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EXTRACTION AND CHARACTERIZATION OF BITTER GOURD OIL FOR THE DEVELOPMENT OF NUTRITIONALLY ENHANCED CONFECTION CHOCOLATE: A COMPREHENSIVE ANALYTICAL STUDY

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Abstract

Momordica charantia, commonly referred to as bitter melon or bitter gourd, is known for its therapeutic and nutritious qualities and has a long history of use as an alternative medicine. Its distinct bitterness is attributable to the non-toxic glycoside momordicine. Bitter gourd oil contains antioxidants, this assists the body in combating free radicals, and other oxidative stress elements. The aim of this research was to develop a stevia-based chocolate using bitter gourd oil which may help in reducing glycaemic index in diabetic rats. The bitter gourd oil (BGO) was extracted and subjected to physicochemical analysis, GC-FID for proper characterization. The extracted components were used in varied ratios for the development of bitter gourd chocolate along with replacement of sugar with natural sweetener stevia that further underwent sensory, physicochemical and storage. As a whole, findings showed that BG chocolate with T_3 treatment (30% BGO 70% coconut oil) was stable in a variety of physicochemical and sensory evaluation. As a result, the relatively best treatment with respect to storage stability i.e., T_3 with a 30% BGO and 70% coconut oil. The findings revealed that chocolate made from bitter gourd oil can be served as a better confectioner product. If developed on a larger scale and commercialized, this product may benefit people effected by diabetes.

Keywords: *Momordica charantia*, Edible Oil, Oil Composition, Chocolate, Food Product Evaluation.

1. INTRODUCTION

Consumption of vegetables is prevalent owing to their numbers of bioactive components including minerals, phenolic compounds, flavonoids, proteins, and dietary fibers whereas being a moderate source of carbohydrates. Among many vegetables, bitter gourd (*Momordica charantia*) has several therapeutic effects and is one of the most nutritionally abundant vegetables. In Asia, Indian subcontinent, Eastern areas of Africa, North America, and South America, bitter gourds are widely grown and are members of the Cucurbitaceae family. Bitter gourd is also known by the other names such as bitter melon, karela, or balsam pear. *Momordica charantia* is a climbing perennial fruity vegetable that may rise to a height of 5 meters and bears cylindrical fruit that varies in length from 9 to

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60 cm and has a warty appearance. It has smoother, shinier, and brighter skin with very few bumps on its surface (Tan *et al.,* 2016). Several illnesses can be treated using bitter gourd supplements and meals made from roots, seeds, leaves, and fruits. It is indeed a good component of natural amino acids, vitamins, carotenoids and minerals as well as it plays a key part in maintaining human nutrition (Sorifa, 2018).

Momordica charantia is a low caloric fruit yet high in health-promoting nutrients. It is rich source of dietary fibers as well as thiamine, riboflavin and niacin. Minerals such as magnesium, folic acid, zinc, phosphorus, and manganese are present abundantly in bitter gourd fruit. *Momordica charantia* has incredibly small number of calories, with only 17 calories per 100 g (Krishnendu and Nandini, 2016). Novel therapeutic research has revealed that *M. charantia* is a "medicine food homology" crop with several beneficial health effects. Furthermore, bitter gourd is used as a useful raw material in tea, beverages, drinks, fruit preservation, and cocktails. Numerous physiologically active substances, which include carbohydrates, lipids, proteins, triterpenoids, saponins, polypeptides, polyphenols, alkaloids, and sterols, are found in bitter gourds (Jia *et al.,* 2017).

Stevia rebaudiana which belongs to the genus Stevia is utilized as a natural sweetener mostly used by diabetic patients. Stevia is widely utilized as a sweetener because it can be cultivated in all regions and has high sweetness with low-calorie value. *Stevia rebaudiana* (Bertoni) is a Paraguayan sweeten plant that possesses organic non-caloric sweetness (Ramesh *et al.,* 2006). Stevia is a plant that has a zero calorie with sweet flavor and has no negative impacts on human health after intake. Stevioside and rebaudioside A are among the glycosides found in stevia; the leaves also include natural diterpene, glycosides, stevioside, rebaudioside A–F, steviolbioside, and dulcoside that gives them their sweet flavor and have significant economic importance all around the globe as a natural sweetener used in foods, drinks, and pharmaceuticals (Ahmad *et al.,* 2020).

Diabetes is the world's leading causes of death. Diabetes mellitus is a growing health concern globally that affects over 285 million people around the world. Diabetes is expected to impact approximately 439 million individuals by the year 2030. Additionally, it is projected that around 75% of those affected will be from countries that are developing. Over 30% of diabetes individuals adopt unconventional medicinal methods because of negative effects and reliance on inexpensive treatments. As a preventive and therapeutic approach, many plant-based therapeutic approaches are used extensively to deal with chronic ailments and infections. As stated by the World Health Organization (WHO), herbal remedies are used by approximately 80% of people globally for their primary healthcare needs. This therapeutic efficacy of plant-based treatment can be attributed to a variety of valuable phytochemicals found primarily in the waste products of these plants. *Momordica charantia* and its different parts and products, for instance, are able to be used in order to low blood glucose levels through biochemical, pharmacological, and physiological causes (Mahwish *et al.,* 2017).

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Momordica charantia is an important medicinal plant that has long been used in Asian countries to combat diseases like diabetes. There has been a recent increase in diabetic patients during recent years due to a sudden change in the diets of people and their lifestyle. This diabetes results in a decrease in the utilization of chocolates, as they contain high sugar content.

However, the consumption of chocolates is still relatively high in the daily lives of people due to their high sensory appeal. In this research, chocolates were developed with the help of stevia and bitter gourds to provide them with high nutritional value while decreasing their sugar content.

2. MATERIALS AND METHODS

The basic materials, including Bitter gourd seeds, were obtained from the Ayub Agriculture Research Institute, while the remaining materials were purchased from Faisalabad's local market.

2.1. Oil extraction

Sample was taken in filter paper was weighed and made into packets. n-hexane was used for extraction. Thimbles consisting of sample were placed in extraction chamber of Soxhlet apparatus. After 7-8 washings, the solvent containing the oil was collected. A rotary vacuum evaporator had been employed to evaporate the solvent that was used (at low temperature and low-pressure boiling occur). After that solvent free oil was collected in a flask and stored till further analysis as described by AOAC (2016).

2.2. Free fatty acid (FFA)

The percentage of fatty acids present in given sample was determined by titration in neutralized ethanol (95%) using a NaOH solution as mentioned in AOCS (2017) Method No. Aa 6-38. Sample was blended with 95% neutralized ethanol in a clean conical flask. The solution was swirled until the complete dissolution of the solution followed by the addition of 3-4 drops of phenolphthalein as indicator. Subsequently, the solution was stirred with 0.1N NaOH until the light pink color remained for thirty-five seconds. The computation of the percentage of free fatty acid was based on the acquired values, ensuring accuracy in the analysis.

$$\text{Free fatty acids} = \frac{\text{Alkali used (mL)} \times \text{N} \times 28.2}{\text{wt. of sample(g)}}$$

Where:

N = Normality of Alkali

2.3. Peroxide value

The peroxide value (PV) was assessed with regard to the iodine developed from the reaction of iodine ion and H_2O_2 using the techniques specified in AOCS (2017), Method No. Cd 8b-90. Sample was added.with chloroform and flask was filled with glacial acetic

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acid and shaken for around 60 seconds to ensure that oil was dissolved. Freshly made potassium iodide solution incorporated to a flask. The resultant mixture was then titrated with 0.1N standardised $Na_2S_2O_3$ solution while being constantly stirred till yellow colour vanished. 0.5 mL starch solution was introduced as an indicator, and the mixture was vigorously stirred until the dark blue colour vansihed. The following formula was used to get the PV:

$$\text{PV} = \frac{(\text{B}-\text{S}) \times \text{N} \times 1000}{wt.\ of\ oil\ taken(g)} \times 100$$

Where:

S = Volume of $Na_2S_2O_3$ utilized for sample

B = Volume of $Na_2S_2O_3$ utilized for blank

N = Normality of $Na_2S_2O_3$

2.4. Iodine value

The grams of iodine absorbed by 100 g of fats and oils as outlined in AOCS (2017), Method No. Cd 1-25. To analyze the fat content in bitter gourd seeds, the extracted fat was discomposed by mixing a solution comprising of carbon tetrachloride (CCl_4) and Wijs solution in a stoppered flask and kept for an hour in a dark place. Afterward, the solution was distilled with 15% KI solution and distilled water.

The resulting mixture was titrated with sodium thiosulphate ($Na_2S_2O_3$) and a freshly prepared starch indicator solution. The reading obtained without the sample running was also recorded. The iodine value was then calculated by subtracting the milliliters used by the sample from the several milliliters of 0.1N sodium thiosulfate, providing the $Na_2S_2O_3$ equivalent of the iodine value as stated in the procedure.

$$\text{Iodine value} = \frac{(\text{B}-\text{S}) \times 12.69 \times \text{N}}{\text{wt. of sample(g)}}$$

Where:

S = Volume of $Na_2S_2O_3$ utilized for sample

B = Volume of $Na_2S_2O_3$ utilized for blank

N = Normality of $Na_2S_2O_3$

2.5. Specific gravity

The determination of specific gravity at ambient temperature was conducted using precision equipment known as a Pycnometer with a capillary drilled closure at its core. The methods used in this investigation were described in AOCS (2017), Method No. Cc 10b-25. Before determining the sample's specific gravity, this instrument was calibrated by filling it with purified water and weighing the entire water mass.

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Following that, the sample's specific gravity at room temperature was calculated as follows:

$$\text{Specific gravity} = \frac{\text{Wt. of oil (g)}}{\text{wt. of water(g)}} = \frac{(\text{C} - \text{A})}{(B - A)}$$

Where:

A = Wt. of empty bottle with specific gravity

B = Wt. of bottle filled with water with specific gravity

C = Wt. of bottle filled with oils with specific gravity

2.6. Saponification value

Saponification value of bitter gourd oil was calculated by following the procedure discussed in AOCS (2017), Method No. Cd 3a-94. Sample were added to a conical flask, and 0.5N alcoholic potassium hydroxide solution (KOH) were used to dissolve it. The resultant mixture was then reflowed for 30 minutes in a water bath using a water condenser. As the resulting solution cooled, phenolphthalein indicator was used as a indicator and titrated with 0.5N HCl. Empty readings were measured separately.

$$\text{Saponification value} = \frac{(\text{B}-\text{S}) \times 0.02805 \times 1000}{\text{weight of sample(g)}}$$

Where:

S = Vol of KOH used for sample

B = Vol of KOH used for blank

2.7. Refractive index

The Refractive index of bittergourd oil was determined by following AOCS (2017), Method Cc 7-25. Adjusted temperature of refractometer to 40℃ for oil samples. The bottom prism was then covered with a few sample drops. Prism was closed and tightened firmly with screwhead and was allowed to stand for about 1–2 minutes or until the sample reached the instrument's temperature. The reading of refractometer was noted along with took several readings and then the average of all the readings was calculated.

2.8. *p*-Anisidine value

The *p*-Anisidine value of bitter gourd oil was evaluated following the Official Method Cd 18-90, specified in AOCS (2017). Oil sample was incorporated with 30 mL of isooctane. The mixture was then mixed with acetic acid (w/v) containing 0.25 percent *p*-anisidine. The solution was completely mixed and was left in dark for 13 minutes.

Following the period of incubation, the amount of absorption was determined at 350 nm with a spectrophotometer. To get the blank reading, five milliliters of isooctane were used in the same way.

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$$p\text{-AV} = \frac{25\times[(1.2S-B)]}{\text{weight of sample(g)}}$$

Where:

S = Sample reading

B = Blank reading

2.9. Thiobarbituric acid (TBA)

AOCS (2017), official method Cd 19-90 was followed to determine the TBA content in bitter gourd oil. 150mg of sample was taken in volumetric flask and sample was dissolved to made it 1 volume with 1-butanol. Regent solution was added in sample along with continuous stirring. After that sample test tube was placed at 90 °C into thermostatic bath. After 2 hours, sample test tube was removed from bath and cool down to room temperature. Absorbance was observed at 530nm in 10mm cuvette with distilled water as reference. Blank sample was done at the same time.

$$\text{TBA} = \frac{50\times(A-B)}{m}$$

Where:

A = Absorbance of test soln.

B = Absorbance of blank

m = Mass of test element

2.10. DPPH

The DPPH radical scavenging assay of *Momordica charantia* oil was performed according to the process as determined by Lopes *et al.* (2018) with minor modifications. DPPH radical solution in methanol (2.0×10^{-4}M) was combined with diluted oil sample in methanol. The final DPPH radical concentration was 1.0×10^{-4}M. Then the reaction mixtures were agitated and incubated for 30 minutes in dark. Thereafter by using a spectrophotometer the absorbance was determined at 517nm.

2.11. Fatty acid profile by GC-FID

The fatty acid composition of bitter gourd oil was examined by the utilization of gas chromatography (GC) equipped with flame ionization detector (FID) according to the Official method Ce 1-62 as determined in AOCS (2017).

2.12. Product development and analysis

The oil extracted from the bitter gourd was used in the preparation of chocolate by replacing sugar with stevia, and after the preparation of chocolate the various analysis was performed to investigate the effect of storage stability.

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2.13. Textural analysis

The **textural analysis** of chocolate developed with different formulations was determined by texture analyzer (TA-HDi; Stable Microsystems, UK) and the triple beam snap (three-point break) method. A 2-mm cylindrical probe with a 5-kg load cell was used to conduct a penetrating test. By the process as described in Jahangir *et al.* (2018).

2.14. Free fatty acids

The FFA contents of chocolate developed with different variations of bitter gourd oil were determined by Official method Aa 6-38 as described in AOCS (2017).

$$\text{FFA} = \frac{\text{Alkali used (mL)} \times \text{N} \times 28.2}{\text{wt. of sample(g)}}$$

Where:

N = Normality of Alkali

2.15. Peroxide Value

The peroxide value of chocolates was estimated by Official method Cd 8b-90 as determined by AOCS (2017). POV was measured by utilizing by formula mentioned below:

$$\text{Peroxide value} = \frac{(\text{B}-\text{S}) \times \text{N} \times 1000}{wt.\ of\ oil\ taken(g)} \times 100$$

Where:

S = Volume of $Na_2S_2O_3$ utilized for sample

B = Volume of $Na_2S_2O_3$ utilized for blank

N = Normality of $Na_2S_2O_3$

2.16. Energy value

The energy value of the chocolate developed with different formulations were measured by Oxygen Bomb Calorimeter (IKA-WERKE, C2000 Basic, GMBH and Co., Germany) as mentioned in AACC (2000).

2.17. Color

Chocolate developed from bitter gourd oil with different formulation were measured for color in the L*, a* and b* color system utilizing a colorimeter (PCM Accuracy Micro sensors. Inc. USA) according to the method determined by **Bouaziz *et al.* (2017).**

2.18. Sensory analysis

Sensory analysis of developed chocolate with various concentration of bitter gourd oil were determined according to the process as described by Meilgaard *et al.* (2007).

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2.19. Statistical analysis

Methodology described by Montgomery (2017) was used for data acquisition, analysis, and statistical examination of the study findings. The complete randomized design (CRD) was applied along with Tukey's HSD posthoc test.

3. RESULTS AND DISCUSSIONS

The objective of the present study was to characterize the significance of bitter gourd oil (BGO) with regards to nutritional and functional properties of the developed edible product (Chocolate) with complete replacement of sugar with stevia.

3.1. Free fatty acid (FFA)

FFA indicates impudence and clarity of fats, oils, and the associated edible food products. These fatty acids are decreased in concentration as double bonds are reduced. The free fatty acid of bitter gourd oil is shown in Table 1. The mean value for the FFAs of bitter gourd oil was 0.88 ± 0.02%. This value was found close to those given by Ali *et al.* (2008) while working on the compositional determination of bitter gourd oil and Anjum *et al*. (2013) also found the free fatty acid content 1.06± 0.04%.

3.2. Peroxide value

Peroxide value provides information regarding the freshness and quality of fats and oils. The mean peroxide value of the extracted oil of bitter gourd was presented in Table 1, which was 7.91 ± 0.21meq/Kg. These results are comparable with the investigation of Anjum *et al.* (2013) who depicted the peroxide value 5.97 ± 0.34meq/Kg for the bitter gourd oil. There are several factors which can affect PV of the oil. Hydrolytic rancidity and a rise in the acidity of changed products can result through spontaneous lipid hydrolysis and enzymatic lipolysis. Another study by Yoshime *et al.* (2016) indicated the peroxide value 8.05meq/Kg of Soxhlet extracted bitter gourd oil.

3.3. Iodine value

Iodine value is an important parameter as it enables to check the unsaturation level of the fats and oil leading to the determination of its stability. The mean iodine value of the bitter gourd oil was 121.54 ± 3.18 g I_2/100g, as given in Table 1. These outcomes align with the conclusions of Anjum *et al.* (2013) who reported iodine value of 124.16 gI_2/100g oil depending upon the methods employed for extraction of oil. Ali *et al.* (2008) also gave the similar results and found 125.21 g I_2/100g of iodine value. Thus, study indicated that bitter gourd oil has increased level of unsaturation.

3.4. Specific gravity

Specific gravity is important in the providing information regarding the oil density, purity, and its suitability in different applications. It is of utmost importance in classification, blending, and formulation of oil-based products as well as enables weight-volume conversions. The bitter gourd oil showed the mean value for specific gravity of 0.847 ±

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0.003, as given in Table 1. This value was shown to be close to the outcomes published by Ali *et al*. (2008). They found that the specific gravity was 0.99 when solvent extraction was used. Another study by Machewad *et al.* (2021) also indicated a value of 0.85 for specific gravity of the bitter gourd oil.

3.5. Saponification value

The key parameter for the molecular weight determination of fatty acid in oil sample is saponification value. The saponification value of bitter gourd oil was 165.02 ± 4.27 mg KOH/g (Table 1). These findings align with those of Anjum *et al.* (2013) who presented the results for saponification value as 186.09 mg KOH/g. Findings presented by Ali *et al.* (2008) determined saponification value in BGO as 190.70 mg KOH/g. Machewad *et al.* (2021) also reported similar findings in which the saponification value of bitter gourd oil was 145.22 mg KOH/g.

3.6. Refractive index

Refractive index measures the bending of light as it passes through the oil sample. The mean value of refractive index of bitter gourd oil was given in Table 1, which was 1.4882 ± 0.0004. Similarly, Ali *et al.* (2008) also reported the refractive index value 1.38. The findings of this inquiry were congruent with those of Machewad *et al.* (2021) who presented the results for refractive index was 1.44.

3.7. *p*-Anisidine value

The *p*-anisidine value is a crucial parameter employed in the analysis of edible oils, providing valuable insights into the oxidative deterioration and overall quality of the oil. In the case of bitter gourd oil, a value of 3.92 ± 0.31 mg/g for the *p*-anisidine value suggests a moderate level of oxidative deterioration as shown in Table 1. Monitoring and interpreting such values are essential for ensuring the quality and safety of edible oils, as well as for making informed decisions regarding their storage, handling, and usage in various culinary applications. Ali *et al.* (2008) reported the similar findings of *p*-anisidine value of bitter gourd oil. Comparable findings were obtained from this specific research by Yoshime *et al.* (2016) who presented the results for saponification value was 3.45 mg/g.

3.8. Thiobarbituric acid (TBA)

Thiobarbituric acid (TBA) is defined as the amount of malondialdehyde present in 1 kg of sample. Thiobarbituric acid (TBA) and MDA can react to yield the quantity of MDA in the oil. The pink-colored TBA-MDA complex is produced in this reaction. Table 1. Shows that the TBA value of bitter gourd oil was 0.56 ± 0.03 mg MDA/kg. Results are according to Padmashree *et al.* (2011) who analyzed antioxygenic activity of bitter gourd showed similar results of TBA of bitter gourd between the range of 0.21 and 0.59 mg MDA/kg.

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3.9. DPPH

An efficient method for evaluating the antioxidant capability of garlic extract and particular bioactive components is the 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) free radical scavenging activity. Table 1 depicts the DPPH activity of the bitter gourd oil. The mean value for DPPH% inhibition of BGO was 22.28 ± 1.56 %. These results of antioxidant activity are supported by Ghaffar *et al.* (2017) who found the antioxidant potential of the bitter gourd oil around 11.92 % based upon the type of solvent used. Anjum *et al.* (2013) also demonstrated similar findings and extended the DPPH assay's mechanism by demonstrating how BGO function as hydrogen or electron donors in the process of reducing DPPH radicals to their reduced forms. Tan *et al.* (2013) also supported the findings which was associated with higher variances in the scavenging activity which showed up to 20.44 %. Ozusaglm and Karakoca (2013) reported the similar findings of DPPH assay of bitter gourd 14.25%.

Table 1: Physicochemical characteristics of bitter gourd oil

Properties	Mean Value
FFA (%)	0.88 ± 0.02
PV (meq O_2/kg)	7.91 ± 0.21
IV (gI_2/100g)	121.54 ± 3.18
SG	0.847 ± 0.003
SV (mg KOH/g)	165.02 ± 4.27
RI	1.4882 ± 0.0004
p-Av (mg/g)	3.92 ± 0.31
TBA (mgMDA/kg)	0.56 ± 0.03
DPPH (% inhibition)	22.28 ± 1.56

3.10. Fatty acid profile by GC-FID

Using gas chromatography, the fatty acid composition of BGO had been analyzed. The fatty acid profile of bitter gourd oil is displayed in Table 2. The saturated fatty acid group includes stearic and palmitic acids. The oil extracted from bitter gourd had 4.58% palmitic acid (C16:0) and 21.04 % stearic acid (C18:0) concentration. Within the category of unsaturated fatty acids are oleic, and linoleic acids, bitter gourd oil had a concentration of 4.72% for oleic acid (C18:1) and contained 5.59 % linoleic acid (C18:2). Among these fatty acids, *Momordica charantia* oil had α-eleostearic acid (ESA) (C18:3n9) content of 52.12%. Lipid peroxidation effectively reduce in animal models during efficacy by the α-ESA which also act as antioxidant. Bitter gourd seed oil contained 31.72 % saturated fat and 62.15 % polyunsaturated fatty acid (PUFA). The findings are consistent with Yuwai *et al.* (1991) who investigated the Fatty acid profile of bitter gourd, which revealed comparable outcomes for bitter gourd wherein eleostearic acid was the most notable fatty acid among all. Results of Nyam *et al.* (2009) showed that eleosteric and stearic was found to be major fatty acid.

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Table 2: Fatty acid profile of bitter gourd oil (BGO) %

BGO	Concentration (%)
Capric acid (C10:0)	0.47
Lauric Acid (C12:0)	0.45
Palmitic acid (C16:0)	4.58
Stearic acid (C18:0)	21.04
Oleic acid (C18:1)	4.72
Linoleic acid (C18:2)	5.59
α-Eleostearic acid (C18:3n9)	52.12
Arachidonic acid (C20:0)	4.91
Saturated fatty acid	31.72
Polyunsaturated fatty acid (PUFA)	62.15

3.11. Product development and analysis

A lab-scale experiment was carried out to develop coconut oil alternative chocolate using bitter gourd oil at substitution rates ranging from 2 to 10 g per 100 g. Cocoa powder, stevia liquid, milk powder, bitter gourd, coconut oil and lecithin were all blended in a blender. The pre-mix was then refined with before being conched for at least 7 hours at 50°C in a runner mill. The slurry was then manually tempered and shaped into little pieces weighing around 12 grams apiece using food-grade plastic. The molded chocolate was put inside a commercial polypropylene sheet. All samples of chocolates were packed in propylene sheets and kept for the purpose of examining its stability at 25 °C. And all over the quality characteristics during the time interval of 0, 15^{th}, 30^{th}, 45^{th}, and 60^{th} days of storage. There have been numerous studies on utilization of bitter gourd extracts and isolates as a nutritious replacement in many food products.

3.12. Textural analysis

Textural characteristic is one of the imperative parameters to assess the physical nature of chocolate. The means of BG chocolate shown in Table 3. As the total number of storage days increased, there was a substantial reduction in the texture hardness ($P \leq 0.05$). This may be attributed to moisture gain of the chocolates over storage period which may have contributed to dampness and thus the softness of the BG chocolate. According to Razavizadeh and Tabrizi (2021), the micro-capsules' surface oil may alter the chocolate's texture and somewhat lessen its hardness. In a similar study, Toker *et al.* (2018) who used compression method to evaluate the hardness of chocolate, discovered a similar trend in that chocolate where the texture was decreased during shelf period.

3.13. Free fatty acids

FFA is a term that is frequently used to describe the quality of an oil and whether it is suitable for human consumption. During the 60 days of storage study the free fatty acid were analyzed in lipid phase extracted from chocolate samples at regular intervals. Findings indicated that the levels of free fatty acids in days were non-significantly impacted by the treatments. T_5 had the highest free fatty acid concentration of 0.64 ± 0.02 % on 60^{th} day as reported in Table 3. With increasing storage days, the FFA percentage

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increased in all the samples. At 60th day, all treatments reached their maximum level. The increasing trend of free fatty acids in chocolate can depend upon number of ingredients including type of oil and powder procured from cocoa beans. During storage, the hydrolysis of fats can lead to fatty acid release within the sample. According to the research by Divya and Baskaran (2016) coconut oil used in showed a free fatty acids level of less than 0.5%, which indicates that it has undergone a negligible amount of oxidative change.

3.14. Peroxide Value

The degree of oxidation on chocolate samples was assessed by measuring PV in the presence of BG oil as a source of natural antioxidants at 25°C for 60 days. Results demonstrated a non-significant difference ($P \geq 0.05$) in storage and its interaction on the amounts of PV in all treatments. At the end of 60 days of storage time, the lowest peroxide value was noticed for the T_0 in which 100% coconut oil was used while highest peroxide value was detected in treatment T_5 in which 50% BG oil and 50% coconut oil was used, as shown in Table 3. The initial PV of BGO based treatments was higher than control owing to higher inherent PV of BGO that was extracted through Soxhlet extraction, which is why incorporation of BGO in combination with coconut oil in higher doses consecutively increased the PV of developed chocolates. The oil type and its chemical composition significantly influence the PO value of chocolate. Peroxide levels for the control sample and the BGO chocolate both increased noticeably over the course of the 60-day storage period, but at the conclusion of the period, these values remained below the permitted levels, indicating the samples' stability during storage. These outcomes closely resemble the research carried out by Muhammad *et al.* (2018) who studied physicochemical properties of chocolate developed by cinnamon nanoparticles found out raise in peroxide value during storage.

3.15. Energy value

The results revealed that as the percentages of the BGO increased, the caloric value gradually decreased as well. As presented, the results showed that T_4 had minimum caloric value as compared to other treatments. An apparent decrease in energy value of BGO based chocolate was observed as compared to control, owing to replacement of sugar with zero-calorie stevia as replacer. Another reason could be lower calorie index of bitter gourd oil (34 kcal/100g) as compared to coconut oil with energy value of 892 kcal/100g (USDA Food Data Central) which results in significantly ($P < 0.05$) lower calorie values of BGO based chocolates in assorted treatments. Verna (2013) also reported that caloric value of the chocolate decrease was observed during storage. Dumbrava *et al.* (2020) observed that energy value increase. A highly significant effect of BGO on treatment levels of chocolates was observed ($P \leq 0.05$). However, a non-significant effect was observed among storage and treatment interaction. As the storage period got to 60 days, a substantial ($P > 0.05$) drop in energy value was detected.

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Table 3: Means for the effect of treatment and storage intervals of BGO Chocolate

	Treatment	Days				
		0	15	30	45	60
Textural hardness (N)	T_0	32.61±1.96	31.01±2.08	30.82±1.77	30.02±1.84	28.87±1.77
	T_1	28.37±1.72	27.95±1.63	27.88±2.07	27.21±1.79	26.58±0.91
	T_2	30.95±1.64	30.12±1.64	29.57±1.89	29.03±1.94	28.78±1.77
	T_3	33.12±0.88	32.74±1.96	32.39±0.93	32.01±1.64	31.83±2.01
	T_4	34.01±1.76	33.85±1.69	33.51±1.72	32.97±0.75	32.51±1.96
	T_5	36.12±1.61	35.89±1.76	35.48±1.86	35.11±1.97	34.94±1.61
Free Fatty Acid %	T_o	0.26 ± 0.02	0.29 ± 0.01	0.32 ± 0.01	0.35 ± 0.02	0.37 ± 0.02
	T_1	0.33 ± 0.03	0.35 ± 0.04	0.37 ± 0.03	0.39 ± 0.03	0.42 ± 0.01
	T_2	0.37 ± 0.01	0.40 ± 0.02	0.42 ± 0.02	0.43 ± 0.01	0.45 ± 0.03
	T_3	0.43 ± 0.04	0.48 ± 0.03	0.50 ± 0.01	0.53 ± 0.04	0.57 ± 0.04
	T_4	0.50 ± 0.03	0.53 ± 0.01	0.55 ± 0.03	0.57 ± 0.01	0.58 ± 0.01
	T_5	0.58 ± 0.01	0.60 ± 0.04	0.62 ± 0.01	0.63 ± 0.02	0.64 ± 0.02
Peroxide value (meq O_2/kg)	T_0	1.55 ± 0.44	2.01 ± 0.40	2.47 ± 0.43	2.89 ± 0.46	3.09 ± 0.37
	T_1	2.71 ± 0.34	2.89 ± 0.37	3.25 ± 0.46	3.88 ± 0.35	4.25 ± 0.41
	T_2	3.16 ± 0.41	3.84 ± 0.32	4.31 ± 0.35	4.91 ± 0.31	5.64 ± 0.35
	T_3	4.21 ± 0.49	4.46 ± 0.43	5.23 ± 0.43	5.81 ± 0.39	6.36 ± 0.47
	T_4	5.89 ± 0.51	6.18 ± 0.39	6.87 ± 0.35	7.23 ± 0.41	7.99 ± 0.51
	T_5	6.72 ± 0.41	7.01 ± 0.43	7.43 ± 0.45	7.36 ± 0.49	7.92 ± 0.43
Energy value (kcal/100g)	To	535.01±10.93	532.25±12.39	534.81±11.53	536.13±10.88	535.11±9.84
	T1	441.23±12.21	440.45±8.66	439.59±12.87	439.12±11.76	437.57±14.73
	T2	425.18±8.24	424.82±7.62	424.34±8.53	422.92±12.01	421.43±12.56
	T3	404.82±8.32	404.19±8.97	403.93±11.86	402.51±9.72	401.91±13.25
	T4	390.34±7.36	389.12±13.34	389.91±12.41	387.18±12.19	385.87±9.39
	T5	378.21±9.89	375.42±11.72	374.82±10.96	371.92±10.14	370.15±12.32

3.16. Color

Color is the most important quality attribute in all kind of food processing industries. It is the first quality parameter evaluated and mainly influences the consumers choice, preference and product acceptance. The F-value predicted that interaction between storage period and treatment had a highly significant influence ($P<0.05$) while its interaction affected significantly results for the color description variables L* of chocolate. Chocolates with varying concentration of bitter gourd oil were stored at room temperature for 60 days. Findings from the investigation showed that the average values of L* of bitter gourd chocolate ranged from 27.16 to 27.42. The highest value of L* was observed in T_1 (29.49 ±0.46) and lowest value of L* was found in T_5 (27.96±0.51) at 0 day. The color description variable of L* decreased during storage. The results (Table 3) depicted those average values of a* of chocolate developed by bitter gourd oil ranged from 7.25 to 6.35. The F value predicted that storage, treatments and its interaction affected highly significantly ($P<0.05$) results for the color description variables a* of bitter gourd chocolate. The highest value of a* was found in T_0 (7.67±0.24) and lowest value was observed in T_5 (6.77±0.23) at 0 day followed by T_1, T_2, T_3, and T_4. It was observed that a value decreased with the increase of bitter gourd oil. The color description variable of a* increased during storage. The results (Table 3) revealed that the average values of b*

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ranged from 7.27 to 5.40. The F value predicted that storage, treatments and its interaction affected highly significantly (P<0.05) results for the color description variables b* of bitter gourd chocolate. T_0 has highest b* value (7.85±0.18) while T_5 has lowest value (5.84±0.23) at 0 day. The color description variable of b* decreased during storage. A study was done by Ekantari *et al*. (2019) on fortification of chocolate with nano-capsulated with dietary blue green algae in which color L*, a* and b* values decreased during the storage.

Table 3: Means for the effect of treatment and storage intervals of L*, a* and b* in BGO Chocolate

	Treatment	Days				
		0	15	30	45	60
L*	T_0	29.70±0.49	26.98±0.45	26.74±0.38	26.37±0.46	26.03±0.46
	T_1	29.49±0.46	29.21±0.28	28.97±0.48	28.61±0.26	28.27±0.41
	T_2	29.01±0.22	28.89±0.41	28.61±0.30	28.37±0.30	28.21±0.24
	T_3	28.85±0.34	28.64±0.38	28.41±0.53	28.25±0.36	28.11±0.58
	T_4	28.23±0.53	28.01±0.26	27.89±0.31	27.68±0.44	27.35±0.47
	T_5	27.96±0.51	27.68±0.37	27.36±0.55	27.13±0.22	26.98±0.35
a*	T_o	7.67 ± 0.24	7.43 ± 0.23	7.26 ± 0.23	7.01 ± 0.25	6.92 ± 0.22
	T_1	7.08 ± 0.21	6.96 ± 0.19	6.75 ± 0.13	6.43 ± 0.19	6.29 ± 0.19
	T_2	7.02 ± 0.14	6.91 ± 0.15	6.71 ± 0.22	6.39 ± 0.24	6.26 ± 0.18
	T_3	6.96 ± 0.24	6.84 ± 0.22	6.64 ± 0.25	6.34 ± 0.19	6.23 ± 0.15
	T_4	6.83 ± 0.15	6.71 ± 0.22	6.58 ± 0.18	6.28 ± 0.21	6.04 ± 0.24
	T_5	6.77 ± 0.23	6.51 ± 0.17	6.39 ± 0.22	6.14 ± 0.13	5.95 ± 0.23
b*	T_0	7.85 ± 0.18	7.41 ± 0.21	7.19 ± 0.21	7.03 ± 0.13	6.89 ± 0.21
	T_1	6.15 ± 0.19	6.92 ± 0.17	6.68 ± 0.18	6.31 ± 0.17	6.21 ± 0.22
	T_2	6.08 ± 0.18	5.86 ± 0.21	5.62 ± 0.21	5.36 ± 0.24	5.17 ± 0.24
	T_3	6.01 ± 0.25	5.73 ± 0.24	5.58 ± 0.25	5.29 ± 0.18	5.11 ± 0.24
	T_4	5.92 ± 0.16	5.67 ± 0.15	5.45 ± 0.22	5.23 ± 0.19	5.07 ± 0.16
	T_5	5.84 ± 0.23	5.62 ± 0.24	5.39 ± 0.25	5.16 ± 0.22	5.01 ± 0.24

1.1. Sensory analysis

The chocolates of various treatments were scored by the taste panel using the 9-point hedonic scoring scale (where 1=Extremely dislike and 9= Like excellent) (Hooda and Jood, 2005). They were tasked with assessing the qualities of the finished product in order to express their perspectives like color, flavor, taste, texture and overall acceptability. Chocolates with different treatments were placed on clear dishes and labelled with random codes during the sensory assessment. Panelists were given access to cold water and crackers to rinse their mouths in between assessments of the samples. In the case of color, no marginal variation was observed between treatments by panelists. Chocolates with increasing levels of bitter gourd oil gives dark color but the sensorial evaluation had favorable remarks for this color change. The color profiles of T_3 were higher owing to unique color appearance of the chocolate. During storage, no significant change in sensory profile of chocolate color was seen as shown in the Figure 3.1. Results demonstrated particularly significant results among treatments, days, and their

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collaborations shown in Figure 3.2. The flavor was assessed from the information the judges set where T_3 was rated slightly better. BGO chocolate developed with higher concentrations T_4 and T_5 had slightly unpleasant flavor, as expressed by panelists. Flavor, texture, and their composition had a great effect on taste which is defined by the tongue. They had shown highly significant distinction with treatments and days. The means for the taste of chocolates were demonstrated in Figure 3.3 with BGO at different levels for taste. T_3 received the highest taste score. Bitter gourd oil chocolate texture is a sign of good quality characteristics that contribute in spread factor and thickness. Mean values of texture of chocolates for the treatments having maximum score for T_3 which considered as best in all treatment while last for T_5 as revealed in Figure 3.4. The findings made it clear that there are considerable differences in texture across various treatments. The mean values for overall acceptability of BGO chocolate had the highest score for T_3 having 15% bitter gourd oil. Mean values for over acceptability of chocolates prepared by bitter gourd oil were shown in Figure 3.5. Also, the findings make it clear that the overall acceptance changed significantly among different treatments. The outcomes support the conclusions of Razavizadeh *et al.* (2021) who described that these sensory characteristics i.e. overall acceptability of chocolate improved due chia seed oil concentration. In another study by Divya *et al.* (2016) overall acceptability of chocolate based on consumer preference was improved by the addition of coconut oil.

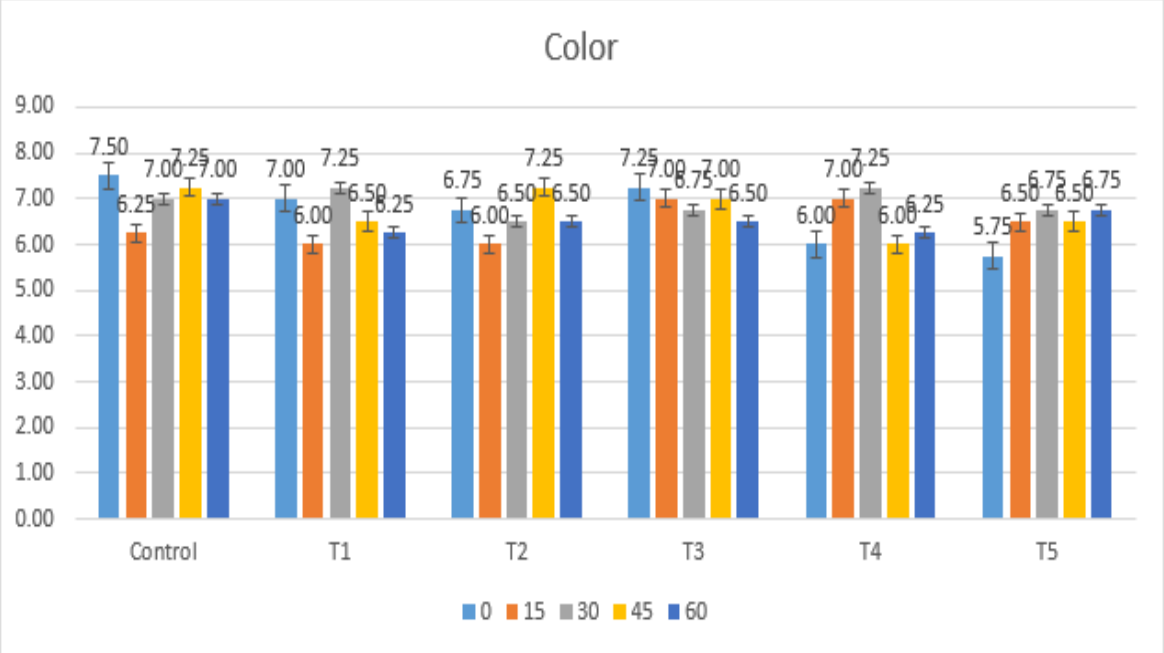

Figure 3.1: Means for the effect of treatment and storage intervals on the color of BGO chocolate

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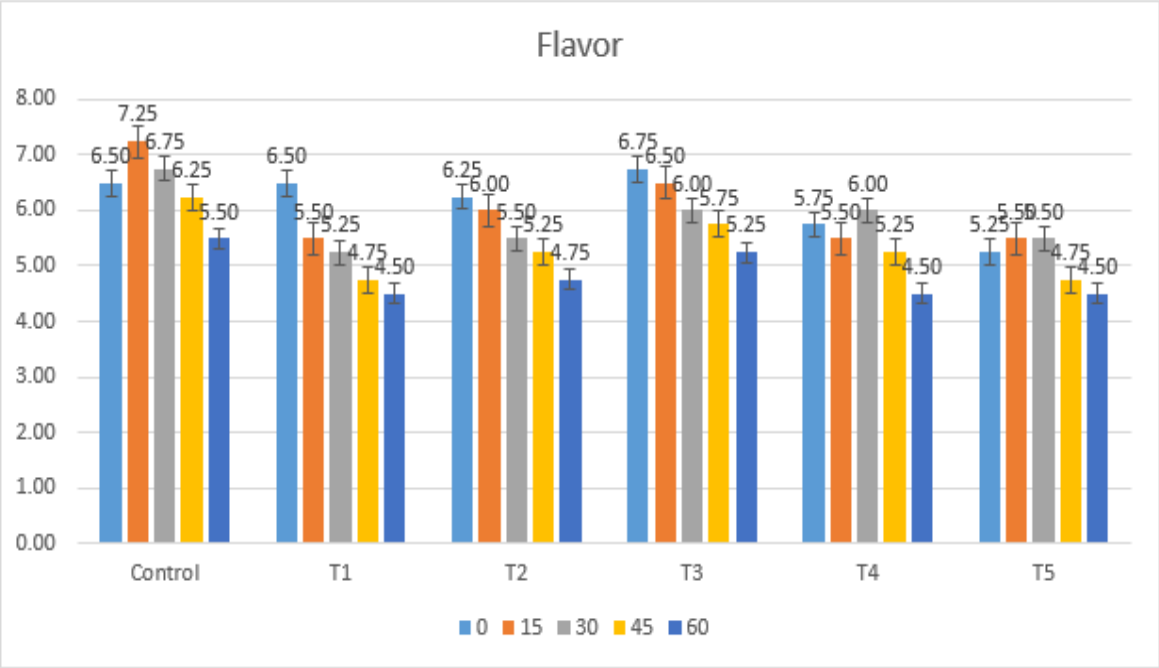

Figure 3.2: Means for the effect of treatment and storage intervals on the flavor of BGO chocolate

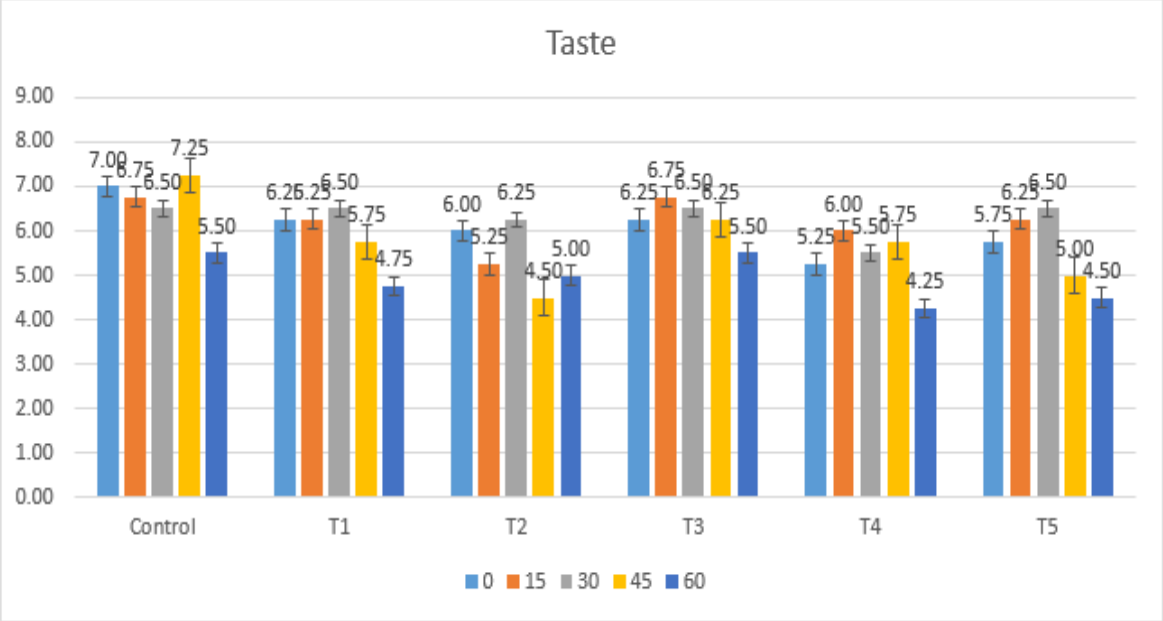

Figure 3.3: Means for the effect of treatment and storage intervals on the taste of BGO chocolate

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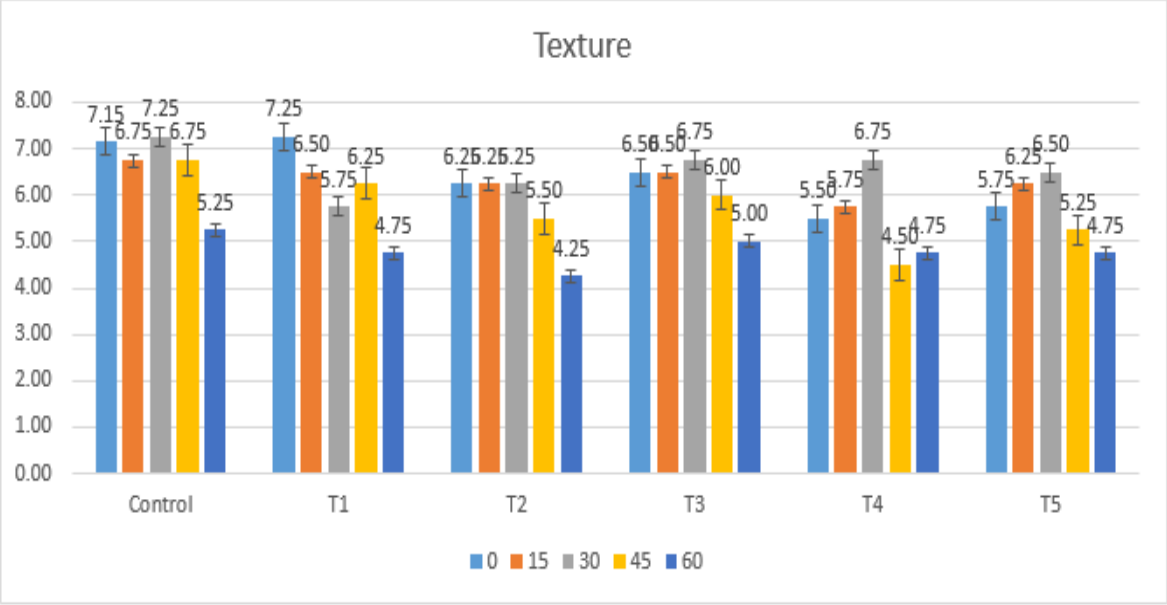

Figure 3.4: Means for the effect of treatment and storage intervals on the texture of BGO chocolate

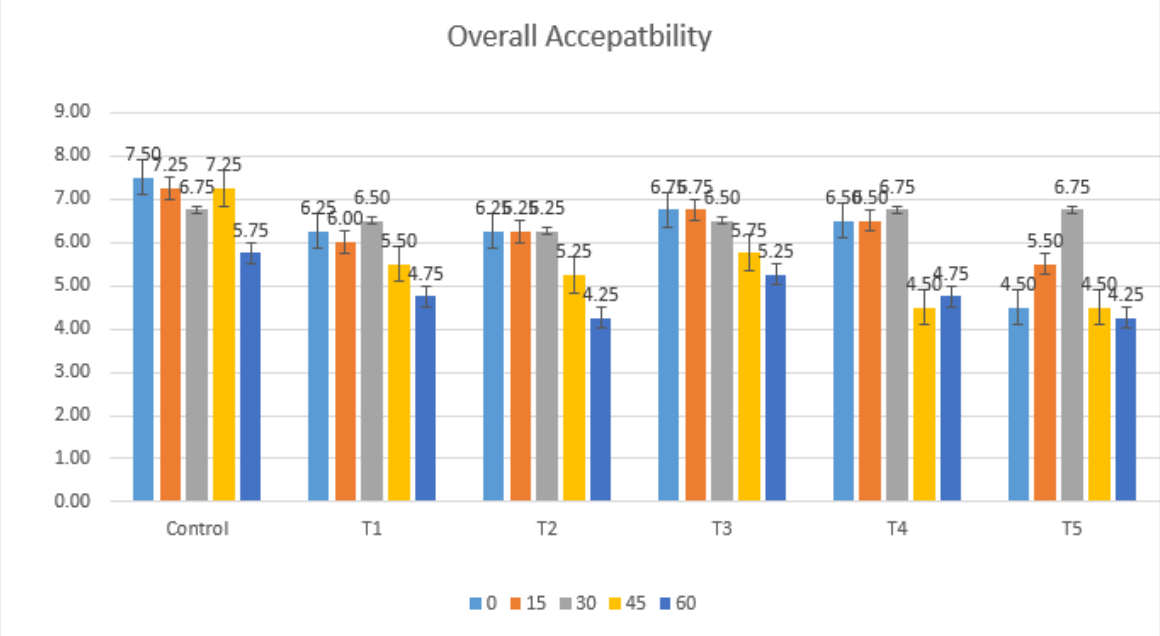

Figure 3.5: Means for the effect of treatment and storage intervals on the overall acceptability of BGO chocolate

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CONCLUSION

Overall, the results concluded Chocolate developed with bitter gourd oil exhibited stability in multiple physicochemical and sensorial analyses and may also serve as a functional food if consumed with appropriate concentrations of bitter gourd oil replaced with coconut oil. This product, if developed at larger scale and commercialized, would aptly benefit the population with anti-diabetic effect and also contribute to value addition of bitter gourd oil into a marketable and healthy commodity.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

Syed Muhammad Abrar Ul Haq and Muhammad Asim Shabbr conceived the work, collected raw material, carried out experimentations, analyzed and interpreted data, and wrote the article. Rana Muhamad Aadil assisted in experimentations, and read the article. Muhammad Anjum Zia supervised the work and read the article. All authors have approved the final article.

Conflict of interest

The authors declare no potential conflict of interest.

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