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Physicochemical Properties, Bioactive Compounds Degradation Kinetics, and Microbiological Counts of Fortified Pomegranate Gummy Candy (GC) during Ambient Storage

Sifat Fisikokimia, Kinetika Degradasi Senyawa Bioaktif dan Jumlah Mikrobiologis Gummy Candy (GC*) Fortifikasi Buah Delima selama Penyimpanan Suhu Ruang*

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Abstract

This study explored the potential of fortified pomegranate gummy candies (GC) as a solution for insufficient vitamin C and phenol intake. It entailed two objectives: assessing the impact of ambient storage (20±2 ℃) on various GC properties and developing kinetic models to predict vitamin C and phenol degradation during storage. The study involved the preparation of GC using a modified formulation and examined moisture content, water activity, texture, vitamin C, total phenolic content, and microbiological counts during 15-day-storage. Findings revealed that the moisture content decreased from 21.82% to 13.41%, potentially affecting texture. Water activity remained high (0.84-0.86), posing potential microbiological risks. The textural analysis indicated high hardness, springiness, gumminess, and chewiness, which may impact consumer acceptance. Adhesiveness remained minimal. Vitamin C decreased from 2,176.90 mg AA/100g to 1,419.10 mg AA/100g, possibly influenced by moisture and oxygen. Phenolic content decreased from 2,845.97 mg GAE/100g to 2,183.70 mg GAE/100g, remaining remarkably high. Kinetic modeling revealed that zero-order kinetics best described degradation, with constant degradation rates. In conclusion, fortified pomegranate GC demonstrate potential as functional food. However, further research is necessary to optimize texture properties, improve formulation and understand complex interactions between gelatin and phenolic compounds for enhanced consumer acceptance and health benefits.
Keywords: gummy candy, kinetic modelling, phenol, physicochemical properties, vitamin C

Abstrak

Studi ini mengeksplorasi potensi gummy candy *(GC) fortifikasi buah delima sebagai solusi kekurangan asupan vitamin C dan fenol. Penelitian ini bertujuan untuk menilai dampak penyimpanan suhu ruang (20±2 ℃) pada berbagai sifat GC dan mengembangkan model kinetik untuk memprediksi degradasi vitamin C dan fenol selama penyimpanan. Pembuatan GC pada penelitian ini menggunakan formulasi yang dimodifikasi dan kemudian dilakukan pemeriksaan kadar air, aktivitas air, tekstur, vitamin C, kandungan fenolik total, dan jumlah mikrobiologis selama 15 hari penyimpanan. Hasil menunjukkan bahwa di akhir penyimpanan, kadar air menurun dari 21,82% menjadi 13,41%, berpotensi memengaruhi tekstur. Aktivitas air tetap tinggi (0,84-0,86), sehingga menimbulkan potensi risiko mikrobiologis. Analisis tekstur menunjukkan kekerasan,* springiness, gumminess, *dan* chewiness *yang tinggi, yang mungkin berdampak pada daya terima konsumen. Daya rekatnya tetap rendah. Vitamin C menurun dari 2.176,90 mg AA/100g menjadi 1.419,10 mg AA/100g, kemungkinan dipengaruhi oleh kelembapan dan oksigen. Kandungan fenolik menurun dari 2.845,97 mg GAE/100g menjadi 2.183,70 mg GAE/100g, namun masih sangat tinggi. Pemodelan kinetik mengungkapkan bahwa kinetika orde nol paling tepat menggambarkan degradasi, dengan laju degradasi yang konstan. GC yang difortifikasi buah delima menunjukkan potensi sebagai pangan fungsional. Penelitian lebih lanjut diperlukan untuk mengoptimalkan sifat tekstur, meningkatkan formulasi dan memahami interaksi kompleks antara gelatin dan senyawa fenolik untuk meningkatkan daya terima konsumen dan manfaat kesehatan.*
Kata Kunci: *fenol,* gummy candy*, pemodelan kinetik, sifat fisikokimia, vitamin C*

INTRODUCTION

Gummy candies (GC) are confectionery products with a gel-like structure, which are commonly composed of fruit extract, sugar, acids, aroma, food colorants, flavorings, and gelling agents such as gelatin (Teixeira-Lemos et al., 2021). Due to the chewy texture, GC was well-liked by children under seventeen (Periche et al., 2014). Recently, the use of pomegranate fruits as an ingredient for food products has gained interest because of its substantial antioxidant properties sourced from its phenolic compounds, including for confectionery production (Alyas et al., 2020; Kong et al., 2020). Besides having high antioxidant activity, pomegranate has a significant pectin content, making it an ideal ingredient for producing confectionery products such as GC and jellies (Garrido et al., 2015). Also, pomegranate was a natural colorant that gave a red-purple color to gummies, thereby making the gummies appealing to consumers, especially children. A growing number of jellies and hard candies products utilize pomegranate as the main ingredient (Belcaro et al., 2020; Pandey et al., 2022). However, limited pomegranate-flavored gummy products are available in the market (Belcaro et al., 2020).

Interestingly, GC could be used as a carrier to incorporate essential vitamins into daily diets, particularly among children who widely consumed them. In recent years, the global COVID-19 pandemic has caused increased focus on personal health, resulting in awareness and demand for functional foods, including vitamin-fortified products (Vishwakarma et al., 2022). Consequently, there has been a notable surge in popularity for vitamin C-fortified GC due to their ease of consumption, nutritional composition, visually attractive appearance, and tasty flavor. GC serves as an excellent option for children, especially those who struggle with swallowing supplement tablets or have particular dietary preferences. It was estimated that the global fortified GC market would grow at a compound annual growth rate (CAGR) of 6.1% from 2021 to 2031 (Albany, 2022; Yan et al., 2021).

Previous studies showed consumer demand turned from traditional confectionary products to low-sugar or healthier products (Periche et al., 2014). Yan et al. (2021) have reported on the escalating popularity of vitamin-fortified gummies in recent years due to their ease of swallowing, appealing appearance, and tasty flavors. Nevertheless, there was limited understanding regarding the impact of using pomegranate juice as a source of phenol enrichment, coupled with vitamin C fortification, on gummies' key physicochemical and textural attributes. Conversely, studies on various food products revealed that the stability of vitamin C and phenolic compounds could be compromised during storage, which was not ideal for consumers seeking optimal bioactive compound content (Basha et al., 2020; Kong et al., 2020). Therefore, assessing these specially formulated gummies holds significant importance, as it may serve as a reference point for manufacturing gummies with desirable qualities and functional value derived from vitamin C and phenols, which act as antioxidants for potential health benefits.

Given the background mentioned above, the potential of fortified pomegranate GC to serve as an effective and stable functional food has become this study interest. This study's objectives were twofold: evaluate the effect of ambient storage on the moisture content, water activity, texture, vitamin C content, total phenolic content, and microbiological counts of the gummies, as well as to develop kinetic models for predicting the degradation of vitamin C and total phenolic content at ambient storage. The study was mainly motivated by the recent reports of lower intake of phenols and vitamin C among the population, especially children (Hemilä, 2017; Hussain et al., 2022). Therefore, this study results are expected to be beneficial for improving the production of functional GC toward combatting phenols and vitamin C deficiency. The study adopted the latest definition presented by the Functional Food Center (FFC) in 2018, which described functional food as natural or processed food containing components that positively affect health (Alongi & Anese, 2021). In this study, the preparation of functional GC was conducted using pomegranate concentrate that was rich in phenols and also included the fortification process to add vitamin C. Fortifying the gummy mixture with vitamin C was imperative due to the loss of vitamin C in the primary raw material, pomegranate concentrate. This loss occurred during the previous juice-concentrate processing, which involved thermal processing, packaging, and food storage, as outlined in the study by Nielsen (2017). Additionally, it was necessary to investigate important quality attributes of the prepared pomegranate gummy to assess its overall quality during ambient storage. An ambient temperature of 20 ℃ is among the common storage temperatures for confectionary products such as GC. By understanding key physicochemical, functional, and microbiological properties, valuable insights could be gained to ensure the market potential and shelf-life stability of the pomegranate GC fortified with vitamin C during ambient storage. The development of a

reliable kinetic model becomes important to accurately assess the rate of decrease in vitamin C and total phenol content over time. Such a model would provide valuable insights into the potential degradation and loss of health benefits associated with these bioactive compounds. Additionally, the manufacturers could make informed decisions regarding product formulation and strategize effectively for time-to-market considerations.

METHODS

The GC was prepared by modifying the formulation proposed by Charoen et al. (2015). The ingredients used were pomegranate concentrate (21.8%), reverse osmosis water (43.6%), gelatin (10.9%), glucose syrup (10.9%), brown sugar (10.9%), citric acid (0.2%), and ascorbic acid (1.7%). The preparation of GC began by diluting the pomegranate concentrate with reverse osmosis water. The diluted pomegranate juice was then stirred and heated using a hot plate. The rest of the ingredients were added to the juice in the appropriate proportions, according to Charoen et al. (2015). The mixture was heated to 95 ℃ for 30 seconds and subsequently cooled to 40 °C. The high temperature was used for heating since this enabled the release of membrane-bound phenolic content in the juice following the study by Kong et al. (2020). Finally, vitamin C was fortified by adding vitamin C into the cooled mixture, reducing the pH from 4.11 to 3.20. pH below four was found to help slow down microbial growth, as evidenced by the study conducted by Kong et al. (2020). The mixture was then poured into molds, and as it cooled further, it began to set and take the shape of the molds. Once the GC mixture was fully set, the candies were removed from the molds and packaged in airtight containers. The GC was stored at ambient and dark storage with a temperature of 20 ± 2 ℃ for 15 days. The relative humidity in the storage is approximately 60%. The measurement of the essential qualities and bioactive compounds was conducted at an interval of 3 days. Based on a previous study on the properties of GC during storage, the control sample was represented by the initial condition of the food before the storage time had elapsed (Handayani et al., 2020). Hence, the GC samples on Day 0 in this study were used as the control sample, which acted as a reference point for comparison.

Measurement of Moisture Content

The moisture content of GC samples, weighing approximately 2 g each, was determined using a moisture analyzer (XM 50, Precisa, Switzerland) at 105 °C. The data were collected in triplicate for each sample to obtain the average moisture content value.

Measurement of Water Activity

The GC samples' water activity (aw) was measured using a water activity meter (Pawkit, Meter brand, USA). The meter was first calibrated using sodium hydrochloride solution with aw of 0.760 before it was used. The water activity of each GC sample was measured in triplicate, and the resulting average value was recorded.

Measurement of Texture Properties

Texture profile analysis was conducted at 25 ± 2 ℃ using a texture analyzer (TA XT Plus, Texture Technologies, USA). The cylindrical probe with a diameter of 6 mm was used to compress 75% of the GC samples from their original height. The samples were compressed twice to replicate (Lee et al., 2016). The texture analyzer settings were as follows: pre-test speed 1 mm/s, test speed 5 mm/s, post-test speed 5 mm/s, waiting time between cycles 5 s, and trigger force 5g. Textural parameters such as hardness, springiness, chewiness, gumminess, cohesiveness, and adhesiveness of each GC sample were measured in triplicates.

Measurement of Vitamin C Content

The procedures for determining vitamin C were based on titration using a 2,6-dichlorophenolindophenol (DCPIP) solution. Firstly, a standard vitamin C solution (ascorbic acid) is prepared by accurately weighing 0.5 g Celin tablet and dissolving it in 100 ml of distilled water. Next, the resulting solution underwent filtration using a Whatman Filter Paper No. 1. and was collected in a beaker. Subsequently, the solution was transferred to the burette using a funnel while adjusting it to the zero mark. Then, a measured amount of DCPIP solution was placed in a conical flask and titrated against the Celin

tablet solution until the color transitioned from blue to pink (endpoint), ultimately becoming colorless. The recorded measurement was designated as the standard reading.

The measurement of vitamin C in GC began with crushing the GC and dissolving 1 g of GC samples, 40 ml of water, and 10 ml of ethanol. The resulting solution was filtered using Whatman Paper No. 1. This solution was then transferred to a burette and titrated against a measured amount of DCPIP solution in a conical flask until the color changed to pink and eventually became colorless. The titration was conducted in triplicate, and the average value of the required volume was recorded. Based on the principle of molar equivalence in the stoichiometry of the reaction between vitamin C and DCPIP, the concentration of vitamin C in the GC sample was determined as mg of ascorbic acid (AA) per mL and subsequently determined for 100 g of GC sample (mg AA/100 g).

Measurement of Total Phenolic Content (TPC)

The total phenolic content determination followed the procedures outlined by Kong et al. (2020) and Herlina et al. (2022). A gallic acid stock solution was prepared by dissolving 100 mg of gallic acid powder in 500 ml of distilled water. Dilutions of gallic acid in distilled water were then made to obtain standard solutions with concentrations ranging from 20 mg/ml to 180 mg/ml. The standard gallic acid solutions were placed in centrifuge tubes and mixed with 5 ml of diluted Folin Ciocalteu's reagent. After a 5-minute incubation at 25 ± 2 ℃ in the dark, 4 ml of 7.5% sodium carbonate solution was added to the tubes, followed by a 2-hour incubation at the same temperature and darkness. The phenolic content of the gallic acid was measured at 765 nm using a UV-visible spectrophotometer (Genesys 180, Thermo Fisher Scientific, Malaysia). A standard calibration curve for gallic acid was plotted using the equation y = 0.011x - 0.020 (R^2 = 0.994).

The GC samples were dissolved in a solution containing 10 mL of ethanol and 100 mL of distilled water, then further diluted with an additional 100 mL of distilled water. Next, 1 mL of the diluted GC samples was mixed with 5 mL of diluted Folin Ciocalteu's solution in centrifuge tubes. After a 5-minute incubation at 25 ± 2 ℃ in the dark, 4 mL of 7.5% sodium carbonate solution was added, followed by a 2-hour incubation under the same conditions. The phenolic content in the GC samples was determined by measuring the blue intensity at 765 nm using a UV-visible spectrophotometer (Genesys 180, USA). The total phenolic content of the GC samples was expressed as mg gallic acid equivalents (GAE) per mL and subsequently determined for 100 g of GC sample (mg GAE/100 g). Measurements were taken in triplicate for each GC sample every three days during a 15-day storage period.

Determination of Microbiological Counts

The GC samples underwent microbiological analyses to assess their aerobic plate count (APC), yeasts, and mold count (YM). The APC was determined using the pour-plate technique on plate count agar. First, the GC samples were diluted and plated on agar plates. These plates were then incubated for 48 hours at a temperature of 36 ± 1 ℃, following the method described by Aljahani (2020). Furthermore, the yeasts and molds in the GC samples were quantified using pour plates of acidified potato dextrose agar. After dilution, the GC samples were plated on agar plates containing acidified potato dextrose agar. The plates were then incubated for three to five days at a temperature of 25 ± 2 ℃, as described by Aljahani (2020).

Kinetic Modelling of Vitamin C and Total Phenolic Content

Concentration was plotted against the storage period to observe the kinetic changes in the concentration of fortified vitamin C and phenols during storage at 20 ± 2 ℃ over 15 days. These changes were assessed for their fit to three different models, namely zero-order, first-order, and second-order kinetics. Regression analysis was employed to identify the optimal mathematical model that best fits the experimental data, whereas the goodness of fit data assesses the most appropriate kinetic model for describing the vitamin C and total phenolic content degradation. The analysis involved expressing the zero-order, first-order, and second-order kinetic models following Demir et al. (2019) using integrated law equations 1 to 3, respectively.

Zero-order reaction: $[A]_t = -kt + [A]_0$ (1)

First-order reaction: $\ln[A]_t = -kt + ln[A]_0$ (2)

Second-order reaction: $1/[A]_t = kt + 1/[A]_0$ (3)

Where,
A = Measured concentration of bioactive compounds in the samples (mg/100g)
A_0 = Initial concentration of bioactive compounds in the samples (mg/100g)
k = Rate constant (mg ● $100g^{-1}day^{-1}$)
t = Number of storage days (days)

The accuracy of each kinetic model was evaluated based on the Coefficient of Determination (R^2) and the Root Mean Sum of Errors (RMSE). The Coefficient of Determination (R^2) is the proportion of the variance in the dependent variable (measured concentration of bioactive compounds in the samples, A) that could be predicted from the independent variable (Number of storage days, t). It indicates how well the linear regression model predicts the actual data, whereby the closer it R^2 to 1, the better the model. Meanwhile, RMSE measures the difference between predicted and actual values (the standard deviation of the residuals). The model with maximum R^2 and minimum RMSE was deemed the best.

Statistical Analysis

A Tukey's test was conducted using the Minitab Statistic 16 Edition (Minitab, LLC, USA) to examine the significant differences between mean values over time. All the experiments were conducted with triplicate measurements at three-day intervals. The results were expressed as the mean ± standard deviation. A confidence level of 95% was used to assess the statistical significance. The differences were considered significant when the p-value < 0.05 (Herlina et al., 2022).

RESULTS AND DISCUSSION

This section provides the findings of the study conducted to assess the effect of ambient storage, controlled at 20 ± 2 ℃, on physicochemical properties, bioactive compounds degradation kinetics, and microbiological counts of fortified pomegranate GC.

Effect of Ambient Storage on the Moisture Content

Moisture content measures the total quantity of bounded and free water present in a food product, usually expressed as a percentage by weight on a wet basis (Zambrano et al., 2019). It was essential for GC candies to have a certain amount of moisture to maintain their soft, chewy texture. However, too much moisture could lead to issues like stickiness or mold growth, while too little moisture could result in a hard and unpleasant texture. According to Gunes et al. (2022), in principle, the moisture content of chewable products and marshmallows usually varies between 10 and 20% and is mostly recommended at <20% (Periche et al., 2014). According to Tireki et al. (2023), the moisture content could be controlled by setting the glucose syrup to sucrose ratio. This study showed in Figure 1 that the moisture content was slightly higher than the required moisture content, as reported by Gunes et al. (2022). On Day 0, moisture content was 21.82±1.05% but significantly decreased to 13.41% on Day 15 ($p<0.05$). Although the moisture content was still appropriate on Day 15, the moisture's significant loss could lead to quality variations in the product, such as premature crystallization, processing difficulties, and undesirable changes in textural properties, especially the hardness parameters (Gunes et al., 2022).

Moisture loss was one of the major concerns during the production of GC. The moisture loss occurred due to a diffusion mechanism driven by the partial water-vapor pressure difference between the product's inside and surface turning the water into vapor. An important aspect to be considered for controlling the final product's moisture content was the formulation, whereby gelatine, a type of hydrocolloid, was used in this study, and thus, the moisture content would depend on the gelatin's amount with the recommended percentage of 6% to 10% by Hartel et al. (2018). It was discovered that formulations that used gelatin had a higher initial moisture content and were more susceptible to water loss (Patel et al., 2018). During storage, the water trapped within the gelatin network structure gradually evaporated, causing the gummies to lose moisture. For future research, it is recommended to investigate various methods, including using additives like humectants and smart packaging, to protect against moisture migration. Such approaches have the

potential to offer substantial benefits in terms of extending the shelf life and preserving the quality of GC products (Subramaniam, 2016).

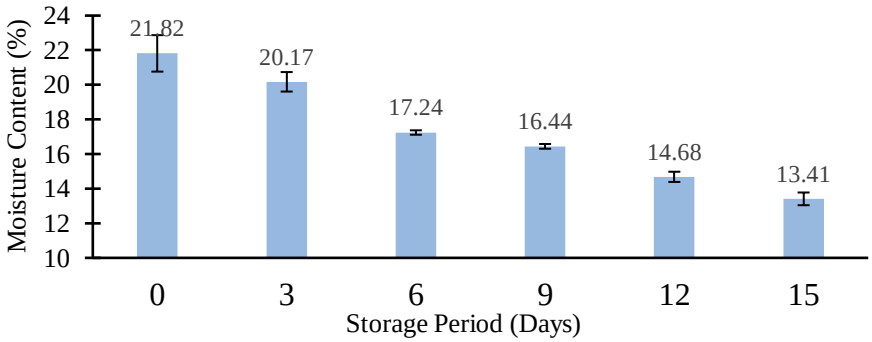

Figure 1: Moisture Content of the Fortified Pomegranate GC during 15 Days of Storage

Effect of Ambient Storage on Water Activity

Water activity (a_w) is the measurement of water availability for biological reactions, which also refers to the ratio of water vapor pressure in food to pure water's vapor pressure. It was a critical parameter that directly influenced microbial stability and the overall quality of GC products. Labuza et al. (1972) reported that the a_w below 0.70 was common for the confectionary products, whereby in principle, the bacteria could not grow below a_w of 0.85, yeasts and molds growth were slowed down around a_w of 0.7, and no growth occurred below 0. However, Labuza et al. (1972) also pointed out that a high reduction in a_w could result in a much harder product; thus, evaluating its texture and other physic properties and consumer acceptance would be necessary. In this study, throughout the storage period, the a_w of the GC samples was high yet stable with no significant difference ($p<0.05$), as shown in Figure 2, ranging from 0.84 to 0.86. The a_w was considered higher than a previous study by Periche et al. (2014), who reported that the developed GC with commercially similar quality had a_w of 0.79. While Gunes et al. 2022) listed that common a_w was in the range of 0.45 and 0.75, these levels were below the critical values for microbial growth. Meanwhile, Buerman et al. (2019) and Tarahi et al. (2023) reported that the a_w of confectionery products usually ranged from 0.54–0.66. However, the GC products should have higher a_w because of their soft and jelly nature, which ranges acceptably from 0.7 to 0.8 (Periche et al., 2014).

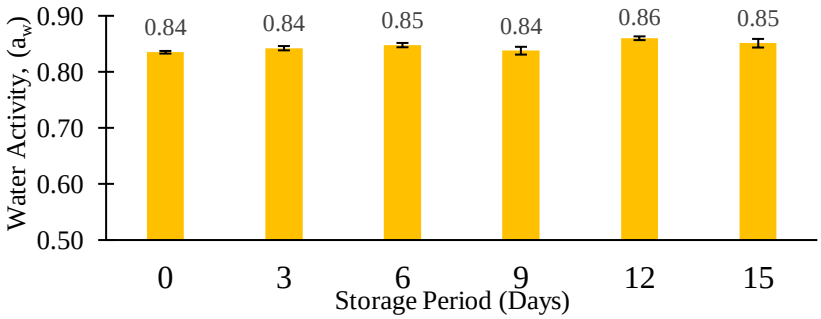

Figure 2: Water Activity (a_w) of the Fortified Pomegranate GC during 15 Days of Storage

High a_w (>0.80), as obtained in this study, indicated that the free water could encourage the growth of microorganisms and promote chemical reactions that might affect the stability of these products (Jiamjariyatam, 2018; Periche et al., 2014). The high a_w in this study was due to the major water proportion from the dissolved pomegranate juice (>65%) that provided the phenolic compound with functional properties for the gummies. Gunes et al. (2022) reported that the percentage of water in the GC formulation should be only 10% to 20%, while 50% of the formulation was originally from sucrose that could bind with water and, thus, reduced the availability of free water (a_w). Furthermore, the high water activity of GC (a_w

> 0.8) further contributed to moisture loss during storage, which was maintained under a relative humidity of approximately 60% in this study (Basha et al., 2021; Patel et al., 2018).

Effect of Ambient Storage on Texture Properties

Table 1 presents the textural parameters, including hardness, adhesiveness, springiness, cohesiveness, gumminess, and chewiness of the GC samples over 15 days of ambient storage. Hardness also referred to as firmness, is considered the primary sensory parameter as it plays a crucial role in assessing the mouthfeel of a product. Hardness is the force needed to achieve a given deformation (Garrido et al., 2015). The harder the GC, the more rigid its structure will be. The result showed that the GC samples fluctuated from 470.73N to 893.66N, with the highest hardness observed at the end of the storage period. The overall hardness was considered high and undesirable compared to the previous study by Nasir et al. (2022), who reported that preferred GC candy should have a small hardness scale around 300N. According to Tireki et al. (2021), the hardness of GC candy was affected significantly by ingredients (glucose syrup to sucrose ratio, starch, and gelatine level). The initial rise in hardness could be attributed to the decrease in moisture content (Siddique & Park, 2019). The gelatin content may plausibly explain the remaining fluctuating trend of hardness. According to Siddique & Park (2019), the subsequent decrease in hardness may be linked to a reduction in the number of pores caused by protein matrix breakage (gelatin) and fusion, leading to a softer texture. The further reaction potentially enhanced the binding between the added microparticles and the fused protein, consequently leading to an increase in the hardness of the GC, which could be observed at the end of the storage period (Siddique & Park, 2019).

Table 1: Textural properties of pomegranate fortified GC during 15 days of storage

Storage Period (Days)	Hardness (N)	Adhesiveness (Ns^{-1})	Springiness (mm)	Cohesiveness	Gumminess (N)	Chewiness (J)
0	617.38 ± 16.85^{c}	0^{a}	2.14 ± 0.95^{b,c}	0.93 ± 0.04^{a}	573.31 ± 12.75^{c}	1240.88 ± 57.97^{b}
3	715.45 ± 22.73^{b}	0^{a}	2.41± 0.73^{a,b,c}	0.93 ± 0.03^{a}	660.33 ± 9.60^{b,c}	1539.78 ± 55.73^{b}
6	632.54 ± 30.99^{c}	-0.08 ± 0.14^{a}	1.95 ± 0.69^{b,c}	0.92 ± 0.01^{a}	578.39 ± 34.70^{c}	1491.40 ± 321.50^{b}
9	857.67 ± 34.97^{a,b}	-0.08 ± 0.08^{a}	4.70 ± 0.88^{a}	0.95 ± 0.01^{a}	813.20 ± 70.32^{a,b}	3717.03 ± 101.39^{a}
12	470.73 ± 9.26^{c}	-0.83 ± 0.74^{a}	3.91 ± 1.27^{a,b}	0.98 ± 0.02^{a}	466.40 ± 109.82^{c}	902.23 ± 187.74^{b}
15	893.66 ± 41.54^{a}	-0.51 ± 0.88^{a}	1.52 ± 0.36^{c}	0.93 ± 0.04^{a}	825.90 ± 21.17^{a}	1250.51 ± 281.18^{b}

The data are presented in triplicates (± standard deviation). Different letters in the same column indicate significant differences between the samples, based on Tukey's test ($p < 0.5$)

Meanwhile, adhesiveness, or stickiness, refers to the work needed to overcome the attractive forces between the food surface and the material surface with which the food comes into contact, such as the tongue and teeth (Mutlu et al., 2018). Adhesiveness, as highlighted by Hamedi et al. (2018), is influenced by surface properties and the combined impact of adhesive and cohesive forces, which are associated with the product's molecular structure (Mutlu et al., 2018). In this study, the range of adhesiveness was from -0.08 Ns^{-1} to -0.83 Ns^{-1}. The range was comparable with results obtained by Nasir et al. (2022), with an average of -0.44 Ns^{-1} for the preferable gummy and considered minimal. The adhesiveness of acceptable gummies should be minimal, as high adhesiveness indicates their potential to stick to the teeth, palate, and tongue (Sumonsiri et al., 2021). The GC showed higher adhesiveness on Day 0 and Day 3 compared to the remaining storage duration since the moisture content at this time was high, as Hartel et al. (2018) described.

The following textural parameter of interest was springiness. Springiness, also known as elasticity, is the mechanical textural attribute relating to the rapidity and degree of recovery from a deforming force (Chen & Rosenthal, 2015). The fortified pomegranate GC exhibited high springiness (all values above 0.95) compared to previously studied gummy candies by Periche et al. (2014) and Nasir et al. (2022). Springiness is known to be closely associated with the concentration of gelatin (Hamedi et al., 2018). Therefore, the

higher proportion of gelatin used in this study (>10%) contributes to the relatively high springiness compared to the previous study's findings. This springiness enhancement is attributed to gelatin's intrinsic nature, a protein derived from collagen, which can create a three-dimensional network upon dissolving in water and subsequent cooling (Hamedi et al., 2018). Water is effectively trapped within this network, yielding a distinctive gel-like structure, whereby a higher proportion of gelatin results in a denser and more tightly interconnected network (Hamedi et al., 2018).

Cohesiveness refers to the mechanical textural attribute relating to the degree to which a food can be deformed before it breaks (Chen & Rosenthal, 2015). Kawano et al. (2017) highlighted that cohesiveness is crucial for consumer acceptance across all age groups. The fortified pomegranate GC samples showed cohesiveness, ranging from 0.92 to 0.98 during storage, with no significant difference. These results closely align with the findings of Charoen et al. (2015) and are similar to the gummy sample preferences highlighted in Nasir et al. (2022), where cohesiveness values ranging from 0.90 to 0.98 were favored. Within this range, cohesiveness implies the desirable ability of the gummy candy to withstand rapid disintegration during chewing, thus ensuring a pleasurable and prolonged chewing experience for consumers. The observed cohesiveness of the GC samples could also be attributed to viscous effects originating from the pomegranate concentrate.

Gumminess, a secondary parameter, is determined by multiplying hardness and cohesiveness and is defined as the energy required to disintegrate a semi-solid food into a state suitable for swallowing (Garrido et al., 2015). In this study, the observed range of gumminess (466.40 to 825.90 N) was notably higher when compared to the acceptable gummy values produced by Nasir et al. (2022), which recorded a value of 331.26 N. This discrepancy can be attributed to the broader range of hardness observed in Table 1, resulting in a higher range of gumminess. As suggested by Mutlu et al. (2018), an increase in hardness leads to an increase in gumminess due to the higher energy required to disintegrate a semi-solid food product into a swallowable state.

The final textural parameter was chewiness, reflecting the energy needed to chew a solid food into a swallowable state and arising from a combination of hardness, cohesiveness, and springiness (Chen & Rosenthal, 2015). Santos et al. (2014) highlighted the advantages of adjusting chewiness to lower values to reduce the energy required during chewing, especially for enhancing sensory acceptance in softer candies. Notably, in this study, chewiness values frequently exceeded those reported by Nasir et al. (2022) and Charoen et al. (2015), who recorded preferred GC candy values of 1314 J and 1226 J, respectively. Similar to gumminess, the elevated chewiness of the GC samples primarily resulted from their high hardness, suggesting increased chewing effort.

Effect of Ambient Storage on Vitamin C Content

The stability of vitamin C in GC was vital due to its sensitivity to degradation, impacting nutritional value, flavor, appearance, and bioavailability. Vitamin C's susceptibility could lead to degradation pathways that reduce its effectiveness, potentially compromising health benefits. The initial analysis indicated that the GC was fortified with 2176.90 mg AA/100g at the outset of storage. Subsequently, as demonstrated in Figure 3, a significant decline ($p < 0.05$) in vitamin C concentration was observed within the GC samples over a 15-day storage interval, reaching 1419.10 mg AA/100g. Regarding a single sample of GC, the results suggested an initial measurement of 29 mg AA/GC on Day 0, which subsequently decreased to 18.90 mg AA/GC by Day 15. Despite the degradation, the Vitamin C content aligns positively with the Recommended Dietary Allowance (RDA) guidelines and was considered high compared to other vitamin C-rich foods (Hosseinifarahi et al., 2020). These guidelines recommend a daily vitamin C intake of 75–90 mg/day for adults (19+ years old) and 45–75 mg/day for children and teenagers (≤18 years old) (Yan et al., 2021). Consequently, individuals within these age groups would need to consume roughly 3 to 5 pomegranate-fortified GC per day, each containing 18.90 mg of vitamin C, to meet the recommended intake (Yan et al., 2021).

The decreasing amount of vitamin C in fortified food agreed with the previous findings by Herbig & Renard (2017). The high a_w in the studied GC might have accelerated vitamin C degradation by facilitating chemical reactions that lead to the breakdown of vitamin C over time. Another possible degradation mechanism was due to the oxygen accessibility in the headspace of storage containers. Oxygen molecules could react with vitamin C molecules, causing them to lose electrons and transform into oxidized forms. One common oxidation product was dehydroascorbic acid, an oxidized form of vitamin C (Herbig &

Renard, 2017). Dehydroascorbic acid reduced antioxidant activity compared to vitamin C and was less effective in providing the health benefits of vitamin C consumption.

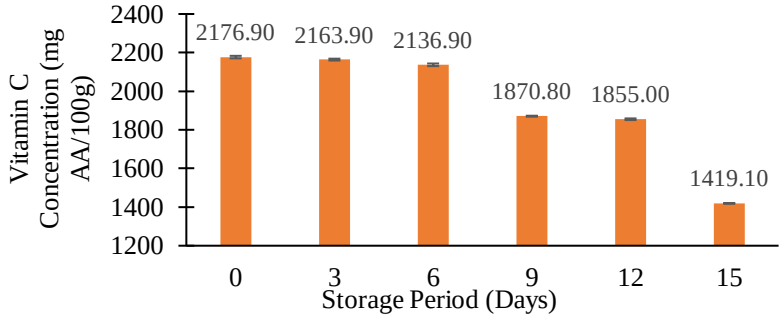

Figure 3: Vitamin C's Concentration of The Fortified Pomegranate GC during 15 Days of Storage

Effect of Ambient Storage on Total Phenolic Contents (TPC)

The stability of phenolic content in the GC is vital for preserving their health benefits, antioxidative activity, bioavailability, sensory properties, and shelf life (Sukardi et al., 2022). Maintaining stable phenolic levels ensures that consumers can reap the intended health advantages of these compounds while enjoying a consistent and high-quality GC. Figure 4 shows the decreasing trend of TPC of the GC samples, indicating the degradation of phenols during ambient storage. Initially, the GC was found to have 2,845.97 mg GAE/100 g of phenols. At the end of 15 days, the concentration of phenols was reduced significantly ($p<0.05$) to 2,183.70 mg GAE/100g. Regarding a single sample of GC, the results suggest an initial measurement of 38.53 mg GAE/GC on Day 0, which subsequently decreased to 30.05 mg GAE/GC by Day 15. Notably, despite undergoing degradation, the phenolic compound content remained remarkably high, surpassing 2000 mg GAE/100 g. This surpassing of levels observed in previous studies is of particular interest, whereby Ali et al. (2021) reported 299 mg GAE/100 g in strawberry and red beetroot candy, while Tarahi et al. (2023) documented 520 mg GAE/100 g in gummy candies formulated with Jaban watermelon exocarp powder. As a result, our pomegranate gummy candy exemplified a potential sample showcasing an abundant phenolic profile.

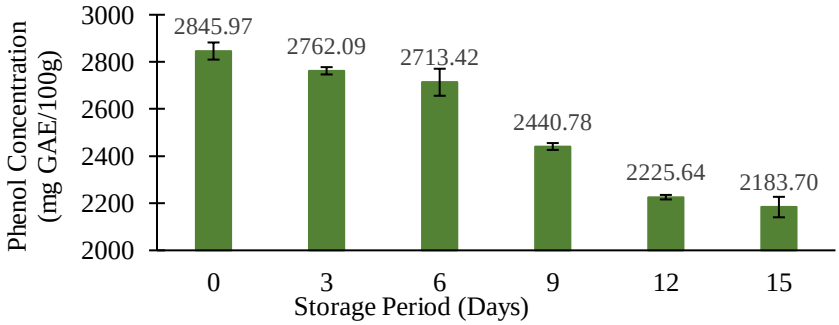

Figure 4: Concentration of Phenolic Compound in The Fortified Pomegranate GC during 15 Days of Storage

The degradation of phenols followed earlier investigations conducted on gummy candies (Casas-Forero et al., 2022; Guo, 2015; Mandura et al., 2020). Casas-Forero et al. (2022) reported that the high a_w favors reactant mobility, contributing to the degradation of phenolic compounds. Another possible reason for the reduction of phenolic compound bioavailability was due to the interaction between gelatin and phenolic compound, leading to lower availability of polyphenols for hydrolysis and oxidation reactions (Tutunchi et al., 2019). Likewise, Kia et al. (2020) indicated that the gelatin triple helix could retain antioxidant compounds due to cross-linking and the formation of three-dimensional networks. While gelatin can influence the bioavailability of phenolic compounds, the extent of this impact can vary based on factors

such as the type of phenolic compound, the specific gelatin matrix, the processing methods used, and the overall composition of the food product. As such, there is a clear need for further research to understand the intricate interactions between gelatin and phenolics comprehensively. Manufacturers of functional foods should consider these interactions when designing products to maintain the desired bioavailability and health benefits of phenolic compounds, thereby warranting the exploration of these factors in future investigations.

Effect of Ambient Storage on Microbiological Counts

Table 2 shows the GC samples' microbiological qualities at the ambient storage's beginning and end. The standard plate count of the GC samples at Day 0 was 4.0 × 10 CFU/g, suggesting the presence of microorganisms, possibly due to contamination during gummy preparation. By the end of the storage period, the GC samples had a moderate standard plate count of 3.0×10^4 CFU/g. The microbiological growth was feasible in the GC samples since the a_w was relatively high on Day 12, reaching 0.86, as in Figure 2. Although the count did not exceed the microbiological limit set at 1×10^6 CFU/g by the Ministry of Health Malaysia, this finding indicates poor hygiene practices and could lead to higher microbial growth (Khalid et al., 2019). A reformulation was also needed to reduce a_w to below or around 0.7 to inhibit microbial growth.

Table 2: Microbiological qualities of fortified pomegranate GC during 15 days of storage

Day of Storage	Day-0	Day-15
Standard Plate Count (CFU/g)	4.0 ×10	3×10^4
Total Yeast Count (CFU/g)	Not detected	3.8×10^4
Total Mold Count (CFU/g)	Not detected	9.0×10^3

Initially, there was no detectable growth of yeast and mold in the freshly prepared GC samples. However, after being stored for 15 days at 20±2 ℃, the yeast count significantly increased to 3.8×10^4 CFU/g, while the mold count reached 9.0×10^3 CFU/g. These counts exceeded the maximum allowable limit of 1.0×10^2 CFU/g for yeast and mold in ready-to-eat foods, as stated in the Food and Drug Administration Food Code (Hasnan & Ramli, 2020). The growth of yeast and mold in the GC samples was feasible since their acid/alkaline requirement for growth was quite broad, ranging from pH 2 to above pH 9. Their moisture requirements were relatively low, whereby most species could grow at an a_w of 0.85 or less, although yeasts generally required a higher water activity (Labuza & Altunakar, 2020). Moreover, the gummy candy's high sugar content, which reaches as high as 37% per gummy according to the formulation used in this study, could also create favorable conditions for yeast and mold proliferation (Kong et al., 2020).

Kinetic Modelling of the Degradation of Vitamin C and Total Phenolic Content

The kinetics of vitamin C and total phenolic content (TPC) of the fortified pomegranate GC samples were evaluated to determine the degradation rate of these valuable bioactive compounds. The reaction order estimations achieved from fitting the zero, first, and second-order kinetic models for vitamin C and TPC are shown in Table 3. The reaction order of these bioactive content changes was determined according to the R^2 and RMSE as a function of storage days at ambient storage temperatures, controlled at 20 ± 2 ℃.

By comparing all the kinetic models, the zero-order model exhibited the highest R^2 value and the lowest RMSE value, indicating that it provided the best fit for describing the degradation kinetics of vitamin C in the GC samples. The degradation kinetics of vitamin C in GC was described by the zero-order kinetics model, denoted as $C=C_0-47.446t$. In this model, the rate of vitamin C degradation was constant over time and independent of its initial concentration. This implies that the degradation rate constant, k, remained steady throughout the storage period, regardless of the initial vitamin C concentration. With a degradation rate constant of 47.446 $\frac{\text{mg AA}}{\text{100g.day}}$, it could be interpreted that 47.446 mg of vitamin C would degrade each day for every 100 g of gummy candies. Additionally, based on this best-fit model, the calculated half-life of 24.163 days indicated that the initial vitamin C concentration in the gummy candies would decrease by half in approximately 24 days due to degradation.

Table 3: Kinetic loss rates constants, R^2 and RMSE values according to zero-order, first order and second-order kinetic models fitted to the experimental data of vitamin C content and TPC in fortified pomegranate GC during 15 days of storage

Kinetic parameters and models	Zero-order			First-order			Second-order		
	k (mg/(100 g.day)	R^2	RMSE	*k* (mg/(100 g.day)	R^2	RMSE	*k* (mg/(100 g.day)	R^2	RMSE
Vitamin C	47.446	0.828	110.886	-0.026	0.793	124.008	1×10^{-5}	0.754	232.634
TPC	44.341	0.973	38.221	0.017	0.969	39.303	7×10^{-6}	0.962	319.209

Similarly, the degradation TPC in the GC was best described by the zero-order kinetics model, denoted as $P = P_0 - 44.341t$. This model suggests that the total phenolic content degradation rate was not influenced by its concentration. Similar to the degradation of vitamin C, the degradation rate of phenol remains constant throughout the storage period, regardless of the initial phenolic compound concentration. The degradation rate constant 44.341 $\frac{\text{mg GAE}}{\text{100g.day}}$ indicated an average daily degradation of 44.341 mg of phenol for every 100 grams of GC candies. However, the half-life of 32.596 days indicated that the initial phenol concentration in the GC candies would take around 33 days to reduce by half.

CONCLUSIONS

In conclusion, this research delved into the impact of ambient storage, maintained at 20 ± 2 °C, on various facets of fortified pomegranate gummy candies (GC). The findings revealed significant dynamics in key attributes. The moisture content is initially slightly higher than recommended and decreased notably during storage, potentially affecting texture characteristics. The water activity (a_w) that was consistently high, primarily due to dissolved pomegranate juice, raised concerns about microbial growth and chemical reactions. The GC displayed elevated hardness levels, exceeding preferred ranges, with fluctuating trends possibly linked to gelatin interactions and moisture changes. On the positive side, adhesiveness and springiness fell within acceptable ranges, aligning with consumer preferences. However, gumminess and chewiness values surpassed preferred levels, primarily driven by high hardness. Additionally, the study observed vitamin C and phenolic compound degradation during storage, though they remained within recommended intake levels. Moisture content and oxygen accessibility were identified as degradation contributors. Notably, the GC retained exceptionally high phenolic concentrations, suggesting its potential as a source of abundant phenolic compounds. The research has also successfully applied the zero-order kinetic model for accurate compound degradation prediction under the defined storage conditions.

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