Effect of honey and lemon juice on the physicochemical, nutritional, minerals, color, bioactive compounds, antibacterial and antioxidant properties of guava-pineapple jelly

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Abstract

Background

Jellies are usually preserved by artificial preservatives where they have harmful side effects and health hazards especially to infants. Honey and fresh lemon juice work as natural preservative due to their hygroscopic, high sugar, low pH and antibacterial properties. Hence, the honey and fresh lemon juice were undertaken to formulate the synthetic preservative free guava-pineapple jelly for extended shelf life with higher retention of nutrients and antioxidants.

Results

Best formulation found using honey and fresh lemon juice treated jelly (T_2 and T_3) by the sensory evaluation, nutrients, bioactive compounds, phenolics, antioxidant and antibacterial properties. Storage study was conducted at ambient condition and the shelf life of the jelly was remained upto 8 months without any quality deterioration. All the physicochemical, nutritional, minerals, bioactive compounds, antioxidants activities and phenolic acids were decreased with progression of storage periods. The highest vitamin-C, energy, total phenolic, flavonoid, carotenoid, ß-carotene, and anthocyanin, were recorded by the combination of guava-pineapple jelly formulated using honey and fresh lemon (T_3) juice; values were 42.94-41.00 mg/100 g, 406.35-406.94 cal/g, 4.15–4.01 mg GAE/100g, 0.91 – 0.84 mg QE/g, 0.94 – 0.84 mg/100g, 12.44–12.20 mg/100g, and 4.06–4.01 mg/100g, respectively. Phenolic acids, i.e. gallic acid, vanilic acid, caffeic acid, ferulic acid, catechin acid and syringic acids were identified and quantified higher in sample T_3; values ranged 5.41–5.30 mg/100 g, 4.66–4.54 mg/100 g, 9.07–8.93 mg/100 g, 0.46 – 0.35 mg/100 g, 81.70-81.43 mg/100g, 3.23–3.10 mg/100 respectively. Microbial study confirmed that the jelly was free from microbes upto 6 months of storage. After 8 months, the Aspergillus, Shigella and E-coli were detected but they were within acceptable limit. Initial color was retained upto 8 months of storage but after 8 months, the color faded out and turned into dark.

Conclusion

Honey and fresh lemon juice are valuable source of natural preservative for formulation of fruit jelly with extended shelf life upto 8 months by preventing microbial activities. The processors could process and preserve the fruit jelly with decent aroma by applying this technology as an alternative to artificial food additives.

1 Introduction

Guava (*Psidium guajava*) is known throughout every tropical region worldwide. The fruit is eaten as a dessert, but the numerous minute seeds give it an unpleasant “sandy” texture. Its juice is very acidic and is too tart and strongly flavored for direct use and consumption due to its hard shell. In Bangladesh, it is a seasonal and perishable fruit. It is mainly cultivated in the hill areas but recently its cultivation is tremendously increasing year by year all over the country. The total production of guava fruit is 243957 metric ton (MT) from a cultivable land of 58618 acres [1]. Its leading growing areas are Chattragram, Dhaka, Barishal, Rajshahi, Khulna, and Pirojpur. The fruit contains 250 IU/100g of vitamin A, 75–265 mg/100g of vitamin C, 17.80–30 mg/100g of thiamin, riboflavin, niacin, and phosphorus [2]. Short storage life (6–8 days) is the major problem that does not allow to keep the fruit in marketable life for a long time [3]. Therefore, processing of guava into jelly may be one of the ways to make value added nutritious products with its prolonged storage life [4].

The pineapple (*Ananas cocomus Merr.)* is a large fruit composed of number six-sided berries arranged spirally and embedded in the juicy pulp of the swollen stem. The socalled fruit carries a crown of spiny leaves and may grow to a weight of 1–3 kg per fruit. The plant is a native of Brazil and its early Spanish name “pina” due to its ressembles a pine cone. The plant bear one fruit, which may take up to two years to mature. It is cultivated throughout the tropical regions of

the world with major commercial plantations in Hawaii, the Phillipines, Taiwan, Malayasia, South Africa and Austratlia, with other centers in Florida, Puerto Rico and East Africa. In Bangladesh, it is a major fruit and its predominant growing areas are concentrated to Modhupur, Sreemongal, Rangamati, Khagrachari, and Chattagram. At present, it is cultivated on 34246.66 acres of land with a production of 208141 MT [1]. The fruit contains adequate amount of vitamins A, B, and C. Every year the fruit goes to postharvest loss due to lack of proper processing and preservation techniques.

Food is an elementary requirement of humans and may contribute to play a vital role to make Bangladesh self-sufficient. The country achieved improvement in the production of food but safe food is still a major problem. Various processed products are made from guava and pineapple fruits worldwide including jam, jelly, leather, nectar, squash, dried powder, toffee, ice-cream, candy, syrup, juice, concentrated puree, canned fruit segments, ready to serve drinks, etc. [5] but these are meager in the country. Synthetic guava jelly can be found in the market made by using a huge amount of pectin (E-440), citric acid (E-330), sodium benzoate (E-211), potassium metabisulphite (E-224), permitted food colors (Allura Red AC E-129 and Ponceau 4R E-124) [6]. Current research indicates that synthetic chemicals and preservatives have various side effects. Their use could be the foundation of the long-term effects on health, especially the manifestation of kidney diseases, liver injury, type-2 diabetes, coronary heart disease, and stroke, which is tremendously increasing across the globe [7]. Most of the synthetic food contains a significant amount of heavy metals that accumulate in the human body. The buildup of heavy metals in the body is the leading cause of developing the above-mentioned diseases and this can also develop abnormality among children [8]. Recent studies have shown that the process of soaking, washing, boiling, grilling and cooking significantly decrease the the concentration of arsenic and heavy metal residue in processed food [8–12]. Organic citric acid is also found to be more effective to reduce the heavy metal residues due to its working ability as a chelating agent [8]. The acid is also used as a flavoring and acidifying agent [13].

Natural methods of preservation include**s** boiling, freezing, pasteurizing, dehydrating, smoking, pickling, adding sugar, lemon juice, honey, *etc*. Jam and jelly are usually preserved by using high sugar content. Lemon juice is a very popular method to preserve fruits and vegetables. Honey is a amazing foundation of nutrients having antimicrobial properties that have been proven by the several researchers [14–19]. It subsidizes energy, immunity system and remedy for several diseases. Natural and cultured both honey production is increasing gradually by the financial and technical assistance of Bangladesh Government, Bangladesh Institute of Apiculture, Bangladesh Small and Cottage Industries Corporation (BSCIC), Palli Kormo Sahayak Foundation (PKSF) and many NGOs like Proshikkhan Shikkha Karmo (PROSHIKA), Mouchas Unnayan Sangstha (MUS) etc. Now a days honey is considered as a new addition to the modernization and commercialization of agriculture in Bangladesh where the country received an export order for 400 metric tons of honey from Japan [20]. Considereing its present production trend, medicinal and antimicrobial activities, hence, an attempt has been taken to utilize the honey for processing of agricultural commodities as well as industrial utilization to make the process economically viable to the honey producers and utilizers.

Among natural substances obtained from the plants, sugar, lemon juice and honey very efficiently decrease the growth of bacteria, increase flavor, and impart attractive color to food [6]. Nowadays, people prefer to find out alternative natural foods instead of synthetic foods. Recently, there has also been much concern by the food scientists, medical scientists, and nutritionists to discover natural foods for the promotion of safe food production, processing, preservation, and distribution. Therefore, the objective of the current research was to develop guava-pineapple jelly through natural and bioactive ingredients such as honey and fresh lemon juice to minimize the use of synthetic chemicals and preservatives in jelly processing.

2 Materials And Methods

2.1 Collection of fruit

Matured local variety of guava was harvested from the farm of the Farm Division, Bangladesh Agricultural Research Institute (BARI), Gazipur-1701, Bangladesh. Ripe pineapple was collected from the farmer's field of the hilly area of Chattagram, Bangladesh. After collection, the fruits were shifted to the laboratory of Postharvest Technology Division, BARI, Gazipur, Bangladesh for pre-processing.

2.2 Processing of guava-pineapple jelly

After pre-processing i.e. cooling, sorting and grading, the guava fruits were processed according to the process flow chart as presented in the Fig. 1.

2.3 Characteristics of Citric acid, Potassium metabisulphite (KMS) and Honey in processing of jelly

Citric acid (E-330) is an acid which occurs naturally in fruits such as lemon and lime. It is used for fruit canning, fruit juices, ice cream, marmalade, pickle and jelly manufacturing, flavoring and acidifying agent. It is also used to suppress browning in fruits and vegetables and as a synergetic compound for antioxidants [13]. The final jelly should contain at least 0.50–0.75% of citric acid but not more than1.00% because a large quantity of acid may cause syneresis (Engineers India Research Institute, EIRI). The citric acid differs from lemon juice due to a chelating agent. As a chelating agent, it is more potential soaking and heating tool for mitigation of heavy metal residues from fruits, vegtables and cooked foods [8]. On the other hand, the fresh lemon juice differ from citric acid due to its aroma. The fresh lemon juice contain limonene but when it gets ready as citric acid form by drying, this can be the result of big losses of acetaldehyde by peroxidation of limonene.Thus in this study, the fresh lemon juice is used as strong natural flavoring agent. Although the storage life of the fresh lemon juice is only 3 weeks at 4ºC [13] but its year round production is available in the country with cheap price.

Potassium metabisulfite (KMS), also known as the food additive E224 or potassium pyrosulfate, is a food preservative which preserves the natural colour of food and protect against bacteria. It does not have residual effect upto 1000 ppm. The concentration of the KMS residue decrease gradually with the advancement of storage periods [5]. Honey is a sweet food made by bees using nectar from flowers. In its undiluted form, it is a rich source of nutrients and is selfpreserving. It is a natural energy-booster, builds immunity and is a natural remedy for many ailments [6]. The research on honey has been carried out by several researchers [6, 17, 21] confirmed to be effective as antibacterial properties and fight against several human pathogens including E.coli, S.aureus, Salmonella, Salmonella typhimurium, Enterobacter aerogenes.

2.4 Data recording and documention technique

Physicochemical, nutritional, bioactive compounds and antioxidant properties of the fresh fruits were recorded. On the day of processing into jelly, the physicochemical and nutritional properties were determined to select the best sample out of the different treated samples. The best sample was also selected following standardization procedure of sensory evaluation. Then the standardized jelly was stored for the future study of color, texture, physicochemical and nutritional properties, minerals analysis, bioactive compounds, total antioxidant properties, phenolic acids compositon and microbial counts during different storage periods (0–8 months).

During processing, total soluble solids (TSS) content was checked by a digital hand refractometer and it was maintained from 67.03ºB-67.31ºB in the experimental sample. Alternatively sheet/drop test was performed for more reliable confirmation of the formation of jelly. According to Kuchi et al.[3], this method is more reliable and easy, if a refractometer is not available on hand. In this process, a little amount of jelly is taken in a tablespoon (wooden ladle may be preferable) and then allowed to drop off on transparent water glass. When the jelly drops scatteredly to the bottom of the filled glass, then boiling should be continued. On the other hand, when the jelly falls in the form of a flake or sheet, it indicates that the endpoint has been reached, thus confirming the formation of jelly. However, the jelly was formulated according to the following treatments.

Treatments

T_1 = 100% guava fruit extract juice + 75% sugar + 0.70% citric acid + 0.1% potassium metabisulfite (KMS) (Traditional)

T_2 = 100% guava fruit extract juice + 75% sugar + 10% fresh lemon juice + 0.5% honey

T_3 = 75% guava fruit extract juice + 25% pineapple extract juice + 75% sugar + 10% fresh lemon juice + 0.50% honey

T_4 = Control sample

2.5 Sensory evaluation

The sensory evaluation was done for standardization of treatments following the procedure of Joshi[22] based on 9-point hedonic scale. A judgment panel was formed comprising thirty expert members from the Bangladesh Agricultural Research Institute (BARI) inter-divisional Scientists to evaluate color, flavor, texture, mouthfeel, spreadable capacity, and overall acceptability of the formulated jelly. The score obtained by the panelists was analyzed by statistical analysis.

2.6 Color measurement

The color of experimental guava-pineapple natural jelly and collected market jelly was assessed according to the method described by Dervisi et al.[23] with little modification using a Chroma Meter (Model CR-400, Minolta Corp Japan). International Commission on Illumination (CIE) lightness (L*), Chroma (C*), and hue angle (H*) values were documented using D65 illuminates and a 10E standard viewer as an orientation method. The equipment was calibrated on a standard white tile. Then, it was adjusted to measure the values of L*, C*, and H* and was replicated three times for each treatment.

2.7 Texture Analysis

Texture of the jelly was measured by a texture analyzer (Stable Micro System, Godalming, UK). The analyzer probe (p-5) was directly inserted in the bottle of jelly by the back extrusion method. The instrument working parameters were determined by the test mode compression with test speed at 1mm/s, and a distance of 2.50 cm. The analysis of the data was measured by Texture Exponent Lite version 6.1.14.0 software (Stable Micro System, Godalming, United Kingdom) to determine the rupture force and expressed in the unit N.

2.8 Physicochemical and nutritional analysis

The physicochemical properties of the jelly concerning moisture, protein, ash, vitamin-C, total, and reducing sugar content were determined according to the procedure described by Ranganna [24]. ß-carotene was analyzed according to the method described by Molla et al. [25] and the value was expressed in the unit µg/100g of the jelly. pH data was recorded by a digital pH meter (Delta 320, Mettler, Shanghai). Total acidity (%) was measured using Auto Titrator (Metrohm 814, USB Sample Processor, Switzerland). Total soluble solid (°Brix) was recorded using a digital hand refractometer (Model NR151). The water activity of the sample was recorded using Lab Touch-aw (Novasina, AG, CH-8853, Switzerland).

2.9 Minerals Analysis

The minerals analyzed in this study were: sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P), sulfur (S), boron (B), copper (Cu), manganese (Mn), iron (Fe), and zinc (Zn). Atomic absorption spectrophotometry (Model-AA-7000S, Shimadzu, Tokyo, Japan) was used to assess Na, Fe, Cu, Zn, B, Mn, Ca, and Mg. K was measured using flame photometry, while P and S were assessed with the spectrophotometric method. Individual minerals were quantified by comparing the corresponding protocol procured from the Sigma Chemical Co., USA.

2.10 Determination of bioactive compounds

2.10.1Total phenolic content

The total phenolic content of the jelly was determined according to the Folin-Ciocalteu method [26] with gallic acid (GAE) as the standard and expressed in the unit mg as gallic acid equivalents (GAE)/g of the jelly.

2.10.2 Determination of total flavonoid content

The total flavonoid content (TFC) of the jelly was measured by the aluminum chloride method [27]. The absorbance was recorded at 415nm using a UV-vis spectrophotometer and expressed as milligram quercetin equivalent per gram of jelly (mg QE/g of jelly).

2.10.3 Determination of total carotenoid content

The determination of total carotenoid content was performed according to the method described by Thaipong et al. [28]. The results were expressed as beta-carotene equivalent in milligram per 100g of jelly (mg/100g).

2.10.4 Determination of ß-carotene content

β-carotene content of the jelly was measured accordinf to the method described by Holden et al. [29] and the value was noted as µg/100g of jelly.

2.10.5 Determination of total anthocyanin

Total anthocyanin of the jelly was measured according to the the method described by Burgos et al. [30]. The results were expressed in mg/100g of jelly.

2.11 Determination of antioxidant activity

2.11.1 Total antioxidant activity

The total antioxidant activity was evaluated by the phosphomolybdenum system based on the technique described by Prieto et al.[31]. The result was expressed in the unit microgram ascorbic acid (AA) per gram (µg AA/g) of the jelly.

2.11.2 Reducing power assay

The reducing power of the jelly was calculated using the approach of Guo et al. [32] and the result was expressed in the unit microgram ascorbic acid per gram (µg AA/g) of the jelly (µg AA/g).

2.11.3 Ferric reducing antioxidant power (FRAP)

FRAP activity was measured following the scheme outlined by Benzie & Strain [33] and the value was expressed as µM Fe (II) per 100g of the jelly.

2.11.4 DPPH radical scavenging activity (DPPH-RSA) and IC_{50}

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical quenching property was measured using procedure described by Brand-Williams et al.[34]. The result was expressed in percent radical scavenging activity.

2.11.5 Metals chelating capacity

The metal chelating capacity (MCC) was measured according to the method of Bahadori et al. [35] and the result was expressed in percent metal chelating capacity (%).

2.12 Assessment of phenolic acids by HPLC

Phenolic compounds were considered based on the method described by Pandey & Negi [36] using high-performance liquid chromatography (Shimadzu SPD-M10A) coupled with a photodiode array detector and autosampler at 280 and 320nm. Six phenolic standards (gallic acid, vanillic acid, caffeic acid, ferulic acid, + catchin acid, and syringic acid) were used for the identification of respective phenolics, and quantification was accomplished using a standard curve prepared by injecting the mixture of all the standards (0.1-0.7mg/ 100g).

2.13 Storage studies

The prepared jelly was poured into a pre-sterilized auto lug cap glass bottle and stored at room temperature up to 8 months. Data were recorded at 0,3,6 and 8 months for the analysis of physicochemical, nutritional, color and texture changes, minerals, bioactive compounds, antioxidant and antibacterial activities, phenolic acids and sensory evaluation.

2.14 Statistical analysis

All data was expressed in triplicate as means ± standard deviation. One-way analysis of variance (ANOVA) with post-hoc using Tukey's Multiple Comparison Test was performed to analyze the data. The connotation was distinct at the 95% confidence level. SPSS 17.0 (IBM INC., New York) software was used for statistical analysis.

3. Results

3.1 Physicochemical, nutritional, bioactive compounds and antioxidant properties of the fresh guava and pineapple

The physicochemical, nutritional, bioactive compounds and antioxidant properties of the fresh experimental guava and pineapple are shown in Table 1. The findings revealed that the bioactive compounds and antioxidant activities of the fresh guava and pineapple samples were statistically highly significant whereas the total sugar, reducing and nor-reducing sugar, total soluble solid and moisture content were statistically non-significant (NS).The variation might be due to the nature of different fruit orchard, classification, genus and family, soil type, and texture [37].

Table 1
Physicochemical, nutritional, bioactive compounds and antioxidant properties of fresh guava and pineapple

Parameter	Guava	Pineapple	LSD
Total sugar (%)	8.12 ± 0.62	7.92 ± 0.01	NS
Reducing sugar (%)	3.45 ± 0.31	3.57 ± 0.01	NS
Non-reducing sugar (%)	4.66 ± 0.42	4.33 ± 0.02	NS
Total soluble solid (°B)	9.86 ± 1.87	12.21 ± 0.01	NS
Acidity (%)	0.31 ± 0.01	0.63 ± 0.01	**
pH	4.02 ± 0.02	3.52 ± 0.01	**
Vitamin-C (mg/100 g)	73.43 ± 1.64	39.49 ± 0.01	**
ß-carotene (µg/100g)	58.44 ± 14.04	20.43 ± 0.01	**
Moisture content (%)	80.51 ± 1.11	79.23 ± 0.00	NS
Ash	3.39 ± 0.28	0.88 ± 0.00	**
Total phenolic (mg GAE/100g)	48.87 ± 0.43	52.20 ± 0.90	*
Total flavonoid (mg QE/g)	0.96 ± 0.01	55.21 ± 2.10	**
Total antioxidant capacity (µg AA/g)	315.22 ± 2.52	179.23 ± 4.92	**
Ferric reducing antioxidant power (µM Fe_2SO_4/100g)	52.56 ± 1.07	30.70 ± 0.59	**
DPPH radical scavenging activity (%)	42.98 ± 0.57	83.90 ± 2.46	**
IC_{50}	18.42 ± 0.11	16.37 ± 0.46	**

All values are means of triplicate determinations ± SD. * and ** indicate significant results at p 0.05 and p 0.01level. NS denotes non-significant difference.

3.2 Physicochemical and nutritional properties of the prepared jelly on the day of storage

Moisture, total sugar, reducing sugar, non-reducing sugar, TSS, acidity, pH, water activity, vitamin-C, ß-carotene, crude protein, crude fat and energy of the different treated jelly were analyzed on the day of storage and are shown in Table 2. The lowest moisture content was recorded for the sample T_1 (21.95%), T_2 (21.75%) and T_3 (21.85%), while the highest moisture content was recorded in the sample T_4 (31.62%). The analysis of variance for ash contents show that the differences were highly significant (Table 2). Lowest ash content was found in the sample T_4 while the highest was in the sample T_1, T_2, and T_3. In the case of sugar content, samples T_1, T_2 and T_3 were found to have lower total sugar, reducing and non-reducing sugar whereas the sample T_4 was found to be higher as 63.44%, 23.11% and 40.34% respectively (Table 2). TSS of the jelly ranged from 67.10 ± 0.10 to 68.70 ± 0.02 °B. The lower TSS (°B) content was calculated for T_1 (67.31°B), T_2 (67.20°B) and T_3 (67.10°B), while the highest was found in sample T_4 (68.70°B).

The lowest a_w was recorded for the treated jellies T_1 (0.59), T_2 (0.56) and T_3 (0.58), while the highest a_w (0.76) was recorded for T_4 jelly. The lower value of pH (2.54) was recorded in the T_4 jelly whereas the higher value was recorded in all the treated jellies (3.38–3.48) (Table 2). In this study, acidity varied significantly in the treated jelly T_1, T_2 and T_3 and T_4.

The highest vitamin-C and ß-carotene contents were observed in the treated jelly T_1, T_2 and T_3 than the controlled jelly T_4. The highest vitamin-C content of the treated jelly T_1, T_2 and T_3 was recorded as 15.29 ± 0.02 mg/100 g, 34.13 ± 0.15 mg/100 g and 43.01 ± 0.10 mg/100 g respectively whereas the T_4 jelly was calculated as 12.91 ± 0.15 mg/100 g (Table 2).

The ß-carotene content of the treated jelly T_1, T_2 and T_3 ranged from 6.31 ± 0.01 to 12.78 ± 0.05 µg/100g whereas the T_4 jelly was found 8.40 ± 0.40 µg/100g. The results indicate that treated jelly T_2 and T_3 possessed higher value of ß-carotene content than the T_4 jelly (Table 2). The significant difference of the crude protein was observed among the jelly T_1, T_2,T_3 and T_4. The treated jelly T_2 and T_3 possessed higher value of crude protein as compared to the T_1 and T_4 (Table 2). The crude fat content of the different treated jelly was statistically significant on the day of storage (Table 2). The less amount of crude fat was found in all treated jelly T_1, T_2,T_3 and T_4. The energy content of the treated jelly T_1, T_2 and T_3 ranged from 303.08 ± 0.01 to 374.29 ± 0.02 cal/g while the T_4 jelly possessed 300.53 ± 0.02 cal/g (Table 2). All the treated jelly was significantly differed and the lower energy content was found in the jelly T_1 and T_4 than T_2 and T_3.

However, the above results conclude that the jelly treated with honey and fresh lemon juice (T_2 and T_3) found nutritionally superior followed by the traditionally treated jelly (T_1 and T_4).

Table 2
Physicochemical and nutritional properties of guava-pineapple natural jelly on the day of preparation

Parameter	Treatment				LSD
	T_1	T_2	T_3	T_4	
Total sugar (%)	61.84 ± 0.06b	61.84 ± 0.05b	61.88 ± 0.05b	63.44 ± 0.06a	**
Reducing sugar (%)	21.05 ± 0.03b	21.05 ± 0.04b	21.05 ± 0.04b	23.11 ± 0.05a	**
Non-reducing sugar (%)	40.79 ± 0.03	40.79 ± 0.01	40.83 ± 0.01	40.34 ± 0.01	NS
TSS (°B)	67.31 ± 0.02b	67.20 ± 0.10b	67.10 ± 0.10b	68.70 ± 0.02a	**
Acidity (%)	0.42 ± 0.01d	0.65 ± 0.01c	1.01 ± 0.01a	0.71 ± 0.01b	**
pH	3.48 ± 0.02a	3.39 ± 0.02b	3.38 ± 0.05b	2.54 ± 0.00c	**
Water activity (a_w)	0.59 ± 0.01b	0.56 ± 0.03b	0.58 ± 0.05b	0.76 ± 0.03a	**
Vitamin-C(mg/ 100g)	15.29 ± 0.02c	34.13 ± 0.15b	43.01 ± 0.10a	12.91 ± 0.15d	**
ß-carotene (µg/100g)	6.31 ± 0.01d	9.13 ± 0.02b	12.78 ± 0.05a	8.40 ± 0.40c	**
Moisture (%)	21.95 ± 0.07b	21.75 ± 0.02b	21.85 ± 0.26b	31.62 ± 1.00a	**
Ash (%)	0.28 ± 0.02c	0.30 ± 0.02b	0.33 ± 0.02a	0.02 ± 0.00d	**
Crud protein	1.90 ± 0.02c	2.97 ± 0.02b	3.11 ± 0.01a	1.91 ± 0.02c	**
Total Fat	0.04 ± 0.02b	0.004 ± 0.00b	0.009 ± 0.00a	0.004 ± 0.00b	**
Energy	303.08 ± 0.01c	353.08 ± 0.01b	374.29 ± 0.02a	300.53 ± 0.02c	**
All values are means of triplicate determinations ± SD. ** indicates highly significant result (p 0.05).					

3.3 Standardization of the jelly by sensory evaluation

The guava-pineapple jelly was standardized based on the sensory evaluation consisting of 30 expert panel members following a 9-point hedonic scale and shown in Table 3. The highest overall score gained by the treatment T_2 (8.19 ± 0.38) and T_3 (7.84 ± 0.27) in terms of color, flavor, texture, mouthfeel, and overall acceptability. The highest vitamin-C, β-carotene,

crude protein and energy value was also obtained by the treatment T_2 and T_3 (Table 2). Hence, based on the sensory evaluation, physicochemical and nutritional properties, the treatment T_2 and T_3 were selected as best formulation for guava-pineapple jelly. Finally the formulated jelly T_2 and T_3 were stored at room temperature for their future storage studies.

Table 3
Standardization of the guava-pineapple natural jelly by sensory evaluation

Treatment	Color	Flavor	Texture	Mouthfeel	Spreadability	Overall acceptability
T_1	5.43 ± 0.814^{b}	6.80 ± 1.03^{b}	6.60 ± 0.96^{b}	6.50 ± 0.97^{c}	6.20 ± 1.03^{b}	6.30 ± 0.63^{b}
T_2	8.38 ± 0.32^{a}	8.20 ± 1.03^{a}	8.30 ± 0.95^{a}	8.40 ± 0.52^{a}	7.70 ± 1.63^{a}	8.19 ± 0.38^{a}
T_3	8.00 ± 0.66^{a}	7.70 ± 0.48^{ab}	8.10 ± 0.56^{a}	7.50 ± 0.53^{b}	7.90 ± 0.56^{a}	7.84 ± 0.27^{a}
T_4	$5.10 \pm 0.0.99^{b}$	5.20 ± 1.03^{c}	4.90 ± 1.19^{c}	5.30 ± 0.82^{d}	8.10 ± 0.96^{a}	5.72 ± 0.66^{c}

All values are means of triplicate determinations ± SD. Means within columns with different letters a, b, c indicate significant result (p 0.05).

3.4 Storage studies of the standardized jelly

After standardization, the jelly was stored upto 8 months for its physicochemical, nutritional, color, texture, minerals, bioactive compounds, antioxidant properties, phenolic acids and microbial count studies during 3, 6 and 8 months.

3.5 Physicochemical and nutritional properties of the standardized jelly after storage

Table 4 shows the physicochemical and nutritional properties of the standardized jelly during 3,6 and 8 months of storage. The study indicates that total sugar, reducing, and non-reducing sugar of the stored jelly were non-significantly changed with the progression of storage periods (Table 4). The highest sugar (total, reducing, and non-reducing sugar) content was recorded in treated sample T_3, while the lowest sugar content was recorded in T_2 sample (Table 4).

TSS of the fresh guava fruit was recorded as 9.86°B, while it was recorded 67.30°B to 67.57°B in the standardized jelly (Table 4). The TSS content of the treated jelly T_2 and T_3 significantly increased during the storage periods (3 to 8 months) (Table 4). The acidity of the fresh guava and pineapple was recorded as 0.31% and 0.63% respectively (Table 1), while it was 0.42–1.01% on the day of preparation of the jelly (Table 2). After storage, the acidity ranged from 0.53 ± 0.04% to 0.57 ± 0.04% and 0.77 ± 0.02 to 0.82 ± 0.02% for the stored jelly T_2 and T_3. Results revealed that the acidity significantly varied with the advancement of storage periods. The pH of the fresh guava was 4.02 (Table 1), while it was recorded as 3.38–3.48 on the day of preparation (Table 2). After storage, the highest acidity was observed in the treated jelly T_3 as compared to T_2 (Table 4). However, the pH of both standardized stored jelly decreased with increasing storage periods (Table 4). It was noted that there was an inverse relationship of pH between the two standardized jelly (T_2 and T_3) (Table 2 and Table 4).

Vitamin-C content of the fresh guava was found 73.43 mg/100g (Table 1), while it was recorded as 34.13 and 43.00 mg/100g for the treated jelly T_2 and T_3, on the day of storage (Table 2). Higher vitamin-C content was recorded in T_3 as compared to T_2. It might be due to the use of a mixture of guava and pineapple juice during the preparation of the jelly. However, the vitamin-C content was significantly decreased during the entire storage periods (3 to 8 months).

Moisture content range of the treated jelly T_1, T_2 and T_3 was recorded as 21.75–21.95% on the day of storage (Table 2) but after 8 months of storage, the moisture content decreased from 21.64–20.03% and 21.78–20.26% for the standardized

jelly T_2 and T_3 (Table 4). The results indicate that the moisture content of the standardized jelly was significantly decreased over the storage periods (3 to 8 months). Ash content of the treated jelly was increased with the advancement of storage periods.

The growth of microorganisms depends on water activity (a_w). The cells of the microorganisms become dormant in the presence of low a_w and osmotic stress conditions. It has been documented that the microorganisms could not be eliminated but the activities of the microorganisms can be stopped by limiting a_w. On the day of preparation, the lowest a_w was calculated as 0.56 and 0.58 (Table 2) but it was increased over the storage periods (3 to 8 months) from 0.57–0.60 and 0.60–0.63 for T_2 and T_3 jelly (Table 4). The higher a_w activates boost microorganisms like bacteria, yeast, and mold to grow in food. The a_w for the jelly ranged from 0.57–0.63 indicating that microorganisms were generally inhibited to grow within this range, suggesting that the standardized jelly T_2 and T_3 can be considered safe in terms of microbial stability and quality, and are shelf-stable up to 8 months. Herein, the study also confirmed that there was a highly significant relationship between the moisture content and a_w (Fig. 2), i.e., the presence of low moisture in jelly may contribute to achieve lower a_w (0.56).

The crude protein content was significantly changed with the advancement of storage periods and it ranged from 3.10 to 3.21% respectively (Table 4). The standardized jelly T_3 possessed the higher amount of crude protein as compared to T_2 jelly over the storage periods (3–8 months).The hiher protein content in the standerdized jelly T_3 followed by the T_2 jelly. The crude fat content of the standerdized jelly T_2 and T_3 insignificantly changed during the storage periods (3–8 months) (Table 4). It is noteworthy that the less amount of crude fat was found in both standerdized jelly over the storage periods (3–8 months). The energy content of the standerdized jelly T_2 and T_3 ranged from 374.40 ± 0.01 to 374.74 ± 0.05 cal/g and 406.35 ± 0.12 to 406.94 ± 0.01 cal/g over the storage periods (3–8 months).The results indicate that the higher energy content was found in the standardized jelly T_3 and followed by the T_2 jelly..

Table 4
Physicochemical and nutritional properties of the guava-pineapple standardized jelly during storage periods

Parameter	T_2			T_3			LSD
	3 month	6 month	8 month	3 month	6 month	8 month	
Total sugar (%)	61.87± 0.06	61.92± 0.04	61.95± 0.04	61.91± 0.03	61.93± 0.03	61.94± 0.02	NS
Reducing sugar (%)	13.02± 0.00	13.64± 0.03	13.43± 0.02	13.36± 0.01	13.45± 0.02	13.48± 0.01	NS
Non-reducing sugar (%)	48.85± 0.04	48.28± 0.01	48.52± 0.02	48.55± 0.02	48.48± 0.01	48.46± 0.01	NS
TSS (°B)	67.30± 0.03	67.44± 0.03	67.45± 0.04	67.43± 0.02	67.56± 0.03	67.57± 0.04	**
Acidity (%)	0.53± 0.04	0.59± 0.04	0.57± 0.04	0.77± 0.02	0.82± 0.03	0.81± 0.00	**
pH	3.18± 0.00	3.19± 0.02	3.10± 0.00	3.35± 0.01	3.30± 0.00	3.31± 0.01	**
Vitamin-C (mg/ 100g)	33.87± 0.04	33.12± 0.01	32.12± 0.01	42.94± 0.03	42.05± 0.04	41.00± 0.00	**
Moisture (%)	21.64± 0.35	20.56± 0.35	20.03± 0.15	21.78± 0.32	20.53± 0.47	20.26± 0.02	**
Ash (%)	0.04± 0.00	0.04± 0.01	0.06± 0.00	0.04± 0.00	0.04± 0.00	0.06± 0.00	NS
Water activity (a_w)	0.57± 0.00	0.59± 0.01	0.60± 0.01	0.60± 0.00	0.63± 0.00	0.63± 0.00	NS
Crud protein (%)	3.10± 0.00	3.11± 0.00	3.19± 0.00	3.12± 0.01	3.17± 0.02	3.21± 0.00	*
Total Fat (%)	0.01± 0.00	0.02± 0.00	0.02± 0.00	0.01± 0.00	0.02± 0.00	0.03± 0.00	NS
Energy (cal/g)	374.40± 0.01	374.49± 0.00	374.74± 0.05	406.35± 0.12	406.45± 0.12	406.94± 0.01	**

All values are means of triplicate determinations ± SD. * and ** indicate significant results at p 0.05 and p 0.01levels. NS means non-significant difference.

3.6 Color of the standardized natural jelly during different storage periods

Appearance is the most common phenomenon used to measure the quality of any product whereas color and surface conditions performance a fundamental role in the appearance of the product. L* is an approximate quantity of lightness that can be well-thought-out as compared to the member of the greyscale, between black and white [38]. Chroma (C*) is a measurable characteristic of colorfulness used to measure the variance of a hue in contrast to a grey color by a similar lightness. The result obtained from the storage studies showed that the values of L* and C* were decreased with the advancement of storage periods and statistically the values were insignificant (Table 5). The lower L* and C* values indicate that the color lightness and intensity of the jelly T_2 and T_3 were lowered gradually up to 8 months of storage. H* value of the T_2 and T_3 jelly was significantly increased up to 6 months of storage. But after 8 months, the value (H*) was insignificantly increased as 120.90 ± 5.59 (T_2) and 119.11 ± 5.89 (T_3) respectively, indicating that the jelly is within 180° and 270° region and started to lose its initial color. The stored jelly (T_2 and T_3) finally faded out and turned into dark after 8 months of storage.

Table 5
Color of the guava-pineapple standardized jelly during different storage periods

Storage period	Parameter	T_2	T_3	LSD
0 day	L*	34.03 ± 7.91	41.90 ± 0.60	NS
	C*	5.33 ± 0.45	5.25 ± 0.64	NS
	H*	70.96 ± 8.53	87.36 ± 1.68	*
3 month	L*	33.03 ± 7.91	41.15 ± 0.73	NS
	C*	5.24 ± 0.46	5.19 ± 0.68	NS
	H*	72.56 ± 7.04	88.00 ± 1.27	*
6 month	L*	32.01 ± 7.82	39.69 ± 0.43	NS
	C*	4.81 ± 0.55	4.96 ± 0.85	NS
	H*	77.56 ± 7.19	89.59 ± 0.30	*
8 month	L*	31.20 ± 7.46	38.57 ± 0.64	NS
	C*	4.72 ± 0.58	4.93 ± 0.86	NS
	H*	120.90 ± 5.59	119.11 ± 5.89	NS

All values are means of triplicate determinations ± SD. * indicates significant result at p 0.05; NS means non-significant result.

3.7 Texture of the standardized natural jelly after 8 months of storage

The rupture forces (FR) of the standardized natural jelly are illustrated in Fig. 3. The initial FR of the jelly T_2 and T_3 were 0.14 N and 0.101 N, respectively which increased to 0.617 N and 0.606 N, respectively. The increase in FR value of the standardized jelly (T_2 and T_3) partially contributed to the jelly hardness; hence, the jelly samples were slightly disliked by the panel of judges after 8 months of storage. In comparison between two standardized sample (T_2 and T_3), T_2 was relatively harder than T_3.

3.8 Minerals of the standardized natural jelly during different storage

Minerals are the inorganic components present in food and turn into ash when food is consumed to produce energy. Generally, two forms of minerals are present in foodstuff, macro and micro minerals, both playing important metabolic roles in the functions of our body [39] and contribute to our daily dietary requirements. In this study, eleven (11) minerals of

the standardized jelly were assessed as shown in Table 6. Results revealed that Ca, Mg, Na, and K were highly present in T_3, while S, Fe, Mn, Zn, B, Cu, and P were impressively present in T_2. It is noteworthy that all the minerals decreased with the progression of storage periods and statistically non-significant differences (except B) were observed during the storage period (0–8 months).

Table 6
Mineral contents of the standardized natural jelly during different storage periods

Minerals	T_2				T_3				LSD
	0 day	3 month	6 month	8 month	0 day	3 month	6 month	8 month	
Ca	0.11± .07	0.10± 0.07	0.09± 0.00	0.08± 0.03	0.12± 0.07	0.11± 0.07	0.11± 0.01	0.10± 0.06	NS
Mg	0.14± 0.02	0.13± 0.02	0.12± 0.00	0.10± 0.00	0.15± 0.02	0.13± 0.01	0.11± 0.01	0.11± 0.00	NS
S	0.24± 0.14	0.19± 0.10	0.16± 0.08	0.13± 0.06	0.23± 0.14e	0.22± 0.14	0.18± 0.11	0.15± 0.09	NS
Fe	12.34± 0.51	12.24± 0.51	12.13± 0.52	11.78± 0.37	12.30± 0.50	11.96± 0.55	11.76± 0.55	11.57± 0.54	NS
Mn	0.69± 0.21	0.68± 0.23	0.61± 0.19	0.55± 0.15	0.68± 0.24	0.65± .24	0.60± 0.20	0.50± 0.20	NS
Zn	3.78± 0.55	3.64± 0.50	3.62± 0.28	3.61± 0.50	3.76± 0.55	3.71± 0.52	3.69± 0.52	3.60± 0.50	NS
Na	0.07± 0.00	0.07± 0.00	0.06± 0.00	0.05± 0.00	0.42± 0.37	0.32± 0.28	0.29± 0.26	0.29± 0.26	NS
K	0.40± 0.20	0.32± 0.16	0.35± 0.15	0.24± 0.12	0.41± 0.21	0.33± 0.18	0.31± 0.18	0.25± 0.15	NS
P	0.47± 0.14	0.40± 0.10	0.35± 0.10	0.30± 0.10	0.45± 0.15	0.40± 0.15	0.35± 0.15	0.22± 0.07	NS
B	0.36± 0.00	0.32± 0.00	0.31± 0.00	0.21± 0.00	0.32± 0.00	0.30± 0.00	0.26± 0.01	0.20± 0.00	*
Cu	0.02± 0.00	0.02± 0.00	0.02± 0.00	0.015± 0.00	0.02± 0.00	0.02± 0.00	0.01± 0.00	0.01± 0.00	NS

All values are means of triplicate determinations ± SD. Ca, Mg, K, Na, P and S expressed as mg %; B, Cu, Fe, Mn and Zn expressed as ppm. * indicates significant result at p 0.05; NS letter means non-significant result.

3.9 Bioactive compounds and antioxidant activity of the standardized jelly during storage

The bioactive compounds e.g., total phenolic content, ascorbic acid, flavonoid, carotenoid, ß-carotene, and anthocyanin content present in guava-pineapple natural jelly are listed in Table 7, showing the values on the day of storage and after 8 months of storage at ambient condition. The highest total phenolic content, flavonoid, carotenoid, ß-carotene, and anthocyanin content in T_3 jelly were observed in the range of 4.15–4.01 mg GAE/100g, 0.91 – 0.84 mg QE/g, 0.94 – 0.84 mg/100g, 12.44–12.20 mg/100g, and 4.06–4.01 mg/100g, respectively. The lowest total phenolic content, flavonoid, carotenoid, ß-carotene, and anthocyanin content in T_2 jelly were recorded in the assortment of 4.07-4.00 mg GAE/100g, 0.79 – 0.70 mg QE/g, 0.90 – 0.79 mg/100g, 9.43–9.01 mg/100g, and 4.03–3.59 mg/100g, respectively. The highest total phenolic, flavonoid, carotenoid, ß-carotene, and anthocyanin content were recorded in standardized jelly T_3.

In this investigation, the antioxidant properties of the standardized jelly were analyzed and shown in Table 8. It can be seen that the standardized storage jelly exhibited potent antioxidant properties. Total antioxidant capacity values of the T_2 and T_3 sample have been found to be 105.84–104.40 µg AA/g and 109.11-107.88 µg AA/g respectively. Results indicate that total antioxidant capacity were statistically significant and decreased with the advancement of storage periods. On the day of storage and after 8 months of storage, the maximum total antioxidant activity and DPPH was recorded in T_3 sample followed by T_2. The reducing power assay (RPA) of T_3 and T_2 showed values of 7.23–7.06 and 6.17-6.00 µgAA/mg, respectively during the storage periods, indicating that T_3 and T_2 are capable of reducing different metallic ions by making a stable chemical bond to scavenge free radicals. The FRAP assay is frequently used to quantify the antioxidant potential of foodstuff and determine the capacity to convert ferric (Fe^{3+}) into ferrous iron (Fe^{2+}) in FRAP reagent (Garzon et al., 2010). As regards to the capacity of T_3 and T_2 to reduce Fe^{3+}-Fe^{2+}, the values were found as 29.51–28.55 and 27.99–26.40 µM Fe_2SO_4/100g, respectively.

Table 7
Bioactive compounds of the standardized jelly during different storage periods

Parameter	T_2				T_3				LSD
	0 day	3 month	6 month	8 month	0 day	3 month	6 month	8 month	
Total phenolic (mg GAE/100g)	4.07± 0.35	4.05± 0.35	4.01± 0.35	4.00± 0.35	4.15± 0.26	4.11± 0.25	4.09± 0.25	4.01± 0.20	**
Total flavonoid (mg QE/g)	0.79± 0.01	0.77± 0.03	0.74± 0.03	0.70± 0.01	0.91± 0.02	0.89± 0.02	0.86± 0.01	0.84± 0.01	**
Total carotenoid (mg/100g)	0.90± 0.03	0.86± 0.03	0.82± 0.02	0.79± 0.02	0.94± 0.03	0.91± 0.02	0.86± 0.03	0.81± 0.04	NS
Total ß-carotene (µg/100g)	9.43± 0.08	9.40± 0.10	9.25± 0.05	9.01± 0.00	12.44± 0.33	12.40± 0.30	12.26± 0.25	12.20± 0.20	**
Anthocyanin (mg/100g)	4.03± 0.06	4.01± 0.05	3.95± 0.06	3.59± 0.40	4.06± 0.19	4.05± 0.16	4.04± 0.15	4.01± 0.10	**

All values are means of triplicate determinations ± SD. * and ** indicate significant results at p 0.05 and p 0.01; NS letter means a non-significant result.

Table 8
Antioxidant activity of the standardized jelly during different storage periods

Parameter	T_2				T_3				LSD
	0 day	3 month	6 month	8 month	0 day	3 month	6 month	8 month	
Total antioxidant capacity (µg AA/g)	105.84± 0.33	105.76± 0.35	105.60± 0.36	104.40± 0.51	109.11± 0.22	109.06± 0.25	108.88± 0.32	107.88± 0.32	**
DPPH radical scavenging activity (%)	40.02± 0.68	39.35± 1.2	38.13± 1.19	36.58± 0.79	41.44± 0.05	41.35± 0.05	41.27± 0.06	40.08± 0.07	*
Ferric reducing antioxidant power (μMFe_2SO_4/100g)	27.99± 0.10	27.92± 0.14	27.65± 0.35	26.40± 0.52	29.51± 0.40	29.45± 0.35	29.24± 0.15	28.55± 0.54	**
Reducing power assay (µg AA/g)	6.17± 0.06	6.12± 0.06	6.07± 0.06	6.00± 0.09	7.23± 0.08	7.16± 0.05	7.11± 0.02	7.06± 0.05	**
IC_{50} (µg/g)	15.53± 0.04	15.46± 0.05	15.27± 0.05	14.19± 0.00	17.98± 0.20	17.91± 0.20	17.70± 0.39	16.65± 0.45	**

All values are means of triplicate determinations ± SD. * and ** indicate significant results at p 0.05 and p 0.01; NS letter means a non-significant result.

3.10 Phenolic acids of the standerdized natural jelly

Six key phenolic acids were observed and displayed in Table 9. All the phenolic acids were significantly differed (except syringic acids) on the day of storage and after 8 months of storage. Findings depict that the standardized natural jelly T_2 and T_3 had abundant phenolic acids. The higher gallic acid, vanilic acid, caffeic acid, ferulic acid, (+) catchin acid and syringic acid have been found in T_3 sample followed by T_2 sample. Among the well-known phenolic acids, the (+) catchin acid was the leading phenolic compounds in both T_2 and T_3 sample followed by other acids.

Table 9
Phenolic acids of the guava-pineapple standardized jelly during different storage periods

Phenolic acids (mg/100 g)	T_2				T_3				LSD
	0 day	3 months	6 months	8 months	0 day	3 months	6 months	8 months	
Gallic acid	5.16± 0.05	5.06± 0.06	5.04± 0.05	4.95± 0.12	5.41± 0.05	5.40± 0.11	5.35± 0.08	5.30± 0.10	*
Vanilic acid	4.16± 0.05	4.20± 0.10	4.17± 0.09	4.06± 0.04	4.66± 0.05	4.65± 0.24	4.61± 0.24	4.54± 0.25	*
Caffeic acid	8.54± 0.17	8.49± 0.18	8.43± 0.17	8.33± 0.13	9.07± 0.04	9.03± 0.01	9.01± 0.01	8.93± 0.06	**
Ferulic acid	0.25± 0.05	0.23± 0.05	0.20± 0.04	0.16± 0.03	0.46± 0.05	0.44± 0.05	0.41± 0.05	0.35± 0.05	**
(+) Catchin acid	75.62± 2.53	75.62± 2.52	75.59± 2.51	75.33± 2.51	81.70± 0.57	81.61± 0.52	81.58± 0.51	81.43± 0.51	*
Syringic acid	3.09± 0.09	3.08± 0.08	3.05± 0.08	2.94± 0.10	3.23± 0.09	3.20± 0.09	3.17± 0.09	3.10± 0.10	NS

All values are means of triplicate determinations ± SD. * and ** indicate significant result at p 0.05 and p 0.01; NS means non-significant result.

3.11 Sensory evaluation of the jelly after 8 months of storage

Table 10 represents the evaluation of the sensory attributes of the standardized jelly after 8 months of storage. Results revealed that maximum score were secured by the T_2 sample for its flavor, texture, mouthfeel, color, and overall acceptability, although insignificant variations were observed between T_2 and T_3. The results are similar to the previous sensory results (Table 3). Although T_3 was observed to have better attributes considering its nutritional, physicochemical, minerals, bioactive compounds, and antioxidant activity values, T_2 achieved the highest sensory score by the panelists. Most of the panel judges declared that the color of the stored jelly turned into light-dark color after 8 months of storage.

Table 10
Sensory evaluation of the standardized natural jelly after 8 months of storage

Treatment	Color	Flavor	Texture	Mouthfeel	Spreadability	Overall acceptability
T_2	7.00 ± 0.00	6.70 ± 0.67	6.60 ± 0.69	7.00 ± 0.66	6.50 ± 1.17	6.74 ± 0.49
T_3	6.80 ± 0.78	6.90 ± 0.73	6.20 ± 0.42	6.90 ± 0.87	6.40 ± 0.84	6.66 ± 0.28
LSD	NS	NS	NS	NS	NS	NS
All values are means of triplicate determinations ± SD. NS means no significant difference.						

3.12 Microbial count of the jelly

Table 11 shows the microbial count of the jelly after 8 months of storage. No microorganism was observed initially due to the higher dilution used for the enumeration. After 6 months of storage, no Aspergillus, Shigella and E-coli was detected in the stored jelly. But after 8 months of storage, few Aspergillus, Shigella and E-coli was detected but they were within the range of acceptable limit (Table 11).

Table 11
Microbial count of the jelly after 8 months of storage

Treatments	Microbial counts			
	0 Day	3 Months	6 Months	8 Months
Aspergillus (cfu/g)				
T_2	ND	ND	ND	$10x\ 10^9$
T_3	ND	ND	ND	$13\ x\ 10^9$
Shigella (cfu/g)				
T_2	ND	ND	ND	$11\ x\ 10^9$
T_3	ND	ND	ND	$12\ x\ 10^9$
E-coli (cfu/g)				
T_1	ND	ND	ND	$4.1\ x\ 10^{10}$
T_2	ND	ND	ND	$4.1\ x\ 10^7$
ND = Not Detected				

3.13 Processing effect and the mechanism to produce natural jelly

Free radicals are hazardous for human health and are produced by oxidation during processing, packaging, and storage of processed food products. Considering that processed food should be produced in a safe and healthy way that is beneficial for humans, free radicals must be prevented. The addition of antioxidants in processed food may be a useful way to prevent the production of dangerous free radical compounds. In this study, there was added total phenolic (201.78 mg GAE/100g) and flavonoid (6.04 mg QE/g) enriched honey bearing antioxidant potential. Honey, used in the study, was obtained from the natural sources having antioxidant and antimicrobial activities, while it was used instead of synthetic chemicals and additives (Sodium benzoate, KMS, Acetic acid, *etc.*). Fresh lemon juice was used instead of synthetic citric acid (100 mL of fresh lemon juice contains 5-7g of citric acid). Thus, a combination of natural honey and fresh lemon juice contributed to impart the jelly with improved attractive color, flavor, and internal metabolism. Moreover, the natural ingredients honey and lemon juice may also act as energy and immunity system builder, and health beneficial for human to combat various illnesses including headache, allergy, asthma, and dermatitis, and even cancer [40].

4. Discussion

The shelf life of the fresh and processed products be contingent on the moisture content. Higher the moisture content enhances the water activity of the products. Maximum moisture content was recorded at on the day of preparation for all the treated jelly. In this study, the moisture content was slightly decreased over the storage periods (0–8 months). Several

similar findings have also been described by the Mehta & Bajaj [41]; Tripathi et al. [42] for candy preparation, those reported that the moisture content may decreased during the storage periods. The slight decrease in moisture content could be due to moisture loss by the process of evaporation, thus increasing the total solids of the jelly.

Herein, the study also confirmed that there was a highly significant relationship between the moisture content and a_w (Fig. 2), i.e., the presence of low moisture in jelly may contribute to achieve lower a_w (0.56). The a_w was found in lower throughout the storage periods where it ranged from 0.60 – 0.56 in the standerdized natural jelly. The results indicating that the formulated jelly was within the range of a_w, thus it generally inhibited to grow microorganisms, suggesting that the standardized jelly can be considered safe in terms of microbial stability and quality, and are shelf-stable up to 8 months. The decreased pH value after storage [43, 44] and the jelly prepared using bee honey acts as a prebiotic due to contained fructose and oligosaccharides which might be contributed to inhibition the growth of microorganisms [45]. Another reason, the diluted honey in jelly might be generated H_2O_2 by the process of oxidizes glucose to gluconic acid have been found to be more effective [14]. The antibacterial properties of honey was more effective due to its high sugar concentration, low moisture content, along with its acidic values that all the characteristics were present in the stored jelly to inhibit the growth of microbial loads. However, the antimicrobial properties of honey have been proven and fully agreement by the sevral researchers [14–19] those reported that honey acts against pathogenic bacteria, oral bacteria as well as food spoilage bacteria. Moreover, the microbial data obtained after 8 months of storage (Table 10), conclud that the lower a_w and presence of honey in jelly can't abolish the microorganism but can stop the activities of microorganisms like Aspergillus, Shigella, and E-coli within the acceptable range of limit (Table 10).

Ash content of a foodstuff represents inorganic residue remaining after destruction of organic matter [46]. It represents minerals like calcium, phosphorus and iron. When the jelly was stored for storage studies up to 8 months, the ash content was found to be increased significantly over the storage periods. The increase in ash content during storage has also been found in pitanga jam [47], conventional and light blackberry jam [48]. The increases of ash content indicate that the products were stable during the storage periods.

Water activity (a_w) is only of limited use as an indicator for the storage life of foods with low water content. Minor changes in water content lead to major changes in a_w [13]. In this study, the effect of a_w in processing of jelly influenced the food compositions that are shown in Table 2 and Table 4. In Fig. 2, highly correlation was found between the a_w and moisture content where the a_w decreased, the moisture content was also decreased. Foods with a_w values between 0.60 and 0.90 are largely protected against microbial spoilage. In this study the a_w values were found between 0.60 and 0.63 after 8 months of storage, which was favorable to increase the storage life of the naturally treated jelly by the inactivation of Aspergillus, Shijella and E-cloi during the storage periods. However, the results obtained from the study disclose that the decreased a_w between the range 0.60–0.63 retarded the growth of microbial activities (Table 10) through slowing down the enzymatic catalyzed reactions.

Total sugar, reducing and non-reducing sugar significantly increased from fresh sample to processed sample even during entire storage periods. An increase in sugar content was reported by several researchers for the guava jelly, fruit bar, and different fruit candies [37, 49–55]. The increasing of total sugar, reducing and non-reducing sugar content between the fresh and the processed samples might be due to variation of sugar content and the formulation variation among the samples. The increased total sugar content may be different in the samples because of insoluble polysaccharides and other starch converted into soluble sugars completely during the storage periods [3]. Another reason might be the increasing of total soluble solids (TSS) entire the storage periods (0 to 8 months) contributed to increase the sugar content in jelly sample [56].

In this study, the range of the TSS value was recorded from 67.10 ± 0.10 to 68.70 ± 0.02 °B. Nurani et al.[57], reported that TSS of the prepared jam and jelly should be ranged from 50–70°B, indicating that the TSS obtained from this study was

within the range of the jelly. The substantial changes ($P < 0.01$) of TSS during storage might be for the degradation of polysaccharides into soluble compounds [41, 58]. Another reason might be due to additional sugar and other ingredients during the preparation of the jelly.

One important feature of storing jelly is the high acidity which usually prevents the growth of food poisoning bacteria and also helps maintain the color and flavor of most jelly, jamd and marmalade. In this study, the acidity of the fresh guava and pineapple were 0.31 ± 0.01% and 0.63 ± 0.01% but after processing into jelly the acidity was increased. The increasing trend of the acidity was also observed during storage periods from on the day of preparation to 8 months of storage. It has been reported by several researchers that the acidity augmented with the advancement of storage [50]. The increased acidity might be due to the combination of mixed fruit juice (guava and pineapple) used during the preparation of the jelly. The significant increase of the acidity with the advancement of storage periods might be due to the conversion of pectic constituents into soluble solids [2, 50]. There was inverse relationship between the acidity and the pH of the formulated jelly. The variation of pH and acidity might be occured due to the variation of the formulation of the manufacturing process and during processing the pH decrease and total acid content may increase [59]. pH of the treated jelly was gradually decreased with the advancement of storage periods. A decrease in pH thus may promote an inhibitory effect on the growth of microorganisms in the standardized jelly after 8 months of storage (although microbial data is not shown here). A small reductions in pH after 320 days of storage in pitanga jams reported by Tobal and Rodrigues [47] was also similar with the present findings. Reduction in pH during 90 days of storage also have been recorded by the Nachtigall et al. [48] those reported that these reductions might be associated to the processing conditions of jam.

Vitamin-C is present in all animals and plant foods, mostly in free from, and it is probably bound to protein as well. It is fully absorbed and distributed throughout the body with the highest concentration in adrenal and pituitary glands. The daily requirement of the vitamin-C for an adult is 100 mg/day. The intake of the vitamin-C is essential to recover the scurvy disease and lower level in blood plasma but in the opposite the high intake of vitamin-C can increase the oxalic acid level that may interrupt the kidney functions [13]. In this study, it is well reported the Vitamin-C content of fresh guava and pineapple fruits were recorded as 53.43 ± 1.64 mg/100 g and 39.49 ± 0.01 mg/100 g but after processing into jelly it was noted as 43.01 ± 0.00 and 34.13 ± 0.15 mg/100 g in the best combination of T_2 and T_3. The highest vitamin-C content obtained in fresh guava than the pineapple have been reported by the several researchers due to their fruit nature and environmental factors [3, 60, 61]. Apart from, the results indicating that the vitamin-C content dramatically decreased due to processing into jelly and after storage entire the storage priods (3–8 months). The decreased vitamin-C content during the entire storage periods might be due to thermal destructions during heat processing, leaching of vitamin-C into water during heating, and its subsequent oxidation during storage [51]. The loss of vitamin-C activates to reduce immediately after harvest and destroys steadily during storage and other processes. Similar results also has been found by the Singh and Harshal [62] for processing of leafy vegetables where they reported that the loss of Vitamin C in green leafy vegetables might be due to the processing method employed in its preparation and subjected to boiling and microwave heating as well as blanching.

Vitamins are required for the normal growth, maintenance and functioning of human body. Hence, their preservation during processing and storage in jelly is of far reaching importance. Vitamin A not occurs in plant origin foods, it occurs in animal tissue. But in plant originated food it is found as β-carotene. β-carotene is the major dietary precursor of vitamin A. Food processing and storage can lead to 5–40% destruction of β-carotene [13]. In this study, the ß-carotene was drastically lost from fresh to processing into jelly even entire the storage periods (0 to 8 months).The results obtained from this study are strongly supported with the findings of Jane et al. [49] those reported that 4053 % loss of ß-carotene might occur during the process of boiling lettuce and carrot. Hackett et al. [52] reported that the conversion of trans form into cis form could be the reason for the loss of ß-carotene during processing. The loss might be occurred due to absence of oxygen and at higher temperature during cooking, boiling and sterilization of jelly.

The lower L* and C* values for color measurement indicate that the color lightness and intensity of the jelly T_2 and T_3 were lowered gradually up to 8 months of storage. The decrease in L values might be due to the reduction of anthocyanin content (Table 7) and the occurrence of the Maillard reaction during the storage of the jelly. The findings are also supported by Maskan et al. [63], who showed that a* and b* values were improved and L* values were reduced during the processing and preservation of grape juice. The stored jelly (T_2 and T_3) finally faded out and turned into dark color after 8 months of storage. This might be due to an increase in water activity (0.57–0.60 and 0.60–0.63; Table 5), reduction of carotenoid (Table 7), and the development of browning compounds. Similar results were obtained by Rhim and Hong [64] while studying red pepper. They reported that the red color of the pepper paled and tarnished black due to an increase in a_w and temperature.

H* value indicates Hue angel value of the stored jelly. The H* value was statistically significant and increased up to 6 months of storage. However, after 8 months, the value (H*) of the standardized jelly T_2 and T_3 was insignificantly increased as 120.90 ± 5.59 and 119.11 ± 5.89 respectively, indicating that the jelly is within 180° and 270° region and started to lose its initial color. Hue angle represents the overall colour of the sample. All the jelly samples were found to have decreased red color values (as the hue angle increased within 180° and 270° region). The decreased hue angle in this study are also fully agreement with the findings of Tijskenset et al. [65] those reported that the color change could be attributed to the air removal around the surface, the air expulsion between the cells and its replacement with water and cell juice that was released from the deteriorated membranes that occurred during storage. Anotherthing is, the color change could be attributed to enzymatic or non-enzymatic browning (Maillard reactions) [66]. In fact, the presence of a higher amount of reducing sugars after inversion of sucrose during cooking, and/or higher pH, could contribute to these browning reactions.

Pectin is the main factor to determine the the jelly consistency and its content and type have an effect on gel hardness [23, 67]. Thus, in this study, the texture profile of the storage jelly was investigated to evaluate the softness and hardness entire the storage periods as most of the consumer preferences high spreadable jelly. The results shown that T_2 jelly found slight harder than the T_3 jelly, could be due to the use of only guava juice which contains solid pectin, whereas T_3 was diluted with pineapple juice. Thus, the presence of solid pectin in T_2 might have worked as a gelling agent following different mechanisms during the storage periods. The softness of T_3 jelly might be due to internal metabolism and enzymatic and non-enzymatic degradation of pectin [38, 67]. The hardness found by the T_2 sample are consistent with the findings of Raj et al.[68] those reported that papaya jam gained more hardness throughout the storage periods due to gell properties nature and the capability of water retention. The gel strength deceased as well as softness gained by the T_3 sample might be due to dilution to the extracted acidic pineapple fruit juice. These results are fully agreement with the findings of Morris et al.[69] and Korus et al.[70] those reported that the decreased gel strength could be due to the decomposition of pectin compounds by the presence of acids in gooseberry jam.

All the minerals were found to be decreased with increasing of storage periods. The minerals value Na, K, Ca and Mg was found to be highest in the sample T_3 whereas Fe, Mn, Zn, B,Cu, P and S was found to be highest in the T_2 sample. The variation of the T_2 and T_3 jelly could be due to their treatment effect using different concentration extracted juice. Similar variations had also been recorded by Mumtaz et al. [71] on different jams and jellies. The researchers determined Fe, Zn, Na, and K as 0.52–0.910 mg/100g, 0.02–0.09 mg/100g, 44.62-71.45mg/100g and 26.10-50.11 mg/100g, respectively; however, no Mn was detected in the their jelly, while the guava-pineapple natural jelly (T_2 and T_3) contained Mn in the range of 0.68 – 0.50 and 0.69-0.55ppm, respectively. The results indicate that the values (Fe, Zn, Na, and K) obtained by the Mumtaz et al. [71] were higher than the treated sample T_2 and T_3. It has been claimed that these variations could be due to the nature of the product, soil structure, soil fertility, orchard type, orchard geographical conditions, the method of processing and preservation (as their sample was collected from the market), and experimental error. T_3 sample had the highest Na content followed by T_2 sample. The results are similar to the apricot and buberry jam that was reported by

Naeem et al. [72]. The differences in Na content between the samples might be associated with the presence of sodium citrate during jelly preparation. Sodium citrate is the sodium salt for citric acid and functions as an acidity regulator in jelly. The average daily requirement of Na intake for the male 3.30 g and female 2.50 g. From a nutritional stand point, the daily Na intake should be limited to 2.30 g (equivalent to 6 g NaCl). As the Na absorption in the human body is rapid and starts within 3–6 min after intake and is completed within 3 hrs, therefore its too much intake can result in serious disorders [13]. K, Ca and Mg content had significantly higher in the T_3 sample whereas the lower was recorded by the T_2 sample. The results are similar to the findings of Giampieri et al. [73]. The higher K, Ca and Mg content for T_3 sample found in the present study could be attributed to the dilution of guava juice with pineapple juice during preparation of T_3 sample jelly [74]. The intake of K, Ca and Mg in normal diet to be ranged from 2.0-5.9 g/day (minimum 782 mg), 0.80–1.50 g/day and 0.30–0.50 g/day respectively [13]. The highest Fe content of T_2 sample have similar levels with blueberry and strawberry jams as reported by Naeem et al. [72]. The higher content of Zn in this study are similar to the grape and strawberry jams [72]. The higher Cu level of T_2 sample found similar to the apricot jam [72]. Other minerals Mn, B, P and S found to be higher in T_2 sample. The possible destruction of Fe, Cu, Mn, Zn, B, P and S found in the T_3 sample might be caused by processing with different treatments, material separation, dilution and thermal heat treatment during processing of jelly [13]. The daily intake of Fe, Cu, Mn, Zn, B, S and P in normaldiet to be from 1.50–2.20 mg/ day, 1.00-1.50 mg/day, 2.00–5.00 mg/day, 5.00–10.00 mg/day, 1.30–4.30 mg/day, 0.80-1.00 mg/day and 0.80–1.20 mg/day respectively [13]. However, all the minerals were present more or less present in both jelly sample (T_2 and T_3). The identified 11 minerals obtained by this study not have only nutritional and physiological imporatance but also contribute to increase the food flavor and activate or inhibit the enzyme-catalyzed and other reactions in the jelly [13].

Numerous bioactive compounds compounds and antioxidant activities such as total phenolic, total flavonoid, total carotenoid, anthocyanin, antioxidant activity, DPPH free radical scavenging activity, ferric reducing antioxidant power, reducing power assay and IC_{50} have carriedout in the stnderdized jelly on the day of preparation and after storage. Results revealed that all the bioactive compounds decreased with increasing of strage periods. In case of anthocyanin, it pigments are very sensitive to temperature and heat treatment during processing of jelly might be contributed to greatly reduce the content of pigments in the jelly. Storage temperature is the another main factor for retention and destruction of anthocyanin content [67]. As the final jelly was stored at room temperature therefore, it might be contributed to decrease the anthocyanin content. Anthocyanin content depend on the degree of pectin esterification. In this study, a higher anthocyanin content was found in the lower degree of pectin and lower energy value of the sample T_3.

Carotenoid has a crucial part in human nutrition and health, which can lessen the risks of cancer and heart diseases because of the activity of pro-vitamin A [75]. The carotenoids extremely present in the diet as β-carotene and α-carotene are involved in the reduction of the incidence of type 2 diabetes [76]. Here, the results obtained from this study indicates that T_3 sample have been found with higher ß-carotene and total carotenoid content followed by T_2. Almost similar observations were made by the Dars et al.[77], those reported that mango juice contain 578 µg/100 g and 1.95 mg/100 g of total carotenoid and ß-carotene content. The variation of the total carotenoids and β-carotene content observed in T_2 and T_3 sample might be affected by the heat processing and storage temperature. The highest total phenolic, flavonoid, carotenoid, ß-carotene, and anthocyanin content in the sample T_3 probably be due to the use of a mixture of guava and pineapple fruit juice during preparation. On the other hand, the guava and pineapple fruits individually contain different bioactive compounds [78]. Vukoja et al. [79] calculated total phenolic content as 1.69 g GAE/kg (dw) and total anthocyanin content as 98.48 mgcya-3-glu/kg in cherry jam, whereas these contents were higher in the standardized jelly (T_2 and T_3). The presence of higher total phenolic and anthocyanin content in this standardized jelly was probably because of adding natural honey during the preparation. Besides, the experimental honey contained 201.78 mg GAE/100g of total phenolic content and 6.04 mg QE/g of total flavonoid content. Therefore, the findings confirm that the standardized jelly (T_2 and T_3) contain a great variety of bioactive compounds.

The maximum total antioxidant activity significantly present T_3 sample might be due to the abundance of phenolic components highly present in T_3 as compared to T_2. T_2 and T_3 showed a sturdy capability to scavenge free radicals as their total antioxidant capacity values were found to be 105.84–104.40 µg AA/g and 109.11-107.88 µg AA/g respectively. The determination of IC_{50} is a generally well established technique to judge the antioxidant activity of foodstuff and its lower value indicates higher free radical quenching ability [80]. Results revealed that both T_3 and T_2 showed potential antioxidant capacity due to their lesser assessment of IC_{50} (17.98–16.65 and 15.53–14.19 µg/g, respectively). The less amount of IC_{50} present in the T_3 and T_2 sample contributed to gain maximum amount of total antioxidant activity that might be accredited to the existence of significant quantities of phenolic compounds and flavonoids. The presence of FRAP values in the sample T_3 and T_2 could donate an electron to decrease the yellow ferric complex to a blue ferrous complex. The high FRAP values of T_3 and T_2 indicate that phenolic composite is the leading provider of the high antioxidant ability of the standardized natural jelly.

Phenolic compounds are an important bioactive compounds that preserve against dissimilar lethal chemical responses and diseases, and their association in antioxidants rely on their structure [81]. The difference of phenolic acids in T_2 and T_3 depends on the food matrix and chemical structures, extraction techniques used, solvent used, and the solubility of individual phenolic acid [81]. However, the results indicate that the standardized natural jelly (T_2 and T_3) were a rich source of phenolic compounds which were decreased slightly with the advancement of storage periods. The slightly decreased phenolic acids still now remains unknown. But the possible reason might be due to the fluctuation of room temperature during the storage periods. Another reason might be oxidisability of the studied phenolic acids with fluctuation of room temperature. Reblova [82] reported that the acivity of phenolic acids for pork lard decreased with increasing temperature. They also found inverses linear correlation between the relative decrease in phenolic antioxidant activity with increasing temperature and the oxidisability of the studied phenolic acids.

The standardized natural jelly (T_2 and T_3) was the abundant source of bioactive compounds and antioxidant activities due to combined processing effect of natural honey and fresh lemon juice.The best combination of these natural ingredients thus contributed to impart the jelly with improved attractive color, flavor, and texture.

5. Conclusions

The highest overall acceptability score (6.74 ± 0.49) was secured by the natural treated jelly using 100% guava extracted juice with natural honey and fresh lemon juice (T_2). But the jelly formulated with 75% guava and 25% pineapple extracted juice using natural honey and fresh lemon juice (T_3) found superior considering physicochemical, nutritional, minerals, bioactive compounds, phenolic acids, and antioxidant properties studied. Based on sensory evaluation, bioactive compounds and antioxidant properties, both T_2 and T_3 could be deliberated as an effective formulation for processing of natural jelly which could serve as an alternative to chemical and synthetically treated jelly. The shelf life of the jelly (T_2 and T_3) could be extended up to 8 months without any significant quality deterioration. Moreover, these findings will provide a valuable source of natural ingredients i.e. honey and fresh lemon juice for the formulation of natural jelly to make the product color more attractive, impressive natural flavor, enriched bioactive compounds and antibacterial properties. Off flavor of fruit pulp also may be treated using these technology during processing into jelly and jam products. The limitation of the main findings was to determine the glycemic index (GI) of the developed jelly. Therefore, future study could be continued to identify the GI of the developed products and their randomized control trial on human.

Abbreviations

BBS
Bangladesh Bureau of Statistics
MT

Metric Ton
BSCIC
Bangladesh Small and Cottage Industries Corporation
PROSHIKA
Proshikkhan Shikkha Karmo
MUS
Mouchas Unnayan Sangstha
BARI
Bangladesh Agricultural Research Institute
KMS
Potassium metabisulphite
EIRI
Engineers India Research Institute
TSS
Total Soluble Solid
L*
Lightness
C*
Chroma
H*
Hue angle
Na
Sodium
K
Potassium
Ca
Calcium
Mg
Magnesium
S
Sulfur
Cu
Copper, Mn:Manganese
Fe
Iron
Zn
Zinc
ANOVA
Analysis of variance
NS
Non-Significant
ND
Not detected
a_w
Water activity
FRAP
Ferric Reducing Antioxidant Power

GAE
Gallic Acid Equivalent
AA
Ascorbic Acid
MCC
Metal Chelating Capacity
DPPH-RSA
2,2-diphenyl-1-picryl hydeazil-Reducing Scavenging Activity
RPA
Reducing Power Assay
UV-Vis Spectrophotometer
Ultra-Violet Visible Spectrophotometer
HPLC
High Pressure Liquid Chromatography
BGD
Bangladesh
APPT
Agricultural Products Processing Technology
AFACI
Asian Food and Agriculture Cooperation Initiative
RDA
Rural Development Administration.

Declarations

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Authors' contributions MMM collected experimental raw material resources; Conceptualization; Experiment design; Experiment performer; Data record, Data analysis and was a major contributor in writing the manuscript. AAS Conducted field experiment on pre and post-harvest management of guava and pineapple and was a contributor for analysis of minerals. MHHK performed analysis of phenolic acids of the sample, release fund and advisory work. MGFC assisted to collect chemicals and reagent and contributed to analysis total carotenoid of the sample. MM supervised the whole research activities and contributed to interprete the patient data of the storage sample. MA contributed to analysis of sample color and format the manuscript. AK contributed to analyze crude protein, total fat and microbial count of the sample. All authors have read and approved the manuscript.

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Code availability Source code is available upon request from the corresponding author

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Figures

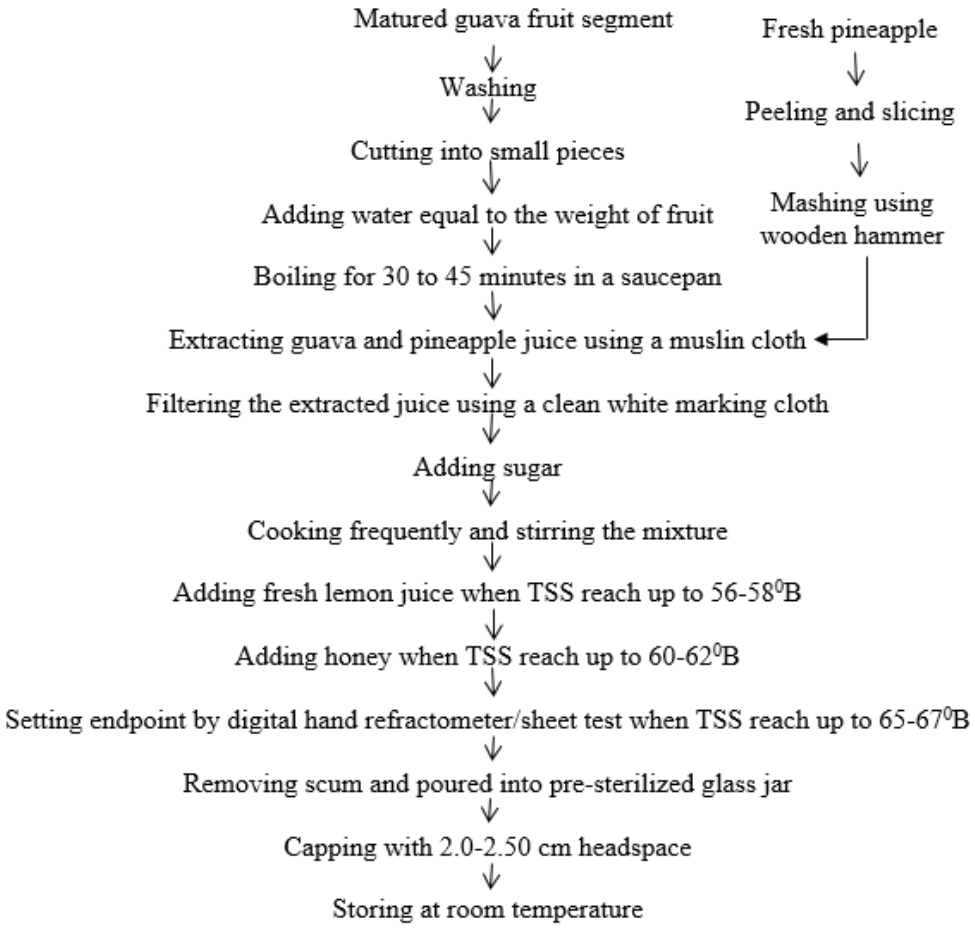

Figure 1

Processing flow chart of guava-pineapple natural jelly.

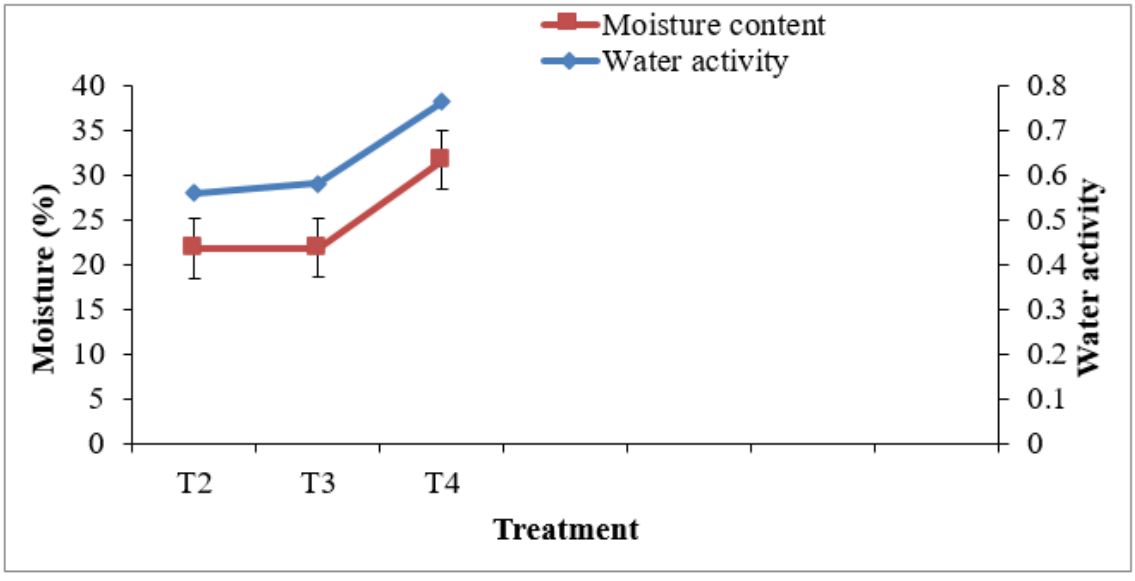

Figure 2

Correlation between water activity and moisture content

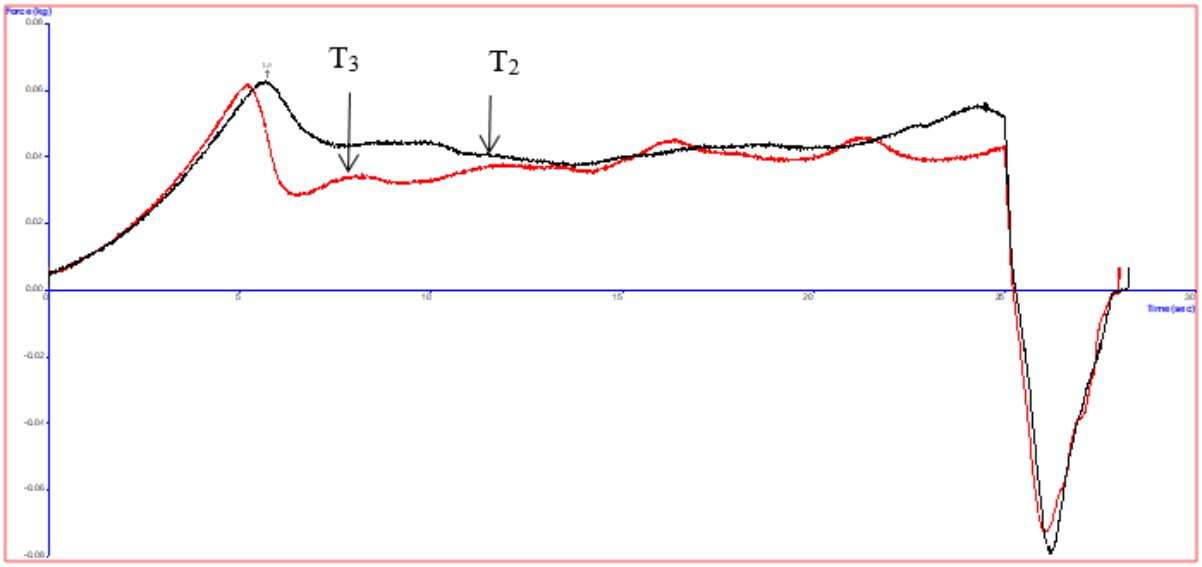

Figure 3

The texture of the standardized natural jelly after 8 months of storage