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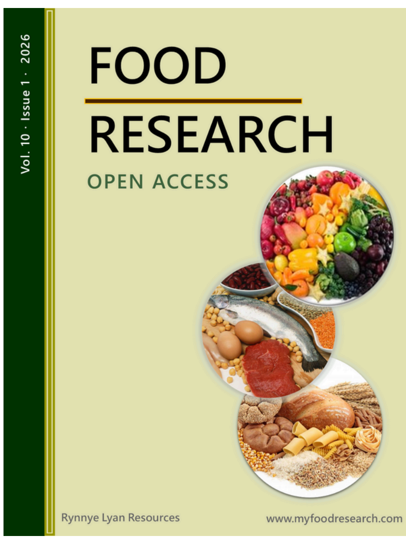

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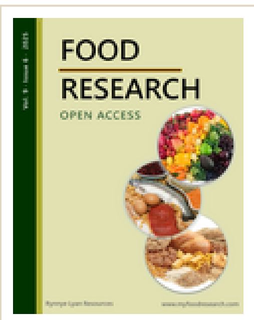

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FOOD RESEARCH

RESEARCH PAPER

Effects of storage temperature and packaging method on the physical characteristics of gelatin-based chewable gummies made from moringa leaf extract

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Abstract

Moringa oleifera leaf extract contains antioxidants and, therefore, has the potential to be designed and marketed as nutraceutical products like chewable gummies. While previous research suggests using 10% gelatin as the optimal formula, the favorable storage temperature and packaging remain unknown. This study aimed to optimize both factors using different conditions: stored at cool temperatures (8-15°C) or controlled room temperatures (15-30°C) in single-serve or bulk packaging in a squeezable bag. Also, the effects of their interactions on the product's required physical characteristics (dispersion time, swelling ratio, and syneresis) were examined using a factorial randomized control trial to be later considered in the optimization. Chewable gummies showed a generally less accepted quality when kept at controlled room temperatures instead of cool ones, as indicated by a higher swelling ratio (1.17-1.37%) and higher syneresis (0.64-0.69%). Also, increased hardness and decreased chewiness and gumminess were observed in gummies stored at controlled room temperature for both packaging methods. From these results, it can be inferred that storage temperature significantly influences the physical characteristics of chewable gummies, while the packaging method and its interaction with storage temperature have less significant effects. Therefore, storage at cool temperatures (8-15°C) was recommended to maintain the physical quality.

1. Introduction

Moringa oleifera contains medicinal substances, nutrients, and vitamins. Many parts of this plant, including leaves, flowers, seeds, and pods, have been developed into functional food, traditional medicine, cosmetics, and industrial products (Coppin *et al*., 2013). Because of their flavonoid and phenolic contents, moringa leaves are known as a great source of antioxidants. Using high-performance liquid chromatography combined with ultraviolet and electrospray ionization-mass spectrometric detector (HPLC-UV-ESI-MSD), previous research identified 12 flavonoids in the leaves, including quercetin and kaempferol glycosides (malonyl glycosides, acetyl glycosides, and succinyl glycosides) (Coppin *et al*., 2013). Meanwhile, the ethanol extract contains phenolic compounds like gallic acid, itaconic acid, catechin, and catechol (Sohaimy *et al*., 2015). With these phenolic and flavonoid contents, moringa leaves can be formulated into nutraceutical and pharmaceutical products that potentially prevent disease and maintain or improve health.

Chewable gummies, or chewable oral gels according to the U.S. Pharmacopeia (USP), are a type of nutraceutical product that is rapidly developed as a health supplement (Davydova, 2018). This product has a chewy structure and is made of gelatin or other suitable gelling agents and several excipients. It is consumed as a health supplement, not regular food (Badan Pengawas Obat dan Makanan (BPOM), 2019). According to the USP Chewable Gels Monographs, it contains 5-10% gelatin, 15-20% water, 28-50% sucrose, and 40-55% corn syrup (Davydova, 2018). Chewable gummies can address problems in supplement intake by pediatric and geriatric patients who often experience difficulty swallowing. Their rapid disintegration time and high public acceptability also increase demand and thus

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positively affect this product's development.

Gelatin is a scientifically proven gelling agent that can be used to make chewable gummies from *M. oleifera* leaves (Rani *et al*., 2021). With its excellent gelling ability, gelatin forms thermos-reversible gels that are stable at a wide range of pH and unaffected by ionic strength. Because gelatin can thicken the structure of chewable gummies (Bagal-Kestwal *et al*., 2019), adding it at an optimized concentration of 10% can produce the desired physical appearance, elastic and gummy texture, a low swelling ratio, and no syneresis during storage (Rani *et al*., 2021). In addition, to ensure the stability of nutraceutical chewable gummies made from moringa leaf extract, it is necessary to study and optimize storage conditions, including temperature and packaging, which potentially cause physical and chemical modifications.

Storage temperature affects water activity and shelf-life (Matulyte, Marksa and Bernatoniene, 2021). Previous research found that chewable gummies of *Myristica fragrans* were less vulnerable to weight loss and texture change when kept at cool temperatures than at controlled room temperatures (Matulyte *et al.,* 2021). However, a stability study discovered that storing chewable gummies at room temperature was sufficient to maintain water activity, moisture content, and microbial parameters (bacterial and mold contamination) (Matulyte *et al.,* 2021). Chemical quality assessments must also calculate active ingredient contents and monitor dissolution during storage to ensure good quality (Čižauskaite *et al*., 2019).

Packaging materials and methods control product stability. Single-serve or bulk packaging in squeezable bags or closed plastic boxes determines weight variation and water loss during storage. Previous research on the shelf-life of chewable gel-based tablets of *Myristica fragrans* explained that storage conditions and selected containers affected weight, texture, and bacterial contamination (Matulyte, Marksa and Bernatoniene, 2021). A similar study on chewy Santol candies revealed that packaging helped maintain color, water activity, moisture content, total acidity, pH value, and sensory evaluation (Renumarn and Choosuk, 2020).

Therefore, using a factorial random design, this preliminary stability study aimed to explore the effects of storage temperature and packaging on the physical stability of chewable gummies made from *M. oleifera* leaf extract. To investigate if different physical contacts between gummies affect stability and shelf-life, two packaging methods were used: single-serve in a plastic bag and bulk packaging (10 tablets) in a squeezable bag. Further, storage at cool temperatures (8-15°C) and controlled room temperatures (15-30°C) were observed in this study as a parameter that significantly influences water activity and weight.

2. Materials and methods

2.1 Materials

Moringa oleifera leaf powder was obtained from Bogo Village, Kapas District (Bojonegoro, Indonesia). All the excipients used in this study were pharmaceutical grade. Gelatin, mannitol, citric acid, benzoic acid, and Saccharum lactis were purchased from Planet Kimia (Indonesia). Other ingredients were sucrose (Sugar Group Companies, Indonesia), propylene glycol (Down Chemical Company, Thailand), flavoring agent (KH Roberts, Singapore), and coloring agent (PT. Anggana Catur Prima, Indonesia).

2.2 Preparation of Moringa oleifera leaf extract

Moringa leaf extract was prepared by maceration. First, 500 g of the leaf powder was weighed and macerated using 5000 mL of 70% ethanol with a 1:10 ratio. After 3×24 h, the macerated mixture was filtered. The filtration result (filtrate I) was macerated again using 2500 mL of 70% ethanol at a 1:5 ratio for 24 h. Then, the resulting filtrate II was evaporated using a rotary evaporator at 50°C (Indonesia Ministry of Health, 2017).

2.3 Preparation of chewable gummies

Table 1 shows the formulas used to produce chewable gummies from moringa leaf extract using gelatin as the gelling agent. The formula was composed of 10% gelatin, according to the optimized concentration in the previous study (Rani *et al*., 2021), and 2% moringa leaf extract as the active ingredient. The chewable gummy was designed to weigh 3 g and have a square shape with a dimension of 1.5 cm × 1.5 cm (length, width). Each manufacturing batch produced 250 chewable gummies. The preparation was conducted in triplicate.

All the components were weighed based on the percentage shown in Table 1. The first step was preparing the sucrose solution and gelling agent. Sucrose was dissolved in purified water, and the temperature was maintained at 80°C until a sucrose solution was obtained. It was then gradually added with gelatin powder until a gel-like structure was formed. In the next step, the gel mixture was added with mannitol and then gradually with propylene glycol, a sodium benzoate solution, and a citric acid solution. This mixture was then stirred and heated at 80°C while being added with the flavoring and coloring agents. After lowering the temperature to 60°C, the ethanol extract of moringa leaves was added little by little while being stirred for 10 min until a dough mass of

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chewable gummy was formed. Afterwards, the dough mass was poured into the mold slowly until fully filled, stored in an air-tight container at 25-30°C for 24 h, and then unmolded. The gummies were allowed to set for 12 h before further tests.

Table 1. Composition of chewable gummies made from *Moringa oleifera* leaf extract.

Component	Function	Percentage (%)
Moringa oleifera leaf extract	Active ingredient	2
Gelatin	Gelling agent	10
Mannitol	Sweetening and firming agent	10
Sucrose	Bulking agent	30
Citric acid	Acidulant	1
Sodium benzoate	Preservative	0.5
Propylene glycol	Plasticizer	4
Melon flavor	Flavor	2
Coloring agent (green)	Coloring agent	0.01
Purified water	Solvent	40.49
Saccharum lactis	Coating agent	-

2.4 Design of storage temperature and packaging method

The prepared chewable gummies were divided randomly into four groups to receive different treatments for physical stability tests, i.e., storage temperature and packaging method. Groups 1 and 2 were kept at controlled room temperatures (20-25°C) but with different packaging: single-serve in plastic bags and bulk in squeezable bags, respectively. Similarly, groups 3 and 4 were packed in single-serve plastic bags and in multiple-share squeezable bags and stored at cool temperatures (8-15°C). Details on each of these groups are presented in Figure 1.

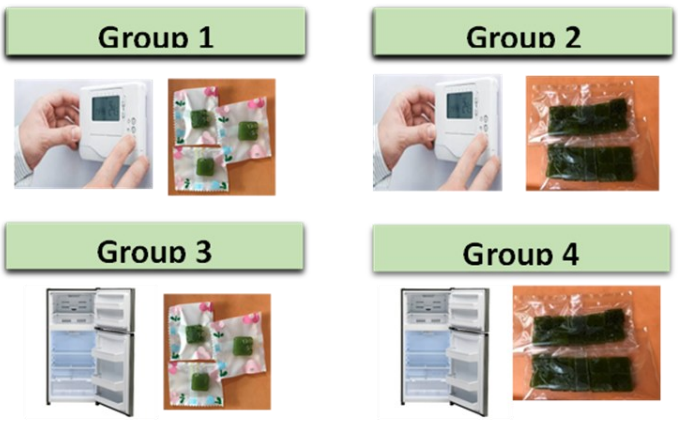

Figure 1. Designs of storage temperature and gummy packaging method for physical stability evaluation.

2.5 Physical stability evaluation

Samples of the grouped chewable gummies were observed organoleptically for any changes in their organoleptic features after 0 (initial condition), 1, 4, and 6 weeks of storage. The characteristics observed were shape, odor, taste, and color. Their textures were also evaluated by mildly rubbing the chewable gummy between the thumb and index fingers to determine stickiness and grittiness (Prakash *et al*., 2014). Other physical stability parameters evaluated in this study included product dimension, weight variation, swelling ratio, dispersion time, syneresis, and texture properties (chewiness, gumminess, and hardness), as explained in the sections below.

2.5.1 Measurement of product dimension

The chewable gummy's length, width, and thickness were measured using a vernier caliper to evaluate stability and estimate the size of the primary or most favorable packaging. It is required that the dimension should not deviate more than 5.0% during storage (Augsburger and Hoag, 2008).

2.5.2 Weight variation test

This test aimed to assess the uniformity of active ingredients among the prepared chewable gummies. Firstly, twenty chewable gummies were weighed individually, and the average weight was then calculated. These products are accepted if the weight of each chewable gummy does not change by more than 7.5% of the average. If this specification was not met, an additional test of another 20 samples of chewable gummies should be conducted. However, the acceptable limit for the second test would be no more than 10% of the average weight (Davydova, 2018).

2.5.3 Swelling ratio assessment

The swelling ratio estimated the extent to which the product's matrix could absorb water into its gel structure. First, the chewable gummy was weighed (Wd, initial weight) and immersed in 100 mL of purified water for 10 s at room temperature (25-30°C). Upon removal, the surface was cleaned with filter paper to remove any remaining water molecules (Kowalski *et al*., 2019). As seen in the equation below, a swelling ratio is a function of the product's initial weight (Wd) and weight after immersion (Ws):

$$\text{Swelling ratio} = \frac{Ws - Wd}{Wd} \times 100\%$$

2.5.4 Determination of dispersion time

This test aimed to observe the disintegration profile of chewable gummies in an aqueous medium (Rani *et* *al*., 2021). The dispersion time was determined by placing a chewable gummy in a beaker containing purified water, and the temperature was maintained at 37°C during the evaluation. The chewable gummy was constantly stirred during this test using a magnetic stirrer until dispersed homogenously in the medium (purified water). The time it took for the chewable gummy to disperse completely was recorded as dispersion time (Matulyte, Marksa and Bernatoniene, 2021).

2.5.5 Syneresis evaluation

Syneresis refers to the shrinking or contraction of gel when water molecules are extracted or expelled from the gel structure during storage, which can be used as an indicator of gel stability. Syneresis was evaluated at a controlled room temperature (Kadhim and Ali, 2019). A filter paper was weighed (A, initial weight), placed on the surface of a chewable gummy to absorb any existing water, and then weighed again (B) (Kaya *et al*., 2015). The syneresis was calculated using this equation:

$$\text{Syneresis} = \frac{B-A}{A} \times 100\%$$

2.5.6 Texture analysis

Texture analysis comprised evaluation of the product's chewiness, gumminess, and hardness. These parameters determined if the chewable gummy produced in this research was easy to chew and swallow, which is a determining factor in public acceptance (Mahat *et al*., 2020). A chewable gummy was placed in the sample compartment of TA.XTplusC texture analyzer. The probe, weighing 5 g, was set to apply 100 g of force at a speed ranging from 50 to 100 mm/s. Two-pressure action was conducted to measure chewiness and gummies (slow press 2) and the hardness test (slow press 1). The test ended when the probe was back in its initial position. A curve that correlated force and time was generated to explain chewiness, gumminess, and hardness (Matulyte *et al.,* 2021).

2.6 Data analysis

Descriptive analysis was employed to process data acquired from organoleptic observation, weight variation, dimension measurement, and texture analysis. ANOVA using a factorial completely randomized design was used to statistically analyze data on swelling ratio, dispersion time, and syneresis. The results of the statistical analysis could determine the predominant factors and describe the interaction between the components.

3. Results and discussion

After unmolding, the chewable gummies made from *M. oleifera* leaf extract were allowed to stabilize for 12 h prior to the visual and sensory examination, and samples from each group were observed in weeks 0, 1, 4, and 6. The parameters observed included organoleptic properties, weight variation, dimension, swelling ratio, dispersion time, syneresis, and texture. Results showed that the chewable gummies had a square shape, light green color, melon odor, and sweet taste with a subtle sour flavor. Also, they were elastic, chewy, and non-sticky when rubbed between fingers in week 0. Table 2 shows the dimensions of chewable gummies in all groups during the six-week storage. Except for group 1 (room temperature, single-share packaging), where changes in shape and dimension were observed, all chewable gummies maintained their physical qualities, including shape, texture, color, and dimension (deviations were below the accepted limits, 5.0%). In group 1, the chewable gummies became softer and thinner after six weeks of storage. The thickness decreased from 0.87±0.02 cm in Week 0 to 0.80±0.10 cm in Week 6. Such a decrease might stem from moisture loss that can be attributed to the nature of gelatin, which tends to lose more moisture over time than other gelling agents. Besides, gelatin is more sensitive to moisture loss at higher temperatures (Čižauskaite *et al*., 2019). Moreover, single-share packaging was believed to

Table 2. Dimension measurements of chewable gummies during storage.

Group	Dimension	Week 0 (cm)	Week 1 (cm)	Week 4 (cm)	Week 6 (cm)
1 (room temperature, single-serve)	Length	1.54±0.03	1.54±0.13	1.55±0.53	1.55±0.53
	Width	1.55±0.03	1.55±0.23	1.56±0.83	1.56±0.83
	Thickness	0.87±0.02	0.87±0.12	0.80±0.52	0.80±0.10
2 (room temperature, bulk in squeezable bag)	Length	1.53±0.02	1.53±0.02	1.55±0.02	1.55±0.03
	Width	1.55±0.03	1.55±0.03	1.53±0.03	1.54±0.02
	Thickness	0.92±0.01	0.92±0.01	0.94±0.02	0.93±0.02
3 (cool temperature, single -serve)	Length	1.52±0.02	1.52±0.02	1.54±0.02	1.54±0.02
	Width	1.53±0.01	1.53±0.01	1.54±0.01	1.54±0.01
	Thickness	0.92±0.02	0.92±0.02	0.94±0.02	0.93±0.02
4 (cool temperature, bulk in squeezable bag)	Length	1.52±0.01	1.52±0.01	1.54±0.02	1.54±0.02
	Width	1.52±0.01	1.52±0.01	1.53±0.02	1.54±0.02
	Thickness	0.97±0.01	0.97±0.01	0.95±0.02	0.93±0.02

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contribute to the dimension change because the space between the gummy and the packaging layer could not sufficiently accommodate water loss from the gummy's structure.

According to the USP Chewable Gels Monographs, a weight variation test ensures the homogeneity of the active ingredient in this product (Davydova, 2018). Table 3 shows the weight variations of all groups during storage. The prepared chewable gummies weighed between 2.80 and 3.05 g, and the individual weight did not vary by more than 7.5% of the average weight even after six weeks of storage. Based on this standard deviation, it can be concluded that all the chewable gummies uniformly contain moringa leaf extract. This also applies to group 1, whose weights substantially decreased from 2.8124±0.01 g in week 0 to 2.8041±0.00 g in week 6 due to moisture loss, or about 0.29% reduction.

A swelling ratio was calculated to determine the ability of the product's matrix to swell in an aqueous medium (Park *et al.*, 2010). It was based on the percentage of water molecules that penetrated the internal structure of its hydrogel, and immediately initiated the diffusion process (Kipcak *et al.*, 2014). Figure 2 compares the swelling ratios of all the chewable gummy groups. Groups 1 and 2 (room temperatures) had a swelling ratio of greater than 1.0% for both packaging methods, while groups 3 and 4 (cool temperatures) had a swelling ratio in the range of 0.43-0.52%. ANOVA revealed a significant difference between the swelling ratios of chewable gummies kept at room and cool temperatures ($p<0.05$). Higher swelling ratios in groups 1 and 2 can be attributed to the ability of gelatin molecules to entrap more water molecules from the environment at room temperatures that promote higher humidity (Matulyte, Marksa and Bernatoniene, 2021). This condition leads to syneresis and dimensional instability (Witono *et al.*, 2014). In contrast, the swelling ratios of groups 3 and 4 remained stable because gelatin can form and maintain stable hydrogen bonds with water molecules at cool temperatures, resulting in a stable three-dimensional gel structure (Chandra and Shamasundar, 2015).

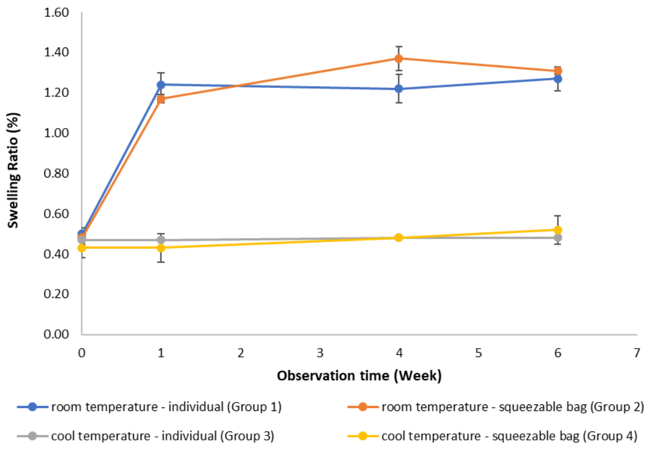

Figure 2. Swelling ratios of chewable gummies stored at cool and room temperatures with different packaging designs.

Dispersion time represents the rate at which a chewable gummy disintegrates in an aqueous medium. The faster the dispersion time, the faster the active ingredient is released from the product (Ruheena and Sirisha, 2018). Figure 3 compares the dispersion times of the prepared chewable gummies. It shows that all the groups required not more than 15 min to disintegrate, but those kept at cool temperatures broke down more slowly than the ones stored at room temperature. This difference was further confirmed to be statistically significant ($p<0.05$). In contrast, the packaging methods do not influence the dispersion time because the gelatin's hydrogel network is more sensitive to temperature than to friction. The hydrogel network becomes more rigid at cool temperatures, making it more difficult for water

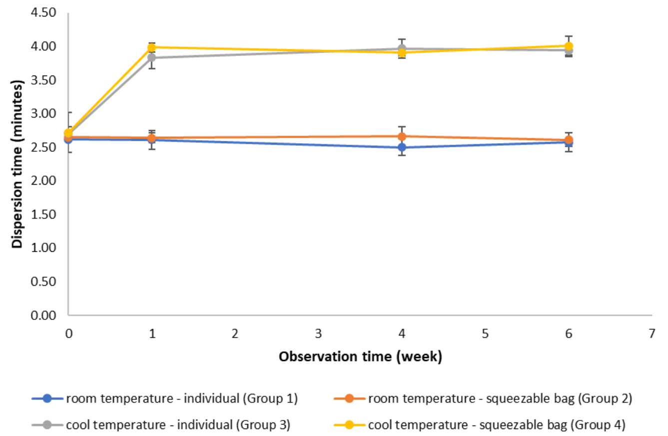

Figure 3. Dispersion times of chewable gummies stored at cool and room temperatures with different packaging designs.

Table 3. Weight evaluation of chewable gummies during storage.

Group	Week 0 (g)	Week 1 (g)	Week 4 (g)	Week 6 (g)
1 (room temperature, single-serve)	2.8124±0.01	2.8122±0.13	2.8112±0.00	2.8041±0.00
2 (room temperature, bulk in squeezable bag)	2.8134±0.01	2.8130±0.02	2.8070±0.01	2.8060±0.03
3 (cool temperature, single -serve)	2.8124±0.02	2.8122±0.01	2.8110±0.02	2.8100±0.05
4 (cool temperature, bulk in squeezable bag)	2.8154±0.01	2.8054±0.01	2.8062±0.01	2.8042±0.03

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molecules to penetrate (Park *et al*., 2020).

Syneresis is the shrinking of a gel-like structure due to the loss of water molecules (Kadhim and Ali, 2019), which is expressed as a percentage. The higher the percentage, the more quickly the gel structure loses moisture and softens, and the poorer the quality will be (Prakash *et al*., 2014). Therefore, syneresis is often used as the main parameter to measure the physical stability of chewable gummies. Figure 4 compares the groups' syneresis profiles during six weeks of storage. It showed that the syneresis remained below 1.0%, which further confirmed that the combination of storage temperature (particularly cool temperatures) and packaging method can protect chewable gummies against moisture loss and quality decline during storage. Because the gelatin's polymer hardens at cool temperatures, water molecules cannot easily escape (Dille *et al*., 2018), and, similarly, moisture in the environment cannot penetrate the polymer structure (Matulyte *et al.,* 2021). On the contrary, higher moisture at room temperatures makes the gelatin network easily contract, causing changes in water activity and syneresis. Decreased free energy in the system affects the gel's ability to retain water (Prakash *et al*., 2014).

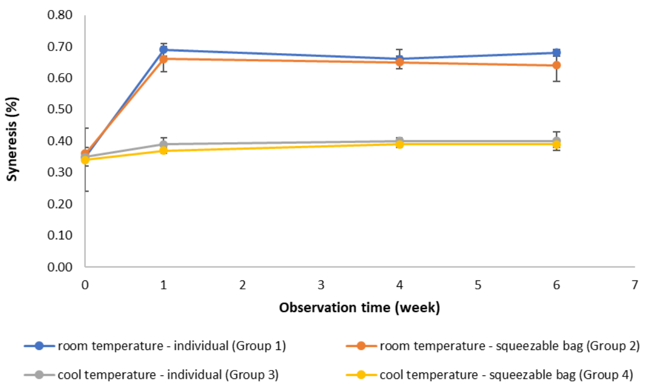

Figure 4. Syneresis profiles of chewable gummies stored at cool and room temperatures with different packaging designs.

The texture analysis used two compressive cycles to imitate mastication in the mouth cavity to obtain information related to the product's hardness, gumminess, and chewiness (Mahat *et al*., 2020). Hardness is the required amount of force exerted on an object to cause deformation (Kusumaningrum *et al*., 2016). Chewiness, as the combined result of hardness, cohesiveness, and springiness (Čižauskaite *et al*., 2019), refers to the energy required to chew a chewable gummy before swallowing. Gumminess is a deformation characteristic related to the hardness and cohesiveness of a gel matrix. Chewable gummies are sticky when made with a high adhesion force, but can be compact due to a high cohesion force (Kusumaningrum *et al*., 2016). Table 4 summarises the texture analysis results of different groups of chewable gummies. It shows that groups 3 and 4 (cool temperatures) were more chewy, sticky, and compact than groups 1 and 2 (room temperatures). This finding can be linked to the thermoreversible character of gelatin. Gelatin molecules assume a strong coil conformation at cool temperatures, increasing hardness, chewiness, and gumminess/ stickiness (Gilsenan and Ross-Murphy, 2000).

Furthermore, Table 4 shows an increase in hardness and a decrease in chewiness and gumminess of chewable gummies in groups 1 and 2 (room temperatures) after four and six weeks of storage. This condition can be associated with gelation and dehydration. While the extensive interaction between gelatin, sucrose, and water resulted in a firm and chewy structure, chewable gummies lose moisture over time and thus cause an increase in hardness (Delgado and Bañón, 2015). In contrast, groups 3 and 4 (cool temperatures) showed a decrease in hardness, chewiness, and gumminess during storage, which can be linked to the cooling process of the gelatin matrix. With faster cooling and higher holding temperatures, the gels produced will be less elastic, chewy, and sticky (Fonkwe *et al*., 2003).

Table 4. Texture analysis of chewable gummies made from *Moringa oleifera* leaf extract.

Group	Parameter	Week 0	Week 4
1 (room temperature, single-serve)	Chewiness (Nmm)	18.01	51.31
	Gumminess (Nmm)	44.03	128.29
	Hardness (N)	542.00	1427.00
2 (room temperature, bulk in squeezable bag)	Chewiness (Nmm)	36.89	24.24
	Gumminess (Nmm)	73.77	60.61
	Hardness (N)	597.00	974.67
3 (cool temperature, single-serve)	Chewiness (Nmm)	111.07	91.72
	Gumminess (Nmm)	271.50	229.31
	Hardness (N)	2249.00	2174.67
4 (cool temperature, bulk in squeezable bag)	Chewiness (Nmm)	76.77	62.90
	Gumminess (Nmm)	170.66	157.25
	Hardness (N)	1317.00	1592.33

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The statistical analysis (factorial ANOVA) revealed that storage temperature significantly affected the physical characteristics of chewable gummies made from moringa leaf extract, particularly swelling ratio, dispersion time, and syneresis ($p<0.05$). In contrast, these characteristics did not differ significantly between the two packaging methods and between the storage temperature-packaging interactions. Storage temperature only slightly influenced the gelation process, polymer network mobility, and the ability of the gel matrix to absorb moisture from the environment. When kept at cool temperatures, chewable gummies maintain their initial swelling ratio, dispersion time and have a lower percentage of syneresis than those stored at room temperatures.

4. Conclusion

Compared to the packaging method, storage temperature was the most influential factor on the physical stability of chewable gummies made from *M. oleifera* leaf extract. In contrast, the packaging method and the interaction between storage temperature and packaging method have less significant effects. Storage at cool temperatures (8-15°C) is recommended to maintain the desired physical characteristics of the chewable gummies.

Conflict of interest

The authors declare no conflict of interest.

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