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Comparison of antioxidative activity of extracts from three parts of two pumpkin varieties *(Cucurbita moschata)* grown in Saudi Arabia

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Abstract

The macronutrients, phenol, flavonoid contents, antioxidant activity, and lipid oxidation inhibitory were evaluated in peel, pulp, and seed of sakaka and nagdi pumpkin. The study was carried out on the freeze-dried samples. The results revealed that the fat and protein

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in the seed of two pumpkins were higher than those of peel and pulp, whereas pulp and peel had the highest fiber and carbohydrate contents, respectively. Composition of macronutrients differed among the pumpkin parts and varieties. The highest phenol content was found in extracts of nagdi peel and pulp. The first two samples had the highest flavonoid content were nagdi and sakaka peel. In the case of antioxidants activity, the extract from nagdi seed displayed the highest antioxidants activity. In the lipid oxidation inhibitory test, the seed extracts, and sakaka peel showed the most potent in inhibiting the formation of reactive substances which showed the least TBA values at the end of the storage period. Furthermore, the TBA values of other pumpkin extracts were lower than the TBA values of the control. Therefore, the extracts of different parts of pumpkin could potentially in nutraceuticals preparation and functional food formulations.

Keywords: Pumpkin, macronutrients, phenolics, Antioxidant activity, Oxidation inhibitory

INTRODUCTION

Oxidation reactions involve molecules, called free radicals, which are very unstable, free radicals are react with other molecules in our bodies including membrane lipids, proteins, and DNA., the unchecked activities of free radicals are related to health disorders such as cancer, diabetes mellitus, gastric ulcers, hypertension, neurodegenerative, arthritis, reperfusion, and inflammatory diseases (Oboh & Ademosun., 2011). In foods, the oxidation reactions can lead to loss of nutritive value and rancidity through from the destruction of essential fatty acids and vitamins and the potential formation of colored products and toxic compounds (Samarin et al., 2012). However, a practical approach to reduce the oxidation process is through consuming foods that are rich in antioxidants (Oboh & Rocha., 2007). Antioxidants are well known to neutralize free radicals and interrupting the radical chain reaction of lipid peroxidation, thus inhibiting oxidative damage (Halliwell., 1999). Overall, although synthetic antioxidants such as butylated hydroxyanisole tertiary,

tertiary-butylhydroquinone, butylated hydroxytoluene, and gallic acid esters have the potential to scavenge free radicals, they have been criticized due to their potential toxic effects (Oboh & Ademosun., 2011). thus, the application of natural antioxidants derived from natural sources is a dramatic increase (Omar et al., 2010).

Fruits and vegetables have high concentration of antioxidants, such as phenolics compounds, ascorbic acid, vitamins, and carotenoids (Jacobo-Valenzuela et al., 2011). Pumpkin (*Cucurbita moschata)* is an important vegetable in the agricultural system (Cao et al., 2005). It is widely planted in many countries due to its high nutritional value and its health benefits (Indrianingsih et al., 2019). Pumpkin can be used for nutritional food as well as medicine in many countries. It is used in processed foods such as syrups, jams, jellies, and purees, This vegetable very popular with its abundant nutrition, low-calorie, and soft texture (Zhou et al., 2017). There are found that the three parts of pumpkin are a good source for phenol, flavonoid, a-tocopherol, carotenoids, vitamin C, vitamin A, carbohydrates, and amino acids (Dini et al., 2013). What is more, it has extensive bioactivities, such as anti-diabetes (Jiang et al., 2011), and antioxidant properties (Indrianingsih et al., 2019). Therefore, it widely used in foods and pharmaceutical applications. Overall, due to the potential application of this vegetable more scientific evidence about their antioxidant activity is needed. To the best of our knowledge, no studies were carried out on the antioxidant properties of *Cucurbita moschata* cultivated in Saudi Arabia. *Cucurbita moschata* is planted in different environments and areas which display different properties and composition of nutrients (Zhou et al., 2017). thus, the current study was examined macronutrients, phenol, flavonoid contents in the peel, pulp, and the seed of two varieties pumpkin (sakaka and nagdi) grown in Saudi Arabia and then the antioxidant activity and oxidation inhibitory of pumpkin extracts were examined to understand their medicinal properties.

MATERIALS AND METHODS

Materials

Two varieties (sakaka and nagdi) of the summer pumpkin, (*Cucurbita moschata*) were procured from the Department of Plant Production, College of Food and Agriculture Sciences, King Saud University, Saudi Arabia. Chemicals such as methanol, acetone, sodium chloride, sodium carbonate, Folin Ciocalteau's phenol reagent, TBA (2-thiobarbituric acid) and standards were purchased from Sigma Aldrich USA.

Sample preparation

Pumpkin samples (pulp, peel, and seed) were separated, cut, and freeze-dried (Unitop 600 SL, VirTis, USA). The dried samples were ground and passed through a sieve of 0.5 mm, wrapped in vacuum, and stored at -20 ° C until examined.

Chemical analysis

Ash content was detected by burned the samples at 600 C°. Lipid and protein contents were measured by Soxhlet and Kjeldahl methods, respectively. Fiber content was determined by sequential hot digestion of the defatted samples with acid and alkaline solutions and the carbohydrate was calculated by subtracting 100% - (% ash + % lipid + % protein + crude fiber) (Indrianingsih et al., 2019).
The energy value was calculated by using the following formula:

$$\text{Energy (kcal/100g)} = (\%\ \text{CHO} \times 4) + (\%\ \text{P} \times 4) + (\%\ \text{F} \times 9) \quad (1)$$

where, CHO, P, and F are the carbohydrate, protein, and fat contents, respectively.

Extraction of the polyphenol

10 g of dried pumpkin (peel and seed) was mixed with 150 ml of a mixture of methanol-acetone-water (7:7:6 v/v/v). After ultrasonic for 20 min at 30 C°, the slurries were centrifuged at 4000 g for 5 min and the supernatants were collected. The residue was re-extracted and centrifuged under the same conditions. The combined supernatants

were evaporated under vacuum at 40 C° to remove the organic solvents. The aqueous phase extract was freeze-dried, dissolved in 5 ml methanol (80%) and then it was stored at -20℃ for further analysis.

Phenol measurement

In brief, 0.5 ml of extract was mixed with 0.5 ml of Folin Ciocalteu's phenol reagent (FCR)**.** After mixing, 1ml of sodium carbonate and 10 ml of distilled water were added. Followed by incubation of the mixtures at room temperature for 45 min. After centrifugation for 5min at 4000g, the Absorbance of the supernatants was subsequently detected at 725 nm (MOD. 4050, Biochrom, and Cambridge, UK). The standard curve was prepared using Gallic acid solution.

Total phenol content; C (expressed as mg GAE/100 g dry weight) was calculated using the following formula;

$$C = C_1 \times (V/m) \times 100 \quad (2)$$

where, C1 is the Gallic acid concentration established from the calibration curve in mg/ml; V is the volume aqueous phase extract in ml; m is the weight of sample in grams (Amin et al., 2018)

Flavonoid measurement

Total flavonoid content in the extracts was measured by the aluminum chloride colorimetric assay (Oboh & Ademosun., 2011). 1 ml of extract or standard solution was mixed with 4 ml distilled water, and 0.3 ml of 5% sodium nitrite. The 0.3 ml 10% aluminum chloride and 2 ml of 1 M sodium hydroxide were subsequently added after 5 and 6 min, respectively. After dilution, the absorbance was measured at 510 nm (MOD. 4050, Biochrom, and Cambridge, UK). Total flavonoid content (expressed as mg Quercetin /g 100 dry weight) was calculated as mentioned in phenol calculation.

Antioxidant activities

In brief, each extract 0.5 ml was mixed with 1.5 ml DPPH solution (0.1 mM). Thereafter, the mixture was incubated in the dark for 60 min at room temperature. The absorbance was subsequently tested at

517 nm (MOD. 4050, Biochrom, and Cambridge, UK). The inhibition percentage was calculated using the following formula:

$$[(A_0 - A_1)/A0] \times 100. \quad (3)$$

A_0 is the absorbance of the control, and A_1 is the absorbance of the sample extract (Turkmen et al., 2006).

Lipid oxidation inhibitory test

Lipid oxidation inhibitory test was conducted by determining the 2-thiobarbituric acid (TBA) in the fish model. Firstly, the moisture and fat contents of ground salmon were detected. Secondly, ground fish (80 g) was mixed with deionized water (20 mL) in mason jars and then the pumpkin extracts (containing 200 ppm and 300 ppm phenolics) and 200 ppm of butylated hydroxytoluene (BHT) were individually added. After homogenization, the fish samples with or without pumpkin extracts were heated in a water bath at 80 C° for 40 min. After cooling to room temperature, the fish samples were homogenized and shifted into plastic bags, and then stored at 4 C° for 7 days. The TBA of samples was tested on days 0 and 7. Briefly, 5 g of fish sample was weighed and 50 ml of 10% trichloroacetic acid was added. After blending and filtering, 5 ml of the filtrate was mixed with 5 ml of TBA reagent in the test tube. The mixtures were heated for 35 min using a boiling water bath. After cooling, the absorbance was measured at 538 nm (Albishi et al., 2013).

Statistical analysis

All experiment data in this study were analyzed by using one-way analysis (ANOVA) and Duncan test for comparison between means using the SAS statistical analysis software program (SAS software version 9.2 (SAS Institute Inc., 2008). Significance was determined at $p \leq 0.05$ level. All data were reported as the mean ± standard deviation (S.D.).

RESULTS AND DISCUSSION

Macronutrients composition

Table 1. Macronutrients composition of the freeze dried Pumpkin (%)

		Ash	Protein	Fat	Crude fibre	carbohydrate	Energy(kcal/100g)
	Peel	9.462 ±0.06^{c}	4.452 ± 0.012^{d}	3.172 ± 0.02^{d}	8.444±0.01^{b}	74.729 ±0.30^{b}	345.272
	Pulp	12.935 ±0.01^{a}	7.610 ± 0.06^{c}	2.925 ± 0.16^{e}	13.658±0.01^{a}	62.820 ±0.94^{d}	308.045
Sakaka	Seeds	8.333 ±0.25^{d}	43.605 ± 0.04^{a}	35.012 ± 0.01^{a}	2.524 ± 0.05^{e}	10.499 ±0.83^{e}	531.524
	Peel	8.259±0.04^{d}	2.715 ± 0.01^{f}	1.235 ± 0.02^{e}	5.498± 0.01^{d}	82.276±0.19^{a}	351.079
Nagdi	Pulp	10.286 ±0.03^{b}	4.240 ± 0.009^{e}	5.337 ± 0.04^{c}	6.765± 0.01^{c}	73.347± 0.06^{c}	358.381
	Seeds	6.595 ±0.22^{e}	40.442 ± 0.09^{b}	31.666 ± 0.06^{b}	2.357± 0.01^{e}	2.357 ±0.23^{f}	456.19

A Data are expressed as means ± SD (n = 3). Values in each column having the same letter are not significantly different ($p > 0.05$).

Ash, protein, fiber, and fat contents of sakaka peel were significantly higher than those of nagdi peel, while nagdi peel showed higher carbohydrate ($p \leq 0.05$) than that in sakaka peel. Fat and carbohydrate contents of nagdi pulp were higher ($p \leq 0.05$) compared to those of the sakaka pulp, while nagdi pulp had lower ($p \leq 0.05$) ash, protein, fiber, and fat contents compared to sakaka pulp. The seed of sakaka had higher ($p \leq 0.05$) protein and fat compared to nagdi seed (Table 1). The differences in the of pumpkins macronutrients possibly depended on numerous factors such as climate, soil fertility, and maturity stage (Indrianingsih et al., 2019). Overall, the values of nutrient components in this study were similar to the findings of Jacobo-Valenzuela et al., (2011); Singh et al., (2016). In addition, the quantities of fat and protein in the seed of two pumpkins were higher than those found in peel and pulp, which agrees with the literature (Fila., 2013). On other hands, the carbohydrate and fiber contents of pumpkins pulp and peel were higher than those of pumpkins seed. Therefore, pulp or peel are recommended as part of weight-reducing diets. In addition, the protein of pumpkin peel contains citrulline **(**Amino acid), which plays an important role in the urea cycle (Pons., 2003).

Phenol and flavonoid contents

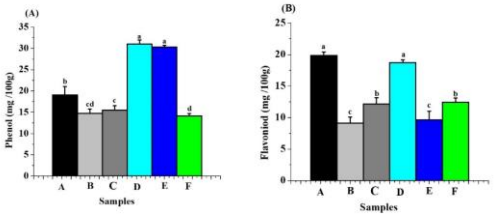

Figure 1. Phenol content (mg GAE /100 g dried sample) and flavonoid content (mg Quercetin /100g dried sample) of freeze dried pumpkin extracts. A, B, C, D, E, F for sakaka peel, sakaka pulp, sakaka seed, nagdi peel, nagdi pulp and nagdi seed respectively. GAE, gallic acid equivalents

The data of the total phenol and flavonoid contents in pumpkins and their by-products are presented in Fig. 1. In this study, the total phenol in the samples ranged from 14.10 to 31.00 mg /100g dry weight, the nagdi peel and pulp contained the highest amount of the total phenol among all samples. It was followed by sakaka peel. The nagdi seed and sakaka pulp showed a similar amount of total phenol (Fig.1A). In the case of flavonoid content, the total flavonoid in the samples ranged from 9.13 to 19.86 mg /100g dried sample, the flavonoid content in the peel of two pumpkins was higher than that in pulp and seed of two pumpkins. Followed by the seed of two pumpkins. The lowest quantity of flavonoid was found in pumpkins pulp (Fig.1B). Combining the phenol and flavonoid data, the nagdi peel contained the highest amount of the total phenol and flavonoid among all samples, while low phenol and flavonoid contents were found in sakaka pulp. Overall, there is found that the contents of phenol and flavonoid in pumpkin and its by-products vary among different varieties (Jacobo-valenzuela et al., 2011; Saavedra et al., 2015) as it was also found in our study, this difference may be mainly attributed to harvest location and genotypes which affect the accumulation of the phenolics by synthesizing different types and quantities of phenolics (Albishi et al., 2013). However, affect the growing conditions (season, fertilizer, soil type, amount of sunlight received, and climatic conditions), storage and analysis methods on the content phenolics cannot be ruled out. Besides, the degree of vegetable maturity when harvested also affect polyphenol

concentrations. Generally, the phenol and flavonoid contents in the analyzed samples as shown in Fig.1 agrees with the phenol and flavonoid contents in the literature (Jacobo-valenzuela et al., 2011; Singh et al., 2016).

DPPH radical scavenging activity

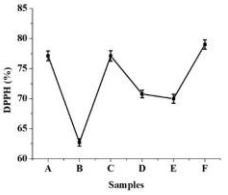

Figure 2. DPPH scavenging activity of freeze dried pumpkin extracts. A, B, C, D, E, F for sakaka peel, sakaka pulp, sakaka seed, nagdi peel, nagdi pulp and nagdi seed respectively.

In this study, DPPH radical scavenging capacity test was used to assess the scavenging activities of phenolics extracts because the DPPH radical was more stable than hydroxyl radicals and superoxide (Chen et al., 2017). DPPH radical scavenging activities of extracts are given in Fig. 2. DPPH radical scavenging capacity test showed that the extracts of nagdi seed displayed the highest scavenging activity as compared to those of tested samples. It was followed by sakaka peel and seed, whereas sakaka pulp extract had the lowest scavenging activity. In this experiment, the extracts of two verities and their by-products have different capacities in the DPPH test probably associated with the differences components in the extract. What is more, although nagdi seed had the least phenol and flavonoid as compared to nagdi and sakaka peel, it exhibited the highest scavenging activity. This may be due to the differences in the chemical constituents which contribute to the scavenging activity (Chen et al., 2017). What is more, the antioxidant activity of the extracts in this study was higher than that found by Indrianingsih et al., (2019). This difference possibly attributed to the content of antioxidants components in the pumpkin extracts which influence by several factors as mentioned above. Generally, the data of this experiment indicated that the pumpkin or its by-products are a good

source of antioxidant compounds (62.721% - 79.007%), which agrees with the antioxidant activity of many vegetables and fruits (Peschel et al., 2006).

Lipid oxidation inhibitory test

Table 2. TBA values of soluble extracts of pumpkin on days 0 and 7 of storage at 4 ℃. TBA, 2-thiobarbituric acid.

	0 days	days 7
Control	2.998 ±0.03^{a}	6.041±0.11^{a}
Sakaka peel-200 ppm	2.242±0.03^{d}	3.2.083±0.07^{h}
Nagdi peel-200 ppm	1.833±0.06^{fg}	4.290±0.03^{d}
Sakaka pulp -200 ppm	2.733±0.06^{b}	4.808±0.06^{b}
Nagdi pulp -200 ppm	1.930±0.11^{f}	4.418±0.03^{c}
Sakaka seed -200 ppm	2.125±0.01^{e}	3.299±0.09^{h}
Nagdi seed -200 ppm	1.404±0.03^{h}	2.991±0.03^{i}
BHT -200 ppm	1.635±0.08^{g}	2.712 ±0.018^{j}
Sakaka peel -300 ppm	2.116±0.03^{e}	3.069±0.33^{i}
Nagdi peel -300 ppm	1.400±0.17^{h}	4.025±0.03^{e}
Sakaka pulp -300 ppm	2.418±0.12^{c}	3.759±0.17^{f}
Nagdi pulp -300 ppm	1.587±0.03^{gh}	4.036±0.04^{e}
Sakaka seed -300 ppm	1.844±0.08^{fg}	2.749±0.07^{j}
Nagdi seed -300 ppm	1.271±0.07^{i}	2.476±0.04^{k}

A Data are expressed as means ± SD (n = 3). Values in each column having the same letter are not significantly different ($p > 0.05$).

The TBA test was used in this work to evaluate the efficacy of extracts to delay the development of oxidative rancidity in a fish model. The salmon fish used had 11.73 ± 0.27% total lipids and 57.73 ± 0.45% moisture. The TBA values of antioxidant-treated fish samples stored at 4°C for 7 days are shown in Table 2. In comparison with the control, the extracts of pumpkin and their by-products were effective in suppressing the salmon oxidation, which showed the least TBA values at the end of the 7-day storage period (Table 2). The order of effectiveness in inhibiting the formation of TBA was: Nagdi seed -300 ppm> BHT-200 ppm> sakaka seed-300 ppm > nagdi seed -200 ppm > sakaka peel-300 ppm > sakaka peel-200 ppm> sakaka seed -200 ppm > sakaka pulp-300 ppm > nagdi peel-300 ppm > nagdi pulp -300 ppm > nagdi peel-200 ppm > nagdi pulp -200 ppm > sakaka pulp-200 ppm > control. Compared to control, the extracts of pumpkin and their by-products displayed percentage inhibition ranging from 20.41%-

59.013%, the nagdi seed -300 ppm inhibited the TBA formation by 59.013%. What is more, the TBA value of nagdi seed at level of 300 ppm was lower ($p < 0.05$) than that of BHT (synthetic antioxidant) at concentration of 200 ppm. Therefore, the better efficacy of pumpkins seed compared to pulps in lowering TBA values is of interest. Pumpkins seed also displayed better antioxidant capacity as reflected in lower TBA values than TBA values of pumpkin pulp, probably due to synergistic capacity of different antioxidants compounds present in the extracts. Overall, similar results were found by Kim et al., (2013), who confirmed that the oxidation of ground beef was reduced by supplementation pumpkin extract.

CONCLUSION

The antioxidant activity and antioxidative activity of pumpkin seed were higher than those of pumpkins pulp. This study revealed that both of nagdi and sakaka pumpkins (peel, pulp, and seed) contain antioxidants which showed DPPH radical scavenging activity and antioxidative activity. Therefore, pumpkins by-products could be considered as a good source for natural antioxidants and may be beneficial for inhibiting diseases of oxidative stress and preparing ingredients of functional food.

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