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Amla Extract: A Natural Antioxidant and Antimicrobial for Cupcake Quality

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Abstract

Lipid oxidation and microorganism growth cause a reduction in cupcake qualities. Because of the harmful effects of synthetic preservatives, there is a growing demand for preservatives and natural antioxidants. In this study, the phytochemical properties and antimicrobial activity of *Emblica oficinalis* (amla) were investigated. Then the effect of adding amla extract at levels 1, 2, and 3% on the qualitative attributes of cupcakes was examined. In the produced cupcake samples, the antioxidant activity was compared by assessing DPPH%, peroxide values, and free fatty acid over 3 weeks of storage at room temperature. Also, the total aerobic bacteria and fungi in cupcake samples were counted. Besides, the color, odor, texture, taste, and overall acceptability were evaluated. The amla extract was shown to be high in phytochemical components (ascorbic acid, flavonoids, phenolics, and DPPH). Therefore, the oxidation rate was slow during the storage of cupcakes containing amla extracts. Also, amla extract led to an improvement in the quality attributes of the cupcake while maintaining good sensory and physiochemical properties. Amla extract at 3% recorded the lowest peroxide value, and its antioxidant activity was close to that of the BHT sample. As a result, it is possible to propose that the cupcake with amla extract at level 3% provides the greatest antimicrobial activity, while levels 1 and 2% provide the best acceptability in other properties.

***Keywords**:* amla; extract; antioxidant activity; antimicrobial activity; cupcake .

1. Introduction

One of the oldest and most widely consumed baked products in the world is the cupcake; due to its nutritional content, variety, and low cost. The main problems with cake production are lipid oxidation and the growth of microbes, which can reduce the shelf life of the finished product. This problem can be avoided by utilizing antioxidants and preservatives. In food, the manufacturer uses antioxidants and antimicrobial substances to keep products fresher [1]. Numerous foods have been found to contain synthetic antioxidants, such as butylated hydroxyl toluene (BHT) and butylated hydroxyl anisole (BHA) [2]. But because of their harmful effects on human health [3], the use of these synthetic substances has started to be controlled, which has boosted the demand for natural antioxidants. This has increased curiosity about finding more affordable, safe, and efficient natural antioxidant and preservative sources [4].

The extracts of plants can provide biological properties, making them viable substitutes for synthetic preservers [5,6]. Many international research teams are studying natural antioxidants, and the body of knowledge in this area is expanding quickly [7]. Phytochemicals are used as functional components in dietary supplements, functional foods, medicines and a variety of other uses. Plant sources such as berries, seeds, herbs, spices, and other are rich in natural antioxidants [8]. Antimicrobial food additives are applied to food to prevent microorganism deterioration and to extend the shelf-life of the foods [9]. Plant extracts include a diverse spectrum of metabolites and phytochemicals, which

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contribute to their antimicrobial properties. Plant extracts antimicrobial efficacy, vary depending on the concentration of active components present, extract synthesis process, and the application method [10].

Amla (*Emblica oficinalis*) is a small fruit in the Euphorbiaceae family [11]. Because of its high concentration of phenols, amla might be considered a source of antioxidants, nutraceuticals or therapeutic components [12]. It is considered the wonder fruit for health and is one of the India oldest fruits. It is also a potential food additive [13-15]. Tannins, phyllembilic acid, rutin, curcuminoids, ascorbic acid and numerous minerals are among the nutrients and phenolic components found in amla [16]. A small amla fruit has the equivalent amount of vitamin C to two orange fruits [17]. A number of researches on the use of amla powder and extracts in bakery products have been published. According to Reddy et al. [18], the addition of amla extract to biscuit had a superior antioxidant effect when compared to the impact of BHA, as the percentage rise in both peroxide and acid values after 6 weeks was lower than that of the control and BHA treated samples. Abid et al. [19] reported that the addition of 10% amla powder to cupcakes' improved the nutritional, and physical qualities as well as sensorial properties when compared to other formulations. Also, Patel et al. [20] use industrial waste of amla to make functional biscuits. The best functional biscuit in terms of dough rheology, microstructure, texture, and sensory qualities is the one produced by replacing 10% of wheat flour with amla pomace. Studies on the antibacterial and antioxidant qualities of amla extracts and their effect on cupcake qualities are nevertheless scarce.

Therefore, the study objectives are to: (i) assess the phytochemical characteristics and antimicrobial activities of amla extract; (ii) evaluate the influence of amla extracts as natural antimicrobial and antioxidant agents on cupcake qualities; and (iii) examine the storage stability of cupcakes.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals

Butylated hydroxytoluene (BHT), gallic acid, 2.6 dichlorophenol indophenol, other chemicals were provided by El-Gomhouria Trading Chemicals and Drugs Company in Egypt.

2.1.2. Microorganism's strains

Gram-positive bacteria like *Staphylococcus aureus* (ATCC 25923) and *Bacillus subtilis* (ATCC 6635), gram-negative bacteria like *Escherichia coli* (ATCC 25922) and *Salmonella typhimurium* (ATCC 14028), and one yeast strain Candida albicans (ATCC 10231) were used in the test.

2.1.3. Wheat flour

Wheat flour (72 % extraction) without antioxidant (Egyptian-French Company for Trade and Mills, Cairo, Egypt).

2.1.4. Additives materials

Sucrose, fresh eggs, skim milk powder, baking powder, vanilla, shortening and salt were obtained from local market

2.2. Methods

2.2.1. Preparation of amla extract

Phytochemicals were extracted from amla powder using ethanol alcohol according to Alshallash et al. [21] with slight modification. In brief, 5 gram of powder was extracted in triplicate with 50 mL of ethanol using magnetic stirring for 12 hours, then filtered using Whatman filter paper (No. 1).

2.2.2. Preparation of cupcake

Cupcake was made according to the recipe supplied by Khalifa et al. [22] A.A.C.C. [23] with minor modification by using the formulas displayed in Table (1). The first sample basic formula was prepared without additives (control), the second sample basic formula with the addition of 200 ppm BHT, and the other samples were prepared with the addition of different levels of amla extracts (1, 2 and 3 %). The cupcake were baked at 195 °C for 20 minutes and left to cool for roughly an hour on racks before being evaluated.

Table 1: Ingredients used in producing different cupcake blends

Ingredients	Control	Treatments			
		BHT	A1	A2	A3
Wheat flour (72%)	250	250	250	250	250
Fresh egg (g)	110	110	110	110	110
Sucrose (g)	125	125	125	125	125
Salt (g)	3.5	3.5	3.5	3.5	3.5
Baking powder (g)	12.5	12.5	12.5	12.5	12.5
Vanilla (g)	2.0	2.0	2.0	2.0	2.0
Skim milk powder (g)	25.0	25.0	25.0	25.0	25.0
Shortening (g)	53.5	53.5	53.5	53.5	53.5
BHT (ppm)	-	200	-	-	-
Amla extract (%)	-	-	1	2	3

Where: BHT: Butylated hydroxyl toluene; A1, A2 and A3: Amla extracts at levels 1, 2 and 3 % respectively.

2.3. Analytical methods

2.3.1. Characterization of amla

2.3.1.1. Phytochemicals evaluation

Analysis of total phenolic, total flavonoid and antioxidant activity

Extraction:

Extracts for total phenolics, total flavonoids and antioxidant activity were prepared using methanol. One ml from sample was mixed with 100 ml methanol and homogenized using the Ultra-Turrax homogenizer. The homogenates were kept at 4°C for 12 hrs and then centrifuged at 10,000 rpm for 20 min. The supernatants were recovered and stored at -20 °C until analysis.

Analysis of total phenolic content

The total phenolic content was determined according to the Folin-Ciocalteu procedure [24]. Briefly, the extract (500 µl) was transferred into a test tube and oxidized with the addition of 250 µl of Folin-Ciocalteau reagent. After 5 min, the mixture was neutralized with 1.25 ml of 20% aqueous Na_2CO_3 solution. After 40 min, the absorbance was measured at 725 nm against the solvent blank. The total phenolic content was determined by means of a calibration curve prepared with gallic acid, and expressed as mg of Gallic acid equivalent (GA) per g of sample.

Flavonoid content was measured according to reference [25]. 2 mL of extract was mixed with 0.2 mL of 5% sodium nitrate. After 5 minutes, 0.2 mL of aluminum chloride was added, then 2 mL of 1N NaOH was added, and the volume was increased to 5 mL with water. After 15 minutes, the absorbance was read at 510 nm, and the flavonoid was expressed in mg Quercetin equivalents (mg QE/g).

The 2.6 dichlorophenol indophenol titration methods [26] were used to measure the ascorbic acid, and the findings were expressed as mg / 100 g.

Free radical scavenging activity was determined using the stable 1, 1-Diphenyl-2-picryl-hydrazyl (DPPH) as reported by Lim et al. [27]. The final concentration was 50 µM for DPPH and the final reaction volume was 3.0 mL. The absorbance was measured at 517 nm against a blank of pure methanol at 60 min. Percent inhibition of the DPPH free radical was calculated by the following equation:

$$\text{Inhibition } (\%) = 100 \times (A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}$$

Where:

- A_{control} is the absorbance of the control reaction (containing all reagents except the test compound).

- A_{sample} is the absorbance of the test compound.

2.3.1.2. Antimicrobial assay

By agar disc diffusion approach, the amla extract was screened for its antibacterial activity against gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and gram-negative (*Escherichia coli* and *Salmonella typhimurium*) pathogens [28]. The discs were placed on agar plates, and each disc in each Petri plate containing the corresponding pathogens received an injection of amla extract (10 µL). This was followed by a 24-hour incubation period. Next, using a metre ruler, the zone of inhibition surrounding each disc was measured in millimetres and recorded.

Candida albicans was used as a test for amla extract's antifungal properties. The discs were placed on PDA agar plates, and 10 µL of amla extract per disc was provided as needed. The discs were then incubated for 72 hours at room temperature. Next, unambiguous zones of inhibition were measured and recorded in millimetres using a metre ruler.

2.3.2. Characterization of cupcake samples

2.3.2.1. Antioxidant activity

Free radical scavenging activity was determined using the stable 1, 1-Diphenyl-2-picryl-hydrazyl (DPPH) as reported by Lim et al. [27]. The final concentration was 50 µM for DPPH and the final reaction volume was 3.0 mL. The absorbance was measured at 517 nm against a blank of pure methanol at 60 min. Percent inhibition of the DPPH free radical was calculated by the following equation:

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Where:

- A_{control} is the absorbance of the control reaction (containing all reagents except the test compound).

- A_{sample} is the absorbance of the test compound.

2.3.2.2. Peroxide value (PV)

PV of cupcakes was calculated using an AOAC method [29]. In a mechanical shaker, 10 g of ground cake was treated with 50 mL chloroform for 30 minutes. Then 10 mL of the extract was mixed with 15 mL of glacial acetic acid and 500 µL of potassium iodide solution (saturated). After 10 - 15 minutes, a 1 % starch indicator was added and the mix was titrated against sodium thiosulfate, and the result was given in g O_2 /100mg.

2.3.2.3. Free fatty acid (FFA)

FFA were assessed using the IUPAC standard method [4], and the results were represented as percentage of oleic acid.

2.3.2.4. Color analysis

Using a CR-400 Chroma Metre (Konica Minolta, Japan), the color index of the baked cupcake was measured and reported as L* (lightness/darkness), a* (redness/greenness), and b* (yellowness/blueness) in CIE units [30]. Five measurements were made at various locations on the cupcake surface, and the values were noted.

2.3.2.5. Microbiological analysis

Microbiological activity was measured as the total count of bacteria, mold and yeast in cupcake stored at

room temperature for three weeks using the plate methods of Onuorah and Akinjede [31].

2.3.2.6. Sensory evaluation

According to the AACC [32], the sensory evaluation of cupcake treatments was estimated. Every sample was presented in duplicate, arranged at random on a plate, and then evaluated by 25 staff members from the Food Science and Technology Department and Biochemistry Department, Faculty of Agriculture, Al-Azhar University. The general appearance, color, odour, taste, texture, and overall acceptability were the aspects that were assessed.

2.3.2.7. Statistical analysis

Using SPSS software, the variance examination (ANOVA) was performed (version 22.0) at the level of $p<0.05$, and Duncan's multiple range test was employed to determine mean separation. The results are presented in terms of mean value and standard deviation (SD).

3. Results and Discussion

3.1. Characterization of amla extract

3.1.1. Antimicrobial activity

The inhibition zone diameter was used to determine the impact of amla extract on the development of strains of *Candida albicans, Bacillus subtilis, Salmonella typhimurium*, and *Escherichia coli*. Figure 1 tabulates the millimeter-accurate measurements of the inhibitory zones that appear on the medium after the incubation time.

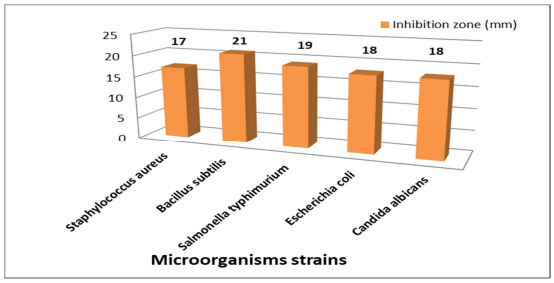

Figure 1: Inhibitory effects of amla extract against tested microorganisms calculated as diameters of inhibition zones (mm).

Amla extract inhibited the growth of investigated microorganisms strains to varying degrees, with *Bacillus subtilis* having the highest inhibition zone (21 mm), and *Staphylococcus aureus* having the lowest (17 mm) compared to *Salmonella typhimurium* (19 mm) and *Escherichia coli* (18 mm). For *Candida albicans*, the inhibitory zone induced by amla extract was 18 mm. The capacity of phytochemicals to bond with microbial cell walls, preventing cell division and growth, may explain amla extract's antibacterial effect. Antimicrobial potential of amla extracts is due to the presence of phenolic compounds, flavonoids, and organic acids [33]. Antibacterial compounds destroy their cytoplasmic membrane and cell wall, cause damage to proteins, and negatively affect the synthesis of DNA and RNA [34,35]. Many research investigations shown that, possesses antimicrobial activity, including antibacterial, antifungal, and antiviral activities [36].

3.1.2. Phytochemical contents

The content of total polyphenols, total flavonoids, ascorbic acid and % of DPPH inhibition of the amla is presented in Figure 2. Amla has been discovered to be high in phytochemical substances. It has around 181.21 mg GA/g of total phenols and 33.38 mg QE/g of total flavonoids. This outcome was in line with the information provided by Malik et al. [37] who discovered that amla had a total phenolic content ranging from 75.75 to 200.93 mg GA/g and flavonoids content ranging from 5.57 to 30.94 mg QE/g. Polyphenolic compounds as the most powerful phytochemicals with antioxidant properties [12,38,39].

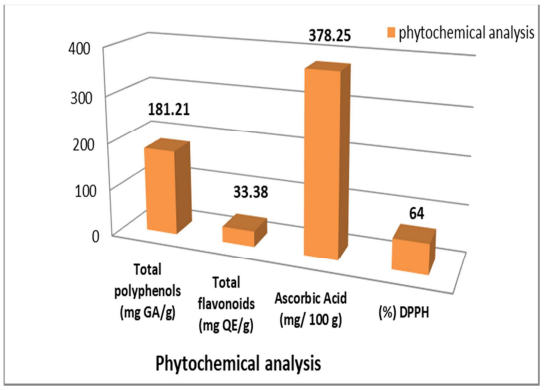

Figure 2: Phenolic, flavonoid, ascorbic acid content and DPPH (%) in amla

Ascorbic acid is an essential nutrient for human health. In this study, ascorbic acid concentration of 378.25 mg/ 100 g of amla. Aayush [40] discovered nearly similar trends in vitamin C content in dried amla (362.67 to 440 mg/100 g). Our result higher than the amount of vitamin C published by Parvez et al. [41], who find that the vitamin C of amla powder was 298 mg/100 g; in contrast, this result was lower than the values reported by KC et al. [42], who found that the vitamin C content was 573 mg/100 g. The variance in the findings may be due to the variation in the extraction solvents.

DPPH is typically employed to assess antioxidant activity, amla contains a lot of antioxidants. Amla powder has an antioxidant capacity of 64.3 %. Goraya and Bajwa [43] discovered a similar trend in antioxidant activity. The DPPH% was lower than the

results noted by KC et al. [42], but higher than those found by Parvez et al. [41].
These findings generally agree with those of other researchers [12, 37]. They discovered that there is a linear link between polyphenol level and antioxidant function and that a high total polyphenol concentration enhances antioxidant performance.

3.2. Characterization of cupcake

3.2.1. Sensory evaluation

Sensory evaluation is a key aspect in determining the quality of foodstuffs. Cupcake samples were assessed in order to determine consumer acceptance. Table 2 shows how adding 200 ppm of BHT, 1, 2, and 3% of amla extract influenced the organoleptic aspects of the evaluated cupcakes. The appearance, color, odor, taste, and acceptability of the cupcake samples differed significantly, while no significant difference between the textures of the cupcakes was observed ($p < 0.05$). At 1 and 2% of amla extract, results showed improvement in all sensory properties as compared to the control and those with BHT and 3% amla extract (Figure 3).

The highest appearance scores were attributed to the cupcakes containing amla extract 1 and 2% (14.86 and 14.98 with non-significant), followed significantly by cupcakes with 3% amla extract, BHT, and control (13.58, 13.51, and 13.41 with non-significant). Cupcake color changed as the amount of amla extract added increased; the crust became darker, especially at 3%. Similarly, surface cupcake downs were also observed with the increased amla extract addition. Whereas, amla extract added a brown color to the finished product. The highest color scores were attributed to the cupcakes containing amla extract 2% (28.86) and 1% (28.56) with non-significance, followed by cupcakes with BHT (26.65) and control (26.22). On the other hand, the cupcake with 3% amla extract had a significantly lower color score (25.55).
For odor score, the cupcake with amla extract had the highest scores (18.88, 18.82 and 18.22, respectively for A1, A3 and A2) followed by cupcake with BHT sample (16.90). The control sample received the lowest odor score (16.73). Concerning the taste, the higher score was recorded for cupcakes prepared with 2 and 1 % (18.63 and 18, respectively) followed by cupcake containing BHT (17.62). The cupcake with 3 % amla extract had lower taste score (16.21).

The mean scores for the texture attribute of the samples showed that control obtained the highest score (14.05), followed by sample 3 % amla extract (13.98), BHT (13.96), A2 (13.94) and A1 (13.87) respectively with non-significant difference between them. In terms of overall acceptability, the cupcake with amla extract at 2 and 1 % received the highest consumer acceptability (94.63 and 94.17 respectively). Whereas cupcake with BHT was recorded 88.64 %, and cupcake with amla at 3% recorded 88.14 % compared to 87.05 % for the control sample. It was found that, amla extract may be incorporated at 1 and 2 % in the formulation of cupcakes.

Table 2: Sensory evaluation of cupcake samples

Samples	General appearance (15)	Color (30)	Oder (20)	Taste (20)	Texture (15)	Overall acceptability (100)
Control	$13.41^{B}\pm0.70$	$26.22^{AB}\pm1.40$	$16.73^{B}\pm0.85$	$16.64^{BC}\pm0.60$	$14.05^{A}\pm0.68$	87.05
BHT	$13.51^{B}\pm0.65$	$26.65^{AB}\pm1.50$	$16.90^{B}\pm0.90$	$17.62^{AB}\pm0.55$	$13.96^{A}\pm0.70$	88.64
A1	$14.86^{A}\pm0.72$	$28.56^{A}\pm1.25$	$18.88^{A}\pm0.98$	$18.0^{A}\pm0.68$	$13.87^{A}\pm0.75$	94.17
A2	$14.98^{A}\pm0.66$	$28.86^{A}\pm1.60$	$18.22^{AB}\pm0.96$	$18.63^{A}\pm0.70$	$13.94^{A}\pm0.68$	94.63
A3	$13.58^{B}\pm0.93$	$25.55^{B}\pm1.3$	$18.82^{A}\pm0.95$	$16.21^{C}\pm0.74$	$13.98^{A}\pm0.65$	88.14

No significant difference ($p<0.05$) in the same letter in the same column.
The table displays each value as mean ± SD

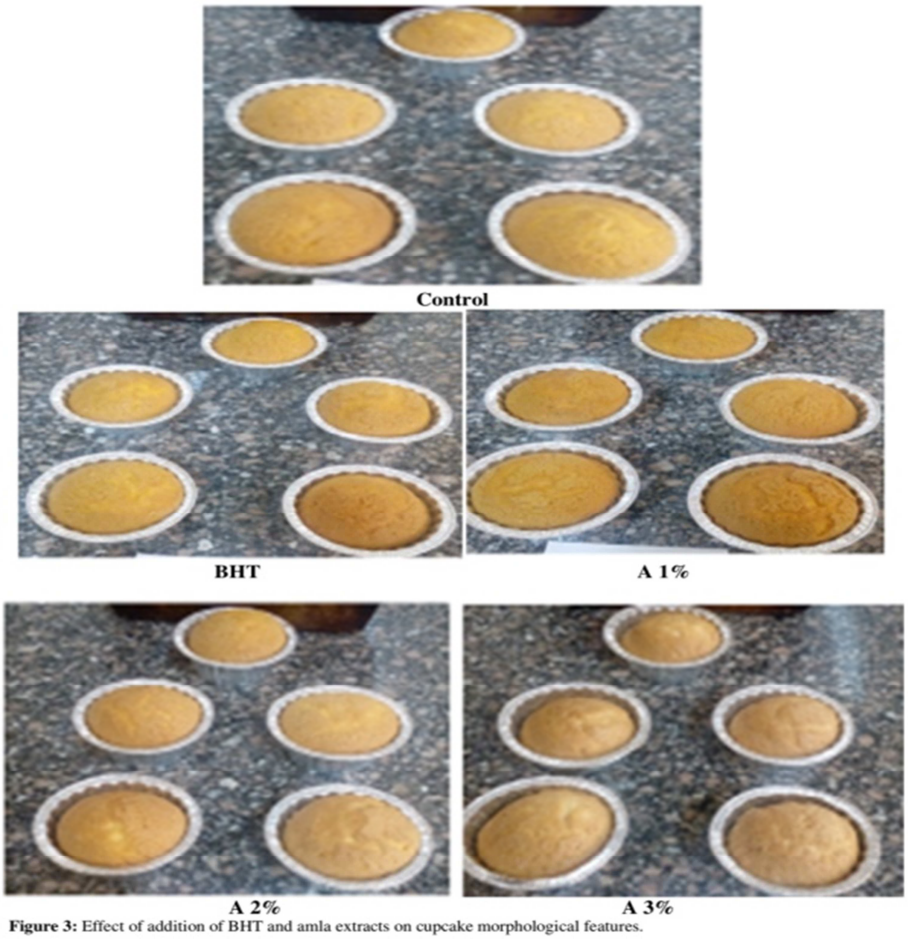

Figure 3: Effect of addition of BHT and amla extracts on cupcake morphological features.

Figure 3: Effect of addition of BHT and amla extracts on cupcake morphological features.

3.2.2. Antioxidant activity

The effects of antioxidants on the cupcakes stability were assessed by measuring DPPH %, FFA and PV periodically during the storage of cupcakes for 21 days.

The DPPH % of cupcakes are shown in Figure 4. The supplemented cupcakes had higher DPPH % than the control (untreated), with the cupcake containing BHT having the highest DPPH %. The DPPH % at zero time in untreated cupcake was significantly lower ($P < 0.05$) than in cupcake containing BHT and amla extracts. The values of DPPH were 36, 68, 52, 57 and 64 % respectively, for control and cupcake with BHT and amla extract at 1, 2 and 3 % respectively.

During storage, the antioxidant activity was observed to decrease in all cupcakes samples. This decrease was marginal in cupcake prepared with BHT and 3 % amla extract, moderate in cupcake containing 1 and 2 % amla extracts and high in control cupcake. After 21 days, the antioxidant activity of the control and cupcake treated with BHT and amla extract at 1, 2 and 3 % decreased to 10, 54, 35, 42 and 51 % respectively. Compared to the remaining samples, the antioxidant activity percentage in samples containing BHT and 3% amla extract was considerably greater ($P < 0.05$) during storage. The results reveal that there is a positive association between the phenolic content of the cupcakes and their DPPH %. When compared to BHT, the addition of 3 % amla extract resulted in the highest lipid stability after 21 days at room temperature. The amount of active compounds naturally present in the amla extract used in this study, as well as their quantity, may be the cause of the variation in antioxidant activity. Previous studies by other researchers have also found that extracts of plant from amla have a high capacity to DPPH % [44,45]. This result are consistent with those of Gul et al. [46] who repoted that high antioxidant activity, notably the ability to scavenge free radicals like DPPH radical, has also been linked to the polyphenol content of amla fruit

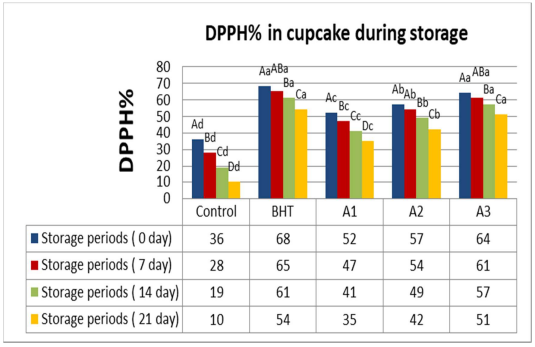

Figure 4: DPPH (%) of cupcake samples during storage.
Different letters vertically indicate a significant difference at ($p<0.05$). Each value in graph is represented as mean ± SD.

PV was measured in all cupcakes to evaluate the degree of peroxide generation owing to fat oxidation (g equivalents of O_2/100 g sample) over the 21 days storage period as indicated in Figure 5. From the figure, the PV was clearly higher in the control cupcake sample than in the BHT and amla extracts treated cupcakes. The PV in all samples was initially very low with values of 0.62, 0.57, 0.62, 0.60 and 0.59 g equivalents of O_2/100 g for the control, BHT, A1, A2 and A3 samples. After 21 days of storage, these values increased in all samples reaching 1.80, 0.99, 1.23, 1.10 and 0.95 g equivalents of O2/100 g. The control sample had much greater increases during storage when compared to samples that were produced with either natural or BHT (1, 2 and 3 % of amla extract).
In addition, increasing the content of amla extract from 1 to 3 % reduce the PV of the cupcake. The cupcake with 3 % amla extract had the lowest ($p < 0.05$) PV when compared to other cupcake samples. This result indicates that amla extract at 3 % has the strongest antioxidant activity, surpassing BHT at 200 ppm. These results are consistent with those of Reddy et al. [18].

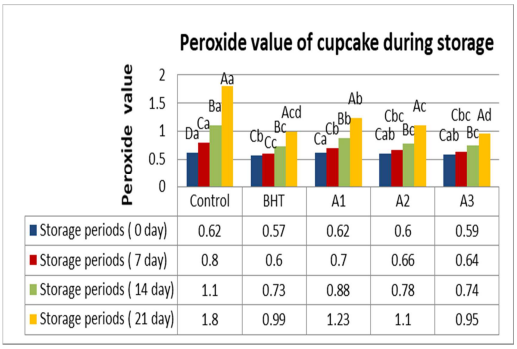

Figure 5: Peroxide value (g equivalents of O_2/100g) of cupcake samples during storage.
Different letters vertically indicate a significant difference at ($p<0.05$). Each value in graph is represented as mean ± SD.

FFA is produced by the breakdown of triglycerides [4] and may be developed by enzymatic activities caused by microorganism's growth. Changes in the FFA contents of the cupcake variants throughout storage are given in Figure 6. From figure, there were significant differences ($p <0.05$) in the FFA content. During storage, the FFA for cupcake samples containing BHT or amla extract (1, 2 and 3 %) were lower than that of the control sample. Over storage, all cupcake samples showed a gradual increase in FFA value. After 21 days of storage, the increase was significant higher in control sample (0.94 % as oleic acid) compared to cupcake samples containing synthetic BHT (0.75 % as oleic acid) or amla extract (0.73, 0.66 and 0.48 % as oleic acid, respectively for A1, A2 and A3 samples). Cupcakes with different levels of amla extract had lower FFA values. Amla extracts at higher levels (3 %) showed a better effect on inhibiting the emergence acidity. Amla extract's phenolic components and flavonoids are very effective at lowering libid levels and glucose [47]. These findings align with the findings of Reddy et al. [18], who reported that amla extract worked to controlling lipid oxidation during storage periods by reducing the values of peroxide and free fatty acid compared to the values under control conditions and butylated hydroxyl anisole in the prepared biscuits.

3.2.3. Color values

Figure 7 shows the color outcomes for cupcakes prepared by adding 200 ppm BHT, 1, 2 and 3 % amla extracts. From statistical analysis, there are no significant differences in the crust and crumb color between the BHT treatment and the control sample

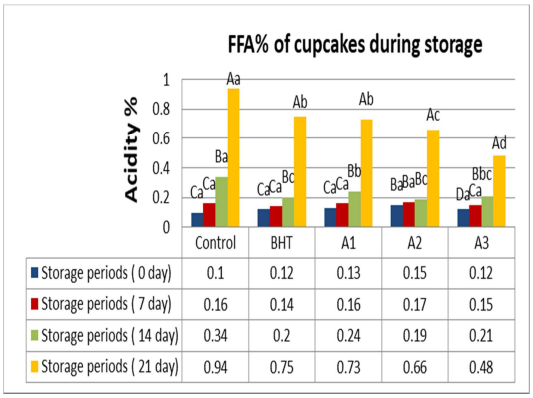

Figure 6: FFA % of cupcake samples during storage.
Different letters vertically indicate a significant difference at ($p<0.05$). Each value in graph is represented as mean ± SD.

However, the addition of amla extracts had a significant effect on the crust and crumb color of cupcake samples as the level of amla addition increased from 1 to 3 %. The crust color changed with the quantity of amla extract changed. It grew darker as indicated by the lower L* values increased

compared to the control and BHT cupcake values. The redness (a*) value of the crust varied, but all of its values were lower than the control crust. Lower L* values imply that the crumb color grew darker. The redness values (a*) of the crumb dramatically decreased ($p<0.05$) as the amount of amla extract was added in increasing amounts. The cupcake's yellow hue was lessened by the addition of amla extract, as seen by a drop in b* values. The yellowness of the cupcake crumb was indicated by b* values, which decreased when the amount of amla extract was increased. The colour of the cupcake crust is created during baking by the caramelization of sugars and the maillard reactions that occur between sugars and amino acids. The degree of ascorbic acid present in amla fruit extracts may be a significant factor in the crumb's colour change. These findings align with those reported by Alkandari et al. [48].

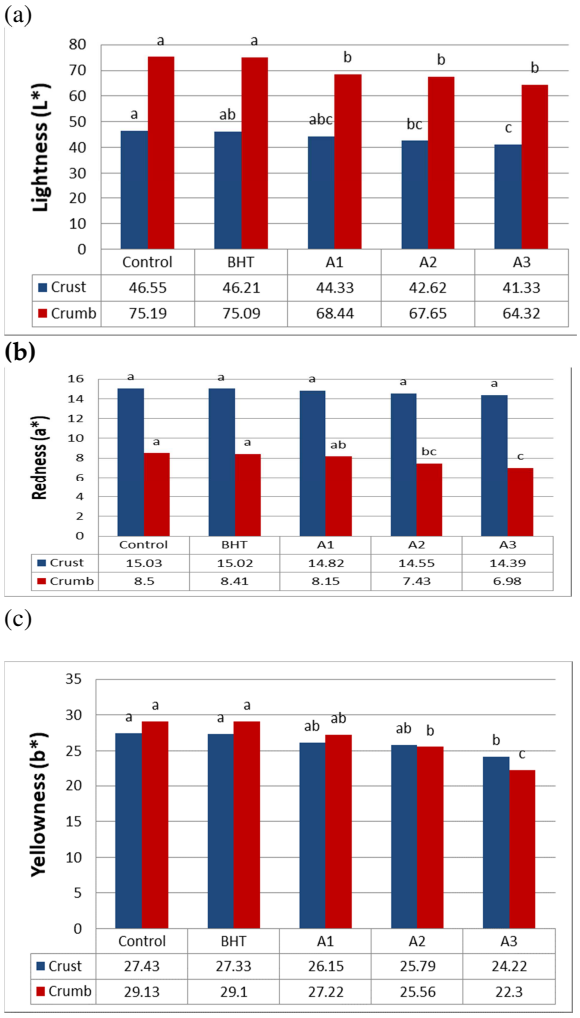

Figure 7: Color values of different cupcake samples at zero time

3.2.4. Microbial evaluation

The development of spoilage macrobiotic in foods is not hazardous to human health, but it has an impact on the shelf-life, textural qualities and overall acceptability of the final products, which influences customer choices. As a result, inhibiting microbial development in foods is critical for food production. Similarly, novel strategies to reduce or completely remove food borne pathogens and spoilage bacteria may be required [49]. Over the course of 21 days of storage, the microbiological status of cupcake samples was assessed every seven days. The results are displayed in Figures 8 and 9. The findings showed that while the amla count rose during storage, the overall number of microorganisms in the cupcakes reduced. In the control, BHT, A1, A2, and A3 samples, the total count of bacteria was 1.60, 1.61, 1.58, 1.59, and 1.56 log cfu/g at the beginning of storage, according to Figure 8. After 21 days of storage, the counts climbed to 6.13, 5.04, 4.77, 4.70, and 4.07 log cfu/g, respectively. When compared to BHT and control samples, the samples containing amla extract exhibited reduced bacterial count. Amla extracts have antibacterial potential and are an important source of new antibiotics and can therefore be used in pharmaceuticals and nutritional supplements which help control infectious disorders through chemotherapy [50]. These results are also compatible with those of [20,51,52], who reported that applying amla inhibited the growth of aerobic bacteria.

In comparison to the BHT and control samples, cupcakes treated with varying amounts of amla extract showed the greatest and highest results in minimising the mold and yeast load after storage (Figure 9).

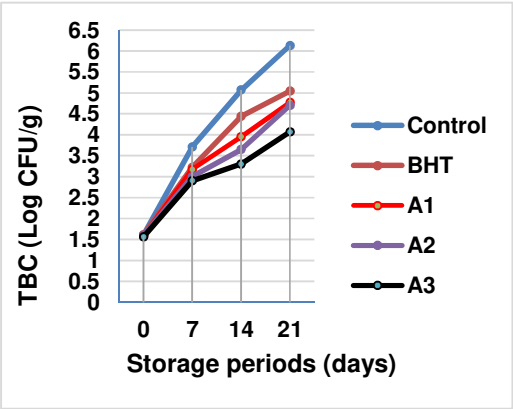

Figure 8: Total bacteria count (log cfu/g) of cupcake samples during storage

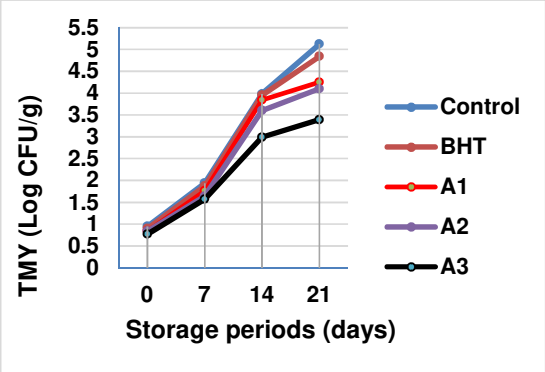

Figure 9: Mold and yeast count (log cfu/g) of cupcake samples during storage

The counts of molds and yeasts for the control, BHT, A1, A2, and A3 samples were 5, 4.84, 3.99, 3.7, and 3.39 log cfu/g at the end of storage. In addition, it is clear that, when compared to all other samples, cupcake containing 3% amla extract had the best and greatest results in reducing the microbial load and preventing the growth of mold and yeast over the storage period. These results are consistent with those of Zaghlool et al. [12], who discovered that applying amla powder to catfish during storage greatly decreased the microbiological load of mold and yeasts and prevented their growth.

4. Conclusion

Amla is an excellent source of bioactive components such as ascorbic acid, polyphenols and flavonoids. When amla extracts were added to cupcake recipes, they had an outstanding antioxidant impact when compared to the effect of synthetic antioxidant (BHT). The increased efficacy of the amla extracts might be attributed to the natural antioxidant stability during baking. Cupcakes containing amla extracts were able to slow the oxidation rate during storage, the 3 % of it had the lowest peroxide value. Furthermore, amla extract improved the qualitative attributes of the cupcake as well as maintaining good physiochemical and sensory qualities. Moreover, amla extract at 3 % proved more efficient than synthetic preservatives in controlling growing microorganisms during storage. Finally, cupcakes with amla extract at levels 1 and 2 % had the highest acceptability, whereas cupcakes with amla extract at level 3 % have the highest antimicrobial effect. Therefore, this study recommended that the amla extract at levels of 1 and 2 % be included in cupcake formulations to replace synthetic antioxidants, because these extracts had no influence on the organoleptic qualities.

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