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Antioxidant activity of extract from gamma irradiated guava (*Psidium guajava* L.) seeds

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ABSTRACT

The objective this study was to study the antioxidant activity efficiency of extracts from irradiated defatted guava (*Psidium guajava* L.) seeds at dose levels of 0, 2, 4 and 6 kGy. The non-irradiated and irradiated defatted guava seeds samples were extracted with acetone: water: acetic acid (90:9.5:0.5). Immediately after irradiation, the antioxidant activity was studied. Gas chromatographic-Mass spectrum was applied to identify and quantify the constituents (%) of extracts and the amino acids composition was determined in all samples under investigation of defatted guava seeds powder. The measurements of the antioxidant activity, using a β-carotene-linoleate model system and radical scavenging capacity effect on 2, 2-diphenyl-1- picrylhydrazyl (DPPH) radical, was determined in the extracts of non-irradiated and irradiated defatted guava seeds powder. Meanwhile, noodles (homemade strips macaroni) were prepared from blends of 72% wheat flour containing 0, 2.5, 5 and 7.5% levels of non-irradiated defatted guava seeds powder. The results showed that samples of extracts from non-irradiated and irradiated defatted guava seeds had contained a considerable total polyphenolic compounds and marked scavenging activity on DPHH radical. On the other hand , the gas chromatography (GC) and mass spectroscopy (MS) separation technique led to identification of 26 components the of extract non-irradiated and irradiated samples. Also, the data revealed that guava seeds powder samples under investigation passes the most important essential amino acids and for human health such as trace elements iron, zinc and manganese. Noodles prepared from 2.5% level of guava seeds powder-wheat flour blend had high acceptable quality. Thus, guava seeds, a waste from guava industry can be utilized improved nutritional properties of noodles or used its extracts as natural antioxidant in food industry field.

Key words: Guava Seeds/ Antioxidant/ Extract/ Gamma irradiation.

INTRODUCTION

The involvement of active oxygen and free radicals in the pathogenesis of certain human diseases, including cancer, aging and atherosclerosis is increasingly being recognized [(1)]. Active oxygen and free radicals, such as superoxide anion ($^{*}O_2$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{*}OH$), are constantly formed in the human body by normal metabolic process, their action is opposed by a balanced system of antioxidant defenses, including antioxidant compounds and enzymes. Upsetting this balance causes oxidative stress, which can lead to cell injury and death [(2)]. Therefore, much attention has been focused on the use of antioxidants, especially natural antioxidants, to inhibit lipid peroxidation, or to protect against the damage of free radicals [(3)].

Recently, research on phytochemicals and their effects on human health have been intensively studied. In particular, research has focused on a search for antioxidants, hypoglycemic agents, and anticancer agents from vegetables, fruit, tea, spices and medicinal herbs. Natural antioxidants, particularly in fruits and vegetables have gained increasing interest among consumers and the scientific community because epidemiological studies have indicated that frequent consumption of natural antioxidants is associated with a lower risk of cardiovascular disease and cancer [(4)]. The defensive effects of natural antioxidants in fruits and vegetables are related to three major groups: vitamins, phenolics, and carotenoids. Ascorbic acid and phenolics are known as hydrophilic antioxidants, while carotenoids are known as lipophilic antioxidants [(5)], guava fruits (*Psidium guajava*, L.) staffed with these type of antioxidants, alongside anti-inflammatory, analgesic, antipyretic, spasmolitic and anti-bacterial activities from guava [(6 & 7)]. Also, guava (*Psidium guajava* L.) fruit is considered a highly nutritious fruit because it contains a high level of ascorbic acid (50–300 mg/100 g fresh weight), which is three to six times higher than oranges. [(8)] reported that Indonesian guava is an excellent source of provitamin A carotenoids. Phenolic compounds such as myricetin and apigenin, ellagic acid, and anthocyanins are also at high levels in guava fruits [(9)]. Therefore, producing guava specially bred for higher levels of antioxidant compounds is a realistic approach to increase dietary antioxidant intake. Related to guava seeds, only few studies were performed about its use.

Therefore, the objectives of this work were carried out to investigate the antioxidant activity of mixed solvent extraction from defatted irradiated guava seeds at dose levels 0, 2, 4 and 6 kGy to determine the total phenolic compounds and to invostigate its potential as a natural preservative. In addition, the chemical composition and amino acids analysis of non-irradiated and irradiated defatted guava seeds were also determined. Moreover, investigate the effect of enriched defatted guava seeds powder at different levels (2.5, 5.0 and 7.5%) on farinograph properties of dough made of wheat flour (72% extraction) to prepare homemade noodles.

MATERIALS & METHODS

Materials:

Guava seed samples

Guava seeds about 25 kgm were collected from a Guava processing industry (Abu-Kabier, Sharkia Governorate, Egypt). The guava seeds were spread over filter papers sheets in trays and dried in an air tunnel drier at 50±2°C. Then, dried guava seeds (ca 8.8%.water) were packaged in tightly sealed polyethylene bags, (each bag weighing Ca. 500g) and equally divided into four groups and stored in freezer till irradiation treatments.

Irradiation treatment

For irradiation treatments, samples of guava seed were subjected to gamma rays at dose levels of 2, 4 and 6 kGy. The irradiation locally experimental ^{60}Co Russian gamma chamber belonging to Cyclotron Project, at Nuclear Research Center, Atomic Energy Authority, Inshase, Egypt.

Methods:

Extraction

Irradiated and non-irradiated dried samples of guava seeds were ground to fine powder in a coffee grinder (Toshiba El Araby MX-5100/5200 Sample Mill, Egypt) for 2 min, but at 15 s intervals the process was stopped for 15 s to avoid heating the sample. Then, powder guava seeds were extracted in a Soxhlet extractor with petroleum ether at 60°C for 6 h to remove the fatty and waxed materials. The defatted guava seed powder was reextracted in a Soxhlet apparatus for 8 h with 300 ml acetone: water: acetic acid (90:9.5:0.5 v/v) at 60°C, as described by [10]. The extracts were concentrated by rotary evaporate under vacuum at 70°C to a final volume of 5 ml. The concentrated extracts were stored in desiccators until use.

Proximate composition

Moisture, lipid, protein, ash, crud fiber were determined according to Official Method [11] and total carbohydrates contents by difference.

Determination of minerals

Iron, copper and manganese were determined on dry weight basis according to Nation et al., [12]. The minerals were determined after solubilizing 0.8g sample in 5ml sulphoric acid, heated for 15 min, one ml of perchloric acid was added and 60-80 the digestion process was continued till the end point (light green color) then the sample was transferred to a standard flask and the volume was adjusted to 25 ml by deionized water. The samples were analyzed by atomic absorption. (BUCK Scientific 210 VGP). Results were expressed as mg/100g.

Determination of amino acids composition

Amino acids composition was determined according to Spackman and Dtein [13], using Eppendrof- Germany LC3000, amino acid analyzer.

Determination of polyphenols

The total phenolic compounds present in extracts of non-irradiated and irradiated defatted guava seed powder samples at 2, 4 and 6 kGy were determined spectrophotometrically by using the Folin-Denis reagent described in according to the standard method of antioxidant activity The concentration of total phenolic compounds in the extracts of non- irradiated or irradiated defatted guava seed powder samples were determined by comparison with the absorbance of the standard, catechin at different concentrations [11].

Antioxidant activity study (DPPH free radical-scavenging assay)

The scavenging effect on 2, 2-diphenyl-1- picrylhydrazyl (DPPH) radical was determined by modifying methods of Brand-Williams,et al., [14] and Gamez,et al., [15]. The extracts of non- irradiated and irradiated at (2, 4 and 6 kGy) defatted guava seeds powder samples were separately mixed with ethanol to prepare test solution of 1 mg/ml sample. DPPH was dissolved in ethanol and mixed with the extracts of guava seeds. The solution was adjusted to a final DPPH concentration of 100 µM. The mixture was shaken vigorously and left to stand for 5 - 60 min in the darkness at room temperature. The amount of DPPH remaining in each period of stand was determined spectrophotometrically at 540 nm, using a microtiter plate reader (Biorad 680, USA). The extracts of RGS test solution was diluted to different concentrations (0.1- 1.0 mg/ml). After vigorous shake, the mixtures were left to stand for 30 min. Tert-Butylhydroquinone (TBHQ) was used to compare the scavenging activity. The radical scavenging activity was calculated as % inhibition from the following equation:

% inhibition as OD= $\{(OD_{smple} - OD_{blank}) / OD_{blank}\}$ x 100.

Antioxidant assay with β-carotene–linoleic acid system

The antioxidant activity of samples assayed using the β-carotene–linoleic acid system was measured as in the method of a literature report with some modification [16]. Briefly, 4 ml of a solution of β -carotene in chloroform (1 mg/ ml) were pipetted into a flask containing 40 mg of linoleic acid and 400 mg of Tween-40. The chloroform was removed by rotary evaporator under vacuum at 45°C for 4 min, and 100 ml distilled water were added slowly to the semi-solid residue with vigorous agitation to form an emulsion. A 96-well microtitre plate (polystyrene) was charged with 50 µl of test sample and 200 µl of the emulsion, and the absorbance was measured at 450 nm, immediately, against a blank consisting of the emulsion without β -carotene. The plate was

stored at room temperature (20–23°C), and the absorbance measurements were conducted again at 30 min intervals up to 420 min. All determinations were carried out in triplicate. The antioxidant activity of the extracts was evaluated in terms of bleaching of β-carotene using the following formula:

Antioxidant activity = [1- (A_0 _ A_t)/ ($À_0$ _ $À_t$)] x 100

Where A_0 and $À_0$ are the absorbance of values measured at zero time of the sample and the control, respectively, $À_t$ and $À_t$ are the absorbance measured in the test sample and the control, respectively, after 420 min.

GC-MS analysis of the extract from defatted guava seeds

The GC-MS analysis of the extracts from defatted guava seeds samples were performed on a Hewlett-Packard model 6890 Series GC System equipped with a HP 5973 MS detector (EI mode, 70 eV). A column type, HP-5 (5% phenyl dimethylsiloxane) with a length of 30 m, an inside diameter of 0.25 mm and a film thickness of 0.25µm, was used. The temperature of the column was programmed to increase after 5 min from 70 to 150°C at the rate of 2°C/min and then after 5 min from 150 to 250°C at the rate of 1°C/min. Helium was used as a carrier gas at a flow rate of 1 ml/min. The injector and detector temperatures were 250 and 280°C, respectively. The components in extract of samples under investigation were identified by comparing on the basis of gas chromatographic retention indices [(17)], mass spectra from Wiley MS Chemstation Libraries (6 th ed., G 1034, Rev.C.00.00, Hewlett-Packard, Palo Alto, CA).

Rheological characteristics

Guava seeds powder blends at 0, 2.5, 5 and 7.5% levels were prepared by replacing wheat flour. The effect of guava seeds powder on the mixing profile of the dough was studied using farinograph (Brabender, Duisburg, Germany) according to the standard AACC methods [(18)].

Raw noodle-making

Noodle samples were prepared from blends containing 0, 2.5, 5 and 7.5 % of defatted guava seeds powder by the micromethod of Zhang,et al.,[(19)] with some modifications. The flour (100 g, 14% moisture basis) and tap water (35 mL) were mixed into noodle dough in a Hobart N50 mixer (Hobart, North York, Canada) for 30 s at slow speed and then for 4 min at medium speed. The stiff dough obtained was allowed to rest 30 min in sealed containers at room temperature. Next, the dough was sheeted eight times in a noodle-making machine (6YM-220-250, Chongqing, China). For the initial pass the roll gap was 2 mm, the sheeted dough was doubled over and passed through the same gap again, and then the roll gap was adjusted to 3.5 mm for another pass. Following that, five more passes were made reducing the gap progressively to 1 mm. Finally, the dough was cut into 2 mm wide noodles. The raw noodles were placed in a zip-lock bag and stored at 4°C for no longer than 24 h before cooking.

Cooking quality

For cooking quality analysis, noodles (20 strips, 22 cm in length) were weighed and then cooked for 4 min in 400 mL boiling tap water, drained for 1 min and weighed. Water absorption was calculated as the weight increase of noodles and expressed as a percentage
of the uncooked noodles. Cooking loss was determined by collecting 100 mL cooking water (thoroughly agitating the total cooking water before sampling) following drainage of the noodles and drying the cooking water in an air oven at 105°C. The residue was weighed and results expressed as a proportion of the uncooked noodles on a dry basis. Three replicates of cooked noodles at each level of lipids content were prepared. Three determinations for each replicate were performed to obtain the mean values.

Extraction of bioactive compounds

Homemade noodles enriched with defatted guava seeds powder were made into powder using mortar and pestle and the powdered samples (1 g) were extracted with 20 ml of 80% acetone for 1 h with occasional stirring using a vertex mixer. The extract was centrifuged at 8000×g. The supernatant obtained was subjected for the estimation of bioactive compounds such as total phenolics, carotenoid and antioxidant activity.

Sensory evaluation

Sensory evaluation of noodles (homemade macaroni) samples were carried for their color, texture, taste, aroma and overall quality. Scores were obtained according to Wierbicki [(20)] by rating the above quality characteristics using the following rating scale: 9= excellent, 8= very good, 7= good, 6= below good-above fair, 5= fair, 4= below fair-above poor, 3= Poor, 2= very poor and 1= extremely poor.

Statistical analysis

The obtained data were exposed to analysis of variance. Duncan's multiple range test at 5% level was used to compare between means. The analysis was carried out using the PROCGLM procedure of SAS [(21)].

RESULTS & DISCUSSIONS

The chemical composition(protein, fat, ash, crude fiber and total carbohydrates contents) of non-irradiated and irradiated guava seeds samples at dose levels of 2, 4 and 6 kGy on dry weight basis are presented in Table (1). The results showed no significant differences between non-irradiated and irradiated guava seed samples.

Meanwhile, the data revealed that the guava seed samples had high percentage of crude fiber and had a considerable amount of iron, copper and manganese in all samples under investigation.

Table (1): chemical composition (on dry weight basis) of non-irradiated and irradiated guava seeds (*Psidium guajava*,L.).

Treatments (kGy)	Chemical composition (%)						Mineral content(mg /100 gDW)		
	Moisture	Protein	Fat	Ash	Crude fiber	*carbohydrates	Iron	copper	Manganese
0	8.80^{A}±0.12	7.49^{A} ±0.29	11.39^{A} ±0.15	1.56^{A}±0.09	63.54^{A} ±0.27	7.22^{A} ±0.32	9.53^{A}±0..33	0.94^{A}±0.8	1.86^{A}±0.05
2	8.87^{A}±0.07	7.84^{A} ±0.31	11.59^{A} ±0.27	1.52^{A}±0.03	63.45^{A} ±0.16	6.73^{A} ±0.25	9.47^{A}±0.07	0.99^{A}±0.02	1.83^{A}±0.04
4	8.83^{A}±0.18	7.51^{A} ±0.04	11.81^{A} ±0.0.9	1.53^{A}±0.26	63.54^{A} ±0.05	6.78^{A} ±0.07	9.69^{A}±0.03	0.98^{A}±0.17	1.87^{A}±0.32
6	8.91 ±0.25	7.25^{A} ±0.09	11.38^{A} ±0.18	1.54^{A} ±0.12	63.47^{A} ±0.09	7.45^{A} ±0.19	9.52^{A}±0.28	0.96^{A}±0.09	1.84^{A}±0.24

Means with the same letters with in the columns are not significantly different (p>0.05).
* by difference

Amino acids composition of non-irradiated and irradiated defatted guava seeds.

Amino acids composition of defatted guava seed powder are tabulated in Table (2). The results illustrated that the defatted guava seeds had most essential and nonessential amino acids. Leucine acid was the highest essential amino acid followed by phenylalanine, isoleucine, arginine and histidine, lysine, methionine, valine and threonine in non-irradiated samples. Meanwhile, the highest nonessential amino acid content was glutamic & proline followed by aspartic and tyrosine, cystine, glycine and alanine and tyrosine in non-irradiated samples.

On the other hand, subjecting defatted guava seed samples to gamma irradiation at dose levels of 2, 4 and 6 kGy reduced the amount of most essential amino acids, except of threonine amino acid increased to about fold compared with non-irradiated samples. Also, the results presented that the dose level of 4 kGy increased the amount of leucine and valine amino acids in comparison with control samples, but the dose level of 6 kGy was completely lost the methionine amino acid. In contests, it could be noticed that the irradiated samples at different doses decreased the amount of nonessential amino acids, except of glycine and serine amino acids were increased by using gamma irradiation at dose levels of 2, 4 and 6 kGy compared with non-irradiated samples. In addition, the results showed the dose level of 4 kGy increased the amount of alanine and aspartic amino acids in comparison with non-irradiated samples.

On the other hand, the data showed that the essential amino acids of defatted guava seeds powder constitute 67.93, 31.52, 41.85 and 30.98 mg/g of total amino acids, whereas nonessential make up 32.06, 21.74, 26.09 and 20.65 mg/g of total amino acids in non-irradiated and irradiated samples at dose levels of 2, 4 and 6 kGy, respectively.

Table (2): Amino acids composition of non-irradiated and irradiated defatted guava seeds (mg/g).

Amino acids (mg/g)	0	Gamma irradiation doses (kGy)		
		2	4	6
EAA*				
Histidine	2.6	1.0	1.4	1.0
Isoleucine	3.6	0.6	0.8	0.6
Leucine	5.8	4.4	6.0	4.4
Lysine	1.8	0.8	0.8	0.6
Methionine	1.2	0.2	0.2	-
Phenylalanine	4.4	1.2	1.4	1.2
Threonine	0.4	0.8	1.0	0.8
Valine	0.8	0.8	1.0	0.8
Arginine***	4.4	1.8	2.8	2.0
Total	25	11.6	15.4	11.4
NEAA**				
Alanine	0.6	0.6	0.8	0.6
Aspartic acid	1.8	1.6	2.0	1.6
Cystine	1.2	0.6	0.8	0.6
Glutamic acid	2.4	1.8	2.0	1.6
Glycine	1.0	1.4	1.6	1.4
Proline	2.4	0.2	-	-
Serine	0.6	1.2	1.4	1.2
Tyrosine	1.8	0.6	1.0	0.6
Total	11.8	8	9.6	7.6
EAAI***	34.76	15.75	19.62	8.57
B.V****	31.22	9.54	13.95	1.35

* Essential amino acids
** Nonessential amino acid
*** Essential only in certain cases
*** Essential amino acid index
****Biological value

The values of essential amino index (EAAI) and biological value (B.V) for non-irradiated and irradiated defatted guava seeds at dose levels 2, 4 and 6 kGy were calculated using a computer software developed by Farag [22]. The data revealed that values of EAAI and B.V for non-irradiated defatted guava seed samples were 34.76 and 31.2, respectively. While, subjecting defatted guava seed samples to gamma irradiation at dose levels 2, 4 and 6 kGy much decreased the values of EAAI and B.V to 15.75, 9.54; 19.62, 13.95 , 8.57 and 1.35, respectively. Also, the results showed that values of EAAI and B.V of irradiated defatted guava seeds at dose level 4 kGy more than the same values of irradiated defatted guava seeds at dose levels 2 and 6 kGy.

Consequently, these results illustrated that irradiated defatted guava seeds samples at dose level of 4 kGy had higher percentages of nonessential and essential amino acids compared with non-irradiated and irradiated samples at dose levels of 2 and 6 kGy. These may be due to the effect of gamma irradiation caused of degradation, oxidation and radiolysis of essential and nonessential amino acids at the higher radiation dose(4kGy).

Total phenolic compounds

The results in Table (3) illustrated the total phenolic compounds (mg/100g gallic acid) in extract of non-irradiated and irradiated guava seed samples. The results indicated that the extract of non-irradiated guava seed samples posses significant total phenolic compounds in comparison with extracts from irradiated defatted guava seed samples at dose levels 2, 4 and 6 kGy. Meanwhile, the results obvious that significant differences in the values of total phenolic compounds between extracts from irradiated defatted guava seed samples under investigation. Where, the results showed that the extracts from irradiated guava seeds samples at dose level 6 kGy had higher total polyphenolic compounds than extracts from irradiated samples at dose levels 2 and 6 kGy. This phenomena may be due to that the higher irradiation dose facilitate certain polyphenolic compounds to be more extractable Gil,et al.,[23] have also, reported that white pulp guavas had higher total polyphenolics than pink pulp guavas were 247.3 and 126.4 mg gallic acid/ 100 g in white and pink pulp, respectively. The ranges of total polyphenolic contents (mg/100 g) were 14–102 in nectarines, 21–111(mg/100 g) in peaches and 42–109 in plums, 142.9 (mg/100 g) in star fruit, 47.9 in pineapple, 56.0 in mango, 57.6 in papaya, 28.8 in litchi [24].

Table (3): Total phenolic compounds (mg/100g as Gallic acid) in extract of non-irradiated and irradiated defatted guava seeds.

Total phenolic compounds(mg/100 g as gallic acid)	Control	Gamma irradiation doses (kGy)		
		2	4	6
Defatted guava seeds	35.15^{A}±0.34	27.81^{B}±0.27	31.45^{C}±0.19	33.67^{D}±0.28

Capital letters were used for comparing between means in the rows and columns, respectively.
Means with the same letters are not significantly different (p>0.05).

Radical- scavenging activity

The Scavenging activities (%) on DPPH radical of extracts from non-irradiated and irradiated defatted guava seeds are presented in Table (4). The results indicate that the extract from non-irradiated guava seeds posses marked scavenging ability from 51.4% to 99.2% with increasing extract concentration from 50 to 200µg/ml on DPPH radical. Moreover, the data revealed that the extracts of irradiated defatted guava seed at dose levels 2 and 4 kGy, except of extracts from irradiated samples at dose level 6 kGy significantly diminished the antioxidant activity to seem smaller when compared with the extracts from control samples. Meanwhile, the results illustrated that increasing the efficiency extracts of non-irradiated and irradiated defatted guava seeds samples as antioxidant activity on DPPH radical was observed with increasing concentration of samples under investigation from 50 to 200 µg/ml. Generally, the results exhibited that the extracts of non-irradiated defatted guava seeds had marked scavenging activity on DPHH radical compared to extracts of irradiated samples at dose levels 2, 4 and 6 kGy. Therefore, the scavenging effect on DPPH radical of extract from non-irradiated samples could be attributed to the phenolic content Chen and Yen [25] reported that the extracts from leaves of various guava cultivars exhibited more scavenging effects on free radicals than did commercial guava tea extracts and dried fruit extracts. The data indicated that guava such extract, which appeared to be responsible for their antioxidant activity Song et al.,[26] reported that the total phenols analyzed in irradiated kale juice immediately after the irradiation, was significantly lower than the control. They added that the phenolic compound level of the irradiated sample became higher after day 1 than that of the control. This phenomenon was attributed to the immediate oxidation of the phenolic compounds, thus playing an antioxidant role by reducing the free radicals and the reactive oxygen species induced by irradiation.

Table (4): Scavenging activity (%) on DPPH radical of extracts from non-irradiated and irradiated defatted guava seeds.

Concentration of samples (µg/ml)	TBHQ	Gamma irradiation doses (kGy)			
		0	2	4	6
50	85.0^{A}±1.9	51.4^{B}±1.9	43.8^{C}±2.1	46.3^{D}±1.4	49.6^{B}±2.4
100	99.2^{A}±2.3	66.4^{B}±2.1	61.2^{C}±1.8	61.2^{C}±1.6	61.2^{C}±2.3
150	99.2^{A}±2.4	73.3^{B}±2.3	66.9^{C}±2.3	74.2^{B}±1.9	71.6^{D}±2.6
200	99.2^{A}±1.8	91.9^{B}±1.8	88.3^{C}±2.0	82.0^{D}±1.7	83.0^{D}±2.3

Capital letters were used for comparison between means in the rows.
Means with the same letters are not significantly different (p>0.05). **TBHQ=** Tetr-Butylhydroquinone.

β-Carotene/linoleic acid bleaching assay

In this assay, oxidation of linoleic acid produces hydroperoxidederived free radicals that attack the chromophore of β-carotene, resulting in bleaching of the reaction emulsion. An extract capable of retarding/inhibiting the oxidation of β-carotene may be described as a free radical scavenger and primary antioxidant (Liyana- Pathirana and Shahidi, 2006).The antioxidant activity of extracts from non-irradiated and irradiated defatted guava seeds by β- Carotene bleaching assay are showed in Table (5). The results showed that the extract of non-irradiated defatted guava samples had marked scavenging activity of inhibiting the bleaching of β-carotene by scavenging linoleate derived free radicals compared with extracts of irradiated defatted guava seed samples at dose levels 2, 4 and 6 kGy. Moreover, the data revealed that no significant differences in the

extract of control samples at higher concentration (150µg/ml) compared with extracts of irradiated defatted guava seed samples at dose levels of 4 and 6 kGy were capable of inhibiting the bleaching of β-carotene by scavenging linoleate derived free radicals. In addition, the results showed that the extracts of non-irradiated and irradiated defatted guava seed samples at dose levels 2, 4 and 6 kGy had higher marked scavenging activity in comparison with artificial antioxidant TBHQ at higher concentration (200µg/ml) of inhibiting the bleaching of β-carotene by scavenging linoleate derived free radicals. Therefore, the extracts of control samples were better than extracts of irradiated defatted guava seed samples at dose levels 2, 4 and 6 kGy.

Table (5): Antioxidant activity of extracts from non-irradiated and irradiated defatted guava seeds by β-Carotene bleaching assay.

Concentration of samples (µg/ml)	TBHQ	Gamma irradiation doses (kGy)			
		0	2	4	6
50	$85.0^{A}\pm1.6$	$52.6^{B}\pm2.9$	$42.4^{C}\pm2.1$	$45.0^{D}\pm1.4$	$48.0^{E}\pm2.4$
100	$99.7^{A}\pm1.3$	$64.4^{B}\pm2.1$	$60.4^{C}\pm1.8$	$59.6^{C}\pm1.6$	$60.0^{C}\pm2.3$
150	$99.7^{A}\pm1.6$	$72.6^{B}\pm2.3$	$65.6^{C}\pm2.3$	$72.3^{B}\pm1.9$	$72.3^{B}\pm2.6$
200	$99.7^{A}\pm1.7$	$93.3^{B}\pm1.8$	$86.3^{C}\pm2.0$	$83.6^{D}\pm1.7$	$82.0^{E}\pm2.3$

Capital letters were used for comparison between means in the rows.

Means with the same letters are not significantly different ($p>0.05$).

TBHQ= Tetr-Butylhydroquinone.

Effect of gamma radiation on constituents of the defatted guava seeds extracts.

To identify the effects of gamma irradiation on the composition of extracts from irradiated samples of defatted guava seeds at doses of 2,4 and 6kGy as shown in (Table 6), dipropyl disulfide was identified in extracts from defatted guava seeds was the most predominate component as its percentage was 18.75%,18.43,17.87 and 15.62 in control and irradiated samples at doses 2,4 and 6 kGy ,respectively. These results in accordance with those obtained by Gyawali,et al., [(27)].

Some components were decreased such as1-Methoxy2-benzene,Dipropyl trisulfid,Di-2propenyl propyl disulfied. From these results, it obvious that gamma irradiation due to some slight increase in their some individual fractions as shown in (Table 5). But, the more effect of gamma irradiation was observed on the most of sulfur fractions such as bis1-methyl ethyl disulfide, methyl propyl disulfide, dipropyl disulfide and dipropyl trisulfide, where the irradiation induced decreases in these fractions proportionally to the applied doses in comparison with control. Also, the amount of total compounds belonging to acid, alcohol, aldehyde, ester, furan, and ketone was also increased after irradiation.Pyun and Shin [(28)] reported that nine S-containing compounds were reduced by irradiation at 20 kGy and six at 10 kGy doses and found that the compounds related with the same chemical group, some of them were increased whereas some were decreased in volatile compounds of dried onion. The high dose rate irradiation splits the chemical bonds in molecules to form the free radicals and then promotes the combination of free radicals to exclude their reaction with food components and forms stable radiolytic products. On the other hand, some components were increased such as pentanal, 2-methyl pentanal, 1-Ethyl thio methyl propen, octylaldehyde phenyl acetaldehyde,1-methoxy2- benzene, isobuthlisothiocyante and decane in irradiated samples of guava seeds at doses 2,4 and 6kGy. Thus, chemical changes in different doses of irradiation will therefore not only produce different amounts of new molecules, but also different of kinds. Dieh, [(29)] reported that several aldehydes produced in irradiated oil emulsion containing amino acids were increased in a dose-dependent manner up to 10 kGy.

Table (6): Effect of gamma irradiation on constituents (%) of extracts from non-and irradiated defatted guava seeds samples.

Compounds	Irradiation dose (kGy)			
	control	2	4	6
Pentanal	1.76	1.92	2.07	2.53
Bis 1-methyl ethyl disulfide	0.34	0.31	0.21	0.13
2-Methyl pentanal	0.08	0.19	0.32	0.42
2,4-Dimethyl thiophene	0.04	0.05	0.09	0.06
2,5-Dimethyl thiophene	0.63	0.89	0.74	0.93
Methyl propyl disulfide	1.37	1.26	1.18	1.08
Methyl trans-propenyl disulfide	1.94	1.85	1.67	1.32
1-Ethyl thio methyl propene	0.13	0.22	0.37	0.56
Dimethyl trisulfide	2.19	2.07	1.83	1.58
2-Pentyl furan	0.05	0.09	0.07	0.07
Octyl aldehyde	0.06	0.11	0.15	0.25
Limonene	0.04	0.07	0.05	0.09
Phenyl acetaldehyde	1.58	1.97	2.45	3.14
γ-Terpinene	0.04	0.07	0.18	0.22
3-Ethyl methyl phenol	0.12	0.16	0.21	0.34
Di-2-propenyl propyl disulfide	0.17	0.14	0.13	0.09
Dipropyl disulfide	18.75	18.43	17.87	15.62
Trans-propenyl propyl disulfide	14.76	14.25	14.06	13.88
Methyl 2-propenyl trisulfide	0.28	0.23	0.18	0.12
1-Methoxy 2-benzene	6.68	8.41	9.04	9.81
Methyl propyl trisulfide	8.51	7.42	7.06	9.25
3-Methyl thio 1-propene	4.55	4.65	4.56	5.18
4,5-Dimethyl thiazole	1.35	1.16	1.02	0.08
Isobuthyl isothiocyanate	10.06	10.04	10.15	10.24
Dodecane	10.04	10.08	10.12	10.32
1,4-Dimethyl tetrasulfide	1.48	1.42	1.28	1.03
Total unknown	12.2	12.9	11.97	11.66

Influence on guava seeds powder on sensory characteristics of noodles

The sensory attributes of noodles fortified with different levels of guava seeds powder were tabulated in Table (7). Results illustrated that no significantly different in color of noodle samples fortified with 2.5% of guava seeds. However, the data showed a slight decrease and significantly different in color noodle samples were recorded between control and samples of enriched with 5 and 7.5% of guava seeds. The color of noodles was changed from creamiest to yellow color in noodles, as a fruit of rectification rate.

On the other hand, the results exhibited a significantly differences in the texture, taste and aroma of noodle samples fortified with 2.5, 5 and 7.5% guava seeds powder they recorded high scores as compared with the control samples. These may be due to natural compounds, such as flavonoids and essential oils. Nevertheless at 2.5% guava seeds powder level, noodles showed that had high scores compared with noodles in replacement levels 5 and 7.5% of guava seeds powder samples. Thus, it could be concluded that 2.5% replacement level of guava seeds powder was the most effective level. Sudha,et al.,[30] found that addition of bran as a source of fiber from cereals (wheat, rice, oat and barley) to wheat flour effected the rheological characteristics in various ways. Biscuits containing wheat bran (20%), oat bran (30%) and barley bran (20%) were highly acceptable. [31] revealed that addition of apple pomace in cake making can avoid the addition of other flavoring ingredients as the cakes prepared with apple pomace had pleasant fruity flavor. Apple pomace also has the potential for use in cake making as a good source of polyphenlos which has antioxidant properties.

Table (7): The sensory attributes of noodles formulated with different levels of raw and irradiated guava seeds powder.

Quality attributes	Substitution levels (%)			
	Control	2.5	5	7.5
Color	$9.7^{A}\pm0.05$	$9.6^{A}\pm0.03$	$9.1^{B}\pm0.09$	$8.9^{C}\pm0.04$
Texture	$9.6^{A}\pm0.02$	$9.1^{B}\pm0.07$	$8.4^{C}\pm0.05$	$7.8^{D}\pm0.04$
Taste	$9.5^{A}\pm0.07$	$8.9^{B}\pm0.06$	$8.1^{C}\pm0.02$	$7.6^{D}\pm0.03$
Aroma	$9.6^{A}\pm0.09$	$9.2^{B}\pm0.04$	$8.8^{C}\pm0.03$	$7.9^{D}\pm0.06$

Capital letters were used for comparing between means in the rows.
Means with the same letters are not significantly different ($p>0.05$).

Effects of replacing wheat flour (72% extraction) with defatted guava seeds powder on farinograph parameters

At the beginning, the result exhibited that defatted guava seeds powder contained all essential amino acids that are important for human health, contained phenolic acids that responsible for their antioxidant activity and some trace elements such as iron, copper and manganese. The effects of addition of guava seeds powder at replacement of levels at 0, 2.5, 5 and7.5% on farinograph properties of dough made of wheat flour (72% extraction) are shown in Fig (1) and Table (8).

The results showed that water absorption of control dough (100% wheat flour) free from defatted guava seeds powder added was 58.3%. The water absorption increased, as the level of replacement with defatted guava seeds powder. Mixture with 2.5, 5 and 7.5% of guava seeds powder caused an increase in water absorption being 62.7, 66.4 and 68.9%, respectively (Fig 1). The increase in water absorption value is due to the ability of defatted guava seeds powder added to absorb more water and swell. Also, results showed that the control dough (100% wheat flour) had arrival time of 1.0 min. It could be observed that the mixtures with 2.5, 5 and 7.5% of guava seeds powder had the same value of arrival time (1.0 min) of control samples. Also, the same data presented that the control and mixtures 2.5, 5 and 7.5% dough recorded the same value of development time1.5 min.

On the other hand, results showed that dough stability time of the control dough was 2 min. It could be noticed that, this value increased with addition of guava seeds powder, it reached to 3 and 6 min in both of mixtures 2.5, 5 and 7.5%, respectively. This may be due to the water holding capacity of defatted guava seeds powder which caused the stiffness and stickiness of the dough (Fig 1). Meanwhile, results illustrated that the degree of softening to control dough was 120 B.U. It could be noticed that doughs which had low stability value, had high degree of softening. This value declined to 110 and 90 B.U in both of mixtures with 2.5, 5 and 7.5 % of guava seeds powder, respectively (Fig 1).

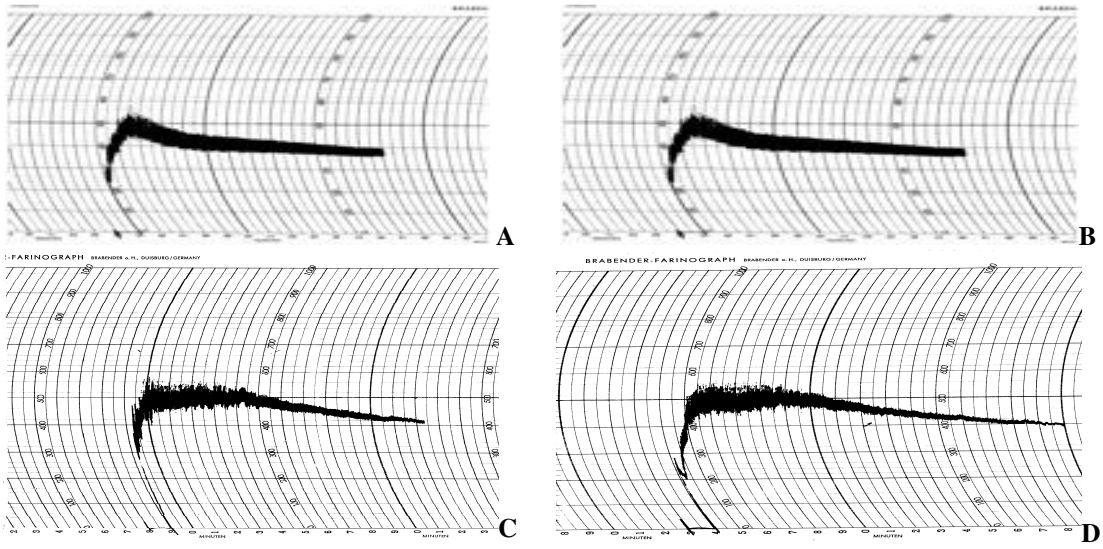

Fig (1): Farinogram of wheat flour (72% extraction) dough mixed with non-irradiated defatted guava seeds powder (guava seeds meal), A (100% wheat flour (72% Extraction) (Control), B (97.5% wheat flour + 2.5% guava seeds powder), C (95% wheat flour + 5% guava seeds powder) and D (9.5% wheat flour + 7.5% guava seeds powder).

Table (8): Farinograph properties of wheat flour (72% extraction) dough mixed with defatted guava seeds powder.

Dough mixture		Water absorption (%)	Arrival Time (min)	Dough development (min)	Stability Time (min)	Degree of softening (B.U)
Wheat flour (%)	Guava seeds powder					
100% (Control)	-	58.3	1.0	1.5	2.0	120
98	2.5	62.7	1.0	1.5	3.0	11
95	5	66.4	1.0	1.5	6.0	90
93	7.5	68.9	1.0	1.5	6.0	90

Bioactive compounds in non-irradiated defatted guava seeds powder incorporated noodles

Incorporation of guava seeds powder as an ingredient in noodle samples significantly improved the content of total polyphenols and free radical scavenging activity in comparison with noodles made from wheat flour (72% extraction) as seen in Table (9).

Defatted guava seeds powder contains a significant amount of polyphenols, while wheat flour contains significant activities of peroxidase and polyphenol oxidase which catalyses the formation of cross-linking of polysaccharides and proteins via phenolic acids. Thus, an increase in the insoluble dietary fiber content may be due to (i) the formation of resistant starch and (ii) formation of cross-linked polysaccharides/ protein, which are resistant to digestive enzymes. Also, the increase in the free radical scavenging may be attributed to the increase in the content of polyphenols as a result of incorporation of guava seeds powder. The results suggested that macaroni processing did not have significant impact on the antioxidant compounds. Thus, the guava seeds powder enriched noodles not only increased the nutritional quality of the product but also increased the nutraceutical property by increasing their antioxidant activity.

Table (9): Total polyphenol content and free radical scavenging activity (%) on DPPH radical of noodles enriched with different levels of non-irradiated defatted guava seeds powder.

Level of addition	Total polyphenols (mg/100g as Gallic acid)	Scavenging activity at concentration of samples (50 µg/ml)
Control	0.46^{A}±0.11	11.97^{A}±0.14
2.5%	1.38^{B}±0.15	15.52^{B}±0.12
5%	1.67^{C}±0.19	22.68^{C}±0.19
7.5%	1.91^{D}±0.17	26.47^{D}±0.17

Capital letters were used for comparing between means in the columns.
Means with the same letters are not significantly different (p>0.05).

CONCLUSION

In general, samples of defatted guava seeds powder are a good source of phytochemicals including polyphenols and essential amino acids. Incorporation of guava seeds powder increased the polyphenol contents in noodles and it also enhanced antioxidant activity. The studies on cooking quality, textural and sensory evaluations showed that the noodles incorporated with guava seeds powder up to 2.5% level resulted in products with good acceptability. Therefore, the guava seeds powder enriched noodles not only increased the nutritional quality of the product but also increased the nutraceutical property by increasing its antioxidant activity. Development and utilization of such functional ingredients can be used to improve the nutritional status of the population, which can impart health benefits by preventing degenerative diseases.

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النشاط المضاد للأكسدة لمستخلص بذور الجوافة المعاملة بأشعة جاما

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ملخص البحث

تعتبر بذور الجوافه مخلف ناتج من صناعة عصائر الجوافة ومنخفض فى قيمته الإقتصادية يمثل هذا المخلف مشاكل بيئية متعددة. ولذلك تهدف هذه الدراسة إلى دراسة تأثير معاملة بذور الجوافه بأشعة جاما بجرعات 2 ،4 و 6 كيلو جراى على نشاط المواد المضادة للأكسدة والمواد الفينولية لمستخلص بذور الجوافه وكذلك على محتواها من الأحماض الأمينية. كما تم إعداد نودلز (شرائط مكرونة رفيعة مصنعة منزلياً) بإستخدام نسب خلط من بذور الجوافة المطحونة 2,5، 5 ، 7,5% فى دقيق قمح 72% ومقارنتها بالكنترول وقد تم دراسة الصفات الريولوجية لهذه الخلطات. وقد أظهرت نتائج مستخلص بذور الجوافه انه يحتوى على مواد فينولية بنسب واضحة وأن لها فعالية كمضاد أكسدة. كما أظهرت ايضا أن العينات المقارنة والمعاملة بالإشعاع تحتوى على جميع الأحماض الأمينية الأساسية والألياف والعديد من العناصر المعدنية النادرة مثل الحديد والنحاس والمنجنيز . وقد أظهرت النتائج أن عينات مستخلص بذور الجوافه الغير معاملة بالإشعاع أن لها فعالية كمضاد أكسدة أكبرمقارنة بباقى المعاملات تحت الدراسة. كما أظهرت النتائج أن أفضل نسب الخلط كانت 2,5% من بذور الجوافة المطحونة الغير المعاملة إشعاعياً . وبناءاً على ذلك يمكن التوصية أن مستخلص بذور الجوافه يمكن استخدامه كمضاد أكسدة طبيعى فعال فى مجال حفظ الأغذية، كما يمكن إستخدام مطحون بذور الجوافة كمصدر للإحماض الأمينية الأساسية التى يحتاجها جسم الإنسان أيضا كمصدر للفينولات العديدة والألياف وعديد من العناصر المعدنية للعجائن التى تضاف إليها مع عدم التأثير السلبى على الصفات الريولوجية.

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