Production of functional pita bread using date seed powder

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Abstract Functional foods represent a novel approach to prevent diet-related diseases. Due to its excellent nutritional and antioxidant properties, date seed was used to develop functional pita bread. Flour was replaced by 5, 10, 15 and 20 % date seed powder. Regular and whole wheat pita breads were the references. Results clearly showed that date seed powder containing bread contained comparable dietary fibers levels as in whole wheat bread and higher levels of flavonoids and phenolics. Date seed powder containing breads were particularly rich in flavan-3-ols whereas reference breads did not contain any of them and only a limited amount of other phenolic compounds. They also exhibited a much higher antioxidant capacity. Additionally, compared to regular bread, acrylamide level was significantly lower in 5 % date seed powder containing bread, and lower in all date seed powder containing breads compared to whole wheat bread. Date seed powder supplemented bread appears as a promising functional ingredient to prevent chronic diseases.

Highlights - Date seed bread has similar fiber level as whole wheat pita bread

- Date seed bread has higher antioxidant capacity than regular and whole wheat breads
- Great amount of flavan-3-ols in date seed bread but not in regular or whole wheat breads
- Date seed bread has reduced acrylamide than whole wheat pita bread
- Date seed bread is shown as a promising functional food

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Introduction

In the context of the alarming worldwide public health problems, including increasing prevalence of nutrition-related diseases (Malik and Razig 2008), functional ingredients/foods are emerging as a new mode of thinking about the relationship between food and health in daily life. Hence, the attempts to develop functional foods with elevated nutritional quality, capable of preventing nutrition-related diseases, are becoming a major focus in the human nutrition area (Magrone et al. 2013).

Fibers, whose beneficial effects on intestinal health, cholesterol and glucose metabolism have already been demonstrated (Anderson et al. 2009), are considered as a promising functional food ingredient (Pelucchi et al. 2004; Trigueros et al. 2013). The consumption of fiber-rich food products has already been shown to help in preventing many of the nutrition-related pathologies, including obesity and type 2 diabetes (Fardet 2010; Beliveau and Gingras 2007).

Moreover, the level of antioxidants is another rising nutritional property targeted in the development of new functional food products (Pelucchi et al. 2004). Oxidative stress, which is defined as an imbalance between the systemic manifestation of reactive oxygen species/reactive nitrogen species and a biological system's ability to readily neutralize these compounds using antioxidant systems, leads to cell damage and cellular mechanisms disruptions. This has been associated with the development of nutrition-related chronic diseases. In addition, more recently, oxidative processes have been postulated to be involved in the formation of carcinogenic compounds in food, during food processing like baking (Hedegaard et al. 2008; Ou et al. 2010). One of these undesirable compounds is acrylamide (AA). AA has been classified

as “probably carcinogenic to humans” by the International Agency for Research on Cancer, evoking an international health alarm. Human exposure to AA should be controlled as low as possible with regard to its inherently neurotoxicity, genotoxicity, carcinogenicity and reproductive toxicity properties (Arwa 2008; Morales et al. 2010; Stadler and Scholz 2004; Zhang et al. 2009). AA is widely found in carbohydrate-rich foods like bread and potato chips (JECFA 2002). The currently observed daily intakes were estimated at 0.3–2.0 $\mu\text{g/kg}$ of body weight/day with a mean of 1 $\mu\text{g/kg}$ of body weight/day by FAO/WHO in 2007 (El Ziney et al. 2009). Interestingly, a daily intake of 0.86 $\mu\text{g/kg}$ of body weight/day has been recently reported in Saudi Arabia, with pita bread being the major contributor (El Ziney et al. 2009). Hence, an increase of the antioxidant level in food matrix might be a way to first increase the antioxidant intake of the population and then to decrease the exposure to AA by limiting the production of AA during food processing.

For this purpose, date seeds represent a strong candidate. Date seeds are produced in huge amounts in the Middle East as dates byproducts but are most often wasted. It has recently been shown that they possess excellent nutritional qualities and represent a good source of bioactive components (Habib and Ibrahim 2008; Habib et al. 2014). The determination of the macro- and micro-nutrient profiles of eighteen varieties of date seeds from date fruits cultivated in the UAE showed that date seeds contain high amounts of fiber (67.6–74.2 g/100 g) and considerable amounts of some minerals, vitamins, lipids and protein (Habib and Ibrahim 2008; Habib et al. 2013). Additionally, date seeds were shown to be rich in antioxidants, for which antihyperlipidemic, anticancer and antimutagenic properties were identified. The determination of their polyphenolic profile by UPLC-DAD-ESI-MS, (Habib et al. 2014) revealed a total amount of polyphenols of 50.2 mg/g, with the primary compounds being epicatechin and catechin, whose antioxidant properties are well established (Fraga and Oteiza 2011). Date seeds have also been shown to exert *in vitro* and *in vivo* antioxidant effects (Habib and Ibrahim 2011). By comparison to whole wheat, published values indicate a much higher phenolic content in date seeds (Adom and Liu 2002; Adom et al. 2003; Mattila et al. 2005). Phenolic contents of 452.7 mg/100 g, 134.2 mg/100 g and 11.1 mg/100 g were determined in whole wheat bran, whole wheat flour and white whole wheat bread, respectively (Mattila et al. 2005).

Since pita bread is the major staple food in the UAE and the region and identified as the major contributor to daily AA intake in Saudi Arabia, the addition of date seed powder to the recipe of pita bread might enable the development of a new functional bread, containing relatively higher amounts of fiber and antioxidants as well as lower amounts of acrylamide in comparison to the regular pita bread and whole wheat pita bread.

Materials and methods

Date palm seeds of the Khalas variety were used in this study. Five kg samples were collected randomly from tamr (fully ripe dates) batches at the end of the season, with no preference to size, color, appearance or firmness. The seeds were first soaked in water, washed to get rid of any adhering date flesh, and then air-dried and ground into coarse powder using a hammer mill. The powder was further ground into fine material in a heavy duty grinder (IKA M 20 Universal Mill; IKA werke GmbH Co. KG, Staufen, Germany) to pass through a 0.5 mm screen using an Udy cyclone mill. The fine powder was then separated into two fractions using sieves of 0.5 mm and 0.25 mm openings. The powder from the 0.25 mm fraction was stored at $-80\text{ }^{\circ}\text{C}$ for bread preparation.

Bread recipe and baking

The dough contained flour (100 parts), sugar (2.4 parts), salt (1 part), yeast (0.8 parts) and distilled/deionized water (50 parts). The ingredients were mixed in a dough mixer at low speed to obtain smooth continuous dough. Dough was fermented at $40\text{ }^{\circ}\text{C}$ for 25 min. The dough was then divided into small balls ($75\pm 3\text{ g}$) and proofed at $40\text{ }^{\circ}\text{C}$ for 25 min. The balls were flattened into sheets 1.7 mm thick, proofed at $40\text{ }^{\circ}\text{C}$ for 15 min and baked at $500\text{ }^{\circ}\text{C}$ for 1 min. The bread loaves were cooled to room temperature, placed in polyethylene bags and stored at $-20\text{ }^{\circ}\text{C}$ until used. Date seed powder replaced a part of the flour. Different replacement levels were considered, according to the amount of fibers found in whole wheat bread (around 7.4 %, according to the USDA National Nutrient Database for Standard Reference, 2012) and to not exceed the current recommendations of 25 g/day for adult woman and 38 g/day for adult man: 5, 10, 15 and 20 % date seed powder. The regular pita bread and the whole wheat pita bread were used as controls.

Bread crushing

Each sample of bread was first crushed in a mixer (20 min) till they formed fine bread crumbs and was transferred into new packs with labels.

Extraction

A weight of 10 g, weighed out on a balance and put in labeled tubes was used to perform the extraction of each bread sample. After weighing, 40 ml of water and methanol was added. The ratio of water:methanol extract solution was 1:1. The samples

were then left in a shaker for 1 h and centrifuged at 5000 rpm for 20 min after that. The solutions were filtered using filter funnel, filter paper and conical flask. The extract solutions were stored at -80 °C for further analysis.

Nutrient composition

Macronutrient contents (protein, fat, fibers and moisture) were assessed in the different samples of date seed powder bread and compared to the contents in the regular pita bread and whole wheat flour pita bread. All assessments were done in triplicate except fibers, which were measured in duplicate. Methodology has been described in details somewhere else (Habib and Ibrahim 2008).

The Kjeldahl method was used to determine the protein content. A weighted sample of each bread was digested/hydrolyzed using concentrated sulphuric acid in presence of copper. Nitrogen in amino acids was converted to ammonium ions and then converted to ammonia gas. The gas was heated, distilled and trapped in a solution in the form of ammonium ions again. The amount of ammonia trapped was determined by titrating with an acid.

To measure fat content, 10 g from each sample of bread was collected. The samples were measured out in thimbles. Meanwhile the cups to be used for the soxhlet apparatus were dried at 106 °C for 1 h and weighed. Once the drying was completed, the samples in the thimbles and the cups were loaded into the soxhlet apparatus, which ran for 1.5 h for fat extraction. Then the cups were left again for dry for 20 min and put in the desiccator to cool and be reweighed. The residue was dried at 103 °C and the fat content determined gravimetrically.

Neutral detergent fiber (NDF), was analyzed by using the Ankom220 fiber analyzer (Ankom®, Tech. Co., Fairport, NY, USA).

Moisture was determined according to the Association of Official Analytical Chemists (method 934.01) (AOAC 2003).

Total phenolics

Total phenolics were evaluated in the methanol:water bread extract using the spectrophotometric analysis with Folin–Ciocalteu's phenol reagent (Singleton and Rossi 1965). The standard curve for total phenolics was made using gallic acid standard solution (0–100 mg/L) and total phenolics were expressed as mg of gallic acid equivalent (GAE)/100 g of bread.

Total flavonoids

An aliquot (250 µl) of each bread extract or standard solution was mixed with 1.25 ml of H₂O and 75 µl of 5 % NaNO₂ solution. After 6 min, 150 µl of 10 % AlCl₃ solution was added. After 5 min, 0.5 ml of 1 M NaOH solution was added and then the total volume was made up to 2.5 ml with H₂O. The absorbance against blank was read at 510 nm. The results were expressed in terms of mg rutin equivalent (RE)/100 g bread (Kim et al. 2003).

Antioxidant capacity

FRAP depends on the reduction of ferric tripyridyltriazine (Fe (III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe (II)-TPTZ) by a reductant (antioxidants or other reducing agents) at low pH. Fe (II)-TPTZ has an intensive blue color and can be monitored at 593 nm. The FRAP reagent included 10 mM TPTZ solution in 40 mM HCl, 20 mM FeCl₃ solution and 0.3 M acetate buffer (pH 3.6) in proportions of 1:1:10 (v/v/v). 50 µL of diluted methanol : water extracts were mixed with 3 mL of freshly prepared FRAP reagent and the reaction mixtures incubated at 37 °C for 30 min. Absorbance was determined at 593 nm against distilled water blank. Aqueous solutions of ferrous sulfate (100–2000 µM) were used for calibration. Triplicate measurements were taken and the FRAP values were expressed as µmol of ferrous equivalent (FE)/100 g of date seed.

The antioxidant activities of the bread samples were also studied through the evaluation of the free radical-scavenging effect on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. DPPH is a stable free radical at room temperature, which produces a violet solution in ethanol. Reduction of DPPH by antioxidants results in a loss of absorbance. The degree of discoloration of the solution indicates the scavenging efficiency of the added substances. The use of DPPH provided an easy and rapid way to evaluate antioxidant activity.

An aliquot (10 µl) of extracted samples was mixed with 90 µl of distilled water and 3.9 ml of 0.25 mM DPPH in methanol. The mixture was thoroughly vortex-mixed and kept in the dark for 30 min. The absorbance was measured later, at 515 nm, against a blank of methanol without DPPH. Results were expressed as percentage of inhibition of the DPPH radical. Percentage of inhibition of the DPPH radical was calculated according to the following equation:

$$\% \text{inhibition of DPPH} = [(\text{Abs control} - \text{Abs sample}) / \text{Abs control}]$$

$$\times 100 \text{ where Abs control is the absorbance of DPPH solution without extracts.}$$

Nitric oxide radical inhibition was estimated by the use of Griess reaction. The reaction mixture 3 ml containing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline, extracted samples were incubated at 25 °C for 150 min. After the incubation, 0.5 ml of the reaction mixture mixed with 1 ml of the sulfanilic acid reagent (0.33 % in 20 % glacial acetic acid) and allowed to stand for 5 min for completion of the reaction process of diazotization. Further, 1 ml of the naphthyl ethylene diamine dihydrochloride was added, mixed and was allowed to stand for 30 min at 25 °C. The concentration of nitrite was assayed at 540 nm. Percentage of inhibition of the Nitric oxide was calculated according to the following equation:

% inhibition of Nitric Oxide

$$= \frac{\text{Abs control} - \text{Abs sample} \times 100}{\text{Abs control}}$$

Where Abs control is the absorbance of the solution without the tested sample.

The stock solutions included 7 mM ABTS solution and 2.4 mM potassium persulfate solution. The working solutions were then prepared by mixing the two stock solutions in equal quantities and left for 14 h at room temperature in the dark. The solution was then diluted by mixing 1 ml ABTS solution with 60 ml methanol to obtain an absorbance of 0.706 ± 0.02 units at 734 nm using a spectrophotometer. Fresh ABTS was prepared for each assay. Sample extracts (1 ml) were allowed to react with 1 ml of the ABTS solution and the absorbance was taken at 734 nm after 7 min using a spectrophotometer. The percentage inhibition calculated as ABTS radical scavenging activity: (%) = $[(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}) / (\text{Abs}_{\text{control}})] \times 100$ where $\text{Abs}_{\text{control}}$ is the absorbance of ABTS radical in methanol; $\text{Abs}_{\text{sample}}$ is the absorbance of ABTS radical solution mixed with sample extracts.

Polyphenols composition of breads

Extraction procedure has already been detailed for the analysis of monomers, dimers, hydroxycinnamic acids, flavanols and flavones in date seeds powder (Habib et al. 2014). Preliminary extraction was done using 150, 300 and 500 mg of solid material. Determination of proanthocyanidin composition was performed directly on the powder of dried bread under the same condition as those described by Habib et al. 2014. Preliminary extraction was performed using 15, 30 and 50 mg of solid material.

Acrylamide

The procedure of sample preparation involved an automated extraction method using accelerated solvent extraction unit ASE® 350 (DIONEX, CA, USA). Samples (5 g) were extracted for 20 min using 10 mM formic acid in 34 ml cells of the

ASE 350. The conditions for the ASE extraction unit were set at 70 °C, 1500 psi, with heat up time of 5 min and static time of 4 min, followed by 3 static cycles, flush volume 60 %, and a purge time (N_2) of 120 s (DIONEX, CA, USA, application note 409). Quantification of acrylamide in the above extracted bread samples was performed on a UPLC-MS/MS with the electrospray positive ionization (ESI+). In detail, an ACQUITY UPLC quaternary pump system equipped with the micro vacuum degasser, thermostated autosampler and thermostated column compartment (Waters, Milford, MA, USA) was coupled with a Micromass Quattro Ultima triple-quadrupole mass spectrometer from Micromass Company Inc. (Manchester, UK). The analyte elution (injection volume 10 μl) was carried out on a UPLC BEH C_{18} column (50 mm length, 2.1 mm i.d., 1.7 μm particle size) (Waters, Milford, MA, USA) maintained at 25 °C with a run time of 3 min. The mobile phase was 10 % methanol/0.1 % formic acid in water with a flow rate of 0.2 ml/min. The conditions used for electrospray source were as follows: capillary voltage, 3.5Kv; cone voltage, 50 V; source temperature, 100 °C; desolvation gas temperature, 350 °C; desolvation gas flow, 400 L/h nitrogen; cone gas flow, 45 L/h nitrogen; argon collision gas pressure to 3×10^{-3} mbar for MS/MS, which gave a highest acrylamide response in this study. The collision energy (CE) was optimized for each multiple reaction monitored (MRM) transition.

Statistical analysis

Statistical analysis was performed using SPSS for windows (version 19; SPSS Inc., Chicago, Illinois, USA). All analytical determinations were performed either in triplicate or in duplicate. Values were expressed as the mean $\pm$ standard deviation. Multiple means comparisons were performed by using the nonparametric Tukey test. Statistical significance was set at $p < 0.05$.

Results

Table 1 shows the moisture, protein, fat and fiber contents of the different bread samples. The moisture content ranged from 27.40 to 34.21 %, with the whole wheat pita bread having the lowest value (27.40 ± 1.61) and the 5 % date seed powder pita bread having the highest one (34.21 ± 1.82). However, no statistically significant differences were detected among the samples. Similarly, the protein content did not significantly differ among the samples, but ranged from 12.36 % in 20 % date seed powder bread to 14.33 % in regular pita bread. In terms of fat content, it was quite similar in regular and whole wheat pita breads, and increased in a linear manner in date seed powder bread from 5 to 20 % of date seed powder, reaching

Table 1 Moisture, protein, fat and fibers content of regular pita bread, whole wheat pita bread and date seed powder containing pita breads (5, 10, 15 and 20 %). Means $\pm$ s.d. are presented

	Moisture (%)	Protein (%)	Fat (%)	Fibers (%)
Regular Pita	32.51 $\pm$ 2.54 ^a	14.33 $\pm$ 0.11 ^a	0.08 $\pm$ 0.10 ^a	1.01 $\pm$ 0.10 ^a
Whole wheat pita	27.40 $\pm$ 1.61 ^a	13.64 $\pm$ 2.42 ^a	0.09 $\pm$ 0.12 ^a	6.18 $\pm$ 0.30 ^b
5 % date seed powder pita	34.21 $\pm$ 1.82 ^a	13.26 $\pm$ 0.58 ^a	0.14 $\pm$ 0.18 ^{a,b}	2.44 $\pm$ 0.07 ^c
10 % date seed powder pita	32.92 $\pm$ 1.68 ^a	13.36 $\pm$ 0.03 ^a	0.34 $\pm$ 0.27 ^{a,b}	5.26 $\pm$ 0.05 ^d
15 % date seed powder pita	30.95 $\pm$ 2.49 ^a	12.87 $\pm$ 0.13 ^a	0.40 $\pm$ 0.32 ^{a,b}	8.11 $\pm$ 0.18 ^e
20 % date seed powder pita	31.24 $\pm$ 0.57 ^a	12.36 $\pm$ 0.19 ^a	0.64 $\pm$ 0.52 ^b	8.94 $\pm$ 0.0 ^f

Comparisons were done between bread samples by using non-parametric Tukey test. Different letters in a column denote significant difference ($p<0.05$)

a maximum of 0.64 %. Not surprisingly, fiber content of whole wheat pita bread was higher compared to regular pita bread. It remained lower in 5 % date seed powder bread, was quite similar in 10 % date seed powder bread, and increased to 8.94 % in 20 % date seed powder bread compared to whole wheat pita bread.

As shown in Fig. 1, the total phenolics content of date seed powder containing bread 5 % and date seed powder containing bread 10 % were similar to regular pita bread and whole wheat pita bread, respectively. From a date seed content of 15 %, total phenolics reach higher levels compared to any other type of bread. By contrast, all date seed powder pita breads contained a higher total flavonoids content compared to regular pita bread (Fig. 2). Besides, a significantly higher flavonoids content was observed in date seed powder containing breads 10, 15, and 20 % compared to whole wheat pita bread, with date seed powder containing bread 20 % containing about 3 times more flavonoids than whole wheat pita bread.

All parameters of antioxidant capacity were higher in all date seed powder pita breads compared to regular pita bread. Compared to whole wheat pita bread, FRAP was higher in 10,

15 and 20 % date seed powder breads, and DPPH, NO and ABTS were higher in all date seed powder breads (Fig. 3).

Table 2 shows that date seed powder bread contains a significant amount of total phenolic compounds, mainly flavan-3-ols (1446.36 $\pm$ 135.08, 3995.43 $\pm$ 203.02, 5521.96 $\pm$ 418.03, 6643.28 $\pm$ 542.33 μ g/g dried date seed powder containing bread 5, 10, 15 and 20 %, respectively) whereas both regular bread and whole wheat flour bread did not contain any flavan-3-ols and only a limited amount of other phenolic compounds.

Compared to regular bread, acrylamide level was lower in date seed powder containing bread 5 % and was at a similar level in date seed powder containing bread 20 %. However, compared to whole wheat bread, acrylamide levels were lowered in all date seed powder containing breads (Fig. 4).

Discussion

A new type of bread was developed based on the addition of date seed powder to wheat flour. The date seed powder bread, which was developed, reached similar levels of dietary fibers compared to the whole wheat version, but with a much less

Fig. 1 Total phenolics (mg GAE/100 g bread) in regular pita bread, whole wheat pita bread and date seed powder containing pita breads (5, 10, 15 and 20 %). Means (s.d.) are presented. Comparisons were done between bread samples by using non-parametric Tukey test, Different letters denote significant difference ($p<0.05$)

Fig. 2 Total flavonoids (mg RE/100 g bread) in regular pita bread, whole wheat pita bread and date seed powder containing pita breads (5, 10, 15 and 20 %). Means (s.d.) are presented. Comparisons were done between bread samples by using non-parametric Tukey test. Different letters denote significant difference ($p<0.05$)

Fig. 3 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, Ferric-reducing antioxidant power assay (FRAP), Nitric oxide (NO) and ABTS in regular pita bread, whole wheat pita bread and date seed powder containing pita

breads (5, 10, 15 and 20 %). Means (s.d.) are presented. Comparisons were done between bread samples by using non-parametric Tukey test. Different letters denote significant difference ($p < 0.05$)

undesirable generation of acrylamide and much higher antioxidant capacity.

Whole wheat bread is largely recognized for better health benefits compared to regular white bread, in part due to its higher fiber content (Anderson et al. 2009). The newly developed date seed powder bread presented comparable amounts of dietary fiber, indicating that similar health benefits on cancer and cardiovascular diseases may be expected from date seed powder containing bread consumption. Considering a daily consumption of 5 loaves of bread of 60 g each, which is in accordance with previous estimations of bread consumption in Middle Eastern countries (Nikkila 1976), entire daily dietary fibers requirements could be met by consuming bread containing 10 % of date seed powder.

Overall, considering the proximate analysis of all bread samples, it appears that the nutritional properties of bread were either improved, as discussed above with dietary fiber content, or at least maintained. Protein content was similar in all breads considered in this work. Regarding fat content, this latter was increased by the addition of date seed powder in the bread. Nevertheless, this might be beneficial, according to the type of fatty acids involved. Indeed, about 50 % of the fatty acids in

date seed oil are monounsaturated and about 10 % are polyunsaturated (Habib et al. 2013). Since a relatively high antioxidant capacity of date seed is now well demonstrated, less oxidation of unsaturated fatty acids in the date seed powder bread could be expected, resulting in greater protection of unsaturated fatty acids for which beneficial health effects have been demonstrated (Abeywardena and Patten 2011; Gillingham et al. 2011). Besides, considering again a daily consumption of 5 loaves of Pita bread, i.e. about 300 g of bread, the total amount of fat provided would reach a maximum of 2 g, which is much less than the daily recommendations even for saturated fat only (Lloyd-Jones et al. 2010).

One important finding of the present study is the significant limitation in acrylamide production in date seed powder bread during the baking process. Whatever the date seed powder amount added to the bread, the level of acrylamide was significantly lower compared to the whole wheat bread, with a reduction of at least 50 %, reaching 80 % with 5 % date seed powder bread. This would significantly contribute to keeping the total daily intake of acrylamide within the range of 0.3–0.8 $\mu\text{g/kg}$ body weight, which has been recommended by the FAO/OMS. Indeed, considering a consumption of 5 loaves of

Table 2 Concentration ($\mu\text{g/g}$ pita bread) of major flavonoids and phenolic compounds in regular pita bread, whole wheat pita bread and date seed powder containing pita breads (5, 10, 15 and 20 %). Means in triplicate $\pm$ s.d.. ND, Not Detected

	Regular Pita bread	Whole wheat pita bread	5 % date seed powder pita	10 % date seed powder pita	15 % date seed powder pita	20 % date seed powder pita
Catechin	ND	ND	10.78 $\pm$ 1.14	22.78 $\pm$ 1.90	35.58 $\pm$ 5.12	46.48 $\pm$ 4.66
Epicatechin	ND	ND	7.03 $\pm$ 1.29	14.78 $\pm$ 1.59	26.61 $\pm$ 1.63	35.96 $\pm$ 2.11
Dimer B1	ND	ND	35.69 $\pm$ 2.74	72.48 $\pm$ 4.72	121.03 $\pm$ 4.51	156.58 $\pm$ 4.39
Dimer B2	ND	ND	4.51 $\pm$ 0.52	12.45 $\pm$ 1.07	23.47 $\pm$ 2.54	31.06 $\pm$ 4.86
Trimer	ND	ND	7.30 $\pm$ 1.84	19.09 $\pm$ 4.31	34.45 $\pm$ 2.43	46.32 $\pm$ 6.76
Monomers and oligomers (before depolymerisation)	ND	ND	65.33 $\pm$ 5.24	141.59 $\pm$ 6.43	241.24 $\pm$ 4.17	316.40 $\pm$ 14.18
Total flavan-3-ols ^a	ND	ND	1446.36 $\pm$ 135.08	3995.43 $\pm$ 203.02	5521.96 $\pm$ 418.03	6643.28 $\pm$ 542.33
Caffeoyl shikimic acid 1	ND	ND	1.96 $\pm$ 0.44	4.68 $\pm$ 0.17	7.70 $\pm$ 0.31	10.52 $\pm$ 1.13
Caffeoyl shikimic acid 2	ND	ND	1.13 $\pm$ 0.29	2.86 $\pm$ 0.15	5.09 $\pm$ 0.31	7.02 $\pm$ 0.53
Unknown hydroxycinnamic acid 1	ND	ND	1.75 $\pm$ 0.33	4.20 $\pm$ 0.21	7.25 $\pm$ 0.32	9.61 $\pm$ 0.78
Unknown hydroxycinnamic acid 2	ND	ND	3.51 $\pm$ 0.96	10.25 $\pm$ 1.19	17.37 $\pm$ 1.96	27.95 $\pm$ 2.83
Unknown hydroxycinnamic acid 3	3.36 $\pm$ 0.12	17.27 $\pm$ 1.21	3.51 $\pm$ 0.64	4.08 $\pm$ 0.26	3.93 $\pm$ 0.32	4.44 $\pm$ 0.14
Unknown hydroxycinnamic acid 4	2.53 $\pm$ 0.06	5.79 $\pm$ 0.24	2.41 $\pm$ 0.16	2.58 $\pm$ 0.21	2.50 $\pm$ 0.03	2.85 $\pm$ 0.61
Total Hydroxycinnamic acids	5.89 $\pm$ 0.33	23.06 $\pm$ 0.98	14.27 $\pm$ 2.68	28.65 $\pm$ 1.62	43.84 $\pm$ 3.12	62.39 $\pm$ 2.73
Quercetin derivative	ND	ND	2.26 $\pm$ 0.32	4.86 $\pm$ 0.28	8.19 $\pm$ 0.32	11.08 $\pm$ 0.30
Unknown flavone or flavonol 1	1.82 $\pm$ 0.02	5.86 $\pm$ 0.31	1.37 $\pm$ 0.35	1.08 $\pm$ 0.13	0.97 $\pm$ 0.05	1.01 $\pm$ 0.07
Unknown flavone or flavonol 2	1.73 $\pm$ 0.03	5.28 $\pm$ 0.61	1.48 $\pm$ 0.12	1.26 $\pm$ 0.18	1.23 $\pm$ 0.09	1.27 $\pm$ 0.10
Unknown flavone or flavonol 3	5.03 $\pm$ 0.26	17.79 $\pm$ 0.97	5.15 $\pm$ 0.39	5.50 $\pm$ 0.41	5.10 $\pm$ 0.18	5.29 $\pm$ 0.46
Unknown flavone or flavonol 4	1.48 $\pm$ 0.02	8.62 $\pm$ 0.90	1.50 $\pm$ 0.05	1.51 $\pm$ 0.14	1.43 $\pm$ 0.12	1.30 $\pm$ 0.10
Unknown flavone or flavonol 5	ND	ND	0.87 $\pm$ 0.13	1.54 $\pm$ 0.05	2.69 $\pm$ 0.18	4.04 $\pm$ 0.13
Unknown flavone or flavonol 6	ND	ND	0.60 $\pm$ 0.05	1.40 $\pm$ 0.12	2.15 $\pm$ 0.12	2.90 $\pm$ 0.28
Total Flavones or Flavonols	10.06 $\pm$ 2.27	37.55 $\pm$ 2.66	13.23 $\pm$ 0.57	17.15 $\pm$ 1.06	21.76 $\pm$ 0.45	26.89 $\pm$ 0.51
Total phenolic compounds ^b	15.95 $\pm$ 2.36	60.61 $\pm$ 2.24	1473.86 $\pm$ 132.83	4041.23 $\pm$ 201.05	5587.56 $\pm$ 415.80	6732.55 $\pm$ 544.76

^a Total flavan-3-ols (including monomers and dimers) are determined by HPLC after acid catalysed depolymerisation in the presence of phloroglucinol

^b total phenolic compounds are calculated as the sum of hydroxycinnamic acids, flavones and flavonols determined by HPLC and total flavan-3-ols determined by HPLC after acid catalysed depolymerisation in the presence of phloroglucinol

Fig. 4 Acrylamide level (ppb) in regular pita bread, whole wheat pita bread and date seed powder containing pita breads (5, 10, 15 and 20 %). Means (s.d.) are presented. Comparisons were done between bread samples by using non-parametric Tukey test. Different letters denote significant difference ($p < 0.05$)

bread and a body weight of 70 kg, the intake of acrylamide would be above the recommended range if whole wheat bread was exclusively consumed, but would become about ten times less than the lower limit of this same range if date seed powder containing bread is consumed instead. However, the change in acrylamide content differs according to the concentration of date seed powder added to the bread and is not linear: while the level of acrylamide is significantly lower in 5 % date seed powder bread compared to the regular bread, it is higher in breads containing 10 and 15 % date seed powder and is similar in bread with 20 % date seed powder. Although it is not clear why this is the case; however, this could be related to the increasing content of fat in date seed powder bread, leading to a greater proportion of carbonyl groups likely to react with amino acids and to release acrylamide. This may occur even though the antioxidant content in date seed powder containing bread is higher compared to regular bread. Nonetheless, the antioxidant capacity in 20 % date seed powder bread might be

high enough to counterbalance this effect, since the acrylamide content in this bread is comparable to those observed in the regular bread. Besides, interestingly, our date seed powder containing bread represents a competitive food product in the goal of acrylamide reduction and cancer prevention since the other attempts in developing functional food product with the same purpose, led most often to similar or even less acrylamide reduction. The addition of 1 g/kg of antioxidants from bamboo leaves and 0.1 g/kg of extract of green tea, both rich in phenolics and both with well-known antioxidant properties, led to a reduction of nearly 82.9 and 72.5 % of acrylamide in bread, respectively (Zhang and Zhang 2007). In another work, the addition of 1 % of rosemary extract and 2 tablespoons of rosemary leaves resulted in a 57–67 % reduction of acrylamide levels in bread (Hedegaard et al. 2008).

Another strength and potential health benefit for this newly developed date seed powder-supplemented bread is based on its high antioxidant power. The increase of polyphenols concentration, especially phenolics and flavonoids, occurred to a similar extent compared to what was reported for single polyphenols, like tannic acid or catechin, by other authors working with grape seed flour, with up to 10 % of grape seed (Hoye and Ross 2011; Meral and Dogan, 2013). In our case, phenolics content reached higher levels with bread containing 15 and 20 % date seed powder compared to regular and whole wheat bread. Regarding flavonoids, the content was much higher in all date seed powder containing breads compared to regular bread. Compared to whole wheat bread, the flavonoids content was significantly higher in 10, 15 and 20 % date seed powder containing bread. In addition, the polyphenols profile determination revealed the complete absence of flavan-3-ols in regular and whole wheat flour bread whereas flavan-3-ols were detected in date seeds powder-containing breads at high level even with only 5 % of date seed powder in the bread and were shown as the most abundant polyphenolic compound in these breads. The levels of flavan-3-ols reached in the date seeds powder-containing breads are similar and even higher than in other type of plant product powder-containing bread or biscuits which might have been developed by other authors (Meral and Dogan 2013; Mildner-Szkudlarz et al. 2012). Considering the published polyphenolic profile of date seed powder (Habib et al. 2014), a decrease in phenolics content can be observed in date seeds powder containing bread. Similar observation was done by other authors during baking process (Meral and Dogan 2013). This is unlikely related to oxidation of these compounds since yeast will use oxygen. This could rather be related to the modification of the compounds through destruction and/or generation of new phenolics during baking process, including mixing. Nonetheless, while some phenolic compounds are destroyed during heating, many more new compounds might be introduced into the food system (Nayak et al. 2013). Whereas, it was demonstrated that baking process increased the phenolics' concentration in whole wheat bread

(Gelinas and McKinnon 2006), destruction of phenolics was reported by other authors (Dietrych-Szostak and Oleszek 1999). This could also give some explanation to the similar levels of phenolics which were observed in date seed powder containing bread 10 % and in whole wheat bread.

In addition, as shown by the increased values of FRAP, NO, DPPH and ABTS parameters, the antioxidant power of the newly developed date seed powder containing bread is indisputably high. Interestingly, the inhibition percentage for DPPH appears to be much greater with the addition of date seed in the bread compared to grape seed as recently observed in the work done by Meral and Dogan 2013.

Flavan-3-ols were identified in this study as the most abundant polyphenols in date seeds bread compared to regular and whole wheat breads in which they were absent. In addition, compared to the previous determination of the polyphenols profile of dates seeds powder (Habib et al. 2014), the baking process was associated with a reduction of approximately 20 % of the initial polyphenols content.

Considering the well-recognized antioxidant actions of flavan-3-ols, we can reasonably attribute the antioxidant property of date seed powder breads to these particular compounds.

Since this newly developed bread based on date seed powder is destined to be consumed by humans, the acceptance by the consumer is an important aspect. Quality characteristics and sensory tests have been conducted with our bread (Hashim et al. 2013). These experiments demonstrated that breads containing 5 and 10 % date seed powder had similar quality and sensory characteristics compared to whole wheat bread, and that while those containing 15 and 20 % date seed powder were less desirable than whole wheat bread, however, they were still acceptable.

Considering that enhanced health benefits is the purpose of developing this functional bread, the bioactivity of the bread has to be guaranteed after human consumption, especially with regards to polyphenolic compounds. It is actually well recognized that the most abundant polyphenols in foods are not necessarily the most abundant bioactive metabolites in the target tissues and that polyphenols undergo transformations, methylation, sulfation or glucuronidation in the intestine and liver before release into the bloodstream (Manach et al. 2005). Hence, bioavailability of polyphenols and their metabolites should be studied. Nonetheless, previous results suggest that catechins and flavanones, some of the major compounds identified in date seeds, are among the best absorbed polyphenol. Additionally, recent studies conducted with green tea and apple showed that polyphenols and their metabolites were found in great amount in plasma and urine after oral ingestion in humans (Kahle et al. 2011; Renouf et al. 2013). This is in favor of a good bioavailability of bioactive compounds from date seed-containing bread. In addition, a recent animal study demonstrated that adding date seed into the feeding of rats was

associated with an improved oxidative status and the absence of any sign of toxicity (data under review for publication).

Conclusion

Using a largely wasted by-product, functional pita bread was developed with a strong potential of contributing to increased fiber and antioxidant intake and decreased acrylamide intake, thus providing a promising protection against chronic disease development. Additional studies, especially clinical trials in humans, have to be conducted in order to confirm the health benefits of this food product.

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