Nutritional composition, antinutritional factors, bioactive compounds and antioxidant activity of guava seeds (*Psidium Myrtaceae*) as affected by roasting processes

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Abstract The purpose of this study was to explore the influences of roasting process on the nutritional composition and nutritive value, antinutritional factors, bioactive compounds and antioxidant activity of guava seeds. Roasting process caused significant ($P \leq 0.05$) decreases in moisture content, crude protein, crude fiber, ash and mineral contents, isoleucine, arginine, glutamic and total aromatic and sulfur amino acids, antinutritional factors (tannins and phytic acid) and flavonoids, while oil content increased. Subjecting guava seeds to 150 °C for 10, 15 and 20 min increased the total essential amino acids from 35.19 g/100 g protein in the raw sample to 36.96, 37.30 and 37.47 g/100 g protein in roasted samples, respectively. Protein efficiency ratio (PER) of guava seeds roasted at 150 °C for 10, 15 and 20 min were about 1.08, 1.14 and 1.18 times as high as that in unroasted seeds. Lysine was the first limiting amino acid, while leucine was the second limiting amino acid in raw and roasted guava seeds. Total phenolic contents was significantly ($P \leq 0.05$) increased by roasting at 150 °C for 10 min. However, roasting at 150 °C for 15 and 20 min caused significant decrease in the phenolic content of guava seeds. Guava seeds subjected to roasting process showed higher DPPH radical scavenging and reducing power activities.

Keywords Guava seeds · Roasting · Nutritional composition · Antinutritional factors · Phenolic contents · DPPH radical scavenging activity · Reducing power activity

Introduction

Waste disposal and by-product management in food processing industry pose problems in the areas of environmental protection and sustainability (Russ and Pittroff 2004). Therefore, strategies for the profitable use of these materials are needed. Several studies on the use of waste products generated by the food industry (Liadakis et al. 1995; Ravindran and Siuvakanesan 1996; Bernardino-Nicanor et al. 2001) have shown that these products are an alternative source of oil and protein for human and animal feeding. Examples of such products are tomato seed (Yaseen et al. 1991; Liadakis et al. 1995) and sesame seed (El-Adawy 1997). Guava seeds are another example, since two of the biggest processors of guava in Mexico discard about 120 t/year of this material. Besides causing contamination problems and transfer expenses, guava seeds may represent an important protein source (Bernardino-Nicanor et al. 2001). The seeds are 6 to 12 % of the fruit weight (Córdoba 1994). Their proximate composition, on a wet weight basis is: 7.6 % protein, 16.0 % fat, 61.4 % crude fiber, 0.93 % ash and 4.1 % water. The fat contains 11.8 % saturated fatty acids and 87.3 % unsaturated fatty acids (76.5 % polyunsaturates). The fatty acids are 0.1 % myristic, 6.6 % palmitic, 4.6 % stearic, 10.8 % oleic, 76.4 % linoleic, 0.3 % arachidonic and 0.1 % linolenic (Prasad and Azeemoddin 1994). The in vitro digestibility of storage proteins of guava seeds is higher than that of the soybean isolate (94.8 g/100 g vs. 89.9 g/100 g). Except for lysine content, the essential amino acid profile is above that recommended in the FAO/WHO (1985) pattern for adults (Bernardino-Nicanor et al. 2001). Several thermal and hydro thermal processing techniques were used for improving the nutritive values and removing antinutritional factors of seeds. These include: dry heating (Papadopoulos 1987; ASA 1997; Mridula et al. 2008), toasting (Tamiyu 2001), cooking (Kaankuka et al. 1996), extrusion (Asiedu 1989; ASA 1997), autoclaving (Balogun

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1989) and infrared (Horani 1987; Ebrahimi-Mahmoudabad and Taghinejad-Roudbaneh 2011; Rathnayaka 2012). Roasting is heat treatment used to induce the development of the typical colour, taste and flavour; it also changes the chemical composition, modifying nutritional value and shelf life (Ozdemir and Devres 2000). The aim of the present investigation was to explore the influences of roasting process on the nutritional composition and nutritive value, antinutritional factors, bioactive compounds and antioxidant activity of guava seeds.

Materials and methods

Chemical reagents

Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate and aluminum chlorid were purchased from Sigma Chemical Co., Ltd (St. Louis, MO, USA). Gallic acid, chloroform, ethanol, acetone, and methanol were purchased from E. Merck Co. (Darmstadt, Germany), and distilled before use. All other reagents were of analytical grade.

Collection of seeds

Guava seeds were collected from Paste and Juice (P&J) company, Sadat city, Monufia, Egypt. Guava seeds were washed with excess water to remove adhering materials and sun-dried.. The dried seeds were packed in plastic bags and stored in a freezer (−25 °C) until further use.

Preparation of guava seeds

Roasting

Guava seeds were arranged a single layer in a Pyrex Petri dish (12×8.0 cm) and roasted in an electric muffle furnace (NEY, Model No M525 S II, U.S.A.) at 150 °C for 10, 15 and 20 min. The roasted seeds were allowed to cool to ambient temperature, milled (Braun, Model 1021, Germany) and stored in glass containers at 4 °C for further analysis (Mariod et al. 2012).

Analytical methods

Chemical composition

The treated and untreated samples were analyzed for moisture, crude oil, crude protein ($N \times 6.25$), crude fiber and ash as described in A.O.A.C (2000). The nitrogen free extracts (NFE) was calculated by difference. The minerals, i.e., Ca, Fe, Zn, K, Cu, P, Mg and Na were determined in a dilute

solution of the ashed samples by atomic absorption spectrophotometer (3300 Perkin – Elmer) as described in A.O.A.C (2000).

Antinutritional factors

Total tannins were determined colorimetrically as described in AOAC (1990). Phytic acid was determined according to the method of Wheeler and Ferrel (1971).

Amino acids

Amino acids were determined according to method of Moore and Stein (1963). Hydrolysis of the samples was performed in the presence of 6 M HCl at 110 °C for 24 h under a nitrogen atmosphere. Sulfur-containing amino acids were determined after performic acid oxidation. Tryptophan was chemically determined by the method of Miller (1967). The amino acid score (AAS) was calculated for each essential amino acid using the WHO/FAO (1991) reference pattern as follows:

$$\text{AAS} = \frac{\text{Concentration of essential AA in the protein under test}}{\text{Concentration of essential AA in the FAO}} \times \text{WHO standard} \times 100.$$

Values for AAS lower than 100 indicate a deficiency of that amino acid. The limiting amino acid (LAA) was defined as that showing the lowest AAS value Chavan and Heigaard 1981). Protein efficiency ratio (PER) was estimated using the regression equation proposed by Alsmeyer et al. (1974):

$$\text{PER} = -0.468 + 0.454(\text{leucine}) - 0.105(\text{tyrosine}).$$

Extraction and quantification of total phenolics and flavonoids

Preparation of the extract

Extraction was carried out according to the method of Kim et al. (2006). Raw and roasted seeds were ground to a fine powder in a laboratory (Perten, USA) grinder. Guava seeds (5 g) were extracted overnight with 100 mL of 70 % ethanol solution in a shaking incubator (100 rpm) at room temperature. Then the extracts were centrifuged at 3500 rpm for 15 min. The supernatants were filtered through a Whatman No.1 filter paper. Extract solutions were concentrated to dryness in a vacuum evaporator at 45 °C. Dried extracts were kept in the dark at −20 °C until further analyses.

Determination of total polyphenols

Total phenolic content of the extracts were determined using the Folin-Ciocalteu assay system (AOCS 1990). A 0.5 ml extract was added with 2.5 ml of Folin-Ciocalteu reagent followed by addition of 2 ml sodium carbonate (Na_2CO_3) (75 g/l). The sample was then incubated for 5 min at 50 °C. The absorbance was measured at 760 nm. Phenolic contents were calculated on the basis of the standard curve for gallic acid (GAL). The results were expressed as mg of gallic acid equivalent per 100 g DW.

Determination of total flavonoid

The total flavonoid content was determined using the Dowd method (Meda et al. 2005). 5 mL of 2 % aluminium trichloride (AlCl_3) in methanol was mixed with the same volume of the extracts solution (0.4 mg/mL). After 10 min the absorbance was measured at 415 nm using PerkinElmer UV–VIS Lambda. Blank sample consisting of a 5 mL extract solution with 5 mL methanol without AlCl_3 . The total flavonoid content was determined using a standard curve with catechin (0–100 mg/L) as the standard. Total flavonoids content is expressed as mg of catechin equivalents (CE)/100 g DW.

Determination of antioxidant efficiency

a) Free radical scavenging capacity

The free radical scavenging capacity of various solvent extracts of guava seeds was measured by using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay (Juntachote and Berghofer 2005). Aliquot (100 μL) of extract was mixed with 5 ml of 6×10^{-3} M methanolic solution of DPPH radical. The mixture was shaken vigorously and left to stand for 30 min in the dark. The absorbance was then measured at 517 nm against a blank. The control was prepared, as above, without any extract and methanol was used for the base line correction. The radical-scavenging activity was expressed as percentage of inhibition and calculated using the following formula:

% Radical scavenging activity

$$= \frac{[(\text{Abs control} - \text{Abs sample}) / (\text{Abs control})] \times 100}{1}$$

Where, Abs control is the absorbance of DPPH radical + methanol; Abs sample is the absorbance of DPPH radical + sample extract/standard.

b) Determination of reducing power

The ability of the extracts to reduce Fe^{3+} was assayed by the method of Chou et al. (2009). Briefly, 1 ml of extract was mixed with 2.5 ml of phosphate buffer

(0.2 mol/L, pH 6.6) and 2.5 ml of $\text{K}_3\text{Fe}(\text{CN})_6$ (1 g/100 ml). After incubation at 50 °C for 25 min, 2.5 ml of trichloroacetic acid (10 g/100 ml) were added and the mixture was centrifuged at $650 \times g$ for 10 min. Finally, 2.5 ml of the upper layer was mixed with 2.5 ml of distilled water and 0.5 ml of aqueous FeCl_3 (0.1 g/100 ml). The absorbance was measured at 700 nm. BHT was used as reference standard. Higher absorbance of the reaction mixture indicated greater reducing power.

Statistical analysis

All analyses were performed in triplicate and data reported as mean $\pm$ standard deviation (SD). Data were subjected to analysis of variance (ANOVA) ($P \leq 0.05$). Results were processed by Excel (Microsoft Office 2007) and SPSS Version 18.0 (SPSS Inc., Chicago, IL, USA).

Results and discussion

Chemical composition

Chemical compositions of raw and roasted guava seeds are presented in Table 1. Moisture content of raw seed was 8.06 ± 0.31 %. Roasting process caused significant decreases in moisture content. The percentage of decrements ranged from 29.15 to 44.78 %. This decrease was gradually and significantly increased with increasing roasting time. These decreases could be attributed to the water evaporating during roasting process. These results are in good agreement with those reported by Adegoke et al. (2004) and Mariod et al. (2012) who reported that roasting processes decreased moisture content of peanut and safflower seeds, respectively. Unroasted guava seeds have significantly the higher level of protein 8.41 %. Roasting process caused significant reductions in crude protein. The protein content decreased significantly from 8.41 (g per 100 g) in raw samples to 8.02, 7.72 and 7.52 in those samples roasted at 150 °C for 10, 15 and 20 min, respectively. These reductions in protein content may be attributed to the protein denaturation during roasting process (Bradbury et al. 1984; Sahni et al. 1997). In this regard maillard reactions between protein and sugars reduce the nutritional value of the protein, depending on the raw material types, their composition and process conditions (Singh et al. 2007; Adeyeye 2010). These results are agreement with EL-Beltagi 2011 who observed significant protein denaturation in peanuts after 45 min roasting at 180 °C. Oil content of raw guava seeds was 15.35 %. Oil content of roasted seeds was higher than that of raw seeds. Roasting process caused significant increases in oil content. This increase was gradually and significantly increased with increasing roasting time. The oil

Table 1 Proximate composition of raw and roasted guava seeds (g/100 g dry weight basis)

Treatment	Moisture	Crude protein (Nx6.25)	Ether extract	Crude fiber	Ash	Total carbohydrate*
Raw	8.06 ^a ±0.31	8.41 ^a ±0.24	15.35 ^c ±0.41	46.21 ^a ±0.27	1.43 ^a ±0.05	28.60 ^a ±0.26
Roasting at 150 °C						
10 min	5.71 ^b ±0.15	8.02 ^b ±0.13	17.70 ^b ±0.27	44.86 ^b ±0.11	1.27 ^{ab} ±0.11	28.15 ^{ab} ±0.45
15 min	4.88 ^c ±0.12	7.72 ^{bc} ±0.24	19.21 ^a ±0.08	44.07 ^c ±0.06	1.21 ^b ±0.02	27.79 ^b ±0.08
20 min	4.45 ^d ±0.09	7.52 ^c ±0.09	19.61 ^a ±0.14	43.70 ^d ±0.10	1.16 ^b ±0.13	28.01 ^{ab} ±0.16
LSD at 0.05	0.353	0.352	0.486	0.295	0.168	0.506

Data are expressed as mean ± standard deviation (SD). Values given represent means of three determinations. Values followed by the same letter are not significantly different ($p < 0.05$)

*By difference

content of guava seeds roasted at 150 °C for 10, 15 and 20 min were about 1.15, 1.25 and 1.27 times as high as that in unroasted seeds. The increase in oil content in guava seed is seemed to be as result of subjection of samples to temperature of roasting process (Mariod et al. 2012). Untreated seeds had significantly the higher level of crude fiber (46.21±0.27 %) therefore; guava seeds could be considered as a good source of dietary fiber. This is an interesting finding since the consumption of dietary fiber has been related to prevention of cardiovascular disease, diabetes, and digestive tract diseases, considering that it lowers the glycemix index of food as well as serum cholesterol levels (Vadivel et al. 2012). Roasting process caused significant reductions in crude fiber. Roasting is a common heating processes applied to legumes, which generally leads to a significant reduction in insoluble dietary fiber and total dietary fiber but an increase in soluble dietary fiber (Mahadevamma and Tharanathan 2004). Ash content of raw guava seeds was 1.43±0.05 %. Roasting treatments significantly ($P \leq 0.05$) decreased the ash contents of guava seeds. This decrease was gradually and significantly increased with increasing roasting time. These decreases could be attributed to the loss of vegetable part of the seed (kernels) during roasting (Mbah et al. 2013). The carbohydrate content decreased from 28.60 % in raw samples to 28.15, 27.79 and 28.01 in guava seeds roasted at 150 °C for 10, 15 and 20, respectively. These decreases in carbohydrate content during roasting process can be attributed to Maillard's reaction (Adeyeye 2010).

Mineral composition

Mineral contents of raw and roasted guava seeds are presented in Table 2. Potassium (K) is predominant element in guava seeds 564.01 mg/100 g dry weight basis. Potassium, sodium, Phosphorus, calcium and Magnesium constituted the major minerals in guava seeds 564.01, 326.81, 212.14, 143.37 and 119.72 mg/100 g dry weight basis, respectively. Guava seeds contain low levels of Iron, Zinc, copper and manganese. These levels of minerals were lower than those obtained by El-Safy

et al. (2012). These differences can be attributed to soil, climate, strain, variety and agronomic practices. No significant ($P \leq 0.05$) differences in zinc, copper and manganese contents were observed between raw and roasted seeds. Concentrations of major elements such as Na, Ca, K and P in raw guava seeds were significantly ($P < 0.05$) higher than roasted seeds. Roasting process significantly ($P \leq 0.05$) decreased the mineral contents of guava seeds. These results are in good agreement with those reported by Mariod et al. (2012) who reported that roasting processes decreased the sodium and calcium content of raw safflower seeds by about 39.2 and 18.1 %, respectively.

Amino acid composition and protein quality of raw and roasted guava seeds

The nutritional quality of a protein is principally governed by its amino acid composition. Data presented in Table 3 show the amino acid composition of raw and roasted guava seeds. Eight amino acids are generally regarded as essential for humans: phenylalanine, valine, threonine, tryptophan, isoleucine, methionine, leucine and lysine (Young 1994). Additionally, cysteine (or sulphur-containing amino acids), tyrosine (or aromatic amino acids), deficiency in these amino acids may hinder healing recovery process (Mat Jais et al. 1994). Results indicated that total essential amino acids of guava seeds protein formed 35.19 % of the total amino acid content. Guava seeds protein was rich in essential amino acids such as isoleucine, total sulfur amino acids, total aromatic amino acids, Threonine and tryptophan compared with the FAO/WHO (1991) reference. However, leucine and lysine were slightly deficient in Guava seeds protein compared with the reference pattern. Total amount of nonessential amino acids represented 64.81 % of the total amino acid content. Glycine, Glutamic and arginine acids were found to be the major non-essential amino acids in guava seeds protein 18.75, 9.06 and 8.52 %, respectively. Subjecting guava seeds to 150 °C for 10, 15 and 20 min increased the total essential amino acids from 35.19 g/100 g protein in the raw sample to 36.96, 37.30 and 37.47 g/

Table 2 Mineral composition of raw and roasted guava seeds (mg/100 g dry weight basis)

Minerals	Raw	Roasting			LSD at 0.05
		10 min	15 min	20 min	
Sodium	326.81 ^a ±11.27	226.41 ^b ±7.89	214.12 ^{bc} ±12.30	196.19 ^c ±9.35	19.475
Potassium	564.01 ^a ±7.25	563.08 ^a ±6.38	563.00 ^a ±13.46	560.17 ^a ±11.54	19.006
Calcium	143.37 ^a ±3.56	120.20 ^b ±4.44	105.87 ^c ±4.21	97.32 ^d ±5.23	8.285
Magnesium	119.72 ^a ±2.19	115.47 ^a ±2.36	105.36 ^b ±5.37	102.85 ^b ±3.91	6.949
Phosphorus	212.14 ^a ±1.07	212.19 ^a ±3.14	213.05 ^a ±3.83	212.07 ^a ±4.39	6.311
Iron	13.94 ^a ±2.21	9.06 ^b ±0.59	7.14 ^b ±0.87	4.18 ^c ±0.96	2.474
Zinc	3.15 ^a ±0.41	3.15 ^a ±0.70	3.12 ^a ±0.09	3.02 ^a ±0.24	0.800
Copper	1.91 ^a ±0.13	1.91 ^a ±0.19	1.93 ^a ±0.04	1.95 ^a ±0.12	0.247
Manganese	1.17 ^a ±0.17	1.18 ^a ±0.08	1.17 ^a ±0.16	1.17 ^a ±0.19	0.273

Values are means ± standard deviation (SD) of three determinations. Values followed by the same letter are not significantly different ($p < 0.05$)

100 g protein in roasted samples, respectively. The roasting caused native protein aggregation, which might simply result from the typically loss of tertiary structure followed by

Table 3 Amino acid composition of raw and roasted guava seeds (g/100 g protein)

Amino acid	Raw	Roasting at 150 °C			FAO/WHO pattern ^a
		10 min	15 min	20 min	
Isoleucine	4.05	3.92	3.89	3.81	2.8
Leucine	5.93	6.25	6.35	6.50	6.6
Lysine	0.91	0.93	0.94	0.96	5.8
Cystine	2.13	1.98	1.90	1.90	—
Methionine	3.52	3.41	3.39	3.34	—
Total sulfur amino acids	5.65	5.39	5.29	5.24	2.5
Tyrosine	4.12	4.00	3.94	3.93	
Phenylalanine	2.61	2.46	2.39	2.37	
Total aromatic amino acids	6.73	6.46	6.33	6.30	6.3
Threonine	3.93	5.10	5.50	5.66	3.4
Tryptophan	2.64	2.31	2.25	2.20	1.1
Valine	5.35	6.60	6.75	6.80	3.5
Total essential amino acids	35.19	36.96	37.30	37.47	
Histidine	2.02	2.90	2.95	2.95	1.9
Arginine	8.52	7.10	6.68	6.65	
Aspartic acid	5.49	6.50	7.05	7.63	
Glutamic acid	9.06	9.00	8.75	8.55	
Serine	5.98	6.15	6.30	6.63	
Proline	8.65	8.40	8.20	8.00	
Glycine	18.75	17.50	17.30	17.20	
Alanine	6.34	6.10	6.00	5.97	
Total non-essential amino acids	64.81	63.50	63.78	63.20	

^a FAO/WHO pattern (FAO/WHO 1991)

(reversible) unfolding, loss of secondary structure, cleavage of disulphide bonds, formation of new intra-/inter-molecular interactions, rearrangements of disulphide bonds and the formation of aggregates. These modifications reflect a progressive passage to a disorganized structure with denaturation of the proteins that adopt an unfolded the denatured molecules associate to form aggregates (Luis et al. 2005). Roasting process decreased the concentration of isoleucine, arginine, glutamic and total aromatic and sulfur amino acids. The composition of the amino acids varies dependent on their thermal stability and reactions involved. For instance, changes in glutamic acid content are less dramatic as compared to cysteine and arginine. The latter amino acids tend to deplete rapidly during roasting due to their involvement in Maillard browning reactions (Illy and Viani 2005). Protein efficiency ratio (PER) of unroasted guava seeds was 1.79. Protein efficiency ratio (PER) was improved by roasting treatments. Protein efficiency ratio (PER) of guava seeds roasted at 150 °C for 10, 15 and 20 min were about 1.08, 1.14 and 1.18 times as high as that in unroasted seeds. The improvement of protein efficiency ratio (PER) is attributed to the increase in the levels of Leucine amino acid and the reduction of tyrosine amino acid during roasting process. Lysine was the first limiting amino acid, while leucine was the second limiting amino acid in raw and roasted guava seeds. The results of the current study agree with the findings of Bernardino-Nicanor et al. 2001, who found that lysine was the first limiting amino acid in guava seeds. The chemical score, an index of protein quality was estimated by comparing the essential amino acid contents of raw and roasted guava seeds with a reference amino acid pattern (FAO/WHO (1991). chemical score of raw and roasted guava seeds are given in Table 4.

Chemical score of raw guava seeds was 15.68. Chemical score of guava seeds protein was improved slightly by roasting process. These results are in good agreement with

Table 4 Protein nutritional quality of raw and roasted guava seeds

Treatment	PER ^a	CS ^b (%)	Limiting amino acids	
			First	Second
Raw	1.79	15.68	Lysine	Leucine(89.84)
Roasting				
10 min	1.94	16.03	Lysine	Leucine(94.69)
15 min	2.05	16.20	Lysine	Leucine(96.21)
20 min	2.13	16.55	Lysine	Leucine(98.48)

^a Protein efficiency ratio^b Chemical score

those reported by Alajaji and El-Adawy (2006) who reported that the chemical score and limiting amino acid varied considerably depending on treatment.

Antinutritional factors

Antinutrients, commonly found in plant food, have both adverse effects and health benefit. One common example is phytic acid, which forms insoluble complexes with calcium, zinc, iron and copper. Another particularly widespread form of antinutrients are the flavonoids, which are a group of polyphenolic compounds that include phenolic compounds (tannins), saponins and enzyme (amylase and protease) inhibitors (Reyden and Sel Vendran 1993). The antinutritional factors of raw and roasted guava seeds are shown in Table 5. Tannins and phytic acid content of raw seeds were 325.67 and 273.63 mg/100 g) on dry weight basis, respectively. Roasting process caused significant decreases in Tannins and phytic acid content. These decreases were gradually and significantly increased with increasing roasting time. Tannins (11.89–52.01 %) and phytic acid (52.00–61.36 %) in guava seeds were significantly ($P \leq 0.05$) reduced by roasting process. The highest reductions were caused by roasting at 150 °C for 20 min. The reduction of tannins and phytic acid content of roasted seeds may due to the effect of heat treatment. Heating destroys the naturally occurring antinutritional factors and is employed for reduction of anti-nutrients in the plant based foods thus enhances the nutritional value of isolated protein (Seena et al. 2006). Phytic acid is relatively heat-stable, hence,

significant and prolonged inputs of energy are requires for its destruction (Sharma and Sehgal 1992). Similar reductions in phytic acid and tannins levels in whole beans processed under various conditions, including soaking, roasting, autoclaving, and pressure-cooking have been reported (Alonso et al. 2000; ElMaki et al. 2007; Ramírez-Cárdenas et al. 2008).

Total phenolic content, total flavonoids content, DPPH and FRAP of raw and roasted guava seeds

Phenolic compounds are widely distributed in fruits and vegetables (Li et al. 2006), which have received considerable attention because of their potential antioxidant activities and free radical-scavenging abilities, which potentially have beneficial implications in human health (Lopez-Velez et al. 2003; Li et al. 2006; Govindarajan et al. 2007). Total phenolic contents (mg/100 g DWb) of raw and roasted guava seeds presented in Table 6. Phenolic content of unroasted or raw guava seeds was 973.80 ± 42.06 mg/100 g DWb. Total phenolic contents was significantly ($P \leq 0.05$) increased by roasting at 150 °C for 10 min. However, roasting at 150 °C for 15 and 20 min caused significant decrease in the phenolic content of guava seeds. Total phenolic content increased from 973.80 ± 42.06 mg/100 g DW in raw guava seeds to 996.23 ± 33.85 in those samples roasted at 150 °C for 10 min. This increase could be due to release more bound phenolics from the breakdown of cellular constituents in function of thermal treatment (Gallegos-Infante et al. 2010) and to degradation of polymerized polyphenols, specifically hydrolysable tannins, and the hydrolysis of other glycosylated flavonoids (Monagas et al. 2009). Likewise, in a study on acorn nut polyphenols, gallic acid was increased after thermal treatment to almost 2-fold content in a dry powder extract (Rakic et al. 2006). Pyrroles and furans which are major compounds formed by the Maillard reaction may contribute to the increased in total phenolic compounds of roasted samples (Yanagimoto et al. 2002). Subjecting guava seeds to 150 °C for 15 and 20 min decreased the total phenolic contents from 973.80 ± 42.06 mg/100 g DW in unroasted or raw guava seeds to 664.12 ± 17.63 and 424.26 ± 28.83 mg/100 g DW in roasted samples, respectively. This might be due to the disintegration of phenolic compounds by increasing roasting time during roasting

Table 5 Effect of roasting process on the antinutritional factors of guava seeds (mg/100 g) on dry weight basis

Treatment	Phytic acid	Reduction (%)	Tannins	Reduction (%)
Raw	273.63 ^a ±8.24	00.00	325.67 ^a ±23.50	00.00
Roasting at 150 °C				
10 min	131.34 ^b ±11.02	52.00	286.94 ^b ±13.49	11.89
15 min	123.10 ^{bc} ±6.28	55.01	216.47 ^c ±9.17	33.53
20 min	105.73 ^c ±12.44	61.36	156.28 ^d ±4.63	52.01
LSD at 0.05	18.436		27.297	

Values are means ± standard deviation (SD) of three determinations. Values followed by the same letter are not significantly different ($p < 0.05$)

Table 6 Total phenolic content, total flavonoids content, DPPH and FRAP of raw and roasted guava seeds

Treatment	Total phenolic content (mg/100 g dw)	Total flavonoids content (mg/100 g dw)	DPPH radical scavenging (%)	Reducing power (absorbance at 700 nm)
Raw	973.80 ^a ±42.06	290.30 ^a ±16.03	63.74 ^b ±6.21	0.567 ^d ±0.09
Roasting at 150 °C				
10 min	996.23 ^a ±33.85	221.13 ^b ±15.04	77.29 ^a ±5.38	0.624 ^c ±0.04
15 min	664.12 ^b ±17.63	201.16 ^{bc} ±17.21	78.14 ^a ±6.13	0.691 ^b ±0.06
20 min	424.26 ^c ±28.83	173.27 ^c ±16.03	78.34 ^a ±4.19	0.740 ^a ±0.03
LSD at 0.05	59.962	30.306	10.425	0.011

Values are means ± standard deviation (SD) of three determinations. Values followed by the same letter are not significantly different ($p < 0.05$)

Total polyphenols are expressed as mg gallic acid/100 g of dry plant material

Flavonoids are expressed as mg catechin/100 g of dry plant material

process. Changes in the polyphenols content after thermal treatment might result in the binding of phenolics with other organic materials present (Alonso et al. 2001). Xu et al. (2007) reported that the total amount of phenolic acids in huyou peel extract decreased after heat treatment, which indicated that some phenolic acids probably were destroyed by heat treatment. Flavonoids are a group of naturally occurring polyphenolic compounds ubiquitously found in the plant kingdom (Grotewold 2006). They are widespread in vegetables, fruits, flowers, seeds, and grains (Dragan et al. 2007). Total flavonoids content in raw and roasted guava seeds are presented in Table 6. Total flavonoids of unroasted guava seeds was 290.30±16.03 mg/100 g DWb. Total flavonoids content in raw guava seeds was significantly ($p \leq 0.05$) higher compared to roasted seeds. Total flavonoids in guava seeds were significantly ($P \leq 0.05$) reduced by roasting process. This decrease was gradually and significantly increased with increasing roasting time. Total flavonoids decreased significantly from 290.30 mg/100 g DWb in raw seeds to 221.13, 201.16 and 173.2703 mg/100 g DWb in those samples roasted at 150 °C for 10, 15 and 20 min, respectively. These reductions might be attributed to flavonoid breakdown during roasting process (Dietrych-Szostak and Oleszek (1999). These results were in good agreement with those indicated by different authors (Alvarez-Jubete et al. 2009; Chlopicka et al. 2012) who reported that the flavonoids content decreased as a result of heating process. Antioxidant activities of raw and roasted guava seeds, as determined by the DPPH radical scavenging method, are shown in Table 6. In DPPH scavenging assay, the antioxidant activity was measured by the decrease in absorbance as the DPPH radical received an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule (Juntachote and Berghofer 2005). DPPH radical-scavenging activity expressed in % inhibition of raw and roasted guava seeds ranged from 63.74 to 68.34 %. Raw guava seeds had significantly ($p \leq 0.05$) lower DPPH radical-scavenging activity compared to roasted seeds. DPPH radical-scavenging activity increased significantly

from 63.74 % in raw samples to 67.29, 68.14 and 68.34 % in those samples roasted at 150 °C for 10, 15 and 20 min, respectively. These increases could be due to the formation of Maillard products such as HMF (5-hydroxymethyl-2-furaldehyde), which render high antioxidant activity (Duenas et al. 2006; Siddhuraju 2006). Nicoli et al. 1997 showed that medium dark roasted coffee brews had the highest antioxidant activity due to the formation of Maillard reaction products. These results are in good agreement with those reported by Rocha-Guzman et al. 2007 who reported that higher values of DPPH activity in cooked common beans (Bayo Victoria) than in uncooked beans, but this effect was related to the cultivar. The reducing power assay is often used to evaluate the ability of an antioxidant to donate an electron (Yildirim et al. 2000). In reducing power assay, antioxidants cause the reduction of the Fe³⁺ into Fe²⁺, thereby changing the solution into various shades from green to blue, depending on the reducing power of the compounds (Ferreira et al. 2007). Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity (Oktay et al. 2003). Results of reducing power activity of raw and roasted guava seeds are presented in Table 6. The reducing power of raw and roasted guava seeds ranged from 0.567 to 0.740 (absorbance value). Significantly greater reducing powers of roasted seeds compared to unroasted ones. Roasting process caused significant increases in reducing power activity. The reducing power of guava seeds increased from 0.567 in raw samples to 0.624, 0.691 and 0.740 (absorbance value) in guava seeds roasted at 150 °C for 10, 15 and 20, respectively. The reducing power of certain compounds is associated with antioxidant activity (Jayaprakasha et al. 2003). Duh (1998) reported that the reducing properties of antioxidants are generally associated with the presence of reductones. Gordon (1990) reported that the antioxidant action of reductones is based on the breaking of the free-radical chain by donating a hydrogen atom.

In conclusion, the results obtained in the present study have shown that roasting process affect the nutritional composition

and nutritive value, antinutritional factors, bioactive compounds and antioxidant activity of guava seeds. Roasting process caused significant ($P \leq 0.05$) decreases in moisture content, crude protein, crude fiber, ash and mineral contents, isoleucine, arginine, glutamic and total aromatic and sulfur amino acids, antinutritional factors (tannins and phytic acid) and flavonoids, while oil content increased. To obtain the highest phenolic content and antioxidant activity, guava seeds should be roasted at 150 °C for 10 min. This study showed roasting process can be used as a tool to increase nutritive value, and antioxidant activity of guava seeds.

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