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Utilization of Beetroot As A Natural Antioxidant, Pigment and Antimicrobial in Cupcake During the Storage Period

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Abstract **- The objective of this study was to evaluate the beetroot powder as chemical analysis, total phenolics, flavonoids compounds, antioxidant activity, minerals content and identification of betalains pigments by HPLC. Four concentrations of beetroot powder (2.5, 5.0, 7.5 and 10%) were substituted with wheat flour 72% extraction to prepare cupcakes, all different cupcakes were baked at 180°C for 30-35 min then cooled before evaluation. The sensory evaluation, physical properties and color characteristics for different cupcake samples were estimated. Meanwhile, microbiology attributed was determined in cupcake substituted with beetroot powder during the storage period (21 days).**

The results observed that the beetroot powder had contained rich source from crude fiber (20.4%), total phenolics, flavonoids compounds, and antioxidant activity. As well as the minerals content, potassium and sodium are the best sources in beetroot, and also, betalain was the highest amount pigment during identification of betalains pigment extracted from beetroot powder.

The results from the sensory evaluation for all different cupcakes showed that the cupcake was detected that replacement at levels of 7.5 and 10% with beetroot powder produced acceptably cupcakes which did not in a sufficiently great differ from wheat flour 72% extraction as control cupcakes. The physical properties recorded that weight increased significantly in a substituted cupcake with 7.5 and 10% beetroot powder which reached 53.00g for both when compared with control which reached 50.0 g. Moreover, the results showed that all cupcakes increased in red color in crust and crumb by increasing beetroot may be able to be the beetroot had contained the betalain pigment. In addition the examination microbiological of different cupcakes the results found that the total count of bacteria and fungi and molds were slightly decreased in cupcake fortification with beetroot than cupcake control during the storage period.

From the obviously results it could be recommended that the beetroot had contained the best amounts from fiber minerals natural antioxidant and antioxidant activity and pigment. Furthermore, the substituted cupcake up to 10% from beetroot powder gives the best sensory characteristics, color (crust and crumb) and inhibition of bacteria and fungi and molds in cupcake until 10% substituted with beetroot. Therefore, it may be concluded that the acceptable cupcakes can be substitute by beetroot red up to 10 % without affecting its quality adversely.

Kew words : Beetroot (Beta vulgaris L.), natural antioxidant, pigment, antimicrobial

I. INTRODUCTION

Beetroot (*Beta vulgaris* L.) are elevated significant sugar content, but it had contained the lowest in calories. The beetroot is from the *Chenopodiaceae* family, and also It was contained vitamins, saponins, betacyanins, betanin, and natural antioxidants. Thus, beetroot intake can be significant a factor in cancer protection. The betalains are obtainable in beetroot is a nutritional factor for the protection and therapy of high blood pressure and cardiovascular diseases and also can prevent the reproduction of cells in human tumors. Moreover, bakery products, candy and ice creams, etc were prepared from beetroot [1].

Beetroot is a natural red food colorant which utilized to make in dry mixes as soups, jellies, etc. The bright red color could be due to the red natural color was known as betalains [2].

Color of food is the main feature regards to consumer attraction and it acts as an indicator of quality and acceptability [3]. Over the last few decades a large number of synthetic colorant is used in food products as coloring ingredient of food the industry which causes severe health problem to the consumer e.g. carcinogenic effect. The uses of synthetic red pigments as additives in food may could be is probably adverse influences on human health [4]. Thus, lots of efforts are going on for use of natural food (bio-colorants) color like betalain (betanin and betacyanin) to be used in dairy and food product. Betalains in red beet have two types of pigments, the betanin with red-violet color and vulgaxanthin with yellowish color [5].

Beetroot is as a natural therapeutic for clinical pathologies connected with oxidative stress and inflammation. Therefore often the betalain pigments are as a display potent antioxidant, anti-inflammatory and chemo-protective activity *in vitro* and *in vivo*. [6].

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Beetroot (*Beta vulgaris L.*) is one of the usually generally produced vegetables around the world. The extracts had to contain phenolic acids as natural antioxidants have been screened to add to produce new natural food recipes. The antioxidant activity as a phenolic compound is may be able to play a significant role in their efficiency scavenging free radicals and decompose peroxides. Thus, the natural antioxidants from beetroot are significant safer alternative to synthetic antioxidants for food protection [7]. Also, beetroot is observed as a significant source of polyphenols in addition to betalains pigments, which are compounds that have to a great antioxidant effect and radical scavenging capacity [8].

Cakes are the greatest consumed bakery product may be due to unique products [9]. It's usually made from soft wheat flour 72% extraction caused decrease in fibers and phytochemicals. Recently, substitutional fibers from various sources are obtainable which has contained rich amounts from fibers and phytochemicals as natural components, like fruit, and vegetable [10].

The target of this study was achieved to determine the chemical composition, minerals content, and fractionation of natural polyphenolic in dry beetroot powder. Moreover, it was used as a natural antioxidant and color to prepare cupcake at 2.5, 5.0, 7.5 and 10.0% levels substituted with wheat flour to reducing the rancidity during the storage period.

II. MATERIALS AND METHODS

A. *Materials*

Soft wheat flour (72% extraction) and baking ingredients such as sugar (sucrose; a commercial grade), salt, skimmed milk powder, shortening, fresh whole egg, baking powder and vanilla were obtained from local supermarket, Taif City, Saudi Arabia.

Red beetroot (*Beta vulgaris* L) was obtained from the local market, Taif City, Saudi Arabia. Fresh beetroots were washed, blanched, peeled and reduced to size (1-3 mm) using a sharp knife. These slices were dried in tray dryer at 60-65 OC for about 7-8 h. The dried beetroot slices were subjected to grinding in a grinder. Then ground material was passed through 60 mesh sieve and packed in plastic bags and stored a refrigerator at 4ºC for further use.

B. *Methods*

- *Proximate chemical composition of beetroot powder*

Proximate composition as protein content, ether extract, ash, and crude fiber were determined of beetroot powder using the methods of the AOAC [11]. Total carbohydrates content was calculated by differences according to Mathew *et al*. [12].

- *Determination of total phenolic content*

Total phenolic (TP) content of samples was determined using Folin-Ciocalteau reagent, according to the method modified from Singh *et al.* [13]. Absorbance was read at 765 nm, using a UV-Vis spectrophotometer (Shimadzu UV-1700; Shimadzu Corporation, Kyoto, Japan). Results were given as mg gallic acid equivalents (GAE) per 100 g fresh weight sample.

- *Determination of total flavonoids compounds*

Total flavonoid (TF) content was determined based on the method described by C˘anadanovic´-Brunet, *et al.* [14] The absorbance was measured at 510 nm and the TF contents of extracts were given as rutin equivalents (RE) per 100 g fresh weight sample.

- *Determination methods of antioxidant activity in beetroot powder*

ABTS (2, 2-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt)

The ABTS assay was performed according to the method used by Miller and Rice-Evans [15]. For the analysis, 1 mL ABTS reaction solution was added to 100 µL sample extract, and the absorbance was measured at 734 nm immediately after 1 min of initial mixing.

- *DPPH (1,1-Diphenyl-2-picrylhydrazyl)*

The DPPH assay was performed as described by Ravichandran *et al.* [16] the absorbance of the mixture was measured at515 nm.

- *FRAP (Ferric Reducing Antioxidant Power)*

The FRAP assay was performed according to the procedure of Benzie and Strain [17]. The absorbance of the reaction mixture was recorded at 593 nm.

- *Minerals content of beetroot powder*

Minerals content as Na, K, P, Ca, Mg, Mn, Fe and Zn were determined of beetroot flour using the flam determined by flame photometer (Galienkamp, EGA 330, England) and Perkin Elmer atomic absorption spectrophotometer (model 80, England) as described in AOAC [11]. Meanwhile, total phosphorus was determined by spectrophotometer at 650 nm according to the method described in AOAC [11]. Extraction and concentration of betalains pigment from red beetroot:

Red beetroot (about 200 g) was mixed shake vigorously with one liter of ethanol (acidified with 2% citric acid) for 15 min at room temperature and left for 24 hours. Then the extract was filtered and concentrated under vacuum by a rotary vacuum evaporator at 40.0 ºC according to the method described by Francis [18].

- *Identification of betalains pigments by High performance Liquid Chromatography (HPLC).*

The identified of betalains pigments by HPLC Merck Pump L- 7100 according to the method reported by Stintzing *et al.* [19] using a LC18 column (250 mm × 4.6 mm, i.d). The flow rate was 1.0 ml / min. and elutes was

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monitored by visible spectrometry at 538nm and 476nm for betalain.

- *Preparation of cupcake substituted with beetroot powder*

All formulae of substituted wheat flour at different substitution levels by beetroot powder were summarized in Table (1). The processing method of the cupcake was taken typically according to AACC [20]. Additionally, substituted WF with beetroot at 2.5, 5.0, 7.5, and 10%, to give four formulae which compared with control sample was prepared with wheat flour 72% extraction. This mixture was mixed gently until got homogenous dough using a Hand mixer (MK-H4-W, Panasonic Co, Malaysia). After getting appropriate texture the dough was poured into paper cups and backed at 180˚C ± 5˚C for 30 - 35 min. The baked cupcakes were cooled down at room temperature, then packed into aluminum foil bags then stored refrigerator at 4C to analysis.

Table (1): Raw ingredients processed of cupcake.

Ingredients	Weight (g)
Soft wheat flour (72% extraction)	250.0
Sugar	125.0
Salt	3.50
Skimmed milk powder	25.0
Shortening	53.50
Fresh whole egg	110.0
Baking powder	12.50
Vanilla	2.0

- *Organoleptic properties of cupcake product*

The organoleptic properties of different substituted cupcakes were determined by properly 20 well-trained panelists from Food Science and Nutrition Department, College Science, Taif University, Saudi Arabia according to AACC [21]. Cupcake samples were left to cool at room temperature for 1 h after baking, then cut with a sharp knife and subjected to panel test. The score was distributed as taste (20), odor (20), texture (15), crust color (15), crumb odor (15), general appearance (15) and overall acceptability (100).

- *Physical parameters*

Physical parameters were studied after cooling of cakes at room temperature .Weight of cakes was recorded in g. Volume was measured by the Rapeseed displacement method AACC [21]. Specific volume was measured by the method given in AACC [21].

- *Color measurement:*

The color of cupcake products were determined by measuring tri-stimulus *L** (brightness), *a** (redness) and *b** (yellowness) values I crust and crumb cupcake with a colorimeter (CIELAB) [22].

- *Microbiological attributes of cupcake product*

Total bacteria count (TBC), and yeast and molds (Y and M) were evaluated periodically in different substituted cupcakes during different storage periods for three weeks. All plates were incubated at 37˚C for 48 h for TBC while at 28˚C for 3 - 5 days for Y and M. All microbiological examinations were performed in triplicates and determined at zero time, 7, 15 and 21 days, respectively according to the procedures described by Difco Manual [23].

- *Statistical analysis:*

All chemical analyses were performed in three replicates and the results were statistically analyzed. Statistical analysis was performed using the GLM procedure with SAS [24] software. Duncan's multiple comparison procedure was used to compare the means. A probability to $p \leq 0.05$ was used to establish the statistical significance.

III. RESULTS AND DICUSSION

- *Proximate composition of beetroot powder*

Chemical composition as protein, ether extract, crude fiber, ash content, and total carbohydrates was determined in beetroot powder and the results found that 12.8, 1.36, 20.40, 11.30 and 54.06%, respectively, as in Table (2).

As well as, polyphenolic (total phenolic, flavonoids compounds) and antioxidant activity (ABTS, DPPH, and FRAP) were determined in beetroot powder and the outcomes are put in Table (2). From the results, the beetroot powder had contained rich amounts from total phenolic and flavonoids compounds were 255.0 mg gallic acid equivalent (GAE)/100 g sample and 260 mg rutin equivalent (RE)/100 g sample, respectively. Meanwhile, the antioxidant activity as ABTS, DPPH, and FRAP were 137, 190 and 181 mg trolox equivalent antioxidant activity (TEAA)/100 g sample, respectively. These means the beetroot had contains rich amounts from nutritional compounds, a natural antioxidant, and antioxidant activity. Red beetroot had contained rich amounts from phenolic compounds, which have antioxidant characteristics. These pigments help to prevent chronic heart disease and some cancers [25].

Zitnanova *et al.* [26] illustrated that the red beetroot is had contained the highest from antioxidant activity. Therefore, red beet can be utilized as natural

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antioxidants [27]. Netzel *et al.* [28] showed that the intake of one dose of red beetroot juice may cause to elevate of natural antioxidant compounds containing betalains in urinary excretion. Both of betalains pigment and phenolic compounds in red beet are happened reducing oxidative damage of lipids and become better antioxidant status in humans. Antioxidant activity in red beet is connected participation of antioxidants in the scavenging of free radicals and as results in the protection of cancer and cardiovascular diseases [29]. Antioxidant activity was also illustrated to increase low-density lipoproteins by betalains pigment which elevates resistance to oxidation [30].

Table (2): Percent of chemical composition of beetroot powder (mg/100g dry weight)

Chemical composition %	Percent beetroot flour	Antioxidant content and activity	Beetroot extract
Protein	12.88±1.25^{c}	Total phenolic (TP)	255±10.48^{a}
Ether extract	1.36±0.04^{d}	Total flavonoids (TF)	260±11.25^{a}
Crude fiber	20.40±1.79^{b}	DPPH	137±8.29^{d}
Ash content	11.30±1.68^{c}	ABTS	190±10.19^{b}
Total carbohydrates	54.06±5.34^{a}	FRAP	181±7.83^{b}

Data represent average values, standard deviation of three independent extractions from each sample on a dry basis. Different letters in the columns represent statistically significant differences ($p < 0.05$). TP results were given as mg gallic acid equivalent (GAE)/100 g sample; TF Contents of extracts were given as mg rutin equivalent (RE)/100 g sample; (DPPH (1,1-diphenyl-2-picrylhydrazyl), ABTS ((2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) di-ammonium salt), and FRAP (Ferric Reducing Antioxidant Power) were given as mg trolox equivalent antioxidant activity (TEAA)/100 g sample).

- *Minerals content of beetroot powder*

Minerals content as potassium, phosphorus, calcium, magnesium, manganese, iron and zinc were determined in beetroot powder and the results are recorded in Table (3). The results showed that the potassium is the major compound in beetroot powder was 26.0 mg/g followed by sodium and phosphorus was 6.26 and 3.50mg/g, respectively. Calcium, magnesium, and manganese were the medium amounts in beetroot and found 2.28, 1.84 and 1.05 mg/g, respectively. Meanwhile, the iron and zinc were the minor compounds in beetroot powder.

Beetroot (*Beta vulgaris* L) is a significant raw material of plant origin and they can be eaten raw, boiled, steamed and roasted. Red beetroot had contained rich amounts of minerals like manganese, sodium, potassium, magnesium, iron, and copper [25]. As well as Pinki and Awasthi [31] found that the level of calcium, phosphorus, and iron in the beetroot powder was 0 32.0, 310 and from 0.14 mg/100g, respectively. In addition, Uma [32] reported that the zinc was 0.96 mg/100g contents of beetroot powder.

Table (3): Minerals content of beetroot powder mg/g

Minerals content	Mg/g	Minerals content	Mg/g
Potassium	26.0±1.25^{a}	Magnesium	1.84±0.02^{d}
Sodium	6.26±0.12^{b}	Manganese	1.05±0.02^{d}
Phosphorus	3.50±0.03^{c}	Iron	0.06±0.001^{e}
Calcium	2.28±0.01^{d}	Zinc	0.03±0.00^{e}

Data represent average values, standard deviation of three independent extractions from each sample on a dry basis. Different letters in the columns represent statistically significant differences ($p < 0.05$).

- *Identification of betalains pigment from beetroot powder*

Betalains pigments are water-soluble nitrogen-containing and it was found with a great percent in the red beet *(Beta vulgaris*). Betalains consist of two subs--classes: betacyanins (red-violet pigments) and betaxanthins (yellow-orange pigments) [33].

Identification and separation of betalains pigment from red beetroot were determined by HPLC and the results are recorded in Table (4) and Figure (1). Four components were identified by HPLC; the major constituent of red beetroot was Betalain 62.6% followed by 15-decarboxy-betanin and isobetalain were found 25.3 and 17.1%, respectively. The vulgaxanthin compound was the minor compound (5.0%) during the identification of betalains. These results are confirmed with that of Wybraniec [34] who observed that the major components of pigments in red beetroot are betalain and isobetalain.

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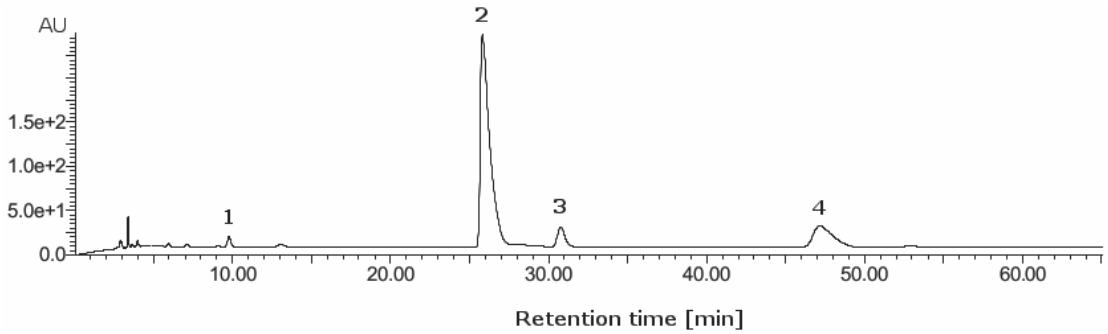

Figure (1) HPLC chromatogram of betalains from beetroot powder extract

Table (4): Identification of betalains extracted from beetroot powder extract

Identified pigment	Retention time(min)	Relative abundance area %
Vulgaxanthin	9.70	5.0±0.15^{c}
Betalain	25.0	62.6±2.64^{a}
Isobetalain	31.0	17.1±1.05^{b}
15-decarboxy-betanin	46.9	25.3±1.29^{ab}

Data represent average values, standard deviation of three independent extractions from each sample on a dry basis. Different letters in the columns represent statistically significant differences ($p < 0.05$)

- *Organoleptic properties of cupcake product*

The average of sensory properties was characteristics and the outcomes are tabulated in Table (5) and Photo (1). The results showed the cupcake was slightly decreased gradually when beetroot powder was increasing from a control sample to cupcake which had contained 10% beetroot powder. While formulating the cupcakes it was observed that at 10% level of beetroot powder incorporation, the crust of cake became semi-hard may be caused the beetroot rich amounts from crude fiber (20.4%). There was a slight difference in overall acceptability of cupcakes as the level of beetroot was increased to 10%. However, up to 10% level of beetroot incorporation, the overall acceptability was recorded.

Photo (1): Shown the cupcake substituted with beetroot powder at 2.5, 5.0, 7.5 and 10.0% levels

Table (5): Organoleptic properties of cupcake product

Types of additions	Taste 20	Odor 20	Texture 15	Crust color 15	Crumb color 15	General appearance 15	Overall acceptability 100
Control	19.50^{a} ±1.06	19.10^{b} ±0.93	14.35^{a} ±0.97	14.65^{a} ±0.74	14.65^{a} ±0.61	14.50^{a} ±0.99	96.75
2.5	19.20^{a} ±0.09	19.20^{b} ±0.63	14.35^{a} ±0.97	14.60^{a} ±0.74	14.60^{a} ±0.72	14.30^{a} ±0.04	96.65
5.0	19.10^{ab} ±0.23	19.30^{a} ±0.12	14.15^{a} ±0.28	14.15^{b} ±0.48	14.30^{b} ±0.09	14.10^{ab} ±0.67	95.10

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7.5	19.00^{b} ±0.56	19.40^{a} ±0.13	14.00^{b} ±0.66	14.00^{b} ±0.11	14.10^{b} ±0.55	14.00^{b} ±0.99	94.50
10.0	19.00^{b} ±0.21	19.40^{a} ±0.35	14.00^{b} ±0.35	13.50^{c} ±0.34	13.50^{c} ±0.87	14.00^{b} ±0.43	93.40

Data represent average values, standard deviation of three from each sample. Different letters in the columns represent statistically significant differences ($p < 0.05$)

- *Physical characteristics of beetroot powder incorporated cupcake*

Weight, volume and specific volume were determined in cupcake substituted beetroot at levels 2.5, 5.0, 7.5 and 10.0% compared with control cupcake, and the results are given in Table (6). The results showed that the weight was increased when the beetroot powder was increasing from 51.0 g at 2.5 % beetroot to 53.0 g at10.0 % beetroot compared with control made from wheat flour was 50.0g, respectively. Meanwhile, the volume cupcakes were decreased when the beetroot was increasing may be due to decreasing level of gluten protein in the blend on increasing amount of beetroot powder and may be the beetroot had rich amounts from crude fiber.

Table (6): Physical characteristics of beetroot powder incorporated cupcake

Physical parameters	Addition of beetroot powder				
	Control	2.5	5.00	7.5	10.0
Weight (g)	50.0±1.25^{a}	51.0±1.38^{a}	52.0±1.18^{ab}	53.0±1.24^{b}	53.0±1.43^{b}
Volume (cc)	109±8.21^{a}	108±7.61^{a}	105±8.45^{b}	102±6.27^{b}	100±7.36^{c}
Specific volume (cc/g)	2.18±0.01^{a}	2.12±0.01^{a}	2.02±0.01^{ab}	1.92±0.01^{b}	1.89±0.01^{c}

from wheat flour 72% and substituted with 2.5 % beetroot powder which reached (4.62 and 32.72) and 10 % pumpkin powder which reached (7.20 and 36.92) when compared with control which reached (3.24 and 30.18) respectively. Meanwhile, brightness was gradually decreased significantly ($P \leq 0.05$) in cupcake from 73.56 at level 2.5% substituted beetroot to 65.11 at substituted level 10% compared with control sample was 77.28, respectively. These results showed the cupcake increased in red color in crust and crumb cupcake by increasing beetroot may be able to be the beetroot had contained the betalain pigment. Betalains are water-soluble pigments found in the vacuoles of plant cells. Betalains are water-soluble pigments and a simple, greatly efficient and low-cost method for crude betalain extraction and increase the best state of being stable for the pigments [3, 36).

Food color is one of the parameters affect food quality and consumer acceptance to an important level. Synthetic food pigments are being utilized in much food products which are a great concern to human health, having allergic and carcinogenic influences. Thus, potentials are being made to utilize natural food pigments from natural sources. Beetroot is one such material which is a source of betalain and had contained from many bioactive and phytochemical compounds which are known to supply various health benefits [37].

Data represent average values, standard deviation of threes from each sample. Different letters in the columns represent statistically significant differences ($p < 0.05$).

- *Measurement of color cupcake product*

Colors are significant quality indicates that decide the consumer agreement of foods. In recent days market for the usage of synthetic pigments has reduced in favor of natural pigments [35].

The color was measured in the crust and crumb cupcake made from wheat flour 72% and substituted at levels 2.5, 5.0, 7.5 and 10 % with beetroot powder by brightness, redness, and yellowness values and the results are reported in Table (3). The results reveal that brightness, and yellowness values in crust were gradually decreased significantly ($P \leq 0.05$) in cupcake made from wheat flour 72% and substituted with 2.5 % beetroot powder which reached (57.52 and 42.34), to cupcake substituted with 10 % beetroot powder which reached (52.13 and 32.82) when compared with control which recorded (59.74 and 45.28) respectively. Meanwhile, redness was gradually elevated significantly ($P \leq 0.05$) in cupcake from 25.56 at level 2.5% substituted beetroot to 34.22 at substituted level 10% compared with control sample was 23.13, respectively

As well as, the results in the same table showed that the redness and yellowness values in crumb cupcake were increased significantly ($P \leq 0.05$) in cupcake made

Table (7): Effect of different substitute of beetroot powder on color characteristics of crust and crumb cupcake

Substitution Level (%)	Crust			Crumb		
	*L**	*a**	*b**	*L**	*a**	*b**
Control	59.74±3.45^{a}	23.24±1.95^{c}	45.28±3.11^{a}	77.28±4.25^{a}	3.24±0.23^{d}	30.18±2.04^{c}
2.5	57.52±3.38^{a}	25.56±1.84^{b}	42.34±2.97^{a}	73.56±4.39^{ab}	4.62±0.38^{c}	32.72±2.34^{b}
5.0	56.34±3.61^{b}	28.73±2.01^{b}	38.19±2.28^{b}	70.24±3.56^{b}	5.71±0.28^{b}	34.64±2.65^{a}
7.5	54.46±3.14^{b}	31.53±2.14^{a}	35.68±2.68^{b}	68.35±3.28^{c}	6.32±0.36^{a}	34.29±2.22^{a}
10.0	52.13±3.33^{c}	34.22±2.16^{a}	32.82±2.39^{c}	65.11±3.94^{c}	7.20±0.64^{a}	36.92±2.31^{a}

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Data represent average values, standard deviation of three independent extractions from each sample on a dry basis. Different letters in the columns represent statistically significant differences ($p < 0.05$).

- *Microbiological of cupcake product*

Microbiological spoilage is usually large agents shorten the shelf life of bakery products. Spoilage from microbial growth reasons economic damage. These damages could be due to more person status like packaging, sanitary practice in manufacturing, storage conditions and product turnover [38].

Biological activity as total bacteria and fungi count were estimated in cupcake made from wheat flour 72% extraction as a control sample and its substituted cupcake at levels 2.5, 5.0, 7.5 and 10%, respectively from beetroot powder, during storage period for three weeks and the outcome are put in Table (8). The results have shown the total count of bacteria and fungi were inhibition in cupcake fortification with beetroot during the storage period compared with control cupcake made from wheat flour 72% extraction. Total count of bacteria for cupcake at level 10% made from beetroot powder was from 2.10×10^{-1} CFU at zero time to 5.60×10^{-1} CFU after three weeks compared with control cupcake was ranged from 2.30×10^{-1} CFU to 7.80×10^{-1} CFU after three weeks. The results from the total count of fungi were parallel and confirmed to the results from the bacteria count. Cupcake prepared from beetroot powder products is good sources of total dietary fiber and betalin pigment as natural antioxidant and the results obtained from the microbial analysis of cupcake product

Mold growth is by away the great agent determining the shelf life of elevating and average bakery products. In general, mold growth on bakery products is a dangerous problem that outcomes in economic damages. Moreover, damages of products may be caused to mold spoilage is among from one to five percent by a controlled basis on the kind of product and the method of processing [39]. Dried beetroots are consumed immediately in the form of chips as a replacement for traditional snacks [40], or as an ingredient of instant food [41]. Lowering the moisture content of fresh foods to make them fewer perishable, that is an easy way to protect these foods.

Table (8): Microbiological attributes of different cupcakes during storage periods.

Substitution level (%)	TBC (log CFU g−1)/ week				Y and M (log CFU g−1)]/week			
	Zero	7	15	21	Zero	7	15	21
Control	2,30 ±0.13^{a}	3.50 ±0.15^{a}	5.10 ±0.32^{a}	7.80 ±0.73^{a}	nd	2.20 ±0.11^{a}	2.90 ±0.24^{a}	11.00 ±1.42^{a}
2.5	2.25 ±0.11^{a}	3.10 ±0.14^{b}	4.60 ±0.29^{ab}	7.10 ±0.54^{ab}	nd	2.10 ±0.12^{a}	2.80 ±0.24^{a}	10.90 ±1.28^{ab}
5.0	2.20 ±0.11^{a}	2,80 ±0.16^{bc}	4.10 ±0.21^{b}	6.60 ±0.86^{b}	nd	2.0 ±0.14^{a}	2.70 ±0.27^{ab}	10.80 ±1/52^{b}
7.5	2.10 ±0.12^{b}	2.50 ±0.12^{bc}	3.80 ±0.25^{bc}	6.10 ±0.62^{bc}	nd	1.90 ±0.13^{b}	2.60 ±025^{ab}	10.70 ±1.37^{c}
10.0	2.10 ±0.12^{b}	2.20 ±0.11^{c}	3.10 ±0.22^{c}	5.60 ±0.43^{c}	nd	1.95 ±0.13^{b}	2.65 ±0.25^{b}	10.75 ±1.64^{c}

Data represent average values, the standard deviation of three from each sample. Different letters in the columns represent statistically slightly significant difference ($p < 0.05$).

IV. CONCLUSION

Beetroot is an interesting ingredient for the production of bakery especially cupcakes. Beetroot is a nutritive value from mineral contents, a natural antioxidant and pigment. Cupcake prepared with 10 % beetroot powder incorporation had better physical and sensory properties color, taste, texture and inhibition of bacteria and fungi. Therefore, it might be concluded that the acceptable cupcakes can be substituted by beetroot red powder up to 10 % without affecting its quality adversely.

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