Food Production, Processing and Nutrition

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Roasted fox nuts (*Euryale Ferox* L.) contain higher concentration of phenolics, flavonoids, minerals and antioxidants, and exhibit lower Glycemic Index (GI) in human subjects

Marrium Liaquat^{*}[®], Imran Pasha, Muhammad Ahsin and Abdullah Salik

Abstract

Fox nut (*Euryale ferox*) is categorized among superior quality foods because of its highly nutritious profile. It also serves as a good protein source for vegetarian diets. However, it is not widely consumed, due to soggy texture and bland flavor as compared to other nuts. In this study, we used roasting and seasoning to enhance its consumer acceptance. Furthermore, we have also compared biochemical, nutritional and physical properties of the roasted and unroasted fox nuts; and their Glycemic Index (GI) in human subjects. Results showed that roasting significantly ($P \le 0.05$) improved the minerals content (from 0.58 to 0.66%), protein content (from 11.40 to 14.57%), total phenolics (from 346.02 to 470.62 mg GAE/100 g), flavonoids (from 4.15 to 4.43 mg CE/g), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (from 48.54 to 79.13% inhibition) and Ferric Reducing Antioxidant Power (from 662.46 to 957.14 µmol FeSO₄/g). Consumer acceptance was evaluated with and without seasoning; both salt and spice seasoned roasted nuts had greater overall acceptability as compared to plain roasted and unroasted nuts. Fox nuts also reported low GI (37%) in human subjects (ethical approval #1772/01-06-2020). Thus, roasted fox nuts can be consumed as nutrient rich and low GI snack; not only as a component of healthy diet but can also be served to patients with metabolic disorders like obesity and diabetes.

Keywords: Euryale ferox seeds, Fox nuts, Roasting, Nutritious snack and low glycemic index foods

Introduction

Euryale ferox is an important cash crop of aquatic origin. It belongs to family Nymphaeaceae and is known worldwide with various names like "fox nut", "gorgon nut", "prickly water lily" and in the native language of Indian Sub-continent as "Makhana" or "Phool Makhana" (Goren-Inbar et al. 2014). Some scientists have referred Makhana seeds as "Black Diamonds" and "Black Gems of Wetlands" because of their black outer seed coat

* Correspondence: marium.liaquat1@gmail.com

(Sundaram et al. 2014). *Euryale ferox* plant has edible seeds that are processed into white puffed nuts and are the main reason for the popularity of the plant. These nuts are highly nutritious and counted as a superior food item in dry fruit category, based on its high Essential Amino Acid Index (EAAI) of 89–93% (Kumar et al. 2011). It makes them an amazing reserve of essential amino acids which are not synthesized in the human body, and need to be part of diet.

Non-Communicable Diseases (NCDs) constitute a prime issue around the globe. The main reason behind their rapid prevalence is globalization and westernization of lifestyle and a dramatic shift and liking towards the



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National Institute of Food Science and Technology, Faculty of Food, Nutrition and Home Sciences, University of Agriculture, Faisalabad 38000, Pakistan

consumption of processed and refined products (Korrapati et al. 2018). One of the coping strategies was presented long before by Dr. David Jenkins (1981) as a concept of Glycemic Index (GI) of foods, that could be used as a controllable factor for prevention of chronic diseases. Low GI foods have provided evidence for improving insulin sensitivity and fasting triglyceride levels along with maintenance in healthy body weight, and an appreciable improvement in health risk markers (Livesey et al. 2008). Fox nuts, being a low GI food, fits perfectly into the criteria of preferred foods for these Non-Communicable Diseases. Complex carbohydrate content of these nuts is responsible for low GI, and makes them an amazing snack for patients with NCDs. Nutrition therapy for diabetic patients by Canadian Diabetes Association has also included recommendations for foods having low glycemic index (Lawson et al. 2005).

Besides these nutritional properties, a wide range of medicinal properties of fox nuts has also been documented in ancient literature of Chinese and Indian medicine. Apart from its macro and micronutrients composition, it's the phenolic constituents and antioxidant properties, that impart specific medicinal attributes to fox nuts (Lee et al. 2002). Nuts rank third after spices and fruits for containing the most phytochemicals (Chang et al. 2016). Phenolic and flavonoid compounds are important determinants of the antioxidant activity, and overall nutritional quality of food stuff. Several studies have supported their role in managing human diseases (Lin et al. 2016). Antioxidant properties exhibited by the phenolic compounds are very much potent as compared to those of other nutrients (Shahidi & Naczk 2003). Nuts are no doubt convenient and tasty snacks, and consuming one serving of nuts a day prevents the development of chronic diseases like cardiovascular disorders, type II diabetes, cancer, high blood pressure and neurodegenerative diseases (Chang et al. 2016). In addition to a bioactive-rich profile, fox nuts have higher concentration macro- (Ca, P, K, Na and Mg) and microminerals (Fe and Zn) (Kumar et al. 2016).

The phytochemicals in nuts are affected by various factors like storage conditions, cultivars, plant location, and processing methods like roasting, irradiation, bleaching etc. (Bolling et al. 2011). Among these, roasting is a technique being used to improve these phytonutrients in food. It is widely being used for processing of nuts, to improve their nutritional quality and overall acceptability. Fox nuts, being soggy in texture, require roasting as a pretreatment to consumption. To gain maximum benefit from *Euryale ferox*: a wonder food, its phenolic and antioxidant profile needs to be preserved or enhanced during processing. The changes happening in the kernels when exposed to roasting include

browning reactions like Maillard reaction or nonenzymatic browning (Mendes et al. 2001). These reactions are responsible for changes in color, texture, phenolic and antioxidant profile (Somporn et al. 2011).

The literature on numerous health benefits of fox nuts and its use in ancient medicines inform us to include this wonder food in our daily diet. Moreover, in comparison to other nuts, fox nuts contain a significantly lower fat content, which makes it a comparatively good choice. However, its high carbohydrate content raises concern whether fox nuts can be recommended to people with diabetes and other metabolic disorders. Soggy texture and bland taste also contributes to its limited consumption. The purpose of this study was to highlight the nutritional importance of fox nut as a low GI snack with additional benefits. There is little data on fox nuts in this regard. That is why, in addition to the GI study, we chose the parameters that contribute to consumer acceptance, which include minerals, phenolics, flavonoids, and antioxidant activity.

To the best of our knowledge, this study is the first one to introduce roasting and seasoning as a pretreatment to fox nuts' consumption to enhance consumer acceptability; and investigated possible compositional changes in roasted fox nuts. We also determined its glycemic index in human subjects to ensure if it could be consumed safely by people with diabetes and other metabolic issues. Salt and spice seasoned snacks were also prepared and evaluated for organoleptic properties.

Materials and methods

Chemicals and reagents

Nitric acid, perchloric acid, HPLC grade acetone, HPLC grade methanol, Folin-Ciocalteu reagent, sodium bicarbonate, gallic acid, sodium nitrite, aluminium chloride, sodium hydroxide, catechin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-S-triazine (TPTZ), iron (III) chloride hexahydrate and iron (II) sulfate were purchased from Sigma (St. Louis, MO). Unless otherwise stated, all the reagents were of analytical grade and purchased from a certified vendor.

Sample collection and roasting process

Unroasted white puffed nuts were purchased from a grocery store at Faisalabad, Pakistan. A brief survey (n = 50) was conducted to ascertain consumer attitudes toward unroasted fox nuts as a potential snack. The most common attributes associated with consumer reluctance to consume this nut as a snack were its soggy texture and bland flavor (Data not given). For roasting, nuts were evenly distributed in a single layer over aluminium plate of preheated (130 ± 2 °C) hot air oven (UFB-400, Memmert, Germany) for 10 min. After roasting, samples were allowed to cool at ambient temperature and packed in polythene zip bags until further analyses.

Chemical compositional analysis

The chemical composition of both unroasted (control) and roasted nuts was analyzed by approved methods of AOAC International (Latimer Jr. 2016). The moisture content was calculated by drying the samples in hot air oven at 105 ± 2 °C (method no. 934–01). The moisture-free samples were completely burned on Bunsen burner followed by incineration in muffle furnace at 525 °C for crude ash content (method no. 942–05). Nitrogen content was determined and fixed conversion factor for nuts (5.30) was used for crude protein calculations (method no. 981–10). Crude fat was determined by method no. 920.39 (A) and crude fiber by method no. 978–10. Nitrogen-free extract also referred to as carbohydrate content was calculated by subtracting all the above-retrieved fractions from 100 (Latimer Jr. 2016).

Mineral analysis

Samples were wet-digested by di-acid mixture (HNO_3 : $HClO_4$) in ratio of 3:1, respectively. Digests were analyzed by Atomic Absorption Spectrophotometer (AA240, Varian, Australia) for minerals analysis (Estefan et al. 2013).

Extraction of phytochemical compounds

The ground samples were subjected to the solid-liquid extraction method (Bonoli et al. 2004) with some modifications. Briefly, 4 g of the sample was allowed to interact with 40 mL of acetone-water extraction mixture (4:1, v/v) for 3 h while agitation (250 rpm) over an orbital shaker (KS-260, Kika-Werke, Germany) followed by 30 min sonication (E60H. Elma, Germany) at 30 °C. The supernatant was harvested and residues were again treated with the fresh extraction mixture as discussed above. The supernatants were reconstituted and centrifuged at 1000 g for 10 min. The solvent was evaporated from samples by rotary evaporator at low temperature (-40 °C) and reduced pressure (EV311H, LabTech, China) near to dryness. Residues were reconstituted in 4 mL (1 mL/g ground sample) HPLC grade methanol.

Determination of total phenolic content (TPC)

The phenolic content was determined by Folin-Ciocalteu method (Sharma et al. 2017). About 0.5 mL extract aliquot was mixed with 0.5 mL of Folin-Ciocalteu reagent and volume was raised to 9.5 mL by distilled water. After 3 min, 1 mL of 25% sodium bicarbonate was added to the reaction mixtures and incubated for 60 min at 25 °C, and absorbance was measured by UV-Vis Spectrophotometer (U2020, IRMECO, Germany) at 725 nm. The results for TPC were calculated against the standard

curve for gallic acid and results are expressed as mg of gallic acid equivalent (GAE) per 100 g of sample.

Determination of total flavonoid content (TFC)

Total flavonoid content was evaluated by following the protocol given (Rebaya et al. 2015). For each sample, about 125 μ L of methanolic extract was allowed to react with 75 μ L of sodium nitrite (5% w/v) for 6 min followed by the addition of 150 μ L AlCl₃ (10% w/v). After 5 min incubation, 750 μ L NaOH (1 M) was added to each sample, and volume was raised to 2.5 mL by distilled water. The final incubation time was ~ 15 min and absorbance was measured at 510 nm by UV-Vis Spectrophotometer. The results are expressed in mg catechin equivalent (CE) per gram of sample.

Antioxidant analysis

Determination of DPPH radical scavenging activity

Stable free radicals of 2,2-diphenyl-1-picrylhydrazyl (DPPH) were used to evaluate the free radical scavenging antioxidant properties of the extracts. DPPH solution (0.1 mM) was prepared by mixing 3.94 mg DPPH reagent in 100 mL HPLC grade methanol. About 150 μ L of methanolic extract was manually homogenized with 2 mL of DPPH solution. Homogenized samples were incubated for 30 min and absorbance was measured at 515 nm by UV-Vis Spectrophotometer (Prado et al., 2013).

Determination of ferric reducing antioxidant power (FRAP)

Ferric reducing antioxidant power of extracts was quantified as reported (Benzie & Strain 1996). Fresh regent (FRAP) was prepared by mixing 2,4,6-tripyridyl-S-triazine (10 mM in 40 mM HCl), FeCl₃:6H₂O (30 mM), and acetate buffer (300 mM, pH 3.6) in the ratio 1:1:10, respectively. The solution was placed in a water bath at 37 °C for 3 h. Afterwards, 300 μ L methanolic extract volume was raised to 480 μ L by distilled water and allowed to react with 1.8 mL of FRAP reagent for 4 min at 37 °C. Absorbance was measured at 593 nm by a UV-Vis Spectrophotometer and results are expressed in μ M FeSO₄ per gram sample.

Color analysis

The color analysis of nuts was performed by a colorimeter (Minolta, Milton Keynes, UK). Samples were evenly spread on a white surface and the instrument was calibrated by D65 illuminant. Results were obtained in the chromaticity coordinate (L*, a* and b*) values (Hojjati et al. 2015; Shakerardekani et al. 2011).

Texture analysis

Roasting impact on firmness (hardness) of the nuts was quantified by a texture analyzer (TA-XT2, Plus, Stable

Microsystems, Surrey, UK) equipped with Ottawa cell HDP/90 arm (Piga et al. 2005).

Bulk and calorie density analysis

Bulk density of nuts was measured by filling a cylinder of known volume with nuts and removing excess kernels by a scale after tapping the cylinder twice (Kumar et al. 2013). The results were calculated by following expression:

$$\rho b (kg/m3) = \frac{Total \ mass \ of \ seeds}{Total \ volume \ of \ seeds}$$

Calorie density was calculated according to the Atwater general system for energy content of foods, based on energy-yielding substrates, regardless of the food type (Capuano et al. 2018). Following expression was used to calculate the results:

Total energy content (Kcal/100g) = $(9 \times \% fat) + (4 \times \% protein)$ + $(4 \times \% carbohydrate)$

Study design for Glycemic Index trial

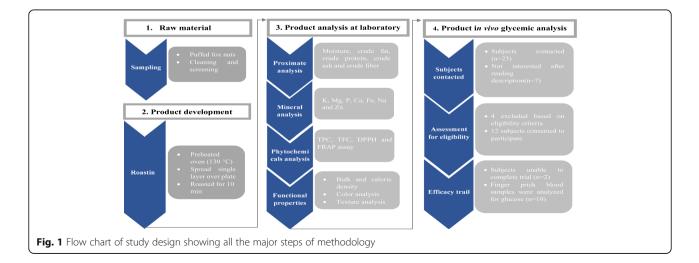
The study was planned to compare the glucose responses of roasted fox nuts (test food) with glucose (reference food), both containing an equal amount of available carbohydrates (25 g). Blood glucose responses were monitored in human subjects in the morning, after overnight fasting of about 8 h. The trial continued for a week, both (test and reference) foods were tested twice with a washout period of 2 days. The study was conducted at Nutrition and Hi-Tech laboratory, University of Agriculture, Faisalabad.

Recruitment of subjects

Twenty-three healthy volunteer graduate students were contacted for the study and ten subjects were finally recruited, based on exclusion criteria (Fig. 1). Recruited human subjects were healthy adults {males (n = 5) and females (n = 5)} aged between 18 and 30 years with a normal Body Mass Index (BMI) (19-25 kg/m²) and without any co-morbidity. Those engaged in shift works; having sleep disorders, impaired fasting blood glucose (>6 mmol/L), any metabolic disease, food allergy or hypersensitivity, smoking, or those taking any sort of medication that can interfere with carbohydrate metabolism, were excluded (Wolever et al. 2019). All the recruited participants had normal sleeping pattern (7-9 h daily) and regular eating habits with a daily routine of having three proper meals and two snack meals. Eligibility was confirmed through an interview and screening session at the laboratory prior to inclusion. Written consent was taken from all the participants, informing them about the protocols, importance and harmlessness of the study.

Determination of Glycemic Index

International Standardization Organization's (ISO) developed standardized method for determining the glycemic index of food components was employed to quantify the glycemic index value (ISO 2010). Capillary blood (Finger prick samples) was analyzed for glucose estimation using Accu-Chek Active glucometer (Roche Diabetes Care, Inc. Chicago). Fasting blood glucose was monitored twice; 15 min and immediately prior to glucose consumption (– 15 and 0 min). Glucose solution (25 g glucose in 250 mL water) was provided to the participants and blood samples were taken at 15, 30, 45, 60, 90 and 120 min after first sip of the drink. The same procedure was repeated for 33 g of fox nuts (containing 25 g of available carbohydrates) with 250 mL water.



Incremental Area Under the Curve (iAUC) for glucose responses by both standard glucose solution and test food were computed as proposed by Food and Agriculture Organization (FAO 1998). Glycemic index was calculated by following expression:

$$GI (\%) = \frac{iAUC \text{ for test food}}{iAUC \text{ for reference food}} \times 100$$

Development and sensory evaluation of snacks

Fox nuts were roasted $(130 \pm 2 \,^{\circ}\text{C})$ and seasoned with different flavors and compared with control (unroasted and bland nuts) for various sensory attributes. A 9-point hedonic scale was used with scores ranging from 1 (dislike extremely) to 9 (like extremely). Twenty semitrained panelists consisting of post-graduate students and faculty members were briefed about the scoring system and sensory characteristics to be evaluated. They evaluated the snacks for their aroma, texture, flavor, crunchiness, bite strength and overall acceptability (Mehyar et al. 2012; Meilgaard et al. 2006).

Analytical quality control and statistical analysis

For quality control, samples were extracted/digested in duplicates (Vaux et al. 2012) with two sample blanks. Minerals (K, Mg, P, Ca, Fe, Na and Zn) samples were run in triplicates on pre-calibrated Atomic Absorption Spectrophotometer (AA240, Varian, Australia). Mineral standards were purchased from Sigma (St. Louis, MO) and used for calibration of the equipment. Samples for proximates, TPC, TFC, DPPH, FRAP, color, texture and bulk density analysis were analyzed in triplicates. The results were expressed as mean values \pm standard deviation (SD).

Independent T-test was used to measure the impact of roasting on chemical composition, minerals content (K, Mg, P, Ca, Fe, Na and Zn), TPC, TFC, DPPH, FRAP, color values (L*, a*, and b*), texture, bulk density and calorie density. $P \leq 0.05$ was set for statistical significance. Analysis of variance (ANOVA) test was used to measure the effect of roasting and seasoning on sensory attributes of fox nut snacks; means were ranked by Tukey's Honestly Significant Difference (HSD) test ($P \leq 0.05$). All the statistical analyses were performed on software IBM-SPSS Statistics for Windows (Version 23.0. Armonk, NY: IBM Corp).

Results and discussion

Changes in chemical and mineral composition of fox nuts by roasting

Fox nuts exhibit a nutritious profile that significantly enhanced upon roasting (Table 1). Roasted nuts contained significantly lower moisture content (4.17%) and higher

 Table 1
 Chemical composition of control (unroasted) and roasted fox nuts (% dry basis)

Treatments	Control	Roasted
Moisture	12.07 ± 0.21^{a}	4.17 ± 0.15 ^b
Crude Ash	0.58 ± 0.04^{b}	0.66 ± 0.03^{a}
Crude protein	11.40 ± 0.44^{b}	14.57 ± 0.29^{a}
Crude fat	0.63 ± 0.05^{b}	0.68 ± 0.02^{a}
Crude fiber	0.41 ± 0.01^{b}	0.51 ± 0.02^{a}
Nitrogen free extract	74.91 ± 0.95^{b}	79.41 ± 0.82^{a}

Values are means±SD (n = 3). Mean values sharing different superscripts in rows show significant difference between the variables based on Independent T-test at $P \le 0.05$

crude protein (14.57%), crude ash (0.66%), crude fat (0.68%) crude fiber (0.51%) and nitrogen-free extract (79.41%) as compared to control (unroasted nuts). This nutrient condensation is mainly due to moisture loss from roasted nuts (65% as compared to control), when exposed to high temperature. Similar decline in moisture content to below 3% was also reported in pulses and nuts after roasting (Açar et al. 2009).

The crude protein content of nuts increased by 28% upon roasting (Table 1), which is comparable to the crude protein in walnuts, hazelnuts and pistachio nuts (13.7-17.1%) and higher than that in chestnuts (3.5%) (Kalogeropoulos et al. 2013). This rise imparts a lot to its health benefits, as protein rich diets are highly appreciated because of their satiating effect with moderate rise in blood glucose levels. Moreover, the protein content of fox nuts mainly consists of essential amino acids with Essential Amino Acid Index (EAAI) of 89% (Goren-Inbar et al. 2014). This makes fox nuts a good source of protein for vegetarian diet. This increase in protein content of fox nuts upon roasting is also supporting the increase in almonds (3%), peanuts (9%) (Süvari et al. 2017), sunflower seeds (37%) (Adesina 2019) and groundnuts (5%) (Kumar et al. 2013) after roasting.

An increase of 14% in ash content was observed upon roasting of fox nuts (Table 1) which also supports the

Table 2 Mineral profile of both control (unroasted) and roasted fox nuts (μ g g⁻¹ on d.w. basis)

Tox Traces (µg g = off a.w. basis)					
Minerals	Control	Roasted			
Potassium	450 ± 21^{b}	472 ± 18^{a}			
Magnesium	244 ± 16^{b}	263 ± 23^{a}			
Phosphorus	530 ± 28^{b}	564 ± 31^{a}			
Calcium	179 ± 09^{b}	192 ± 12^{a}			
Iron	109 ± 07^{b}	127 ± 10^{a}			
Sodium	220 ± 11^{b}	238 ± 14^{a}			
Zinc	15 ± 2^{b}	18 ± 3^{a}			

Values are means±SD (n = 6). Mean values sharing different superscripts in rows show significant difference between the variables based on Independent T-test at P \leq 0.05

increase in ash content of almonds (5%), peanuts (15%) and sunflower seeds (7%) upon roasting (Süvari et al. 2017). Significant rise in crude fat (8%), fiber (24%) and carbohydrate content (6%) was also reported in fox nuts upon roasting, which is also in accordance with the trend observed during roasting of groundnuts (Kumar et al. 2013). Carbohydrate content in fox nuts is significantly greater than other nuts (11.2 to 29.3%) which is a compositional compensation for a strikingly low fat content. As, fat content in different seeds and nuts ranges from 1.9 to 66.5% (Kalogeropoulos et al. 2013), fox nuts have comparatively lower fat (0.63–0.68%), which makes it a better choice for snacks over other high fat nuts, and also a good option for weight loss and frequent snacking.

Minerals are the nutrients that don't provide us with energy but are still crucial for various physiological mechanisms. Mineral profile of fox nuts is not exactly similar to other nuts. We chose to analyze these minerals (K, Mg, P, Ca, Fe, Na and Zn) in fox nuts based on their greater importance in the scenario of mineral deficiencies in developing countries like Pakistan (Ahsin et al. 2020; Government of Pakistan 2018). Almonds, cashews, pistachios, walnuts and macadamia nuts contain significantly higher content of potassium (3680 to $10,250 \,\mu g \, g^{-1}$), magnesium (1210 to 2920 μ g g⁻¹), phosphorus (188 to 5930 μ g g⁻¹) and calcium $(370 \text{ to } 2690 \,\mu\text{g g}^{-1})$ as compared to fox nuts. However, these nuts contain prominently lower concentration of iron $(29.1 \text{ to } 66.8 \,\mu\text{g g}^{-1})$ and sodium $(10 \text{ to } 120 \,\mu\text{g g}^{-1})$ in comparison to fox nuts. Whereas, Zn in fox nuts is somewhat around the range in other nuts (13 to 57 μ g g⁻¹) (Amarowicz et al., 2017). Roasted fox nuts reported significantly greater proportion of potassium (472 μ g g⁻¹), magnesium $(263 \ \mu g \ g^{-1})$, phosphorus $(564 \ \mu g \ g^{-1})$, calcium $(192 \ \mu g)$ g^{-1}), iron (127 µg g^{-1}), sodium (238 µg g^{-1}) and zinc $(18 \ \mu g \ g^{-1})$ as compared to control (unroasted fox nuts) (Table 2). K and Ca present in fox nuts plays a vital role in maintaining cardiovascular health. Whereas, Ca along with P and Mg is important for bone mineralization and musculo-skeletal health. Increase in mineral profile of fox nuts (~ 5 to 20%) after roasting is supported by increase in potassium (5%) and phosphorus (7%) in roasted groundnuts (Kumar et al. 2013) and calcium (36%), magnesium (18%), potassium (6%) and phosphorus (15%) in roasted brown sesame seeds (Tenyang et al. 2017). Therefore, consumption of fox nuts can contribute in combating micro nutrient deficiencies.

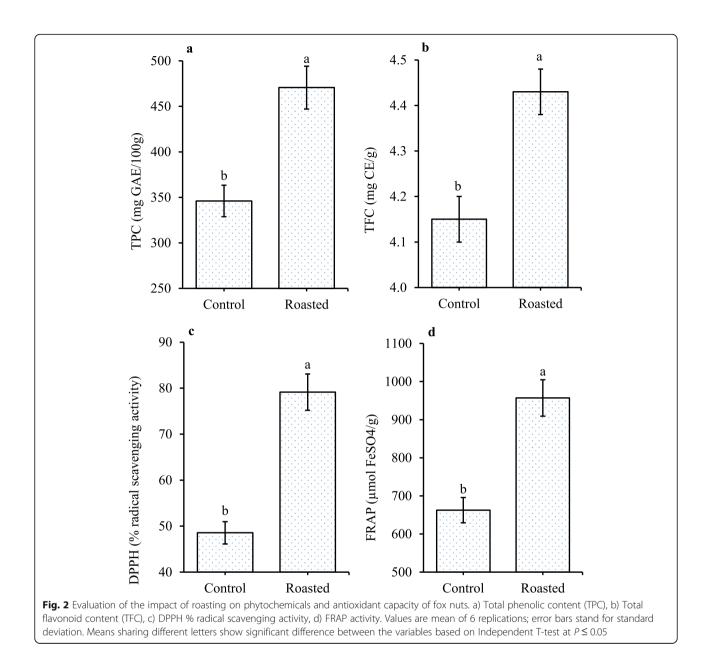
Roasted fox nuts contain higher phytochemicals with enhanced antioxidant activity

Fox nuts, in comparison to other nuts, exhibit higher total phenolics content (ranged from 346.02 to 470.62 mg GAE/100 g) than macadamias (46 mg GAE/100 g), cashews (137 mg GAE/100 g), brazil nuts (112 mg GAE/100 g) and almonds (239 mg GAE/100 g). However,

pistachios (867 mg GAE/100 g) and walnuts (1625 mg GAE/100 g) reported an opposite trend (Kornsteiner et al. 2006). Whereas, the fox nuts' flavonoids content (4.43 mg CE/g) was lower as compared to cashews (5.20 mg QE/g), almonds (4.58 mg QE/g) and pistachios (13.74 mg QE/g) (Ghazzawi & Al-Ismail 2017). Roasting significantly affected the TPC and TFC of fox nuts, which ultimately altered the antioxidant activity exhibited by nuts after roasting (Fig. 2). TPC was 36% higher in roasted nuts (470.62 mg GAE/100 g) as compared to control (346.02 mg GAE/100 g). Similar increase in TFC (7%) was also reported in roasted nuts as compared to control (4.15 mg CE/g) {Fig. 2(a,b)}. When we compare roasted fox nuts with other nuts in terms of antioxidant activity, it exhibits higher ability to scavenge DPPH free radicals (79.13%), as compared to roasted cashews (59.99%), pistachios (74.20%) and almonds (78.1%) (Ghazzawi & Al-Ismail 2017). In conclusion, total phenols and flavonoids are variable among different nuts but roasted fox nuts exhibit higher antioxidant capacity than most of the widely consumed nuts (i.e., cashews, pistachios, and pistachios).

The increase in TPC, TFC, DPPH and FRAP profile of roasted fox nuts was found to be significant, which might be due to release of bound phenolics and flavonoids or making the available phenolics more extractable and assayable (Duodu 2014). The heat applied to foods of plant origin causes evaporation of intracellular water, which triggers various chemical reactions and promotes protein denaturation resulting in greater availability of plant phenolics in the matrix (Rizki et al. 2015). A variety of byproducts and brown pigments (melanoidins) are also produced when amino acids and reducing sugars react at high temperature (maillard reaction). These Maillard Reaction Products (MRPs) contribute to the phenolic content of roasted product, exhibit high antioxidant activity and cause flavor changes; and also contributed to a significant increase in the antioxidant activity of cashew nuts' skin and kernels upon roasting (Chandrasekara & Shahidi 2011). Most of these products exhibit phenol or flavonoid like structures and are detected by the chemical reagents used in the analysis (Şahin et al. 2009). Melanoidins produced from these maillard reactions also improves the oxidative stability, aroma and flavor of oils extracted from roasted nuts (Suri et al. 2019).

Furthermore, the total antioxidant activity after roasting is the net activity exhibited by both the naturally occurring antioxidant compounds and newly formed Maillard reaction products. Therefore, roasting can affect both the nutritional and bioactive characteristics of foods (Rizki et al. 2015). The findings of this study supports increase in total antioxidant activity of cashew nuts (up to 60%) after roasting, in contrast to other nuts. This increase was reported due to greater amount of starch

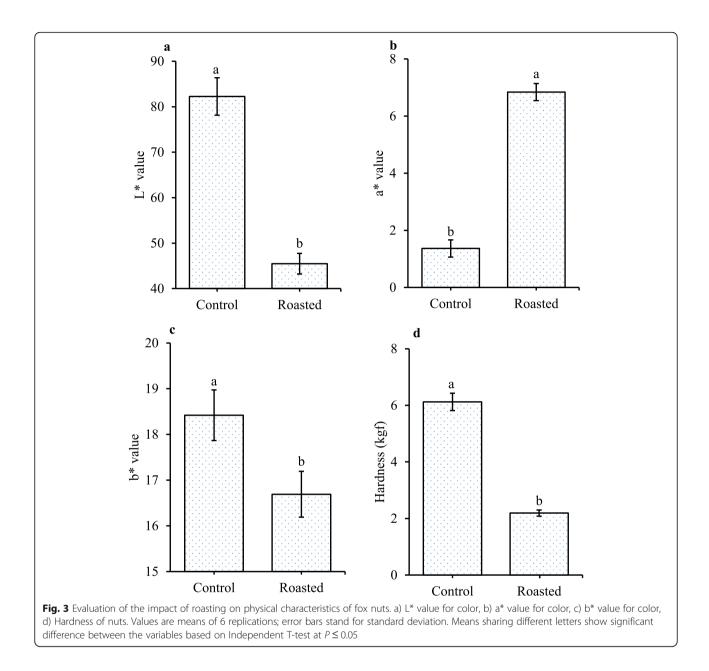


(23%) in cashew nuts, as compared to almonds (0.74%), hazelnuts (0.48%), pistachio (1.67%) and pine nuts (1.43%), which leads to formation of more antioxidant compounds through Maillard reactions (Açar et al. 2009).

Impact of roasting on color and texture of fox nuts

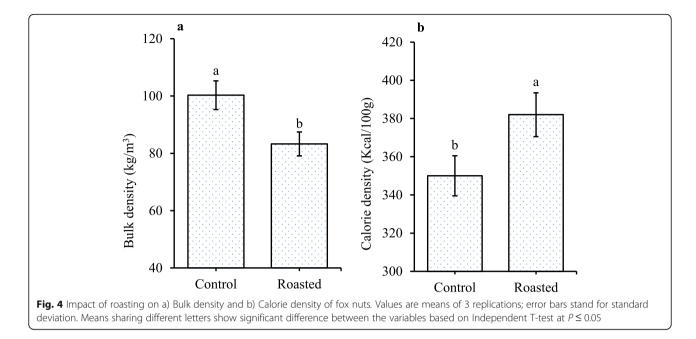
Significant ($P \le 0.05$) impact of roasting was observed on color and texture of fox nuts (Fig. 3). Color of the nuts was measured in terms of lightness L* and chromaticity co-ordinates a* and b*. Color is one of the important characteristics of foods that attracts the consumer. Lightness (L*) was reported to be 45% lower in roasted nuts as compared to control (82.25), which is due to increase in brown color {Fig. 3(a)}. Similar decrease in L* value (up to 41%) was reported in corn kernels (Chung et al. 2014) due to increase in production of brown colored compounds with increase in temperature and time of roasting. Approximately, 12% decrease in L^* value of pistachio nuts is also reported after roasting (Hojjati et al. 2015).

Roasted fox nuts showed 9% decrease in b* value as compared to control (18.42) and ~ 400% higher red tint (a*) than control (1.37) {Fig. 3(b and c)}. This increase in red tint occurred as a result of caramelization and browning reactions as reported in pistachio nuts (~ 350% higher) after roasting (Hojjati et al. 2015). Similar increase in redness was also observed in corn kernels, ranging from 200 to 600% upon exposure to different roasting temperatures (Chung et al. 2014).



A significant decline of ~64% was noted in the force required to break the kernels after roasting {Fig. 3(d)}. Means for hardness of nuts decreased from 6.12 kgf to 2.19 kgf upon roasting. This decrease in breaking force is due to increase in crispiness of fox nuts upon roasting. As moisture loss reduced its soggy texture and increased crispiness. Pistachio nuts also showed a decline (55%) in hardness after roasting (Moghaddam et al. 2016). This declining trend is also in agreement with the findings for walnuts (Kita & Figiel 2006) and pistachio nuts (Nikzadeh & Sedaghat 2008), which confirms the use of roasting in decreasing the hardness of nuts.

Impact of roasting on bulk and calorie density of fox nuts Loss of moisture content in roasted nuts led to a 17% decrease in its bulk density {Fig. 4(a)}. Roasting significantly ($P \le 0.05$) decreased the bulk density of fox nuts from 100.30 kg/m³ to 83.31 kg/m³. The results were quite in accordance to literature (Jha & Kachru 1998), which reported bulk density of makhana ranging from 70 to 90 kg/m³ that varied with its moisture percentage. This decrease in bulk density was accompanied by a significant ($P \le 0.05$) rise (~ 9%) in calorie density from 350 kcal/100 g to 382 kcal/100 g after roasting {Fig. 4(b)}. Thus, equally weighed roasted nuts will give more calories as compared to unroasted ones.



Fox nuts exhibit low glycemic index (GI) in human subjects

A positive correlation ($r^2 = 0.81$) was observed between GI and BMI of the participants (Table 3). Glycemic index of roasted fox nuts was 37.05%. Incremental areas under the glucose response curve are presented for both test food (Fox nuts) and reference food (glucose powder), having an equal amount of available carbohydrates (Fig. 5). Based on GI, foods are classified into three categories; low GI foods (GI < 55%), medium GI foods (70% > GI > 55%) and high GI foods (GI > 70%) (Widanagamage et al. 2009).

According to a recent systematic review on international tables of glycemic index values, nuts reported the lowest average GI values (22 ± 2) amongst all other food groups, because they constitute very low amount of carbohydrates (Atkinson et al. 2021). However, fox nuts make an exception as they contain comparatively higher carbohydrates and a higher GI, but still remain classified as low GI (37%) snacks and ultimately preferable over other widely consumed moderate to high GI snacks like popcorns (55–89%), potato crisps (57%) jelly beans (80%) and muesli bars (61%) (Foster-Powell et al. 2002).

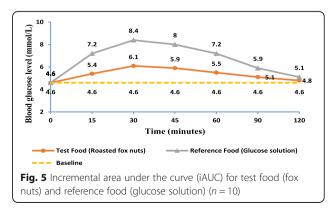
Table 3 Characteristics of participants (n = 10) enrolled and glycemic responses reported in glycemic index (Gl) study

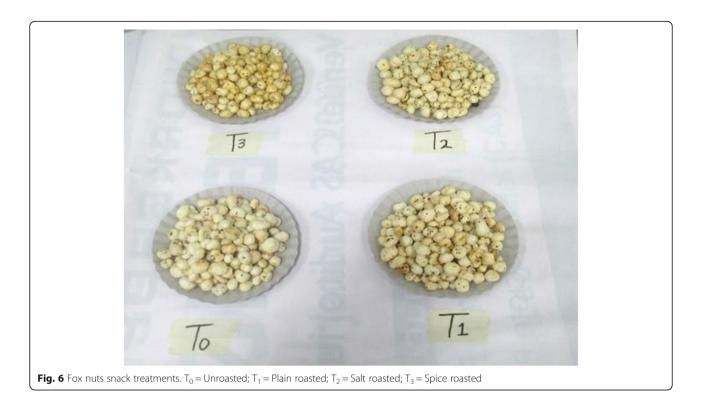
Parameters	Values
BMI (kg/m²)	22.1 ± 1.60
iAUC test food (mmol.min/L 2 h)	86.30 ± 6.74
iAUC reference food (mmol.min/L 2 h)	232.67 ± 6.41
Glycemic Index (%)	37.05 ± 1.95
Glycemic Load	9.26 ± 0.49

Low GI foods have a potential beneficial impact on the prevention and management of metabolic syndrome (Livesey et al. 2008). Low GI foods are digested and absorbed slowly thus preventing a sudden rise in postprandial blood glucose levels. Apart from all these, low GI diets are also helpful in weight management and are in fact better than low-fat diets, as low GI diets are also associated with increasing the rate of resting energy metabolism (Pereira et al. 2004). Due to their slow digestion and absorption, foods with a low GI are associated with reduced hunger, increased satiety, decrease in voluntary food intake, reduction in insulinemia and ultimately promoting fat oxidation as a source of fuel for energy (Brand-Miller et al. 2009).

Development and sensory evaluation of fox nuts snacks

In typical omnivorous diet, nuts and seeds are either consumed as snack items or added to savory and sweet dishes as minor ingredients, but they are widely consumed in vegetarian diets as important source of protein





and other nutrients (Gray 2013). Fox nuts being high in protein, low in fat and low in GI offers numerous health benefits.

Fox nuts were seasoned with salt and spices (Fig. 6). Roasted nuts gained significantly ($P \le 0.05$) higher scores for aroma and texture after seasoning as compared to control and plain roasted samples. A significant ($P \le 0.05$) change was also observed among the flavor, crunchiness, bite strength and overall acceptability of the snacks (Table 4). Aroma (7.47), texture (7.74), flavor (7.68) and crunchiness (7.84) of spice roasted snacks were liked the most by consumers. The best response for bite strength (7.68) and overall acceptability (7.68) was observed for both salt and spice roasted nuts. Roasting improved the crunchiness (82%), bite strength (36%) and texture (82%) of fox nuts with plain roasting leading to 23 times higher overall acceptability of nuts. Whereas, addition of salt and spice for seasoning improved the flavor (8%) and overall acceptability (7%) of fox nuts, in comparison to plain roasting.

Conclusion

The results shed light on the efficiency of roasting in making crispy and nutritionally superior product from raw fox nuts. Roasted fox nuts were figured out to contain higher phenolic and flavonoid content with enhanced antioxidant activity as compared to unroasted nuts. Roasting also increased the protein and mineral content of the nuts, thus increasing its nutrient density. High protein content of fox nuts also makes it a good source of protein for vegetarian diet. Furthermore, the prominent outcome of the study is the low GI of fox nuts, which makes it a healthy and nutritious snack in comparison to other widely consumed processed and energy-dense snacks. Roasting with added salt and spice increased the consumer acceptability of the nuts. Thus it is concluded that roasted fox nuts are better alternative for widely used high-fat and high-calorie snacks. Its low GI offers various health benefits including reduced hunger, increased satiety and obesity management. Hence it

Table 4 Mean values for sensory attributes of fox nuts snack treatments

Treatments	Aroma	Texture	Flavor	Crunchiness	Bite Strength	Overall Acceptability		
To	6.37 ± 1.42^{b}	5.84 ± 2.14^{b}	5.58 ± 1.92 ^b	4.10 ± 2.42^{b}	5.47 ± 2.22 ^b	5.84 ± 1.64 ^b		
T ₁	7.00 ± 1.11^{ab}	7.32 ± 1.29^{a}	7.11 ± 1.10^{a}	7.47 ± 1.47^{a}	7.42 ± 1.07^{a}	7.16 ± 0.96^{a}		
T ₂	7.42 ± 1.02^{a}	7.63 ± 1.07^{a}	7.37 ± 1.07^{a}	7.63 ± 0.83^{a}	7.68 ± 0.82^{a}	7.68 ± 1.00^{a}		
T ₃	7.47 ± 0.90^{a}	7.74 ± 1.10^{a}	7.68 ± 1.06^{a}	7.84 ± 1.01^{a}	7.68 ± 1.06^{a}	7.68 ± 1.00^{a}		

Values are expressed as means \pm SD. Means sharing different superscripts along the columns are significantly different based on Tuckey's HSD test at $P \le 0.05$. T0 = Unroasted; T1 = Plain roasted; T2 = Salt roasted; T3 = Spice roasted

is a safe and healthy option for vegetarians and those with metabolic disorders, as well as for general population as a healthy snack. Nutrition data of unroasted and roasted fox nuts derived from this research open new avenue for researchers to explore its prospective health benefits. A lot of research still needs to be done to explore its nutritional composition at a deeper level including the impact of roasting on amino acids, individual carbohydrates and characterization of flavor active compounds formed as a result of roasting.

Abbreviations

GAE: Gallic acid equivalent; CE: Catechin equivalent; DPPH: 2,2-diphenyl-1picrylhydrazyl; FRAP: Ferric reducing antioxidant power; GI: Glycemic Index; EAAI: Essential amino acid index; NCDs: Non-communicable diseases;; HPLC: High performance liquid chromatography; AOAC: Association of official analytical chemists; TPC: Total phenolic content; TFC: Total flavonoid content; BMI: Body mass index; ISO: International Standardization Organization; iAUC: incremental area under the curve; FAO: Food and agriculture organization.

Acknowledgements

This work is part of a master's thesis by Marrium Liaquat. We are thankful to all the graduate students and faculty members who volunteered for sensory evaluation and efficacy trial.

Funding

No funding was received to assist with the preparation of this manuscript.

Authors' contributions

ML, IP and AS conceived and designed the study. ML and MA performed the experiments and worked on data analysis and interpretation. ML drafted the manuscript and all authors revised the manuscript and approved the final version to be submitted.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Declarations

Ethical approval and consent to participate

All the procedures followed for glycemic index study were according to the guidelines of National Biosafety Committee 2005 and Punjab Biosafety Rules 2014 and human ethical clearance was taken from Institutional Biosafety and Bioethics Committee (IBC) of University of Agriculture, Faisalabad, with protocol number 1772/01-06-2020. Informed consent was obtained from all individual participants included in the study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Received: 24 November 2021 Accepted: 26 December 2021 Published online: 18 January 2022

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