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RESEARCH ARTICLE

PHYSICOCHEMICAL PROPERTIES AND FOOD APPLICATION OF MARIGOLD FLOWER EXTRACTS PREPARED BY CONVENTIONAL AND SUPERCRITICAL CO_2 METHODS

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Abstract

Marigold (*Tageteserecta*Linn) flower extracts prepared by conventional and supercritical Marigold (*Tagetes erecta* Linn) flower extracts prepared by conventional and supercritical CO_2 extraction were evaluated and compared in terms of chemical composition, antioxidant activity and color attributes. Biscuit enriched with marigold flower extract was prepared and evaluated for antioxidant activity, specific volume, color attributes and sensory properties. The results of marigold flower composition showed that the moisture content was 68.32±0.14%, crude protein 2.34±1.07%, total carbohydrate 16.03±0.85%, total dietary fiber 10.98±0.43 %, total fat 0.62±0.10% and ash 1.30±0.21%. Iron was the most abundant element among the estimated minerals recording 1.058±0.041 mg/100g. From the extraction results it could be conclude that the methods of extraction affect clearly on the contents of biological active compounds of marigold flowers. Using supercritical CO_2 for marigold flower extraction increases the content of total phenols, total flavonoids and oleoresins in the resulted extracts. Marigold extract prepared by supercritical CO_2 technique had approximately DPPH inhibition equal to that for ascorbic acid. Biscuit enriched with marigold flower extract had higher DPPH inhibition and specific volume. Finally, the addition of marigold flowers extract to biscuit improved the color, odor and overall acceptability of the resultant products.

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Introduction:-

Tagetes is a genus (family *Asteraceae*) including about 50 species of annual or perennial herbaceous plants. The plant *Tagetes erecta* Linn. in India known as Genda Phul (Marigold). It is stout, branching herb, native to Mexico and some warmer parts of America and neutralized elsewhere in the tropics and subtropics including India and Bangladesh (**Khulbe, 2015**).

The major phytochemical antioxidants in marigold flowers extracts were reported to be phenolics and carotenoids. Phenolics in the extract mainly constituted gallic acid and quercetin, whereas lutein is a major carotenoid present in marigold petals (**Rivas, 1989, Kaisoon *et al.*, 2011, Ingkasupart *et al.*, 2015; Kushwaha and Verma, 2017**).The two major classes of pigments present in the *Tagetes* spp. are the flavonoids and carotenoids (**Vasudevan *et al.*, 1997**). The lutein ester carotenoids, in particular, have been identified as the principal pigment components in marigold flowers (**Gong *et al.*, 2012**). Flavonoids are a class of secondary plant metabolites that are thought to exert several effects beneficial to human health through their antioxidant and chelating properties **(Ciz*et al.*, 2010**). These are commonly found in both edible and non-edible plants, and have been reported to exert multiple biological effects, including antioxidant activity (**Kahkonen*et al.*, 1999**).

Tagetes spp. has a long history of human use as food, perfumes, medicines, ornamentals, and in ritual and sociocultural ethno practices, depending on geographical location and ethnic background (**Navarro-González *et al.* 2015**).A number of marigold species are reported to possess therapeutic usage in various ailments, such as skin complaints, wounds and burns, conjunctivitis and poor eyesight, menstrual irregularities, varicose veins, hemorrhoids, duodenal ulcers, etc. (**Cetkovic *et al.*, 2004**). Additionally, the lethal dose and toxicity determination of the color extracted from marigold showed that it is safe for consumption as no clinical symptoms were observed in rabbits after giving the maximum dose of 1000ml of extracted color (**Alim-un-Nisa *et al.*, 2018).**

The fresh flowers of *Tagetes* spp. may be added to salads or used as an edible garnish; they provide a bitter taste, which can be helpful to balance out an overly sweet dish. (**Prakasa *et al.*, 2000; Kaul *et al.*, 2005; Singh *et al.*, 2016).** Moreover **Tiwari *et al.* (2016)** reported that *Tagetes minuta* L. is reputed as a source of 'Tagetes oil' of trade that finds an extensive use in food, flavoring, pharmaceutical, perfumery and cosmetic industry.The orange-yellow carotenoid lutein substance found in the florets of *T. erecta* and many other marigolds (*T. minuta* included) has been identified, isolated, and approved by the European Union for use as a food coloring agent and nutrient supplement (food additive) in a wide range of baked goods and baking mixes, beverages and beverage bases, breakfast cereals, chewing gum, dairy product analogs, egg products, fats and oils, frozen dairy desserts and mixes, gravies and sauces, soft and hard candy, infant and toddler foods, milk products, processed fruits and fruit juices, soups and soup mixes in levels ranging from 2 to 330 mg/kg **(Martinez *et al*, 2009; Navarro-González *et al.*, 2015; Tiwari *et al.*, 2016; Rajvanshi and Dwivedi, 2017; Alim-un-Nisa *et al.*, 2018).**

The extraction method and solvent used to obtain a biological active compounds-enriched extracts can greatly influence the biological activity. A successful extraction technique combines the optimal solvent or mixture of solvents with a convenient technique. In order to select an extraction method it is necessary to evaluate the efficiency, the stability of the extracted substances, the availability of resources and processing costs, leading towards a biological application of the extract (**Louzada *et al.*, 2001**).

Supercritical fluid extraction (SFE) is an innovative, clean and environmental friendly technology with particular interest for the extraction of biological active compounds from plants and herbs (**Felfoldi-Gava *et al.*, 2012**). Supercritical CO_2 is selective, there is no associated waste treatment of a toxic solvent, and extraction times are moderate. Further supercritical extracts were often recognized of superior quality when compared with those produced by hydro-distillation or liquid-solid extraction (**Campos *et al.*, 2005**)

Some authors investigated SFE on triterpenoids of marigold. **Hamburger *et al.* (2003)** applied supercritical fluid extraction for purification of faradiol esters under 500 bar/50°C. The extraction yield was 5% and approximately 85% of faradiol esters were extracted. Also, SFE under different conditions was investigated on marigold oleoresin by **Campos *et al.* (2005).**

Biscuits are one of the important bakery products, it is greatly consumed. It has certain advantages, such as being cheaper than the conventional bakery products, a very palatable vehicle of nutrition and energy, easy to use everywhere. Moreover, it has a good taste, good shelf life at ambient temperature (**Ahmad and Ahmed, 2014; Al-Marazeeq and Angor, 2017**). Biscuit consumption is considered one of the top ten daily consumed foods. Generally, efforts were needed in recent days to improve the physicochemical properties, sensory attributes and nutritional qualities biscuits (**Masoodi and Bashir, 2012; Jauharah *et al*., 2014**).

The aim of this research was to evaluate and compare the marigold flower extracts prepared by conventional and supercritical CO_2 extraction in terms of chemical composition, antioxidant activity and color attributes, as well as the effect of marigold flowers extract addition on the antioxidant activity, specific volume, color attributes and sensory evaluation of biscuit.

Materials And Methods:-

Materials:-

Marigold (***Tageteserecta*** Linn**)** flowers were collected during summer 2015 from a private farm located in Astrakhan state, Russia and washed properly under running water to remove the dust. The flowers were naturally dried at room temperature (28±2 °C) for 72 h then milled and frozen (-18±2°C) stored till use.

Wheat flour (72%), butter 80% fat, cow milk 3.9% fat, eggs, sugar, vanillin, baking powder and salt were purchased from local market, Zagazig city, Egypt. Chemicals of analytical grade were purchased from Algomhourya Company, Zagazig branch, Egypt.

Method:-

Extraction of marigold petals:-

Conventional method:-

Dried marigold petals were extracted with 95% ethanol by continuous shaking at 120 rpm at 25°C for 24 h. The sample was then filtered through a 0.45 μm membrane, and the filtrate was freeze dried and stored at 4±1ºC in a refrigerator in the absence of light (**Ingkasupart *et al*., 2015**).

Supercritical CO_2 method:-

Supercritical CO_2 extraction process was performed as described by **Felfoldi-Gava *et al.* (2012).** Dried marigold petals were extracted using carbon-dioxide in a high pressure pilot plant equipped with 5 L volume extractor vessel (delivered by NATEX Austria). The extraction vessel was supplied with 800 g of raw material. The designed extraction pressures were 300 bar. The temperature was set to 60 °C. The ethanol concentration was 10%. The extraction was performed in triplicate. After adjusting the desired temperature and pressure, the CO_2 feed was started with a flow rate about 7 kg h^{-1}. The accumulated product samples were collected and weighed at certain time intervals. The extraction was carried out until the amount of the product sample collected for 1 h decreased to under 0.1% of the raw material.

Recipes and preparation of biscuits:-

Recipes and preparation of biscuits were made as described by **Al-Marazeeq and Angor (2017)** with slight modifications. The formula used to prepare the biscuits was as follows: 500 g wheat flour (72%), 200 g butter (80% fat), 200 ml cow milk (3.9% fat), 2 eggs, 250 g powdered sugar, 2 g salt,4 g vanillin and 6 g baking powder. This formula served as a control ($Bis_{control}$). The marigold flower extract (prepared from supercritical CO_2 method) was added in the percent of 0.5, 1.0 and 1.5% of total biscuit ($Bis_{control}$) formula and named $Bis_{0.5}$, $Bis_{1.0}$ and $Bis_{1.5}$, respectively. The ingredients were manually mixed, formed and baked at 180 °C for 15 min and cooled at room temperature for 2 hrs. The resulted biscuits were packaged in polyethylene bags and cold stored (4±1ºC) till used.

Proximate chemical composition:-

The proximate composition (moisture, total solids, protein, fat, ash and carbohydrates) was analyzed by following the Association of Official Analytical Chemists (AOAC) official methods (**AOAC, 1990**) in triplicates. The moisture was obtained by drying the flowers in an oven at 110 °C until constant weight was achieved. The crude protein content of samples was estimated by the macro-Kjeldahl method (N × 6.25). The total fat was determined using a Soxhlet procedure. The ash content was quantified after incineration of the samples at 525 °C for 24 h. The total carbohydrates were calculated by difference. Total dietary fiber (TDF) was determined by following the

enzymatic and gravimetric method described by **Prosky*et al*. (1988)**. The samples were digested consecutively with α-amylase (thermo-stable), protease and amyloglucosidase to obtain the residue resistant to *in vitro* intestinal digestion.

The chemical compounds of the dietary fibers were precipitated by adding 90% ethanol; after one hour, samples were filtered through glass filters using a Fibertec System E 1023 (Högänas, Sweden). The residues were desiccated overnight and then weighed to determine the residue amount. The protein and ash contents were analyzed in the residues to eliminate the amounts of these compounds and to obtain the final weight of the residue, which was expressed as percentage of total dietary fiber (TDF).

Mineral composition determination:-
The mineral composition was analyzed by inductively coupled plasma optical emission spectroscopy (ICP-OES), using an ICAP 6500 Duo Thermo model (Thermo Scientific, Cambridge, UK), after microwave-assisted digestion (Ultra CLAVE, Milestone Inc., Shelton, CT, USA) with H_2O_2 and HNO_3 (1:4 *v*/*v*) (**Navarro-González *et al.*, 2015**).

Determination of total phenols content:-
The total phenol content of marigold flowers was determined as described by **Kushwaha and Verma (2017).** 0.10 g of powdered sample was mixed with 3 ml distilled water, followed by Folin ciocalteau reagent 0.5 ml (1:1 with water). It was mixed for 3 min. then 2 mL of Na_2CO_3 (20%) was added and incubated for a further 30 min. at room temperature (22±2ºC) in dark. A dark blue color was developed and estimated at 650 nm. Gallic acid was used as the standard for the formation of calibration curve to the calculation of total phenol content in mg/g of the used material.

Determination of total flavonoids
The total flavonoids content of the samples was determined by the aluminum chloride ($AlCl_3$) method as described by **Ahmad *et al.* (2015)**. Quercetin was used as the standard for the calculation of the calibrated curve. The different aliquots of sample and standard were taken, and made up the volume to 1 ml with methanol followed by $AlCl_3$ (0.1 mL) and sodium acetate (0.1 mL). The reaction mixture was diluted by 1 mL distilled water and the absorbance was measured at 510nm.

Oleoresins, total carotenoids, lutein and β-carotene determination:-
Oleoresins were measured according to **Mbaeyi-Nwaoha *et al.* (2013)** by extracting the finally milled marigold flowers with acetone to exhaustion in a Soxhlet apparatus. The extract (Oleoresins) was poured in rotary evaporator flask and evaporated at 65°C till all the solvents were expelled. The difference between the empty flask and flask with the separate concentrated extracts was used in obtaining the oleoresin content yield. Total carotenoids were measured by UV/VIS spectrophotometry at 445 nm (**Rodriguez-Amaya, 1999**). Lutein and β-carotene were measured by HPLC using the method of **Giuffrida*et al.* (2007**).

DPPH radical scavenging activity assay:-
The reaction mixtures contained 200 µL of 150 µM DPPH (2, 2-diphenyl-1-picrylhydrazyl) in 95% ethanol, and 22 µL of diluted marigold extract or 1% ascorbic acid solution. The mixture was incubated in the dark at room temperature for 30 min, and the absorbance recorded at 517 nm as detailed in a previous study (**Fukumoto and Mazza, 2000**). The percentage scavenging activity of the DPPH radicals was calculated as follows: $[(Abs_{blank} - Abs_{sample})/Abs_{blank}] \times 100$, at marigold extract concentration of 20 mg/mL.

Specific volume determination:-
Biscuits samples were weighted (gm.) after 2 hours of cooling at room temperature. The volume (cm^3) was measured by rapeseed replacement method. Specific volume was obtained by dividing the volume of sample by their weight according to the method described in the **AACC (1983).**

Color measurement:-
The color attributes of the tested samples expressed as lightness (L), redness (a) and yellowness (b) were measured using the Hunter Lab color analyzer (Hunter Lab Color Flex EZ, USA). All tests were measured in triplicate samples and the means were recorded.

Sensory evaluation:-

Sensory evaluation of control biscuits and biscuits enriched with marigold flower extract was performed according to the method described by **Chioma and Chizoba (2015)**. Biscuits samples were judged by 10 panelists from the teaching staff, graduated students and technicians of the Department of Food Science, Faculty of Agriculture, Zagazig University. The panelists were from different sexes and ages. The samples were evaluated for desirability in color, taste, odor, texture and overall acceptability. A numerical basis as a sort of evaluation from 1 to 9 was used where (1 =dislike extremely, 2= dislike very much, 3= dislike moderately, 4= dislike slightly, 5= neither like nor dislike, 6= like slightly, 7= like moderately, 8, like very much, 9= like extremely). Water was used to neutralize the taste between samples testing.

Results And Discussion:-

Proximate composition of marigold flowers:-

The fresh flower of marigold (*Tagetes erecta Linn.*) was subjected to proximate analysis to evaluate their quality. Proximate composition of marigold flowers is presented in Table 1. The obtained results showed that the moisture content was 68.32±0.14%, crude protein 2.34±1.07%, total carbohydrate 16.03±0.85%, total dietary fiber 10.98±0.43%, total fat 0.62±0.10% and ash 1.30±0.21%. These results are similar to those obtained by **Navarro-González *et al.* (2015).**

Table1:-Proximatecompositionof marigold flowers:

Components	Moisture	Crude protein	Total			Ash
			carbohydrate	dietary fiber	fat	
Percentage (%) on fresh weight basis	68.32±0.14	2.34±1.07	16.03±0.85	10.98±0.43	0.62±0.10	1.30±0.21

Table 2 summarizes the mineral composition of the edible marigold flowers. The flower sample was subjected to various mineral analyses. Iron was the most abundant element among the studied minerals recording 1.058±0.041 mg/100g, the second was copper being 0.977±1.002 mg/100g and the rarest was sulphur reaching 0.028±0.065 mg/100g. It is notably that the marigold flowers showed higher content of potassium (0.198±0.022 mg/100g) than sodium (0.016±0.009 mg/100g). These data of marigold flower minerals contents recorded in this study are in harmony with those obtained by **Navarro-González *et al.* (2015**) who found that marigold **(***Tagetes erecta Linn*) flowers collected from Spain contains Iron 1.026 ± 0.052 mg/100g, Copper 0.104 ± 0.025 mg/100g, Potassium 0.215 ± 0.007 mg/100g, Sodium 0.015 ± 0.007 mg/100g.

Table2:-Mineral composition of marigold flowers

Mineral	*Percentage (mg/100g) on fresh weight basis*
Sodium (Na)	0.016±0.009
Magnesium (Mg)	0.081±0.010
Calcium (Ca)	0.099±0.004
Potassium (K)	0.198±0.022
Iron (Fe)	1.058±0.041
Phosphorus (P)	0.075±0.016
Sulphur (S)	0.028±0.065
Manganese (Mn)	0.401±0.101
Copper (Cu)	0.977±1.002

Yields of total phenols, total flavonoids and oleoresins:-

Yields of total phenol, total flavonoids and oleoresins contents of marigold flowers and extracts can be seen in Table 3. Total phenol content of dried marigold flowers was 20.02±2.09 mg gallic acid/g; this concentration was increased to 60.34±1.43 and 75.87±0.95 mg gallic acid/g for conventional marigold extract and supercritical CO_2 marigold extract, respectively. Total flavonoids recorded 72.90±1.41 mg quercetin /g for marigold flowers, 110.13±1.98 quercetin /g for conventional marigold extract and 140.06±0.27 quercetin /g for supercritical CO_2 marigold extract. Finally, oleoresins contents of marigold such products ranged between 28.32±1.34 to 67.02±2.94 mg/g. From above mentioned data it could be conclude that the methods of extraction affect clearly on the contents of biological active compounds of marigold flowers.

Table3:-Yields of Total phenol, total flavonoids and Oleoresins

component	Marigold flowers	Marigold flowers extracts prepared by	
		Conventional method	Supercritical CO_2
Total phenol content (*mg Gallic acid/g*)	20.02±2.09	60.34±1.43	75.87±0.95
Total flavonoids (*mg Quercetin /g*)	72.90±1.41	110.13±1.98	140.06±0.27
Oleoresins (*mg/g*)	28.32±1.34	49.74±0.65	67.02±2.94

Total carotenoids, lutein and β-carotene contents:-

Total carotenoids, lutein and β-carotene contents of marigold flowers and extracts are presented in Table 4. Marigold flowers contain 8.07±0.43% carotenoids (of total oleoresins). On the other side, the data revealed that marigold carotenoids include 3.87±0.55% free lutein and 0.39±0.76% β-carotene. The percent of total carotenoids was increased in marigold extracts prepared by conventional and supercritical CO_2 methods being 15.56±0.95% and 25.45±1.43 %, respectively. Lutein content was 9.45±1.0 and 18.76±1.21% of total carotenoids for marigold extracts prepared by conventional and supercritical CO_2 methods, respectively. Moreover, β-carotene accounted 0.71±0.76 and 1.73±1.06% of total carotenoids for marigold extracts prepared by conventional and supercritical CO_2 methods, respectively. It is clear that using supercritical CO_2 increase the extraction of carotenoids from marigold flowers. For lutein content in marigold flowers, the same trends were reported by **Piccaglia *et al*. (1998)**.

Table 4: Total carotenoids, lutein and β-carotene contents

Component	Marigold flowers	Marigold flowers extracts prepared by	
		Conventional method	Supercritical CO_2
Total carotenoids (% total Oleoresins)	8.07±0.43	15.56±0.95	25.45±1.43
Lutein (% total carotenoids)	3.87±0.55	9.45±1.03	18.76±1.21
β-carotene (% total carotenoids)	0.39±0.76	0.71±0.76	1.73±1.06

DPPH inhibition (%) of marigold products compared with ascorbic acid:-

Many tests and techniques have been reported and developed to determine the presence and activity of antioxidants in food systems, nutraceuticals and dietary supplements. The DPPH assays method was conducted to determine the antioxidant activity of marigold products. The results of the DPPH assays of marigold flowers, extracts and ascorbic acid are presented in Table 5. The DPPH inhibition (%) of marigold flowers ranged between 54.56 to 61.77% during incubation times 0, 30, 60, 90 and 120 min. The DPPH inhibition (%) of marigold extract prepared by conventional method reached 87.65 % at incubation time 120 min while reached 89.18% for marigold extract prepared by supercritical CO_2 at the same time of incubation. This leads to the fact that the marigold extracts have a high antioxidant capacity than marigold flowers. On the other side, marigold extract prepared from supercritical CO_2 had more antioxidant capacity than that prepared by conventional method. Moreover, marigold extract prepared from supercritical CO_2 had approximately DPPH inhibition equal to that for ascorbic acid.

Table 5:- DPPH inhibition (%) marigold products compared with ascorbic acid

sample		DPPH Inhibition (%), during incubation time (min)				
		0	30	60	90	120
Marigold flowers		54.56	56.98	59.63	60.12	61.77
Marigold flowers extracts prepared by	**Conventional method**	79.74	82.42	85.07	85.64	87.65
	Supercritical CO_2	81.43	83.68	87.71	88.32	89.18
Ascorbic acid		85.43	878.47	88.87	89.09	90.88

Color attributes of marigold products:-

Generally, marigold flowers color ranges from yellow and gold to orange, red. However, the color attributes of marigold products (flowers and extracts) were illustrated in Table 6. The L (lightness) value was 38.65±3.98 for marigold flowers, 42.32±2.68 for marigold extract prepared by conventional method and 41.98±3.65 for marigold

extract prepared by supercritical CO_2. While a value (redness) was 1.34±1.96 for marigold flowers, 1.21±1.54 for marigold extract prepared by conventional method and 1.31±.91 for marigold extract prepared by supercritical CO_2. The b value (yellowness) was 16.05±2.01 for marigold flowers. This value was increased in the case of marigold extracts prepared by conventional and supercritical CO_2 methods being 21.61±3.04 and 22.90±2.83, respectively. From these results, it could be recognize that the color get more yellowness in the marigold extracts than flowers as a result of the high content of carotenoids in the extracts (specially that prepared by supercritical CO_2) than the flowers.

Table6:-Color attributes of marigold products

Color value	Marigold flowers	Marigold flowers extracts prepared by	
		Conventional method	Supercritical CO_2
L, lightness	38.65±3.98	42.32±2.68	41.98±3.65
a, redness	1.34±1.96	1.21±1.54	1.31±.91
b, yellowness	16.05±2.01	21.61±3.04	22.90±2.83

DPPH inhibition, specific volume, color and sensory attributes of biscuits containing marigold flower extracts:-

Table 7 shows the DPPH inhibition (%) of biscuits samples containing different levels of marigold flowers extract prepared by supercritical CO_2 methods. The marigold flowers extract prepared by supercritical CO_2 method was chosen for biscuit manufacturing due to his high content of total phenols, total flavonoids and Oleoresins. The addition of marigold flowers extract to biscuits improved the antioxidant activity of the resultant products. Where, the DPPH inhibition during 120 min of incubation time ranged from 42.76 to 48.12 for $Bis_{control}$, 45.57 to 51.05 for $Bis_{0.5}$, 48.79 to 58.79 for $Bis_{1.0}$ and 52.04 to 61.82% for $Bis_{1.5}$. These results show that the % inhibition of DPPH was increased when the added extracts of marigold flowers in the biscuits was increased. Moreover the increase in % inhibition of DPPH was directly related to the incubation time. $Bis_{1.5}$ sample had the highest percent of DPPH inhibition.

Table 7:- DPPH inhibition (%) of biscuits containing marigold flower extracts

Biscuit samples	DPPH Inhibition (%), during incubation time (min)				
	0	30	60	90	120
$Bis_{control}$	42.76	42.82	45.01	46.35	48.12
$Bis_{0.5}$	45.57	45.90	47.28	49.23	51.05
$Bis_{1.0}$	48.79	50.58	51.17	53.45	58.79
$Bis_{1.5}$	52.04	53.32	57.34	58.71	61.82

$Bis_{control}$: biscuit free from marigold flower extract.
$Bis_{0.5}$: biscuit containing 0.5% marigold flower extract.
$Bis_{1.0}$: biscuit containing 1.0% marigold flower extract.
$Bis_{1.5}$: biscuit containing 1.5% marigold flower extract.

Specific volumes of biscuits enhanced with marigold flower extract samples as well as control are presented in Table 8. Specific volumes of all tested samples ranged between 3.05- 3.83cm^3/g. These results revealed that the specific volume of biscuits was slightly increased by increasing the addition of marigold flower extract.

Table 8:-Specific volumeof biscuits containing marigold flower extracts

Characteristic	Biscuit samples			
	$Bis_{control}$	**$Bis_{0.5}$**	**$Bis_{1.0}$**	**$Bis_{1.5}$**
Weight, g	8.24	8.08	7.97	8.64
Volume, cm^3	25.13	27.88	28.21	33.09
Specific volume, cm^3/g	3.05	3.45	3.54	3.83

$Bis_{control}$: biscuit free from marigold flower extract.
$Bis_{0.5}$: biscuit containing 0.5% marigold flower extract.
$Bis_{1.0}$: biscuit containing 1.0% marigold flower extract.
$Bis_{1.5}$: biscuit containing 1.5% marigold flower extract.

Colorimetric parameters (L, a and b values) and graphic photos of biscuits samples containing different levels of marigold flowers extract as well as control sample are presented in Table 9 and Figure 1, respectively. It was clearly noticed that yellowness (b) were gradually increased due to the increase in marigold flower extract addition to biscuit samples. $Bis_{1.5}$ had the highest L value (39.70±1.37) followed by $Bis_{0.5}$ (38.87±1.43), $Bis_{control}$ (36.65±0.94) and $Bis_{1.0}$ (36.06±2.01). $Bis_{1.5}$ recorded the highest a, redness and b, yellowness being 2.10±82 and 12.64±2.07, respectively.

Table 9:-Color attributes of biscuits containing marigold flower extracts

Color value	Biscuit samples			
	$Bis_{control}$	$Bis_{0.5}$	$Bis_{1.0}$	$Bis_{1.5}$
L, lightness	36.65±0.94	38.87±1.43	36.06±2.01	39.70±1.37
a, redness	1.03±0.85	1.30 ±0.79	1.64±0.95	2.10±82
b, yellowness	5.51±1.03	8.50±1.43	10.31±1.29	12.64±2.07

$Bis_{control}$: biscuit free from marigold flower extract.
$Bis_{0.5}$: biscuit containing 0.5% marigold flower extract.
$Bis_{1.0}$: biscuit containing 1.0% marigold flower extract.
$Bis_{1.5}$: biscuit containing 1.5% marigold flower extract.

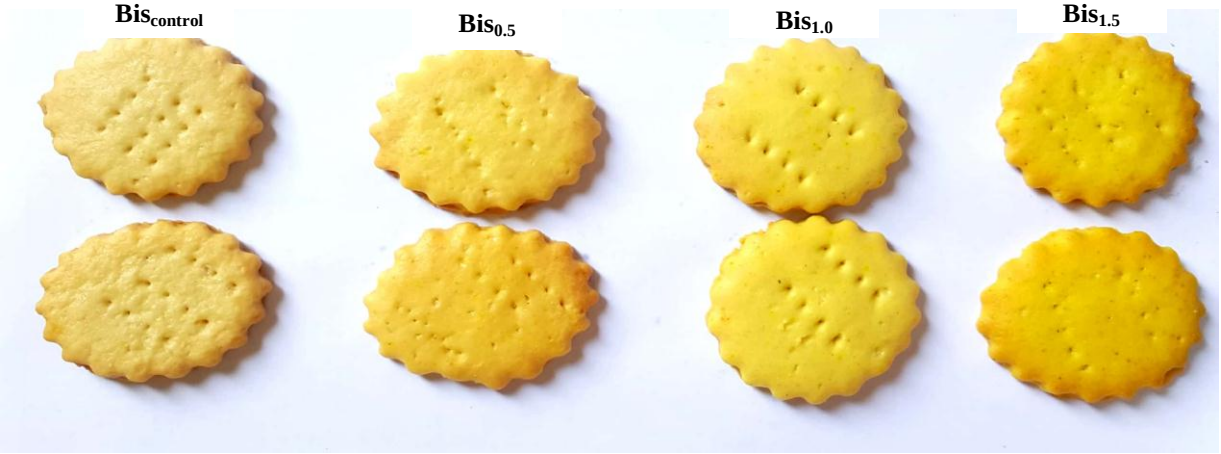

Figure 1:-Graphic photos of biscuits containing marigold flower extract.
where:
$Bis_{control}$: biscuit free from marigold flower extract.
$Bis_{0.5}$: biscuit containing 0.5% marigold flower extract.
$Bis_{1.0}$: biscuit containing 1.0% marigold flower extract.
$Bis_{1.5}$: biscuit containing 1.5% marigold flower extract.

Table 10 illustrates the sensory attributes of biscuits samples containing different levels of marigold flowers extract as well as control sample. Generally no significant variations were recorded between biscuits samples containing marigold flowers extract and control samples regarding taste and texture values. The addition of marigold flowers extract to biscuit improved the color, odor and overall acceptability of the resultant products. $Bis_{1.5}$ was judged as the best sample for color, taste, odor, texture and overall acceptability recording 8.85±1.56, 8.64±2.09, 8.61±1.62, 8.34±1.03 and 8.75±1.63, respectively. Natural edible color was extracted from T. erecta gave lemon yellow color shade which when applied on candies increased the attractiveness (**Alim-un-Nisa *et al*., 2018**).

Table 10:-Sensory attributes of biscuits containing marigold flower extracts

Biscuit samples	Sensory attributes				
	Color	Taste	Odor	Texture	Overall acceptability
$Bis_{control}$	6.32±1.45	8.23±1.85	7.25±1.67	8.12±2.49	7.19±2.30

$Bis_{0.5}$	7.85±1.12	8.43±1.34	7.35±2.43	8.24±1.87	8.24±1.73
$Bis_{1.0}$	8.05±1.39	8.52±1.24	8.46±2.75	8.02±1.78	8.57±1.34
$Bis_{1.5}$	8.85±1.56	8.64±2.09	8.61±1.62	8.34±1.03	8.75±1.63

$Bis_{control}$: biscuit free from marigold flower extract.
$Bis_{0.5}$: biscuit containing 0.5% marigold flower extract.
$Bis_{1.0}$: biscuit containing 1.0% marigold flower extract.
$Bis_{1.5}$: biscuit containing 1.5% marigold flower extract.

Conclusion:-

The using of supercritical CO_2 method for preparing marigold flowers extract enhance its color as well as the content of bioactive compounds such as phenolics, flavonoids and oleoresins comparing with conventional method. On the other side, the addition of marigold flowers extract to biscuit recipes up to level 1.5% (w/w) enhanced its antioxidant activity, specific volume, color attributes. Moreover, the acceptability of sensory properties was improved by this fortification. Therefore, it is recommended to utilize the marigold flower extract in production of biscuits to improve the quality of this product.

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