

Physicochemical Characteristics, Calcium Content, and Storage Stability of Anchovy (*Stolephorus commersonnii*) Extract Gummy Candies

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Calcium is an essential micronutrient for bone growth and development during childhood. Anchovy (*Stolephorus commersonnii*) is a local marine resource containing calcium and protein and may be incorporated into food products. This study aimed to evaluate the physicochemical characteristics, calcium content and proximate composition, microbiological quality, heavy metal contamination, and storage stability of anchovy extract gummy candies. Anchovy powder was prepared by drying and milling, followed by aqueous maceration extraction. Gummy candies were formulated using gelatin and taro starch as gelling agents. Five formulations were prepared, and the formulation with the most favorable physical characteristics was selected for further evaluation. The selected formulation contained 25% gelatin and 7.5% taro starch and exhibited a chewy texture, acceptable aroma, and pH of 4.7. The product remained physically stable during 14 days of refrigerated storage at 8–15°C, whereas softening, liquefaction, and fungal growth were observed at room and elevated temperatures. Proximate analysis showed moisture content of 54.42–54.68%, protein content of 31.68–31.96%, carbohydrate content of 13.01%, ash content of 0.61–0.63%, and calcium content of 28.15–28.44 mg/100 g. Lead and mercury were not detected, while total plate count and yeast and mold counts were within the applicable limits under the tested conditions. These findings demonstrate the feasibility of incorporating anchovy extract into a gummy candy matrix with measurable calcium and protein content; however, further optimization is required to increase calcium content and improve stability at room temperature.



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Calcium is an essential micronutrient that plays a crucial role in children's growth and development, particularly in bone formation and mineralization. More than 99% of the body's calcium is stored in bones as hydroxyapatite, which provides structural

strength and serves as a calcium reservoir for maintaining physiological functions [1]. During early childhood, particularly between 1 and 5 years of age, calcium requirements increase substantially due to rapid growth. Consequently, inadequate calcium

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intake during this critical period may adversely affect growth and bone development [2].

Calcium deficiency among children remains a significant public health concern in Indonesia. Evidence indicates that the consumption of calcium-rich foods among Indonesian children remains below recommended dietary intake levels [2]. Furthermore, national data indicate that approximately 8.2% of children under five years of age experience calcium deficiency. Inadequate calcium intake contributes to various nutritional problems, including stunting, which continues to be a major public health issue in the country [3].

Stunting is a condition of impaired growth resulting from chronic undernutrition over a prolonged period and commonly affects children under five years of age. Low calcium intake has been shown to be significantly associated with the incidence of stunting among children [4]. In addition, calcium, together with vitamin D, plays a vital role in bone mineralization; therefore, deficiencies in these nutrients may hinder linear growth in children [5]. Micronutrient deficiencies, including calcium deficiency, remain a major public health concern among Indonesian children [6].

Efforts to improve calcium intake in children can be achieved through food fortification strategies. Calcium fortification has been demonstrated to enhance health status and support normal metabolic functions [1]. Furthermore, dietary interventions involving foods rich in animal protein, calcium, and zinc have been associated with significant improvements in children's height growth. Therefore, the development of calcium-rich functional foods represents a promising strategy for addressing calcium deficiency among children [7].

One potential natural source of calcium and an important local marine resource in Indonesia is anchovy (*Stolephorus* sp.), which contains high levels of calcium and protein because it is typically consumed together with its edible bones [15]. Incorporating anchovy into innovative food products, such as gummy candies, may enhance children's acceptance of this calcium source. Previous studies have shown that gummy candies formulated with anchovy extract contain high levels of calcium while maintaining desirable physical characteristics [8]. Gummy candies are confectionery products

characterized by their chewy texture, sweet taste, and appealing appearance, making them particularly attractive to children and suitable as vehicles for nutrient fortification [9,16]. However, in the development of functional food products, physical and chemical stability are critical factors that must be considered to ensure product quality, safety, and shelf life. Physical stability encompasses changes in appearance, color, and texture, whereas chemical stability refers to the preservation of active compounds, such as calcium, during storage [10,16,17].

Previous research by Hermawati et al. (2025) evaluated the calcium and protein content of anchovy (*Stolephorus commersonnii*) extract powder and gummy candy. Although the study demonstrated the incorporation of anchovy extract into a gummy candy formulation, limitations related to physical stability and the relatively strong anchovy odor remained. Therefore, further formulation development was needed to improve the physical characteristics and storage stability of the product. In the present study, the gummy formulation was modified by incorporating taro starch in combination with gelatin as gelling agents. The reformulated product was subsequently evaluated for its physicochemical characteristics, storage stability under different temperature conditions, proximate composition, calcium content, heavy metal contamination, and microbiological quality. Thus, the present study provides additional information on the formulation characteristics and storage stability of anchovy extract gummy candies beyond the evaluation of calcium and protein content reported in the previous study. Therefore, this study aimed to evaluate the physicochemical characteristics, calcium content, proximate composition, microbiological quality, heavy metal contamination, and storage stability of reformulated anchovy (*Stolephorus commersonnii*) extract gummy candies.

Results and Discussion

Results of Anchovy Powder and Extract Preparation

The observations showed that the anchovy powder exhibited a greyish-white color, whereas the extract appeared gray. This color change may be attributed to the extraction and drying processes, which may have induced nonenzymatic browning

reactions and increased the concentration of mineral and protein components in the extract. Heat treatment during the production of gel-based food products can affect color characteristics through structural changes in bioactive compounds and protein–carbohydrate interactions [10,18].

The drying loss of the anchovy powder was 93.43%, indicating that most of the water present in the fresh material was removed successfully. Drying is an essential step in reducing water activity, thereby inhibiting microbial growth and extending the shelf life of the material. The powder yield (6.56%) and extract yield (20.08%) indicated relatively efficient processing and extraction. Extraction yield is influenced by several factors, including particle size, solvent type, extraction time, and the concentration of compounds soluble in the extraction solvent. The use of water as the extraction solvent resulted in a relatively high yield, likely due to the abundance of

protein and mineral components that can be dispersed in an aqueous medium [11].

The ash content of the anchovy powder and extract was 10.70% and 16.42%, respectively, exceeding the standard limit ($\leq 10\%$). The high ash content reflects the substantial mineral content of anchovies, particularly because they are commonly consumed with their bones, which are rich sources of calcium and phosphorus. The moisture content of the anchovy powder (4.47%) and extract (1.85%) complied with the established quality requirements, as both values were below the maximum permissible limit of 10%. Low moisture content is important for preventing microbial proliferation and minimizing enzymatic reactions that may deteriorate product quality during storage.

Table 1. Physicochemical Characteristics of Anchovy Powder and Extract

Test Parameter	Powder	Extract	Standard
Organoleptic Properties (taste, color, appearance, and aroma)	Taste: Bland Color: Grayish white Appearance: Powder Aroma: Characteristic anchovy odor	Taste: Bland Color: Gray Appearance: Dry extract Aroma: Characteristic anchovy odor	-
Drying Loss	93.43%	-	-
Yield	6.56%	20.08%	-
Ash content	10.7%	16.42%	Not more than 10%
Moisture content	4.47%	1.85%	

Organoleptic Evaluation of Anchovy Gummy Candies

The anchovy gummy candies were reformulated based on a formulation reported in a previous study [8]. Although the previous formulation successfully produced gummy candies, the product exhibited limited stability and retained a strong anchovy extract odor. Therefore, the present study focused on improving the physical stability, taste, and aroma of the gummy candies through formulation modification. Taro starch was combined with gelatine

to enhance the physical stability of the gummy matrix [16,19]. Five formulations were prepared and evaluated. Among them, Formula 5 (F5), containing 25% gelatine and 7.5% taro starch, was selected as the optimal formulation because it exhibited superior shape retention, reduced stickiness, adequate physical stability, and a less pronounced anchovy odor compared with the other formulations.

These findings suggest that increasing the gelatine concentration and incorporating taro starch improved the gel structure of the gummy candies,

resulting in enhanced elasticity and better physical stability during storage. Furthermore, taro starch may have acted as a filler and moisture-binding agent, thereby reducing excess moisture and preventing product stickiness. The pH of the selected gummy candy formulation was 4.7, indicating a mildly acidic environment that may contribute to product stability and microbiological quality.



Figure 1. Physical appearance of Formula 5 (F5) anchovy gummy candies. F5 was selected as the

optimal formulation based on the overall evaluation results.

Weight Uniformity of Anchovy Gummy Candies

Weight uniformity reflects the homogeneity of ingredient mixing and the consistency of the manufacturing process. Minor variations among individual gummy weights may be attributed to differences in mold-filling volume, variations in gummy mass viscosity, or product shrinkage during the cooling and setting processes. The homogeneity of the gelatine matrix and active ingredients plays an important role in determining weight uniformity and maintaining consistent gummy texture during storage [9]. The relatively small weight of the gummies (approximately 1–2 g per unit) provides a convenient unit size for further product development. Product size and weight may also influence consumer acceptance and handling, particularly for products intended for children.

Table 2. Weight Uniformity of Anchovy Gummy Candies

No.	Weight (g)	No.	Weight (g)
1.	1.3869	11.	2.2045
2.	1.7043	12.	1.7087
3.	1.6214	13.	1.4603
4.	1.8090	14.	2.0559
5.	2.4537	15.	1.8851
6.	1.8133	16.	1.8909
7.	1.8045	17.	1.5999
8.	2.0705	18.	1.6179
9.	1.9725	19.	1.7196
10.	1.8333	20.	1.6018
Median		1.8107 g	

Stability Evaluation of Anchovy Gummy Candies

The stability test results for the selected anchovy gummy candy formulation under different storage conditions are presented in Table 3. Previous studies have also shown that formulation and storage temperature can influence gummy texture and stability [6,17]. Storage temperature markedly affected the physical stability of the gummy candies.

The product remained physically stable throughout 14 days of refrigerated storage (8–15°C). At room temperature (15–30°C), progressive softening and partial liquefaction were observed by day 12, followed by visible fungal growth on day 14. More rapid deterioration occurred under elevated temperature conditions (30–40°C), with liquefaction observed by day 7 and fungal growth by day 9. These

findings indicate that storage temperature strongly influenced the physical and microbiological stability of the gummy candies.

These findings are likely associated with the relatively high moisture content of the gummy candies (approximately 54%), which may make the gel matrix sensitive to temperature fluctuations. At higher temperatures, hydrogen bonds within the gelatin network may weaken, resulting in gel softening and loss of structural integrity. Storage temperature is therefore an important factor affecting the textural stability of gummy products. Low-temperature storage helps maintain gel strength, preserve product structure, and inhibit microbial growth.

The findings of the present study complement previous research on anchovy extract gummy candies by providing additional information on formulation characteristics and storage stability. The incorporation of gelatin and taro starch resulted in a selected formulation with favorable physical characteristics, while the stability evaluation demonstrated that storage temperature affected the physical integrity of the product. These findings address limitations identified in the previous formulation and provide additional information on the stability of reformulated anchovy extract gummy candies.

Table 3. Stability Test Results of Anchovy Gummy Candies

Storage condition	Observation period	Physical changes observed	Stability status
Refrigerated (8–15°C)	Day 0–14	No apparent changes in shape, texture, liquefaction, or visible fungal growth	Stable
Room temperature (15–30°C)	Day 0–14	Softening and partial liquefaction observed on day 12; visible fungal growth observed on day 14	Unstable
Elevated temperature (30–40°C)	Day 0–14	Liquefaction observed on day 7; visible fungal growth observed on day 9	Unstable

Proximate Composition of Anchovy Gummy Candies

The proximate composition and calcium content of the anchovy gummy candies are presented in Table 4. The formulation showed a relatively high protein content of 31.68–31.96%, while fat content was below 0.02%. The moisture content ranged from 54.42% to 54.68%, reflecting the gel-based characteristics of the gummy candies. The calcium content was 28.15–28.44 mg/100 g, indicating a measurable but relatively limited contribution as a calcium source. The ash content of the gummy candies ranged from 0.61% to 0.63%, indicating the presence of mineral components derived from the anchovy extract. The relatively lower ash content compared with that of the extract suggests that the

minerals were dispersed throughout the gummy matrix along with other formulation ingredients.

The moisture content ranged from 54.42% to 54.68%, reflecting the gel-based nature of the product and its soft texture. The relatively high moisture content may increase susceptibility to microbial growth if storage conditions are not properly controlled. This observation is consistent with the stability study, in which microbial growth and physical deterioration were observed at higher storage temperatures. The gummy candies contained relatively high protein levels (31.68–31.96%) and measurable calcium levels (28.15–28.44 mg/100 g), while fat content was below 0.02%. The relatively high protein content was mainly associated with the anchovy extract and gelatine used in the formulation.

The relatively low calcium content indicates that further optimization of the extraction process and/or the proportion of anchovy extract may be needed to increase the calcium content of the final product. Adequate calcium intake plays a crucial role in supporting skeletal growth and development during childhood [15]. Nevertheless, the calcium content

obtained in the present study remains relatively low compared with the recommended daily calcium intake for children. Therefore, further optimization of the extraction process and/or an increase in the proportion of anchovy extract may be necessary to enhance the calcium contribution of the product.

Table 4. Proximate Composition of Anchovy Gummy Candies

No	Parameter	Unit	Simplo	Duplo	Limit of detection	Method
1	Ash content	%	0.61	0.63	-	SNI 01-2891-1992 point 6.1
2	Energy from fat	kcal/100 g	0	0	-	18-8-9/MU
3	Total fat content	%	<0.02	<0.02	-	18-8-5/MU
4	Moisture content	%	54.42	54.68	-	SNI 01-2891-1992 point 5.1
5	Total energy	kcal/100 g	179.88	178.76	-	18-8-9/MU
6	Carbohydrate	%	13.01	13.01	-	18-8-9/MU
7	Protein	%	31.96	31.68	-	18-8-31/MU
8	Calcium (Ca)	mg/100 g	28.15	28.44	-	18-13-1/MU

Heavy Metal and Microbiological Contamination Analysis of Anchovy Gummy Candies

The results of the heavy metal and microbiological contamination analyses of the anchovy gummy candies are presented in Tables 5 and 6. No detectable levels of lead (Pb) or mercury (Hg) were found in the tested samples, indicating that

these contaminants were below the detection limits under the analytical conditions used in this study. Heavy metals such as lead and mercury can accumulate in fish tissues because of aquatic environmental pollution. Therefore, the absence of detectable Pb and Hg indicates that the tested gummy candy samples did not show detectable contamination by these two heavy metals.

Table 5. Heavy Metal Contamination Analysis of Anchovy Gummy Candies

No	Parameter	Unit	Simplo	Duplo	Limit of detection	Method
1	Lead (Pb)	mg/kg	Not detected	Not detected	0.0023	18-13-14/MU (ICP-MS)
2	Mercury (Hg)	mg/kg	Not detected	Not detected	0.002	18-13-14/MU (ICP-MS)

The total plate count (TPC) ranged from 7.9×10^2 to 8.9×10^2 CFU/g, while the yeast and mold count (YMC) was <10 CFU/g. These values were within the applicable microbiological criteria for the tested product. The low microbial counts indicate good microbiological quality under the tested conditions. According to the applicable requirements of the Indonesian National Standard, microbiological contamination testing is an important parameter for evaluating the microbiological quality of food

products. Overall, the tested gummy candy samples met the evaluated heavy metal and microbiological criteria. These findings should be interpreted within the scope of the parameters assessed in this study and should not be considered evidence of overall product safety or safety for consumption by children.

Table 6. Microbiological Contamination Test Results of Anchovy Gummy Candies.

No	Parameter	Unit	Simplo	Duplo	Limit of detection	Method
1	Total plate count (ALT)	CFU/g	7.9×10^2	8.9×10^2	-	ISO 4844-1:2013 Amd 1:2022
2	Yeast and mold count	CFU/g	<10	<10	-	SNI ISO 21527-2:2012

Conclusion

Anchovy (*Stolephorus commersonnii*) extract was successfully incorporated into a gummy candy formulation using gelatin and taro starch. The formulation containing 25% gelatin and 7.5% taro starch was selected based on its favorable physical characteristics, including shape retention, reduced stickiness, and acceptable texture and aroma. The selected formulation remained physically stable for 14 days under refrigerated storage conditions (8–15°C), whereas storage at room (15–30°C) and elevated (30–40°C) temperatures resulted in progressive physical deterioration and visible fungal growth. The gummy candies contained 31.68–31.96% protein and 28.15–28.44 mg/100 g calcium, while lead (Pb) and mercury (Hg) were not detected under the analytical conditions used. The microbiological results showed total plate counts and yeast, and mold counts within the applicable limits under the tested conditions (20). Overall, the findings demonstrate the feasibility of incorporating anchovy extract into a gummy candy matrix with measurable calcium and protein content. However, the relatively low calcium content and limited stability at room temperature indicate the need for further optimization of the extraction and formulation processes. Calcium bioavailability, consumer acceptability, and the contribution of the product to dietary calcium requirements were not evaluated in this study and should be investigated in future research

Materials and Methods

Study Design

This study employed experimental laboratory design. The study consisted of four main stages: (1)

preparation of anchovy (*Stolephorus commersonnii*) powder and extract; (2) formulation and preliminary evaluation of anchovy extract gummy candies; (3) selection of the optimum formulation based on its physical characteristics; and (4) evaluation of the selected formulation for physicochemical characteristics, proximate composition, calcium content, heavy metal contamination, microbiological quality, and storage stability under different temperature conditions.

Preparation of Anchovy Powder (Simplicia)

High-quality rice anchovies (*Stolephorus commersonnii*) were used as the raw material in this study. The collected samples were cleaned thoroughly under running water to remove adhering impurities and contaminants. Subsequently, the anchovies were dried in an oven at 45–60°C until a constant weight was achieved. The dried anchovies were then sorted to remove any remaining unwanted materials and ground into fine powder using a blender. The resulting powder was sieved through a 60-mesh sieve to obtain a uniform particle size. The weight of the anchovy powder was recorded to determine the final yield, which was subsequently calculated as the percentage recovery of the dried anchovy powder (8).

Preparation for Anchovy Extract

Anchovy extraction was performed by maceration. A total of 300 g of anchovy simplicia was placed in a brown bottle and macerated with 1 L of distilled water for 24 h, with stirring every 4 h for 15 min. The anchovy solution was filtered using a 300-mesh filter cloth. The filtrate was collected, while the anchovy powder was remacerated twice. The collected filtrate was treated with 12.85 g of NaOH

for 15 min and then centrifuged at 250 rpm for 30 min to collect the sediment. The sediment was oven-dried at 40–50°C until dry. The dried extract was weighed, and its yield was calculated [8].

Determination of Moisture Content and Ash Content of Anchovy Powder

The moisture content of the anchovy powder was determined using both a moisture balance and the oven-drying method. Approximately 3 g of anchovy powder or rice anchovy extract was analyzed for moisture content using a moisture balance in triplicate. For ash content determination, porcelain crucibles were preheated in an oven at 105°C for 30 min, cooled in a desiccator for 15 min, and weighed repeatedly until a constant weight was obtained. Approximately 3 g of anchovy powder or extract was accurately weighed into the pre-weighed crucibles. The samples were initially charred over a burner flame and subsequently incinerated in a muffle furnace at 600°C until complete ashing was achieved. The crucibles were then cooled in a desiccator and reweighed. The heating, cooling, and weighing procedures were repeated until a constant weight was reached. The ash content was calculated from the final residue remaining after incineration [11].

Formulation of Anchovy Gummy Candies

The gummy candy formulation was initially developed based on a previously reported method [8] and subsequently reformulated to improve product characteristics. The reformulation involved modifying the proportions of the gummy candy ingredients and substituting part of the starch component with taro starch. Gummy candies were prepared by dispersing gelatin and taro starch in hot distilled water and allowing the mixture to swell completely. Anchovy extract was then added, and the mixture was homogenized using a magnetic stirrer at 600 rpm for 8 min. In a separate beaker, citric acid, sorbitol, and stevia were dissolved until a clear solution was obtained. This solution was subsequently added to the anchovy extract mixture and stirred using a magnetic stirrer at 600 rpm while being heated at 40°C for 10 min. Food coloring and flavoring essence were

then incorporated into the mixture and stirred until uniformly distributed. The final mixture was poured into molds and stored at 19°C for 24 h to allow gel formation and setting before further analysis.

Organoleptic Evaluation

Each gummy candy formulation was evaluated for taste, color, aroma, and chewiness [12].

Weight Uniformity Test

Weight uniformity was assessed by weighing 20 individual gummy candies and calculating the mean weight. The weight of each gummy candy was then compared with the average weight. The formulation was considered acceptable if no more than two gummies deviated from the specified percentage limit (Column A), and none deviated beyond the stricter limit (Column B), as defined by the applicable pharmacopeial criteria [8].

pH Determination

The pH of the gummy candies was determined by melting three gummy samples and measuring the pH of the resulting solution using a calibrated pH meter [12].

Moisture Content Analysis

The moisture content of the anchovy gummy candies was determined using a moisture balance. Approximately 5 g of gummy candy was placed in the instrument, and the moisture content was recorded as a percentage by weight (% w/w) [8].

Stability Study

The stability study was conducted by placing six gummy candies in a storage container and storing them for two weeks under three different temperature conditions: refrigerated (8–15°C), room temperature (15–30°C), and elevated temperature (30–40°C). Changes in the physical characteristics of the gummies were monitored throughout the storage period (12).

Microbiological Contamination Analysis

The prepared anchovy gummy candies were evaluated for microbiological contamination, including total plate count (TPC) and yeast and mold count (YMC). The yeast and mold count was determined by enumerating colonies that developed after sample inoculation on Potato Dextrose Agar (PDA) and incubation at room temperature for 24 h. Total plate count was determined by inoculating samples onto Nutrient Agar (NA), followed by incubation at 37°C for 24 h. The microbiological quality of the product was evaluated according to the applicable requirements of the Indonesian National Standard (SNI) 01-3840-1995 (13).

Heavy Metal Contamination Analysis

Heavy metal contamination in the anchovy gummy candies was assessed by determining lead (Pb) and mercury (Hg) concentrations using inductively coupled plasma mass spectrometry (ICP-MS). The results were reported in mg/kg and compared with the applicable detection limits.

Determination of Protein Content

Protein content was determined using the Micro-Kjeldahl method. Briefly, 0.5 g of gummy candy sample was weighed into a digestion flask and mixed with 0.5 g of selenium catalyst mixture and 10 mL of nitrogen-free concentrated sulfuric acid (H_2SO_4 , specific gravity 1.84). The mixture was digested in a fume hood for 1–1.5 h until a clear solution was obtained. After cooling to room temperature, the digest was transferred into a 1-L distillation flask, and the digestion flask was rinsed three times with distilled water. A total of 100 mL of distilled water was used for rinsing and transferred into the distillation flask.

Subsequently, 40 mL of 45% NaOH solution was added, and the flask was immediately sealed. An Erlenmeyer flask containing 5 mL of saturated 4% boric acid solution and two drops of mixed methyl red–methylene blue (MR-MB) indicator was used as the receiving solution. Distillation was performed until approximately 40 mL of distillate had been collected. The resulting distillate was titrated with 0.1

N HCl until the endpoint color change was observed. A reagent blank was prepared using 100 mL of distilled water and 40 mL of 45% NaOH, followed by the same distillation and titration procedures. Crude protein content was calculated from the nitrogen content determined by the Kjeldahl method (14).

Determination of Calcium Content

Calcium content was analyzed using atomic absorption spectrophotometry (AAS). Prior to analysis, 10 g of gummy candy sample was weighed into a beaker and mixed with n-hexane to remove lipids. The mixture was stirred, filtered, and separated from the yellow-colored lipid fraction. The resulting residue was dried in an oven at 100°C (13). For calcium determination, 5 g of the dried sample was incinerated in a muffle furnace at 550°C for 4 h until a whitish ash was obtained. The ash was dissolved in 50 mL of 3 N nitric acid (HNO_3) and boiled for 10 min. The solution was filtered through Whatman No. 41 filter paper into a 50-mL volumetric flask and diluted to volume with distilled water. An aliquot of 1 mL of the stock solution was further diluted with distilled water and mixed with 10 mL of 5% lanthanum oxide (La_2O_3) solution as a releasing agent. Calcium concentration was then measured using an atomic absorption spectrophotometer at a wavelength of 422.7 nm.

Statistical Analysis

The results obtained from the physicochemical, proximate composition, calcium, heavy metal, and microbiological analyses were evaluated descriptively. The selected formulation was produced as one independent batch, and analytical measurements were performed in triplicate where applicable. The results were reported as numerical values and, where replicate measurements were available, summarized using appropriate descriptive statistics. The storage stability assessment was conducted descriptively by evaluating observable changes in the physical characteristics of the gummy candies during the storage period.

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