Development of Tuna Fish (*Thunnus Sp*) in Increasing Hemoglobin Levels for Pregnant Women at the Waisai Health Center, Raja Ampat Regency

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Thunnus Sp Cookies, Tuna Processing, Anemi Pregnant Women.

ABSTRACT:
Introduction: Based on Riskesdas 2018, the prevalence of anemia in pregnant women increased from 37.1% (2013) to 48.9% (2018). Iron deficiency during pregnancy can inhibit fetal development and have an impact on the quality of pregnancy outcomes, including the risk of *BBLR*, stunting, and abortion. At the Waisai Health Center, 36.36% of pregnant women experience anemia. Protein has an important role in providing organic iron (haem), and tuna is a high source of protein. Sorong, as one of the tuna-producing regions in Indonesia, offers great potential for food innovation.
Objective: This study aims to develop processed tuna fish (*Thunnus Sp Cookies*) to increase Hb levels of pregnant women.
Method: The research lasted for two years (January 2021-December 2022). The first year was focused on identifying the best formula of Thunnus Sp Cookies through a panelist test using the ANOVA method. The second year, using a pre and post experiment control group design, examined the effect of tuna cookies on the Hb level of pregnant women. The sample was 44 pregnant women, with data analyzed using the paired T-test.
Results: The results of this research support the roadmap of the Sorong Ministry of Health Polytechnic (2019-2023) in the development of appropriate technology based on local culture. The first year's output is in the form of a monograph book draft, while the second year produces ISBN books and articles in nationally accredited journals as well as publication drafts in international journals. The level of technological readiness reaches level 3, namely the Thunnus Sp Cookies formula application.

1. Introduction

One of the major nutritional challenges in Indonesia is ensuring adequate nutrition during pregnancy. Proper nutrition plays a vital role in the development of the embryo and fetus, as well as in maintaining the health of the mother. Since pregnancy is a continuous process, any nutritional deficiency at one stage can lead to various impacts on pregnancy outcomes (Ismail, 2022). One common deficiency seen in pregnant women is anemia. According to the 2018 Riskesdas, the prevalence of anemia among pregnant women increased from 37.1% in 2013 to 48.9% in 2018, indicating that the nutritional intake of pregnant women remains insufficient (Kementerian Kesehatan RI Badan Penelitian dan Pengembangan, 2018). Anemia is often caused by a lack of proper nutrition and iron deficiency. During pregnancy, the body requires an additional 1000 mg of iron, with 500 mg needed to support the increase in red blood cell mass.

Iron metabolism is influenced by various factors, including protein intake. Animal protein sources such as fish, eggs, and meat are excellent sources of high-quality protein. Among these, tuna is especially notable for its high protein content. In addition to protein, tuna is rich in other essential nutrients such as vitamins, calcium, iron, and magnesium.

Tuna is a significant export product from Sorong City, with a promising future. According to the Head of Production Statistics at BPS Sorong City, five types of fish are commonly exported, including tuna, mackerel, grouper, bubara, and red snapper. One potential use of tuna is to process it into fishmeal, which can serve as a key ingredient in food diversification efforts.

The provision of supplementary food (PMT) is an effective strategy to improve dietary diversity, especially among vulnerable groups like pregnant women. Nutrient-fortified foods, such as cookies enriched with multiple nutrients, have been suggested as a potential solution to combat malnutrition in pregnant women (Momongan et al., 2023). Cookies are an ideal choice due to their low moisture content, dry texture, and widespread acceptance. Additionally, their small portions make them convenient snacks that can be regularly consumed by pregnant women (Putra et al., 2021).

Despite the potential of using tuna fish (Thunnus Sp) to enhance hemoglobin (Hb) levels in pregnant women, research on this specific fortification is still limited. One study conducted at the Waisai Raja Ampat Health Center found that tuna-fortified cookies were well-accepted by pregnant women and significantly improved their Hb levels ($p = 0.005$) (Sunaeni, 2018). However, the study's findings have not been extended to other areas in West Papua, and the shelf-life of these fortified cookies remains undetermined. A pre-survey conducted in late December 2021 revealed that 33.33% of pregnant women in Sorong City and Sorong Regency suffered from anemia.

The purpose of this study is to evaluate the food security quality of Thunnus Sp cookies, assess the Hb levels of pregnant women, and examine the difference in Hb levels before and after consuming Thunnus Sp cookies in health centers across Sorong City and Sorong Regency, West Papua.

2. Literature Review

The Basic Concept of Tuna Fish

Tuna is a marine fish that consists of several species from the Scombridae family, especially the genus Thunnus. Tuna fish has a very wide distribution area or almost in all tropical and subtropical areas (Erauskin-Extramiana et al., 2019). Tuna is a type of fish rich in protein, providing between 22.6 to 26.2 grams of protein per 100 grams of meat, and is low in fat, containing just 0.2 to 2.7 grams per 100 grams. It is also a good source of essential minerals such as calcium, phosphorus, iron, and sodium, as well as vitamins like vitamin A (retinol) and B vitamins (thiamine, riboflavin, and niacin). Because of these nutritional properties, tuna is beneficial for promoting adult health and supporting children's growth and development (Bustami, 2012).

a. Morphology Ikan Tuna (*Thunnus Sp*)
   In general, the body of a fish is divided into three parts, namely:

1) Caput or often called the head.
2) Truncus or body parts.
3) Cauda or tail section.

Tuna fish have a streamlined, torpedo-shaped body with a pointed head, designed for efficient swimming. Their bodies are smooth, and they feature curved pectoral fins. The caudal (tail) fin is forked with wide slits, aiding in swift movement. Additionally, small, separate finlets are present along the back near the dorsal and anal fins, contributing to their hydrodynamic shape (Wang et al., 2020).

b. Classification of Tuna fish *(Thunnus sp)*

Tuna fish (*Thunnus sp*) is a group of fish that has a swimming speed of up to 50 km/h, is gigantic in size, and has an average length of more than 1.5 meters and weighs up to hundreds of kilos. Tuna fish belongs to the Scrombidae family. The classification of tuna fish (Forleo et al., 2023) is as follows.

1) Phylum: Chordata
2) Subphylum: Vertebrata
3) Class: Teleostei
4) Subclass: Actinopterygii
5) Order: Perciformes
6) Suborder: Scombroidae
7) Family: Scombridae
8) Genus: Thunnus
9) Species: Thunnus sp.

This classification places Thunnus sp., commonly known as tuna, within the vertebrate group of ray-finned fishes under the order Perciformes.

c. Types of Tuna Fish

Tuna Fish Tuna fish has various types, namely big-eyed tuna, albacore tuna, yellowfin tuna, bluefin tuna and dog's tooth tuna.

1) Big-eyed Tuna (Thunnus Obesus)

Big-eyed tuna can grow up to 2.5 meters and weigh up to 210 kg. The age can reach 11 years. This type of Tuna fish is widespread in the Indian Ocean, Atlantic Ocean, and Pacific in tropical and subtropical regions.

2) Tuna Albakor

Tuna Albakor is one of the smallest types of tuna fish, can grow up to 1.4 meters with a weight of 60 kg, its lifespan can reach 9 years and this type of tuna fish is widespread throughout the tropics.

3) Tuna Sirip Biru

Bluefin tuna has 2 types, namely southern bluefin tuna and northern bluefin tuna. Bluefin tuna can grow up to 245 cm with a maximum weight of 269 kg and can live up to 10 years.

4) Tuna Sirip Kuning

Yellowfin tuna can grow up to 239 cm with a maximum weight of up to 2 quintals, can be up to 9 years old. This fish is widespread in tropical and subtropical waters but is not found in the Mediterranean Sea. The number of eggs produced can reach around 200 thousand eggs.

5) Dog Tooth Tuna

This type of tuna is named as dog tooth tuna because it has a mouth like a dog. This fish can grow up to 2.5 meters but only reaches 1.5 meters on average. This fish is widespread in the tropical waters of the world, can live in the open sea with a depth of 20-300 meters.

d. Benefits of Tuna Fish

Tuna fish, which inhabit the deep sea, are an excellent source of nutrients for the human body. Their meat is packed with protein and essential nutrients, including minerals like selenium, magnesium, and potassium, along with B-complex vitamins and omega-3 fatty acids. These nutrients contribute to overall health and are beneficial for heart and brain function.

The following are some of the benefits of tuna for health:

1) Heart Health

Tuna fish with its high omega-3 is very beneficial for maintaining heart function.

2) Preventing Cancer

Tuna fish can also play a role in preventing cancer, including ovarian cancer, pancreatic cancer, and other types of cancer that attack the digestive tract (mouth, pharynx, esophagus, stomach, and intestinal cancer). The abundant omega-3 content in tuna is also beneficial for preventing breast cancer and lowering the risk of developing leukemia.

3) Improves Brain Cognitive Function

Omega-3 found in tuna can help improve memory function or cognitive function of the brain, so that it can avoid brain function degenerative diseases such as Alzheimer's.

4) Improving Responsiveness

Tuna fish insulin hormone is also recommended to be consumed for people with type-2 diabetes, because of its abundant omega-3 fat content. Various studies suggest that omega-3 in tuna can prevent obesity and increase the body's response to the hormone insulin.

5) Helps with the detoxification process.

Selenium, along with the omega-3s contained in tuna, is an important fuel to produce gluthathione peroxidase, an antioxidant type.

e. Nutritional Content in Tuna Fish

Tuna has an impressive nutritional profile, offering high protein and low-fat content. Its protein levels are nearly double that of eggs, traditionally considered a primary protein source. For comparison, tuna provides 22-26.2 grams of protein per 100 grams of meat, while eggs offer around 13 grams. Additionally, tuna is rich in essential minerals such as calcium, phosphorus, iron, and sodium, as well as vitamins A (retinol) and B-complex (thiamin, riboflavin, and niacin). Typically, 50-60% of the tuna fish's body is edible (Hidayati & Masyhuri, 2015). White tuna contains more protein and less fat compared to red tuna meat (García-Arias et al., 1994).

Tuna is also packed with vital minerals like iodine, with its iodine content 28 times higher than that of freshwater fish. Iodine plays a crucial role in preventing goiter and boosting children's cognitive abilities. Additionally, tuna is rich in selenium, with just 100 grams of tuna fulfilling 52.9% of the daily selenium requirement (Bonaventura et al., 1978; Bookchin et al., 1978; Herawati et al., 2022; Podojoyo et al., 2021).

Tuna is considered heart-healthy when the potassium-to-sodium ratio is at least 5:1. Bluefin tuna has a 6.4:1 ratio, skipjack tuna reaches 11:1, and yellowfin tuna hits 12:1. Potassium supports blood pressure control, treats hypertension, removes carbon dioxide from the blood, and aids muscle and nerve function (Alayash, 2004). High potassium levels also promote oxygen flow to the brain and help maintain fluid balance in the body. Bluefin tuna is also rich in vitamins, particularly vitamin A, with 2,183 IU per 100 grams, covering 43.6% of daily vitamin A needs. It’s also an excellent source of vitamin B6 and folic acid. The World Health Rating by The George Mateljan Foundation categorizes tuna as “very good” for vitamin B6, scoring a nutrient density of 6.7 (with the upper limit of “very good” being 6.7). Vitamin B6 and folic acid can reduce homocysteine, a harmful intermediate byproduct linked to artery damage and heart disease (Wilson & Reeder, 2022).

While tuna contains cholesterol, it is lower than many other animal products, at 38-45 mg per 100 grams. Its high EPA (eicosapentaenoic acid) content stimulates leptin, a hormone that helps regulate food intake, preventing overeating and reducing the risk of obesity (Alayash, 2021).

Tuna Fish Product Processing

a. Product Processing Rules

The processing of food products, including fish, must refer to the recommended Nutritional Adequacy Figure. The development of processed food products is the process of creating or modifying products into new foods. The purpose of developing processed food products is to improve product quality according to consumer demand and regulations, to increase competitiveness, profits and improve nutrition and public health. Principles and procedures for using AKG to develop processed food products:

1) Setting consumer targets. The processed food products to be developed are intended for whom (for example, for the public, infants, toddlers, pregnant women, or breastfeeding mothers) and their nutritional problems (Lin, 2018).

2) Determination of food ingredients and composition to be used, by meeting food safety requirements.

4) The determination of superior nutrients in processed food products and the requirements for labelling processed foods that are developed, for example related to nutritional problems or related to improving the nutritional quality of processed food products to be developed.

5) The use of AKG for the target group of processed food products is appropriate.

6) The selection of food ingredients or nutrient compounds by considering the purpose, availability of technology, interactions between nutrients, bioavailability, and sensory/organoleptic value of the product to be produced (Alayash, 2018).

b. Processing *Thunna Sp* Cookies

Cookies are a form of biscuits that are widely loved by children and adult consumers. The quality standards of cookie products based on SNI 01-2973-1992 can be seen in Table 1.

Table 1. Cookie Quality Standards

Test Criteria	**Classification**
Kalri (Kalri/100 grams)	Minimum 400
Water (%)	Maximum 4
Protein (%)	Minimum 6
Fat (%)	Minimum 18
In carbhidra (%)	Minimum 70
Ash (%)	Maximum 2
Crude Fiber (%)	Maximum 0.5
Metals	Negative
Baud and Taste	Normal And Not Rancid
Color	Usual

Source: National Standardization Agency (1992)

Some of the ingredients that are widely used in the cookie manufacturing industry include wheat flour, water, sugar, fat, development agents, milk powder, yolks, and egg whites (Rondon et al., 2021).

c. Stages of Manufacturing

1) Main composition

In making cookies, the main ingredients are sugar, egg fat and flour. In general, the ingredients that make up cookies can be grouped into binding ingredients, namely flour, water, milk, and eggs as well as chocolate products and softeners including sugar, shortening, baking powder and egg yolks. The ideal baking time is at 180-250° C for 16-20 minutes (Giri et al., 2021).

2) Manufacturing stage

This process begins with beating the eggs, margarine and butter using a mixer until well mixed, adding powdered sugar then beating again until the dough turns pale. After that, cheese and chocolate powder are added and then beaten again. Ingredients in the form of flour such as fish meal, wheat flour, cornstarch, and skim milk flour are mixed until smooth in separate places. Next, the mixture is put into the cream dough and mixed until it is waterproof, then maltodextrin is added (Okagu et al., 2022; Tortorella et al., 2020).

3) After that, the dough is put into a cookie presser and printed with a length of approximately 5 cm. Next, the dough is baked at 150°C for 45 minutes until it reaches a brown color.

4) Next, an initial organoleptic test was carried out on the resulting cookie product. The hedonic test was carried out by 30 semi-trained panelists. The scoring scale for the hedonic test is scale 1 to strongly express dislike, scale 2 to express dislike, scale 3 to indicate normal, scale 4 to express like, and scale 5 to express strongly like.

d. Dosage Cookies Thunna Sp

Consumption of Thunna Sp Cookies is 180 gr (2 cookies) for pregnant women in the first trimester, and 240 gr (3 cookies) for pregnant women in the second and third trimesters (Dąbrowski et al., 2016; Ryšlavá et al., 2013).

Anemia in Pregnant Women

a. Understanding

Anemia is a condition marked by a reduction in red blood cells (erythrocytes) or hemoglobin levels, which hampers the body's ability to transport oxygen to tissues. In pregnant women, anemia is diagnosed when hemoglobin levels drop below 11 g/dl during pregnancy or below 10 g/dl in the postpartum period. This decreased oxygen-carrying capacity can have serious consequences for both the mother and the developing fetus (Hosseini et al., 2015).

b. Classification

The division of anemia in pregnancy according to (Yuan et al., 2023) anemia in pregnancy includes:

1) Iron deficiency anemia.

The most common type of anemia in pregnancy is anemia due to iron deficiency. This deficiency can be caused by a lack of iron in food, due to resorption disorders, impaired use, or because too much iron comes out of the body, for example in bleeding (Gervais, 2016).

2) Megaloblastic anemi

Megaloblastic anemia in pregnancy is caused by folic acid deficiency, rarely due to vitamin B12 deficiency (Miller et al., 2014). Unlike in Europe and in the United States, the frequency of megaloblastic anemia in pregnancy is quite high in Asia, such as in India, Malaysia, and Indonesia. It is closely related to food deficiency.

3) Anemia Hypoplastic

Anemia in pregnant women, which is caused by the bone marrow being less able to make new blood cells, is called hypoplastic anemia in pregnancy.

4) Anemia Hemolytic

Hemolytic anemia is caused because the destruction of red blood cells takes place faster than they are made. Women with hemolytic anemia have difficulty becoming pregnant, when they become pregnant, then their anemia usually becomes more severe.

c. Causes of Anemia

1) Iron deficiency anemia is caused by inadequate iron intake in the diet. During pregnancy, a woman's need for iron increases by 200-300% to support the formation of the placenta and red blood cells. As a result, iron supplementation is essential, even for women who are otherwise well-nourished (Gunnoo & Madder, 2016).

2) Direct causes such as many restrictions on certain foods during pregnancy can worsen the condition of iron nutritional anemia, usually pregnant women are reluctant to eat meat, fish, liver, or other animal foods for irrational reasons.

3) Infectious diseases Infectious diseases such as tuberculosis, intestinal worms and malaria are also the cause of anemia because they cause increased destruction of red blood cells and disruption of erythrocytes.

4) Bleeding The cause of iron anemia is also due to too much iron coming out of the body, such as bleeding (G. Thomas, 2001).

d. Signs and Symptoms of Anemia

The signs and symptoms of iron deficiency anemia are often nonspecific and vague, including paleness, fatigue, palpitations, tachycardia, and shortness of breath. Paleness can be observed in areas such as the palms, nails, and conjunctiva. In some cases, symptoms may be absent or overshadowed by those of an underlying condition, or anemia may present alongside the primary illness. Common symptoms of anemia include dizziness, lightheadedness, lethargy, weakness, fatigue, difficulty swallowing (dysphagia), an enlarged spleen, poor appetite, reduced physical fitness, and delayed wound healing (Bae et al., 2014).

e. Determination of anemia status in pregnant women

The threshold value used to determine anemia status. The classification of anemia in pregnant women can be seen in Table 2.

Table 2. Classification of Anemi in Pregnant Women

Anemia Status	**Rate Hb (g/dl)**
No Anemi	> 11
Mild Anemi	9-10
Medium Anemi	7-8
Anemia Charter	< 7

Source: Irianto, 2014

f. Effect of Anemi on Pregnancy

Anemia during pregnancy poses significant risks, including miscarriage, premature birth, restricted fetal growth and development, susceptibility to infections, heart failure (when hemoglobin is below 6 g%), hyperemesis gravidarum, antepartum hemorrhage, and premature rupture of membranes (PROM). Anemia also increases the likelihood of complications during pregnancy and delivery, raising the risk of maternal and infant mortality as well as low birth weight. The effects of anemia on pregnancy can range from mild discomfort to severe disruptions in pregnancy progression (M. C. Thomas et al., 2006).

g. How to prevent anemoney in pregnancy

There are four approaches to preventing anemia:

1) Give tablets or iron injections or increase iron consumption.
2) Surveillance of infectious diseases.
3) Fortification of staple foods with substances. milk.
4) In pregnant women, by routinely checking their pregnancy at least 4 times during pregnancy to get Iron Tablets and other vitamins from health workers and eating nutritious food 3x1 days with 2 times more portions (Frass, 2015).

3. Methods

This study employed a True Experimental Design, with two phases over two years. In the first year, laboratory experiments were conducted to develop the optimal formula for Thunnus Sp cookies through four rounds of testing (replications). In the second year, clinical trials were carried out using the Pretest-Posttest Control Group Design. The study involved pregnant women, who were randomly assigned to either an intervention group receiving Thunnus Sp cookies or a control group receiving standard PMT (Supplementary Food) biscuits for pregnant women. Both groups underwent a pretest to measure hemoglobin (Hb) levels before the intervention, and a posttest afterwards. Statistical tests were then performed to determine any significant differences between the two groups. Internal controls were applied by clustering experimental units homogeneously to ensure the validity and effectiveness of the study.

The research was conducted between January 2021 and December 2022 at the Waisai Health Center in Raja Ampat Regency. This location was chosen due to the high prevalence of anemia among pregnant women and its status as a practice site for Interprofessional Education (IPE) by the Sorong Ministry of Health Polytechnic. In the first year, preliminary research focused on developing the Thunnus Sp cookie formula and conducting organoleptic testing. In the second year, the clinical trial involved giving Thunnus Sp cookies to pregnant women as a supplementary food, with Hb levels measured before and after 90 days of intervention.

The study population consisted of all 56 pregnant women within the Waisai Health Center's coverage area. The sample included 48 pregnant women, with 3 additional participants as reserves. Data collection instruments included observation forms for organoleptic, proximate, chemical, and clinical tests of Hb levels. To reduce bias, participants were asked to fill out a food record form. Data collection began after obtaining the necessary research permits. Participants were briefed on the benefits and procedures for consuming Thunnus Sp cookies, and informed consent was obtained. The sample was divided into two groups: 24 pregnant women in the intervention group (consuming Thunnus Sp cookies) and 24 in the control group (consuming PMT biscuits). Hb levels were measured before (pre-test) and after (post-test) the 90-day intervention, and results were compared between the groups.

Data analysis occurred after collection and involved several stages. The first stage was editing to ensure the completeness and consistency of the data. Next, coding was done to prepare the data for computerized analysis. For the univariate analysis, Hb level data from the pregnant women was analyzed by calculating the mean and standard deviation, which were then presented in tables. For bivariate analysis, chemical content tests (protein, fat, ash, water, carbohydrates), organoleptic tests, and proximate tests were analyzed using

ANOVA, provided the assumptions for parametric tests were met, or the Wilcoxon sign rank test if the data were not normally distributed. Hb level data from before and after the intervention was analyzed using a paired T-test at a significance level of 95% ($\alpha = 0.05$). The hypothesis was accepted or rejected based on the probability value (p), and the results were presented in table format.

4. Results

Making Tuna Cookies

The process of making tuna cookies is carried out in two stages of research: preliminary and main. In the preliminary research, MP-ASI cookies were made with tuna fishmeal substitutions of 0%, 17%, 25%, 33%, and 41%, which were modified from (Jung et al., 2019) research. The results of the acceptability test show that cookies with a substitution of 17% are the most preferred because they have a unique taste, bright color, distinctive aroma, and good texture. Cookies with 0% substitution are considered too standard, while those with higher substitutions (40% and 50%) are less preferred because they are wet and fishy.

The main research was carried out by reducing substitutions to 10%, 20%, and 30%, aiming to improve quality in terms of taste, color, aroma, and texture. After manufacturing, an acceptability test and solubility test were carried out to determine the level of preference of respondents for various tuna cookie formulas. These cookies are made from basic ingredients such as margarine, eggs, powdered sugar, milk, wheat flour, cornstarch, and tuna fish meal substitution.

Rehydration Power

Rehydration power can be measured using a brew test. The brew test is a physical test carried out on selected products. The brewing test is carried out by dissolving the sample in water until it reaches its consistency.

According to (Desjardins et al., 2012), the brewing test is a method to find out the ratio of the amount of water added to rehydrate the product. The amount of water added is determined by the solubility and water binding capacity of the materials that make up the product. The results of the brewing test can be seen in table 3.

Table 3. MP-ASI Cookies Brewing Test

It	Yellow Pumpkin Flour Substitution Treatment	Repetition			Average ±SD	P value
		I	II	III		
1.	10%	1.4%	1.2%	1.2%	1.27±0.12b	0,016
2.	20%	1.1%	1.3%	1.1%	1.17±0.12b	
3.	30%	1.0%	0.8%	0.9%	0.90±0.10A	

Description: The same notation shows no real difference
Source: Primary Data (2018)

According to Table 3, the solubility values in the substitution treatments with 10%, 20%, and 30% yellow pumpkin flour are 1.27, 1.17, and 0.90, respectively. A One-Way ANOVA test conducted on the solubility indicator, with a 95% confidence level, showed a p-value of 0.016 (< 0.05). This indicates that the null hypothesis (Ho) is rejected, confirming that the substitution of yellow pumpkin flour has a significant effect on the solubility of the cookies.

From Table 3, it is evident that the solubility value for 10% yellow pumpkin flour substitution does not differ significantly from the 20% substitution. However, the 30% substitution shows a significant difference compared to both the 10% and 20% substitutions. The solubility values are further illustrated in Figure 1.

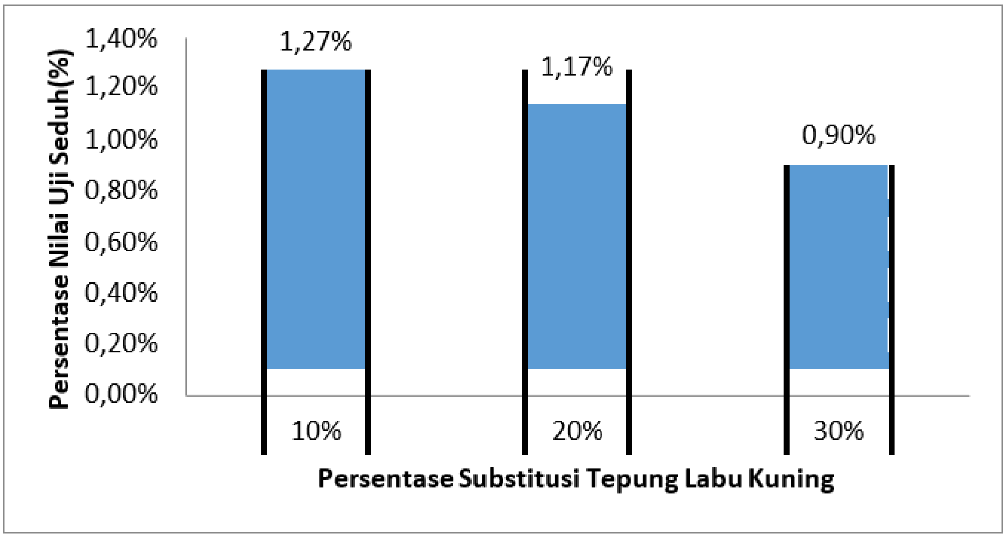

Figure 1. MP-ASI Cookies Brewing Test Chart

Figure 1 illustrates that as the substitution of yellow pumpkin flour increases, less water is required to dissolve the cookies to reach their desired consistency. This reduction in water needed is due to the high fiber content in yellow pumpkin flour, which absorbs water and increases the moisture content of the cookies (Lever-van Milligen et al., 2014). According to (Honda et al., 2017), yellow pumpkin flour contains 13% moisture. Additionally, (Hämäläinen et al., 2018) noted that yellow pumpkin flour has hygroscopic properties, meaning it easily absorbs water, and it contains pectin and fiber, which bind water more effectively than wheat flour.

The water volume for cookies with a 20% substitution of yellow pumpkin flour, which closely resembles the texture of commercial biscuits, is 11 ml per 10 g. This water-to-cookie ratio produces a porridge consistency that is neither too thick nor too thin. According to (Galarneau et al., 2010), the rate of water absorption in cookies is influenced by factors such as particle size and distribution, the mixing process, and the composition of the ingredients.

Acceptability

The preference test is used to determine a person's level of acceptance of a product, which is crucial for ensuring the product's success. According to (Martínez-Martínez et al., 2014), various factors can influence panelists' assessment of acceptability, including age and educational background. It is also important that the panelists are in good health and free from mental disorders.

Acceptability tests measure the degree of liking or disliking a product based on factors such as color, aroma, taste, texture, and overall impression. A higher level of product acceptability indicates greater consumer satisfaction (Kavsaoğlu et al., 2015).
The results of the cookie acceptability test, conducted on 30 panelists with 10%, 20%, and 30% yellow pumpkin flour substitutions, assessing color, aroma, taste, texture, and overall preference, are presented in Table 4.

Table 4. Consumer Acceptance Results of MP-ASI Cookies

Substitutions	Color	Aroma	Taste	Texture	Overall
10%	3.50±0.82	3.47±0.77a	3.33±0.66a	3.30±0.75A	3.43±0.73
20%	3.87±0.73	3.97±0.67b	3.90±0.66b	3.90±0.61b	3.87±0.68
30%	3.90±0.92	3.57±0.90ab	3.73±0.91b	3.67±0.84ab	3.80±0.85
P value	0.464	0.025	0.031	0.047	0.294

Description: The same notation shows no real difference
Source: Primary Data (2018)

Based on the results of the One-Way ANOVA test on the acceptability indicators—color, taste, aroma, and texture—with a 95% significance level, the p-value was found to be 0.00 (< 0.05). This means that the null hypothesis (H0) is rejected, indicating that the substitution of tuna fishmeal in tuna cookies has a significant effect on color, taste, aroma, and texture.
From the table, it is shown that in the aroma test, the 10% tuna fishmeal substitution is significantly different from the 20% substitution, while the 30% substitution does not significantly differ from either the 10% or 20% substitutions. In the taste test, the 10% yellow pumpkin flour substitution differs significantly from both the 20% and 30% substitutions, and the 20% substitution also differs significantly from the 30%. For the texture test, the 10% substitution is significantly different from the 20% substitution, but the 30% substitution does not differ significantly from either the 10% or 20% substitutions.

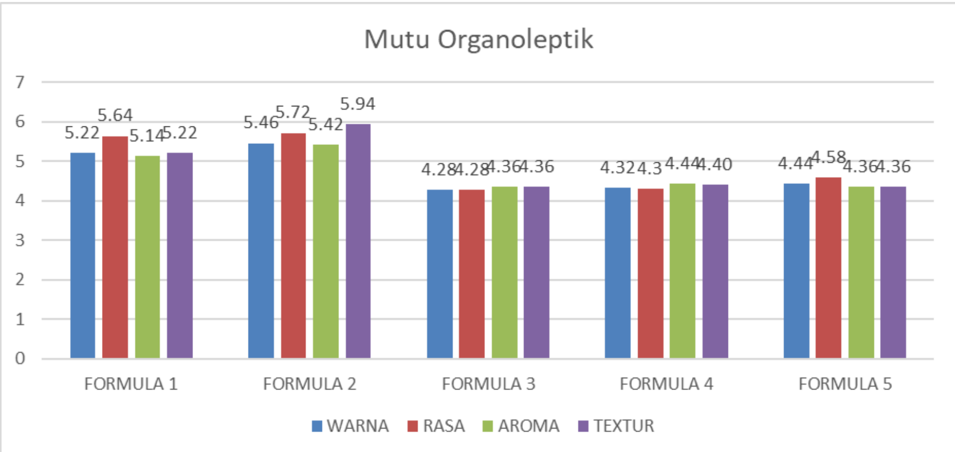

Figure 2. Consumer Acceptability Graph of MP- ASI Cookies with Yellow Pumpkin Flour Substitution

Color
The color of cookies is generally influenced by the caramelization process and the Maillard reaction during baking. However, the type and amount of flour used also play a

significant role in determining the cookie's color. Finer flour particles can result in a brighter appearance of the cookies (Chaudhary et al., 2017). According to a bar chart, cookies in formulas 1 and 2 had the brightest colors compared to those in formulas 3, 4, and 5, based on panelists' preferences. This indicates that as the concentration of fishmeal increases, the brightness of the cookies decreases. Additionally, the length of the baking process also affects the final color of the cookies. Fellows (2000) stated that color is the most immediate sensory response and can leave a strong impression, as it is the first attribute perceived by the eye. Even if a product has excellent taste, texture, and aroma, its visual appeal, particularly color, is a key factor in whether it will be consumed.
Figure 1 shows that cookies with a 10% yellow pumpkin flour substitution had the lowest average acceptability score for color at 3.5, while cookies with a 30% substitution scored the highest at 3.9. This suggests that higher concentrations of yellow pumpkin flour increased the panelists' preference for the cookies' color. (Muady et al., 2016) highlighted that color is a crucial factor in determining food quality, as it is the first characteristic seen and can be highly influential. The color change in cookies due to the yellow pumpkin flour substitution is attributed to the high carotenoid content in yellow pumpkin, which imparts an orange-yellow hue (Kim et al., 2021).
According to (Ganjavi et al., 2010), carotene is the primary pigment responsible for red, orange, yellow, and green hues in fruits and vegetables. In addition to color pigments, other factors influencing food color include heat, which can lead to caramelization. Caramelization occurs when a sucrose solution is heated, causing evaporation and an increase in concentration and boiling point. As the temperature reaches the melting point, caramelization begins. Furthermore, yellow pumpkin flour contains sugars and starches that can trigger the Maillard reaction, a process that causes browning. The Maillard reaction involves the interaction of amino groups from amino acids, peptides, or proteins with sugar’s hydroxyl glycosidic groups, ultimately leading to the formation of melanoidins, which give the food its darker color (Rahmani et al., 2018).

Aroma
Evaluating food through an odor test is crucial, as it helps determine whether a product is appealing to consumers. The aroma of food plays a significant role in influencing how enjoyable it is, making it an important factor in consumer preferences (Mol, 2011). Aroma can be detected through the sense of smell and is a subjective sensation produced by volatile compounds that create scents (Raber et al., 2012).
Figure 1 shows the average acceptability of the aroma of cookies, with the lowest score being 3.4 for cookies with a 10% yellow pumpkin flour substitution, and the highest score of 3.97 for cookies with a 20% substitution.
According to (Sunoko & Huang, 2014), aroma is a key factor in determining consumer acceptance of a product. Before tasting, consumers typically smell the food to decide if it is appealing and suitable for consumption. A pleasant aroma can capture consumers' attention.
The variation in aroma among the cookies is attributed to the differing concentrations of yellow pumpkin flour. Yellow pumpkin flour imparts a distinctive aroma and has specific properties, making it a viable complement to wheat in various processed food products (Mousakhani-Ganjeh et al., 2015). However, when yellow pumpkin flour was used at a higher concentration, such as 30%, panelists found the aroma to be too strong and unpleasant, leading to a lower preference for the cookies' aroma.

Taste

Taste is a key parameter in organoleptic testing and one of the primary factors influencing the outcome of these evaluations. Taste testing engages the sense of taste (tongue), which can detect flavors such as sweet, salty, spicy, and bitter. The taste of a product is shaped by the compounds present in its ingredients and is closely related to a person's preference for a food item. As (Fakhri et al., 2022) noted, food with an appealing and enjoyable taste is more likely to be favored by consumers.

Figure 1 illustrates the acceptability scores for the taste of cookies, with the lowest average score of 3.33 observed in cookies with a 10% yellow pumpkin flour substitution, and the highest average score of 3.9 in cookies with a 20% substitution.

Cookies with a 30% yellow pumpkin flour substitution were less popular among panelists, primarily due to the strong, distinctive flavor of the yellow pumpkin. This observation aligns with the findings of (Sobhanardakani, 2017), which indicated that the more yellow pumpkin flour is used in biscuits, the stronger and more prominent the yellow pumpkin flavor becomes.

The distinctive flavor of yellow pumpkin can negatively impact the acceptability of the taste in products where the flavor is too dominant. According to Hosseini et al. (2015), several factors can influence panelists' taste evaluations, including chemical composition, temperature, concentration, and interactions with other flavor components.

The sensations triggered during taste testing result from stimulation in the mouth caused by the properties of food ingredients. This stimulation activates sensory nerves in the face, tongue, and teeth, which generate specific taste perceptions.

Texture

The taste of a food product can be influenced by its texture, which is shaped by its physical properties. A penetrometer, or simply pressing or touching the product, can be used to measure the hardness and texture of food (Muñoz-Benavent et al., 2018).

Figure 1 shows the texture acceptability scores for cookies, with the lowest average of 3.3 observed in cookies with a 10% yellow pumpkin flour substitution, and the highest average of 3.9 in cookies with a 20% substitution.

Texture refers to the sensation felt in the mouth when food is bitten, chewed, or swallowed, or by touch with the fingers. Each food product has unique texture properties, determined by its physical state, the size and shape of its cells (De Magistris et al., 2015).

The figure illustrates that yellow pumpkin flour substitution impacts the texture of the cookies. This aligns with Isnaini's (2016) findings, which showed that increasing yellow pumpkin flour in a recipe result in a softer, mushier product. Additionally, the texture of cookies is also affected by the ingredients used. Specifically, higher protein content leads to a harder texture in food products (Sadighara et al., 2024).

Overall

Figure 1 illustrates the overall acceptability of cookies, showing the lowest average score of 3.3 for cookies with a 10% yellow pumpkin flour substitution, and the highest average score of 3.9 for cookies with a 20% substitution. The results indicate that as the percentage of yellow pumpkin flour increases beyond 20%, respondents' acceptance of the cookies decreases. This decline in preference is attributed to factors such as a less appealing, overly dense color, an overpowering aroma, a lack of flavor, and a crumbly texture.

Among all the tested parameters, cookies with a 20% yellow pumpkin flour substitution were most favored. These cookies had an attractive color, a pleasing aroma where the yellow pumpkin scent was not too strong, a good taste, and a balanced texture that was neither too soft nor too hard.

Characteristics of Respondent

Table 5. Characteristics of Pregnant Women Based on Age in the Experimental and Control Groups

Age	N	Minimum	Maximum	Mean	Std. Deviation
Exam class	20	18	41	26.20	6.246
Control Classes	20	19	39	26.85	5.112

Based on table 5. Above it is known that the average age of pregnant women in the experimental class is 26 years old, the lowest age is 18 years and the highest is 41 years. Meanwhile, in the control class, the average age was 27 years. The lowest age is 19 years and the highest is 39 years.

Table 6. Characteristics of Pregnant Women Based on Parity in the Experimental and KLPK Control Groups

Parity	N	Minimum	Maximum	Mean	Std. Deviation
Experimental classes	20	1	3	1.90	.968
Control Classes	20	1.00	3.00	1.8500	.74516

Based on table 6. The above shows that the average parity of pregnant women in the experimental class 2. parity is the lowest 1 t and the highest is 3 years. Meanwhile, in the control class, the average parity was 2, and the lowest parity was 1 and the highest was 3.

Average Value Distribution

Table 7. Distribution of the Average Value of Hb Levels in the Experimental Class and the Control Class

No	Class	Up to Hb	N	Min	Max	Mean	SD
1.	Experimental classes	Hb rate before treatment	20	7.8	10.9	9.825	.9262
		Hb levels after treatment	20	10	14	11.54	1.084
2.	Control class	Hb rate before treatment	20	7.5	13.0	9.990	1.0983
		Hb levels after treatment	20	9.0	12.4	10.410	.9095

Based on Table 7, the hemoglobin (Hb) levels of pregnant women in the experimental group averaged 9.8 g/dL before treatment, with a standard deviation (SD) of 0.92. The lowest recorded Hb value was 7.8 g/dL, while the highest was 10.9 g/dL. After treatment, the average Hb level increased to 11.5 g/dL, with an SD of 1.084. The lowest post-treatment Hb value was 10 g/dL, and the highest was 11.5 g/dL.
In the control group, the average Hb level before treatment was 9.99 g/dL, with an SD of 1.1. After treatment, the average increased to 10.41 g/dL, with an SD of 0.91. The lowest Hb value in this group was 9 g/dL, and the highest was 12.4 g/dL.

Data Normality Testing

Table 8. Distribution of Normality of Hb Levels Before and After Treatment in the Experimental and Control Classes

Class	Up to Hb	df	Sig. (2-tailed)
Experimental classes	Hb Rate Before Treatment	20	0.087
	Hb levels after treatment	20	0.238
Control Classes	Hb PMT before	20	0.114
	Hb PMT after	20	0.211

Based on Table 8, the Shapiro-Wilk test results indicate that the hemoglobin values for pregnant women in both the experimental and control groups were normally distributed. In the experimental group, the p-value before treatment was 0.087 and after treatment, it was 0.238. Similarly, in the control group, the p-value before treatment was 0.114, and after treatment, it was 0.211. Since all p-values are greater than 0.05, this confirms that the data in both groups followed a normal distribution.

Difference in Average Hb of Pregnant Women Before and After in the Experimental and Control Classes

Table 9. Difference in Average Hb of Pregnant Women Before and After in the Control Group

No	Variable	Sig. (2-tailed)
1	Hb Experimental class before-after	0,000
2	Hb class Control Before-After	0,006

Based on Table 9, the T-test results for the experimental group show a significance value of 0.000 ($p < 0.05$), indicating a significant difference in the hemoglobin (Hb) levels of pregnant women before and after the treatment. Similarly, in the control group, a significance value of 0.006 ($p < 0.05$) was obtained, which also indicates a significant difference in Hb levels before and after the experiment. This confirms that both groups experienced notable changes in Hb levels following the treatment.

Categorization of Interpretation of the Effectiveness of the N-Gain Score of Hb in Pregnant Women After Treatment in the Experimental Class and Control Class

Table 10. Interpretation of Effectiveness Based on the N-Gain Score of Pregnant Women After Treatment in the Experimental Group and Control Group

Group	N	Mean
Kls Experiment	20	1.9056
Kls Control	20	0.6178

Based on Table 10, the N-gain value test results indicate that the average N-gain score for the experimental group, where pregnant women were given Thunna Sp cookies, was 1.91, which is greater than 0.7. This places it in the category of high effectiveness for increasing hemoglobin (Hb) levels. In contrast, the control group, which received standard PMT cookies, had an average N-gain score of 0.6, which is below 0.7, categorizing it as having moderate effectiveness in improving Hb levels.

Comparison of the Average Difference in Hb Levels of Pregnant Women After Treatment in the Experimental Class and the Control Class

Table 11. Comparison of the Average Difference in Hb Levels of Pregnant Women Before and After Treatment in the Experimental Group and the Control Group

	Levene's Test for Equality of Variances	**Equal variances assumed**
	Sig.	**Sig. (2-tailed)**
Experimental class-Counter class	0,434	0,000

Based on Table 11, the Levene's Test for Equality of Variances yielded a significance value of 0.434, which is greater than 0.05. This indicates that the variance in N-gain data between the experimental and control groups is homogeneous, meaning both groups have similar variance.
Additionally, the independent samples t-test shows a significance value (2-tailed) of 0.000, which is less than 0.05. This result confirms that there is a statistically significant difference in effectiveness between the administration of Thunna Sp cookies and PMT cookies in increasing the hemoglobin (Hb) levels of pregnant women.

5. Conclusion

The substitution of tuna fishmeal and yellow pumpkin flour significantly impacts the quality of MP-ASI cookies in terms of rehydration, acceptability, color, aroma, taste, and texture. The optimal substitution of tuna fishmeal at 17% results in the most favored flavor, while a 20% substitution of yellow pumpkin flour yields the best texture and most preferred aroma. The study also revealed that higher amounts of yellow pumpkin flour reduce the water required to dissolve the cookies, due to the hygroscopic properties of the fiber. Overall, cookies with a 20% yellow pumpkin flour substitution offer the best quality regarding solubility and consumer acceptability.

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