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Research Article
Determination of Antioxidant and Antifungal Activities in Cookies Fortified with Solar Dried Prickly Pear Peels Powder

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Abstract

Background and Objective: The fortification of bakery products by new materials that attain various goals is considered a challenging that finally gains useful health amelioration. This study was planned to assess the effect of incorporation of solar dried prickly pear peels powder in qaraqeesh (Egyptian cookies) with respect to increase shelf life, sensory palatability and nutritional value. Prickly pear cactus (*Opuntia ficus-indica*) beside distributed in arid and semiarid regions proved to have phytochemical compounds with high antioxidants capacity. **Materials and Methods:** Fungi colonies were isolated from prickly pear peels. Three levels (1, 3 and 5%) of dried peels powder were added to wheat flour along with other ingredients to make cookies samples. Mycological analysis was assessed in yeast with the three concentrations of peels powder as well as the fresh peels and negative control. The total phenolics, flavonoids, tannins, anthocyanins and carotenoids as well as the antioxidant activity were evaluated in fresh and dried cactus peels. **Results:** Findings showed that the prickly pear peels powder (PPPP) antioxidant activity was not much affected by the solar drying conditions. The effect of different extracting solvents at different polarties and pH on the phenolic and flavonoids contents of PPPP was studied. Aflatoxins production by aflatoxignicity *A. flavus* (ATCC 28542) was inhibited by adding different concentrations of PPPP to cookies. Sensory evaluation of fortified cookies was done. All the evaluated characteristics of cookies were given nearly the same values for all levels of dried peels powder. **Conclusion:** Addition of 5% dried cactus peel had lower overall quality and color than the control. Adding 3% of PPPP to cookies (qaraqeesh) showed the highest sensory score. Dried cactus peels may improve quality, nutritional value and shelf life of cookies.

Key words: *Opuntia ficus-indica*, prickly pear peels, aflatoxin contamination, antioxidant activity, phenolic compounds, flavonoids, anthocyanins, carotenoids

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In today's world great attention has been paid to isolate phenolics compounds from natural sources, especially from plant source (phytochemical compounds) because of their antioxidant properties. Antioxidants were reported to prevent the formation of peroxides and subsequently delay the process of the food oxidation or formation of free radicals. These free radicals have deleterious effects due to their ability to oxidize macromolecules, such as carbohydrates, lipids, proteins and DNA causing degenerative diseases[1]. Now-a-days development of new functional fortified breads and flour products consider as a challenging task which can affect metabolism and other health-related conditions[2].

Prickly pear cactus (*Opuntia ficus-indica*) belongs to Cactaceae family which is one of angiosperm families. This family contains several species of cactus which originated from Latin America. These species are distributed to different parts of the world regions including Egypt. Besides being a popular fruit from the pre-Hispanic times in Mexico, it is considered a vigorous plant in arid and semiarid regions and protected against soil erosion[3,4].

Many reports indicate compounds that *Opuntia ficus-indica* (*O. ficus*) contains phenolic compounds which have potential roles in preventing many serious health problems such cancer, arteriosclerosis and hypercholesterolemia[5,6]. Hyperglycemia, hypertension and gastric diseases are reported to be cured by cactus phenolic compounds[7]. Cactus also contain flavonoids as a polyphenolic compounds secondary metabolites. Flavonoids have been assessed as anti-oxidant factor as well as having anti-inflammatory and immunomodulating activities. The "diet hypothesis" suggests that healthy diets (lots of fruits and vegetables) may play a vital role in the increase of antioxidants, since foods contain antioxidants are capable of decreasing or preventing degenerative diseases[8].

Aflatoxins (AFs), produced primarily by the fungus *Aspergillus flavus*, are mycotoxins known of their high carcinogenic effects. Within the group of AFs, Aflatoxin B1 (AFB1) has been reported to cause hepatic cancer in humans. A wide range of organisms could be affected by AFB1 as a factor of dermatitis and teratogenicity[9]. Different agricultural commodities were found to be contaminated with either AFs producing fungi or AFs. Although the presence of *Aspergillus* mould does not essentially mean contaminations by aflatoxin, however, there is always an increased risk[10].

Addition of antioxidants is a common and effective way to prolong the shelf life of many food stuffs. Several plant-derived biocompounds have antioxidant activities and may be more effective and safe than many synthetic ones. Synthetic antioxidants have shown evidences of toxicity in animal models[11]. Both the concentration and absorption of natural antioxidants are important to produce maximum effect. The most common group of polyphenolic compounds is flavonoid, it considers as an antioxidant, antimicrobial, anticancer and anti-inflammatory factor. Flavonoids are distributed widely in all plants and are being investigated for its widespread health benefits. Quercetin (a flavonoid) has the ability to reduce oxidative damage caused by LDL cholesterol through scavenging free radicals and chelating transition metal ions leading to cardiovascular protection[1]. Anthocyanins pigments are represented a major group of flavonoids metabolism which is attributed to coloration of fruits peel. These pigments act as powerful antioxidants protecting the skin from UV radiation and free radical damage[12]. Cell membrane is protected against oxidative damage by carotenoids which derived from a 40-carbon polychain and is considered as the backbone of the molecule[13].

The aims of this study are: (1) Assess the phytochemical constituents and antioxidant activity of dried cactus peels, (2) Evaluate the antifungal activity of cactus peels, (3) Prepared bakery products such as qaraqeesh (traditional Egyptian cookies) fortified with PPPP and (4) Discharge the fruits wastes which always cause detrimental environmental effects.

MATERIALS AND METHODS

Study area: The study was carried out at Food Toxicology and Contaminants Department, Molecular Biology Department, Food Technology Department and Quality Control Laboratory, National Research Centre (NRC) Cairo. From May, 2017-February, 2019.

Chemicals: Folin-Ciocalteu's (FC) reagents, were obtained from Merck Company. Ascorbic acid, 2, 2 diphenyl-1-picryl-hydrazyl (DPPH), 3, 4, 5-trihydroxy benzoic acid (gallic acid), catechin, quercetin, vanillic acid and aflatoxins (B_1, B_2, G_1, G_2) were products of Sigma-Aldrich company. Solvents were Merck Company products. All other chemicals were of the highest purity commercially available.

Aspergillus flavus (ATCC28542) has a capability to produce both aflatoxins B (B_1 and B_2) and G (G1 and G2) obtained from MIRCEN, (Microbial Research Center, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

Prickly pear peels: Cactus peels (*Opuntia ficus indica*) were obtained from 6th October, Giza Governorate, Egypt. The peels were washed thoroughly and divided into 2 groups: The first was kept fresh and the second was dried by solar drier at

temperature range from 60-70°C. Both the fresh and the dried peels were ground in an electric grinder, then fresh peels were kept at -4°C for analysis while the dried one were stored at room temperature.

Determination of moisture content: Moisture content of fresh peels, solar dried ones as well as fortified Qaraqeesh was determined. The procedure was fully described in AOAC[14].

Mycological analysis

Isolation and identification of fresh and solar dried peels as mycoflora surface: Fungi was isolated by direct plating method from the fresh and dried peels was described by Toma and Abdulla[15]. Fungi colonies were identified according to morphological and microscopic characteristics[16].

Aflatoxins production by aflatoxignicity *A. flavus*: *Aspergillus flavus* was assessed in yeast extract sucrose broth medium with 1, 3 and 5% of PPPP as well as the fresh peels and negative control[17].

Aflatoxin determination: Aflatoxins (B_1, B2, G1, G2) were extracted and cleaned up in all materials under investigation using the method described in AOAC[14]. Finally, the AFLs analyzed by High Performance Liquid Chromatography (HPLC).

Preparation of cookies fortified with dried prickly pear peels

Baking procedure: Prepared by straight dough method according to the method of Faridi and Rubenthaler[18] and slight modification by Anwar and Sallam[19]. The formula consisted of 500 g wheat flour (72%), 5 g active dry yeast (*Saccharomyces servisia*), 2 g sugar, 3 g sodium chloride, 60 mL corn oil. The PPPP was added to the flour at concentrations 1, 3 and 5%. Control cookies were also backed.

Sensory evaluation: The evaluation for sensory characteristics was made by 15 panelists from staff of Food Technology Department National Research Centre, Cairo. The scoring scheme was done as mentioned by Noor Aziah and Komathi[20].

Phytochemical compounds determination methods: All determinations methods were performed in triplicate).

Determination of total polyphenol compounds: The total phenolic content was determined according to the Zilic *et al.*[21] procedure. Some modification was done to minimize the volume of the reactants according to Hussein *et al.*[22]. An aliquot of 10 µL of each extract (1 mg mL^{-1}) was mixed with 50 µL of Folin-Ciocalteu phenol reagent (10x dilutions) in an ELISA plate (Sigma-Aldrich Company) and allowed to react for 5 min. Then the mixture was neutralized with 40 µL of 20% saturated Na_2CO_3 solution was added and allowed to stand for 40 min in the dark before the absorbance of the reaction mixture was read at 725 nm using a microplate ELISA reader (BioRad). A calibration curve prepared with gallic acid were done to measure the total phenolic content and expressed as milligrams of gallic acid equivalent (mg GAE) per g of sample dry-weight. Additional dilution was done if the absorbance value measured was over the linear range of the standard curve.

Determination of total flavonoid content: The flavonoid content was determined using 2 procedures based on the formation of aluminium-flavonoid complex in acid or alkali medium according to Pekal and Pyrzynska[23] with minor modifications.

Procedure 1: The $AlCl_3$ solution (0.5 mL, 10% w/v) was added to 1 mL of the prickly pear peel extract. Subsequently, acetic acid (0.5 mL, 1 M) was added and the mixture was vigorously shaken and incubated at room temperature for 15 min. Rutin, quercetin and catechin were used as standard controls (concentration range of 5-65 µg mL^{-1}) and the color developed was measured spectrophotometrically at 405 nm.

Procedure 2: Of the test solution 0.5 mL was mixed with 0.25 mL of $NaNo_2$ (5%, w/v). After 5 min, 0.25 mL $AlCl_3$ solution (10% w/v) was added and the mixture was vigorously shaken. 6 min later, 2 mL of 1 M NaOH was added and the mixture was shaken and left for 15 min at room temperature. The color developed was measured spectrophotometrically at 510 nm. Rutin, quercetin and catechin were used as standard controls in concentration range of 5-65 µg mL^{-1}.

Determination of total anthocyanins: Total anthocyanins content was measured according to the pH differential method[24]. Two dilutions of the peel samples, one with 0.025 M potassium chloride buffer, pH 1.0 and the other with 0.4 M sodium acetate buffer, pH 4.5 were prepared. Absorbance of each dilution at 520 nm and 700 nm were measured using distilled water as a blank. The difference in absorbance values at pH 1.0 and 4.5 was directly proportional to the total anthocyanin concentration, which was expressed as cyanidin-3-glucoside equivalents/100 g weight, using a molar absorption coefficient equal 26,900.

Determination of the antioxidant capacity using the free radical scavenging activity method: The bleaching rates of a stable free radical DPPH (2, 2 diphenyl-1-picryl-hydrazyl) by samples were monitored at 520 nm. A series of samples solutions with varying concentrations were prepared, 10 µL from each sample was added to 90 µL of 0.1 mM methanolic DPPH solution in a 96-well microtiter plate. After incubation in the dark for 30 min at room temperature, the absorbance was measured using an ELISA micro plate reader (Bio-Rad Laboratories Inc.). The percentage of remaining DPPH was calculated as:

$$\text{DPPH scavenging effect (\%)} = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100$$

where, $A_{Control}$ is the absorbance of the DPPH reaction and A_{Sample} is the absorbance in the presence of samples[25].

Determination of total tannin: Proanthocyanidinor the tannin content was determined according to Broadhurst and Jones[26] with few modifications, using catechin as a reference compound. The 400 µL of tested samples was added to 3 mL of vanillin solution (4% in methanol) and 1.5 mL of concentrated HCL acid. After 15 min of the absorbance was read spectrophotometrically at 500 nm.

Extraction of phenolics components by different solvents at different polarties and pHs: Solar dried peels powder (PPPP) was extracted with water and four different organic solvents[6,7] (ethanol, methanol, acetone, propylene glycol). Aqueous organic solvents[22] were done by adding water (1:1). Different pHs were prepared by adding acetic acid (10%) or sodium bicarbonate (0.1 M) to the solvents. Samples were extracted for 2 h at room temperature. Extracts were centrifuged then solutions were stored at -20°C for further use.

Statistical analysis: The ANOVA test carried out for statistical analysis using assistant computer programs[27]. A p value of less than 0.05 was considered to be statistically significant.

RESULTS

Isolation and identification of fresh and dried samples as mycoflora surface: Isolation of mycoflora surface of fresh and dried peels revealed the absence of fungi in both samples as shown in Fig. 1. This means that the samples under investigation are safe to use in food.

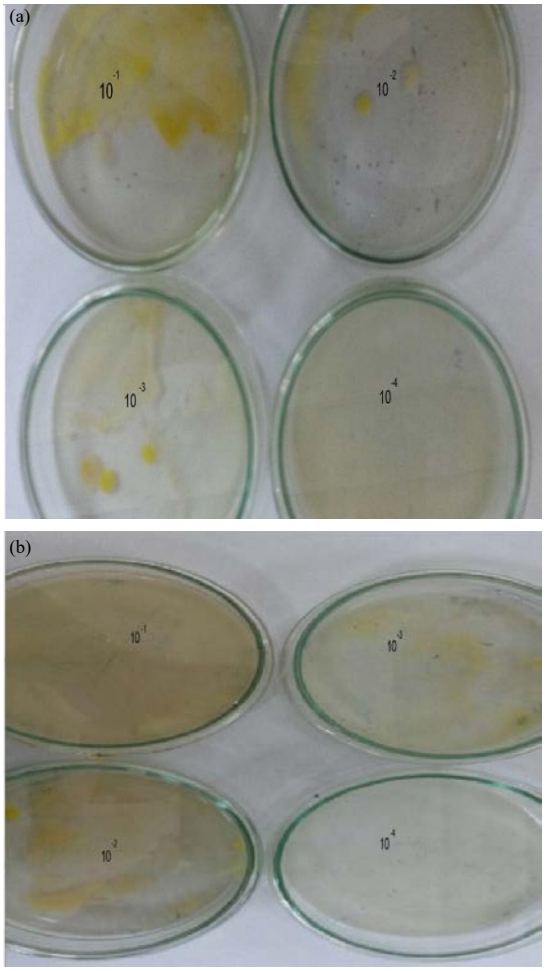

Fig. 1(a-b): No fungi could be isolated from either (a) Fresh and (b) Dried prickly pear peels. Serial Dilution of samples was used (Yellow color due to peel pigments)

Aflatoxin production by aflatoxignicity *A. flavus*: Determination of aflatoxins production ability by *A. flavus* (ATCC28542) was detected in yeast. Extracted sucrose (YES) medium. Aflatoxins were confirmed by extracting the medium then measured by HPLC. Aflatoxins production by aflatoxignicity *A. flavus* (ATCC28542) was decreased by adding either the fresh peels or different concentrations of PPPP as shown in Fig. 2. The growth of the toxic fungi was inhibited by the increased levels of dried PPPP (from left to right).

Moisture and some phytochemicals contents, of fresh and dried samples moisture and total contents of tannins, anthocyanins and carotenoids and DPPH activity were evaluated in absolute methanol extractions of fresh and solar dried samples (Table 1). Tannin content in

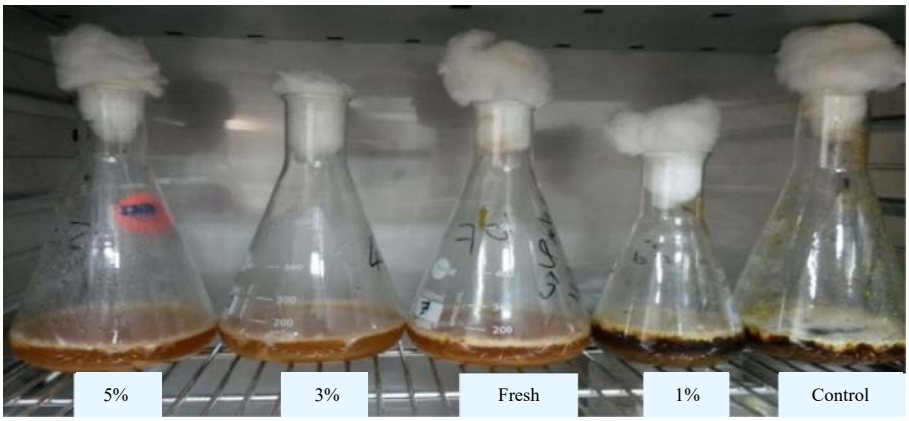

Fig. 2: Aflatoxins production by aflatoxigenicity *A. flavus* (ATCC28542) on fresh peels and different concentrations of PPPP

Table 1: Moisture and some phytochemicals contents, of fresh and dried samples

Cactus peel	Moisture content (%)	Total tannins (mg tannic acid/100 mg)	Total anthocyanin (mg/100 mg)	Total carotenoids (mg/100 mg)	DPPH (gallic acid equivalent/100 mg)
Fresh	90.48	11.70±4.38	1.50±0.40	3.12±0.351	11.5±2.40
Dried	13.25	75.12±5.13	2.16±0.61	8.83±0.695	58.0±8.31

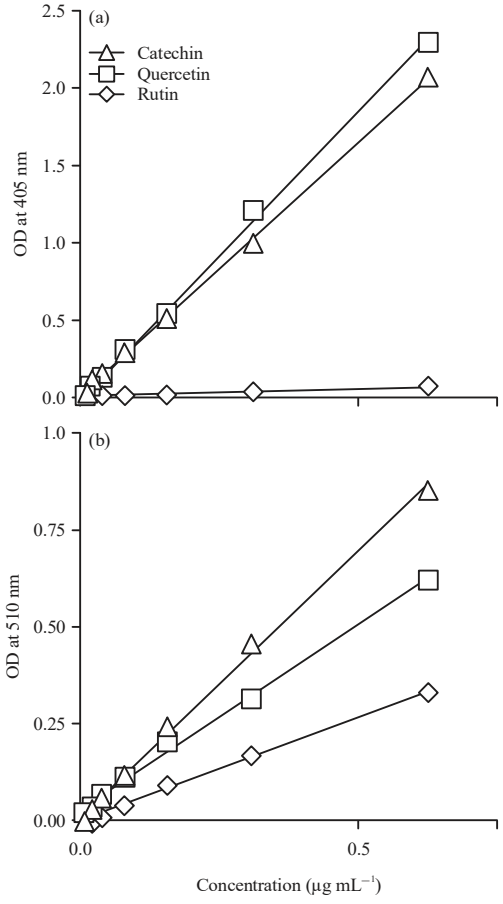

Fig. 3(a-b): Standard curve of 3 different standards (catechin, quercetin and rutin) in two procedures, (a) Acid medium and (b) Alkali medium for determination of flavonoids

solar dried samples dried samples represents 6.4-fold relative to that in fresh samples. This percent coincides with the relative percent between fresh and dried sample humidity (6.8). A slight decrease in DPPH activity of dried samples (58±8.31 gallic acid equivalent) representing 5-fold only of the fresh ones (11.5±2.4) was observed (Table 1). Both anthocyanins and carotenoids were minor constituents in the methanol extract of prickly pear peels and appeared to be much affected during the solar drying process as their contents in the dried sample are only 1.4, for anthocyanins and 2.8, for carotenoids relative to those of the fresh samples.

Polyphenols and flavonoids contents of fresh and dried peels: The phenolic and flavonoid contents of absolute methanolic extract of both fresh and dried samples are shown in Table 2. It is evident that the total phenolics (expressed as mg gallic acid equivalent) are about 6.5 folds higher in the dried (270.3±32.7 mg gallic acid) than in fresh (41.3±9.5 mg gallic acid) samples. The total flavonoids express similar values (6.1, 6.5 folds higher in the dried than fresh sample) using two procedures. Three standards (catechin, rutin and quercetin) were used for estimation of the total flavonoid contents using two procedures at different pHs; procedure 1 in acidic medium and procedure 2 in alkali medium. As shown in Fig. 3, catechin could not be detected under the conditions of procedure 1 even at high concentrations. Therefore, procedure 2, being more selective, was preferred for estimation of the total flavonoid

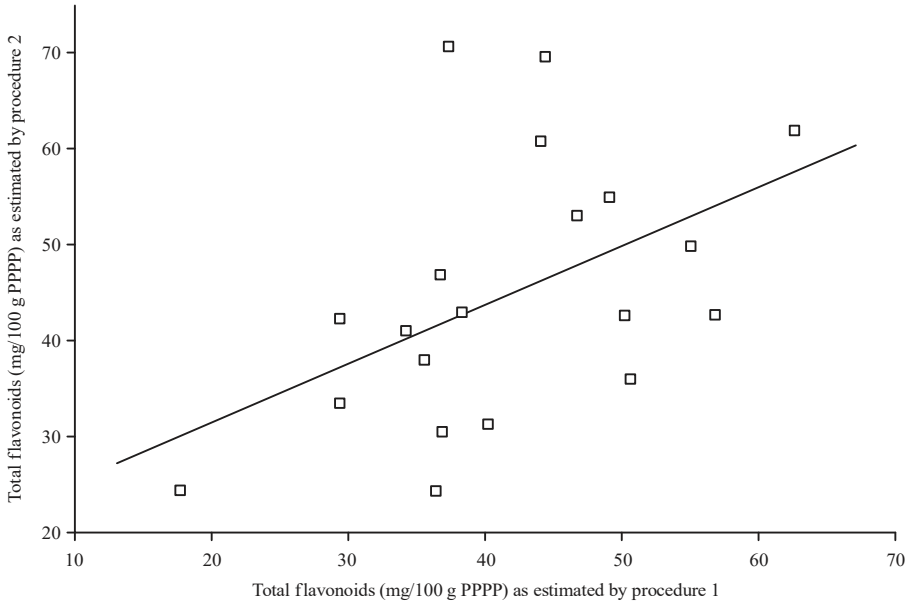

Fig. 4: Correlation between procedures 1 and 2 used to estimate the total flavonoid contents of *Opuntia ficus* peels ($r = 0.479$ at $p<0.05$)

Table 2: Total contents of phenols and flavonoids (estimated in 2 media) in fresh and dried peels

		Total flavonoid (procedure 1) in acidic media			Total flavonoid (procedure 2) in alkali media		
Opuntia ficus peel	Total polyphenol gallic acid	Catechin	Rutin	Quercetin	Catechin	Rutin	Quercetin
Fresh	41.33±9.56	ND	6.38±0.27	7.20±0.23	22.37±2.99	7.55±1.16	8.07±2.04
Dried	270.35±32.7	ND	39.30±10.98	45.56±9.60	112.40±22.95	42.50±8.90	58.07±12.8

ND : Not detected, All data are represented as mg equivalent/100 mg PPPP

contents in further experimentation. When the relationship between procedure 1 and procedure 2 was calculated (Fig. 4), a poor correlation ($r = 0.479$ at $p<0.05$) was observed as expected.

Extractive power of different solvents towards peels' phenolics components: The extractive power ability towards phenolics, flavonoids and anthocyanins of different absolute solvents (water, ethanol, methanol, acetone and propylene glycol) is demonstrated in Fig. 5a. The results showed that flavonoids were most efficiently extracted by methanol while water was the best solvent for phenolics and anthocyanins followed by methanol. On the other hand, the diol propylene glycol (common synthetic food additive) had the least ability for extraction of these compounds. Colored plant pigments, including anthocyanins, seem to dissolve better in pure distilled water as evident from the apparent darker of the water extract (Fig. 5b).

Effect of different pH and polarities on the chosen solvents' extractive power: The levels of phenolic and flavonoid contents extracted by different solvents of different polarities and pHs are shown in Fig. 6. The results showed that when pure water was used as a solvent, adding salt (0.9% NaCl) reduced the percentage of extracted phenolics (~12%) and flavonoids (~18%). Furthermore, rendering the extraction medium weakly acidic or alkaline does not add much to the extractive power of pure water for both phenolics and flavonoids (Fig. 6a).

On the other hand, the contents of phenolics and flavonoids yields were several times more in aqueous organic solvent extracts than in pure solvents; Weakly acidic 50% methanol recorded the highest extract of phenolic contents (483.70±41.18 mg g^{-1} PPPP) for all tested organic solvents (Fig. 6c). In 50% aqueous ethanol solution phenolics and flavonoids extractions increased 3 times than pure ethanol (Fig. 6b). Extraction by 50% acidic acetone was 3 times higher (467.55±17.15), compared with pure acetone (Fig. 6d). Pure propylene glycol (Fig. 6e). was the least solvent for extracting both phenolics (92±20.16 mg g^{-1}) and flavonoids (22±5.5 mg g^{-1}). Whereas, 50% aqueous methanol were the most efficient solvent in extracting flavonoids (76.55±7.57 mg g^{-1} PPPP). It was observed that the alkali solvent extracts always showed darker colors as shown in the accompanying photos (Fig. 6), however, this does not necessarily reflect higher phenolic and/or flavonoid contents.

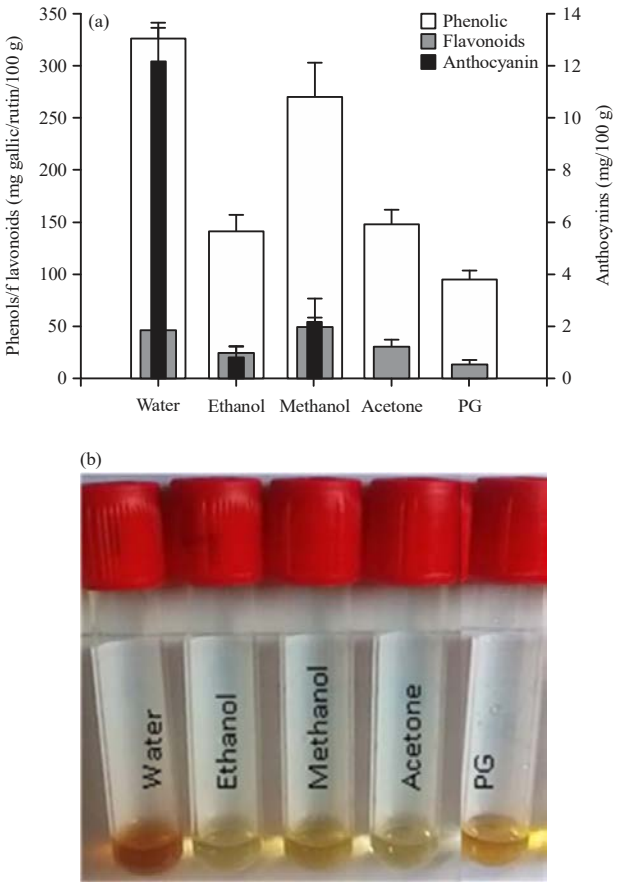

Fig. 5(a-b): (a) Histogram represented the levels of phenolic, flavonoids and anthocyanin contents from solar dried peels extracts by different pure solvents, water, ethanol, methanol, acetone and propylene glycol (PG) and (b) Colors of the different extracts by chosen solvents

Histogram X-axis represented different solvents chosen

Antioxidant activity of backed cookies fortified with PPPP: Backed cookies (qaraqeesh) fortified with (1, 3 and 5%) PPPP and control ones (Fig. 7), were analyzed for their antioxidant activity and weather the final product was affected by the added PPPP after the baking process. The scavenging activity (DPPH) of the 50% aqueous methanolic extract of the different types of cookies (control, 1, 3 and 5%) are shown in Fig. 8. It is obvious that the control cookies have antioxidant activity due to flour 72% and ingredients of the dough other than PPPP. However, this activity is much increased significantly ($p<0.05$) by adding 3 and 5% of PPPP which enhance antioxidant power compared to the original dough. While the observed increase of DPPH activity from 3-5% concentration (Fig. 8) was found to be statistically insignificant).

Sensory evaluation of cookies: The difference between evaluated characteristics of the control cookies (wheat flour 72%) and fortified ones was found to statistically in significant (Table 3). However, cookies (qaraqeesh) containing 5% PPPP had the lowest overall quality and color (Fig. 7). The best taste for all fortified qaraqeesh was with 3% PPPP (Table 3).

Moisture content of cookies after storage: The moisture content in fresh and dried peels recorded 90.48 and 13.25%, respectively (Table 1). The effect of different levels of PPPP and storage duration of cookies (qaraqeesh) on moisture contents are shown in Table 4. From this table it is clear that moisture content was increased by addition of PPPP compared with control. On the other hand, all moisture content values were decreased after a storage period for one year which ranged from 5.26-6.90% for all qaraqeesh samples.

Table 3: Sensory evaluation of cookies

Qaraqeesh	Overall acceptance	Taste	Texture	Colour	Oder
Control	8.18±0.59^{a}	7.90±0.46^{a}	7.40±0.55^{a}	8.50±0.45^{a}	8.40±0.37^{a}
1% PPPP	7.60±0.45^{a}	7.20±0.39^{a}	6.80±0.44^{a}	8.00±0.39^{a}	7.80±0.36^{a}
3% PPPP	7.50±0.69^{ab}	7.40±0.37^{a}	7.40±43^{ab}	7.60±34^{ab}	7.80±42^{a}
5% PPPP	6.05±0.42^{b}	6.80±0.33^{a}	6.60±0.48^{a}	6.80±0.39^{b}	7.20±0.42^{a}

Means with the same letters in the same column are not significantly different ($p<0.05$)

Table 4: Moisture content of cookies after storage period

		Moisture content after storage period		
Qaraqeesh	Level (%)	10 Days	60 Days	One year
WF 72%	0	10.44±1.22	9.44±0.26	6.44±0.82
WF 72% with PPPP	1	16.01±0.06	10.46±0.65	5.26±0.56
	3	19.42±0.43	11.30±0.50	6.90±0.52
	5	21.86±0.11	11.83±0.16	6.72±0.62

WF: Wheat flour, results represent the mean values of three determination±standard deviation

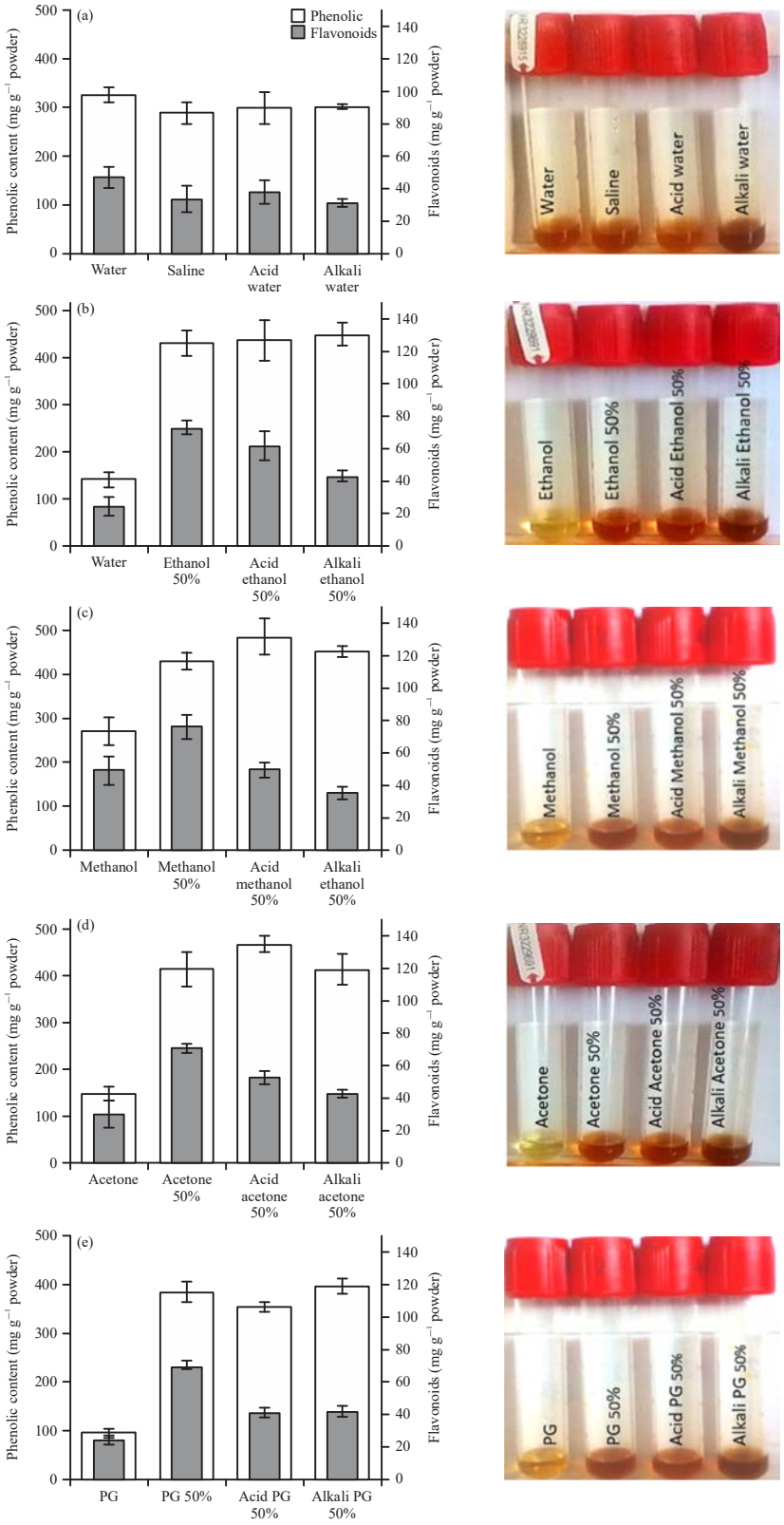

Fig. 6(a-e): (a) Effect of polarity and pH on (a) Water, (b) Ethanol, (c) Methanol, (d) Acetone and (e) Polyethylene glycol as a solvent to extract phenolics and flavonoids from PPPP

X-axis on each histogram represents the solvent different polarities and pHs

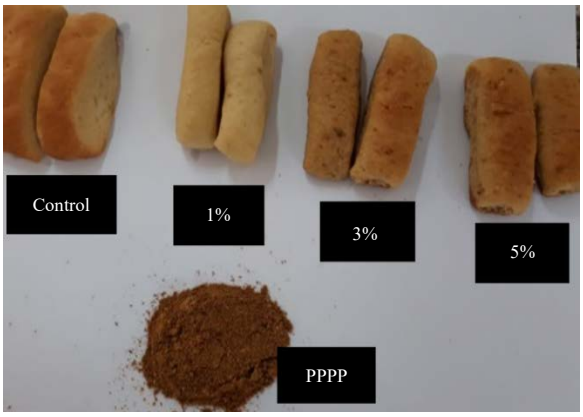

Fig. 7: Cookies (qaraqeesh) after baking control and fortified ones

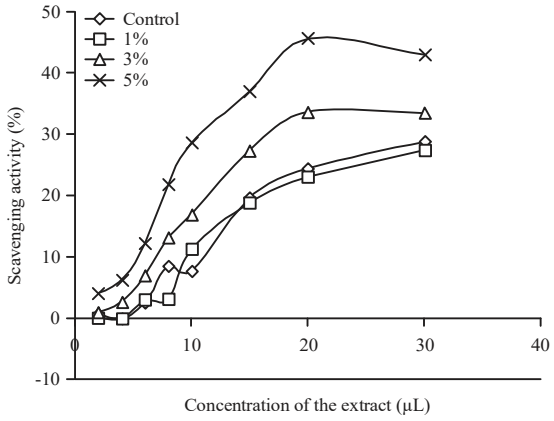

Fig. 8: Antioxidant activity of different types of cookies as measured in the aqueous methanolic extract of cooked cookies

DISCUSSION

This study was undertaken, mainly, to evaluate the nutritional and protective effects of cactus pear peels on cookies. Although cactus peel makes up to ~40% of the fruit weight[19], constituting a major by-product and although it is highly rich in polyphenolic compounds (~200 higher than the fruit bulb)[28]. Cactus peels-in Egypt-often went as a waste product to be finally discarded without being efficiently utilized forming a problem to the environment. In the present study, PPPP was being treated and incorporated in a bakery product "qaraqeesh" to increase the nutritional value and antioxidant properties. Bakery products enriched with natural sources of antioxidants have attracted much attention in the last decades for consumers who care about healthy food[11]. Therefore, industrial utilization of pear peels in producing bakery products will reduce waste, improve quality and extend product's shelf life.

First, isolation of fresh and dried peels as mycoflora surface was done, where no fungi were detected indicating that the peels are safe to use in food. The results showed that the moisture content of fresh peels was 90.48% and reduced to ~13.25 after solar drying. This comes more or less in accordance with Anwar and Sallam[19], who found the moisture content of fresh prickly pear peels at level of 75.8±1.22%. The slight variation in humidity may be due to the difference in thickness of the peel of cactus according to the species. The results also showed that the solar drying conditions did not much affect the antioxidant properties estimated by 2, 2-diphenyl-l-picrylhydrazyl (DPPH) of cactus peels.

Due to its ability to reduce the growth of *A. flavus* (ATCC28542) and subsequently reduce the aflatoxins production as shown in the present study, prickly pear peels could expand shelf life of qaraeesh. It has been reported that cactus peel contains biochemical compounds which have anti-fungi and anti-bacteria effects[29,30]. Among these compounds are phenols and flavonoids[31] which appear and vary in different cactus species. Parts of cactus plant also vary in their biochemical activities where peel has higher antioxidant activity than the pulp and cladodes as reported by Yeddes *et al.*[32].

Anthocyanins are among the most important water soluble groups of plant pigments which significantly reduced oxidative stress[21]. Carotenoids which are lipid-soluble C40 tetraterpenoids have an important role in the protection of lipoproteins and cellular membranes against oxidative damage[33]. Both anthocyanins and carotenoids were found to be minor constituents in the methanol extract of PPPP and affected by drying (Table 1). Anthocyanin lost 80% of its content by drying, representing 0.79% of total polyphenols in PPPP. Whereas, tannins represent about (7.54% of the total polyphenols of the methanol extract of both dried and fresh peels samples.

Flavonoids, sometimes known as vitamin P are one class of secondary plant metabolites[33]. Flavonoids comprise the most abundant group in diet[34,35]. The antioxidant effects encountered in cactus fruits were reported to be due to the major flavonoids[5]. Flavonoids can protect from free radicals by interacting with lipids, proteins and carbohydrates to inhibit their peroxidation[36]. They can also interact with various enzyme systems due to their iron-chelating and iron-stabilizing properties[12]. Inhibition the metabolism of

arachidonic acid is another important effect of flavonoids on enzyme systems which gives flavonoides their anti-inflammatory and anti-thrombogenic properties[37]. Yeddes *et al.*[32] proved that isorhamnetin derivatives flavons were the main polyphenols in *O. ficus indica* peel.

Two widely applied procedures based on Aluminum complex formation are used for determination of flavonoid compounds in peel samples[23]. Results of the present study exhibited that procedure (using $NaNo_2$ in presence of NaOH) gives elevated values in comparison to procedure 1 (using acetic acid). This comes in agreement with Zilic *et al.*[21], who explained that compounds that bear catecholic moieties exhibited considerable absorbance at 510 nm while under the conditions of procedure 1 the absorbance was negligible. Moreover, aluminium complexes formed with catechin had their maximum absorbance at 300 nm[38], thus their high flavonoids contents in some samples could be missed at 400 nm. Therefore, procedure 2 was preferred to apply in further experimentation for total flavonoid estimation because of its broader selectivity, although none of the two methods could detect all flavonoid types. The results was showing a poor correlation ($r = 0.479$ at $p<0.05$) between procedure 1 and procedure 2, indicating that both methods are directed to different flavonoid classes.

One of the most important factors affecting the extraction efficiency of bioactive compounds from plant materials and their consequent health and medicinal benefits is the extraction solvent; type, polarity and pH[39]. Polyphenols are often extracted in higher contents in more polar solvents such as aqueous methanol/ethanol than absolute methanol/ethanol[40]. The current investigation agrees with this finding.

Ghasemzadeh *et al.*[41] declared that the amount of phenolic compounds as well as their antioxidant activities increased by increasing the solvent polarity and recommended methanol as an extractive solvent for routine screening of plant and herb materials. Accordingly, pure methanol (as a common polyphenol solvent) was used in preliminary experimentation for extraction of the major phytochemical components of PPPP.

The effect of solvents polarity and pH on the total extracted phenolic and flavonoid contents of peels was measured (Fig. 6c). The results showed that pure solvents were not the best extractive solvents. Mixing organic solvents with water (1:1) highly increased the levels of phenolic compounds up to 1.5-4 folds. In this investigation, weakly acidic 50% aqueous methanol extract recorded the highest phenolic contents, followed by weakly acidic 50% aqueous acetone (467.55 ± 17.15 mg g^{-1} PPPP). Ngo *et al.*[39] recommended acetone (50% v/v) as solvent of choice for *Salacia chinensis* roots. Flavonoids in this current study were more efficiently extracted by 50% aqueous methanol (76.55 ± 7.57 mg g^{-1}). The addition of water to organic solvents (aqueous solvents) increased the total phenolic content recovery[40], since water allows plant tissues to swell so the solvent penetrates the sample tissues better[42]. It may be worth mentioning that the dark color of aqueous alkali solvent extracts was not accompanied by high phenolic or flavonoid contents as may be expected but might rather be attributed to different plant pigments that change color with varying pHs.

Qaraqeesh enriched with cactus peels is an amazing alternative for traditional cookies, with affordable cost, good taste and high nutritional value.

CONCLUSION

Prickly pear peels (fresh or dried) had high content of antioxidants scavenging activity, beside other phenolic components. Addition of cactus peels to cookies had antifungal effects which could expand their shelf life. Substituting 3% of wheat flour in cookies formulation with PPPP was found to have no significant difference observed as compared to the control sensory evaluation. Further investigations need to be done to identify the unknown phytochemical groups and compounds in prickly pear peel antioxidants and their effects.

SIGNIFICANCE STATEMENT

This study indicated the antifungal effect of cactus peel that can be beneficial for preserving bakery products for long period (1 year). This study will help the researchers uncover the area of antioxidants benefits from fruits peels especially cactus. Thus it may support the trend of fortifications food products by natural antioxidants.

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