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Nutritional Composition and Bioactive Compounds of Bael (*Aegle marmelos*) and Development of Functional Food Products

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Abstract

Aegle marmelos, widely known as bael, belongs to the Rutaceae family. It is one of the most inexpensive and appealing fruits, considered to be an essential source of natural antioxidants and bioactive components. The major purpose of the research study was to investigate the nutritional composition and bioactive constituents of bael pulp, as well as to develop new value-added products that maintain the maximum quantity of nutrients. The developed food products were subjected to evaluate sensory attributes according to a nine-point hedonic scale. It was found that the moisture, protein, fat, crude fiber, and total ash content of bael fruit pulp were 61.20%, 2.48%, 0.47%, 3.04%, and 1.29%, respectively. When compared to the catechin standard, the antioxidant activity of such extract indicated good antioxidant capacity, with an IC_{50} value of 75.68 μg/ml for methanol extract. Vitamin C content was about 10.21 mg/100g. Besides, total flavonoid and phenolic contents were found as 140 mg of Quercetin Equivalent (QE)/g and 106.65 mg of Gallic Acid Equivalent (GAE)/g, respectively. Results of sensory attributes revealed that there was a significant difference ($P < 0.05$) in color, taste, and overall acceptability between bael murabba and bael bar. The overall acceptability of bael murabba (6.7) and bael bar (7.1) is acceptable in quality, but their specific characteristics were found slightly different by the test panelists. These products might be applicable for the treatment of several diseases like atherosclerosis, diabetes, constipation, irritable bowel syndrome, peptic ulcer, tumor, and osteoporosis.

Keywords

Bael, *Aegle marmelos*, Bioactive Compounds, Antioxidant Activity, Bael

Murabba and Bar

1. Introduction

Aegle marmelos belongs to the Rutaceae family. It is a kind of tropical fruit that is native to a geographical area and full-grown throughout Bangladesh. Bael is widely used for unit consumption and employed in traditional medicine for its several therapeutic characteristics [1]. Although mature fruits are the most popular, traditional Ayurvedic medicine employs wholly diverse components of the bael tree, including fruits, blooms and buds, leaves, bark, branches, seeds, and roots [2]. The mature fruit is spherical with firm skin and a dappled reddish-brown color [3]. Ripe fruits have a distinct aroma and are characterized by the release of tannins [4].

Bael pulp contains protein, carbohydrates, fat, and dietary fibers, as well as a variety of vitamins and minerals including vitamin A, B_1, B_2, B_3, calcium, phosphorus, and iron [5] and many biologically active compounds such as carotenoids, flavonoids, alkaloids, terpenoids, and coumarins [2] [6]. Various phytochemical and biological evaluations have been reported in the literature for the importance of the bael. So, it has been used in ethnomedicine to exploit its medicinal properties including antidiabetic, antioxidant, and antihyperlipidaemic [2]. Alcoholic extracts of bael were found to possess antimalarial activity (50% and above) *in vivo* as well as *in vitro* [7]. The *in-vitro* antiviral activity of bael was observed for its efficacy against human coxsackieviruses B1 - B6 [8]. Unripe fruit extract of bael showed gastroprotective and antidiarrhoeal effects in rats. Unripe fruit extract (50 and 100 mg/kg, i.p.) produces significant inhibition of gastric lesion as well as the accumulation of intestinal fluids induced by castor oil in mice was significantly inhibited by raw fruit extract [9]. The leaf extract of bael leaf treatment reduced the symptoms of radiation-induced sickness and increased survival. The radioprotective action might be due to free-radical scavenging and arrest of lipid peroxidation accompanied by an elevation in glutathione [10]. A study revealed that bael showed anti-inflammatory properties by inhibiting the carrageenan-induced paw oedema and cotton-pellet granuloma as well as the antinociceptive and antipyretic activities of the leaves were established [11]. Bael leaves possess antihyperlipidaemic effects in rats with isopropanol-induced myocardial infarction [12]. The usage of bael fruit in the food industry varies by nation, for example, in our country, the ripe fruit is consumed fresh, but in other countries namely, India is also made as nectar, sorbet, marmalade, jam, and cream [13].

The global market for nutraceuticals and functional foods is currently huge and expanding. These are still employed mostly by the unorganized sector, with little attention placed on their commercial application in terms of higher value-added commodities. The functional health food products from bael still de-

mand the scientific community's interest. The selected pharmacological investigations on various parts of the fruit have been undertaken, and it supports the potential of bael to be processed and manufactured to produce a variety of products [13]. So far, we have not seen any report to develop new products from bael in Bangladesh that has inspired us to explore new product development. The study's major goal is to determine the nutritional composition and bioactive substances, as well as to develop innovative products using bael pulp while maintaining the maximum quantity of nutrients. It would also provide an opportunity to explore alternatives to synthetic antioxidants in processed foods, as synthetic antioxidants pose potential health risks. The study's findings might be valuable to the scientific community and beneficial applications of bael for farmers and consumers to be conscious of the worth and proper utilization of the resources.

2. Materials and Methods

2.1. Sample Collection

Naturally, ripen bael and other ingredients required for product development were collected from Tangail Sadar, Tangail.

2.2. Proximate Analysis of Bael

Bael fruit sample was analyzed for moisture, ash, crude fiber, total protein, and total fat content. Moisture, ash, fat, and crude fiber content were determined by the AOAC method [14].

2.2.1. Estimation of Moisture

About 10 g of bael pulp was weighed into a porcelain basin and dried at 100˚C to 105˚C in an oven until the weight of the dish with its content was constant. The dish was cooled in a desiccator each time before weighing.

$$\text{Moisture content}(\%) = \frac{A-B}{W} \times 100$$

where, A = Basin and sample weight; B = Final weight; W = Sample weight.

2.2.2. Determination of Crude Protein Content

The sample was dried first. Then the amount of crude protein was determined based on the AOAC method: 960.52 [14] by the Kjeldahl technique where concentrated sulphuric acid (H_2SO_4), 40% sodium hydroxide (NaOH), and 0.1 M hydrochloric acid (HCl) were taken.

$$\text{Protein content}(\%) = \text{Total nitrogen}(N) \times 6.25$$

2.2.3. Estimation of Crude Fat Content

The moisture-free sample was collected and weighed. The dried sample was then dissolved in a methanol solution overnight. The filtered solution was dried and finally, the quantity of crude fat in the provided sample was determined.

$$\text{Percentage of crude fat} = \frac{\text{Weight of the extract}}{\text{Weight of the sample taken}} \times 100$$

2.2.4. Estimation of the Fiber Content

About 1 gram of fat-free dried sample was taken into a 500 ml beaker and added 200 ml of 0.255N sulfuric acid. Then, it was heated for 30 minutes by the addition in frequent intervals without changing the volume. After completing the task, filtrate the mixture through a thin muslin cloth and neutralize for removing acid with hot water. About 200 ml of 0.313 N sodium hydroxides was added. Further heating for another 30 minutes, and then filtrate again. After neutralizing, the filtrate was dried overnight at 30˚C - 100˚C and weighed. The crucible was heated at 600˚C for 2 - 3 hours in a muffle furnace. After cooling, the estimation of crude fiber content was calculated.

$$\text{Percentage of crude fiber} = \frac{100 - (\text{Moisture} + \text{fat}) \times \text{weight of fiber}}{\text{weight of sample taken}(\text{moisture and fat free})}$$

2.2.5. Estimation of Ash

The dried sample was taken accurately into a porcelain crucible. Then, it was placed in a muffle furnace for heating for about 3 - 5 hours at 600˚C. After completion of ashing, it was cooled in a desiccator. Finally, the amount of crude ash was calculated.

$$\text{Ash content}(\%) = \frac{A - B}{W} \times 100$$

where, A = Final weight; B = Empty crucible weight; W = Sample weight.

2.3. Determination of Bioactive Compound

2.3.1. Determination of Vitamin C Content

In a conical flask, 10 mL of standard vitamin C was titrated with a dye solution. About 5 - 6 grams of bael pulp was chopped into little pieces and vigorously homogenized with 3 percent Meta phosphoric acids before being filtered through a thin layer of muslin cloth. After centrifuging the filter for 10 minutes at 3000 rpm, the supernatant was determined by titrating with a solution of 2, 6-dichlorophenol indophenol. The amount of vitamin C in the extract was evaluated by comparing it to the titration result of a standard solution of vitamin C [15].

2.3.2. Determination of Antioxidant Activity

The radical scavenging capacity of DPPH was used to assess antioxidant activity [16]. A sample of 2 ml of extract at various concentrations (12.5 - 200 g/ml) was mixed with a solution of 3 mL of methanolic DPPH (6.25 - 100 g/ml). The mixture was stirred and left at room temperature for 25 minutes in dark conditions. The absorbance was measured at 517 nm using a suitable blank solution. The DPPH scavenging activity was estimated in mg Quercetin Equivalents (QEs) per gram of crude extract.

2.3.3. Determination of Total Flavonoid Content

The aluminum chloride colorimetric test was involved in the determination of flavonoid content [16]. About 1ml aliquot and 1 ml standard solution of quercetin (50, 75, 100, 125, 150, and 200 µg/ml) were placed in a test tube. Then add 4 ml distilled water and 0.3 ml 5 percent sodium nitrite solution. After passing 5 minutes, 0.3 ml of a 10% aluminum chloride solution was added. Next, 2 ml of 1 M NaOH was added. Finally, the volume was increased to 10 ml with de-mineralized water and well-mixed. After some time, the yellowish-orange color developed. The measurement of sample absorbance was performed at 510 nm by using a UV-visible Jasco V-630 spectrophotometer. Standard quercetin was applied to generate the calibration curve. The total flavonoid was calculated as mg of quercetin equivalents per 100 g dried sample weight.

2.3.4. Determination of Total Phenolic Content

Phenolic contents were calculated using the Folin Ciocalteu technique [16]. The standard curve was calibrated using gallic acid at different concentrations (10 - 100 µg/ml). The results were represented in milligrams of Gallic Acid Equivalent (GAE) 100 per gram of dried sample weight.

2.4. Bael Murabba Preparation

Using a fine cutter or saw, cut the mature bael fruit into 3/4-inch thick slices. Remove the shell, mucilage, and seeds from the slices. Boil the pieces in a 0.2% (1.2 g/600ml) citric acid solution until they are soft. Remove the pieces from the water. Spread sugar and pieces layer by layer and let aside for 24 hours. Remove the fragments the following day and concentrate the sugar solution to 60˚Brix. Add 2.4 g citric acid and 600 mg potassium metabisulphite. Soak the pieces in sugar syrup overnight. Concentrate the syrup to 72˚Brix and dip the pieces again. Repeat this procedure until the solution's TSS reaches 72˚Brix. After preparation, fill the jar in a ratio of 45:55 from pieces of bael and syrup into the jar, pack, and store [17].

2.5. Bael Bar Preparation

First, select ripe bael and wash with fresh water. Then break bael and remove the hard peel and pulping. The seeds were removed from the bael pulp, and the pulp was weighed. The desired amount of water was then added to the Bael pulp and well combined to make a fine bael paste. Heating (80˚C for 10 minutes) bael paste with adequate stirring so that it does not adhere to the bottom and get connected to the pan. Then add sugar, pectin, and flour to the bael mixture and continue cooking while stirring. Dried milk powder and cardamom powder were added and well mixed. Then, as a preservative, potassium metabisulfite ($K_2S_2O_5$) was added. After a few minutes, measure the sugar content at 50˚Brix. Finally, citric acid was added to the mixture to extend the shelf life and a trace quantity of salt was added to enhance the flavor. The final point (TSS-71.5%) was then determined using a refractometer (ABBE). After cooking, pour it into a stain-

less-steel pan that has been coated with Ghee and distribute it evenly. Then, transfer to a micro-oven for 10 - 12 hours of drying at 65˚C to get the final moisture content of 15% ± 2% weight basis. After removing it from the microwave oven, cooled the sheet, and cut it into 3 × 4 cm squares. Finally, it was vacuum-packed in butter paper and kept at room temperature for a longer shelf life.

2.6. Sensory Evaluation Method

The developed food products (bael murabba and bael bar) were subjected to evaluate sensory attributes according to a nine-point hedonic scale [18] judged by 20 panelists among students and teachers of Food Technology and Nutritional Science department of MBSTU, Santosh, Tangail, Bangladesh.

2.7. Statistical Analysis

The data were analyzed using SPSS for windows V.20 and presented as Mean ± SD (Standard Deviation). The statistical significance was determined using the One-way Analysis of Variance (ANOVA). Each value was derived from the average of three observations and the mean value was reported. The significance levels of $P \leq 0.05$ were used for statistical differences.

3. Result and Discussion

3.1. Proximate Composition of Bael

Moisture content was done on the wet basis from ripened fruit. Then, a dried sample of bael pulp was analyzed for ash, fat, and crude fiber content. For this purpose, triplicate measurements were accounted for each test.

The results of the proximate analysis of bael are represented in Table 1. It revealed that the moisture, ash, fat, crude fiber, and protein content of bael fruit pulp were 61.20% ± 2.96%, 1.29% ± 0.07%, 0.47% ± 0.11%, 3.04% ± 0.29%, and 2.48% ± 0.34% respectively. The moisture content is a significant factor that influences the physical attributes of fruits such as size, viscosity, weight, and bulk density. These property evaluations may be useful in fruit harvesting, transportation, storage, and processing activities. The moisture content of bael fruit was

Table 1. Proximate composition of bael.

Parameters	Amount
Moisture (%)	61.20 ± 2.96
Ash (%)	1.29 ± 0.34
Fat (%)	0.47 ± 0.11
Crude fiber (%)	3.04 ± 0.29
Protein (%)	2.48 ± 0.34
Vitamin C (mg/100g)	10.21 ±1.23

Note: Values are mean ± S.D. of triplicate analyses and expressed as percent dry matter.

found to be 61.20% which is in the range reported (61.04%) by Sawale *et al.* [19] and is higher (56.91%) than that reported by Uttarwar *et al.* [20]. The Ash content of bael was 1.29% ± 0.07% which is quite low to the value reported (1.7%) by Sawale *et al.* [19]. Proteins are crucial nutrients that are well-known as body-building biomolecules because they include essential amino acids that are necessary for human health and well-being. Protein content was found to be 2.48% ± 0.34% which is in the range of 2.79% reported by Uttarwar *et al.* [20], however, it is higher than that reported by Anurag *et al.* [21] who found the value 1.6%. The crude fat content of bael was 0.47% ± 0.11% which is found to be similar results of 0.39% as reported by Sawale *et al.* [19]. Uttarwar *et al.* [20] and Sawale *et al.* [19] reported that the fiber content of bael was 5.79%, and 3.07% respectively whereas our study reveals 3.04% ± 0.29% which is close to the value reported by Sawale *et al.* [19] and is less than that reported by Uttarwar *et al.* [20]. The reason for the differences among the values may be due to the geographical differences of the sample, soil, and maturation or due to the varieties of the collected samples. The nutrient content is influence by geographical differences of the sample where grown, soil, and maturation or due to the varieties of the collected samples. Weather conditions like drought, rainfall, salinity, presences of minerals in the soil, the other nutrient in the soil etc. influence the nutrient content of grown product to a great extent.

Vitamin C, also known as ascorbic acid, is an antioxidant that plays a major role in health-promoting metabolic activities. **Table 1** also revealed that the vitamin C content is about 10.21 ± 1.23 mg/100g. The present value of this investigation was close to the previous report [19] which contained 16.80 mg/100g and 8 - 60 mg/100g respectively. The low or high amount of vitamin C content identified in the current study might be ascribed to a variety of factors including physiological and morphological properties of the plant, ambient circumstances, and soil conditions. Vitamin C is an antioxidant that helps protect your cells against the effects of free radicals. Free radicals might play a role in heart disease, cancer and other diseases.

3.2. Antioxidant Activity of Bael

The method of DPPH is based on the decrease of DPPH in the presence of a hydrogen-donating antioxidant owing to the production of diphenyl picryl hydrazine. Because of their hydrogen-donating capacity; extracts lower the color of DPPH. The proportion of DPPH radical scavenging activity was obtained by plotting concentration in a graph, and the IC50 was estimated from the graph (**Figure 1**, **Figure 2**). For Catechin standard, the standard curve equation is $y = 0.7569x + 16.679$ and for methanol extract, the standard curve equation is $y = 0.3871x + 20.703$.

Table 2 showed that the IC_{50} value of standard Catechin and methanol extract were 44.023 µg/ml and 75.683 µg/ml respectively. This finding is in close agreement with the previous report [22]. Rajan *et al.* [22] revealed that the antioxidant

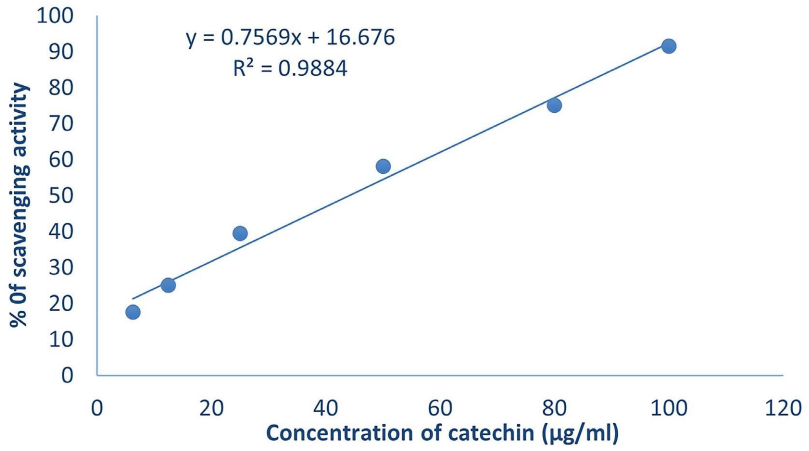

Figure 1. Calibration curve of catechin for scavenging activity.

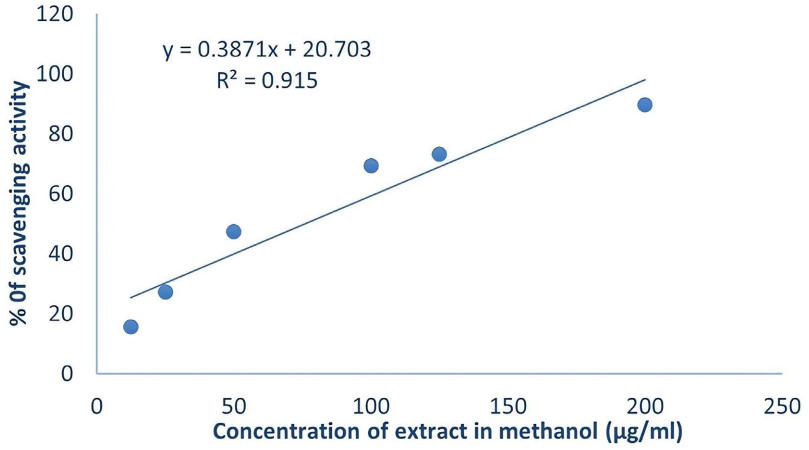

Figure 2. Calibration curve of methanol extract for scavenging activity.

Table 2. Antioxidant activity, flavonoid, and phenolic content of bael.

Parameters	Amount
Antioxidant activity	75.68 µg/ml
Total flavonoid content	140 mg of QE/g
Total phenolic content	106.65 mg of GAE/g

content was about 106.158 ± 25.332 µg/ml of IC_{50} value for methanol extract which showed slight variation in comparison with our present study.

3.3. Determination of Total Flavonoid Content

The total flavonoid content was measured using the aluminum chloride technique. Kamtekar *et al.* [23] used Quercetin as a standard. The quercetin solution of concentration (50 - 200 ppm) abides by Beer's Law at 510 nm with a regression coefficient (R^2) = 0.9889. The plot has a slope (m) = 0.0075 and an intercept = 0.0355. The equation of the standard curve: $y = 0.0075x + 0.0355$ (**Figure 3**).

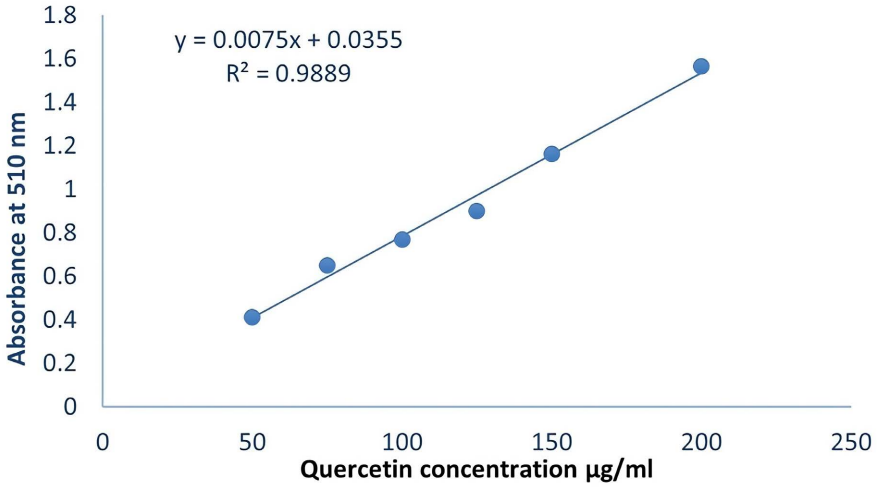

Figure 3. Total flavonoid content for standard quercetin.

Rajan *et al.* [22] revealed that the total flavonoid content was about 166.33 mg of Quercetin equivalent to 1 gm of alcoholic extracts. Our result is about 140 mg, which is consistent with the earlier research [22]. Adisakwattana *et al.* [24] showed that the bael fruit contained 44.57 ± 2.65 mg/g quercetin equivalents which are very lower than the present study.

3.4. Determination of Total Phenolic Content

The reagent used for the estimation of total phenolic content is composed of Gallic acid, which is diminished to a mixture of blue oxide of molybdenum when the phenols are oxidized. The blue hue generated has a maximal absorption in the range of 750 nm and is proportionate to the total amount of phenolic chemicals originally present. At 750 nm, a concentration (10 - 100 ppm) of Gallic acid solution complies with Beer's Law with the regression coefficient (R^2) = 0.9887. The slope (m) of the plot is 0.0109, while the intercept is 0.0631. The standard curve equation: $y = 0.0106x + 0.0542$ (**Figure 4**).

Suvimol and Pranee's [25] findings revealed that bael pulp contained a total phenolic content of 87.34 mg GAE/g dry weight, which is slightly lower than our recent investigation. The present study report is very similar to the previous report by Rajan *et al.* [22] which contained (147.66 mg/g) and is less than that reported by the previous author [26]. Gheisari *et al.* [26] and Adisakwattana *et al.* [24] reported total phenolic content of 336.1 8.1 mg/g and 433.33 mg/g, respectively, which differed from our current investigation.

3.5. Sensory Evaluation of Bael Murabba and Bael Bar

Sensory evaluation is a scientific approach for identifying sensory attributes of food using human senses such as vision, hearing, smell, taste, and touch. All these sensory attributes received varied degrees of acceptance in terms of appearance, flavor, color, taste, texture, and overall acceptability. The sensory scores of bael murabba and bael bar are depicted in **Table 3**.

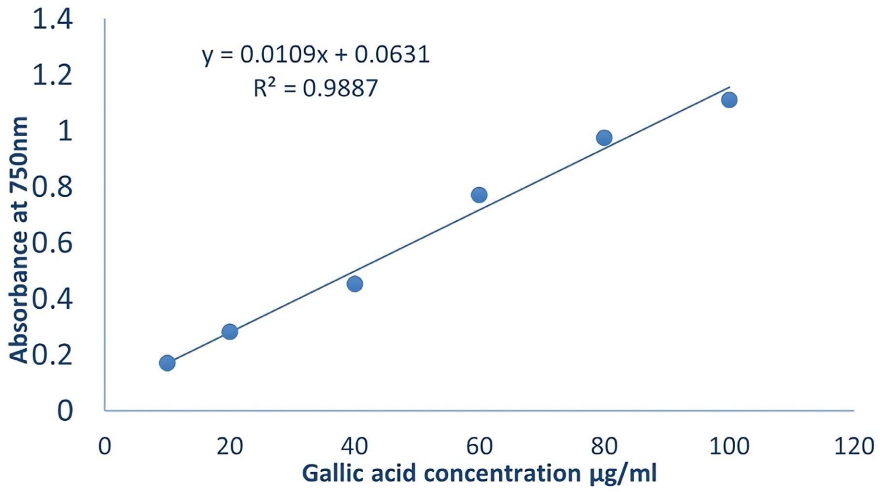

Figure 4. Total phenolic content for standard gallic acid.

Table 3. Sensory evaluation of bael murabba and bael bar.

Quality parameters	Bael murabba	Bael bar
Appearance	7.3 ± 0.470[a]	7.2 ± 0.768[a]
Color	7.0 ± 0.459[a]	7.8 ± 0.894[b]
Flavor	7.1 ± 0.553[a]	7.5 ± 0.946[a]
Taste	6.7 ± 0.657[a]	7.1 ± 0.852[b]
Texture	6.5 ± 0.513[a]	6.9 ± 0.852[a]
Overall acceptability	6.7 ± 0.801[a]	7.1 ± 0.553[b]

Note: Data are presented as mean ± standard deviation, with various superscripts in the row showing statistically significant differences at $p < 0.05$.

Color is a key factor in properly assessing manufactured food products since it not only reflects the appropriate raw material used in preparation but also offers information about the product's formation and quality. Table 3 reveals the mean quality score of the color of the bael murabba and bael bar. The analyzed result of the product color scored 7 and 7.8 for the bael murabba and bael bar respectively, whereas the test panelists judges both the developed products moderately. According to the data, the bael bar was scored significantly ($P < 0.05$) higher than the bael murabba. The primary factor in determining whether a product is acceptable or not, is its flavor. The quality score for the flavor of the generated food products demonstrated that there is no significant difference between the bael murabba and bael bar. The judges accepted both goods in terms of flavor moderately. Bael murabba and bael bar received scores of 7.1 and 7.5 for their flavors, respectively. From the analysis, the taste acceptability of the bael bar was moderately liked whereas the bael murabba was slightly liked by the test panelists. Table 3 shows the texture acceptability of bael murabba was lowest than others. From the analysis, the texture of the bael bar was moderately liked whereas the bael murabba was slightly liked by the test panelists. The result

indicates that the score of the bael bar was significantly ($P < 0.05$) higher than bael murabba in terms of texture. According to the findings, there was a significant difference ($P < 0.05$) in color, taste, and overall acceptability between bael murabba and bael bar. There was no significant difference ($P > 0.05$) observed in the appearance, flavor, and texture of the products. Overall acceptability was determined based on quality scores obtained from the evaluation of different quality traits including color, flavor, and texture of the manufactured food products. The Factors that influence the acceptability of bael products like murabba and bar may include color and taste, found in present study. The mean regarding the overall acceptability of bael murabba and bael bar revealed that the overall acceptability of bael bar was highest while bael murabba has the lowest acceptability. The overall acceptability score of bael bar and bael murabba was found as 7.1 and 6.7, respectively. There was no such report found to compare with our findings.

4. Conclusion

A. marmelos is a good source of fiber and ash content based on the analysis. High fiber content is more effective for patients who have been suffering from constipation and inflammation. Ash content indicates that it is an indispensable source of several minerals. Results also showed that it contains a high amount of vitamin C which exerts antioxidant activity. It possessed a substantial amount of total flavonoid and phenolic contents that might be useful for the treatment of many diseases such as atherosclerosis, diabetes, cancer, constipation, irritable bowel syndrome, peptic ulcer, tumor, and osteoporosis. Both the developed food products (bael murabba and bael bar) were overall acceptable in quality although few specific characteristics were observed as slightly variable. Both products could be used for the treatment of different diseases and the improvement of our health.

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Availability of Data and Material

All data and materials were available in the text of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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