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Chemical, Mineral and Phytochemical Screening Assay of Date Palm Seeds for the Development of Date Palm Seed Coffee

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Abstract

Date palm fruits possess high nutritional and therapeutic value with significant antioxidant, properties. The inedible parts of date palm (date seeds) are often discarded as waste however these are rich source of dietary fiber, phenolics and antioxidants. Present study is an attempt to explore the nutritional and phytochemical profile of date seeds and their utilization in food product development. Purposely, date seeds were analyzed for their proximate and mineral contents. Afterwards, date seeds extracts was obtained using methanol as extraction solvent. The extract was analyzed for total phenolic and flavonoids contents. Finally roasted date seed powder was used for the development of date seed coffee and analyzed for its sensory. The proximate composition of date seed powder showed that it contained 5.65±0.17% moisture, 1.17±0.04% ash, 5.85±0.23% crude protein, 7.95±0.39% crude fat, 64.75±2.59% crude fiber and 14.63±0.44% nitrogen free extracts. Amongst the minerals, potassium is present in maximum amount 375.87±11.7 mg/100g followed by phosphorus 125.58±5.02 mg/100g, magnesium 77.55±2.33 mg/100g, calcium 18.73±0.93 mg/100g and sodium 15.23±0.6 mg/100g. Moreover, the antioxidant profiling of date seed showed that the value of TPC 31.2±0.93 mg GAE/g of dry weight and flavonoids 29.5 mg/g dry weight. The developed date seed coffee was also sensory evaluated.

Keywords

Date Seeds, Chemical Composition, Mineral Contents, Antioxidant, Date Seed Coffee

1. Introduction

Date palm (*Phoenix dactylifera* L.) is considered as one of the oldest and staple crops in Southwest Asia and North Africa. Besides, dates can be grown in Australia, Southern Africa, Mexico, South America and the United States, especially in southern California, Texas and Arizona [1, 2]. Egypt is largest producer of dates while, Pakistan is the 5th largest date producing country [3].

The most significant quality attributes to grade dates are color, flavor, moisture content and absence of defects i.e insect, damage, cracks and surface damage. Date fruit is good source of high nutritional value. It is rich in carbohydrates, dietary fibers, proteins, minerals and vitamin B complex [4, 5]. In more details, carbohydrates forms 70% of date fruit and date proteins are rich in amino acids. Minerals in date fruits are calcium, iron, magnesium, selenium, copper, phosphorus, potassium, zinc, sulfur, cobalt, fluorine, manganese, and boron [1, 6].

The protective effects of fruits against chronic diseases are ascribed to bioactive non-nutrients called phytochemicals. Phytochemicals have gained increased interest among several investigators, including clinicians due to their antioxidant

activity, cholesterol-lowering properties, and many other potential health benefits such as cardiovascular diseases, prevention of diabetes and chemoprevention of cancer [1, 7].

Date seed, an agro-waste encompasses a number of bioactive components that are helpful in mitigating various physiological threats. Ample amount of waste is produced from date processing industries mainly date pit and tip. Date pits are usually discarded as a waste material having merely no use. It considered waste product of date fruit. Rarely, in the Middle East, date seeds are used in animal feed, such as; for camel, sheep, poultry and cattle industries [8]. Seeds are often used in foods, pharmaceuticals and also having ornamental benefit like making beads for decoration [9]. These are rich source of dietary fiber, phenolics and antioxidants and no health use of these by product results in nutritional as well as economic loss [10].

The aim of the current research was to explore the antioxidant potential and nutraceutic worth of date seeds due to its phytochemistry. In low income countries, the development of cost effective natural products is the need of time as numerous researches have yet been reported in this field. Date industries produces large amount of waste in the form of date pit, therefore need of the day is to utilize these agro wastes because of its high antioxidants potential. In this context, conventional solvent extract were prepared and analyzed for their *in vitro* antioxidant potential through phytochemical screening assays. During product development module, date seeds coffee powder and ready to drink date seeds coffee were prepared to assess consumer acceptance using 9-point hedonic scale. The date seeds extracts were further used during storage for their antioxidant profiling through TPC and DPPH assay. The collected data was finally subjected to statistical analysis in order to determine the level of significance.

2. Materials and Methods

The present research was conducted in the Functional and Nutraceutical Food Research Section, National Institute of Food Science and Technology, University of Agriculture, Faisalabad Pakistan. For the purpose, date seed powder was used for the extraction and characterization of its bioactive molecules. Afterwards, roasted date seeds were used for the development of caffeine free coffee to explore its sensory characteristics.

2.1. Procurement of Raw Material

The dates were procured from local market. The analytical reagents were purchased from Merck (Merck KGaA, Darmstadt, Germany) and Sigma-Aldrich (Sigma-Aldrich Tokyo, Japan).

2.2. Preparation of Date Seed Powder

Date flesh and pit was separated. Seeds were washed to remove adherent fruit material. After washing and drying, seeds were roasted and grounded to obtain fine roasted date seeds powder. The powder was stored in plastic jars at ambient temperature prior to analysis.

2.3. Proximate Analyses

The date seed samples were analyzed for moisture, ash, crude protein, crude fat and crude fiber according to their respective methods as described in Association of Official Analytical Chemists (AOAC) [11].

2.3.1. Moisture Content

The moisture content of date seeds powder were determined following method mentioned by AOAC Method No. 934-01 [11]. Accordingly, 10 g sample was dried in hot air oven (Model: DO-1-30/02, PCSIR, Pakistan) at a room temperature of 105±5°C for the duration until weight was constant. Moisture percent was found according to the following equation;

$$\text{Moisture (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

2.3.2. Total Ash

The ash content of date seeds powder was estimated according to the procedure outlined in AOAC Method No. 942-05 [11]. Briefly, 5 g of sample was directly charred on flame in crucible until there were no fumes coming out. Afterwards sample was ignited in muffle furnace (MF-1/02, PCSIR, Pakistan) at 550-600°C for 5-6 hours or until grayish white residues were obtained.

$$\text{Ash (\%)} = \frac{\text{weight of ash}}{\text{weight of sample}} \times 100$$

2.3.3. Crude Protein

The percentage of nitrogen in the sample was determined by following AOAC Kjeldahl's Method No. 984-13 [11]. The sample was first digested with concentrated H_2SO_4 in the presence of digestion mixture (K_2SO_4: $FeSO_4$: $CuSO_4$ 100: 5: 10) for 2-3 hours or until the digested material attained light greenish or transparent color. This material was diluted (250 mL using distilled water) and distillation was done by taking 10 mL of diluted material and 10 mL of 40% NaOH solution in the distillation apparatus. Liberated ammonia was collected in 2% boric acid solution containing methyl red as an indicator. Finally the distillate was titrated against 0.1 N H_2SO_4 till golden brown end point. The crude protein percentage was calculated by multiplying nitrogen (N) with factor as described below:

$$\text{N (\%)} = \frac{\text{volume of 0.1 N } H_2SO_4 \times \text{volume of dilution} \times 0.0014}{\text{weight of sample (g)} \times \text{volume of distillation}} \times 100$$

$$\text{Crude Protein} = \text{N (\%)} \times 6.25$$

2.3.4. Crude Fat

The crude fat content in date seeds powder was calculated following guidelines of Method No. 920-39 in AOAC [11]. Briefly, 3 g sample was refluxed in soxhlet apparatus (Model: H-2 1045 Extraction Unit, Hoganas, Sweden) using n-hexane as a solvent.

$$\text{Crude fat (\%)} = \frac{\text{Intial weight} - \text{Final weight}}{\text{Intial weight}} \times 100$$

2.3.5. Crude Fiber

The crude fiber was estimated by digesting fat free sample using 1.25% H_2SO_4, followed by 1.25% NaOH solutions. The residue was weighed and ignited in a muffle furnace at 550°C till white residue left. Fiber percentage was calculated according to the AOAC Method No. 978-10 [11]. The crude fiber was calculated by using following expression.

$$\text{Crude fiber (\%)} = \frac{\text{weight loss on ignition (g)}}{\text{weight of sample (g)}} \times 100$$

2.3.6. Nitrogen Free Extracts (NFE)

The NFE was calculated through subtraction method following the expression:

$$\text{NFE \%} = 100 - (\text{Moisture} + \text{Crude protein} + \text{Crude fat} + \text{Crude Fiber} + \text{Ash})\%$$

2.4. Mineral Analysis

For mineral determination, wet digestion of all samples was carried out according to the method of AOAC [11]. The sample 0.5 g was taken in a conical flask and was digested with 10 mL HNO_3 at a temperature of 60-70°C for 20 minutes and then digested with 5 mL $HClO_4$ at a temperature of 60-70°C for 20 minutes and subsequently increasing the temperature to 195°C till the volume of the content was reduced to 1-2 mL. The digested sample was transferred to 100 mL volumetric flask and volume was made up to the mark using distilled water and then filtered. After filtration, the digested samples were stored for different mineral determination according to their respective methods.

2.5. Phytochemical Screening Assay

2.5.1. Preparation of Date Seed Extracts

The solvent extraction was carried out using aqueous methanol by following the protocol of Al- Farsi *et al* [12]. Briefly, 50 g of date seeds powder was added in 149 mL of aqueous methanol in 250 mL conical flask, 1 mL of acetic acid and 40 mL of distilled water were added in flask. The mixture of solvent and date seeds powder was placed in orbital shaker for 3-4 hours at 280 rpm with controlled temperature at 20°C. The mixture was filtered through Whatman filter paper No. 2. The solvent was evaporated using rotary evaporator under reduced pressure at 40°C. The final extract was stored at -40°C.

2.5.2. Total Phenolic Contents

The total phenolic contents in date seeds extracts were determined by the Folin-Ciocalteu method [13]. The principle is based on the oxidation of phenolic compounds under alkaline conditions that result in reduction of phosphotungstic acids contained in Folin-Ciocalteu reagent to phosphotungstic blue or blue colored tungsten oxides. The absorbance of phosphotungstic blue is directly proportional to the number of aromatic phenolic groups. Briefly, 1 mL of appropriately diluted samples or a standard solution of gallic acid was added to a 25 mL volumetric flask containing 9 mL of distilled water. A reagent blank was prepared using distilled water. One milliliter of Folin–Ciocalteu phenol reagent was added to the mixture and mixed by shaking. After 5 min, 10 mL of 7% Na_2CO_3 solution were added under shaking. The solution was then immediately diluted to 25 mL with distilled H_2O and mixed thoroughly. After incubation for 90 min at 23°C, the absorbance was read at 750 nm using a spectrophotometer. The total phenolic contents are expressed as milligrams of gallic acid equivalents (GAE) per 100 g of powder. All measurements were performed in triplicate using gallic acid as standard, expressing the results as mg/g of gallic acid equivalent (GAE). The total polyphenols in each extract were measured by using the mentioned formula:

$C = c \times V / m$

C = Total phenolic contents (mg/g plant extract, in GAE)

c = Concentration of gallic acid (mg/mL)

V = Volume of extract (mL)

m = Weight of lemongrass extract (g)

2.5.3. Total Flavonoids

The flavonoid content was measured using the procedure of Al- Farsi and Lee [14]. Briefly, 1 mL of the extracts or standard solutions of catechin was added to a 10 mL volumetric flask. Distilled water was added to make a volume of 5 mL. At zero time, 0.3 mL of 5% (w/v) sodium nitrite was added to the flask. After 5 min, 0.6 ml of 10% (w/v) $AlCl_3$ was added and, then 6 min, 2 mL of 1 M NaOH were also added to the mixture, followed by the addition of 2.1 mL distilled water. Absorbance was read at 510 nm against the blank (water) and flavonoid content was expressed as mg catechin equivalents per 100 g of fresh herb. Samples were analyzed in triplicate.

2.6. Product Development

In the product development module, roasted date seeds powder was utilized for the preparation of date seed coffee powder and ready to drink date seed coffee. According to the treatment plan mentioned in Table 1, T_0 was formulated to have 100% date seed powder, While T_1 and T_2 had date seed coffee powder + traditional coffee in 70:30% and 50:50% respectively. To assess the technological characteristics of date seed coffee, ready to drink date seed coffee was also prepared and analyzed.

Table 1. *Product Development Plan.*

Treatments	Date Seed Coffee Powder	%
T_0	Date seed coffee	100
T_1	Date seed coffee + traditional coffee	70:30
T_2	Date seed coffee + traditional coffee	50:50

2.7. Sensory Evaluation

The sensory evaluation attributes; color, taste, flavor, sweetness and overall acceptability were analyzed. Sensory assessment of prepared product was scored using nine point

hedonic scale system ranging from extremely like to dislike (9 = like extremely; 1 = dislike extremely) in the sensory evaluation laboratory of the NIFSAT, University Of Agriculture, Faisalabad, Pakistan. Various sensory attributes like taste, color, flavor, sweetness and overall acceptability of the prepared products were evaluated during storage by following the protocol of Meilgaard *et al* [15].

3. Results and Discussion

3.1. Proximate Composition

Proximate composition is essential parameter to assess the quality of raw material. The proximate characteristics of date seeds powder which were found during instant research indicated that the roasted date seeds powder contained 5.65±0.17% moisture, 1.17±0.04% ash, 5.85±0.23% crude protein, 7.95±0.39% crude fat, 64.75±2.59% crude fiber and 14.63±0.44% nitrogen free extracts (Figure 1).

The present finding are in line with the previous work of El Sheikh *et al* who analyzed the roasted date seeds powder and results revealed that date seeds contains 5.59% moisture content, 1.21% ash, 5.90% crude protein, 63.81% crude fiber and 7.5% crude fat. The results are similar and in the range of current findings. The results showed the average fat content of date seed powder was 7.95% which is reasonably high. This is an indication that date seeds has a reasonable amount of fat content and allows for high oil recovery from date seeds through various extraction techniques. The high fiber content of the date seeds shows that it is a good source of energy and necessary for digestion of food [16]. Simillarly, it was reported that 75-80% fiber content in date seeds depending upon variety and ripeness [17].

In another investigation, Al Farsi and Lee (2008) compared the comparative study of date pulp by comparing with date seeds and results revealed that date pulp contain 1.5% ash, 10.2% moisture content, 1.2% lipid and 10.9% fiber. While date seeds contains reasonable high value of dietary fiber required for balanced diet and can be considered a good source of fiber. These results also support the present study because values are close to the current investigation regarding all the proximate values [14]. Earlier, Akasha *et al* (2012) also found that date seeds powder contain crude fat, carbohydrates and moisture content 8.14%, 62.71% and 5.39% respectively, which support the findings of present study. The carbohydrate content is high (62.71%). This shows that date seeds are a good source of energy. The food energy of date seeds sample (360.55 cal/100g) is moderate compared with those of other plants [18].

Moreover, Elleuch *et al* (2008) have also revealed that the date seed powder contained moisture content 6.5%, crude protein 5.8% and crude fiber 68.7%, however differ in carbohydrates contents from the previous investigations. But crude protein, crude fiber and moisture content were also in the same range as the present results. The high moisture content indicates that date seeds may be susceptible to microbial growth but the reasonable amount of carbohydrate and fiber shows that it can be part of human diet and also considered as a good source of dietary fiber [19].

The present results regarding chemical composition of date seeds powder are also in strong harmony with findings of Rehman *et al* (2007) who reported that moisture content, protein, lipids and ash are 1.6%, 6.96%, 7.95% and ash 0.97% respectively. However, the difference in carbohydrate and fat content may be attributed to varietal difference and environmental conditions [20].

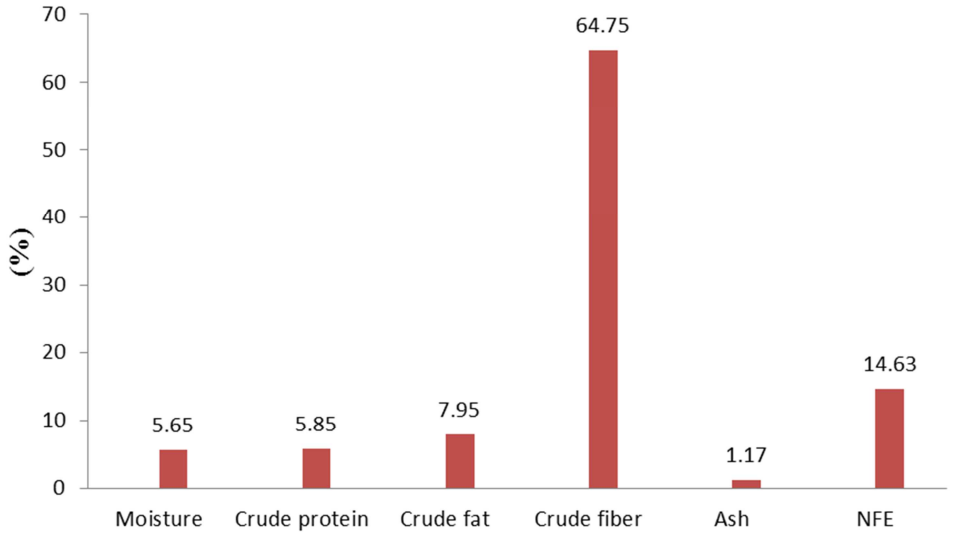

Figure 1. *Proximate Composition of Date Seeds.*

3.2. Mineral Profile

Minerals play an imperious part to evaluate quality characteristics of raw material *i.e.* date seeds. Mineral content (Figure 2) analysis of seeds powder has exhibited that potassium is present in maximum amount 375.87±11.7 mg/100g, followed by phosphorus 125.58±5.02 mg/100g, magnesium 77.55±2.33 mg/100g and calcium 18.73±0.93

mg/100g, sodium 15.23±0.6 mg/100g, while iron, zinc, copper and manganese is also found in considerable amounts. Owing to the rich mineral profile of date seeds powder, extensive research has been carried out to estimate the amount of mineral elements present. Data obtained was comparable to the research findings, with potassium being the most abundant element in green tea. El sheikh *et al* (2014) reported minerals in roasted date seeds samples and estimated the concentrations ranges of potassium, phosphorus, magnesium, calcium, sodium and iron as 375-379, 120-122.5, 75.5-77.5, 18.2-18.4, 14.9-15.9 and 3.58-3.60 mg/100g, respectively [16].

Previously, Besbes *et al* (2004) determined the amount of minerals in date seeds as potassium 229 mg/100g followed by posphorous, magnesium, calcium and sodium as 68.3, 51.7, 38.8 and 10.4 mg/100g, respectively. Date seeds mineral profile varies widely with varieties and growing regions. However, studies concluded that the concentration of potassium in all date seeds sample was higher than phosphorus while magnesium was higher than calcium. [21]. Ali-Mohamed and Khamis, (2004) reported that comparison of mineral content of date seeds and barley seeds revealed that date seed was the good source of minerals and can also be used in the food products in place of barley [22].

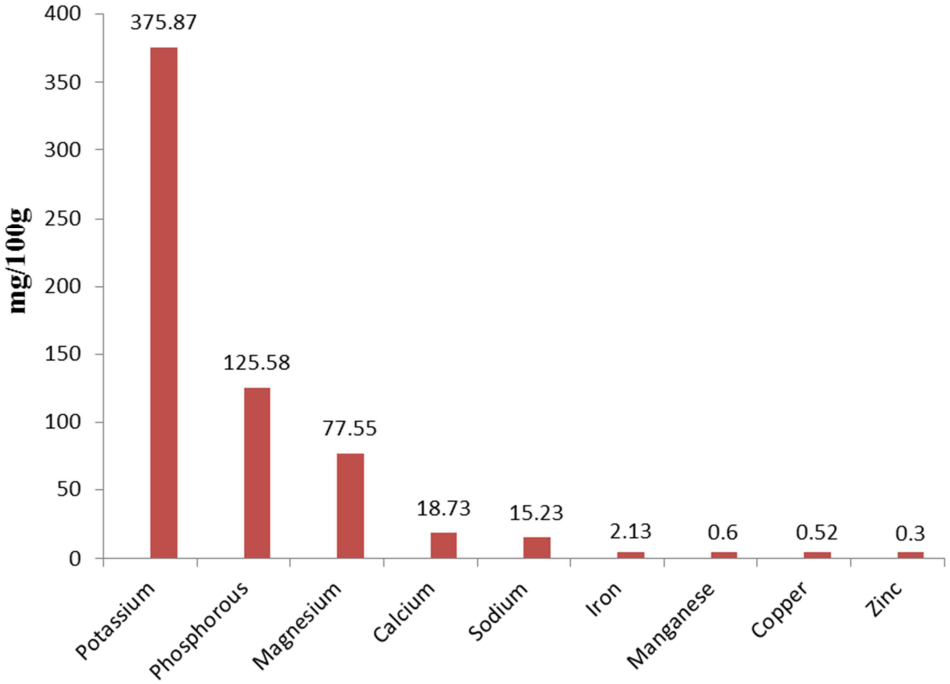

Figure 2. *Mineral Profile of Date Seeds.*

3.3. Phytochemical Screening Assay

3.3.1. Total Phenolic Content (TPC)

Health associated benefits of polyphenols have necessitated their quantification in various food products. The values of TPC acquired from date seeds thorough solvent extraction are represented in table 2 which are 31.2±0.93 mg GAE/g of dry weight.

Earlier, it was measured the total polyphenols extracted through solvent extraction of 14 different varieties of date seed extracts. They measured the polyphenol contents on (mg/100g) basis using dry plant which is 3541 mg/100g in Zahedi variety and 1260 mg/100g in Shahabi variety respectively, ultimately they concluded that the on dry basis different varieties show different range of phenolics contents [23]. Antioxidant effectiveness in largely depends on the bioavailability of responsible compounds and phenolic contents. Besbes *et al* (2004) determined total polyphenols in Maktab and Kabkab dalaki 2 varieties were 3284±10.14 to 2548±75.71 mg/100g [21]. This difference in the result was due to different extraction method.

3.3.2. Flavonoids

The value of total flavonoids observed during the current investigation assimilated from date seed through solvent extraction were as 29.5 mg/g dry weight (Table 2). This is the amount which obtained through methanol extraction. Al-Farsi and Lee carried out the flavonoids compound analysis by comparing aqueous, butanol and acetone extracts. They concluded that maximum value were observed in butanole 50% extract 15.75±0.81 g/100g followed by acetone 50% 15.93±0.98 g/100g and least amount in aqueous extract 8.13±0.70 to 6.39±0.30 g/100g of flavonoids content [14].

Table 2. *Phytochemical Screening and Antioxidant Activity of Date Seeds.*

TPC (mg/g)	31.23±1.24
TFC (mg/g)	29.54±0.88

3.4. Sensory Evaluation of Date Seed Ready to Drink Coffee

The mean squares for sensory evaluation of date seed cold coffee (Table 3) revealed the momentous variation on flavor,

taste, color, sweetness& overall acceptability as a function of storage and treatments. While, non-significant variation was observed on color, flavor, taste, sweetness and overall acceptability regarding their interaction throughout the study.

The highest score (Table 4) for overall acceptability of date seed cold coffee were assigned to T_0 (7.4±0.29), T_2 (7.3±0.26) and T_1 (7.2±0.28), although, the variation was significant. A momentous decrease in overall acceptability (8.1±0.32 to 6.4±0.26) was shown during storage at initiation and termination of study respectively.

Lee and Chamber found that the flavor of coffee or tea changed with different brewing methods. Green flavor of green tea was overtaken by brown flavor due to increase brewing temperature and time [24]. Simillarly, Baggenstoss *et al* (2007) another researcher exhibited the same trend of sensory attributes during storage of cold coffee. [25]

Table 3. *Mean Square for Sensory Evaluation of Ready to Drink Date Seed Coffee.*

SOV	df	Color	Flavor	Taste	Sweetness	Overall acceptabilility
Treatments (A)	2	1.979^{**}	0.6770^{*}	1.541^{**}	6.583^{**}	4.616^{**}
Time (B)	2	1.989^{**}	18.6620^{**}	1.208^{*}	2.083^{*}	0.366^{*}
A x B	4	0.358^{NS}	0.2345^{NS}	0.244^{NS}	0.083^{NS}	0.153^{NS}
Error	18	0.292	0.2322	0.215	0.362	0.188

**= Highly significant, * =Significant, NS=Non significant

T_0 = Date seed coffee 100%

T_1 = Date seed coffee 70% + traditional coffee 30%

T_2 = Date seed coffee 50% + traditional coffee 50%

Table 4. *Mean Values for the Effect of Storage Time and Treatments on Overall Acceptability of Ready to Drink Date Seed Coffee.*

Storage (Days)	Treatments			Means
	T_0	T_1	T_2	
0	8.2±0.32	7.9±0.31	7.8±0.32	$8.1±0.32^{a}$
15	7.7±0.30	7.4±0.28	7.1±0.27	$7.4±0.28^{b}$
30	6.5±0.26	6.5±0.26	6.4±0.26	$6.4±0.26^{c}$
Means	7.4±0.29	7.2±0.28	7.3±0.26	

T_0 = Date seed coffee 100%

T_1 = Date seed coffee 70% + 30% traditional coffee

T_2 = Date seed coffee 50% + 50% traditional coffee

4. Conclusion

Date seed, an agro-waste contains a number of bioactive components that are helpful in mitigating various physiological threats. The inedible parts of fruits and vegetables are often discarded as waste. However, these are rich source of dietary fiber, phenolic and antioxidants. In modern era consumers are more conscious about their diet and demand low caloric foods with better health improving properties. Intake of coffee is one of the most common trends in world, because to support our daily activities. Drinking coffee is considered as a sign of friendship and socialization. As date seeds are source of dietary fiber, phenolic and antioxidants and no health use of these by product results in nutritional as well as economic loss. In low income countries, the development of cost effective natural products is the need of time. Therefore, more research work is needed for further evaluations of its potential and utilization for the development of novel functional foods.

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