**Egyptian Journal of Chemistry**
**http://ejchem.journals.ekb.eg/**

# A Comparative Study Between Different Additives for Date Pits Coffee Beverage: Health and Nutritional Evaluation

**Tamer I. M. Ragab[1*], Nadra S. Y. Hassan[2]**
[1]*Chemistry of Natural and Microbial Products Department, Pharmaceutical Industry Division, National Research Centre, 12622 Dokki, Giza, Egypt.*
[2]*Experimental Kitchen Research Unit, Food Technology Research Institute, Agricultural Research Center, Giza, Egypt.*

DATE pits are the main waste product of the date manufacturing that could present potentially precious material for the production of coffee drink ingredients. Three coffee types were prepared from roasted date pits powder with some valuable additives. The chemical composition was found to comprise the following: protein, fats, flavonoids, tannins, phenolics, dietary fiber, carbohydrates, fatty acid, essential minerals, and caffeine content. The results indicated that C1 (roasted date pits), C2 (date pits 92.5%, cardamom 2.5%, button roses 4%, nutmeg 0.5% and cloves 0.5%), C3 (date pits 61.67%, barley 30.83%, cardamom 2.5%, button roses 4%, nutmeg 0.5% and cloves 0.5%) had high nutritional values in comparison with commercial coffee. Caffeine concentration was estimated and C3 revealed the lowest caffeine content (2.99 mg/g) whereas, control coffee has the highest caffeine content (36.02 mg/g). The sensory evaluation qualified C3 designated the most overall acceptability (8.70) which is better than the control coffee (8.53). In addition, C3 coffee has the highest antioxidant activity 91.72% and control sample was 84.18%. All samples have no cytotoxicity and propagate the normal cells (Bj-1). Based on all these findings, the prepared coffees are recommended to be a new type of an integrated healthy coffee drink.

**Keywords:** Caffeine, Chemical composition, Cytotoxicity, Date pits, Sensory evaluation.

## Introduction

Food industry produces waste residues that could be transformed into useful ingredients. The potential benefit of exploiting food waste, which includes improved profitability, has encouraged intensified research [1]. Date palm fruits (*Phoenix dactylifera* L.) are suitable examples of social and economic growth. Coffee is grown in over 70 countries, mainly in Latin America, Asia, and Africa. Ever since 2010, coffee production has been approximately 8 million tones, Brazil and Columbia contributes to nearly 40% of this production. Annually, Egypt import-approximately 40.000 tons (0.1 kg/person). Brewed coffee is one of the most favorite and consumed beverages in the world owing to its pleasant flavor and taste [2]. The increasing demand for coffees resulted in a segmentation of the coffee market and higher prices are paid for specialty coffees [3]. There are two types of coffee, Arabic and Robusta, which have the highest economic importance. Drinking coffee more than 6 cups/day might cause "caffeinism" (i.e., anxiety or agitation). Coffee containing high ratio caffeine can cause insomnia, nervousness, stomach upset, vomiting, increased heart and breathing rate [4]. Lately, there has been an interest in the investigation of coffee beans alternative to avoid its common side effects. The date pits have been roasted and ground to substitute coffee [5]. A large quantity of date pits could be easily collected from the date processing industries [6]. The world production of dates is 7.9 million tons per year. Egypt, Iran, and Saudi Arabia are the largest producers [7]. Date production of Egypt represented about 20% of the total world production [8]. Pits are about 12-15% of the date palm (wt/wt)-estimating up to 800,000 tones/year [9]. Date pits are rich in nutrient compounds like fats, protein, carbohydrate and essential minerals [10]. In traditional Egyptian medicine, date palm pits are listed in folk remedies for the management

*Corresponding author e-mail: tamerragab2006@gmail.com; Tel: 00201220547472
Received 26/6/2019; Accepted 7/7/2019
DOI: 10.21608/ejchem.2019.14035.1867

of diabetes, liver diseases and gastrointestinal disorders [11]. In addition, its combination with insulin minimizes the toxic effects of diabetes as mentioned by El-Fouhil et al. [12]. Therefore, the need to explore the potential of date pits through product innovation of food and drink stuffs with a high nutritional value was necessary. Barley grass powder is rich in functional ingredients that could be reflected in its biological activities [13]. The aim of this study focused on the preparation of three types of coffee from roasted date pits with different additives. The determined nutritional content was compared with the commercial coffee. Furthermore, the biological activities like antioxidant, antimicrobial, probiotic and *in vitro* cytotoxicity activity were identified.

## Materials and Methods

*Materials*

The date palm pits were obtained from the drying plant of the Technology Food Research Institute of the Commercial Type. Barley powder, Cardamom powder, Nutmeg, Button roses, sugar, Cloves and commercial coffee were purchased from the local market, Giza governorate, Egypt.

*Cell culture*

Human normal fibroblast cell line (BJ-1) was maintained in DMEM-F12 medium. The medium was supplemented with 10% fetal bovine serum and incubated at 37°C in 5% $CO_2$ and 95% humidity. Cells were sub-cultured using 0.15 % trypsin.

*Methods*

*Roasted date pits powder preparation*

The collected date pits were soaked and washed with normal tap water and then with distilled water to remove any adhering date flesh. Date pits were roasted in an oven set at 60-70°C for 4h and then 180°C for 1h until the pits color turned into light brown. Some butter was added during the roasting stage to make the date seeds easier to be ground. Roasted pits were milled using high speed blender (IKA-Laboratechnic, Germany).

*Coffee preparation*

The prepared pits powder was used as the main base for healthy coffee with some additives as shown in (Table 1).

*Date pits coffee drink preparation*

Add the equivalent of hanging tea (6 grams) of C1, C2, C3 and control coffee separately to the water with a little sugar, and then boiling.

*Physiochemical characterization*

Crude protein, fats, moisture, ash content and total dietary fiber were determined according to the standard methods [14].

*Total phenolic content (TPC)*

The total phenolic content was estimated according to Makkar's method [15]. Three mL of the extract was thoroughly mixed with 1.5 mL of Folin Ciocalteu phenol reagent (previously diluted 1:10 in water), and allowed to stand for 5 min. Sodium carbonate solution (20%) 1.5 mL was added and the mixture was gently stirred. After incubation (90 min at room temperature), the absorbance was measured at 725 nm on a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). Readings were calibrated using known concentrations of gallic acid. TPC was expressed as mg of gallic acid equivalents (mg gallic acid equivalent/ 100g extract) and the values are presented as means of triplicate.

*Total flavonoid content (TFC)*

The total flavonoid content was determined according to Al-Farsi & Lee [16]. Briefly, 1 mL of extract was dissolved in 5 mL of $H_2O$ in a 10 mL volumetric flask. Sodium nitrite 0.3 mL (50 g $L^{-1}$ in water) was added; the mixture was allowed to stand for 5 min and then 0.3 mL of aluminum chloride (100 g $L^{-1}$ in water) was added. The mixture was incubated (6 min at 25°C), after that 2 mL of sodium hydroxide (1 M) was added and diluted to volume with water. The absorbance was immediately measured at 510 nm. Measurements were calibrated with a standard curve of known concentrations of catechin. TFC was expressed as mg of catechin equivalents (mg catechin equivalent/ 100g extract).

**TABLE 1. The prepared date pits coffees composition (%w).**

| Additives | C1 | C2 | C3 | Control |
|---|---|---|---|---|
| Date pits | 100 | 92.5 | 61.67 | --- |
| Barley | --- | --- | 30.83 | --- |
| Coffee seeds | --- | --- | --- | 92.5 |
| Cardamom | --- | 2.5 | 2.5 | 2.5 |
| Button roses | --- | 4 | 4 | 4 |
| Nutmeg | --- | 0.5 | 0.5 | 0.5 |
| Cloves | --- | 0.5 | 0.5 | 0.5 |

*Tannins content*

The tannin contents were determined using Folin Denis reagent as described by [17]. In that method, a standard calibration curve was prepared and the Absorbance (A) against the concentration of tannins at specific wavelength was estimated.

*Fatty acid composition*

The extracted crude fatty acids were fractionated and identified using gas chromatography with FID detector (PE Auto System XL) with auto sampler and Ezchrom integration system [18].

*Total carbohydrate determination*

Complete with acid hydrolysis of samples were carried out according to the modified method by Ragab et al. [19].

*Qualitative examination of the hydrolyzed products*

The hydrolyzolate sugars were detected using chromatography on Whatman No.1 paper, using the solvent system: n-butanol-acetone-water (4:5:1). Authentic samples of D-galactouronic acid,D-Galactose, D-glucose, D-Fructose, D-Mannose, L-arabinose and D-xylose were co-chromatographed as reference sugars. After chromatographic separation, the chromatogram was air dried and dipped in 40-50 ml of the color reagent, air dried, and then heated at 105°C for 10 min in an oven for developing the colored spots.

*Quantitative determination of the hydrolyzed products*

Quantitative determination of the hydrolyzed sugars was done. The individual chromatographic spots were cut off, divided into small strips, and dropped into 4 ml eluting agents. The absorbance of the resulting colored solutions was determined at 390 NM Spectrophotometer UNICO 7200 [20].

*Scanning electron microscopy (SEM)*

The surface morphology of samples was examined using scanning electron microscopy (JEOL 5410) microscope with an accelerating voltage conducted at 10 kV. Coffee samples were gold coated using a Hitachi coating unit IB-2 coater under a high vacuum, 0.1 Torr, high voltage, 1.2 kV and 50 mA.

*Energy dispersive X-ray analysis (EDXA)*

EDXA is an x-ray spectroscopic method for determining elemental compositions (qualitative and quantitative analysis).

*HPLC conditions*

HPLC analysis was carried out using an Agilent 1260 series. The separation was carried out using a C18 column (4.6mm x 250mm i.d., 5 μm). The mobile phase consisted of water (A) and 0.02% trifluoroacetic acid in acetonitrile (B) at a flow rate1 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (80% A); 0–5 min (80% A); 5-8 min (40% A); 8-12 min (50% A); 12-14 min (80% A) and 14-16 min (80% A). The multi-wavelength detector was monitored at 280 nm.

*Sensory evaluation*

The sensory evaluation method was conducted according to Mirghani et al. [21]. The samples were prepared hot to a group of 10 trained members were recruited as consumer panelists. The panelists were subjected to sensory evaluation using a 10-point hedonic scale for color, taste, odor, flavor and overall acceptability.

*DPPH radical-scavenging assay*

The DPPH method [22] was used to determine the free radical scavenging potential of each sample.

*Antimicrobial activity*

The antimicrobial activity of samples was investigated against *Staphylococcus aureus* ATCC 6538, *Candida albicans* ATCC 10231, *Escherichia coli* ATCC 8739 and *Heliobacter pylori* pathogenic bacteria by well diffusion method [23].

*Prebiotic activity*

The prebiotic activity of coffee samples was evaluated. Experimentally, the three probiotics *L. Casei, L. reuteri* and *L. helveticus* were grown in the MRS medium, while *E. coli* was grown in nutrient broth medium at 37°C for 24 h [23].

*Cell viability assay*

Cell viability was determined using the MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay as described by Mosmann [24].

*Statistical analysis*

Minitab Statistical Software 16.0 (Minitab Inc., State College, USA) was used to perform all statistical analyses. ANOVA and significant differences were evaluated [25].

## Results and Discussion

*Prepared coffees nutrient content*

Chemical composition results of the dried date pits (C1) were presented in Tables 2 and 3. Moisture, ash, protein, fats, flavonoids, tannins,

total phenolics, and dietary fiber content were determined to be 3.19%, 1.21%, 12.20%, 7.97%, 1615.12mg, 139 mg, 1834.22 mg, and 64.33% respectively. The obtained results of moisture, ash and fat content were within the range of values. On the other hand protein content (12.2%) was higher than (5.9%) obtained by El Sheikh et al. [9]. In contrast, dietary fiber content was 64.33g/100g (soluble dietary fiber 55.84% and insoluble dietary fiber 8.36%) which was lower than that presented earlier in the literature. Al-Farsi et al., reported that the content in three seed varieties ranging between 77.8 and 80.2 g/100g [26]. The high nutritional value of the date pits was based on their dietary fiber content, which makes them suitable for the preparation of fiber-based foods and dietary supplements. Flavonoids are widely distributed in date pits (1615.12 mg/100g), fulfilling many functions as producing or red/yellow pigments. There are 9 flavonoids (Hesperdin, narengin, rutin, hespertinkaempferol, quercetin, rosmarinic, 7-hydroxyflavon and naringinin) fractionated from the date palm pits and identified by El Sheikh et al. [9]. Flavonoids have a wide range of biological and pharmacological activities including antioxidant, anti-inflammatory, anticancer and antimicrobial [27]. The tannin occurred in suitable ratio 139 mg/100g which plays a role in protection from predation and might help in regulating the plant growth.Principal human dietary sources of tannins are tea and coffee. Coffee pulp contains low amounts of tannins. Date pits have high total phenolic content of 1834.22mg/100g which could be reflected in its antioxidant activities.Roasted date pit contained 17 phenolic compounds as follows; pyrogallol, cinnamic acid, benzoic acid, elagicacid, catechol, protocatchoic acid, syringicacid, caffeine, vanillic acid, epicatechins, chlorogenic acid, P-OH-benzoic, ferulic, catechein, gallic acid, caffeic acid, 4-amino-benzoic [9]. The chemical constituents explained that date pits are rich in bio-compounds. Date pits processing industries could be an excellent source of coffee and food ingredients [28].

To improve the nutritional value and the biological activity, different additives have been added to the date pits (C1) as shown in (Table 1). C2 was prepared from a mixture of date pits 92.5%, cardamom 2.5%, button roses 4%, nutmeg 0.5% and cloves 0.5%. The nutritional value was significantly increased, especially protein (14%), fats (10.75%), flavonoids (1615.12 mg/100g) and phenolics (1834.22 mg/100g) content. C3 was also prepared by mixing date pits 61.67%, barley 30.83%, cardamom 2.5%, button roses 4%, nutmeg 0.5% and cloves 0.5%. Likewise, the nutritional value was considerably increased (flavonoids 1445.77 mg/100g and phenolics 1788.33 mg/100gcontent). Commercial coffee (control) purchased from the local market, consisted of coffee seeds 92.5%, cardamom 2.5%, button roses 4%, nutmeg 0.5% and cloves 0.5%. Moisture, ash and fat content of the control sample recorded high values 11.71%, 4.94% and 14.84% respectively. In contrast, protein (10.88%), flavonoids (839.44 mg/100g) and phenolics (886.55 mg/100g) recorded low values. In general, the chemical constituents of the prepared coffees (CI, C2 and C3) were better than the commercial one and we predicted that this could be reflected in their health behavior. The prepared coffees might have the ability to avoid defects and side effects of the commercial coffee in the market.

**TABLE 2. Chemical constituents (g/100g) of C1, C2, C3 and control sample.**

| Samples | Moisture | Ash | Protein | Fat | T.C % | Dietary fiber | Soluble dietary fiber | Insoluble dietary fiber |
|---|---|---|---|---|---|---|---|---|
| C1 | 3.19±0.19$^{b}$ | 1.21±0.20$^{c}$ | 12.20±0.2$^{b}$ | 7.97±0.03$^{c}$ | 11.10±0.10$^{c}$ | 64.33±0.33$^{a}$ | 55.84±0.20$^{a}$ | 8.36±0.06$^{a}$ |
| C2 | 2.10±0.20$^{c}$ | 2.32± 0.02$^{b}$ | 14.00± 0.30$^{a}$ | 10.75±0.05$^{b}$ | 14.30±0.30$^{b}$ | 56.53±0.53$^{b}$ | 49.18±0.18$^{b}$ | 7.35±0.05$^{b}$ |
| C3 | 2.39±0.09$^{c}$ | 2.23±0.03$^{b}$ | 11.32±0.32$^{c}$ | 7.41± 0.02$^{d}$ | 24.8±0.40$^{a}$ | 51.85±0.15$^{c}$ | 45.10±0.10$^{c}$ | 6.75±0.05$^{c}$ |
| Control | 11.71±0.20$^{a}$ | 4.94±0.04$^{a}$ | 10.88±0.08$^{c}$ | 14.84± 0.04$^{a}$ | 16.00±2.00$^{b}$ | 41.63±0.20$^{d}$ | 36.21±0.03$^{d}$ | 5.42±0.02$^{d}$ |
| LSD | 0.331 | 0.199 | 0.460 | 0.069 | 1.943 | 0.633 | 0.275 | 0.089 |

**TABLE 3. Flavonoids, tannins and phenolic content (mg/100g) of C1, C2, C3 and control sample.**

| Samples | Flavonoids | Tannins | Phenolics |
|---|---|---|---|
| C1 | 1615.12±5.12[b] | 139.00±0.005[a] | 1834.22±4.06[b] |
| C2 | 1677.33±6.67[a] | 137.01±0.1[a] | 1888.55±3.89[a] |
| C3 | 1445.77±7.72[c] | 130.70±0.06[a] | 1788.33±5.37[c] |
| Control | 839.44±3.44 [d] | 157.02±0.04[a] | 886.55±2.73[d] |
| LSD | 11.227 | 0.116 | 7.763 |

*Fatty acid composition*

The extracted crude fatty acids were fractionated and identified in C2, C3 and control coffee samples using a gas chromatography technique as shown in Table 4. Fatty acids are carboxylic acids have a long aliphatic chain, which either saturated or unsaturated. C2 results showed that saturated fatty acids (Lauric, Myristic, Palmitic, Stearic, Arachidic and Behenic) represented 37.46%, however the unsaturated fatty acids (Palmitoleic, Oleic, Linoleic, Linolenic, Gadoleic and Erucic) were 62.54%. C3 and control sample showed that saturated fatty acids represented 39.59% and 26.09%, however, the unsaturated fatty acids were 60.41% and 73.91%, respectively. The roasted date pits showed that unsaturated fatty acids were 48.66%, however, the saturated fatty acids were 51.34% [9]. The main mono-unsaturated fatty acid was oleic acid found in C2 (48.49%) and C3 (47.43%), linoliec acid as a polyunsaturated fatty acid was 8.10% and 8.30%, respectively. These results were in agreement with the findings of Biglar et al. (2012) [29], they stated that the oleic acid is about half the value of the date pits fatty acids and ranged from 33.38% to 51.40%. The control coffee sample has the lowest ratio of oleic acid (16.52%) in comparison with C2 and C3. The quality and uses of the fatty acids related to the oleic and linoleic acid amount. The major saturated fatty acids in C2 and C3 were lauric acid 16.80% and 17.04%, myristic acid 8.83% and 9.18% and palmitic acid 9.62% and 9.67%, respectively. Control coffee has no lauric and myristic acid. Lauric acid generates an improvement in the fat profile and decrease the hazard of the cardiovascular diseases [30]. Consequently, the two prepared coffee samples may possibly introduce a new valorization in this field. Fatty acids results indicated the overpowered of C2 and C3 on the control sample.

*Total carbohydrates*

The results verified the linearity, precision and common sugars in samples ranging from C1 11.1%, C2 14.3%, C3 24.8%, and commercial coffee 16%. The prepared coffee has a common sugars of interest in food and beverages, especially C3 as shown in Table 5. The total carbohydrate content is a good tracer for assessing the authenticity of soluble instant coffee.Coffee carbohydrates constitute the main part (at least 10-25% of the dry weight) of raw coffee beans.The carbohydrates in coffee supply the flavor of the drink as they undergo complex changes to react with amino acids during the roasting process. Carbohydrates perform aroma binders, foam stabilizers and impart viscosity to the coffee beverage [31]. The quantitative and qualitative test indicated the presence of essential sugar ratios in sample C1, C2 and C3 better than the commercial coffee. The three coffee samples included D-glactouronic acid 5%, 2.5% and 2.5%, D-Galactose 15%, 12.5% and 20%, respectively. Control sample has no D-galactouronic acid and high amount of D-Galactose 45%. D-galactouronic acid is one of the most important uronic acidic sugars, playing important roles in the metabolic activities of the human body. The control coffee sample has a high glucose content 10%, while C1 has nil glucose, C2 5% and C3 6.5%. Subsequently, C1, C2 and C3 coffee samples are saved and suitable for diabetic patients. L-arabinose represented half of the total sugar content C1 50%, C2 40% and C3 40%. Arabinose is an inhibitor of sucrose, which breaks down sucrose into fructose and glucose in the small intestine. Foods and drinks that contain arabinose are usually designed for prediabetic and diabetic patients. Arabinose could be used as a potential prebiotic, because it cannot be absorbed by the intestine and could be utilized by probiotics such as bifidobacteria [32]. D- Mannose was the second available sugar C1 30%, C2 40% and C3 30%, while the control sample has nil. Mannose is essential in human metabolism, mainly in the glycosylation of definite proteins [33]. Monosaccharide constituents of C1, C2 and C3 indicated the presence of essential sugars and their absence from control coffee.

**TABLE 4. Fatty Acids Content of C2, C3 and control sample (%).**

| Fatty acids | C2 | C3 | Control |
|---|---|---|---|
| Lauric | 16.80 | 17.04 | 0.00 |
| Myristic | 8.83 | 9.18 | 0.00 |
| Palmitic | 8.62 | 9.67 | 25.30 |
| Palmitoleic | 1.53 | 2.17 | 7.66 |
| Stearic | 2.96 | 3.12 | 0.00 |
| Oleic | 48.49 | 47.43 | 16.52 |
| Linoleic | 8.10 | 8.30 | 37.61 |
| Linolenic | 1.39 | 2.14 | 10.58 |
| Arachidic | 0.02 | 0.36 | 0.35 |
| Gadoleic | 0.19 | 0.37 | 0.28 |
| Behenic | 0.23 | 0.22 | 0.44 |
| Erucic | 1.97 | 0.00 | 0.13 |

**TABLE 5. Monosaccharide constituents after acid hydrolysis (% w/w).**

| Samples | Monosaccharide constituents (% w/w) | | | | | | |
|---|---|---|---|---|---|---|---|
| | D-glactouronic acid | D-galactose | D-glucose | D-fructose | D-mannose | L-arabinose | D-xylose |
| C1 | 5 | 15 | - | - | 30 | 50 | - |
| C2 | 2.5 | 12.5 | 5 | - | 40 | 40 | - |
| C3 | 2.5 | 20 | 6.5 | - | 30 | 40 | t |
| Control | - | 45 | 10 | 45 | - | - | - |

*Surface morphology determination*

The surface morphology and crystal size of coffee samples were determined using scanning electron microscopy (SEM). Electron beam focused on the sample surface and producing the image about the topography and composition. C1 image indicated aggregates have no definite shape and crystal size ranging from 4.47µm-476nm (Fig. 1). C2 and C3 coffee samples, images reflected also no specific morphology. Crystals have size ranging from 1.84-4.19 µm and 2.55 µm -556 nm. The control coffee sample has a crystal size of 3.51-10.55 µm. In general, all prepared coffee samples have the same morphology and crystal size less than control sample, which could be reflected on the solubility degree.

*Qualitative and quantitative elemental composition*

EDXA is a surface analysis technique where the qualitative and quantitative coffee sample's elemental composition was determined using an x-ray spectroscopic method (Fig. 2). EDXA analysis showed that C1 and C3 contained mainly from C, O, N, Na, Mg, P, Cl, K, Ca and Fe elements. However, C2 contained from C, O, N, Na, Mg, P, Cl, K, Ca and Se elements with different ratios as listed in Table 6. Ca, Mg, K, P, Fe, Zn, Mn, Cu and Na elements were found at high concentrations in date pits [9]. The results revealed that, the prepared coffee samples include a variety of vital elements, which could be valuable in the human body and participate in numerous metabolic reactions. The human body's content of Se is in the range of 13-20 mg. According to the Food and Drug Administration (FDA) the prepared coffee has the most minerals deemed essential for proper human nutrition.

*Caffeine concentration*

Caffeine concentration was estimated using High Performance Liquid Chromatography (HPLC) analysis Agilent 1260 series (Fig. 3). The obtained results revealed that C3 has the lowest caffeine content (2.99 mg/g) and control coffee has the highest caffeine content (36.02 mg/g). Also, C1 and C2 have low caffeine content 5.70 and 4.47 mg/g, respectively (Fig. 4). There are many factors affecting−the caffeine content of coffee, such as: Type of coffee beans, roasting conditions and serving size. Caffeine content results indicated that the roasting conditions were ideal for date pits. Based on the data reviewed, it could be concluded that for the healthy adult population, moderate daily caffeine intake could be at a dose level up to 400 mg. The overdose might be associated with adverse effects such as toxicity, cardiovascular disease, bone status and calcium balance, changes in adult behavior, increased cancer incidence

and male fertility [34]. Also, reproductive-aged women should consume daily less than 300 mg caffeine, while children should consume 42.5 mg. Consequently, the prepared coffees are safe and healthy.

*Sensory evaluation of prepared coffee*

Sensory evaluation is human senses (color, taste, odor and flavor) for evaluating the prepared coffee samples. The qualification of sensory evaluation of the prepared coffee was given in (Table 7). The obtained results indicated that C3 coffee was the most overall acceptability (8.70) and better than control coffee (8.53). C2 also has good sensory parameters (8.33). Sensory evaluation was increased from 5.73 (C1) by the addition of spices to induce better taste and higher nutritional and health benefits. The results of sensory evaluation revealed that the date pits powder can be successfully used as a unique natural alternative to coffee, cocoa and food components [21].

*Antioxidant activity*

The di(phenyl)- (2,4,6-trinitrophenyl) iminoazanium (DPPH) radical scavenging activity of the prepared coffee samples was determined and compared−with control coffee (Table 8). C3 coffee has the highest antioxidant activity 91.72%, followed by C2 was 88.13%. A roasted date pits C1 was 79.56% and the control sample antioxidant activity was 84.18%. Such results supported the total phenolic content obtained in Table 3. High level of phenolic compounds in date pits were reported and ranged from 3100-4400 mg gallic acid equivalents/100 g and 580-930 µM Trolox Equivalents Antioxidant Activity. Pyrogallol is the most abundant phenolic compound in date pits and it is more efficient at scavenging $O_2$ than catechol [35]. Based on the above results, prepared coffee has beneficial effects as antioxidants or anti-nutritional by acting as metal scavengers reducing the bioavailability of iron.

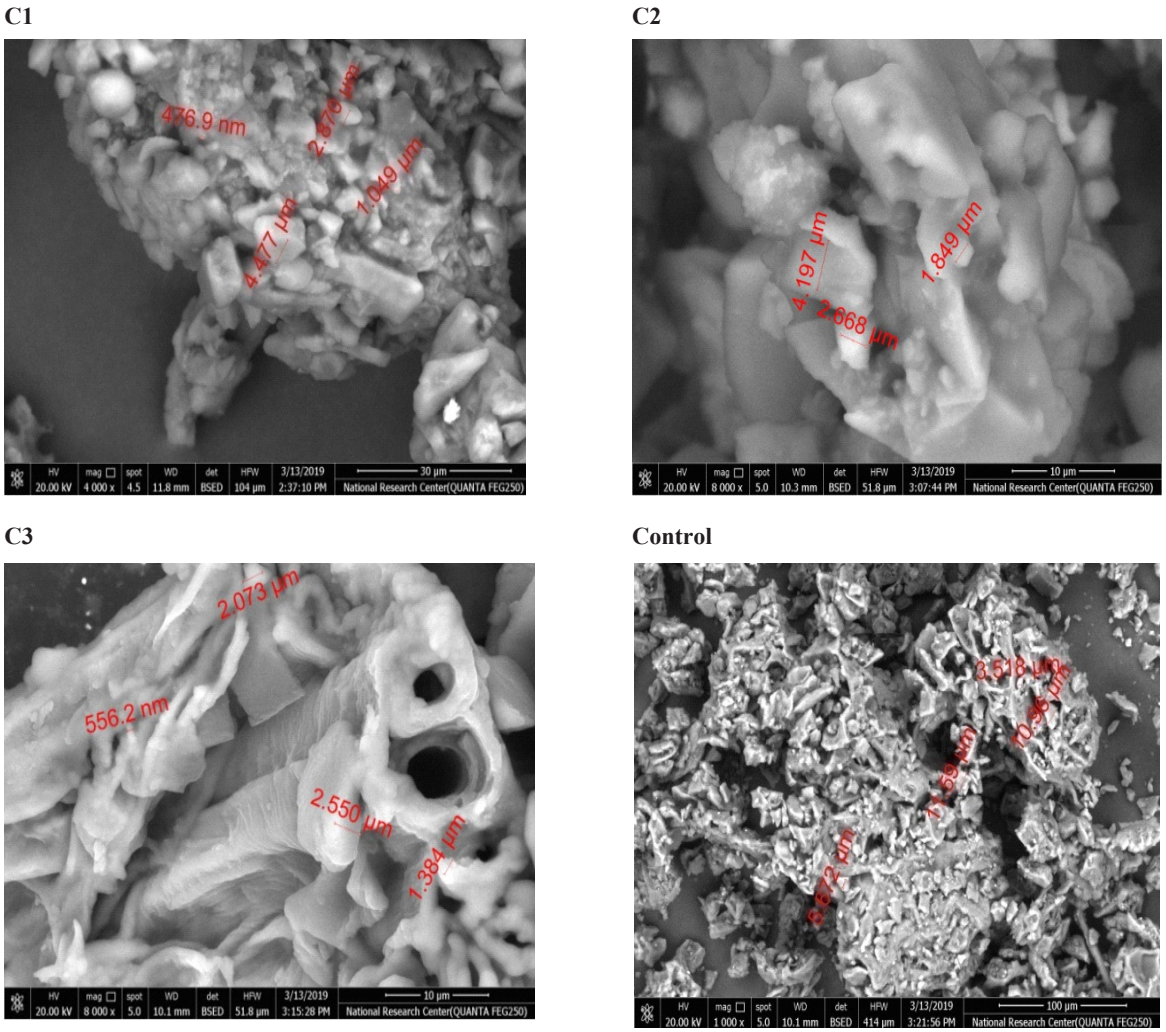
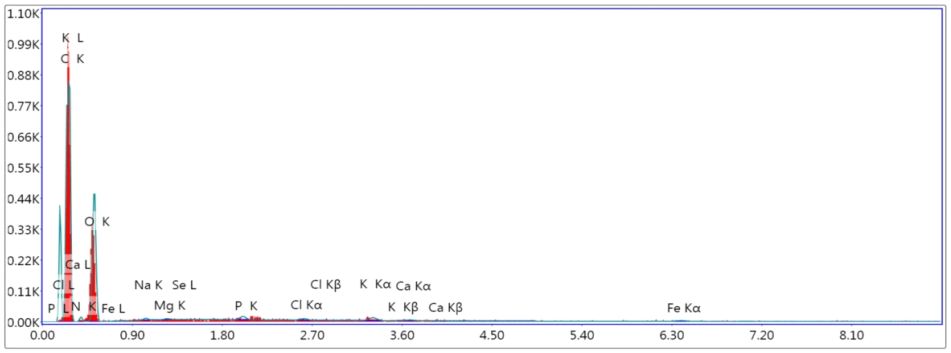
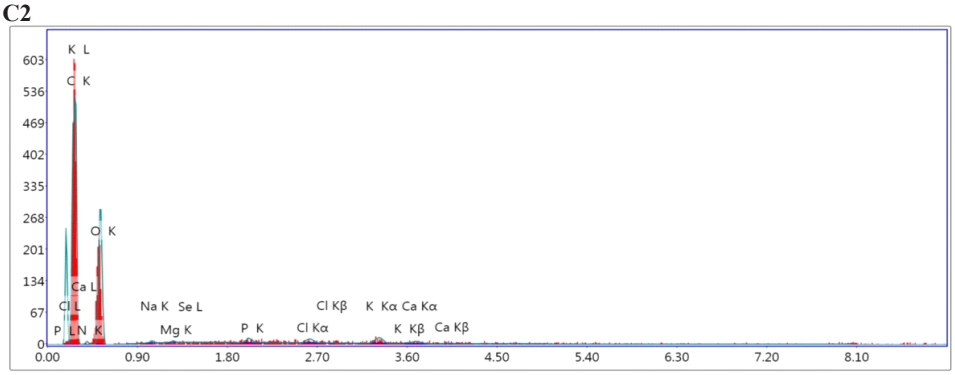



**Fig. 1. Scanning electron microscopy (SEM) of C1, C2, C3 and control coffee samples.**

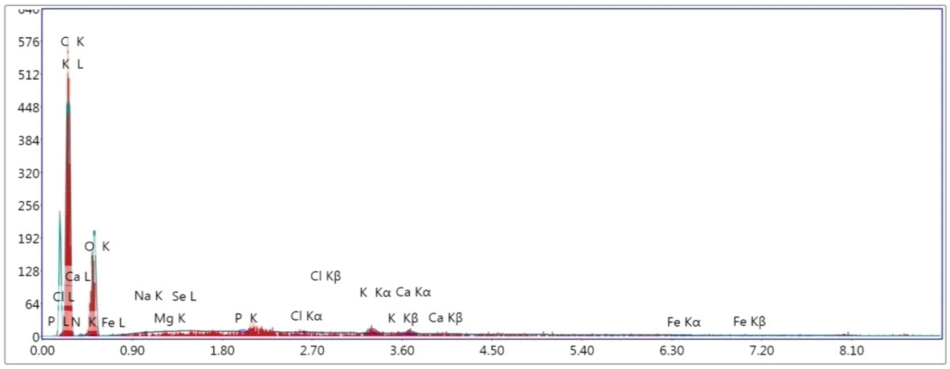
**C1**




**C2**




**C3**




**Control**

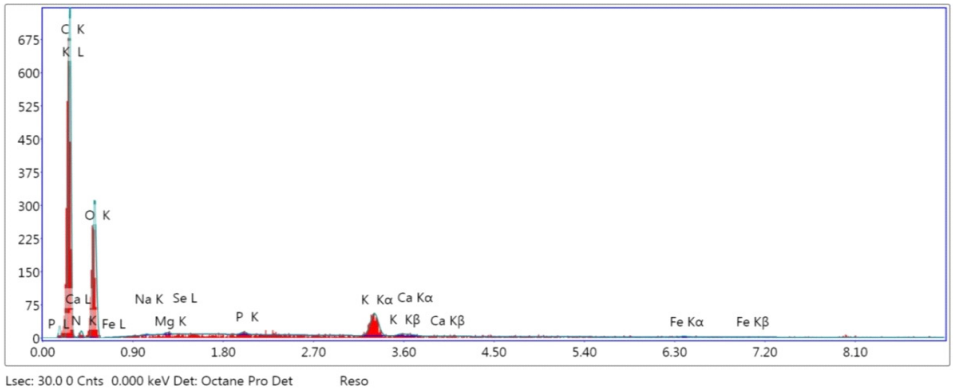
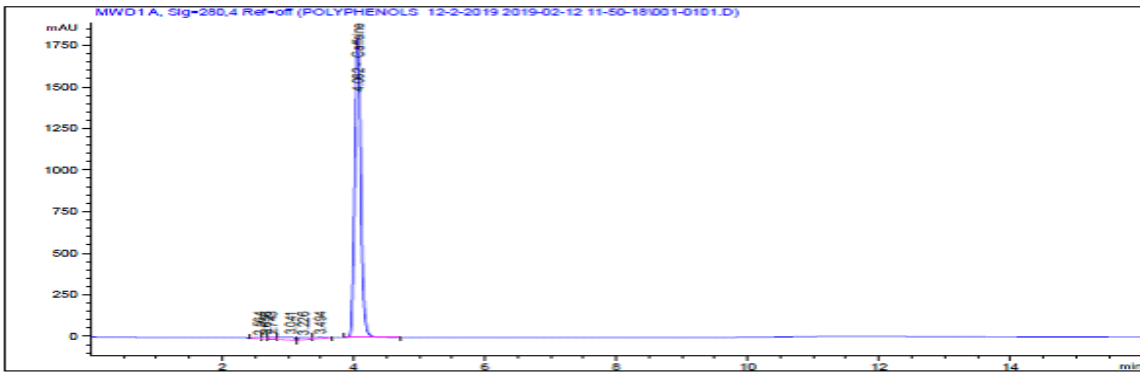



**Fig. 2. Energy dispersive X-ray analysis (EDXA) of C1, C2, C3 and Control.**

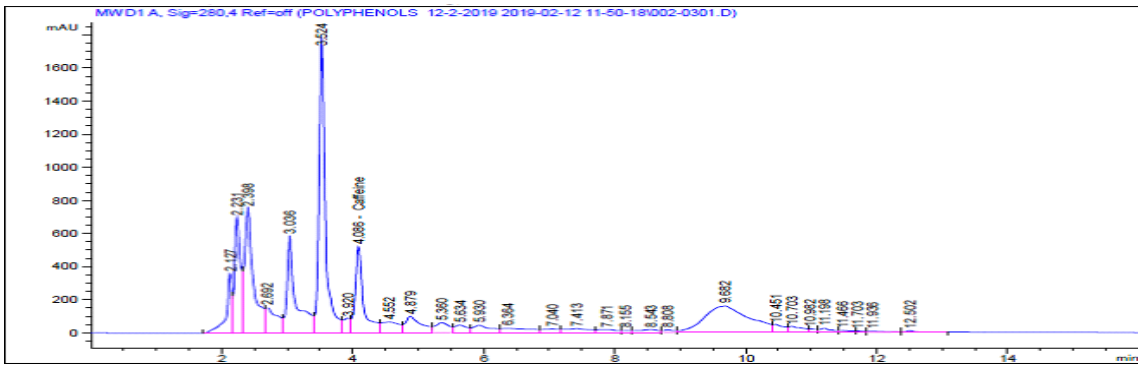
**TABLE 6. Qualitative and quantitative coffee samples elemental composition (Weight %).**

| Element | Weight % | | | |
|---|---|---|---|---|
| | C1 | C2 | C3 | Control |
| C | 46.94 | 47.53 | 51.39 | 48.40 |
| O | 44.89 | 44.32 | 41.54 | 41.23 |
| N | 4.6 | 3.24 | 1.56 | 5.81 |
| Na | 0.41 | 0.37 | 0.14 | 0.15 |
| Mg | 0.08 | 0.08 | 0.02 | 0. 13 |
| P | 0.32 | 0.39 | 0.24 | 0.18 |
| Cl | 0.17 | 0.44 | 0.17 | 2.76 |
| K | 0.42 | 0.82 | 0.82 | 0.19 |
| Ca | 0.13 | 0.39 | 0.56 | 0.34 |
| Fe | 0.43 | -- | 0.32 | 0.34 |
| Se | -- | 2.43 | -- | 0.82 |

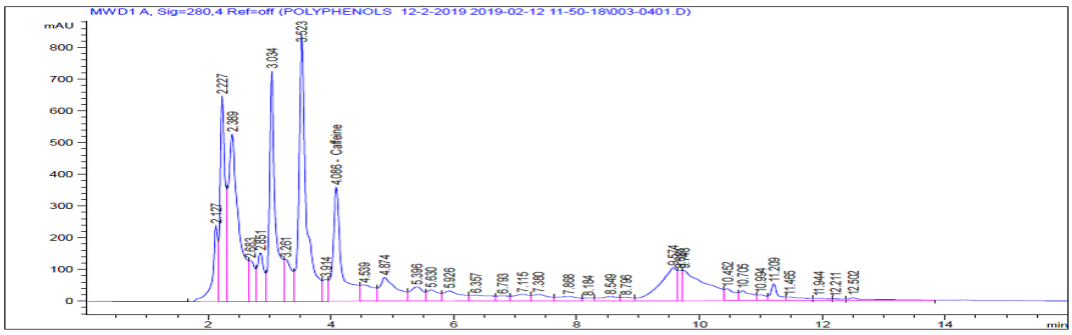
**Caffeine standard**



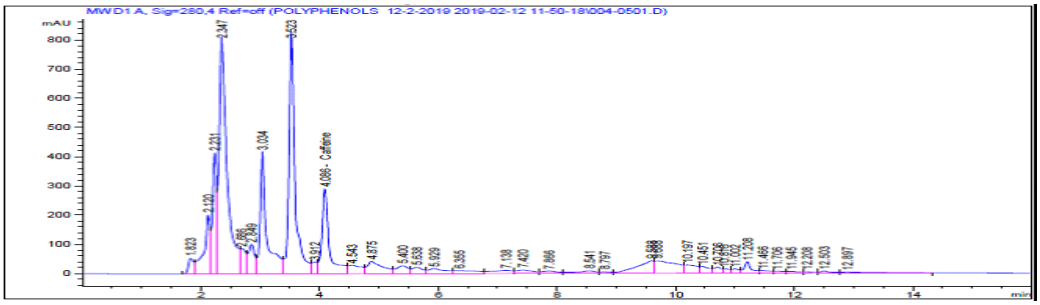

**C1**



**C2**




**C3**




**Control**

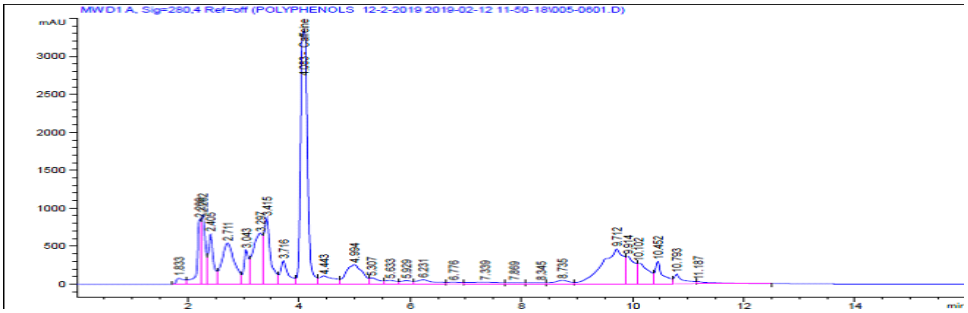



**Fig. 3. HPLC chromatograms for caffeine content in standard caffeine, C1, C2, C3 and control coffee.**

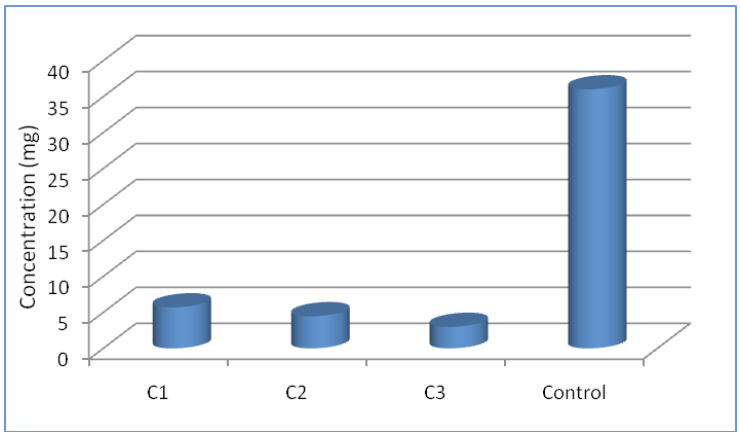



**Fig. 4. Caffeine content (mg/g) in C1, C2, C3 and control coffee.**

**TABLE 7. Sensory evaluation (10) of prepared coffee C1, C2, C3 and Control.**

| Samples | Color | Taste | Oder | Flavor | Overall acceptability |
|---|---|---|---|---|---|
| C1 | 5.80±0.65[b] | 5.66±0.25[b] | 5.26± 0.47[b] | 5.40± 0.40[c] | 5.73± 0.35[b] |
| C2 | 7.97±0.21[a] | 8.46±0.35[a] | 7.97± 0.21[a] | 7.63± 0.31[b] | 8.33± 0.51[a] |
| C3 | 8.77±0.15[a] | 8.50±0.26[a] | 8.40±0.3[a] | 8.53± 0.35[a] | 8.70± 0.10[a] |
| Control | 8 .00±0.40[a] | 8.26±0.15[a] | 8.00±0.1[a] | 8.67± 0.25[a] | 8.53 ± 0.31[a] |
| LSD | 0.7628 | 0.498 | 0.570 | 0.624 | 0.658 |

**TABLE 8. DPPH% radical scavenging activity of the prepared coffee samples.**

| Samples | DPPH% | | | |
|---|---|---|---|---|
| | 100 µg/mL | 50 µg/mL | 25 µg/mL | 12.5 µg/mL |
| C1 | 79.56±1.00[c] | 47.30±1.18[c] | 29.00±1.32[c] | 18.50±1.88[c] |
| C2 | 88.13±0.64[b] | 56.90±0.70[b] | 35.00±1.0[b] | 29.20±2.0[a] |
| C3 | 91.72±1.73[a] | 86.30±2.06[a] | 42.00±2.0[a] | 24.20±1.0[b] |
| Control | 70.00±1.53[d] | 41.20±1.0[d] | 23.20±0.77[d] | 17.30±2.46[c] |
| LSD | 2.444 | 2.51423 | 2.55271 | 3.60117 |

*Antimicrobial activity*

All the tested samples did not exhibit inhibition zone at low concentration (10 and 100 µg/mL) against the four pathogenic microbes (*Staphylococcus aureus*, *Candida albicans*, *Escherichia coli* and *Heliobacterpylori*). Roasted coffee has antibacterial activity against a wide range of bacteria; this activity mainly depends on the degree of roasting. Alcoholic extracts of date pits are effective in inhibiting bacteria as compared with antibiotics [36].

*In vitro cytotoxicity assay*

The samples have no cytotoxicity activity and accelerated the normal cells (Bj-1) as shown in Table 9. The detected cytotoxic effect of coffee samples owing to its phenolic content and antioxidant activity. Phenolic and flavonoids, compounds were responsible for the protective cells effect [37].

*Prebiotic activity*

The three prepared coffee samples and control were evaluated for prebiotic activity with *L. helveticus, L. reuteriand L. casei* probiotics bacteria. The obtained results showed that coffee samples characterized by their higher prebiotic activities as shown in (Table 9). C3 coffee revealed the best prebiotic index toward *L. helveticus* 220, *L. reuteri* 205 and *L. casei* 180, respectively. On the other hand, the results indicated that C2 had a good probiotic index against (*L. helveticus* 210, *L. reuteri* 198 and *L. casei* 179) while C1 had slightly lower activity against (*L. helveticus* 155, *L. reuteri* 135 and *L. casei* 79). Prepared coffee samples gave high prebiotic activity owing to their chemical composition (protein, fats, flavonoids, carbohydrates, tannins and total phenolics).

**TABLE 9. *In vitro* cytotoxicity activity % at100µg/mL and Prebiotic activity at (150 mg) of coffee samples.**

| Samples | Cytotoxicity | Prebiotic index | | |
|---|---|---|---|---|
| | | *L. helveticus* | *L. reuteri* | *L. casei* |
| C1 | 6.9% | 155 | 135 | 79 |
| C 2 | 0 % | 210 | 198 | 179 |
| C3 | 0 % | 220 | 205 | 180 |
| Control | 0 % | 202 | 199 | 170 |

## Conclusion

Based on the previous results, it is clear that the research team has reached different types of coffee drink characterized by valuable nutrients and avoids the side effects of commercial coffee, and thus reduce the risk of communities drinking large quantities of coffee.

## Conflicts of interest

No conflict of interest to be disclosed by authors.

## Acknowledgment

The authors are grateful to the deanship of the NRC.

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# دراسة مقارنة بين الإضافات المختلفة لمشروبات قهوة نوي التمر: التقييم الصحي والتغذوي

**تامر رجب[1] و نادرة يوسف[2]**

[1]قسم كيمياء المنتجات الطبيعيه والميكروبيه- شعبة بحوث الصناعات الصيدليه والدوائيه- المركز القومي للبحوث-١٢٦٢٢ الدقي- الجيزة- مصر.

[2]وحدة بحوث المطبخ التجريبي -معهد بحوث تكنولوجيا الأغذية- مركز البحوث الزراعية- الجيزة- مصر.

تُعد نوي التمر مخلفا رئيسيا أثناء عملية انتاج التمور والتي يمكن ان تقدم موادا ثمنية تتدخل ضمن مكونات مشروب القهوة. تم تحضير ثلاثة أنواع من القهوة من بودرة نوي التمر المحمصة مع بعض الإضافات القيمة. تم تعيين المكونات الكيميائية التالية: البروتين، الدهون، الفلافونويدات، الفينولات البسيطة والمركبة، الألياف الغذائية، الكربوهيدرات، الأحماض الدهنية ، المعادن الأساسية و تم تقدير محتوى الكافيين. وقد أشارت النتائج إلى مشروب القهوة الأول والمحضر من بودرة التمر المحمصة دون اي إضافات, مشروب القهوة الثاني (بودرة نوي التمر المحمصة بنسبة ٩٢,٥ ٪ ، الحبهان ٢,٥ ٪ ، زهور الورود ٤ ٪ ، جوزة الطيب ٠,٥ ٪ والقرنفل ٠,٥ ٪), ومشروب القهوة الثالث (بودرة نوي التمر المحمصة ٦١,٦٧ ٪ ، الشعير ٣٠,٨٣ ٪ ، الحبهان ٢,٥ ٪ , زهور الورد ٤٪ وجوزة الطيب بنسبة ٠,٥ ٪ والقرنفل بنسبة ٠,٥٪( تحتوي علي قيمً غذائية عالية مقارنة بالقهوة التجارية. تم تقدير تركيز الكافيين وكشفت النتائج أن مشروب القهوة الثالث يحتوي علي أدنى محتوى للكافيين (2.99 ملغم / جم) ، في حين أن القهوة التجارية تحتوي على أعلى محتوى للكافيين (36.02 ملغم / جم). كم تم تقييم التقييم الحسي لمشروبات القهموة المحضرة مقارنة بالتجارية وقد أثبتت النتائج ان مشروب القهوة الثالث الأكثر قبولاً (٨,٧٠) وهو أفضل من القهوة التجارية (٨,٥٣). بالإضافة إلى ذلك ، تتمتع مشروب القهوة الثالث علي أعلي نشاط مضاد للأكسدة بنسبة ٩١,٧٢٪ ، وكانت عينة القهوة التجارية ٨٤,١٨٪. جميع العينات ليس لها سمية علي خلايا الجلد الطبيعية بل ساعدت علي نمو الخلايا بشكل صحي. و بناءً على كل هذه النتائج ، يوصى بأن تكون القهوة المعدة نوعًا جديدًا من مشروب القهوة الصحي المتكامل.