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BIOCHEMICAL AND TECHNOLOGICAL STUDIES ON GREEN TEA

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ABSTRACT: The present study was designed to investigate the phenolic contents in green tea, to evaluate the antioxidant activity of plant extract and to studying the potential effect of green tea extract to increase protective effect against oxidative stress, and study the effect of plant on chemical properties of oil used in cupcakes. The main component of such green tea was methyle gallate, the extracts under study was found to have antioxidant activity in vitro and in vivo, green tea methanol extract recorded 0.645 at concentration of 100µg/ml in reducing power assay. In DPPH assay green tea methanol extract recorded inhibition percent of 90.4% at concentration of 100 µg/ml comparing with ascorbic acid 97.7%. Green tea have biological effect on liver functions (ALT and AST activity, ALB and TP levels), kidney functions (urea and creatinine levels) and antioxidant markers (CAT activity and MDA level). The treatment with green tea extracts led to reduction in ALT and AST activity, and an increase in total protein and albumin levels accompanied by reduction in creatinine and urea levels, meanwhile treatment with H_2O_2 caused an elevation in CAT activity and MDA level but treatment with green tea extracts affected this elevation, also green tea effected on food technology during storage period of cupcakes and effected on the acid value and peroxide value of cupcakes oil under study.

Key words: Liver, Green tea, Histology, ALT, AST, ALP, CAT, MDA, Sensory, cupcake.

INTRODUCTION

Throughout the world, plants have been a rich source of nutrients and antioxidants as they contain lot of bioactive molecules and compounds. In terms of the bioactive molecules, most of them produce chemical defense against stresses or infections. Native plants usage in traditional as well as modern medicine is gaining a lot of attention nowadays, and the recent studies showed that a number of plant products and herb extracts exert potent antioxidant actions. There are several foods or food components that provide health benefits.

These foods, also known as “functional foods,” provide benefits beyond basic nutrition and may play a role in reducing or minimizing the risk of certain diseases and other health conditions "International food information council foundation" (2011). Functional foods may be whole, fortified, enriched or enhanced. Functional components of food include beta carotenes which are known to scavenge free radicals; calcium reduces the risk of osteoporosis; potassium which reduces the risk of high blood pressure; flavonoids and fatty acids which known to reduce coronary heart diseases and dietary fiber supports gastro intestine health etc. With the rapidly changing socio-economic status, consumers have become aware and are looking for products that provide benefits beyond nutrition. Functional food market is the fastest growing segment in the food market all over the world.

One of such natural food source which can be utilized to add functionality to other foods or act as functional food itself is Green tea which has become one of the most popular and consumed beverages, widely recognized for its healthy influences, since ancient times Cabrera *et al.*, (2006). The terminology is sometimes misleading with respect to the classification and scope of the different ingredient. The tea plant (Camellia sinensis) is a species of plant that

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leaves and leaf tops used to make tea. Lately, the benefits of green tea, such as its antiarthritic, antibacterial, anti angiogenic and antioxidative effects, have been extensively reported.

The benefits are mainly attributed to the polyphenols, of which catechins is the major component, including epicatechin (EC), epigallo-catechin (EGC), epicatechin-3-gallate (ECG), and epigallocatechin gallate (EGCG). Jian *et al.*, (2004). Catechins are widely considered to be preventive agents against mammary cancer post-initiation, degenerative diseases, oxidative stress, cardiovascular and neurological disorders, and hepatotoxicity. They are also considered as antitumorigenic agents and immune modulators in immunodys function caused by transplanted tumors or cancer treatments**.** Many of these health beneficial effects are credited to the most abundant catechin: EGCG Mandel *et al.,* (2004). While a plethora of sources have joined the advocacy for the magic of tea extracts.

MATERIALS AND METHODS

The plant Green tea chines tea (Camellia sinensis) was obtained from the local market English mark named Ahmed tea.

Preparation of extracts

Methanolic extract

500 grams of plant sample powder were steeped in 5000 ml of methanol 80% and the mixture was then kept in shaker incubator for 24 hours at room temperature. then filtered through filter paper and centrifuged at 3000 rpm for 15 min. the filtrate was placed in rotary vacum evaporator to evaporate alcohol from it, to obtain a dried powder as by (Mukhtar and Ghori, 2012).

Aqueous extract

500 grams of plant sample powder were steeped in 5000 ml of distilled water and the mixture was then kept in shaker incubator for 24 hrs at room temperature then filtered through filter paper and centrifuged at 3000 rpm, for 15 min. The filtrate was placed in freeze drier to evaporate the water from it. the dried powder was transferred to a sterile universal flask in the refrigerator for later usage (Dorman e*t al.,* 2003).

Determination of phenolic compounds

The concentration of phenolic compounds in each extract was determined colorimetrically by the method of Folin-Ciocalteu as described by (Gulcin *et al.,* 2002)**,** in which the extract was employed for the chemical determination of phenolic compounds as follows:

Reagents

a) Folin –Ciocalteu was diluted 1:1 with distilled water.
b) Na_2CO_3 solution 20%

Procedure

A volume of 1 ml of each extract was diluted to a total volume 25 ml with distelled water and 1ml of the solution extract was pipetted into a flask then 46 ml of distilled water and 1ml of Folin – ciocalteu were added and mixed thoroughly. The mixture was left to stand for 3 min to which 3 ml of 20% sodium carbonate solution was then added. After 120 min of incubation at ambient temperature with constant shaking, the absorbance was measured at 760 nm against reagent blank. Measurements were carried out in duplicate and a calibration curve was formed using gallic acid as mg GA/ g extract.

Determination of flavonoids contents

The flavonoids contents were determined using the method reported by (Dewanto *et al.,* 2002). Briefly, an aliquot (250 µL) of each extract or standard solution were mixed with 1.25 mL of deionized water followed by 75 µL of a 5% $NaNO_2$ solution. after 6 min, 150 µL of a 10% $AlCl_3.6H_2O$ solution were added to each mixture after 5 min, 0.5 ml of 1M NaOH was added, and the total volume was adjusted to 3.0 ml with deionized water. Catechin was used as a standard. The absorbance at 510 nm, which was corrected using a blank, was then determined and the results were expressed as mg of catechin equivalents (CE) / g extract.

Quantitative determination of phenolic compounds by HPLC

A modified method of (Zeo *et al.,* 2002) was used. A Shimadzu LC 20 AT HPLC filtered with a SIL 20 A auto sampler and a SPD-20 UV-visible detector with a class LC 10 chromatography workstation was used for the analysis of the prepared sample. A luna TM 5 µM C18, 25cm ×4.6 mm i.d (phenomenex, Torrance, CA, USA) column with a Ryeodyne precolumn filter 7335 model was used. All solvents were filtered through a 0.45 µM millipore membrane filter disk and degassed before injection into a HPLC system. A gradient elution was carried out using the following solvent systems: Mobile phase A (acetonitrile / acetic acid /double distilled water -9/2/89 v/v/v). Mobile phase B (acetonitrile /acetic acid /double distilled water -80/2/18 v/v/v). The mobile phase composition for a binary gradient condition was started at 100% solvent A for 10 min, then over 105 min .at linear gradient to 60% mobile phase A ,32% mobile phase B and held at this composition for 10 min. before the next injection. the flow rate of mobile phase was 1mL / min and the temperature at the column was performed at 35 ±0.5 o C. the quantification of catechins was performed at 278 nm and was achieved using a caffeine external standard with a calibration curve $R_{2=}$ 0.9984 in conjunction with the consensus individual catechin relative responce factor (RRF) values with respect to caffeine calculated on dry matter basis. Total catechins as percentage by mass a sample dry matter basis was given on the summition of individual catechins .

Antioxidant activity

Reducing power assay

Reducing power ability of the samples were determined using the method of Adesegun *et al.,* (2008) by mixing 2.5 ml of extact samples at various concentrations (25 ,50 ,75 ,100) µg mL^{-1} with 2.5 ml of 1% potassium ferricyanide and incubate at 50°c for 20 min. 2.5mL of trichloroacetic acid 10% was added and centrifuged (1000 ×g ,10 min) the supernatant (2.5 ml) was mixed with equal volume of distilled water and ferric chloride (0.5 ml , 0.1%) the absorbance was measured at 700 nm against a reagent blank**.** Vit Cwas used as a standered.

Determination of free radical scavenging activity by DPPH assay

The antioxidant activity of extracts was measured on basis of the scavenging activity of the stable 1, 1 diphenyl-2-picrylhydrazyl (DPPH) free radical according to Lee *et al.,* (1996) with slight modifications, 1 ml of 0.1 mM DPPH solution was mixed with 1 ml of various concentrations (25, 50, 75, 100) µg/ ml of plant extracts, corresponding blank sample were repaired and ascorbic acid was used as reference , standard mixture of 1ml methanol and 1ml DPPH solution was used as a control. The reaction was carried out in triplicate and the decrease in absorbance was measured at 517 nm after 30 minutes in dark using UV-vis spectrophotometer, the inhibition % was calculated using the following formula

Inhibition % = Ac – As/Ac×100

The Ac is the absorbance of control and As is the absorbance of sample.

Biological effect

Expermintal animals

Adult male albino rats (100-120gm) were obtained from Memorial Institute of Ophthalmology in Giza, Egypt. All the animals were kept in plastic cages and placed in a well-ventilated rat house, temperature was (20-25±2°c) and lighting conditions were natural light from large windows during the night and were acclimatized to laboratory conditions for one week perior to the start of experiment. Rats were kept on a balanced diet throughout the expermintal period.

Expermintal designs

The expermintal animals (20 rats) were divided into 5 groups, each have 4 rats as follow

1- The first group was used as (negative control) where rats received tape water as a drinking water with food.
2- The second group (positive control) where rats treated with 0.5%H_2O_2 in drinking water with food.

3- The third group: rats were treated with 0.5% H_2O_2 in drinking water and received green tea aqueous extract (300mg/kg/day)
4- The fourth group: rats were treated with 0.5% H_2O_2 in drinking water and received green tea methanol extract (300mg/kg/day)
5- The fifth group: rats were treated with 0.5% H_2O_2 in drinking water and received selmarine (100mg/kg/day)

Blood sampling

Blood samples were collected from orbital sinus veins technique using heparinized capillary tubes at the end of experimental period, into clean, dry, and labeled eppendorf tubes (2.5ml), the tubes contained heparine as anticoagulant (7.5 I.U/ml blood) according to Schalm (1986). Because of disturbance stress which can alter the metabolic profile, several precautions to minimize stress during sampling were taken into account. Samples were centrifuged to separate plasma. Plasma samples were divided into aliquots to avoid repeated freezing and thawing then kept in a deep freezer at (-20°C), till the different assays were carried out.

Biochemical analysis

Liver function tests

Determination of alanine and aspartate transaminase activities:

Alanine transaminase (ALT/GPT) and aspartate transaminase (AST/GOT) were determined in plasma as described by Young (1990).

Determination of albumin level

Albumin was determined in plasma as described by Canon *et al.*, (1974).

Determination of total protein level

Total protein was determined in plasma as described by Schultze and Heremans, (1966).

Determination of kidney functions

Determination of urea and creatinine levels

Urea and creatinine were measured according to the method of Young, (2001).

Antioxidants biomarkers

Determination of catalase (CAT) activity

Catalase activity was determined in plasma as described by Aebi (1984).

Determination of (MDA) malondialdehyde level

Lipid peroxidation was measured by determining MDA using thiobarbituric acid reactive substances (TBARS) by a spectrophotometric assay according to a previous report (Ohkawa *et al.*, (1979).

Technological study

Preparation of cupcake

Cupcakes were prepared according to Sudha *et al.*, (2007). The formula included 25.84% wheat flour (72% extraction), 25.84% sugar, 31.01% whole egg, 6.46% shortening, 10.34% corn oil, 0.13% baking powder and 0.39% salt. Cupcake batter was prepared in a Hobart mixer (N-50) using flour batter method. The flour, shortening, salt and baking powder were creamed together to get a fluffy cream; eggs and sugar were whipped together until semi-firm foam resulted. The sugar-egg foam was mixed with the creamed flour and shortening, after which the vegetable oil was added in small portions. For each cupcake variation, 50 g portions of batter were weighed and placed in paper baking cups in an aluminum muffin pan. The cupcakes were baked at 160°C oven for 45 min. Cupcakes were cooled to room temperature before sensory evaluation**.**

Sensory properties of flat bread and cupcake

Sensory evaluations of flat bread and cupcakes were performed using 15 panelists of staff members of Food Science and Technology Department, Menoufia University. Panelists were selected on the basis of their interest and availability. Sensory quality properties were evaluated using a 9 point hedonic rating scale from 1 for dislike extremely to 9 for like extremely for each property. Flat bread was evaluated for appearance, crust color, crumb

colors, taste, aroma and overall acceptability. Cupcakes were evaluated for crust color, crumb color, flavor, softness and eating quality (Attia-Afaf, 1986).

Separation of oil from cupcake

Cupcakes were soaked in n-hexane (bp 40-60 °C) at room temperature (~ 25 °C), with shaking for 36 h with several changes of solvent (eight times). Evaporation of n-hexane was performed using a rotary evaporator (ROT. VAC. EVA. RVA. 64, Prague Czech Republic) under vacuum. The oil was dried over anhydrous sodium sulfate, filtered, stored in closed dark brown bottles without any further purification in a deep freezer at -18°C until used. AOCS, (1989).

Determination of specific gravity

The specific gravity of the oils was determined using a pycnometer (1 ml-capacity) as described by Gunther (1960).

Determination of refractive index

The refractive index was determined using abbe refractometer, model 60 according to the procedure described in A.O.A.C. (1975).

Determination of acid value

Oil collected (1 to 2) g using a method specified in Korean food code was placed into a flask, and 100 ml mixed solution of methanol and ether (2:1) was then added. With 1-2 drops of phenolphthalein indicator injected, pink color was chosen as the determination of endpoint through titration by 0.1 N KOH Kim *et al.*, (2015).

Determination of peroxide value

1 to 2 g of oil was recovered using a method specified in Korean food code, 25 ml of a mixed solution of acetic acid and chloroform (3:2, v/v) was added. On top of this, 1 ml of KI saturated solution was also added. Then, it was left in a dark room for 10 mins after being shaken for 1 min. With 30 ml of distilled water and starch indicator solution injected, the endpoint through titration by 0.01 N $Na_2S_2O_3$ was observed when the solution turned colorless. (Ministry of Food and Drug Safety, 2017).

Statistical analysis

The Data were analyzed using a completely randomized factorial design SAS (1988) where a significant main effect was detected,the means were separated with the student Newman –Keuls test. Defferences between treatments of ($p \leq 0.05$) were considered significant using Costato programe. Biological resuls were analyzed using analysis of variance ANOVA.

RESULTS AND DISCUSSION

Total phenolic and total flavonoid content of green tea extract

Data in Table (1) showed total flavonoids and total phenols in methanolic extract of green tea leaves, total phenolic content recorded (69.75 mg/g), while total flavonoid, (23.16 mg/g), the above data were in accordance with that obtained by Chacko *et al.,* 2010). where they reported that polyphenols are the main compounds in green tea, moringa tea, and banaba tea. Banaba tea had the highest TPC (20.54±0.31 mg/g), followed by green tea (14.62±0.44 mg/g), moringa tea that its leaves dried at 50 ºC (12.23±0.40 mg/g), and moringa tea that its leaves dried at 60 ºC (11.41±0.67 mg/g).

HPLC analysis for phenolic compounds of green tea

Data in Table (2) showed that methyl gallate, coumaric acid, gallic acid, catechin and narningenin were the major phenolic compounds in green tea, The high content of phenolic compounds, such as flavonoids and phenolic acids, are the main promotor of antibacterial, anti-inflammatory, and antitumor activity. As a matter of fact, cell apoptosis derived from cancer development can be cured with green tea because of its antioxidant potential, which scavenges the reactive oxygen radicals, and, thus, is avoided cell damage Özcan (2018).

Table (1) : Total phenolic compounds and total flavonoids in green tea leaves.

Plant	Total phenols (mg GAE/g)	Total flavonoids (mg catchin/ g)
Green tea	69.75	23.16

Table (2): Phenolic compounds of green tea.

Phenolic compounds	Conc. (µg/ml)	Phenolic compounds	Conc. (µg/ml)
Gallic acid	156.49	Vanilline	3.67
Chlorogenic acid	29.53	Ferulic acid	6.67
Catechin	80.90	Naringenin	41.87
Methyl gallate	1147.06	Querectin	1.89
Syringic acid	37.48	Cinnamic acid	0.05
Rutin	122.47	Kaempferol	0.60
Ellagic acid	40.24	Hesperetin	1.51
Coumaric acid	185.96		

The antioxidant activity in vitro

Free radical scavenging activity

Data in Table (3) showed the free radical scavenging level using DPPH assay % in different concentrations (25 µg/ml, 50µg/ml, 75µg/ml and 100µg/ml) of tested extracts, Green tea recorded 90.4% at the same conc. comparing with ascorbic acid which recorded 97.7% at concentration 100µg/ml. Our results were in consistent with that obtained by Tandoro *et al.,* (2020), who reported that green tea have antioxidant activity due to its phenolic and flavonoid compounds.

Reducing power

Data in Table (4) showed the result of reducing power level assay in different concentration of tested extracts (25 µg/ml, 50µg/ml, 75µg/ml and 100µg/ml). It is clear that the reducing power of green tea increased by increasing its concentration and reached the maximum (0.645) with concentration of 100µg/ml.Our results are similar with that obtained by Stankovic *et al.,* (2011) who reported that green tea have antioxidant activity in vitro and in vivo due to its content of phenolic and flavonoid s compounds.

Effect of green tea extracts on liver function

ALT and AST activity

Data in Table (5) showed ALT and AST activites, ALb and T.P level in plasma in all studied groups after 30 days of treatment .It can be noticed that negative control group recorded 25.17 IU/L for ALT activity while positive control group recorded 45.9 IU/L, groups which treated with methanolic extract and aqueous extract of green tea were decrease to 29.76 and 33.45 IU/L respectively.

The negative control group for AST activity recorded 79.41 IU/L while positive control recorded 112.85 IU/L,but treatment with green tea aqueous extract decreased it to 95.5 IU/L , while green tea alcoholic extract recorded 89.21 IU/Lcomparing to silymarin group 89.99 IU/L .

From data in Table (5), it can noticed that albumin level for negative control group recorded 3.72 mg/dl while positive control group recorded 3.53 mg/dl. Meanwhile, the group which treated with green tea methanolic extract recorded (3.36 mg/dl).

Table (3): DPPH assay of methanol green tea leaves extract:

Samples	DPPH % inhibition			
	25µg/ml	50µg/ml	75µg/ml	100µg/ml
Green tea	62.02	72.83	86.75	90.40
Ascorbic acid	96.35	96.75	97.43	97.70

Table (4) Reducing power of green tea leaves extracts

Sample	Reducing power m Mol.ascorbic eq.			
	25µg/ml	50µg/ml	75µg/ml	100µg/ml
Green tea	0.029	0.172	0.388	0.645

Table (5): Effect of green tea extracts on liver functions

Groups	ALT(IU/L)	AST(IU/L)	T.P(mg/dl)	ALB (mg/dl)
Negative group	25.17^{a}± 2.17	79.41^{a} ± 0.94	7.112^{b}±0.97	3.722^{c}± 0.23
Positive group	45.90^{e} ± 2.91	112.85^{f} ± 1.45	6.056^{a}±0.15	3.533^{bc}±0.27
Green tea aqu. E.	33.45^{d}± 2.17	95.50^{d} ± 2.51	6.34^{ab} ± 1.1	3.188^{ab}±0.05
Green tea M. E.	29.76^{bc}±1.91	89.21^{b} ± 1.68	6.71^{ab} ±0.52	3.363^{ab} ± 0.2
Silymarin group	27.26^{ab}±2.16	89.99^{bc} ± 1.94	6.75^{ab}± 0.18	3.782^{c}± 0.33

Mean values of n=3 ± Standard Deviation. a-bMeans followed by different superscript letters in each column indicate significant differences at p<0.05. A-B Means followed by different superscript letters in each row indicate significant differences at p<0.05
Green tea Aqu. E: Green tea aqueous extract
Green tea M.E: Green tea methanolic extract

From data in Table (5), it can be noticed that total protein for negative control group recorded the highest value 7.11 mg/dl, while the positive control group recorded the lowest value 6.05 mg/dl, the silymarine group have a moderate value 6.75 mg/dl. On the other hand green tea water and methanolic extract have recorded 6.34 and 6.71 mg/dl respectively. Our findings are in line with that obtained by Bakr and Header (2014) who confirmed that oral intake of green tea had a significant effect in improving total protein in all treated groups compared to control group.

Kidney functions

Plasma creatinine and urea level

Data in Table (6) showed creatinine and urea levels in plasma for all studied groups after 30 day of treatment .It can noticed that positive control group recorded the highest value 1.91 mg/dl for creatinine while negative group recorded the lowest value 0.92 mg/dl, green tea aqueous extract recorded 1.05 mg/dl and green tea alcoholic extract recorded 1.002 mg/dl .As for urea the positive group recorded the highest value 57.59 mg/dl while negative group recorded 38.95 mg/dl ,green tea aqueous extract recorded 44.22 mg/dl while green tea alcoholic extract recorded 38.92 mg/dl, our results are in accordance with that obtained by **S**en *et al.,* (2018), who reported that administration of ethanolic extract of green tea and Pesiduim guajava leaves at high dose (200 mg/kg b.wt.) significantly caused a decrease in creatinine, urea and uric acid. In line with these results, Saleh *et al.,* (2018) stated that green tea has renoprotective effect and lowering effect on kidney functions parameters duo to bioactive compounds as beta-carotene, vitamin C, vitamin E, and polyphenols which are a good source of natural antioxidants which can protect against oxidative damage.

Table (6): Effect of green tea on kidney functions

Groups	Creatinine	Urea
Negative group	$0.927^{a} \pm 0.092$	$38.95^{a} \pm 2.3$
Positive group	$1.910^{b} \pm 0.15$	$57.59^{b} \pm 3.8$
Green tea aqu. E.	$1.055^{a} \pm 0.15$	$44.22^{a} \pm 3.6$
Green tea M. E.	$1.002^{a} \pm 0.23$	$38.92^{a} \pm 2.5$
Silymarine group	$0.922^{a} \pm 0.098$	$41.85^{a} \pm 4.1$

Mean values of n=3 ± Standard Deviation. a-b Means followed by different superscript letters in each column indicate significant differences at $p<0.05$. A-B Means followed by different superscript letters in each row indicate significant differences at $p<0.05$

Green tea aqu.E: Green tea Aqueous extract

Green tea M.E: Green tea Methanolic extract

Antioxidant parameters

Catalase activity and Malondialdehyede level

Data in Table (7) showed catalase activity and MDA level in plasma for studied groups after 30 days of treatment. For CAT activity, it can be noticed that positive control group recorded the highest value 113.5 IU/L comparing with negative control 35.75 IU/L. Green tea aqueous and methanolic extracts recorded 83 and 54 IU/L respectively. MDA in positive group recorded 57.25 mg/dl but negative group recorded 14.25 mg/dl, meanwhile green tea aqueous and alcoholic extract recorded 40 mg/dl, and 33.75 mg/dl respectively. Finally, silymarine group recorded 24.50 mg/dl. The results are in the line with that of Gangarapu *et al.*, (2013), who revealed that, H_2O_2 administration induces oxidative stress which was manifested by a significant increase in MDA levels in blood. Some studies have shown that green tea leaves extract increase the SOD and CAT activities as well as GSH content and significantly reduced elevated MDA in rats intoxicated by acetaminophen, cadmium chloride and alcohol. Bahashwan *et al*., (2014).

Food application

Sensory evaluation of cupcake

Data presented in Table (8) show sensory properties of cupcake prepared by adding green tea leaves powder with different concentrations (0.1%,0.3% and 0.5%) to cupcake, green tea additives levels were similar ($p>0.05$) to the control cupcake in all sensory properties under study which had lower ($p \leq 0.05$) mean scores for all sensory properties compared to control and cupcake prepared with No significant ($p \leq 0.05$). Appearance, flavor Texture and other all acceptability of cupcakes were not significantly ($p \leq 0.05$) affected by adding green tea of different. conc. At higher concentrations (0.5%) these attributes were significantly ($p \leq 0.05$) reduced as compared with the control cupcake. our findings were in accordance with Beswa *et al.*, (2016) who suggested that cupcake fortified with green tea showed no effect on the physical and sensory properties. the fortification must be readily available and accessible without producing a substantial change in the fortified meal's sensory characteristics or consumer acceptability. Under the current experiment, locally available were used as fortification to develop fortified cake, their nutritional, sensory evaluation, and consumer acceptability were assessed. The cakes became darker in color than the unfortified cake with the increase of concentration in the formulations The darker color was expected with the addition of the formulations, A similar color change trend was also reported in several studies in cookies and snacks due to the chlorophyll concentration of the leaves used for fortification. This dark color can adversely affect the acceptability of the fortified cake by consumers, as it is more attractive in terms of appearance. However, nutritional and other sensory attributes of fortified cake may outweigh that limitation as people are more concerned today about health benefits rather than appearance.

Table (7): The antioxidant activity of green tea extracts.

Groups	CAT IU/L	MDA mg/dl
Negative group	$35.75^{a} \pm 1.7$	$14.25^{a} \pm 2.2$
Positive group	$113.50^{g} \pm 3.4$	$57.25^{f} \pm 1.7$
Green tea Aqu. E.	$83.00^{e} \pm 4.3$	$40.00^{d} \pm 1.4$
Green tea M. E.	$54.00^{c} \pm 2.5$	$33.75^{c} \pm 3.5$
Silymarine group	$45.00^{b} \pm 5.7$	$24.50^{b} \pm 2.6$

Mean values of n=3 ± Standard Deviation. a-b Means followed by different superscript letters in each column indicate significant differences at $p<0.05$. A-B Means followed by different superscript letters in each row indicate significant differences at $p<0.05$

Green tea aqu. E : Green tea water extract

Green tea M.E : Green tea Methanolic extract

Table (8): Sensory properties of cupcake containing different concentrations of green tea and storage at 4°C.

Sensory Properties	Storage period (days)					
	0	7	14	21	28	LSD
Appearance						
Control	8.73^{a}	7.73^{b}	7.36^{b}	6.13^{c}	4.26^{d}	0.56
Green Tea 0.1 %	8.20^{a}	8.36^{a}	7.88^{a}	6.46^{b}	5.09^{c}	0.67
Green Tea 0.3 %	8.66^{a}	8.53^{a}	8.20^{ab}	7.80^{b}	5.68^{c}	0.60
Green Tea 0.5 %	8.70^{a}	7.66^{b}	6.06^{c}	5.40^{d}	4.26^{e}	0.61
Texture						
Control	8.66^{a}	8.59^{a}	8.14^{ab}	7.53^{b}	6.46^{c}	0.64
Green Tea 0.1 %	8.73^{a}	8.65^{a}	8.26^{b}	7.40^{c}	6.93^{d}	0.62
Green Tea 0.3 %	8.86^{a}	8.53^{a}	8.32^{ab}	7.60^{b}	7.81^{b}	0.69
Green Tea 0.5 %	8.73^{a}	8.33^{a}	8.26^{ab}	7.26^{b}	5.23^{c}	0.59
Flavor						
Control	8.56^{a}	7.28^{b}	$6.46b^{c}$	$6.29b^{c}$	5.17^{c}	0.76
Green Tea 0.1 %	8.58^{a}	7.26^{b}	7.81^{c}	5.66^{d}	5.25^{d}	0.57
Green Tea 0.3 %	8.66^{a}	8.16^{b}	6.45^{c}	6.27^{c}	5.86^{d}	0.64
Green Tea 0.5 %	7.59^{a}	7.40^{a}	6.05^{b}	5.86^{c}	5.13^{c}	0.62
Overall acceptability						
Control	8.47^{a}	7.72^{b}	6.47^{c}	5.13^{d}	4.85^{e}	0.67
Green Tea 0.1 %	8.34^{a}	7.47^{b}	7.24^{b}	6.45^{c}	5.07^{d}	0.52
Green Tea 0.3 %	8.81^{a}	7.95^{b}	7.58^{b}	6.75^{c}	5.61^{d}	0.59
Green Tea 0.5 %	8.32^{a}	7.24^{b}	7.12^{b}	6.29^{c}	5.04^{d}	0.64

Mean values of n=3 ± standard deviation. A-B Means followed by different superscript letters in each column indicate significant differences at $p<0.05$. A-B means followed by different superscript letters in each row indicate significant differences at $p<0.05$

Specific gravity of cupcake

Data presented in Table (9) showed specific gravity of oil it separated from cupcake prepared with different levels of green tea storage at 4°C can be notice that the high value of specific gravity was for control sample after 28 days while green tea leaves powder have the high values of specific gravity in concentration (0.5%) at the end of time. These results agreed with that of Verma *et al.,* (2020). who showed that the stability of the total polyphenolic content (TPC) in the microencapsulated green tea was estimated under different storage conditions and the half-life values of the reactions were calculated. In addition, the degradation kinetics of the polyphenols were monitored during the storage period and the rate constants and the degradation of the polyphenols were calculated. A first-order reaction model was adjusted under all the conditions evaluated.

Refractive index and acid value of cupcake

Data presented in Tables (10 and 11) showed refractive index and acid value of oil cupcake prepared with different levels of green tea storage at 4°c. it can be noticed that no different between control sample and treated samples with green tea for refractive index while for acid value, control sample recorded 0.65 after 28 day, meanwhile samples treated with green tea at concentrations 0.1, 0.3 and 0.5% recorded 0.53,0.34 and 0.36 respectively after 28 day of treatment.

Peroxide value of cupcake

Data presented in Table (12) showed peroxide value of oil cupcake prepared with different levels of stored green tea at 4°C. it can be noticed that the high value of peroxide value was (12.56) in control at 28 day, green tea revealed a good results through the period of storage. Our findings are in accordance with that of Gharby *et al.,* (2015) who reported that, PV measurement is the most common method of determining the content of hydroperoxides. Sponge-fat cakes enrichment with lower concentrations of green tea extracts prolonged the induction time of the lipid fraction when compared to the sample without any added antioxidants. According to Gramza-Michlowska and Bajerska (2007), green tea extract also protects lipids from oxidation for nearly three times and rosemary extract for two times longer than in the case in the control sample.

Table (9): Specific gravity of oil cupcake containing different concentrations of green tea stored at 4°C.

Storage (days)	Specific gravity				
	0	7	14	21	28
Control	0.89	0.91	0.93	0.95	0.97
Green Tea 0.1 %	0.89	0.89	0.90	0.92	0.93
Green Tea 0.3 %	0.90	0.91	0.92	0.92	0.93
Green Tea 0.5 %	0.91	0.91	0.92	0.93	0.94

Table (10): Refractive index of oil cupcake containing different concentrations of green tea stored at 4°C.

Storage (days)	Refractive index				
	0	7	14	21	28
Control	1.468	1.469	1.470	1.472	1.472
Green Tea 0.1 %	1.468	1.470	1.469	1.469	1.470
Green Tea 0.3 %	1.468	1.470	1.470	1.471	1.471
Green Tea 0.5 %	1.469	1.471	1.470	1.472	1.472

Table (11): Acid value of oil cupcake containing different concentrations of green tea stored at 4°C.

Acid value	Storage (days)				
	0	7	14	21	28
Control	0.28	0.34	0.41	0.47	0.65
Green Tea 0.1 %	0.26	0.29	0.35	0.39	0.53
Green Tea 0.3 %	0.24	0.26	0.27	0.31	0.34
Green Tea 0.5 %	0.24	0.27	0.28	0.33	0.36

Table (12): Peroxide value of Cupcake containing different concentrations of green tea stored at 4°C.

Storage (days)	Peroxide value				
	0	7	14	21	28
Control	1.56	5.82	7.25	9.34	12.56
Green Tea 0.1 %	1.53	4.47	6.35	7.68	10.57
Green Tea 0.3 %	1.49	3.82	4.37	5.63	8.29
Green Tea 0.5 %	1.52	4.23	5.18	7.24	9.52

Conclusion

Application of green tea extract in foods technology is of great interest because it is dramatically enhanced the antioxidant status of this bio-product. GCMS/MS analysis of green tea revealed the presence of several compounds with known health potent effects. Present results indicate that green tea extract provide higher antioxidant activities in cupcake. Fortified cupcake had good sensory scores and save the cupcake content of oil during storage period.

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REFERENCES

Adesegun, S.A.; Elechi, N.A. and Coker, H.A.B. (2008). Antioxidant activities of methanolic extract of Sapium elliticum. Pak. J. Biol. Sci., 11: 453-457.

Aebi, H. (1984). Methods in enzymatic analysis. Academic Press. New York.3:276-286.

A.O.A.C (1975) Association of official analytical chemists. 12th Edition., Washington D.C.

A.O.C.S (1989). Official and Tentative Methods and Recommended Practices of the American oil Chemists Society, 4th edition, Edited by D. Firestone, AOCS, Champaign, IL U.S.A.

Attia-Afaf, A. (1986). Physical and chemical studies on the staling of some Egyptian bread. Ph. D. Thesis, Food Sci. Dep. Cairo Univ. pp. 102-106.

Bahashwan, S.; Hassan, M.H.; Aly, H.; Ghobara, M.M.; El-Beshbishy, H.A. and Busati, I. (2014). Antioxidant parameters affected with

treatment with moringa and green tea. Journal of Pure & Applied Sciences & Technology, 23(1): 21-29.

Bakr, E. H. and Header, E. A. (2014). Effect of aqueous extract of green tea (Camellia sinensis L.) on obesity and liver status in experimental rats. International Journal of Pure & Applied Sciences & Technology, 21(2): 53-63.

Beswa, D.; Dlamini, N.R.; Amonsou, E.O.; Siwela, M.; and Derera, J. (2016). Effects of amaranth addition on the pro-vitamin A content, and physical and antioxidant properties of extruded pro-vitamin A-biofortified maize snacks. J. Sci. Food Agric. 96: 287–294

Cabrera, C.; Artacho, R. and Gimenez, R. (2006). Beneficial effects of green tea-a review. J. Am. Coll. Nutr., 25(2): 79-99.

Chacko, S.M.; Thambi, P.T.; Kuttan, R.; and Nishigaki, I(2010). Beneficial effects of green tea: A literature review. *Chin. Med.*, *5*,13.

Dewanto, V.; Wu, X.; Adom, K.K. and Liu, R.H. (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. J. Agric Food chem. 50: 3010-3014.

Dorman, H.J.; Peltoketo, A.; Hiltunen, R. and Tikkanen, M.J. (2003). Characterization of the antioxidant properties of de-odourised aqueous extracts from selected Lamiaceae herbs. Food Chem .83(2): 255-262.

Gangarapu, V.; Gujjala, S. and Korivi, S. (2013). Combined effect of curcumin and vitamin E against CCl4 induced liver injury in rats. Am J Life Sci, 1(3): 117–124

Gharby, S.; Harhar, H.; Matthäus, B.; Bouzoubaa, Z. and Charrouf, Z. (2015). The chemical parameters and oxidative resistance to heat treatment of refined and extra virgin Moroccan Picholine olive oil. *J Taibah Univ Sci.* 10:100–106. doi: 10.1016/j.jtusci.2015.05.004.

Gramza-Michałowska, A. and Bajerska-Jarzebowska, J. (2007). Leaves of *Camellia sinensis*: Ordinary brewing plant or super antioxidant source? Food 1: 56-64.

Gulcin, I.; Oktay, M.; Kufrevioglu, I. and Aslan, A. (2002). Determination of antioxidant activity of lichen cetraria islandica (L.) Ach. J. Ethnopharmacol. 79: 325-329.

Gunther, E. (1960). The essential oils . Vol .1,4 th ed. D.Van Nostrand Company ,Inc Princelon, New Jersy, Toronto, New York, London.

Jian, L.; Xie, L. P.; Lee, A. H. and Binns, C. W. (2004). Protective effect of green tea against prostate cancer: a casecontrol study in southeast China. Int. J. Cancer, 108: 130–135.

Khan, and Mukhtar H, (2011), Green Tea Polyphenols and Metabolites in Prostatectomy, *Natl. Heal. Inst.*, 81 (7): 519–533.

Kim, H.J.; Lee, K.J.; Ma, K.H.; Cho, Y.H.; Lee, S.; Lee, D.J. and Chung, J.W. (2015). Effect of tomato leaf extracts on anti-inflammatory and antioxidant activities. *Journal of Integrative Agriculture*, 27(4): 529-535.

Lee, J.; Park, J. and Choi, J. (1996). The antioxidant activity of Ecklonia stolonifera. Archives Pharmacal Res .,19(3): 223-227.

Mandel, S.; Weinreb, O.; Amit, T. and Youdim, M.B.H. (2004). Cell signaling pathways in the neuroprotective actions of the green tea polyphenol (3)-epigallocatechin-3-gallate: Implications for neurodegenerative diseases. J. Neurochem. 88: 1555–1569.

Ministry of Food and Drug Safety. (2017). Korean Food standards codex. Retrieved July 29, 2020 from Food Safety Korea website: http://www.foodsafetykorea.go.kr/foodcode

Mukhtar, S. and Ghori, I. (2012). Antibacterial activity of aqueous and ethanolic extracts of garlic, cinnamon and turmeric against Escherichia coli ATCC 25922 and Bacillus Subtilis DSM. Int. J. Applied Bio. Pharm. Tech., 3: 132-137.

Ohkawa, H.; Ohishi, N. and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem, 95: 351–358.

Özcan, M.M. (2018). Moringa spp: Composition and bioactive properties. S. Afr. J. Bot. 2018, 129: 25–31.

Saleh, Nahed, S.; Allam, T.S.; El-Rabeaie, Riham, M. and El-Sabbagh, H.S. (2018). Protective effect of some Egyptian medicinal plants against oxidative stress in rats. Alexandria Journal of Veterinary Sciences, 58 (1): 1-14.

SAS (1988). SAS Users Guide: Statics version 5th Ed., SAS. Institute Inc., Cary N.C.

Schalm, O.W. (1986). Veterinary Haematology. 4th ed., Lea Febiger, philadelphia, PP. 21-86.

Schultze, H.E. and Heremans, J.F. (1966). Molecular biology of human protein. Elsevir publishing company, Amesterdam, Saunders: 346-421.

Sen, S.; Chakraborty, R. and Kalita, P. (2018). Dilleniaindica fruit prevents cisplatininduced kidney injury in experimental rats through modulation of oxidative stress, marker enzyme, and biochemical changes. Nutr., 43: 15.

Stankovic, M.S.; Niciforovic, N.; Topuzovic, M. and Solujic, S. (2011). Total phenolic content, flavonoid concentration and antioxidant activity of whole plant and plant parts extractsfrom teucrium montamnum L. var. montanum, F. supinum (L.) Reichenb. Biotechnologyand Biotechnology. 25(1): 2222-2227.

Sudha, M. L.; Baskaran, V. and Leelavathi, K. (2007). Apple pomace as a source of dietary fiber and polyphenols and its effect on the rheological characteristics and cake making. Food Chemistry, 104: 686–692.2017; 2(4): 173-178.

Tandoro, Y.; Widyawaty, P.S.; Budianta, T.D.W. and Sumargo, G. (2020). Phytochemical identification and antioxidant activity of moringa fruits and leaves extracts: a comparative study. International journal of pharmacy and pharmacutecal sciences, 12 (6): 55-58.

Verma, A.K.; Rajkumar, V.; Kumar, M.S. and Jayant, S.K. (2020). Antioxida- tive effect of drumstick (Moringa oleifera L.) flower on the quality and stability of goat meat nuggets.

Young, D.S. (1990). Effects of drugs on clinical laboratory tests,third edition. 3: 6-12.

Young. D.S. (2001). Effect of diseaseon clinical Lab.tests.4th ed AACC American Assosition for Clinical Chemistry. The medicinal plants. 5th.Ed Tehran university publication, Tehran. 312-314.

Zeo, Y.; Chen, H. and Deng, Y. (2002). Simultaneous determination of catechins caffeine and gallic acids in green, oolong, black and puerr teas using HPLC with a photodiode array detector. Talanta, 57: 307-316.

دراسات كيميائية حيويه وتكنولوجيه علي الشاي الأخضر

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الملخص العربي

تم دراسه التركيب الكيميائي للشاي الاخضر ووجد انه يحتوي علي ١٤ مركب أعلي محتوى كان ميثيل جالات (١١٤٧,٠٦٪). كما سجلت كمية الفينولات الكلية للشاي الأخضر (٦٩,٧٥مجم/GAE جم) وأما اجمالي المركبات الفلافونيدية وجد أن أعلي متوسط للشاي (٢٣,١٦مجم/جم GAE) في حين أن فحص قدره DPPH بتركيزات مختلفة ٢٥ مجم/ديسيلتر و٥٠ مجم /ديسيلتر و٧٥مجم/ديسيلتر و١٠٠مجم/ديسيلتر. نجد FRSR الشاي الأخضر نسبة (٨٢,٢٪) في التركيز ١٠٠. نتيجة فحص Reducing power% بتركيزات مختلفة ٢٥ مجم/ديسيلتر و٥٠ مجم /ديسيلترو٧٥مجم/ديسيلتر و١٠٠مجم/ديسيلتر. نجد أن الشاي الأخضر سجل أعلي نسبة (٠,٦٤٥٪) و أوضحت النتائج أن فوق أكسيد الهيدروجين يسبب ارتفاعا في اليوريا والكرياتينين. وأدت المعاملة بالشاي الأخضر الي تقليل اليوريا والكرياتينين في الدم أوضحت النتائج أن فوق أكسيد الهيدروجين تسبب في ارتفاع كل ما يلي في البلازما CAT وMDA. وأدت المعاملة بالشاي الأخضر الي تقليل CAT وMDA في الدم وكان اكثرهم تأثير المستخلص الكحولي للشاي الأخضر. لقد تم عمل تحكيم لعينات الكيك بعد التبريد لمده ٢٨يوم ووجد أنه بزيادة التركيز يحدث تغير طفيف في اللون وتم دراسة الكثافة النوعية لعينات الشاي كل منها بتركيزات مختلفة ومعامل الانكسار سجل أعلي قيمة عند عينة الكنترول بعد ٢٨ يوم. وتم دراسة تأثير الزيوت المستخلصة على رقم الحامض بعد التخزين على درجة ٤ درجة مئوية وسجل رقم الحامض أعلي قيمة للعينة الكنترول عند اليوم ٢٨ (٠,٦٥٪) كما أن الشاي الأخضر أعطي نتائج جيدة في الثلاث تركيزات. أما بالنسبة لرقم البيروكسيد يمكن أن نلاحظ أن أعلي قيمة كانت ١٢,٥٦ للعينة الكنترول بعد ٢٨ يوم وأقل قيمة لرقم البيروكسيد كانت ١,٤٥ ويمكن ملاحظة أن الشاي الأخضر أعطي نتائج جيدة في فترة التخزين.