Egyptian Journal of Food Science
http://ejfs.journals.ekb.eg/

Dried Date Powder Addition in Balady Bread Processing and Its Effect on the Hepatotoxicity Induced by Some Heavy Metals

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TILL now there is no way to avoid human being exposed to harmful chemicals and heavy metals in urban and industrial world. The aim of this experiment was to find out the negative effect of Lead (Pb), Cadmium (Cd) and Mercury (Hg) on hepatic cells and how date fruit could reduce their effects *in vetro*, as well as, evaluate the Balady bread produced by adding different ratios of date powder (0, 0.25, 0.50 and 1%). Results clearly showed that, the most effective tested dose of ethanolic date fruit powder extract against tested heavy metals (Pb, Cd, and Hg) was 400 µg/mL.This dose recorded hepato-protective effect 32.12±4.19 % for Lead, 22.49±3.88 % for Cadmium and 18.77±2.62% for Mercury, respectively. Also, it improved the cellular viability of cultured liver cells to 53.03±4.53, 37.67±2.84 and 27.86±4.21 %, for Pb, Cd, and Hg, respectively. On the other side, addition of date powder to Balady bread had no significant effect ($p > 0,05$) on chemical composition of the produced bread with one exception related to polyphenol content which increased significantly ($p < 0.05$). Also, two date powder ratios (0.5 and 1%) had positive effects on all sensory properties. The obtained results suggested that, date powder could be considerable solution in reduction of heavy metal hepato-toxicity and applicable in Balady bread.

Keywords: Heavy metals Hepato-toxicity, Date powder, Balady bread

Introduction

Several publications have been written about the heavy metal residues in food (Naghipour et al., 2014). Ingestion is the most common way to be exposed to heavy metals. Heavy metal traces are significant in nutrition because of their basic nature as well as their toxicity (Fraga, 2005).

Nowadays, the load of heavy metals in our environment is steadily increasing, and it has become one of the world's most pressing issues (WHO, 2014).The heavy metals problem is mostly linked to increased activity in the industrial, agricultural, and healthcare sectors, among others. Heavy metals are not biodegradable, yet they are toxic to the environment. Toxic minerals are those that are recognized to be harmful to living things.

Probable humaneness sources of toxic metals in the environment include activities such as metal cleaning, paint tubs, refineries, paper and pulp, tanning, and chemical industrial processes (Magomya et al., 2013).

Toxic metal ions are primarily associated with nucleic acids, proteins, and tiny metabolites in living organisms. Cells' basic processes are interrupted when they lose control of the balance of critical metals, resulting in deadly health consequences (Järup, 2003).

Cadmium is used in electroplating and other electronics production processes. Almost all of the world's cadmium output is increasing as a result of zinc smelting, potentially exceeding the World Health Organization's acceptable limit of 3.0 ppb in drinking water (Magomya et al., 2013; WHO, 2014). In humans and other mammals, Cd exposure can result in a variety of adverse effects, such as testicular damage, pulmonary edema, renal and hepatic dysfunction, osteomalacia, etc.

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Received: 30/11/2021; accepted: 24/2/2022
DOI: 10.21608/ejfs.2022.107106.1114

In addition to the direct cytotoxic effects that could lead to apoptotic or necrotic event, Cd have been implicated in the development of cancer and it has been classified as a type I carcinogen (International Agency for Cancer Research, 1993). Chronic Cd intoxication results mainly in renal disease (Diamond et al., 2003; Jin et al., 2004), but acute Cd exposure primarily results in liver accumulation and hepatocellular damage. After acute exposure to inorganic forms of Cd, the preponderance of the dose accumulates in the liver (Zalups, 2000).

Lead pollutant (Pb) is emitted by industrial mining, steel, automobiles, and batteries, laboratory waste, paints, and pollutants produced by growing industrial development. Lead pollution can cause nausea, encephalopathy, headaches, and even mental retardation (Salama & Radwan, 2005).

Previous studies have shown that both Cd and Pb exposure can lead to hepatocyte enlargement, inflammatory cell infiltration (Li et al., 2019) and hepatocyte necrosis, whereas it can also cause elevation in the plasma activity levels of liver enzymes, such as aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) (Cao et al., 2017). Furthermore, Pb can also affect the production of heme, destroy the structure of the cell membrane, cause lipid metabolic dysfunction, and reduce liver detoxification function (Flora et al., 2012).

Methyl mercury is a common type of mercury to which most people are exposed. The most serious adverse effects of methylmercury consumption in humans are neurological consequences and cardiovascular (Carocci et al., 2014). Also, the reproductive and immunological systems are both affected (Garner et al., 2010).

In addition to evidence of mercury effects on various organs, some animal studies have suggested that acute mercury exposure can damage the liver (FilipakNeto et al., 2008 and Ung et al., 2010).

Another study among South Korean adults, which examined the relationship between blood Hg levels and subclinical changes in liver function, identified a positive association indicating the possibility to use this association as a proxy measure of nonalcoholic fatty liver disease among the general population of South Korea (Lee et al., 2014).

A report from Dutczak et al. (1991), indicates that extensive absorption of methyl mercury occurs in the gall bladder and subsequent biliary-hepatic cycling of the compound contributes to its long biologic half life

There is considerable scientific evidence that there is a relation between dietary intake, particularly vegetables and fruits, and diabetes, cardiovascular disease, and cancer risk factors, which their high concentration of phytochemicals, like polyphenols, explains this, at least in part (Randhir et al., 2004). In addition, those polyphenols are classified into several classes, the most important of which being flavonoids, lignin and phenolic acids. Polyphenols, by the action of free radical scavengers, can protect cell components from oxidative damage caused by oxidative stress conditions.

In dry places like the Middle East, the date palm, *Phoenix dactylifera* L., is an important agricultural, historical, commercial, and scientific tree.Since 1974, Egypt has been the world's top producer of dates, with exceptionally high average yields when compared to other nations (Hoffmeister & Khaira, 2011). In Saudi Arabia (Al-Shayeb et al., 1995), Turkey (Aksoy&Öztürk, 1996), Kuwait (Bu-Olayan& Thomas, 2002), and Jordan, the date palm has been utilized as a pollution biomonitor (Al-Khlaifat& Al-Khashman, 2007).

Dates' components have previously been found to be powerful antioxidants, anti-tumor, and anti-inflammatory agents, making them a viable alternative medicine for the treatment of a variety of illnesses (Eid et al., 2014). Dates are a high-nutritional-value dietary source. Date is containing extraordinary levels of both dietary fiber and polyphenols. Dates are rich in calories and carbohydrates, including fructose, glucose, and sucrose and also include a high percentage of dietary fiber. Dates also contain significant amounts of polyphenols such as phenolic acids and flavonoid glycosides (El-Sohaimy& Hafez, 2010). Moreover, date fruit contains also,proteins, minerals, and vitamin B complexes including thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6), and folate (B9) (Eoin,2016). This research paper aims to find out the negative effect of lead (Pb), cadmium (Cd) and mercury (Hg) on hepatic cells and how date fruit could reduce their effects as well as evaluate the Balady bread produced by adding different ratios of date powder.

Materials and Methods

Materials

Dried date fruit powder

Date fruits (*Frahy cultivar*) were purchased from local market, washed, and dried at 60°C in a ventilated oven (Domenica UNOX: XF043, Italy); the dry material was milled into fine powder and stored in an airtight bottle until use. The moisture level was less than 7% (dry basis).

Cell Culture

Chemicals and HepG2 human liver hepatoma cell line were obtained from the Laboratory of the Regional Center for Mycology and Biotechnology, Al- Azhar University. HepG2 cells were cultured in DMEN (Dulbecco's modified's medium), enriched with 10% fetal calf serum, penicillin (100 U) and streptomycin (100 µg).Every two days, the culture media was replaced, and the cells were sub-cultured confluency until they achieved around 90%.

Balady bread dough

Wheat flour (82% extraction), Dry active yeast, Salt (sodium chloride) and sugar were obtained from the local market Cairo, Egypt.

Methods

Date extract preparation:

10g of dried dates were extracted by 100ml ethanol (99%) for 24 hr in dark conditions. Then, the extract was filtered and refrigerated stored in brown bottles until usage.

In Vitro Hepatoprotective activity evaluation of dried date fruit powder against heavy metals

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide or (MTT) assay was done according to a previously published technique to perform the test (Stockert et al., 2012). The MTT assay is used to determine the metabolic state of cellular mitochondrial enzymes in a sensitive manner by reducing tetrazolium salt into blue colored formazan product, which accumulates within the cell. Then the cells degradation is liberating formazan product which can be detected and quantified by simple colorimetric method, which in turn may be represented as a measure of viability and/or number of cells.

According to the experimental treatment, HepG2 cells (1×10^4 cells/well in 96 well plates) were grown at 37°C for 24 hr with 10% fetal calf serum. For evaluating the hepatoprotective effect of dried date fruit powder against heavy metals, cells were treated with the dried date fruit powder (0, 12.5, 25, 50, 100, 200 and 400 µg/mL) and incubated for 24 h,then the toxicants (heavy metals) were added by the following concentration (Hg 20 µg/mL as Mercuric chloride ($HgCl_2$), Cd 50 µg/mL as Cadmium chloride ($CdCl_2$) and Pb100 µg/mL) as Lead acetate ($C_4H_6O_4Pb$)after that they were incubated for another 24 h.,each treatment was repeated 4 times (i.e. 4 wells for each treatment)

Determination of the cell ability to reduce MTT to formazan derivative after exposure to tested compounds shows its hepatoprotective effect. The optical density of formazan formed in the control cells (ODc) was taken as 100%, where the viability of hepatocytes in tested groups (ODt) were represented as a percentage of the control cells. The results were expressed as:

$$\text{Viability \%} = [(ODt/ODc) \times 100\%$$

Where:

ODt: Optical density of wells treated by tested samples.

ODc: Optical density of untreated cells.

Hepatoprotective % = Viability % of treated groups - Viability % of –ve control

Chemical analysis

The moisture content was determined by drying the samples at 70°(C (Domenica UNOX: XF043, Italy). Crude (protein (micro-kjeldahl), crude fat (Soxhlet apparatus) and ash were determined according to AOAC, 2000. While, total carbohydrates were calculated by difference. Total polyphenols of tested samples were determined using Folin-Ciocalteu reagent according to previously published method (Velioglu et al., 1998). A spectrophotometer (Jenway-UV-VIS Spectrophotometer) was used to measure the absorbance at 760 nm. Total polyphenols (mg/100g) were expressed as gallic acid equivalent (Asami et al., 2003).

Phenolic compounds fractionations

High performance liquid chromatography (HPLC) (Agilent-1100 Series) was used to evaluate phenolic components in tested samples, as described by Öztürk et al. (2007). A gradient program was utilized with two solvent systems (A: 0.5 percent acetic acid in 50:50 acetonitrile: water (1:1); B: 2 percent acetic acid in water at a constant solvent flow rate of 1.2 ml/min and injection volume of 20 l on a reversed phase Zorbax Eclipse XDB-C18 column (4.6 x 150 mm, 5 m). The UV-VIS detector caught the signals at 280 nm.

Making balady bread

Balady bread was prepared by mixing 1 kg flour with other ingredients including 1.5% dry active yeast, 1.5% sodium chloride and 700 ml water. The mixture was well mixed for 20 min. The dough was left for fermentation at 30°C and 85% relative humidity for 15 min. After fermentation, the dough was divided into 100 g pieces. Each piece was shaped on a table previously covered with a fine layer of bran and left to ferment 15 min at the same mentioned temperature and relative humidity. The fermented dough pieces were flattened to about 20 cm diameter. The flat loaves were backed at 160-180°C for 3-4 min, in electric oven (Domenica UNOX: XF043, Italy). Bread loaves were allowed to cool at room temperature before sensory evaluation (Yaseen et al., 2007).

Sensory evaluation of balady bread

Balady bread loaves (of all studied samples) were evaluated organoleptically, where, half slice of each bread sample was served for 10 trained panelists on white, odorless and disposable plates,according to El-Farra et al. (1982). The panelists were asked to evaluate Crump texture, taste, odor, crust color, appearance and acceptability, using a score from 1 to10.

Statistical analysis

Results were analyzed by (ANOVA) using SAS statistical package of the general liner model (GLM) as described by Cramer, et al., 1999. The result averages were based on three-replicates ($p \leq 0.05$).

Results and Discussion

Evaluation of in vitro Hepatoprotective activity

To evaluate the hepatoprotective efficacy of ethanolic date fruit powder extract against lead (Pb), mercury (Hg), and cadmium (Cd), the MTT assay is recommended.This technique was commonly used in toxicological research to evaluate and choose established cell lines as sensitive biomarkers for, or indications of, early metal contamination. The cytotoxicity assay's sensitivity varies based on the differences in processes that cause cell death (Goswami et al., 2014). At this study, the cytotoxicity of the three heavy metals against ethanolic date fruit powder extract was determined using the MTT assay.

The cytotoxicity impact was found to be statistically significant ($p < 0.05$) for the concentration range of tested samples employed in this investigation as represented in table 1. The cultivated liver cells (HepG2 cells) were subjected to the following concentrations of heavy metals, Pb(100 µg/mL), Cd (50 µg/mL) and Hg (20 µg/mL) (Podsiki, 2008), which recorded cellular viability (%) 20.91±2.78, 15.18±1.07 and 9.09±1.85, respectively, but none of them recorded any protection (%) for hepatocytes.

TABLE 1. Evaluation of *in vitro* Hepatoprotective activity of date fruit powder extract (µg/ml) against lead (Pb), cadmium (Cd) and mercury (Hg).

	Pb (100 µg/ml)		Cd (50 µg/ml)		Hg (20 µg/ml)	
	Mean viability cells %	Mean hepatoprotective cells %	Mean viability cells %	Mean hepatoprotective cells %	Mean viability cells %	Mean hepatoprotective cells %
0	20.91^{E}±2.78	0	15.18^{E}±1.07	0	9.09^{E}±1.85	0
12.5	21.85^{E}±1.9	0.94^{E}±0.93	15.18^{E}±1.07	0.36^{E}±0.39	9.59^{E}±1.64	0.51^{E}±0.69
25	23.45D^{E}±1.27	2.54^{E}±1.96	16.33^{E}±1.38	1.15^{E}±0.85	10.62^{E}±2.02	1.53^{E}±1.01
50	26.96^{D}±1.71	6.05^{D}±2.82	18.34^{D}±2.17	3.16^{D}±1.77	12.45^{D}±1.94	3.36^{D}±0.89
100	35.49^{C}±1.73	14.58^{C}±2.83	23.98^{C}±2.13	8.79^{C}±1.79	16.95^{C}±2.87	7.86^{C}±1.17
200	43.92^{B}±3.42	23.01^{B}±3.68	30.96^{B}±1.43	15.78^{B}±2.45	21.49^{B}±2.68	12.41^{B}±0.93
400	53.03^{A}±4.53	32.12^{A}±4.19	37.67^{A}±2.84	22.49^{A}±3.88	27.86^{A}±4.21	18.77^{A}±2.62
LSD	3.623	3.303	1.186	2.272	1.859	1.401

Means (±SD) in the same column with different superscripts are significantly different ($p < 0.05$)

The HepG2 cells that had already been exposed to heavy metal samples were treated with different doses (µg/ml) of ethanolic extract of date fruit powder (12.5, 25, 50, 100, 200 and 400). According to Table 1, the most effective tested concentration of ethanolic date fruit powder extract against tested heavy metals (Pb, Cd, and Hg) was 400 µg/mL, which had a hepatoprotective effect (%) 32.12±4.19, 22.49±3.88 and 18.77±2.62, respectively. Also, the same concentration, improved the cellular viability of cultured liver cells (%) by 53.03±4.53, 37.67±2.84 and 27.86±4.21, respectively. Depending on the results shown in Table 1, the relative cell viability in HepG2 cells (following treatment with 400 µg/mL of ethanolic date fruit powder extract) was suggesting that HepG2 cells existed well tolerant to the toxicity of investigated heavy metals. However, relative cell viability increased noticeably as date fruit concentrations increased and reached the maximum value at 400 µg/mL of date powder.This impact is attributable to the date›s antioxidants content, which includes phenols and flavonoids (Idowu et al., 2020).

Chemical composition of dried date fruit powder

Fractionation of phenolic compounds (µg/100 g) of dried date fruit powder

Polyphenols, also known as phenolic compounds, are a broad term that refers to a large number of compounds (over 8000). They have at least one aromatic ring attached to one or more hydroxyl groups and are produced as secondary metabolites in plants.

Figure 1 shows the fractionation of phenolic compounds (µg/100 g.) for 100% Date powder by HPLC analysis, the most abundant are Pyrogallol (16432.75 µg/100 g) and Catechein (9465.92 µg/100 g), then P-OH-benzoic (2391.43 µg/100 g), Chlorogenic (2077.29 µg/100 g),Caffeic (1446.59 µg/100 g), Ellagic (1370.17 µg/100 g), salycillic (1354.87 µg/100 g), Caffeine (1112.33 µg/100 g),however Catechhol (645.23 µg/100 g), Coumarin(440.16 µg/100 g), Vanillic (409.22 µg/100 g), Gallic acid (278.45 µg/100 g), Ferulic (203.38 µg/100 g) and 4-Aminobenzoic (195.68 µg/100 g) are the lowest one.

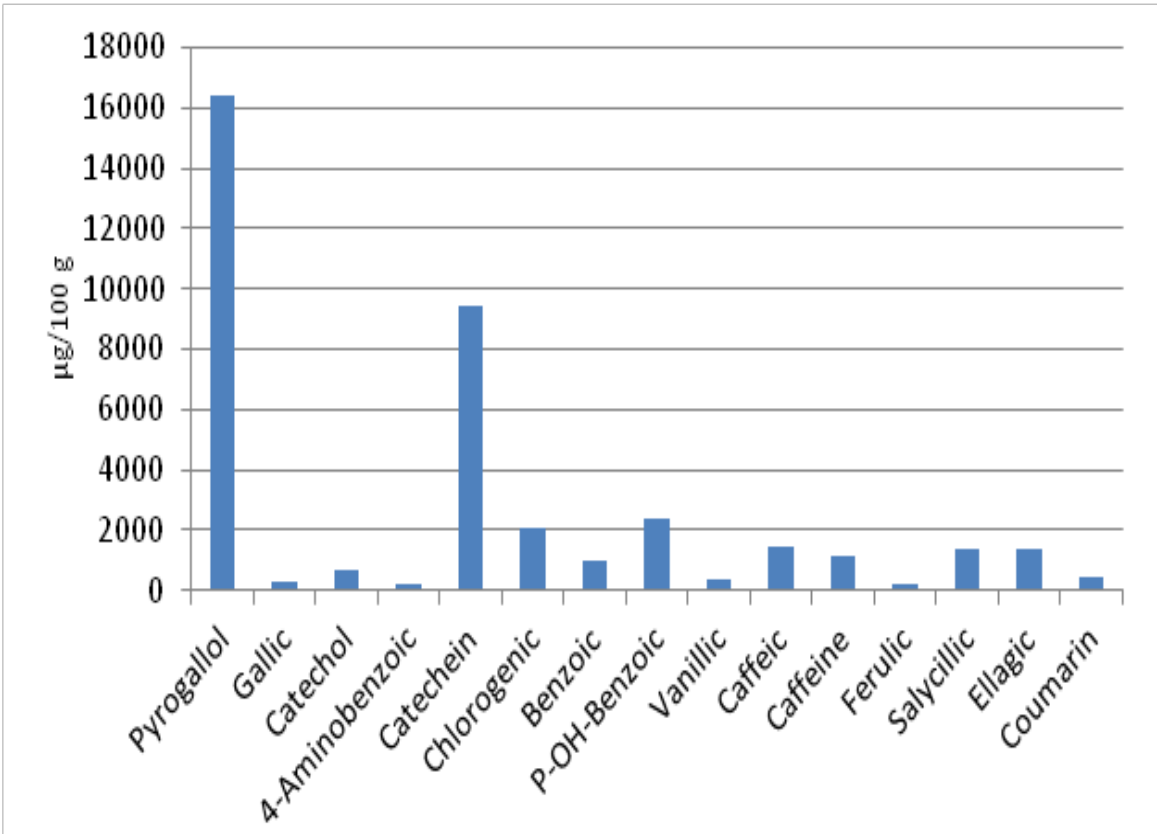

Fig. 1. Phenoliccompunds of date frult poweder.

Although some previous reviews have stated that Pyrogallol is a powerful antioxidant. But beside its beneficial effects, it produces toxicity as a result of its ability to generate free radicals (Upadhyay et al., 2010). In spite of that, a recent study demonstrated the non-toxic properties and high pharmacological similarity of the two bioactive compounds, Gallic acid and Pyrogallol. It is concluded that Gallic acid and Pyrogallol can be used as cell-protective and anti-cancer drugs especially for colorectal cancer (CRC) as they can suppress all CRC-inducing proteins (Mitra et al., 2021). This could be due to polyphenols' dual antioxidant/pro-oxidant effect, or, more specifically, their ability to either scavenge or generate radicals depending on the environment (Touriño et al., 2008), which agree with the obtained data from Table 1 and Fig. 1. Catechins are polyphenol compounds found in many plants that play an important role as powerful anti-oxidants. Catechins have a number of health benefits, including the ability to scavenge free radicals and slow the degradation of extracellular matrix caused by ultraviolet (UV) radiation and pollution (Shi et al., 2016). Gallic acid is a trihydroxybenzoic acid found in plant metabolites. It has strong antioxidant and free radical scavenging properties and can protect cells, tissues, and organs from oxidative stress damage. Also, it influenced a variety of signaling pathways via inflammatory cytokines and enzymic and nonenzymic antioxidants (Ito et al., 2019). 4-Hydroxybenzoic acid exhibits a phenolic derivative of benzoic acid that is antimicrobial, antimutagenic, antiestrogenic, hypoglycemic, anti-inflammatory, anti-platelet aggregating, nematicidal, antiviral, and antioxidant activities. It is also recommended to be used as a preservative in a variety of drugs, cosmetics, pharmaceuticals, foods, and beverages (Oksana et al., 2012). Coumarin (2H-1-benzopyran-2-one) is a natural plant by-product with anti-inflammatory, anticoagulant, antibacterial, antifungal, antiviral, anticancer, antihypertensive, antitubercular, anticonvulsant, antiadipogenic, antihyperglycemic, antioxidant, and neuro protective properties. Finally, the antioxidant activity of dates is attributed to their content of phenolic compounds, such as coumarin, gallic acid, pyrogallol, catechin, 4-hydroxybenzoic acid and other phenolic compounds listed in Fig. 1, and this is confirmed by the results obtained from Table 1 and Fig. 1, which is in agreement with previous studies demonstrated that due to the presence of the phenolic compounds, date palm fruits are potent bioactivities natural antioxidants, which can be used for the management of oxidative stress–related diseases (Ghnimi et al., 2017 and Khan et al., 2016).

Proximate Composition of dried date fruit powder

The moisture content, protein, fat, ash, carbohydrates, and total polyphenols of dried dates (dry basis) are represented in Fig 2. The average of moisture, protein and fat contents of dried dates are 6.8, 3.14 and 1.61%, respectively, however the average of Ash content is 1.3%. Total carbohydrate% was calculated by subtracting the sum of the contents % of moisture, protein, lipid, and ash from 100%. As shown in Fig 2, the average of carbohydrates in dried dates are 87.95%, the carbohydrate content is expected to be high, consisting primarily of sugars and fibers.

The results obtained from Fig. 2 is in agreement with previous studies indicated that dates are a good source of energy because of their high sugar content. Although dates have the lowest protein and fat content of all the dried fruits, they still provide a lot of carbohydrates and energy. As a result, dates play a significant role in the diet and, as a result, their nutritional value is significant (Al-Farsi et al., 2005; Al-Shahib & Marshall, 2002).

Dried date fruit powder application in balady bread manufacture

Referring to data obtained in Table 1, the application was focused on the most effective dose of date fruit powder extract (400 µg/ml) equivalent to (0.5% dried date powder addition), and also lower and higher doses of this concentration, and utilizing it in the manufacture of balady bread samples.

To assess the chemical and sensory properties of bread (100 g weight for each loaf) with the addition of dried date fruit powder as a supplement, four treatments were performed as follows: control 0, 0.25, 0.5, and 1%.

Proximate Composition of the bread samples

Table 2 showed that no significant difference ($p > 0.05$) between treated samples and control sample in moisture, ash, protein, fat and total carbohydrate contents, whereas there was significant difference ($p < 0.05$) in total polyphenols between the treated samples each other and the control as well, so we can notice that, there is an increase of total polyphenol (ppm) by increasing of date powder addition **(%),** the obtained data is in line with those of Sudha et al. (2007); Ismail et al. (2006).

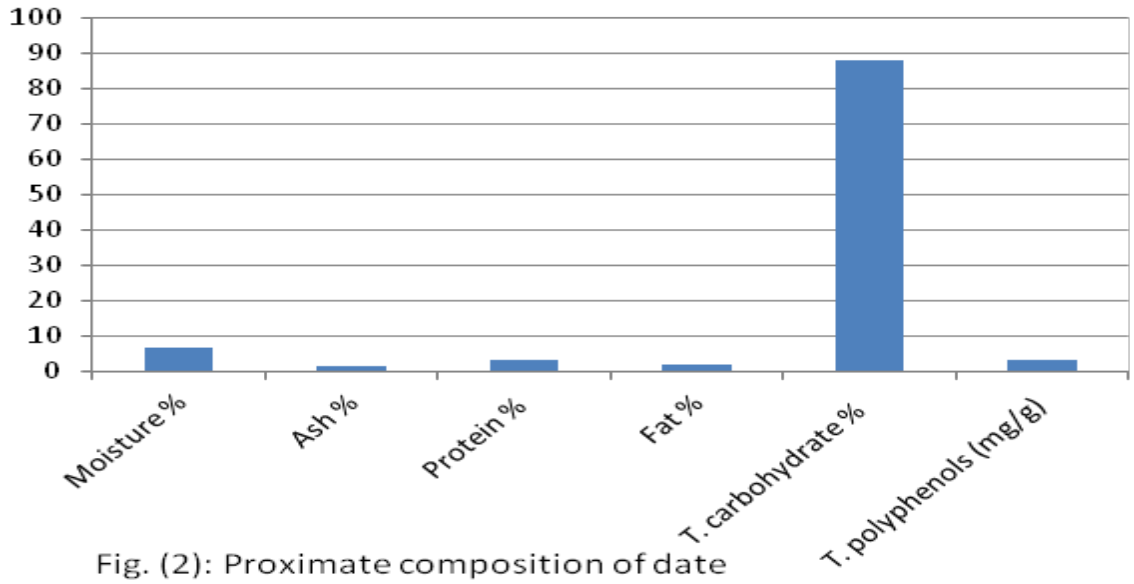

Fig. 2. Proximate compunds of date.

TABLE 2. Chemical composition of studded Balady bread samples produced by adding differentdate powder ratios.

	Control 0% Date powder bread	0.25% Date powder bread	0.5% Date powder bread	1% Date powder bread	LSD
Moisture %	$13.12^{a}\pm 0.22$	$13.24^{a}\pm 0,29$	$13.11^{a}\pm 0.11$	$13.27^{a}\pm 0.25$	0.33
Ash %	$1.38^{a}\pm 0.08$	$1.35^{a}\pm 0.10$	$1.33^{a}\pm 0.05$	$1.30^{a}\pm 0.05$	0.149
Protein %	$11.40^{a}\pm 0.4$	$11.32^{a}\pm 0.22$	$11.25^{a}\pm 0.20$	$11.17^{a}\pm 0.07$	0.307
Fat %	$1.80^{a}\pm 0.20$	$1.76^{a}\pm 0.16$	$1.67^{a}\pm 0,07$	$1.68^{a}\pm 0.13$	0.13
Total carbohydrate %	$72.3^{a}\pm 0.80$	$72.35^{a}\pm 0.35$	$72.64^{a}\pm 0.94$	$72.58^{a}\pm 70$	0.570
Total polyphenols (ppm)	$7.92^{d} + 0.22$	$10.12^{c} + 0.27$	$12.72^{b} + 0.50$	$17.28^{a}+ 0.38$	1.195

Means ($\pm$ SD) in the same row with different superscripts are significantly different ($p < 0.05$).

Fractionation of phenolic compounds (µg/100 g.) of the bread samples

As shown in Table 3, there is a direct relation between the percentage of added date powder and the varied phenolic compounds in bread samples.

Whereas, the Pyrogallol increased from 85.52 (µg/100 g.) in control sample to 131.91, 238.30 and 334.89 (µg/100 g.) in 0.25, 0.50 and 1% treatments, respectively, which means that the phenolic compound increased by increasing dried date powder addition and this applies to the rest phenolic compounds in Table 3.

Sensory Evaluation of Bread Loaves

Data represented in Table 4 showed that all added date powder ratios improved the crust color, appearance and acceptability where the highest positive effect was recorded for 0.5%treatment with corresponding values 8.96, 9 and 8.96, respectively. On the other hand, 0.5 and 1% treatments recorded noticeable positive effect on taste, odor and crump texture, in comparison with control sample.

These findings mean that, when the date's powder ratios are increased, the sensory properties of the product are improved more markedly in comparison to the control. The obtained results are in agreement with Sudha et al. (2007).

TABLE 3. Fractionation of phenolic compounds (µg/100 g) of the bread samples produced by adding different date powder ratios.

	Test of phenolic compounds (µg/100 g.)			
	Control sample	**0.25% Date powder bread**	**0.5% Date powder bread**	**1% Date powder bread**
Pyrogallol	85.5220	131.9179	238.3056	334.8947
Gallic	3.1832	14.5913	17.6976	22.5031
Catechol	3.3459	3.5097	5.6332	17.2577
4-Aminobenzoic	5.5672	8.2171	8.2898	10.6554
Catechein	20.0122	27.2067	44.1544	52.1525
Chlorogenic	26.4394	40.8675	51.1669	68.7669
Benzoic	4.3798	9.0429	10.4698	13.6501
P-OH-Benzoic	8.1739	13.1007	13.4010	24.4022
Vanillic	12.8021	13.5120	14.1625	30.8659
Caffeic	9.8577	20.1030	25.2936	33.5688
Caffeine	27.4944	43.4111	62.6773	67.6945
Ferulic	8.6940	17.1175	26.1906	37.1073
Salycillic	19.4520	29.6947	36.9817	43.7539
Ellagic	49.3161	61.0836	88.2901	123.6545
Coumarin	4.1365	9.0109	9.5870	17.1461

TABLE 4. Sensory evaluation for tested bread samples produced by adding different date powder ratios.

	Control sample	**0.25% Date powder bread**	**0.5% Date powder bread**	**1% Date powder bread**	**LSD**
Taste	$8.90^{c} + 0.015$	$8.50^{c} + 0.012$	$9.20^{a} + 0.020$	$9.00^{b} + 0.015$	0.101
Odor	$8.79^{c} + 0.018$	$8.50^{d} + 0.014$	$8.96^{b} + 0.022$	$9.08^{a} + 0.022$	0.034
Crump texture	$8.71^{b} + 0.018$	$8.67^{c} + 0.018$	$8.88^{a} + 0.020$	$8.88^{a} + 0.028$	0.034
Crust color	$8.13^{c} + 0.036$	$8.92^{a} + 0.03$	$8.96^{a} + 0.032$	$8.63^{b} + 0.021$	0.041
Appearance	$8.30^{c} + 0.11$	$8.60^{b} + 0.12$	$9.00^{a} + 0.13$	$8.50^{b} + 0.12$	0.141
Acceptability	$8.36^{c} + 0.012$	$8.60^{b} + 0.020$	$8.96^{a} + 0.032$	$8.91^{a} + 0.032$	0.044

Means ($\pm$ SD) in the same row with different superscripts are significantly different ($p < 0.05$).

Conclusion

Results clearly showed that, the most effective tested dose of ethanolic date fruit powder extract against tested heavy metals (Pb, Cd, and Hg) was 400 µg/mL. This dose recorded hepato-protective effect 32.12±4.19 % for Lead, 22.49±3.88 % for Cadmium and 18.77±2.62 % for Mercury, respectively. Also, it improved the cellular viability of cultured liver cells to 53.03±4.53, 37.67±2.84 and 27.86±4.21%, for Pb, Cd, and Hg, respectively. In the same time, addition of date powder to Balady bread had no significant effect ($p > 0.05$) on chemical composition of the produced bread with one exception related to polyphenol content which increased significantly ($p < 0.05$). Also, two date powder ratios (0.5 and 1%) had positive effects on all sensory properties. The obtained results suggested that, date powder could be considerable solution in reduction of heavy metal hepato-toxicity and applicable in Balady bread.

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