% *Ulva* sp. with independent formulation ingredients (green bean flour 5%, corn flour 5%, egg yolk 15% coconut oil 5%, butter 5%, fine sugar 13.5%, vanilla aroma 0.5%, salt 0.2%, milk powder 5%, ammonium bicarbonate 0.5%, and maltodextrin 0.3%) are verified and then observed for their nutritional quality.

Table 5. Response, target, limit score and dan importance at optimization step.

Component	Goal	Lower limit	Upper limit	Importance
Wheat flour	is in range	33.00	36.50	+++++/5
Catfish flour	maximize	8.00	11.50	+++++/5
Ulva sp. flour	is in range	0.50	2.00	+++++/5
Texture	maximize	3.27	4.43	+++++/5
Flavour	maximize	3.30	4.10	+++++/5
Hardness	minimize	2001.82	2827.55	++++/4
Protein	maximize	11.22	14.52	+++++/5
Color				
Lightness (L*)	is in range	58.68	71.62	+++/3
Redness (a*)	is in range	4.36	10.60	+++/3
Yellowness (b*)	is in range	20.35	25.48	+++++/5

Proximate analysis, physical, and sensory properties of the optimized biscuit

The results of the proximate analysis, crude fibre, dietary fibre, physical colour, texture profile, and sensory properties of the optimized biscuits compared to commercial product are presented in Table 6. The moisture content of the optimized biscuits in this study was 3.99 ± 0.24%, higher compared to commercial biscuits at 1.23 ± 0.07%. However, the moisture content is lower than the standard moisture content for biscuits according to Indonesian National Standard SNI 2973:2022 (BSN 2022), with a maximum of 5%, and UNICEF biscuits for every child have a maximum of 4.5% (UNICEF 2022).

The ash content of optimized biscuits was 1.75 ± 0.03%, higher than commercial biscuits (1.31 ± 0.04%). Meanwhile, the ash content recommended by UNICEF for every child is 3.5% (Egayanti et al. 2019). Biscuits fortified with catfish have higher protein content (19.38 ± 1.02%) compared to commercial biscuits (8.82 ± 0.97%). Therefore, biscuits formulated based on the recommendations of the MD-DX13® software is better in nutrition compared to commercial biscuits. The fat content of biscuits fortified with catfish flour and *Ulva* sp. flour was 11.12%, lower than commercial biscuits which have a fat content of 17.35%. The fat content in biscuits for supplementary children's food from UNICEF is a minimum of 15%. The high-fat content in biscuits is expected to provide energy value for the biscuits produced as supplementary foods. The fat formulation used in these biscuits was only 10%, coming from butter (5%) and oil (5%), while the fat content in catfish flour is only 11.28%.

The sensory analysis of biscuits was performed on appearance, odour, taste, and texture. The panellists preferred optimized biscuits for their appearance and odour compared to commercial biscuits, while the taste and texture were dominant for the commercial biscuits. The biscuits fortified with catfish and *Ulva* sp. flour have a darker brown colour compared to commercial biscuits, which the panellists did not like. Panellists preferred the aroma of the optimized biscuits, which have a more fragrant vanilla essence and milk compared to commercial biscuits. The texture of commercial biscuits was crispier and easily crumbled when bitten and chewed, compared to biscuits fortified with catfish flour, which tends to be harder. In addition, panellists preferred commercial biscuits for their sweeter taste compared to biscuits fortified with catfish and *Ulva* sp. flour. Overall, panellists gave the same score between biscuits fortified with catfish flour and the commercial biscuits.

Table 6. Proximate analysis, dietary fibre, sensory properties, texture, and colour of the optimized biscuits fortified with catfish flour and *Ulva* sp., compared to commercial biscuits.

No	Parameters	Optimized biscuits	Commercial biscuits
1	Water content (% ww)	3.99 ± 0.24	1.23 ± 0.07
2	Ash content (% ww)	1.75 ± 0.03	1.31 ± 0.04
3	Protein content (% ww)	19.38 ± 1.02	8.82 ± 0.97
4	Fat Content (% ww)	11.12 ± 0.76	17.35 ± 0.78
5	Carbohydrate content (% ww) (by different)	63.52 ± 0.89	71.29 ± 0.76
6	Fiber content (% ww)	1.75 ± 0.06	2.01 ± 0.03
7	Dietary fibre (% ww)	20.83 ± 1.87	22.48 ± 2.1
8	Appearance (sensory)	4.3 ± 1.54	4.1 ± 0.9
9	Odor (sensory)	4.6 ± 2.34	3.6 ± 1.2
10	Taste (sensory)	4.05 ± 1.76	4.3 ± 1.2
11	Texture (sensory)	4.2 ± 1.56	4.8 ± 0.9
12	Hardness (g)	1482.58 ± 275.12	1498.27 ± 166.32
13	Fracturability (g)	44.10 ± 0.37	42.73 ± 0.18
14	Lightness (L*)	62.53 ± 0.5	66.23 ± 0.65
15	Redness (a*)	11.93 ± 0.35	11.76 ± 0.25
16	Yellowness (b*)	34.33 ± 0.35	28.96 ± 0.70

Amino acid profile of optimized biscuit

Table 7 shows the results of the optimized biscuits amino acid analysis in comparison to commercial biscuits. Fortifying with catfish and *Ulva* sp. flour significantly increased essential and non-essential amino acids. The amount of essential amino acids in optimized biscuits was much higher, at 608 mg/g, compared to commercial biscuits, which was only 291 mg/g. The non-essential amino acids in optimized biscuits were 1161 mg/g, higher than the non-essential amino acids in commercial biscuits, which was only 421 mg/g. Fortification with catfish and *Ulva* sp. flour can improve the nutritional quality of the produced biscuits, especially the protein and amino acid composition.

The amount of essential amino acids in these biscuits exceeds the FAO's recommended amino acid for children ages 4–6 years, meanwhile commercial biscuits contain tryptophan phenylalanine and tyrosine with lower amino acid scores (Egayanti et al. 2019) (Table 7). Biscuits fortified with catfish and *Ulva* sp. flour contain lysine and threonine, which are higher than the standards issued by the FAO. As a basic component of blood antibodies, lysine, strengthens the circulatory system, maintains normal cell growth, and lowers excessive blood triglyceride levels (Andri et al. 2020). Threonine is also a second-limiting amino acid, known as a crucial nutrient for cell growth and proliferation, thereby providing beneficial impacts on factors related to health and disease (Vaivada et al. 2020). The biscuits produced also contain a high level of glutamic acid that contributes to the umami taste, influencing the savoury taste of the biscuits (Zhao et al. 2016).

Table 7. Amino acid profile of biscuits fortified with catfish flour and *Ulva* sp. flour.

No	Types of amino acids	Optimized biscuits (mg/g)	Commercial biscuits (mg/g)	FAO/WHO Reference 2013 (mg/g)
Essential amino acids				
1	Histidine	74 ± 2.00	34 ± 2.0	16
2	Leucine	80 ± 2.08	60 ± 1.52	30
3	Lysine	143 ± 1.52	60 ± 1.0	61
4	Methionine +cysteine	89 ± 3.51	48 ± 1.15	48
5	Phenylalanine +tyrosine	54 ± 1.52	21 ± 2.08	23
6	Threonine	138 ± 2.08	51 ± 1.08	41
7	Valine	73 ± 2.51	31 ± 2.51	25
8	Isoleucine	13 ± 2.08	11 ± 1.52	6.6
9	Tryptophan	91 ± 1.52	37 ± 0.57	40
Total of Essential Amino Acids		**608 ± 2.09**	**291 ± 1.42**	–
Non-Essential Amino Acids				
1	Cystine	16 ± 1.52	6.0 ± 0.57	–
2	Arginine	198 ± 3.05	48 ± 2.51	–
3	Aspartat acid	129 ± 3.51	63 ± 0.57	–
4	Glutamic acid	405 ± 152	149 ± 2.08	–
5	Serine	95 ± 3.51	34 ± 0.58	–
6	Glycine	71 ± 1.53	28 ± 2.51	–
7	Tyrosine	49 ± 3.51	21 ± 1.51	–
8	Alanine	83 ± 2.51	36 ± 2.08	–
9	Proline	115 ± 2.64	36 ± 1.52	–
Total of Non Essensial Amino Acids		**1161 ± 2.59**	**421 ± 1.55**	–

Recommended Dietary Allowances (RDA) for nutritious biscuits fortified with catfish and *Ulva* sp. flour

Recommended Dietary Allowances (RDA) is computed as Nutritional content per saving biscuit by dividing nutritional needs of toddlers and multiplied with 100%. The biscuit can satisfy the RDA when the value is greater than 20%. According to UNICEF, a serving of biscuits must have weight of 50 grams, or four pieces, in order to provide 20% of the recommended dietary allowance for children ages 1–5 (UNICEF 2022).

The energy content of optimized biscuits is influenced by the fat, protein, and carbohydrate content since the value is calculated by adding up the multiplication value of fat by 9, protein by 4, and carbohydrate by 5. The energy content of the optimized biscuits was 495.2 kcal/100 g, higher than the standard recommended biscuit set by Indonesian National Standard (SNI) 2973:2022). The energy is also higher than that of biscuits supplemented with vitamin and mineral premix released by UNICEF for every child, which have an energy content of 450 kcal (UNICEF 2022). The protein and dietary fibre content exceeds 20% of the recommended RDA per serving (Table 8). Meanwhile, the fat and carbohydrate values were below 20%, still being limiting factors to meet the RDA for age 4–6 years. Therefore, the formulation of biscuits recommended by the

Table 8. Recommended Dietary Allowances (RDA) for nutritious biscuits fortified with catfish and *Ulva* sp. flour.

Parameters	Nutritional content of 100 g biscuits	Nutritional content per saving biscuit (50 g)	Nutritional needs of toddlers 4–6 years old	Recommended Dietary Allowances (RDA)
Energy (kcal)	495.20	247.60	1400	17.68
Protein (% ww)	19.38 ± 1.02	9.69	25	38.76
Carbohydrates (% ww)	63.52 ± 0.89	31.76	220	14.38
Fat (% ww)	11.12 ± 0.76	5.56	50	11.12
Dietary fibre (% ww)	20.83 ± 1.87	10.42	20	52.10
Calcium (Ca) (mg/kg)	202.21 ± 4.23	101.11 ± 2.11	500	10.11
Phosphorus (P) mg/kg	224.77 ± 4.14	112.39 ± 2.07	95	22.47
Magnesium (Mg) (mg/kg)	105.97 ± 4.63	52.99 ± 2.31	10	55.77
Sodium (Na) (mg/kg)	268.34 ± 2.62	134.17 ± 3.23	900	14.90
Potassium (K) (mg/kg)	327.13 ± 6.47	163.56 ± 0.12	2700	6.05
Iron (Fe) (mg/kg)	7.19 ± 0.27	3.60 ± 0.14	10	36.00
Zinc (Zn) (mg/kg)	3.47 ± 0.49	1.74 ± 0.25	5	34.80
Vitamin A	337 ± 1.52	168.5 + 0.76	450	37.44
Vitamin B1	0.72 ± 0.02	0.36 ± 0.01	7	5.13
Vitamin B2	0.02 ± 0.00	0.012 ± 0.00	0.6	2
Vitamin B6	0.85 ± 0.00	0.425 + 0.00	0.6	70.83
Folic Acid	0.06 ± 0.30	0.285 ± 0.01	0.6	4.75
Vitamin C	0.85 ± 0.00	0.424 ± 0.00	200	2.22
Vitamin E	0.27 ± 0.00	0.136 ± 0.00	45	3
Omega 3	0.25 ± 0.0	0.12 ± 0.00	0.9	13.33
Omega 6	6.96 ± 0.18	3.48 ± 0.09	10	34.80
Omega 9	18.4 ± 0.50	9.2 ± 0.25	–	–

MD-DX13® software can be categorized as biscuits containing high protein.

Minerals play a crucial role in maintaining the body's functions at the cellular, tissue, organ, and overall body levels (Singh and Prasad 2023). These biscuits meet the RDA for macro-minerals, where phosphor and magnesium exceeded 20%, while sodium and potassium contents were below 20% to meet the recommended mineral intake. The micro-mineral Zn and Fe have RDAs for children under five years old in accordance with the Regulation of the Minister of Health of the Republic of Indonesia Number 28 of 2019 (Ministry of Health of the Republic of Indonesia 2019). The macro-mineral (Phosphor and Magnesium) and micro-minerals (Iron and Zinc) in these biscuits come from the catfish flour used as a fortification ingredient (Abdel et al. 2021). However, these biscuits are still unable to compete with UNICEF" s High-Energy and High-Protein biscuits that are combined with vitamins and minerals and provide a minimum of 250 mg/kg of calcium, 10 mg/kg of iron, and 8 mg/kg of zinc for each child (UNICEF 2022). Micronutrients are essential minerals obtained from a variety of foods that are not produced by the body. The supplementation of phosphor, Iron, and Zinc can have a positive growth effect on children. Zinc is crucial for toddlers, as it is involved in various metabolic processes as a catalyst, ion regulator, or structural element of proteins (Singh and Prasad 2023).

The optimized biscuits have vitamin content that meets RDA for vitamin A and Vitamin B6 (Table 8). Both vitamins have RDA" s for children under five years old, per the Regulation of the Minister of Health of the Republic of Indonesia Number 28 of 2019 (Ministry of Health of the Republic of Indonesia 2019). The other vitamins such as Vitamin B1, B2, Folic Acid, Vitamin C, and E, remain a limiting factor, as their RDAs are less than 20%. The vitamin A in this biscuit is still below that in biscuits fortified with a vitamin and mineral premix released by UNICEF for every child, which is 500 mg of vitamin A as retinol (UNICEF 2022). Vitamin A is crucial for vision, immunity, bone growth and reproduction, maintaining the cornea and sclera surfaces, and the integrity of the epithelium in the respiratory, urinary, and digestive tracts (Awasthi and Awasthi 2020). Vitamin B6 is an essential vitamin that is important for the development of the brain, nerves, and skin and participates in the transformation of carbohydrates, lipids, amino acids, and nucleic acids into energy (Stach et al. 2021). Optimized biscuits also contain omega 3 and omega 6 essential fatty acids derived from catfish flour containing 13.6 g/100 g omega-3 (Nurasmi et al. 2018) with a recommended dietary allowance of 34.8%.

Conclusion

The present work has successfully employed Response Surface Methodology using Mixture Design-DX 13® software to obtain the optimum formulation of nutritious biscuits fortified with Catfish flour and *Ulva* sp. flour. The recommended formulation by MD-DX13® for biscuits fortified with catfish flour and *Ulva* sp. flour contained wheat flour (33.18%), catfish flour (10.57%), and *Ulva* sp. flour (1.25%). The fortified biscuits had protein content of 19.38 ± 1.02%, fat content of 11.12 ± 0.76, carbohydrate content of 63.52 ± 0.89%, energy content of 495 kcal, and a dietary fibre content of 20.83 ± 1.87%. These nutritional parameters meet the Recommended Dietary Allowance, which includes energy, protein, dietary fibre, macro minerals (calcium, magnesium, potassium), microminerals (iron and zinc), and vitamin A. The biscuits also contain essential amino acids and omega 3, 6 and 9 fatty acids which impose several biological effects and health benefits in quantities exceeding the standards set by FAO/WHO (2019) for children aged 4–6 years. In future research, it is recommended to study the shelf life of biscuits in various packages and to include business analysis evaluation on a small and medium industrial scale.

Author contributions

Conceptualization, Theresia Dwi Suryaningrum, Ellya Sinurat; methodology, Theresia Dwi Suryaningrum, Fateha, Ellya Sinurat, Agus Supriyanto, Indri Mardiyana, Natalia Prodiana, Nurhayati; software, Fateha; formal analysis, Theresia Dwi Suryaningrum, Waryanto, Ma'muri, Sihono; resources, Theresia Dwi Suryaningrum; Diah Ikasari, Ellya Sinurat; writing-original draft preparation, Theresia Dwi Suryaningrum, Diah Ikasari; writing-review and editing, Hari Eko Irianto, Giyatmi; supervision, Theresia Dwi Suryaningrum, Hari Eko Irianto.

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References

Abdel ME, Abdel-Aal HA, Souzan SL, Nassar AG (2021) Nutritional value of African catfish (*Clarias gariepinus*) meat. Asian Jurnal of Applied Chemistry and Research 8(2): 31–39. https://doi.org/10.9734/ajacr/2021/v8i230190

Adepoju AA, Isinkaye OD, Ofeniforo BE, Adeyiola O, Agada ES, Ayo-Dada DO (2022) Comparative analysis of amino acid composition in the head, muscle and tail of fresh African catfish (*Clarias gariepinus*). Journal of Agricultural Chemistry and Environment 11(4): 231–239. https://doi.org/10.4236/jacen.2022.114016

Andri, Harahap RP, Tribudi YA (2020) Estimation and validation of methionine, lysine, and threonine amino acids from grain feedstuff as protein source. Jurnal Nutrisi Ternak Tropis 3(1): 18–22. https://doi.org/10.21776/ub.jnt.2020.003.01.4

Antoine FR, Wei CI, Littell RC, Marshall MR (1999) HPLC Method for analysis of free amino acids in fish using o-Phthaldialdehyde precolumn derivatization. Journal of Agriculture and Food Chemistry 47: 5100–5107. https://doi.org/10.1021/jf990032+

AOAC (2005) Official methods of analysis of AOAC international 18th edition Association of Official Analytical Chemists.

Arvianto AA, Swastawati F, Wijayanti I (2016) The effect of fortification African catfish (*Clarias gariepinus*) meat meal to lysine content on biscuits. Jurnal Pengolahan Dan Bioteknologi Hasil Perikanan 5(4): 20–25.

Astuti D, Noor R (2023) Technical Instructions for Providing Supplementary Food Made from Local Food for Toddlers and Pregnant Women. Director General of Public Health. Ministry of Health of the Republic of Indonesia.

Awasthi S, Awasthi A (2020) Role of vitamin A in child health and nutrition. Clinical Epidemiology and Global Health 8: 1039–1042. https://doi.org/10.1016/j.cegh.2020.03.016

Bekare AH, Adeola AA, Otesile I, Obadina AO, Afolabi WA, Adegunwa MO, Akerele RA, Bamgbose OOO, Alamu EO (2020) Nutritional, texture, and sensory properties of composite biscuits produced from breadfruit and wheat flours enriched with edible fish meal. Food Science & Nutrition 8(2): 1–21. https://doi.org/10.1002/fsn3.1919

Bekhit AD, Holman BWB, Giteru S, Hopkins DL (2021) Total volatile basic nitrogen (TVB-N) and its role in meat spoilage: A review. Trends in Food Science & Technology 109: 280–302. https://doi.org/10.1016/j.tifs.2021.01.006

BSN (2009) Indonesian National Standard SNI 2354.8: 2009. Procedure of chemical analysis – Part 8: Determination of Total Volatil Base Nitrogen (TVB-N) and Trimetil Amin Nitrogen (TMA-N) in fishery product. National Standardization Bureau.

BSN (2022) Indonesian National Standard SNI 2973: 2022. Standard quality of biscuits. National Standardization Bureau.

Canti M, Kurniady F, Hutagalung RA, Prasetya W (2023) Nutritional, physical, and sensory properties of fish crackers produced from the head of catfish (*Clarias gariepinus*). Food Research 7(1): 51–63. https://doi.org/10.26656/fr.2017.7(S1).17

Damayanti D, Sari IP, Sulaiman TNS, Bestari AN, Setiawan IM (2018) The Formulation of pacing (*Costus speciosus*) extract tablet by using Avicel®Ph 200 as filler-binder and amylum as disintegration agent. Indonesian Jurnal Pharmacy 29(1): 29–34. https://doi.org/10.14499/indonesianjpharm29iss1pp29

Deshpande A, Ramachandran R (2022) Early childhood stunting and later life outcomes: A longitudinal analysis. Economy Human Biology 44: 101099. https://doi.org/10.1016/j.ehb.2021.101099

Egayanti Y, Yuniarti P, Ramadani MP, Achmad HN (2019) Guidelines for evaluation of multi-nutrition and non-nutrition foods. Directorate of Processed Food Standardization. Dep Bidang Pengawasan Pangan Olahan. Badan POM, 70 pp.

Guiné RPF (2022) Textural properties of bakery products: A review of instrumental and sensory evaluation studies. Applied Sciences 12: 8628. https://doi.org/10.3390/app12178628

Hatijar H (2023) Incidence rates of stunting in infants and toddlers. Jurnal Ilmiah Kesehatan Sandi Husada 12(1): 224–229. https://doi.org/10.35816/jiskh.v12i1.1019

Hosjack I, Benninga MA, Hauser B, Kansu A, Kelly VB, Stephen AM, Lopez AM, Slavin J, Tuohy K (2022) Benefits of dietary fibre for children in health and disease. Archives of Disease in Childhood, 1–7. https://10.1136/archdischild-2021-323571

Hustiany R (2016) The Maillard reaction forms flavor and color in food products. https://repo-dosen.ulm.ac.id//handle/123456789/17832

Ikasari D, Hastarini E, Suryaningrum TD (2020) Characteristics of cookies formulated with fish protein concentrate powder produced from snakehead fish (*Channa striata*) extraction by-product. E3S Web of Conferences 147: 1–8. https://doi.org/10.1051/e3sconf/202014703028

Indonesian Standardization Society (2023) Biscuits are mandatory for SNI 2973-2022. [Accessed August 13, 2024]

Istinganah M, Rauf R, Widyaningsih (2017) The level of hardness and acceptability of biscuits from a mixture of corn flour and wheat flour with a proportional volume of water. Jurnal Kesehatan 1(2): 83–93. https://doi.org/10.23917/jurkes.v10i2.5537

Kumoro AC, Wardhani DH, Kusworo TD, Djaeni M, Ping TC, Azis YMF (2022) Fish protein concentrate for human consumption: A review of its preparation by solvent extraction methods and potential for food applications. Annals of Agricultural Sciences 67(1): 42–59. https://doi.org/10.1016/j.aoas.2022.04.003

Kusumawati R, Sinurat E, Sihono, Fransiska D, Subaryono, Nurhayati, Purnomo AH, Utomo BSB, Basmal J (2022) Utilization of *Ulva* spp. in biscuit formulation: feasibility studies at the household scale. IOP Conf. Ser.: Earth Environment Science 978: 012036. https://doi.org/10.1088/1755-1315/978/1/012036

Kuzio M, Zibula L (2023) A Complete Guide to Fiber Analysis. http://fiber-analysis-in-feed-formulation-a-complete-guide [Accessed December 2023]

Majid MIA, Endarti E, Hashim R, Chong A (2003) Direct FAME synthesis for rapid total lipid analysis from fish oil and cod liver oil. Journal of Food Composition and Analysis 18: 161–171. https://doi.org/10.1016/j.jfca.2003.12.007

Ministry of Health of the Republic of Indonesia (2019) Minister of Health Regulation Number 28 concerning Recommended Nutritional Adequacy Rates for Indonesian People.

Moustafa TY, Eladel H (2015) Amino acids profile of *Ulva* sp. macroalgae collected from Alexandria coast in Egypt: A potential food resource. Point Journal of Botany and Microbiology Research 1(2): 15–22. http://www.pjournals.org/PJBMR

Nandiyanto ABD, Ragadhita R, Ana A, Hammouti B (2022) Effect of starch, lipid, and protein components in flour on the physical and mechanical properties of Indonesian biji Ketapang cookies. International Journal of Technology 13(2): 432–443. https://doi.org/10.14716/ijtech.v13i2.5208

Nurasmi, Sari AP, Rusmiati (2018) Analysis of fatty acid content of omega 3, omega 6, and omega 9 from catfish (*Clarias* sp) in improving toddler nutrition. Journal of Borneo Holistic Health 1(1): 96–100.

Nurliyani, Erwanto Y, Rumiyati, Sukarno AS (2023) Characteristics of protein and amino acid in various poultry egg white ovomucoid. Food Sciences and Technology 43: 1–10. https://doi.org/10.1590/fst.101722

Pathare PB, Opara UL, Al Said FJ (2013) Colour measurement and analysis in fresh and processed foods: A Review. Food and Bioprocess Technology 6: 23–60. https://doi.org/10.1007/s11947-012-0867-9

Pinto FR, Duarte AM, Silva F, Barroso S, Mendes S, Pinto E, Almeida A, Sequeira V, Vieira AR, Gordo LS, Gil MM (2022) Annual variations in the mineral element content of five fish species from the Portuguese coast. Food Research International 158: 111482. https://doi.org/10.1016/j.foodres.2022.111482

Rasyid A, Timur A (2017) Evaluation of nutritional composition of the dried seaweed. Tropical Life Sciences Research 28(1): 119–125. https://doi.org/10.21315/tlsr2017.28.2.9

Rauf R, Nurdiana, Sarbini D (2018) Iron content, hardness, and acceptability of biscuits from composite cassava and wheat flours substituted with green spinach flour. Jurnal Kesehatan 11(1): 1–8. https://doi.org/10.23917/jk.v11i1.6998

Rieuwpassa FJ, Karimela EJ, Lasaru DC (2018) Characterization of functional properties fish protein concentrate of Rainbow Runner (*Elagatis bipinnulatus*). Jurnal Teknologi Perikanan dan Kelautan 9(2): 177–183. https://doi.org/10.24319/jtpk.9.177-183

Sabariman M, Sandrasari DA, Azni IN, Permata TD (2021) Application of the mixture design method in the functional drink formulation of ginger powder, red ginger, and brown sugar. Jurnal Teknologi Pangan dan Kesehatan 3(1): 41–48. https://doi.org/10.36441/jtepakes.v3i1.533

Salvatore S, Battigaglia MS, Murone E, Dozio E, Pensabene L, Agosti M (2023) Dietary fibres in healthy children and in pediatric gastrointestinal disorders: A practical guide. Nutrients 15: 2208. https://doi.org/10.3390/nu15092208

Setyawati B, Fuada N, Nazarina N, Rachmawati R, Salimar S, Ernita E (2023) Supplementary biscuits for the recovery of malnourished children in Indonesia. Jurnal Gizi Pangan 18(1): 11–20. https://doi.org/10.25182/jgp.2023.18.1.11-20

Singh P, Prasad (2023) A review on iron, zinc and calcium biological significance and factors affecting their absorption and bioavailability. Journal of Food Composition and Analysis 123: 105529. https://doi.org/10.1016/j.jfca.2023.105529

Sinurat E, Fransiska D, Sihono, Kusumawati R (2021) The effect of *Ulva* biscuit diet on reducing blood glucose levels of sucrose induced rats. Jurnal Pascapanen Dan Bioteknologi Kelautan dan Perikanan 16(1): 63–72. https://doi.org/10.15578/jpbkp.v16i1.682

Stach K, Stach W, Augoff K (2021) Review vitamin B6 in health and disease. Nutrients 13(9): 3229. https://doi.org/10.3390/nu13093229

UNICEF (2022) Product specification sheet biscuit high energy and protein CAR. UNICEF for every child. Material No: S00002.

Vaivada T, Akseer N, Akseer S, Somaskandan A, Stefopulos M, Bhutta ZA (2020) Stunting in childhood: an overview of global burden, trends, determinants, and drivers of decline. American Jurnal Clinic Nutrition 112: 777S–791S. https://doi.org/10.1093/ajcn/nqaa159

Widyaningrum ME, Evawati D, Widiana ME, Saputrai E (2022) Healthy cookies fortification of fish meal as an effort to diversify post-harvest processing of fishery products to increase the economic value of fishermen. European Journal of Business and Management 14(20): 34–38. https://doi.org/10.7176/EJBM/14-20-04

Zhao CJ, Schieber A, Gänzle MG (2016) Formation of taste-active amino acids, amino acid derivatives and peptides in food fermentations – A review. Food Research International 89: 39–47. https://doi.org/10.1016/j.foodres.2016.08.042

Zhang Y-Q, Li H, Wu H-H, Zong X-N (2021) Stunting, wasting, overweight and their coexistence among children under 7 years in the context of the social rapidly developing: Findings from a population-based survey in nine cities of China in 2016. PLOS ONE 16(1): e0245455. https://doi.org/10.1371/journal.pone.0245455