





Development and nutritional value of instant anchovy (*Stolephorus indicus*) soup as vitamin D-rich meal for pregnant women

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ABSTRACT

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Indian anchovy (*Stolephorus indicus*) is widely used as a raw material for sauces, salted fish, food flavoring, and side dishes in Southeast Asia due to its abundant macro and micronutrients, including protein, fat-soluble vitamins A, D, and E, as well as high levels of calcium and iron. Therefore, this study aimed to develop an instant anchovy soup formula to meet the daily vitamin D adequacy of pregnant women. Physicochemical characterization was performed to evaluate the properties of anchovy flour (AF) and the instant anchovy soup formula. The characterization included the assessment of the water solubility index, water absorption capacity, color and bulk density, proximate compositions, as well as calcium, iron, and vitamin D content. Additionally, a sensory evaluation was conducted to determine the best instant soup formula. F2 was selected as the most preferred instant soup formula based on sensory parameters. The nutritional values of F2 comprised protein 12.11%, fat 2.18%, ash 7.78%, moisture 6.79%, carbohydrates 71.14%, iron 9.76 mg/100g, Ca 519.67 mg/100g, and vitamin D 47.2 mcg/100g.

Contribution/Originality: This study presents a novel approach to reducing heavy metal contamination in processed fish by utilizing a natural acidic treatment method. Unlike previous studies that focused on chemical treatments, our research explores the effectiveness of lemon juice and ginger as eco-friendly alternatives. The findings contribute to food safety by providing a sustainable and cost-effective method for reducing toxic metal content in seafood, thereby enhancing public health.

1. INTRODUCTION

Indian Anchovy (*Stolephorus indicus*) is the highest marine fishery commodity in Indonesia. Furthermore, the production value is estimated to be approximately 4.63 billion rupiah from the sales achieved at fish auctions in Banten Province in 2022 (BPS, 2023). Anchovy is abundant in the local market and has an affordable price. This is a type of pelagic fish commonly used as raw material for sauce, salted fish, food flavoring, and side dishes, as well as additional nutrition to enrich various products (Corapci & Guneri, 2019).

Anchovy has a small size but is packed with various levels of incredible nutritional content, influenced by factors including the surrounding water environment, diet, age, and season. This species is rich in protein, vitamins, minerals, essential amino acids, and polyunsaturated fatty acids (PUFAs), making it nutritionally comparable to other fish such

as mackerel. Anchovy provides the best protein intake, excellent iron absorption (Litaay et al., 2023) and has the potential to be a crucial source of PUFA-rich oil containing various fat-soluble vitamins A, D, and E, coenzyme Q10, as well as thiamine, riboflavin, and niacin (vitamins B1, B2, and B3) (Scurria et al., 2020).

A study in Korea reported the successful extraction of vitamin D₃ from European anchovy (*Engraulis encrasicolus*) oil fillet leftovers through UHPLC–HESI–MS (ultra-high performance liquid chromatography heated electrospray ionization mass spectrometry). (Scurria et al., 2020) The results showed 81.5 µg/kg of vitamin D₃, which corresponds with the data indicating that vitamin D content in fish oil ranges from 18 to 350 µg/kg (Scurria et al., 2020).

The fortification of food products with anchovy has been widely implemented to enhance calcium and vitamin D content. The intervention of 50 g of soy-catfish-anchovy-rice (SCAR) porridge supplied daily for 14 days to tuberculosis patients with complications showed a significant increase in calcidiol serum levels. The study on the administration of Anchovy Calcium Extract (ACE) to female Sprague-Dawley rats reported that ACE enhanced calcium levels in both mothers and infants, as well as the bone firmness of mothers. Consequently, anchovy presents a great opportunity as a dietary supplement to fulfill the necessary calcium and vitamin D needs for the health of mothers and infants.

Vitamin D is among the nutrients that support calcium homeostasis and bone mineralization, particularly during growth periods (Agarwal, Kovilam, & Agrawal, 2018). A meta-analysis involving six studies that included 830 pregnant women in Indonesia, with an average age between 27.6 and 30.6 years, showed that 25% experienced vitamin D insufficiency, 63% had a deficiency, and 78% were affected by hypovitaminosis D (Octavius, Daleni, Angeline, & Virliani, 2023). The combined serum vitamin D level is 40.59 nmol/L, which falls below the threshold for 25(OH)D deficiency ((Mitchell, Hena, Finkelstein, & Burnett-Bowie, 2012; Wacker & Holick, 2013). Low levels of vitamin D heighten the likelihood of conditions such as preeclampsia, gestational diabetes, premature birth, low birth weight, small-for-gestational-age infants, impaired bone development, and neonatal infections such as respiratory tract infections and sepsis (Agarwal et al., 2018; Bao et al., 2018; Pusparini, 2014).

The mentioned risk can be reduced by consuming vitamin D-rich and vitamin D-fortified foods or supplements during the preconception period and pregnancy (Looman et al., 2018). Fortifying food products is an effective method for providing nutrients to pregnant women without significantly altering their diet, while preservation helps retain the nutritional value.

Dehydration is one of the earliest and most widely utilized techniques for preserving food, as it lowers moisture levels, decreases microbial activity, and prevents the deterioration of products to extend shelf life (Abraha et al., 2018). Anchovy is a perishable fishery product, suggesting the need to adopt effective preservation or processing methods to prevent spoilage after harvest (Wu et al., 2024). Preprocessing is a crucial initial step that helps prevent the oxidation of antioxidants during drying, enhances drying efficiency, and improves the quality of the final dried products (Wu et al., 2024). The transition from fresh anchovy to acidic immersion, dehydration, and grinding can impact nutrient composition. Therefore, optimizing drying conditions such as temperature and duration is essential for maintaining the quality of the raw material (Kusumaningtyas, Laily, & Sudiarti, 2024).

Anchovy flour (AF) is a commonly used raw material in the formulation of instant soup, serving as both a flavor enhancer and a rich source of minerals and vitamins. Optimizing the formula of instant fish soup is key to achieving desirable physical, chemical, and sensory properties while ensuring it meets the daily vitamin D requirements of pregnant women. Therefore, this study aimed to enhance and assess the physicochemical properties of AF and the sensory quality of the instant anchovy soup formula. The physical characterization included an assessment of yield, bulk density, color, water absorption, and solubility, as well as ash, protein, fat, carbohydrate, calcium, and vitamin D content; then a sensory evaluation was conducted.

2. MATERIAL AND METHOD

2.1. Materials and Equipment

Fresh anchovy was purchased from the fish market at Muara Baru, Jakarta, Indonesia. Gum arabic (GA), maltodextrin, garlic powder, shallot powder, refined sugar, mushroom broth, cornstarch, and dairy creamer used in instant soup formulation were obtained from an online marketplace. Lemons and ginger were bought from a traditional market in Serpong, Indonesia, and vitamin D premix (Premix Vit-Milk SCM003) was purchased from PT Global Nutritech. Meanwhile, the equipment used in this study included oven dehydrators, vortex mixers, horizontal mixers, grinders, analytical scales, centrifuges, a colorimeter, as well as a viscometer.

2.2. AF Preparation

A total of 1000 g of fresh anchovies were prepared by removing the heads and stomach contents, then rinsed under running water and allowed to drain. Around 50 grams of fresh ginger were peeled and sliced thinly, then combined with juice from two lemons (approximately 5-10 mL). This mixture was added to a pan containing 1000 mL of tap water and boiled for 10 minutes on the stove before turning off the heat, followed by soaking the anchovies in the hot ginger-lemon water for 10 minutes.

Anchovy was drained, arranged on a tray, and dried in an oven dehydrator at 40°C for 24 hours, then ground using a grinder with a 60-mesh sieve. The resulting anchovy flour was stored in an airtight container and kept in the refrigerator until use.

2.3. Formulation of Instant Anchovy Soup

The development of instant anchovy soup followed a randomized block design (RBD) with six groups and two replications. The concentration variations in the soup formula included 2, 3, and 4 g of anchovy flour (AF) along with 1.5 and 3 g of gum arabic (GA), formulated in the ratios: F1 (2:1.5), F2 (3:1.5), F3 (4:1.5), F4 (2:3), F5 (3:3), and F6 (4:3). Preliminary experiments indicated that using more than 3 g of AF/150 mL of water led to dark coloration, affecting the soup's appearance. The formula was developed within concentration limits, with variations set one level above and below.

Table 1. Composition of instant anchovy soup formula with variation in the concentration of GA and AF.

Material	Units	F1	F2	F3	F4	F5	F6
AF	g	2	3	4	2	3	4
GA	g	1.5	1.5	1.5	3	3	3
Maizena	g	4	4	4	4	4	4
Onion powder	g	2	2	2	2	2	2
Shallot powder	g	2	2	2	2	2	2
Pepper powder	g	0.5	0.5	0.5	0.5	0.5	0.5
Refined sugar	g	0.5	0.5	0.5	0.5	0.5	0.5
Mushroom broth powder	g	0.25	0.25	0.25	0.25	0.25	0.25
Maltodextrin	g	2	2	2	2	2	2
Dairy creamer	g	2	2	2	2	2	2
Vitamin D premix	g	-	-	-	-	-	-
Total weight (Approximately)	g	20					

Table 1 presents the composition of different formulations (F1 to F6) used in the development of instant anchovy soup. The table lists the ingredients along with their respective quantities in grams. The GA concentration (10%) was based on findings by Aliyah (2019), which identified 10% as the optimal level for improving water solubility. Additionally, a preliminary study showed that adding 3 g of cornstarch per 150 mL of water improved the formula's water absorption capacity, enhancing soup thickness (Aliyah, 2019). Other ingredients, such as maizena, onion powder, shallot powder, pepper powder, refined sugar, mushroom broth powder, maltodextrin, and dairy creamer,

are kept constant across all formulations. Dairy creamer and maltodextrin are included to improve consistency. Additionally, the vitamin D premix is not included in any formulation. The approximate total weight of each formulation is 20 g. The mixing process involved sequentially adding ingredients into a horizontal mixer at the lowest rotation speed.

The vitamin D premix used in this study was encapsulated flour comprising a mixture of 10% vitamin D3, 39% retinyl palmitate (vitamin A), 48% thiamine hydrochloride (vitamin B1), and 3% maltodextrin. One gram of this contains 213.75 mcg of vitamin D3, and the added concentration was adjusted to meet the daily vitamin D requirement, implying that the premix was introduced to complement the vitamin D content present in AF.

2.4. Physicochemical Analysis

The physical analysis of dehydrated AF and instant anchovy soup included determining yield (AOAC, 2007) and bulk density (Widowati, Asni, & Nuraeni, 2020), as well as conducting color tests with a chromameter.

Water absorption capacity (WAC) is an analytical method used to measure the amount of water or aqueous solution added to AF material. The mixture is then subjected to centrifugation, and the amount of water retained by the sedimented material in the centrifuge tube is measured. This measurement serves as a guideline for determining the volume of water required to make dough. The difference between the sample's weight after water incorporation and its original dry weight, expressed per 100 grams, indicates the quantity of water absorbed by the flour (Rauf & Sarbini, 2015). To determine WAC, a sample of AF was mixed with water in a 1:10 (w/w) ratio and shaken thoroughly for five minutes. The mixture was then centrifuged at 1500 rpm for 30 minutes. The weight of the supernatant was recorded, and WAC was calculated using the following formula:

$$\text{Amount of water absorbed} = \frac{(\text{Initial water weight} - \text{final weight}(\text{sediment}))}{\text{Initial weight}} \times 100\% \quad (1)$$

Solubility or dispersibility refers to a material's ability to dissolve in water, including its capacity to break down agglomerates. In this study, a 1 g sample was liquefied in 20 mL of distilled water and heated in a water bath at 60°C for 30 minutes. The supernatant was extracted using a centrifuge at 3000 rpm for 20 minutes. After extraction, 10 mL of the solution was obtained and dried in an oven. The percentage solubility of the raw material was calculated by dividing the measured dry sediment weight by the initial weight of the material, then multiplying by 100% (Aliyah, 2019).

The nutritional makeup of AF comprises protein, fat, ash, moisture, and carbohydrate levels. Protein and fat were measured using the Kjeldahl and Soxhlet methods, respectively; ash and moisture content were assessed with the gravimetric method. Carbohydrate content was determined by calculating the difference (AOAC, 2007). Then, calcium, iron, sodium, and heavy metal contaminants were analyzed through Atomic Absorption Spectrophotometry (SIG, 2023). Vitamin D content was assessed through Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) (SIG, 2023), and Vitamin D levels were assessed in AF samples from three different production batches to evaluate stability.

2.5. Sensory Evaluation

The sensory evaluation uses human senses to assess the level of preference for instant anchovy soup. This was conducted with ethical approval from the Research and Community Engagement Ethical Committee, Faculty of Public Health, Universitas Indonesia (Approval Number: Ket-27/UN2.F10.D11/PPM.00.02/2024).

The sensory evaluation of instant anchovy soup products assessed preference across four parameters, including appearance/color, texture, aroma, and taste. Preference levels were rated on a scale of 1 to 5, ranging from "dislike very much" to "like very much." This test featured 25 untrained panelists, consisting of healthy women aged 18-35 living in Plosokerep village, Pati, Central Java, including one in the first trimester of pregnancy. The results were

recorded numerically and analyzed statistically for categorization, while panelists who completed the evaluation received a goody bag as a token of appreciation.

2.6. Statistical Analysis

The experimental data were assessed using a one-way analysis of variance (ANOVA) with IBM SPSS version 25 software. When ANOVA indicated significant differences between treatments ($p < 0.05$), it was followed by Duncan's Multiple Range Test (DMRT) to determine which groups differed significantly at a certain significance level (5%).

3. RESULT AND DISCUSSION

3.1. Physical Characterization of AF and Instant Anchovy Soup

Fresh anchovies were cleaned by removing abdominal dirt and heads to eliminate bitterness as well as minimize the fishy smell. At this step, the weight of the fresh anchovies without heads decreased by 3.16%. The fresh sample was blanched in hot water containing lemon juice and ginger extract for 10 minutes. The blanching process aimed to inactivate enzymes present and reduce the fishy odor, which affected sensory qualities and nutritional value during storage. The application of lemon juice in the blanching process offered numerous benefits, particularly in reducing heavy metal contamination through its chelating ability, enhancing freshness, and improving the taste and nutritional quality of products (Sipa, Jamaluddin, & Ihwan, 2016). This juice helped enhance food safety by reducing the risk of microbial contamination and naturally extending the shelf life of anchovies (Bhavadharini et al., 2022).

Meanwhile, ginger was incorporated to enhance nutritional value, improve aesthetic appeal and organoleptic properties, and preserve the original anchovy qualities without deterioration during processing. (Laelago Erseido et al., 2023)

The blanched anchovy was dehydrated at 40°C for 24 hours until its moisture content dropped below 10%, the recommended threshold for flour production (Hassoon, Dziki, Miś, & Biernacka, 2020). The drying process was conducted using a convective fish dryer to prevent spoilage. This method ensured that the fish dried in a controlled and protected environment, safeguarding the product from harmful microorganisms, insects, and unpredictable weather conditions (Fitri et al., 2022).

The dried anchovy had a yield of 18.8% dry weight to wet weight (dw/ww) and was subsequently ground using a grinder with a 60-mesh size. During the milling process, the AF achieved a yield of 98.2% dry weight to dry weight (dw/dw) or 14.9% dry weight to wet weight (dw/ww). The flour was stored in an airtight container with silica gel and then placed in a refrigerator until use. The yield was determined by comparing the final product weight to the fresh material weight (AOAC, 2007). The physical characterization results of AF and instant anchovy soup are presented in Table 2 and 3.

Table 2. Physical characterization of AF

Dried from fresh anchovy yield (% dw/ww)	AF from dried anchovy yield (% dw/dw)	AF from fresh anchovy (% dw/ww)	Density (g/cm ³)	WAC (%)	WSI (%)	Color		
						L*	a*	b*
18.8	98.2	14.9%	4.2	1.73 ± 0.39	8.43 ± 0.11	62.27	1.12	7.14

Density is an important factor in assessing food compactness and texture, as well as determining the required container size for storage (Kabir et al., 2022). A compact food texture enhances its ability to withstand pressing processes, leading to strong bonds and minimal air spaces between constituent particles.

A higher bulk density indicates that the flour occupies less volume for the same weight compared to products with lower density. Typically, the bulk density of flour-based food products ranges from 0.3 to 0.8 g/mL, which is crucial for ensuring good physical quality in instant flour production. Instant products should maintain an adequate

bulk density, as a lower value may lead to reduced satiety for consumers and limit the nutritional benefits (Zahra, Budi Pramono, & Priyo Bintoro, 2019). High and low bulk-density flour are respectively suitable as main and complementary ingredients in food products (Kabir et al., 2022).

Table 3. Physical characterization of instant anchovy soup.

Instant anchovy soup formula	WAC (%)	WSI (%)	Color			Viscosity (mPAS)
			L*	a*	b*	
F1	147.50 ^{ab} ±4.20	53.03 ^a ±2.48	109.97	0.90	9.66	11.33 ± 2.31
F2	162.39 ^{cd} ±5.82	50.73 ^a ±1.25	107.96	1.13	10.64	16.67 ± 1.15
F3	168.97 ^d ±5.10	50.45 ^a ±0.89	107.12	1.14	10.23	20.67 ± 3.06
F4	145.14 ^a ±7.67	53.95 ^a ±3.23	110.93	0.80	9.52	20.67 ± 2.31
F5	156.55 ^{bc} ±4.57	53.09 ^a ±1.54	109.62	0.90	9.84	17.33 ± 1.15
F6	167.49 ^d ±3.65	52.10 ^a ±0.96	108.13	1.03	10.14	17.33 ± 4.16

Note: The WAC data for F1–F6 were analyzed using an F-test at the 5% significance level. When significant differences were found, the analysis was continued using Duncan's Multiple Range Test (DMRT) at the same level. The letter 'a' indicates the lowest mean value, which is significantly different from the formula labeled only with 'd'. The label 'ab' indicates no significant difference from either 'a' or 'b'. Similarly, 'bc' and 'cd' indicate no significant differences from 'b' and 'c', and from 'c' and 'd', respectively. The letter 'd' represents the highest mean value of WAC and is significantly different from those labeled 'a' and 'b'. Meanwhile, all WSI values for F1–F6 were labeled with the letter 'a', indicating no significant differences among the formulas based on the DMRT analysis.

The color of AF was evaluated using a chromameter based on the Hunter Lab color system by Hunter (1952). This assessment method consists of three parameters: L*, a*, and b*. The L* value ranges from 0 (black) to 100 (white), representing the amount of reflected light, with higher values indicating greater brightness. The a* value represents chromatic colors between red and green, where positive values (0 to +80) indicate red hues and negative values (0 to -80) indicate green hues. Similarly, the b* value represents chromatic colors between blue and yellow, with positive values (0 to +70) indicating yellow hues and negative values (0 to -70) indicating blue hues (Kusumaningtyas et al., 2024; Swiranata, Mangku, & Rudianta, 2020). In this study, color measurements showed that AF exhibited a grayish appearance with a subtle reddish tint.

Water absorption capacity (WAC) plays a vital role in the food industry, as a decrease in WAC can reduce product humidity, negatively affecting yield and quality. A high WAC is crucial for preserving moisture levels, improving product quality, and ensuring that key properties remain stable during storage, even under extreme heat or freezing conditions (Köhn et al., 2015).

The water absorption capacity (WAC) of anchovy flour (AF) was measured at $1.73 \pm 0.39\%$, which was significantly lower than the values obtained for formulations F1, F2, F3, F4, F5, and F6 in instant anchovy soup. Formulations with higher gum arabic (GA) content (F4, F5, F6) exhibited significantly lower WAC compared to those with lower GA content (F1, F2, F3), with a p-value < 0.05. Increasing the concentration of AF in the instant anchovy soup formula while keeping the GA concentration constant resulted in a rise in WAC. Additionally, increasing the anchovy concentration led to an increase in WAC in instant fish soup formulations. The flour's lower porosity resulted in limited water absorption and made it more difficult to dissolve, which impacted the final soup consistency. However, the addition of GA improved water absorption, allowing the flour to dissolve more rapidly and increasing the overall WAC (Haryanto & Suryati, 2020).

The water solubility index (WSI) is used to measure the ability of a material to dissolve in water, which is crucial for evaluating the solubility of food ingredient particles. Expressed as a percentage, WSI is determined by measuring the amount of material that dissolves in water under specific conditions. A higher WSI value indicates greater solubility. WSI and WAC are interconnected, as both parameters reflect the extent to which a material dissolves in water under specific conditions. The solubility percentage is calculated by measuring the dry sediment weight, dividing it by the initial material weight, and multiplying the result by 100% (Aliyah, 2019).

WAC is essential for the food industry because a decrease in water absorption capacity will reduce humidity, which can negatively affect product yield and quality. A high WAC value is essential for preserving moisture levels in products, enhancing quality, and ensuring that desired traits are maintained throughout the shelf life or during exposure to challenging conditions such as extreme heat and freezing temperatures.

The WAC of AF was $1.73 \pm 0.39\%$, significantly lower than the values obtained for F1, F2, F3, F4, F5, and F6 of instant anchovy soup. Formulas with higher GA content (F4, F5, F6) showed significantly lower WAC compared to those comprising lower GA (F1, F2, F3) with a p -value < 0.05 . Increasing AF concentration in the instant anchovy soup formula with the same concentration of GA led to a rise in WAC. Increasing the concentration of anchovy would lead to a rise in the WAC of the instant fish soup formula. The flour was less porous, leading to limited water absorption and difficulty dissolving. This affected the soup formula, as an increase in AF weight caused the instant soup to settle more quickly. GA addition improved water absorption, which allowed the flour to dissolve more rapidly and increased WAC.

WSI of AF was $8.43 \pm 0.11\%$, which was significantly lower than the value obtained for F1, F2, F3, F4, F5, and F6. Instant fish soup formula with higher GA content had significantly higher water solubility ($p < 0.05$), with F4-F6 showing greater solubility than F1-F3. However, increasing AF concentration in the formula with a constant GA concentration decreased soup water solubility. To improve WSI, maltodextrin should be added to enhance AF dispersion in water, thereby increasing the solubility (Kusumaningtyas et al., 2024).

Increasing AF concentration in the formula generated a darker gray coloration compared to the yellowish-white color of soup without AF. Additionally, higher GA concentration increased the viscosity of the instant soup solution (F4-F6 $>$ F1-F3). Instant soup with a 1.5% GA concentration (F1-F3) showed a rise in viscosity as the AF concentration increased. However, the type consisting of a 3% GA concentration (F4-F6) showed a decrease in viscosity as the AF concentration increased (Aliyah, 2019). A previous study reported that an optimum GA concentration for enhancing water solubility was 10%.

AF is used for flavoring instant soup, and it serves as a valuable source of vitamin D due to its high vitamin content. Therefore, monitoring vitamin D levels during production is essential for stability control.

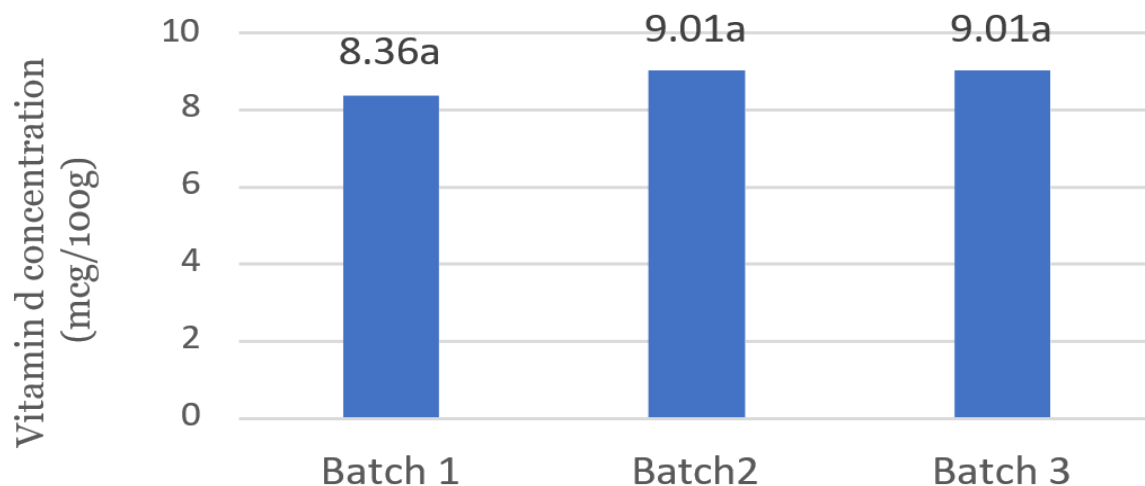


Figure 1. Stability of vitamin D concentration in several AF production batches.

Note: The label 'a' indicates there was no significant difference among these AF production batches based on the DMRT at a significance level of $\alpha < 5\%$.

Figure 1 shows that the vitamin D concentration across production batches remained stable. The vitamin D content in the first sample had an average of 8.275 mcg/100g, which did not significantly differ ($p > 0.05$) from the 9.105 mcg/100g concentration in both the second and third samples. Although there was a slight increase, the vitamin D concentration in AF remained relatively stable throughout the production process.

3.2. Heavy Metal Contamination

Fish is known as a healthy food because it contains high levels of protein, vitamins, and minerals. It also serves as a source of PUFAs and omega-3 fatty acids, specifically EPA and DHA, which are widely recognized for their health benefits for both adults and children. However, concerns about potential contaminants in fish have raised doubts about its safety as a healthy food.

Contamination of the aquatic ecosystem by toxic metals is a global public health concern, as fish and bivalves can bioaccumulate and biomagnify heavy metals to levels harmful to human use (Gbogbo et al., 2018). Lead (Pb), mercury (Hg), cadmium (Cd), and arsenic (As) are classified as harmful heavy metals in foodstuffs by various regulatory agencies, including the National Standardization Body, FAO (Badan Standard Nasional, 2009). These substances are toxic due to their adverse effects on cellular processes and their potential to cause neurological illnesses, kidney damage, skin disorders, circulatory system complications, and an increased risk of melanoma (Gbogbo et al., 2018).

Anchovy pre-treatment was conducted before the drying process to reduce undesirable characteristics, such as fishy odor, lower heavy metal contamination, shorten drying time, and preserve the nutritional composition (Kusumaningtyas et al., 2024). A previous investigation optimized the immersion conditions using alkaline or water treatment for fresh anchovy (Litaay et al., 2023). This study included performing immersion in acidic solutions, specifically lemon juice and ginger, for 10 minutes to reduce heavy metal levels in AF. The findings indicated that the detected concentrations of heavy metals, including arsenic, cadmium, mercury, tin, and lead, were within acceptable limits in fish and processed products, as presented in Table 4. Therefore, AF was safe for consumption and could be used as a fortifying source of minerals and vitamins.

Table 4. Heavy metal content in AF.

Heavy metal	Content (mg/kg)	Permitted limit level (mg/kg)*
Arsen (Ar)	1.0	1.0
Cadmium (Cd)	0.03	0.1
Mercury (Hg)	0.12	0.5
Tin (Sn)	nd	250
Lead (Pb)	nd	0.3

Note: nd; No detection. *Heavy metal contamination limits in fish and processed products according to SNI 7388: 2009 (Badan Standard Nasional, 2009).

3.3. Chemical Characterization of AF

The formulation of instant anchovy soup using AF as the main ingredient, spices, fillers, and thickening agents has been carried out. Formula optimization was conducted using 3 variations of anchovy concentration and 2 variations of GA concentration, leading to 6 formula types. The instant soup formula developed in this study is intended to meet the nutritional needs of mothers during pregnancy. The resulting formula's nutritional value is based on the recommended daily intake for pregnant women and is presented in Table 5.

Table 5. Proximate composition of AF and instant anchovy soup formula.

Parameter	Fresh anchovy*	Dried anchovy	Instant anchovy soup formula						**
			F1	F2	F3	F4	F5	F6	
Ash content (%)		14.58	7.77 ^a	7.78 ^a	7.76 ^a	7.64 ^a	7.66 ^a	7.63 ^a	-
Calory from fat (Kcal/100g)	-	40.01	17.9 ^a	18 ^a	18,2 ^a	16.76 ^a	16.78 ^a	16.75 ^a	-
Total fat (%)	-	4.45	2,2 ^b	2.18 ^b	2,18 ^b	1.87 ^a	1.87 ^a	1.88 ^a	Min. 20
Moisture content (%)	-	8.55	6.76 ^a	6.79 ^a	6,78 ^a	6.75 ^a	6.74 ^a	6.76 ^a	Maks. 8
Total calory (Kcal/100g)	77	329,.1	351.69 ^a	351.72 ^a	353,7 ^a	351.71 ^a	350.72 ^a	351.7 ^a	Min. 450
Carbohydrate (%)	-	0.99	71.46 a	71.14 a	70.62 a	72.54 a	71.5 ^a	71.47 ^a	-

Parameter	Fresh anchovy*	Dried anchovy	Instant anchovy soup formula						**
			F1	F2	F3	F4	F5	F6	
Protein content (%)	16	71.43	11.81 ^b	12.11 ^a	12.66 ^a	11.20 ^b	12.23 ^a	12.26 ^a	Min. 10
Iron(mg/100g)	1	2.73	-	9.76-	-	-	9.74	--	11-18
Calcium (mg/100g)	500	2810.51	481.23 ^b	519.67 ^a	587 ^a	482.1 ^b	519.67 ^a	587.02 ^a	250-450
Sodium (mg/100g)	-	-	555.41 ^a	551.89 ^a	550.19 ^a	550.2 ^a	552.97 ^a	551.8 ^a	Maks. 500
Vitamin (D3 mcg/100g)	-	8.28	46.6 ^a	47.2 ^a	47.7 ^a	46.7 ^a	47.2 ^a	47.74 ^a	Maks 15/day
Total sugar(mg/100g)		-	-	15.48	-	-	15.74	-	Maks.20

Note: * The nutrition composition of fresh anchovy according to a previous study (Aryati & Suci Dharmayanti, 2014).

** Requirements for the nutrition composition of additional food for pregnant women according to the Indonesian National Standard (SNI).

Values followed by the same letter indicate no significant difference based on DMRT at a significance level of $\alpha < 0.05$. The letter 'a' indicates that the nutritional composition is not significantly different from other values labeled 'a', whereas the letter 'b' indicates a significant difference from those labeled 'a'.

The fat content in the formula was low, falling short of the daily recommended intake for pregnant women. Adding vegetable oil might be necessary to meet the nutritional requirements for fat and calories. The sodium content exceeded the specified quality requirements, probably due to the use of mushroom broth in the soup formula, which contained a significant amount of salt, suggesting the need to reduce the mushroom broth. Similarly, the vitamin D content was high, indicating a need to reassess the quantity of vitamin D premix added to the formula. Vitamin D in AF ranged from 8 to 9 mcg per 100g; hence, the consistency and stability of this content should be monitored during the production process. The vitamin D premix should be stored in a cool place and periodically tested to ensure stability.

3.4. Sensory Evaluation of Anchovy

Instant anchovy soup is an example of convenient food products suitable for pregnant women due to its practicality and ease of preparation. This can be enjoyed simply by adding hot water or boiling with additional ingredients such as vegetables, tofu, eggs, and noodles, which allow for a more varied consumption. As a supplementary meal for pregnant women, instant anchovy soup can be consumed as a snack or main dish.

Sensory analysis is an important criterion in the quality evaluation of fishery products by human senses. This is essential for ensuring the quality of fishery products, offering important information to consumers (AOAC, 2007; Widowati et al., 2020). The sensory assessment of instant anchovy soup was conducted based on preference levels for four sensory parameters, including appearance/color, texture, aroma, and taste, as shown in Figure 2. Preference levels were rated on a scale of 1 to 5, ranging from 'dislike very much' (1), 'slightly dislike' (2), 'fair' (3), 'slightly like' (4), to 'like very much' (5).

Panelists rated the F2 formula highest for the appearance parameter, with a score of 4.0, which was significantly different from F1 only ($p < 0.05$). There was a preference for the aroma of F6, which received the highest score of 4.04, although this was not significantly different when compared to F1, F2, F3, F4, and F5 ($p > 0.05$). F2 was the most chosen in taste terms, with the highest score of 4.16, but not significantly different from the ratings for F1, F3, F4, F5, and F6 ($p > 0.05$). F6 was rated the best formula with a texture score of 3.72, which was not substantially different from others ($p > 0.05$).

Based on the sensory effectiveness test results for the instant anchovy soup formula, F2 achieved the highest total favorability score of 15.8 and had a composition ratio of AF = 3:1.5.

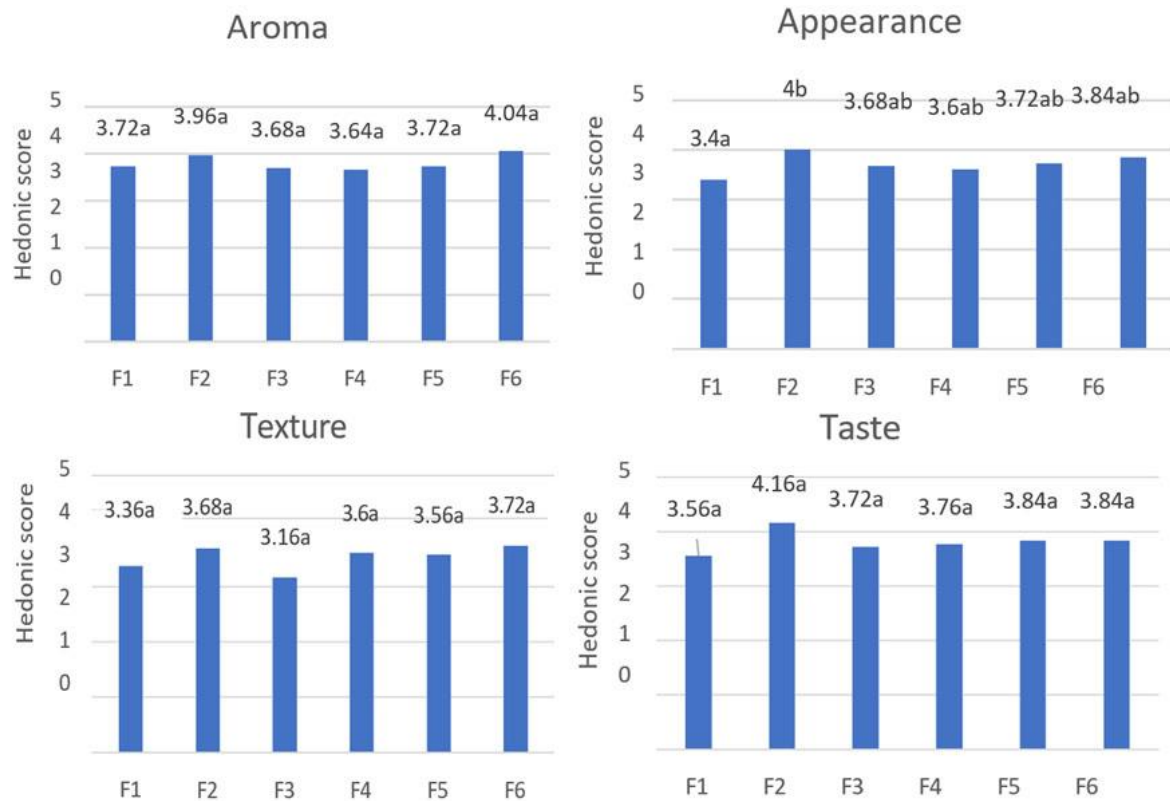


Figure 2. Diagram of sensory evaluation results of instant anchovy soup.

Note: Values followed by the same letter indicate no significant difference based on DMRT at a significance level of $\alpha < 0.05$. The letter 'a' indicates that there were no significant differences ($p > 0.05$) in the hedonic scores for aroma, texture, and taste among formulations F1 to F6. The letter 'b' indicates a significant difference in the appearance parameter compared to those labeled 'a', while the label 'ab' indicates no significant difference from either 'a' or 'b'.

4. CONCLUSION

In summary, this study detects that the treatment and drying process of anchovy are crucial for reducing undesirable characteristics, such as fishy odor and heavy metal contaminants while preserving the nutritional composition of AF. The factors considered when mixing dry ingredients for the instant soup formula are accurate ingredient composition and measurements, consistent and controlled mixing conditions, good solubility and consistency, as well as maintenance of the homogeneity of the components. These aspects are essential to ensure the resulting soup meets the nutritional composition and vitamin D stability expected by the target consumers. Further improvements to the formula are necessary to make the soup a valuable source of vitamin D for pregnant women.

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Transparency: The authors state that the manuscript is honest, truthful, and transparent, that no key aspects of the investigation have been omitted, and that any differences from the study as planned have been clarified. This study followed all writing ethics.

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