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Utilization of the date seed powder in food manufacturing

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Date seeds are considered food waste that most people do not care to reuse because that contains many phenols, vitamins, minerals and fibers. Therefore, these seeds have been used and included in functional foods that have a high percentage of fiber and phenols, which are considered antioxidants and help in preventing diseases, especially cancers. All of this leads to reducing waste without affecting the environment or leading to the depletion of natural resources, All of this is in line with the United Nations Sustainable Development Goals.

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ARTICLE

Utilization of the date seed powder in food manufacturing

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Abstract

This research aimed to use date seeds powder in food manufacture. The date seeds were roasted, powdered (RDSP) and used in manufacture of laboratory chocolate balls (CB), chocolate spread (CS) and dark chocolate (DC), where the cocoa powder (CP) was replaced by 25, 50 and 75% RDSP in each processed product mentioned above. Chemical composition, total phenol content, phenols and flavonoids compounds identification, minerals composition of RDSP, CB, CS and DC were investigated. Moreover, cytotoxicity of RDSP and sensory evaluation of CB, CS and DC samples was estimated. Results showed that RDSP exhibit an excellent content of crude fibre, total carbohydrates, total phenol, potassium, a considerable amount of potassiume, sodium, iron, calcium and magnesium. The RDSP showed doses dependent cytotoxicity, where the cell viability at doses of 62.5, 125, 250, 500 and 1000 µg/ml of RDSP was 95.63, 92.48, 89.99, 75.22 and 52.85% for the MCF-7 cells, respectively and the IC50 value was 1112.28 µg/ml in MCF-7 cells. The usage of RDSP caused reinforcement in the crude fibre total phenol, sodium and potassium content, improvement in phenolic and flavonoids compound of the CB, CS and DC samples, moreover, its overall acceptability was ranged between very good and good grade. Therefore, RDSP could be recommended to be used in chocolate products manufacture and had anti-cancer properties.

Key words: Anticancer, chocolate balls, chocolate spread, cytotoxicity, date seeds powder, dark chocolate, total phenol.

1. Introduction:

Dates (*Phoenix dactylifera L.*) belong to Palmocea family, are a staple food in arid and semi-arid areas. From 10-15% of the weight of a date fruit is date seed, often known as stones or pits [1]. Date seeds contain significant quantities of phenolic, fibre, fat, protein, moisture, and vitamins [2]. Also, it contains high concentrations of minerals (Ca, Mg, K, Na and Fe) [3], oil (5 -13%), which is rich in tocopherols, phytosterols and fatty acids (oleic, linoleic, palmtic, meristic and lauric) [4]. Date seed's dietary fiber has therapeutic applications for a number of illnesses, including colorectal, prostate, and colon cancers, diabetes, obesity, hypertension, coronary heart disease, intestinal problems, and hyperlipidemia. Additionally, people have been used plant bioactive chemicals to fend off diseases like cancer and seed extracts have been discovered to be helpful in preventing live cell death ([1] and [5]). Date seeds are a by-product of dates and are now an environmental problem, as date seeds are disposed as a waste from the manufacture date products sector, where the date palm processing results in a 6.11–11.47% of date seeds as waste products ([5] and [6]). Chocolate is one of the most popular foods all over the world with a sweet-tasting product, a popular ingredient in various culinary applications [7]. It has a variety of potential health benefits, highly nutritious energy source, fertility and sexual activity [8]. It could be prepared with a variety of ingredients to enhance its nutritional value and produce functional foods. Chocolate has been processed from the seeds of the tropical cacao tree. Some people believe that chocolate is the 'food of gods' as it appears to have been utilized as a medicinal remedy by decreasing the heart risks [9]. Therefore, date seeds could be used in the manufacture of many food products with high nutritional value. Thus, the goal of this study was to utilization of the date seed powder in food manufacture where the date seed powder was used in chocolate balls, chocolate spread and dark chocolate processing. Moreover, roasted date seed powder has been assessed for its cytotoxicity, also, sensory properties was estimated for all chocolate balls, chocolate spread and dark chocolate samples.

2. Material and methods:

2.1 Materials:

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Supplementary Information available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

Date seeds (Phoenix dactylifera L.), Saidy variety were obtained from local market, New Valley Governorate, Cairo, Egypt. Cocoa, biscuit, sugar, corn oil, vanilla, milk and sweetened condensed milk were obtained from local market, Cairo, Egypt.

2.2 Methods:

Preparation of roasted date seed powder:

Roasted date seed powder (RDSP) was prepared according to [10] where date seeds were separated manually from the date fruit, washed and roasted in a preheated oven for 1 hr., in order to achieve the steady-state condition, at 160°C for 30 minutes until the seeds color turned to light brown. The seeds were crushed using blender (Laboratory Mill 3100; Perten instruments AB, Postal Address: P.O. Box 5101. S- 141 05 kungens S- 141 05 Kurva, Sweden), then sieved (60 meshes) to obtain the fine seed powder and finally were packed and stored at –18°C.

Preparation of chocolate balls:

The chocolate balls (CB) were prepared according to [11] by mixing crushed biscuit, cocoa and condensed milk together with a proportion that made the mixture smoother with good consistency for rolling in balls shape, then, place the CB in a prepared loaf pan and refrigerate for 2-3 hrs. until it becomes firm and easy sliced. Cocoa powder was replaced with RDSP at levels 25% (CB2), 50% (CB3) and 75% (CB4), where the control sample was prepared by 100% CP (CB1).

Preparation of chocolate spread samples:

The preparation of chocolate spread (CS) was processed according to [12] where cocoa, milk powder, sugar powder, salt was mixed well, then placed in an electric blender then vegetable oil was added gradually until the desired consistency of chocolate spread was obtained. Cocoa powder was replaced with RDSP at levels 25% (CS2), 50% (CS3) and 75% (CS4), where the control sample was prepared by 100% CP (CS1).

Preparation of dark chocolate samples:

The preparation of dark chocolates (DC) was processed according to [13] where cocoa butter was added in a pot and heated over medium low until melted, then add cocoa and sugar powder and whisk until mixture is smooth and no clumps remain, thereafter a chopped nuts were be added . Pour the mixture into a silicone chocolate mold, place it on a tray and keep it in the refrigerator until it sets and ready for analysis. Cocoa powder was replaced with RDSP at levels 25% (DC2), 50% (DC3) and 75% (DC4), where the control sample was prepared by 100% CP (DC1).

Analytical methods:

Cytotoxicity determination by Sulforhodamine B (SRB) assay for RDSP:

The cytotoxicity effect of RDSP was performed in Nawah Scientific Inc., (Mokatam, Cairo, Egypt), which detected against breast adenocarcinoma cells (MCF-7) that maintained in DMEM media supplemented with 100mg/mL of streptomycin, 100 units/ mL of penicillin and10% of heat- inactivated fetal bovine serum in humidified, 5% (v/v) CO2 atmosphere at 37°C. the aliquots of 100 µL cell suspension (5x10^3 cells) were puted in 96-well plates and incubated for 24 hours in full medium. Another aliquot of 100 µL media containing medications at different concentrations was used to treat the cells. Cells were fixed by substituting medium with 150 µL of 10% TCA after 72 hours of drug treatment, and they were then incubated for one hour at 4°C, then get rid of the TCA solution and wash the cells five times with distilled water. After adding aliquots of 70 µL SRB solution (0.4%w/v), the mixture was allowed to sit at room temperature for 10 minutes in a dark area. After three rounds of washing with 1% acetic acid, the plates were left to air dry overnight. The protein-bound SRB stain was then dissolved with 150 µL of TRIS (10 m M); a BMGLABTECH®-FLU Ostar Omega microplate reader (Ortenberg, Germany) was used to detect the absorbance at 540 nm ([14] and [15]).

Chemical composition:

Moisture, crude protein, total ash, ether extract and crude fiber of RDSP, CB, CS and DC samples were estimated according to [16]. Total carbohydrates were determined by differences.

Total phenol content:

The prepared extracts of RDSP, CB, CS and DC samples were carried out for total phenol content determination according to the methodology described by [17]. Two grams of sample were homogenized with 20 mL of 80% ethanol. The mixture was kept in the dark at room temperature overnight, filtered (0.45 µm) and stored at -18°C until determination. One milliliter of alcoholic extract was added to 1 mL 95% ethanol, 5 mL distilled water and 0.5 mL 1N Folin-Ciocalteu reagent. 1 mL 5% Na2CO3 was added after 5 min. and the reagent mixture was kept for 60 min. at room temperature. The quantification of phenolic compounds was performed spectrophotometrically by measuring the absorbance in UV-VIS spectrophotometer Shimadzu 1240, at 725 nm, and a gallic acid (10-100 µg/mL) in 95% ethanol was used for obtaining a standard curve.

Total phenol compounds identification:

Phenolic and flavonoid compounds of RDSP, CB, CS and DC samples were determined for by using high performance liquid chromatography (HPLC) analysis according to [18] in Desert Research Center laboratories, Cairo, Egypt. The instrument Condition was: system Thermo (Ultimate 3000) consisted of: pump, automatic sample injector and DELL-compatible computer supported with Cromelion7 interpretation program. A diode array detector DAD-3000 was used. The Thermo-hypersil reversed phase C18 column 2.5× 30 cm was operated at 25º C. Mobile phase consists of 0.05% Trifluoroacetic acid/Acetonitrile (solvent A) and distilled water (solvent B). The UV absorption spectra of both standards and samples were registered in range of 230–400 nm. Samples, standards solutions and the mobile phase were degassed then purified by using 0.45 µm membrane filter (Millipore). Sample preparation :0.1 gm of sample dissolved in 10 ml methanol, in vortex for 10 minutes, then filtered and stocked under cooling conditions (4°C). Identification of the compounds was done by comparison of their retention's time and UV absorption spectrum with those of the standards.

Minerals composition:

RDSP, CB, CS and DC samples were determined for mineral composition in terms of sodium, potassium, iron, calcium and magnesium by using Inductively Coupled Plasma (Ultimate 2JY plasma) at Soil, Water and Environment Research Institute, Agriculture Research Centre, Giza, Egypt. Minerals concentration was indicated as the average value (mg/kg of dry weight).

Sensory evaluation:

The sensory evaluation of prepared chocolates samples was evaluated organoleptically. Ten panellists in the desert research centre were requested to evaluate the most acceptable samples for sensory attributes of CB, CS and DC were supplemented with RDSP. Sensory evaluation of prepared date seed products was estimation according to [19], whereas every quality received a score ranging from 1 (poor) to 10 (great).

Statistical Analysis:

The collected data were analysed using the SPSS (Statistical Program for Sociology Scientists) Statistics Version 20 for computing the mean values, LSD, ANOVA ($p < 0.05$) and Duncan Multiple Range test [20].

3. Results and discussion:

Nutritional value of RDSP:

Data showed in table (1: a) clarified that, the moisture content of RDSP was 1.11%, meanwhile, crude protein, total ash, ether extract, crude fibre and total carbohydrate contents were 5.36%, 1.78%, 4.37%, 20.80% and 66.58%, respectively, on dry weight basis. Also, data in table (1: a) illustrated that, roasted date seeds powder contains a good proportion of total phenol content was 5070 mg GAE/100g, while, data in table (1: b) illustrated the potassium, calcium, sodium, magnesium and iron contents of RDSP which were 240.83, 11.31, 29.72, 56.88 and 2.11 mg/100g respectively.

[21] found that roasted date seed powder has high nutritional value where protein, carbohydrates, fat, ash and fibre content was found to be 5.92%, 58.62%, 7.5%, 0.96% and 21.25% respectively. [22] mentioned that alwadi algadid dried date seeds contained crude protein (5.62%), crude fibre (18.01%), total ash 1.48%, ether extract (8.5%) and total carbohydrates was 56.76%. moreover, recorded a lower proportion of total phenol content (3010.05 mg GAE/100g) and potassium (215.9 mg/100g), but higher proportion of sodium (30.5 mg/100g), iron (6.53 mg/100g), calcium (136.7 mg/100g) and magnesium (67 mg/100g). [23] recorded a roasted date seeds with lower total ash (1.16%), total carbohydrate content (57.66%) and calcium (18.97 mg/100g) but higher in crude protein, crude fibre and fat with values 7.41, 25.66 and 8.11%, respectively and also higher content of potassium, magnesium, sodium and iron (379.0, 77.00, 15.50 and 3.64 mg/100g) respectively.

Table 1. Chemical composition value of roasted date seed powder (Based on dry weight).

Parameters	RDSP
Moisture Content (%)	1.11±0.01
Crude Protein (%)	5.36±0.01
Total Ash (%)	1.78±0.10
Ether Extract (%)	4.37±0.08
Crude Fibre (%)	20.80±0.06
Total Carbohydrates*(%)	66.58±0.15
Total phenol content (mg GAE/100g)	5070±0.31

*Calculated by differences, ±: standard division, RDSP: roasted date seed powder.

Table 1. b, minerals of roasted date seed powder (Based on dry weight).

Parameters	RDSP
Potassium (mg/100g)	240.83±1.17
Sodium (mg/100g)	11.31±0.01
Iron (mg/100g)	2.11±0.01
Ca (mg/100g)	29.72±0.08
Mg (mg/100g)	56.88±0.01

RDSP: roasted date seed powder.

The resulted total phenol content was close with that observed by [24] who stated that the total phenolic content of date seed powder was 3490.15 mg GAE/100g, but higher than those found with [25] who highlighted that total phenol of date seed powder was 2.53 mg GAE/100g and [26] notified that date seed powder contain total phenolic content ranged from 1.98 to 4.65 mg GAE/100 g. [1] announced that date

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seeds considered to be a good source of phenolic compounds. The cultivar is one element that is likely to have an impact on the profile of polyphenols and antioxidant qualities in plant products and, more broadly, the composition of nutrients [27].

Cytotoxic activity of RDSP:

The Sulforhodamine B (SRB) assay is a colorimetric method used to determine the cytotoxicity effect through assess the cell viability [28].

Figure (1) showed the cytotoxic activity of RDSP against MCF-7 cells examined using SRB assay. A dose-dependent cytotoxic reaction to RSDP was reported against MCF-7 cells after 72 hours of exposure. The percentages cell viability at doses of 62.5, 125, 250, 500 and 1000 µg/ml of RDSP was found 95.63, 92.48, 89.99, 75.22 and 52.85% in MCF- 7 cells, respectively, and the IC50 value was determined at 1112.28 µg/ml. This mean that as the RDSP concentration increased the cell viability decreased. Results were in agreement with Al-Sheddi, (2019) who reported that the percent cell viability at doses of 250, 500, and 1000 µg/ml of sun-dried date seed extract was found as 77%, 51%, and 35%, respectively, while the IC50 value was 769.2 µg/ml. The same trend of action was also observed with [29] who found that roasted date seed recorded an increment in cytotoxic activity against WISH (normal amniotic cells), HepG2 (Human hepatocellular carcinoma) and HCT116 (colon cancer) as the concentration of roasted date seed concentration increased and mentioned that the cytotoxic effect may be due to the phenolic compounds content and its antioxidant effects. Habib et al., proliferation of breast cancer cells dose-dependently. Moreover, [30] referred that flavonoids have a potential role of action to crush tumour cells. [31] suggested that samples that had IC50 value less than 125 µg/mL could be a strong potential cancer therapeutic agent, whilst samples with IC50 value between 125 and 5000 µg/mL was considered to be a moderate potential cancer therapeutic agent. Likewise, [32] notified that date seed extract reduced MCF-7cell viability within 48hr and the IC50 was 678.4 µg/ml.

Chemical composition of chocolate balls, chocolate spread and dark chocolate samples:

The chemical composition of CB, CS and DC samples were clarified in table (2: a, b, c) with significant decreases ($p< 0.05$). For the CB samples, it was found that, moisture content increased from 0.66% in CB1sample to 0.94% in sample CB4. Moreover, increasing roasted date seed powder proportion led to a remarkable increase in both ether extract and crude fibre content, where the values increased from 19.75% and 1.48% for CB1 sample to 20.45 and 15.79% for CB4 sample, respectively. Meanwhile, a decrement was occurred in the content of crude protein and total ash, wherever the crude protein value decreased from 10.94% for CB1 sample to 3.28% for the CB4 sample, while the total ash value was 3.57% and decreased to 0.97%.

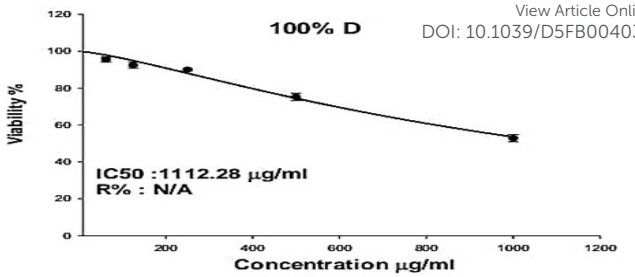


Figure 1. Dose response curve of roasted date seed powder.

Table 2 a. Chemical composition of chocolate balls samples (g/100g DW).

Chemical composition (%)	Samples			
	CB1	CB2	CB3	CB4
Moisture	0.66±0.1^{c}	0.66±0.1^{c}	0.75±0.2^{b}	0.94±0.1^{a}
protein	10.94±0.02^{a}	10.94±0.02^{a}	6.56±0.01^{b}	3.28±0.01^{c}
Total ash	3.57±0.004^{d}	2.85±0.004^{c}	1.48±0.004^{b}	0.97±00.04^{a}
Ether extract	19.75±0.02^{b}	14.94±0.02^{d}	17.46±0.02^{c}	20.45±0.02a
fibre	1.48±0.04^{d}	6.56±0.02^{c}	11.13±0.002^{b}	15.79±0.02^{a}
carbohydrates	64.26±0.01^{b}	64.93±0.01^{a}	63.37±0.03^{c}	59.51±0.01^{d}

CB1:100%CP (control sample), CB2:25%RDSP+75%CP, CB3:50%RDSP+ 50%CP, CB4:75% RDSP+25%CP. Each value represents the mean of three replicates (Mean ± SD). Same letters in each column represents the insignificant difference at p< 0.05.

Table 2 b. Chemical composition of spread chocolate samples (g/100g DW).

Chemical composition (%)	Samples			
	CS1	CS2	CS3	CS4
Moisture	0.25±0.02^{c}	0.25±0.04^{c}	0.26±0.03^{b}	0.29±0.02^{a}
protein	12.69 ±0.02^{a}	7.66± 0.01^{b}	6.68 ±0.03^{c}	6.47 ±0.01^{c}
Total ash	2.99±0.01^{d}	2.10±0.01^{c}	1.01±0.01^{b}	0.97±0.02^{a}
Ether extract	64.88±0.05^{a}	64.00±0.02^{b}	63.62±0.04^{c}	62.46±0.04^{a}
fibre	1.100±0.01^{d}	4.95±0.002^{c}	9.82±0.04^{b}	14.75±0.02^{a}
carbohydrates	18.34±0.02^{b}	21.29±0.04^{a}	18.87±0.03^{c}	13.35±0.04^{d}

CS1:100%CP (control sample), CS2:25%RDSP+75%CP, CS3:50%RDSP+50%CP, CS4:75% RDSP+25%CP. Each value represents the mean of three replicates (Mean ± SD). Same letters in each column represents the insignificant difference at p< 0.05.

Table 2 c. Chemical composition of dark chocolate samples (g/100g DW).

Chemical composition (%)	Samples			
	DC1	DC2	DC3	DC4
Moisture	0.81±0.04^{c}	0.84±0.02^{c}	0.88±0.03^{b}	0.91±0.05^{a}
protein	12.19 ±0.01^{a}	7.20± 0.03^{b}	6.45±0.04^{c}	6.25 ±0.02^{c}
Total ash	3.15±0.05^{d}	2.92±0.02^{c}	1.99±0.07^{b}	1.05±0.06^{a}
Ether extract	63.79±0.2^{a}	63.67±0.1^{b}	63.65±0.1^{c}	63.59±0.3^{d}
fibre	0.07±0.02^{d}	4.35±0.01^{c}	9.24±0.02^{b}	14.55±0.03^{a}
carbohydrates	20.08±0.02^{b}	21.86±0.03^{a}	18.67±0.02^{c}	14.56±0.01^{d}

DC1:100%CP (control sample), DC2:25%RDSP+75%CP, DC3:50%RDSP+ 50%CP, DC4:75% RDSP+25%CP. Each value represents the mean of three replicates (Mean ± SD). Same letters in each column represents the insignificant .difference at p< 0.0.

With regard to the CS samples, there was an increment in crude fibre content and a decrement in crude protein, total ash, ether extract and total carbohydrates occurred as the RDSP proportion increased. As well data in table (2: a, b, c) showed an increment in crude fibre and moisture content with

a slightly decrement in ether extract content, meantime the crude protein and total ash content had a remarkable decline for the DC samples as the RDSP proportion increased. Finally, it could be concluded that, among all the three food products under study, the usage of RDSP positively affect the crude fibre content and this is may be due to the fact that RDSP contain a good proportion of crude fibre as mentioned above.

[24] lighted on an increment in crude fibre content and a decrement in fat content for both uncooked and cooked beef meat balls when the replacement level of date seed powder increased. Same trend of results was observed with [33] who noticed that as the roasted date seed increased as the crude fibre increased, while moisture content, total ash, ether extract content and total carbohydrates decreased three types of coffee processed from roasted date seeds and mentioned that the dietary fibre content of roasted date seeds makes them suitable for the preparation of dietary supplements and high fibre-based food.

[23] registered a decrement in crude protein and total carbohydrates content and increment in crude fibre as the replacement level of roasted date seed powder increased in processed cake samples. [12] found that both crude fibre and total ash content increased in chocolate spread as the replacement level of cocoa powder by roasted date seed powder increased.

Total phenol content of chocolate balls, chocolate spread and dark chocolate samples.

Phytochemical compounds such as phenolic compounds have a healthy because of for their antioxidants and anticarcinogenic, also, for their bioavailability into human body [34]. Therefore, total phenol content (TP) was investigated for CB, CS and DC and results were clarified in figure (2: a, b, c). It could be notified that there was an increment trend in TP content in all samples as the RDSP proportion increased, where the TP values increased from 1020.5 to 1080.33 mg GAE/100g for the CB1 and CB4 samples, from 2030.5 to 2080.5 mg GAE/100g for the CS1and CS4 samples and from 2040 to 3000 mg GAE/100g for the DC1and DC4 samples, respectively. Results were in the same lines with those observed by [12] who found that the total phenol content increased as the roasted date seed powder proportion replacement with cocoa powder increased in chocolate spread and mentioned that because of the high total phenols content of date seed powder. [33] referred that roasted date seed coffee contain total phenolic content higher than two kinds of coffee, one of them contain coffee seeds only and another kind of coffee contained 60% roasted date seed and 30% barley seeds. [24] recorded an improvement in total phenolic content for both uncooked and cooked beef meat balls as the replacement level of date seed powder increased when compared to the control samples.

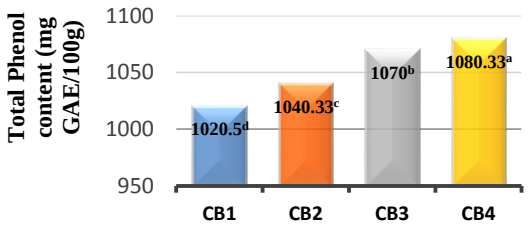


Figure (2: a). Total phenol content of chocolate balls samples p< 0.05.

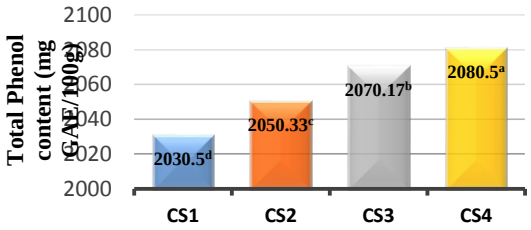


Figure (2: b). Total phenol content of spread chocolate samples p< 0.05.

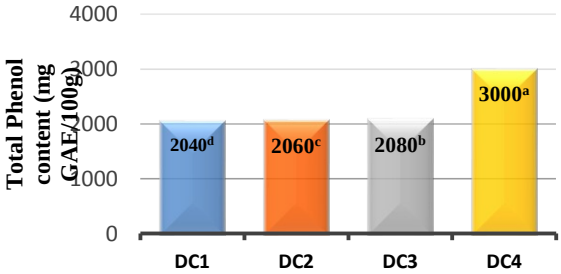


Figure (2: c). Total phenol content of dark chocolate samples p< 0.05.

Total phenol compounds identification of chocolate balls, chocolate spread and dark chocolate samples:

The identification of total phenol compounds for the CP, RDSP, CB, CS and DC samples was determined by HPLC and results were showed in tables (3: a, b and 4: a, b, c, d, e, f, g and h) and figures 3(a, b, c, d, e, f, g and h). A 16 phenol compounds were identified (10 polyphenol and 6 flavonoids). From data illustrated in table (3: a and b) and figure 3(a and b), it was cleared that RDSP contain a highly content of quinic (0.0117mg/100g), ellagic (0.0031mg/100g), chlorogenic (0.0378mg/100g), cinnamic (0.4552mg/100g), resorcinol (0.0199mg/100g), pyrochatechol (0.0013mg/100g), vanillic (0.0007mg/100g), ferulic (0.0007mg/100g), phenantherine (0.0019mg/100g), apeginin (0.8439mg/100g), diosmin (0.5627mg/100g), rutin (7.0620mg/100g), hesperidin (5.3637mg/100g) and kampferol (0.01877mg/100g) as compared to CP.

Regarding the total phenol compounds for the CP, RDSP, CB, CS and DC samples, data in table (4: a, b, c, d, e, f, g and h) and figures 3 (a, b, c, d, e, f, g and h) displayed that, there was an enhancement exhibit in ellagic, chlorogenic, apeginin, diosmin, hisperidin and quercitin for the CB samples as the proportion of RDSP increased. Moreover, CB3 sample found to have a good content of quinic, cinnamic, coumaric and kampferol

when compared to CB1 sample. Also, for the CS samples, it was noticed that ellagic, chlorogenic, cinnamic, diosmin, kampferol and quercitin were improved in CS2 sample as compared with CS1 sample. Concerning DC samples, the ellagic, phenantherine and coumaric were increased in DC4 as the RDSP proportion increased.

Table 3. a. Total phenol compounds identification of cocoa powder and roasted date seed powder.

Total phenol compounds	mg/100g	
	CP	RDSP
Quinic	0.0013	0.0117
Ellagic	0.0004	0.0031
Chlorogenic	0.0016	0.0378
Cinnamic	0.0168	0.4552
Resorcinol	0.0033	0.0199
Pyrochatechol	n.a.	0.0013
Vanillic	n.a.	0.0007
Ferulic	n.a.	0.0007
Phenantherine	0.0002	0.0019

CP: cocoa powder, RDSP: roasted date seed powder.

Table 3.b. Total phenol compounds identification of cocoa powder and roasted date seed powder.

flavonoids compounds	mg/100 g			
	CB1	CB2	CB3	CB4
Apeginin	0.0831	0.8439	Apeginin	0.0831
Diosmin	0.5935	0.5627	Diosmin	0.5935
Rutin	1.4871	7.0620	Rutin	1.4871
Hisperidin	0.9719	5.3637	Hisperidin	0.9719
Kampferol	n.a.	0.1877	Kampferol	n.a.

CP: cocoa powder, RDSP: roasted date seed powder.

[24] and [23] informed that date seed powder contains a good content of phenolic and flavonoids component. [35] mentioned that roasted date seeds manifested a highest overall content of flavonoids compared to the raw date seeds.

Table 4: a. Total phenol compounds identification of chocolate balls samples.

Phenolic compounds	mg/100 g			
	CB1	CB2	CB3	CB4
Quinic	0.0100	0.0015	0.0021	0.0002
Ellagic	0.0024	0.0026	0.0034	0.0031
Chlorogenic	0.0003	n.a.	0.0002	0.0003
Cinnamic	0.0065	0.0002	0.0026	0.0043
Resorcinol	0.0001	0.0032	0.0002	n.a.
Pyrochatechol	n.a.	0.0001	0.0001	0.0000
Vanillic	n.a.	0.0000	0.0001	n.a.
Ferulic	0.0000	n.a.	n.a.	n.a.
Phenantherine	n.a.	0.0000	n.a.	n.a.
Coumaric	0.0088	n.a.	0.0081	0.0001

CB1:100%CP (control sample), CB2:25%RDSP+75%CP, CB3:50%RDSP+50%CP, CB4:75% RDSP+25%CP.

Table 4: b. Flavonoids compounds identification of chocolate balls samples.

flavonoids compounds	mg/100 g			
	CB1	CB2	CB3	CB4
Apeginin	0.0197	0.0809	0.0753	0.0208
Diosmin	0.2007	0.0987	0.0990	0.1009
Rutin	1.7056	0.4452	0.6911	0.3051
Hisperidin	n.a.	n.a.	0.1678	0.4359
Kampferol	n.a.	n.a.	0.2641	n.a.

CB1:100%CP (control sample), CB2:25%RDSP+75%CP, CB3:50%RDSP+50%CP, CB4:75% RDSP+25%CP.

Table 4: C. Total phenol identification of chocolate balls samples.

	mg/100 g			
	CS1	CS2	CS3	CS4
Quinic	0.0004	0.0003	0.0002	0.0002
Ellagic	0.0000	0.0018	0.0009	0.0008
Chlorogenic	0.0000	0.0006	n.a.	n.a.
Cinnamic	n.a.	0.0032	0.0005	0.0004
Resorcinol	n.a.	n.a.	0.0017	0.0020
Pyrochatechol	n.a.	n.a.	n.a.	n.a.
Vanillic	n.a.	n.a.	n.a.	n.a.
Ferulic	n.a.	n.a.	n.a.	n.a.
Phenantherine	n.a.	n.a.	n.a.	0.0001
Coumaric	n.a.	n.a.	n.a.	0.0001

CS1:100%CP, CS2:25%RDSP+75%CP, CS3:50%RDSP+50%CP, CS4: 75 % RDSP+25 % CP.

Table 4: d. Flavonoids compounds identification of chocolate balls samples.

flavonoids compounds	mg/100 g			
	CB1	CB2	CB3	CB4
Apeginin	0.1377	0.1051	0.0994	0.0577
Diosmin	0.5624	0.6593	0.5199	0.3494
Rutin	0.6832	0.6487	0.5562	0.0015
Hisperidin	2.8826	n.a.	0.2327	n.a.
Kampferol	1.5774	4.6104	n.a.	0.0368
Quercitin	0.0478	0.1490	n.a.	0.0925

CS1:100%CP, CS2:25%RDSP+75%CP, CS3:50%RDSP+50%CP, CS4: 75 % RDSP+25 % CP,

Table 4: e. Phenolic compounds identification of chocolate balls samples.

Phenolic compounds	mg/100 g			
	DC1	DC2	DC3	DC4
Quinic	20.9786	14.1253	13.2550	0.6218
Ellagic	3.3903	1.9364	2.2284	4.7255
Chlorogenic	11.5280	7.4094	6.6897	2.5675
Cinnamic	0.4297	0.2087	0.1446	0.0465
Resorcinol	0.7334	0.1972	0.2715	0.0522
Pyrochatechol	0.1643	0.0175	n.a.	0.0130
Vanillic	0.0143	n.a.	n.a.	0.0076
Ferulic	0.0565	n.a.	n.a.	0.0370
Phenantherine	0.1526	0.2888	0.2367	0.2375
Coumaric	4.5590	4.6393	4.7025	5.7115

DC1:100%CP, DC2:10% RDSP+90%CP, DC3:20%RDSP+80%CP, DC4:30%RDSP +70%CP.

Table 4: f. Flavonoids compounds identification of chocolate balls samples.

flavonoids compounds	mg/100 g			
	CB1	CB2	CB3	CB4
Apeginin	0.7278	0.0900	0.6348	0.3374
Diosmin	0.4362	0.3607	0.2261	0.1032
Rutin	4.3042	3.4640	1.6834	0.7770
Hisperidin	n.a	n.a.	n.a	n.a.
Kampferol	n.a.	n.a.	n.a.	n.a.

DC1:100%CP, DC2:10% RDSP+90%CP, DC3:20%RDSP+80%CP, DC4:30%RDSP +70%CP.

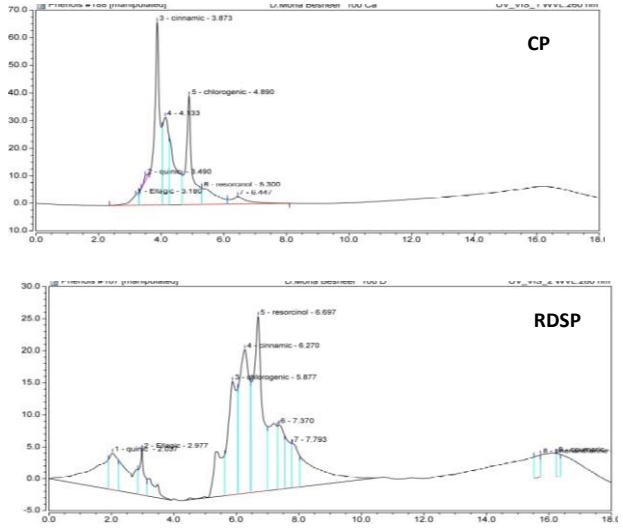
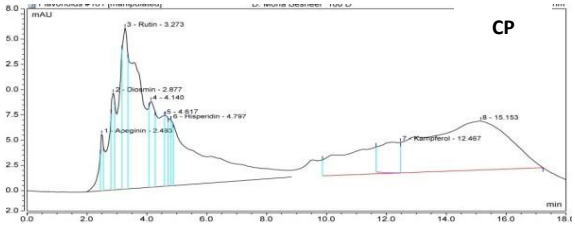


Figure 3a. Total phenol compounds identification of cocoa powder and roasted date seeds powder.



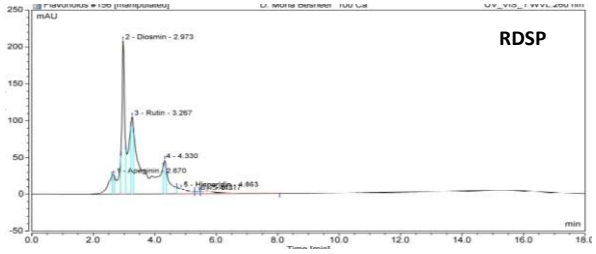


Figure 3b. Total flavonoid compounds identification of cocoa powder and roasted date seeds powder.

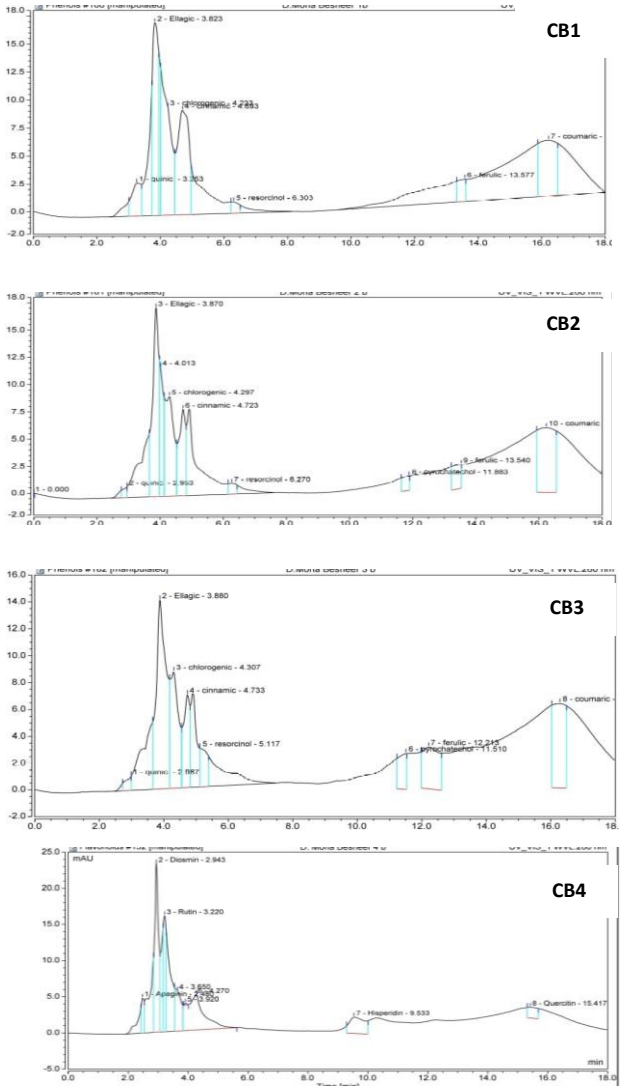
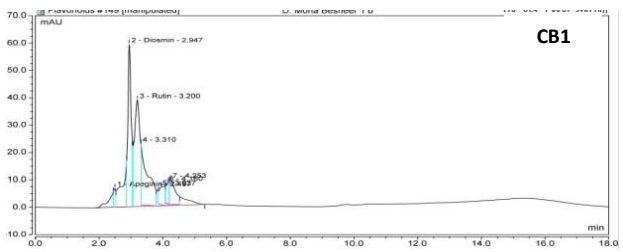


Figure 3c. Total phenol compounds identification of chocolate ball samples.



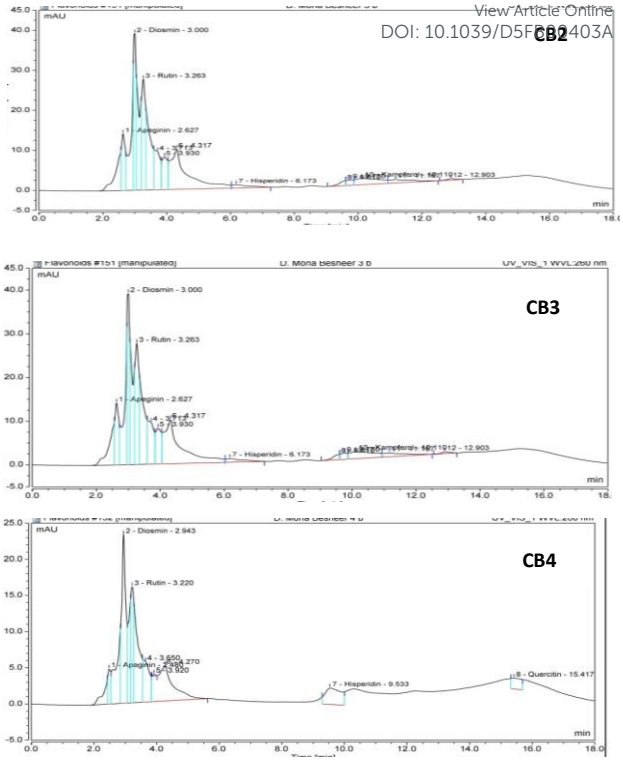


Figure 3d. Total flavonoid compounds identification of chocolate ball samples.

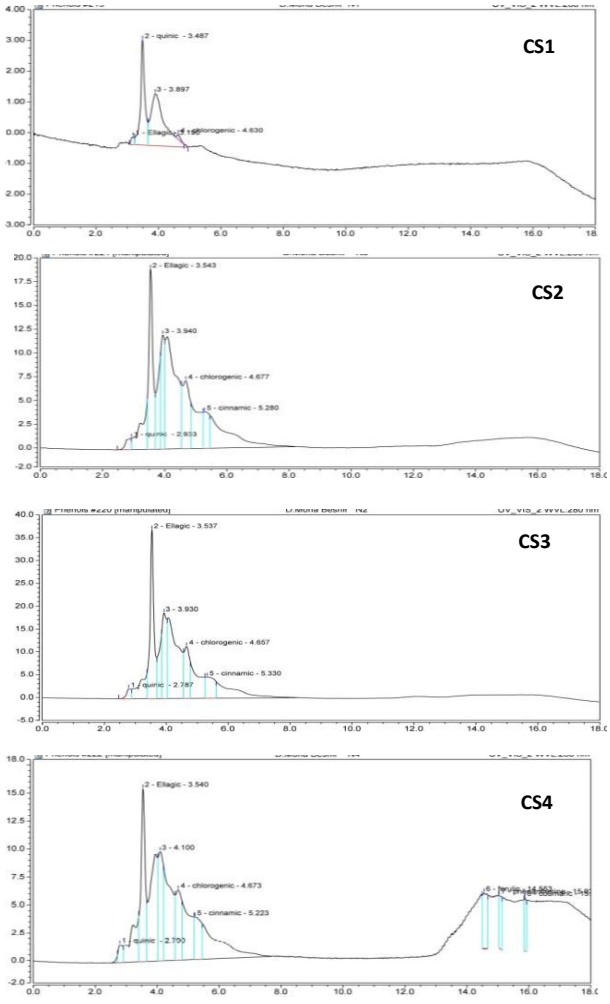


Figure 3e. Total phenol compounds identification of chocolate spread samples

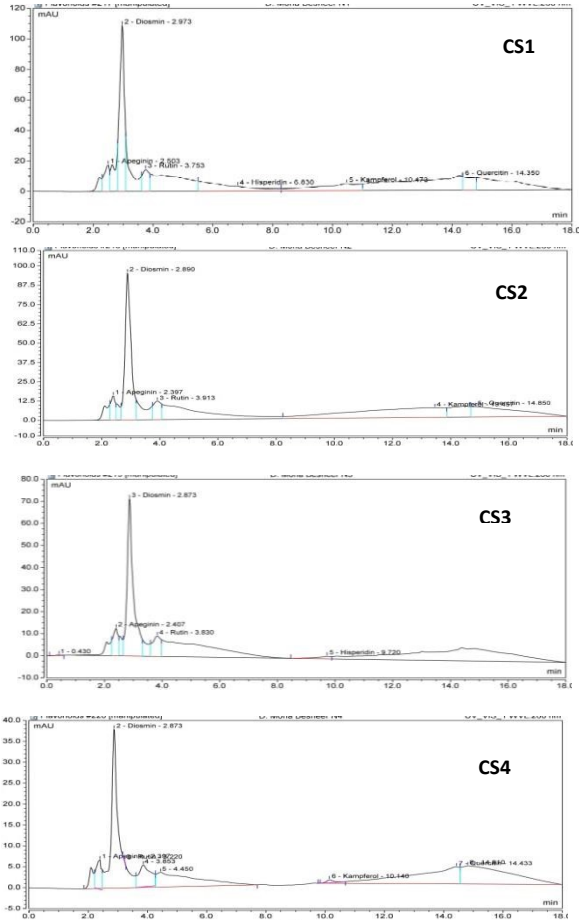


Figure 3f. Total flavonoid compounds identification of chocolate spread samples.

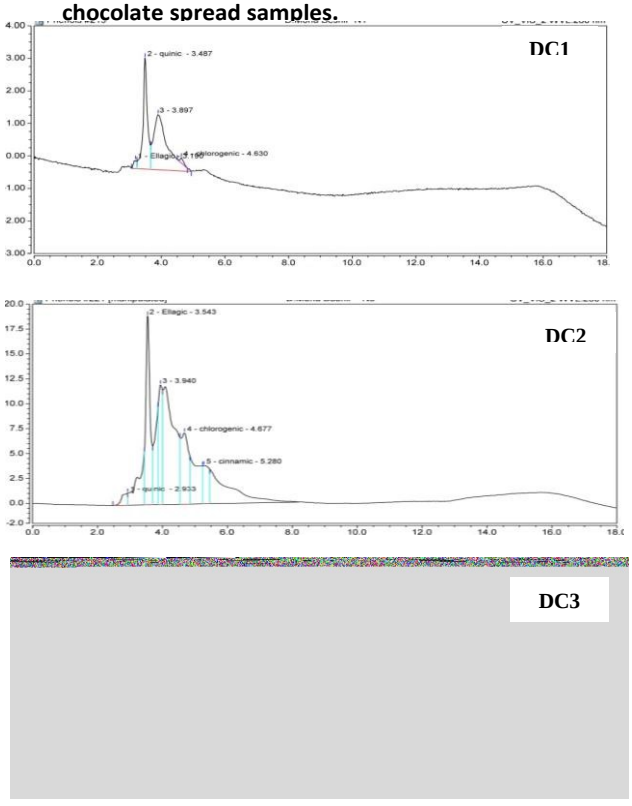


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Figure 3g. Total phenol compounds identification of dark chocolate samples.

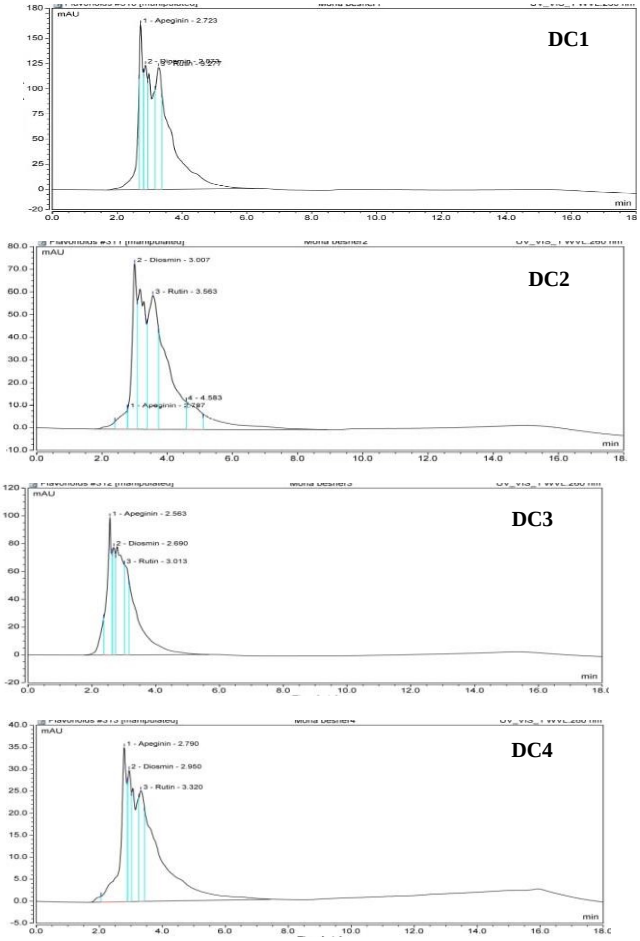

Figure 3h. Total flavonoid compounds identification of dark chocolate samples.

Minerals content of chocolate balls, chocolate spread and dark chocolate samples.

Minerals considered to be a key role for overall mental and physical well-being. It is important for bones, teeth, blood, nerve cells and in immune and brain system. Minerals are also vital to overall mental and physical well-being [36]. Therefore, iron, magnesium, calcium, sodium and potassium were investigated in CB, CS and DC samples. Data of minerals content were listed in table (5: a, b and c) at $p< 0.05$ and showed that, there was an increment trend in both potassium and sodium component as the replacement proportion of cocoa powder by RDSP increased among all CB, CS and DC samples. On contrary, iron, magnesium, calcium was decreased

among all CB, CS and DC samples as the replacement proportion of cocoa powder by RDSP increased and so, it could be concluded that successfully reinforcement both sodium and potassium content of CB, CS and DC samples.

Table 5. a. Minerals components of chocolate balls samples.

Minerals (mg/100g)	Samples			
	CB1	CB2	CB3	CB4
Fe	11.90±0.1^{a}	8.75±0.2^{b}	7.13±0.1^{c}	4.24±0.1^{d}
Mg	189.78±0.5^{a}	165.47±0.5^{b}	159.22±0.3^{c}	105.98±0.3^{d}
Ca	147.91±0.03^{a}	131.64±0.2^{b}	103.24±0.1^{c}	80.13±0.1^{d}
Na	2.70±0.1^{d}	6.60±0.1^{c}	8.11±0.2^{b}	12.43±0.2^{a}
K	102.45±0.2^{d}	155.00±0.3^{c}	195.00±0.5^{b}	274.00±0.6^{a}

CB1:100%CP (control sample),CB2:25%RDSP+75%CP,CB3:50%RDSP+50%CP, CB4:75% RDSP+25% CP, , Each value represents the mean of three replicates (Mean ± SD). Same letters in each column represents the insignificant difference at p< 0.05.

Table 5. b. Minerals components of spread chocolate samples.

Minerals (mg/100g)	Samples			
	CS1	CS2	CS3	CS4
Fe	11.20±0.3^{a}	9.25±0.1^{b}	6.20±0.3^{c}	4.12±0.2^{d}
Mg	153.90±0.4^{a}	135.32±0.1^{b}	129.97±0.2^{c}	75.12±0.1^{d}
Ca	117.00±0.3^{a}	101.70±0.2^{b}	73.10±0.1^{c}	52.05±0.4^{d}
Na	2.20±0.3^{d}	4.30±0.1^{c}	6.70±0.1^{b}	9.35±0.1^{a}
K	95.3.0±0.2^{d}	137.00±0.3^{c}	167.67±0.1^{b}	201.45±0.2^{a}

CS1:100%CP (control sample),CS2:25%RDSP+75%CP,CS3:50%RDSP+50%CP, CS4:75% RDSP+25% CP, , Each value represents the mean of three replicates (Mean ± SD). Same letters in each column represents the insignificant difference at p< 0.05.

Table 5. c. Minerals components of spread chocolate samples.

Minerals (mg/100g)	Samples			
	DC1	DC2	DC3	DC4
Fe	11.2±0.2^{a}	9.10±0.3^{b}	6.15±0.1^{c}	4.32±0.1^{d}
Mg	151.70±0.3^{a}	139.24±0.2^{b}	122.34±0.4^{c}	78.15±0.4^{d}
Ca	117.00±0.3^{a}	103.04±0.2^{b}	77.10±0.2^{c}	50.97±0.1^{d}
Na	2.20±0.4^{d}	4.39±0.1^{c}	6.77±0.0.4^{b}	9.05±0.0.1^{a}
K	95.30±0.1^{d}	135.13±0.1^{c}	161.67±0.3^{b}	204.85±0.4^{a}

DC1:100%CP (controlsample),DC2:25%RDSP+75%CP,DC3:50%RDSP+50%CP, DC4:75% RDSP+25% CP, , Each value represents the mean of three replicates (Mean ± SD). Same letters in each column represents the insignificant difference at p< 0.05.

Sensory evaluation of chocolate balls, chocolate spread and dark chocolate samples:

Data in table (6: a, b and c) presented the sensory evaluation of CB, CS and DC samples with significant differences (p< 0.05). It was noticed that sensory parameters estimated between very good and good level for odor, taste, color and texture parameters for all the CB, CS and DC samples that contain RDSP as compared with each control sample of the three products under study which contain CP only. Moreover, there were no significant differences in overall acceptability for both CB and CS samples when compared with control sample but for the DC samples, the overall acceptability of

Table 6: a. Sensory evaluation of chocolate balls samples.

Sensory parameters	Samples			
	CB1	CB2	CB3	CB4
Odor	9.67±0.21^{a}	9.25±0.36^{ab}	9.17±0.40^{ab}	8.33±0.42^{b}
Taste	9.67±0.21^{a}	9.58±0.27^{a}	9.58±0.27^{a}	9.00±0.26^{a}
Color	10.00±0.00^{a}	9.83±0.17^{a}	9.67±0.21^{a}	9.08±0.20^{b}
Texture	9.67±0.17^{a}	9.58±0.15^{a}	9.50±0.22^{a}	9.42±0.24^{a}
Overall acceptability	9.67±0.21^{a}	9.42±0.27^{a}	9.17±0.31^{a}	8.83±0.31^{a}

CB1:100%CP (control sample),CB2:25%RDSP+75%CP,CB3:50%RDSP+50%CP, CB4:75% RDSP+25% CP, , Each value represents the mean of three replicates (Mean ± SD). Same letters in each column represents the insignificant difference at p< 0.05.

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Table 6: b. Sensory evaluation of spread chocolate samples.

Sensory parameters	Samples			
	CS1	CS2	CS3	CS4
Odor	9.33±0.33^{a}	9.33±0.21^{a}	9.00±0.37^{a}	9.00±0.37^{a}
Taste	9.67±0.21^{a}	9.50±0.22^{a}	9.30±0.22^{ab}	8.58±0.33^{b}
Color	9.33±0.33^{a}	8.75±0.36^{a}	9.00±0.37^{a}	8.83±14.8^{a}
Texture	9.17±0.31^{a}	8.75±0.17^{a}	9.17±0.31^{a}	8.92±0.33^{a}
Overall acceptability	8.92±0.33^{a}	9.17±0.4^{a}	9.25±0.25^{a}	9.17±0.40^{a}

CS1:100%CP (control sample),CS2:25%RDSP+75%CP,CS3:50%RDSP+50%CP, CS4:75% RDSP+25% CP, , Each value represents the mean of three replicates (Mean ± SD). Same letters in each column represents the insignificant difference at p< 0.05.

Table 6:c. Sensory evaluation of dark chocolate samples.

Sensory parameters	Samples			
	DC1	DC2	DC3	DC4
Odor	10.00±0.00^{a}	9.58±0.15^{a}	9.00±0.00^{a}	8.75±0.11^{a}
Taste	10.00±0.00^{a}	9.67±0.11^{b}	9.17±0.11^{c}	9.00±0.00^{c}
Color	10.00±0.00^{a}	10.00±0.00^{a}	9.67±0.11^{a}	8.92±0.24^{b}
Texture	8.92±0.24^{b}	10.00±0.00^{b}	9.67±0.11^{b}	8.92±0.24^{b}
Overall acceptability	10.00±0.00^{a}	9.83±0.11^{a}	9.00±0.00^{b}	9.00±0.13^{b}

DC1:100%CP (controlsample),DC2:25%RDSP+75%CP,DC3:50%RDSP+50%CP, DC4:75% RDSP+25% CP, , Each value represents the mean of three replicates (Mean ± SD). Same letters in each column represents the insignificant difference at p< 0.05.

DC3 and DC4 samples were significantly lower compared to DC1 and DC2 samples with a slightly difference values. However, the overall acceptability of both DC and CS samples rated as a very good samples, whilst the CB samples rated between very good and good as compared with the control sample, which is mean that replacement of CP with different proportion of RDSP didn't negatively affect the sensory properties of CB, CS and [37] evaluated the sensory properties of low-fat ice cream samples containing 3.5 and 2.5% of date seed powder instead of cocoa and observed that the superior sample in taste, texture, and general acceptance was the sample containing 3.5% of date seed powder compared to other studied samples. Also, [38] informed that all cheese spread samples fortified with date seed powder were sensory acceptable. [12] announced that chocolate spread sample processed using 4% date seed powder was the most superior sample as compared with the other treatment samples in terms of taste, texture and aroma, taking into consideration, a decrement in bitterness occurred in the chocolate spread sample containing 100% date seed powder.

4. Conclusion:

Purpose of the present investigation was to assess the usage ability of roasted date seed powder in chocolate products manufacture. Results demonstrated that the RDSP showed doses dependent cytotoxicity in all the testes cell lines, where the cell viability was found to be 95.63, 92.48, 89.99, 75.22 and 52.85% for the MCF-7 cells at doses 62.5, 125, 250, 500 and 1000 µg/ml of RDSP, respectively and the IC50 value was 1112.28 µg/ml in MCF-7 cells. Moreover, RDSP found to be a good source of total phenol, crude fibre and total carbohydrate, also, contains a good proportion of potassium and sodium, considerable amount of magnesium,

calcium and iron. Distinct outcomes were observed when CP was replaced by RDSP, where crude fibre, total phenol, potassium, sodium content, as well, phenolic and flavonoids component among CB, CS and DC samples were enhanced. The overall acceptability of all chocolate products samples recorded a good score. That being so, these findings support the usage of RDSP as an excellent source of crude fibre, total phenol, potassium and sodium with an excellent cytotoxicity effect which mean a positive effect on cancer cells.

Data availability

The data supporting this article have been included within the article.

Author contributions

Manuscript preparation, methodology design, statistical analysis and Sample processing Writing, review, and editing of the document.

Conflicts of interest

The author announces there is no conflict of interest with respect to the content of this article.

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Data availability

The data supporting this article have been included within the article.