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Evaluation of Calcium and Protein Content in Powder and Gummy Candy from Anchovy Extract (*Stolephorus commersonnii*)

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ABSTRACT

Anchovies (*Stolephorus commersonnii*) are a good source of calcium and protein. Intake of these nutrients is associated with child height. According to the Ministry of Health of the Republic of Indonesia's Nutrition Status Study Report, the prevalence of stunting among Indonesian children in 2022 was 21.6%, which is still higher than the World Health Organization's (WHO) standard of <20%. This study aims to optimize the formulation of anchovy extract gummy candies that meet quality standards and contain high levels of calcium and protein. Anchovy powder contains 328.64 mg/kg of calcium, 28.46% protein, 0.12% ash, and 5.3% moisture. The anchovy extract was prepared using the maceration method with a 70% ethanol solvent. Four gummy candy formulations were made based on variations in anchovy extract concentration: 10%, 7.5%, 3%, and 1.5%. Formulation 4 (F4) with 1.5% anchovy extract produced the best physical quality, with a standard deviation (SD) of <5% for the weight uniformity test. The organoleptic test results for F4 are as follows: the anchovy taste is mild, the color is dark purple, the smell is slightly aromatic, the shape is consistent with the mold, and the texture is chewy. F4 gummy candies contain 1,490.64 mg/kg of calcium, 27.49% protein, 9.17% ash, and 3.96% moisture. A serving size of one gummy (1.5 g) contains 2.24 mg of calcium, 0.41 g of protein, 0.06 g of total fat, 0.07 g of carbohydrates, 0.55 g of sugar, and 4.18 mg of sodium. F4 meets quality standards and is high in calcium and protein.

INTRODUCTION

The human body has approximately 1 kilogram of calcium, of which more than 99% accumulates in the bones as calcium phosphate (Zhou et al., 2013). The role of calcium is very important for muscle contraction, nerve activity, cell differentiation, enzyme activation, immune response and programmed cell death. Calcium concentrations are maintained inside cells and outside cells to ensure this role runs well.

According to the Indonesian Ministry of Health (2019), the Recommended Dietary Allowance (RDA) for calcium intake is 650 mg/day for children aged 1-3 years, and 1000 mg/day for children aged 4-6 years. Meanwhile, the RDA for protein is 20 g/day for children ages 1-3 and 25 g/day for children ages 4-6.

Unfortunately, research suggests otherwise. A study in Langkat Regency, North Sumatra (Jayusman et al., 2021), found that the average calcium intake of non-stunted children aged 2-5 years was 233.53±162.68 mg/day. While the average calcium intake of stunted children of the same age was 127.86±53.80 mg/day. Results also show a significant difference in calcium intake between stunted and non-stunted children (p=0.000).

Children over 2 years old who are not drinking breast milk are recommended to consume foods containing calcium so that the AKG daily is sufficient. In addition to spinach,

broccoli, and soybeans, fish is an abundant source of calcium in Indonesia. The main source of calcium in fish comes from its bones.

The anchovy, or *Stolephorus commersonnii*, is a very popular fish that is frequently used as complementary food to breast milk (MPASI) due to its high protein and calcium content. However, anchovies are a food that is easily damaged and rotten, so it needs to be processed into other forms to extend its shelf life.

According to Indonesian National Recearch Agency (BRIN), nutraceutical research and development based on active or functional natural ingredients is very necessary to improve health. The development of variations in anchovies is very promising due to their high nutritional value and potential to increase product added value (Kari et al., 2022).

A dosage form that is both engaging and simple for kids to take, such gummy candies, is necessary to encourage them to eat the anchovy extract nutraceutical. Gummy candies are generally liked by children because of its pleasant taste, aroma, color, shape and texture. It is envisaged that calcium requirements during the child's growing stage will be fulfilled by ingesting supplements in the form of gummy candies made from anchovies extract.

The aim of this study is to optimize the calcium and protein content of gummy candies made from anchovy extract and to ensure their quality. This study has never been conducted because the gummy candy available in Indonesia are usually made from fruits containing multivitamins and omega-3 fatty acids, rather than calcium and protein. Abroad, gummy candy containing calcium are made from calcium carbonate, which is fairly expensive (approximately 150 thousand rupiah). These gummy candy are made from local ingredients, specifically Indonesian marine biological resources, which have the potential for further development.

METHODS

The materials used were ancovies, citric acid (Merck KGaA, Germany), stevia (Berkat Rezeki Utama, Indonesia), violet dye and grape essence (Triefta Aroma Nusantara, Indonesia), selenium reagent mixture, concentrated N-free H_2SO_4 with a specific gravity of 1.84, distilled water, 45% sodium hydroxide (NaOH), 4% boric acid, MR-MB Indicator, hydrochloric acid (HCl), N-hexane, 3 N nitric acid (HNO_3), 5% dilanthanum trioxide (La_2O_3), gelatin, sorbitol (Pharmacy Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Pakuan, Indonesia).

Raw Material Preparation

The anchovy samples were cleaned by rinsing under running water to remove any adhering dirt, then dried using an oven at a temperature of 45-60°C. The dried anchovy was separated from the dirt and ground using a blender, then sieved using a 60 mesh. The weight of the anchovy powder simplicia was weighed to obtain its final weight in order to calculate the yield.

Extraction of Anchovy

The extraction of anchovy was done by maceration. A total 300 g of anchovy simplicia into a brown bottle and macerated with 1 L of distilled water for 24 hours with stirring every 4 hours for 15 minutes. The anchovy solution was filtered using a 300 mesh filter cloth. The filtrate was collected, while the anchovy powder was remacerated 2 times. The collected filtrate was reacted with 12.85 g of NaOH for 15 minutes, then centrifuged at 250 rpm for 30 minutes to take the sediment. The sediment was oven-dried at 40-50°C until dry. The dry extract was weighed and its yield value was calculated.

Gummy Candies Formulation

In this study, four different formulas were used to produce gummy candies from anchovies. The only difference between the formulas is the percentage of anchovy extract (10%, 7.5%, 3%, 1.5%) . The gummy making steps begin with weighing the ingredients according to the formulation (**Table 1**).

Table 1. Gummy candies formula of anchovy extract

Formulation
Anchovy extract
Gummy former (gelatin)
Natural sweetener (sorbitol)
Natural sweetener (stevia)
Acidity regulator (citric acid)
Dye (purple)
Essence (grape)
Distilled water

Then dissolved the dry extract with distilled water, by stirring using a magnetic stirrer at a temperature of 40-50°C. Next, add gelatin, sorbitol, stevia, purple dye, and grape essence.

Stir until completely dissolved and thickened, then pour into the mold.

Characterization of Gummy Candies

Organoleptic Test

These characteristics are carried out by visual observation of the shape, color, and smell of the gummy candies.

Weight Uniformity Test

Several gummy candies were weighed using an analytical balance. It is declared as uniform if there is no significant difference in weight (Standard Deviation < 5%).

Water content test

Using a moisture analyzer, the water content of around 3 g of powdered simplicia or anchovy extract or gummy candies was determined in triplicate (Panca et al., 2023).

Ash Content Test

An oven set to 105°C was used to heat the crucible for 30 minutes. After 15 minutes of cooling in a desiccator, weigh the crucible to ensure that its weight remains constant. Using a weighted porcelain cup, the ancovy powder or dry extract or gummy candies were weighed up to 3 g. Char it over the burner flame, then ash it in a furnace at 600°C. Then use a desiccator to cool. Repeat, until the weight is constant (Panca et al., 2023).

Determination of Protein Content

The protein content test was conducted at the Service Laboratory of the Faculty of Mathematics and Natural Sciences at Pakuan University. The measurement of protein content was done using the Kjeldahl method.

Determination of Calcium Content

The tests for calcium and sodium were done by the IPB Integrated Laboratory. The calcium content of the sample is read using an Atomic Absorption Spectrophotometer (AAS) with a wavelength of 422.7 nm.

RESULT AND DISCUSSION

The physical characteristics of anchovies are short heads, cylindrical morphology, and predominantly white pigmentation. In addition, *Stolephorus commersonnii* also has a distinctive aroma and soft texture.

The research began with the preparation of anchovy powdered simplicia. The amount of anchovy powder obtained was 477.90 g with a yield of 15.92%. The high powder yield is due to the complete utilization of anchovies during processing, without any being wasted.

Table 2. Evaluation results of anchovy powder

Test parameters	Result	Standard
Organoleptic	Taste: tasteless	-
	Color: greyish white	-
	Smell: like anchovies	-
	Shape: powder	-
Drying Shrinkage	84%	-
Rendement	15.92%	-
Protein Content	28.46%	-
Calsium Content	328.64 mg/kg	-
Ash content	0.12%	≤ 10%
Water content	5.3 %	< 10%

Anchovy powder has unique characteristics. The organoleptic profile of anchovy powder are tasteless, smell like anchovies, and grayish white in color. Color acts as a primary determinant of consumer preference for powder products, directly affecting their perception of taste, texture, and overall product value (Luo et al., 2019; Muniz et al., 2023). Anchovy flour's bright coloration is attributed to the raw material's inherent pigmentation. In a comparative study, Rostini (2024) reported that anchovy flour possessed the highest L value (a measure of whiteness/brightness) (Boral et al., 2015), relative to flours from other fish (mackerel, mackerel, lemuru, and yellow mackerel). Consequently, anchovy flour exhibited the closest color proximity to white.

This study employed the Kjeldahl method to ascertain protein levels, as it is a widely utilised technique for protein analysis in food. The Kjeldahl technique quantifies crude protein levels by measuring total nitrogen content, which encompasses non-protein nitrogenous compounds such as urea, nucleic acids, purines, and pyrimidines. This method operates on the principle of converting organic substances into inorganic forms (Rosaini et al., 2015).

The research results in **Table 2** show that anchovy flour contains 28.46% protein. Compared to commercial chicken eggs, which only contain 12.8% protein, anchovies powder has a higher protein level. Nonetheless, anchovies powder's protein concentration is still lower than egg powder, which has a minimum

protein value of 45% (Wulandari and Arief, 2022).

The protein content of anchovy powder in this study is lower than the protein content of black anchovy flour, which is 70.16% (Litaay et al., 2023), and approximately the same as skipjack fish flour 27.57-29.99% (Orlan et al., 2019). Additionally, the protein content of fish powder, as per the National Standardization Agency (SNI 2715:2013) quality standard A, is required to be at least 60% (Domili et al., 2020). However, a review of the literature indicates that the protein content in anchovies powder falls within the established range of protein concentrations (13-28%) derived from marine source (Ermis et al., 2023). **Table 2** demonstrates that anchovies powder has 328.64 mg/kg of calcium. It can be concluded in 100 g of anchovy powder contains 32.864 mg of calcium. Analysis of anchovy powder, as documented in the Indonesian Food Composition Table, shows that it provides 48.8 g of protein, 2381 mg of calcium, and 23.4 mg of iron per 100 g (Indonesia, 2018). It is possible to conclude that the calcium content of anchovy powder in this study is still below the standard.

The calcium content of anchovy powder in this study is lower than the protein content of white anchovy flour, which is 504 mg/g (KB et al., 2020), and tilapia bone powder 187.0-214.8 mg/g (Lekahena et al., 2014). The difference in calcium content is influenced by the type of fish, habitat differences, biological cycles, and powdered fish body parts.

Following high-temperature oxidation (above 450°C), the remaining inorganic material, termed ash content, is analyzed. In this study, the ash content of anchovy powder was 0.12%. This value is within the standard of Indonesian Materia Medika (MMI) and Farmakope Herbal Indonesia, which is below 10% (Ri, 2008). Moreover, the ash content in fish powder, as defined by the Indonesian National Standard (SNI 2715:2013) quality standard A, is limited to a maximum of 20%.

The amount of water in fish powder affects the processing time, the cohesiveness of the dough during production and the stability of the final product. In this study, the water content of anchovy powder was found to be 5.3%. This value is in accordance with the quality standards of the Indonesian National Standard (SNI 2715: 2013) Quality Standard A (Domili et al., 2020), which is less than or equal to 10%.

The anchovy extract obtained from the maceration process was 23.83 g with a yield of 5.34%. The maceration process was selected for extraction due to its simplicity and ease of execution. The solvent used in the extraction stage is distilled water, based on safety considerations and the polarity of calcium and protein that can dissolve in distilled water.

Table 3. Evaluation results of anchovy extract

Test parameters	Result	Standard
Organoleptic	Taste: tasteless	-
	Color: gray	-
	Smell: like anchovies	-
	Shape: dry extract	-
Rendement	5.34%	-
Ash content	0.97%	≤ 10%
Water content	2.6 %	< 10%

According to organoleptical test, the anchovy powder which was originally grayish white turns gray after being extracted. The odor still smells of anchovies and has no taste (**Table 3**).

As reported by Janbakhsh et al. (2018), ash content in fish extract is used to assess the levels of minerals and organic components. In this study, the ash content of anchovy extract was 0.97%. While the water content of anchovy extract was 2.6%. Both chemical parameters measured were consistent with the established standards of the Indonesian Materia Medika (MMI) and Farmakope Herbal Indonesia, which is below 10% (Ri, 2008). To evaluate the characteristics of anchovy extract in gummy form, an initial test was conducted by creating one formula. In formula 1 (F1), a sticky gummy with a very fishy smell was obtained which was thought to come from anchovy extract.

The data from the F1 preliminary test was used to create three other formulations, each with different levels of anchovy extract and gelatin. All four formulations exhibited a consistent dark purple coloration (**Table 4**). But there are differences, specifically in taste and odor. A reduction in anchovy extract concentration within the formulation correlated with more desirable aroma and flavor profiles.

In addition, only F1 presented gummy candies with a less chewy texture and the shape that was inconsistent with the mold's dimensions. The observed phenomena are correlated to the varying gelatin proportions

among the formulations, with F1 demonstrating the the lowest gelatin concentration.

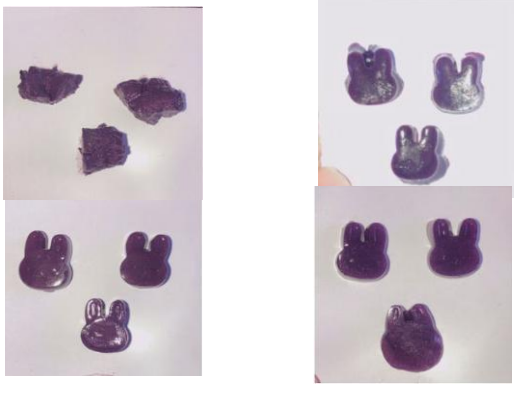
Table 4. Evaluation results of anchovy extract gummy candy preparations

Test parameters	Results				Standard
	F1	F2	F3	F4	
Organoleptic:					
Taste	Anchovies taste are still strong compared to grape essence, and too sour	Anchovies taste are still powerful compared to grape essence, and too sour	The anchovies taste are still quite strong, and the sour taste is exactly right	The taste of anchovies is not strong, and the sour taste is just right	Taste, color, shape and smell can be accepted by kids.
Color	Dark purple	Dark purple	Dark purple	Dark purple	
Smell	Very strong smell of anchovies	Quite strong smell of anchovies	The smell of anchovies is not too strong	Weak aromatic anchovy odor	
Shape	Doesn't follow the mold	Follow the mold	Follow the mold	Follow the mold	
Texture	Less chewy	Quite chewy	Chewy	Chewy	Chewy

Readily observable organoleptic properties (color, odor, taste) significantly impact consumer acceptance of gummy preparations and facilitate initial identification. Quality preparations exhibit unique, appealing characteristics and are free from physical and aesthetic defects, including chips, cracks, fading, and contamination.

Among the four formulas developed (its appearance are shown in **Figure 1**), the optimal selection was F4 since it met the requirement (taste, color, shape and smell can be accepted by kids).

The ash content of gummy candy from anchovy extract is 9.17%. The mineral profile and quantity of this residue are directly influenced by the type of food material subjected to ashing.



F3 **F4**

Figure 1. Anchovy gummy candy appearance

Gummy candy is a semi-moist confection, rendering it susceptible to rapid spoilage. The gummy candy processing concept centers on reducing water activity (aw) to inhibit pathogenic and spoilage microbes, while maintaining a sufficient water content for edibility and storage stability. The standard water content for gummy candy is 10-40%, with an a_w of 0.6-0.9 (Koswara, 2009). A measured water content of 3.96% was observed in the gummy samples. This result was considerably < 10%, which can be explained by the 30% gelatin concentration in the F4. According to Koswara (2009), gelatin plays a multifunctional role in jelly candy processing, acting as a crystallization inhibitor, a gelling agent that transforms liquids into elastic solids, and a texture modifier.

Evaluation of preparation weight uniformity is essential for determining consistent mass, functioning as a standard production control to ensure target weight achievement. The uniform weight of the preparation indirectly signifies the homogeneity of its constituent substances.

The results of the weight uniformity test for F4 of the gummy candy preparation showed an average weight of 1.7578 g for the rabbit-shaped mold and 0.7374 g for the carrot-shaped mold. This variation can be attributed to unstable thermal parameters during the heating phase. Heating significantly impacts the viscosity of the preparation, affecting its pourability into the mold. A decrease in temperature lead to

increased density, thus contributing to weight inconsistencies.

The weight consistency of gummy candies was assessed. This was done by evaluating the permissible deviation from the average weight. The standard deviation obtained in the gummy candy uniformity test was 0.0173 (1.73%) for the rabbit mold, and 0.0264 (2.64%) for the carrot mold. The smaller the standard deviation (< 5%), the more uniform the gummy weight.

Recognized for their high nutrient density, anchovies provide substantial protein, and calcium. Protein is fundamental for tissue repair and growth, and calcium is crucial for cell function and growth regulation. Research in Tegalwangi Village, Cirebon District (TW et al., 2024) concluded that vitamin A, calcium, and zinc deficiencies can cause stunting. Therefore, the formulation of gummy candy utilizing anchovy extract aims to produce a product enriched with protein and calcium.

The results of this study show that each 1.5 g serving of gummy contains 2.24 g of calcium and 0.41 g of protein. Additionally, one gummy contains 0.06 g of total fat, 0.07 g of carbohydrates, 0.55 g of sugar, and 4.18 mg of sodium. These results indicate that one gummy meets the daily calcium and protein requirements for children aged 2-5 years and older.

CONCLUSIONS

Anchovy extract (*Stolephorus commersonnii*) can be formulated into gummy candies but further reformulation is required. Based on this research, it was concluded that F4 (using 1.5% anchovy extract) will produce the best physical quality. The water content and ash content were also within the appropriate parameters for semi-wet food product specifications. Currently, these anchovy extract gummy candies can be consumed once every two days. Further research on stability testing of gummy candy preparations from anchovy extract prior to industrial-scale production is needed. In addition, reformulation is also needed to reduce the percentage of anchovy extract so that anchovy gummy candy can be consumed once a day.

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AUTHORS' CONTRIBUTIONS

All authors conceptualized the research and conducted literature studies related to preformulation. Ema Hermawati developed the formula for the gummy candies, and Marybet Tri Retno Handayani evaluated the results. Dian Farida Ismyama wrote the manuscript and was also responsible for data interpretation and analysis. After carefully examining the findings, all authors approved the final draft of the manuscript.

CONFLICT OF INTERESTS

No conflict of interest in this publication.

ETHICAL CONSIDERATION

This study does not require research ethics approval because it does not involve animal testing or human subjects. The authors guarantee that there is no plagiarism, data fabrication, duplicate publication, or other ethical issues.

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