Evaluation and Application of Some Phytochemicals in Pomegranate Seed Residues and Guava Seeds

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ABSTRACT

Phytochemicals were recommended to reduce coronary heart-related diseases and diabetes incidence. In consequence, the development of foods with high phytochemicals content should be desirable. The aim of the present work was to investigate the possibility of using pomegranate seed residues (PSR) and guava seeds (GS) as a source of phytochemicals. The data revealed that PSR and GS contain high amount of phytochemicals (α-tocopherols, polyphenols, carotenoids and flavonoids). PSR contained significantly higher amounts of α-tocopherols, polyphenols and flavonoids (1.839,412.60 and 91.41mg/ 100g DM, respectively) compared with (1.370, 100.59 and 56.23 mg/ 100g DM, respectively) for GS. On the other hand, GS contained significantly higher amounts of total carotenoids (223.12 mg/ 100g DM) than PSR(107.08 mg/100g DM). Furthermore the results indicated that GS contained significantly higher amounts of trypsin inhibitors ,haemagglutinin activity, phytic acid and oxalate (43.77 TIU/mg DM, 406.88unit/100gDM, 70.35mg/100g DM and 6.93mg/100gmDM,respectively) as compared with PSR (21.18, 286.66,13.09 and 1.87,respectively). The data also indicated that PSR and GS are considered good sources of crude fibers (59.18 and 65.22%, respectively). Furthermore, both seeds contain high amounts of crude oil and omega fatty acids. Linolenic acid was the major unsaturated fatty acid in (PSR) oil (63.84%), while linoleic acid was the most predominant unsaturated fatty acids in (GS) oil (71.63%). The PSR and GS were utilized in formulating novel functional biscuits. Sensory evaluation showed that panelists judged the enriched biscuits as acceptable.

Keyword: Phytochemicals, by-products, pomegranate seed residues, guava seeds, fatty acids composition, functional biscuits.

INTRODUCTION

Nowadays, there is interest by consumer, researches and the food technologists toward how food products can help maintain health and the role that they play in prevention and treatment of many illnesses which has become widely accepted(Hassan *et al*., 2012). Considerable importance is given to functional foods which in principle are a part of their basic national functions, provide phytochemical benefits and play an important role in disease prevention or slow the progress of cancer and chronic diseases. There has been virtual explosion of interest in the vegetable and fruits by-products (seeds and peels) as that contain several nutritional and phytochemical components which have benefit in the human diet. These components in pomegranate and guava seeds are by- products of juice and jam production. They contain antioxidants (polyphenols, pigments and tocopherols) and omega fatty acids (Hea *et al*., 2014, Mekni *et al*.,2014). Due to the large amount residues of pomegranate and guava seeds with their valuable pharmaceutical and nutritional value, more beneficial applications in food industries have been suggested to use as animal feed or in commercial cosmetic products (Chaudhary & Tripathi, 2014, Methew *et al*. ,2014).

Since, several studies have indicated that

pomegranate seed residues and guava seeds contain high amounts of natural antioxidants, crude lipid and omega fatty acids, the present work was carried out to investigate utilization of these by-products to produce novel functional biscuits containing phytochemicals.

MATERIALS AND METHODS

Materials

Pomegranate (*Punica granatum* L.) and guava (*psidium guajava*) fruits were obtained from Alexandria local market. The fruits were medium in size. Biscuit ingredients including, flour (72% extraction), sugar, shortening , egg and baking powder were purchased from Alexandria local market, Egypt.

Methods

The PSR and GS remained after pulping were removed, washed several times with tap water to remove the fibrous artifacts. The seeds were drained and dried in an ordinary dryer at 50°C for 12 hrs. The dried seeds were cleaned and ground to pass through 60 mesh sieve and stored in glass kilner jars at -18°C until analysis.

Chemical Composition

Proximate analysis of PSR and GS including crude fat, crude protein (N×6.25), crude fiber and ash were carried out according to the AOAC (2007) procedures, unless otherwise was stated. The following mineral elements: Na, K, Ca, Mg, Zn, Cu, Fe, Se and Mn were measured in ash solution using Perkin Elmer atomic absorption spectrophotometer (Model 2380) as outlined in the AOAC (2007). Total phosphorus was assayed calorimetrically at 630 nm using Carlzeiss Spekol colorimeter (AOAC,1990).

Vitamins

Ascorbic acid content was determined using 2.6-dichlorophenol indophenol dye as described by AOAC (2007). Vitamins (D, E, B_1, B_2, and B_{12}) were analyzed by Reversed-HPLC. A typical non-aqueous reverse phase (NARP) mobile phase consists of a polar solvent (acetonitrile), a solvent with lower polarity (e.g.dichloromethane) to act as solubilizer and to control retention by adjusting the solvent strength, and a third solvent with hydrogen capacity (e.g. methanol) to optimize selectivity. Vitamins were separated on Acclaim® polar Advantage (PA) II column with a single injection using an aqueous-to non- aqueous mobile phase gradient. HPLC columns (Acclaim PA, PA_2, C_{18}-) were used for the separations with an aqueous mobile phase (phosphate buffer- CH_3 CN) for water soluble vitamins. Detection wavelength- witching mode was applied for sensitivity optimization (Moreno & Salvado, 2000).

Amino acid composition

Amino acids were determined according to the method described by Spackman *et al.* (1958) using a Beckman model 119Cl amino acid analyzer (USA). Ninhydrin was used as a detective compound.

Antioxidant compounds

The total polyphenols of PSR and GS were determined using the Folin– Ciocalteu reagent at 760 nm using a Jasso V–530 UV–Vis spectrophotometer. The total polyphenol content was standardized against tannic acid and expressed as mg per 100g sample (Singleton *et al*., 1999, Meyers *et al*., 2003).

Total carotenoids were determined according to Lichtenthaler (1987), while flavonoids were determined according to the method described by Gitelson *et al.* (2001).

Antioxidant activity

The N,N-Dimethyl-*p*-Phenylenediamine Dihydrochloride (DMPD) assay described by Fernandez-Pachon *et al*.(2004) was used for measuring antioxidant activity. In this method, the sample is added after the radical is formed. The decrease of absorbance of the radical is proportional to the concentration and activity of the sample analyzed. In presence of an oxidant solution of (iron chloride). DMPD, form a coloured radical cation. $DMPD^{\cdot+}$. The reaction is as follows: 1 ml of 100 ml DMPD is added to 100 ml of 0.1 M acetate buffer, pH 2.25, and the radical is obtained by adding 0.2ml of 0.05 M ferric chloride. Scavenging assay proceeds by adding 0.1 ml of sample to 2 ml of radical solution and measuring the absorbance at 505 nm. The results were calculated as a per cent of discoloration according to the formula:

$$\% \text{ Inhibition } (1 - A_{sample} / A_{control}) \times 100$$

Antinutritional factors

Trypsin inhibitor

Trypsin inhibitor was extracted according to the method of Lin & Chen (1989). Ten g sample were blended with 20 ml of distilled water and the suspension was centrifuged at 4000 xg for 5 min. The supernatant was diluted to 250 ml with phosphate buffer (pH 7.6). One ml of trypsin solution (20 mg trypsin / ml) was added to a tube containing 1 ml of 1% casein (pH 7.6). Another 1 ml trypsin solution was added to the second tube which contains 1 ml of trypsin inhibitor extract. The tubes were shacked well and incubated for 20 min at 37° C. 3 ml 5% TCA solution was added and centrifuged after standing for 1hr (3500 xg for 10 min.) The concentration of amino acids in the supernatant was determined by Lowery method (Plumer, 1987). Trypsin inhibitor activity was set as the number of trypsin unit inhibited (TUI).

The haemagglutinating activities of PSR and GS were determined by the method of Liener & Hill (1953).

Phytic acid was determined in PSR and GS according to the method described by Wheeler & Ferrel (1971).

Alkaloids were determined according to Papthanasiou *et al*.(1999).

Oxalate content was determined according to the method described by the AOAC (1990).

In vitro protein digestibility

In vitro protein digestibility of PSR and GS using the proteolytic enzymes pepsin followed by panceatin was determined according to the method described by Prakash & Prakash (1999). The digestibility was calculated as a percentage of digested protein in relation to the analyzed protein content of the sample.

Analysis of PSR and GS oils

Purified crude PSR and GS oils were extracted and determined by the procedure of Folch *et al.*(1957) using chloroform: methanol (2: 1,v/v) as a solvent system. The obtained oil, after removing the solvent using a rotary evaporator at 45 °C, was flushed with nitrogen gas and stored in a sealed glass at- 18 °C until analysis.

Refractive Index (RI) at 25°C, iodine value (IV), saponification value (SV), peroxide value (PV as $mEqO_2$ / Kg oil), free fatty acids (FFA as % oleic acid), unsaponifiable matter (%) and colour using a Lovibond tintometer (Model E) were determined as described in the AOCS (1995).

Fatty acid composition

Preparation of fatty acid methyl esters of oils extracted from PSR and GS were performed according to the procedure of Radwan (1978), using 1% sulphuric acid in absolute methanol. The fatty acid methyl esters obtained were separated by Shimodzu gas chromatograph (GC- 4 CM- PFE) under the following conditions: column, 10 % DEGS on 801100 chromosorb Q III; Detector temperature 270°C: flow, N_2 and chart speed, 5 min. Standard fatty acid methyl esters were used for identification. The area under each peak was measured by the triangulation method as percentage of each fatty acid was regard to the total area.

Fractionation of total lipids and phospholipids

Total Lipid extracts of PSR and GS were fractionated using a TLC technique according to the method of O'Leary and Metthews (1990) on high performance silica gel plates, with 200 um thickness. The developing solvent was; hexane: diethyl ether: glacial acetic acid (75:23:2 by volume). The visualization was carried out by spraying with 8% orthophosphoric acid / Cupric solution followed by charring in an oven at 180 °C for 10 min. Fractionation of phospholipids was carried out using TLC technique according to the method of Borgstrom (1952) using the solvent system of chloroform: methanol: water (65:25:4 V/V/V). The identified classes and phospholipid sub-fractions were quantified by Shimadzu TLC scanner (C.S.910) according to Blank *et al*.(1964). The area under each peak was measured by triangulation and percentage of each class was calculated with regard to the total area.

Preparation of biscuits

Biscuits were prepared according to the method described by Askar (1991). The effect of enriching PSR and GS at 10,15 and 25% levels, based on the weight of wheat flour was studied. The biscuits formula was as follows:

Ingredients	**amounts (g)**
Wheat flour (72% extraction)	250
Shortening	100

Powdered sugar	100
Egg	70
Ammonium bicarbonate	0.50
Baking powder	1.25
Vanillin	4.0
Milk powder	25
Sodium chloride	1.0

Sensory evaluation of biscuit products

Colour, taste, odour, texture and overall acceptability of biscuits enriched with PSR and GS along with control were assessed by 15 panelists of Food Science and Technology Department, Faculty of Agriculture, Alexandria University. The panelists were asked to score the aformentioned attributes according to a standard hedonic rating scale from 9 (like extremely) to 1 (dislike extremely) according to Kramer & Twigg (1973).

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) and Duncan's multiple range test to separate the treatment means (Steel & Torrie, 1980). The analysis was computed using the SPSS V.11.5 program.

RESULTS AND DISCUSSION

Chemical composition of PSR and GS

Table (1) shows the chemical composition of PSR and GS. The results of proximate composition indicated that PSR contained significant higher amounts of crude protein, crude fat and Ash (12.99, 22.34 and 3.07%, respectively) compared with those of GS (10.58, 17.44 and 1.81%, respectively). On the other hand, GS contained significant higher amounts of crude fiber and carbohydrates (65.22 and 4.95%) compared with PSR (59.18 and 2.84%), respectively. Accordingly, both PSR and GS are considered a good source of crude fiber, fat and crude protein. These by-products could be utilized in fortification of foodstuffs. These results are nearly in accordance with those found by Uchoa *et al.* (2009) and Methew *et al.* (2014).

The results of mineral content of PSR and GS are shown in Table (1). The mineral contents can be arranged in a decreasing order based on their concentrations as follows: phosphorus, potassium, calcium, sodium, magnesium, manganese, selenium, zinc, copper and iron. The PSR contained significantly higher amounts of all the aforementioned minerals with zinc and copper being the exception compared with GS . Consequently, such by-products could be considered a good source of phosphorus, potassium, calcium, sodium, magnesium, selenium, copper and iron. These data are more or less in accordance with those reported by EL-Deek *et al.* (2009), Uchoa *et al*. (2009), El-Safey *et al*.(2012) and Methew *et al.* (2014).

In general, it could be concluded that both PSR and GS are characterized with high content of the most nutritive minerals. These data reveal the importance of utilizing such by-products in food from the health point of view.

Vitamins, including vitamins B_1, (thiamine), B_2(riboflavin) and E (α-tocopherol) content of PSR and GS were evaluated and the results are recorded in Table (1). Guava seeds contained significantly higher amounts of vitamins, B_1, B_2 and

Table 1: Proximate chemical composition, mineral and vitamin contents of pomegranate seed residues and guava seeds

Component	PSR*	GS**
A-Proximate composition (%)		
Crude protein	12.99 ± 0.08[a]	10.58 ± 0.10 [b]
Crude fat	22.34 ± 0.11[a]	17.44 ± 0.10 [b]
Ash	3.07 ± 0.08 [a]	1.81 ± 0.11[b]
Crude fiber	59.18 ± 0.18 [b]	65.22 ± 0.12 [a]
carbohydrate ***	2.84 ± 0.11[b]	4.95 ± 0.10[a]
B-Minerals (mg/ 100g DM)		
Phosphorus	990.09 ± 0.58[a]	752.13 ± 0.41[b]
Potassium	761.28 ± 0.35[a]	578.29 ± 0.28[b]
Calcium	498.74 ± 0.27[a]	286.78 ± 0.40[b]
Sodium	385.83 ± 0.19[a]	159.17 ± 0.36[b]
Magnesium	243.39 ± 0.45[a]	187.66 ± 0.28[b]
Iron	3.62 ± 0.12[a]	1.20 ± 0.11[b]
Zinc	12.71 ± 0.15[b]	21.86 ± 0.13[a]
Manganese	61.08 ± 0.16 [a]	42.43 ± 0.19 [b]
Copper	4.18 ± 0.17[b]	6.86 ± 0.13[a]
Selenium	39.45 ± 0.28[a]	25.95 ± 0.15[b]
C- Vitamins (mg/ 100gDM)		
B1 (Thiamine)	0.865 ± 0.09[b]	1.051± 0.11 [a]
B2 (Riboflavin)	0.123 ± 0.05[b]	0.263 ± 0.02 [a]
C (L-ascorbic acid)	2.754 ± 0.13[b]	5.249 ± 0.12 [a]
E (α-tocopherol)	1.839 ± 0.08 [a]	1.370 ± 0.10[b]

* (PSR) Pomegranate seeds residues and **(GS) guava seeds.
*** Carbohydrate calculated by difference. Values followed by the same letter in a row are not significantly different at $P \leq 0.05$.

C (1.051, 0.263 and 5.249 mg /100g Dry matter, respectively) as compared to those of PSR (0.865, 0.123,and 2.754 mg/100g dry matter, respectively). On the other hand, PSR contained significantly higher amount of vitamin E (1.839 mg/100g dry matter) compared with (1.370 mg/100g dry matter) for GS. These results are in accordance with those reported by El-Bedawey *et al*. (2010), El-Safey *et al.* (2012), Chaudhary & Tripathi (2014) and Anahita *et al.* (2015).

The determined vitamins naturally occur in PSR and GS are considered one of the most important phytochemicals having the antioxidants and antimicrobial properties and good stand point in human nutritional (Altunkalya, 2014).

Amino acid composition of PSR and GS

The amino acid composition of PSR and GS are shown in Table (2). It was obvious that PSR is higher than GS in lysine, leucine and histidine, the opposite was noticed for the rest of essential amino acids, since GS had a higher content of methionine, isoleucine, phenylalanine ,cysteine, threonine, tyrosine and valine. It could be noted that both PSR and GS exhibited much higher content of methionine, isoleucine phenylalanine, tyrosine, valine and histidine, than the reference protein pattern of FAO/WHO (2002). On the other hand, both PSR and GS were found to be deficient in some essential amino acids such as lysine. Therefore, addition of such food wastes to biscuit and bread and other foodstuff, especially that are deficient in amino acids containing sulphur, aromatic amino acids, leucine and isoleucine has a great economic value and a good stand point in food technology and human nutrition. The results of amino acid composition presented in the present study are more or less in a good agreement with those reported by El-Safey *et al.*(2012) and Methew *et al.* (2014).

Table 2: Amino acid composition of pomegranate seed residues and guava seeds

Amino acid (g/ 100g protein)	Seeds Protein		FAO /WHO pattern(2002)
	PSR*	GS**	
Essential a. a.			
Lysine	1.84 ± 0.11 [a]	1.16 ± 0.12 [b]	5.2
Methionine	2.75 ± 0.15 [b]	3.45 ± 0.13 [a]	2.2
Leucine	7.09 ± 0.12 [a]	6.87 ± 0.15 [b]	6.3
Isoleucine	4.82 ± 0.10 [b]	5.50 ± 0.11 [a]	4.2
Phenylalanine	2.92 ± 0.17 [b]	3.59 ± 0.19 [a]	2.8
Cysteine	1.08 ± 0.13 [b]	1.56 ± 0.11 [a]	-
Threonine	2.90 ± 0.12 [b]	3.63 ± 0.12 [a]	2.7
Tyrosine	4.36 ± 0.09 [b]	4.84 ± 0.10 [a]	4.1
Valine	4.50 ± 0.07 [b]	5.83 ± 0.11 [a]	4.2
Histidine	2.55 ± 0.06 [a]	2.08 ± 0.11 [b]	1.8
Total essential a. a.	34.81 ± 0.12 [b]	39.51 ± 0.15 [a]	
Non-essential a. a.			
Alanine	6.01 ± 0.11 [b]	8.59 ± 0.12 [a]	
Arginine	7.25 ± 0.18 [b]	10.63 ± 0.14 [a]	
Glutamic	24.55 ± 0.12 [a]	17.60 ± 0.10 [b]	
Glycine	8.19 ± 0.10 [a]	5.94 ± 0.11 [b]	
Aspartic	6.18 ± 0.16 [a]	3.71 ± 0.11 [b]	
Serine	4.04 ± 0.0.13 [b]	6.32 ± 0.17 [a]	
Proline	6.87 ± 0.12 [a]	4.35 ± 0.15 [b]	
Total non-essential a. a.	63.29 ± 0.15 [a]	57.10 ± 0.13 [b]	

* (PSR) Pomegranate seeds residues **(GS) guava seeds. Values followed by the same letter in a row are not significantly different at $P \leq 0.05$.

Antioxidant compounds and antinutritional factors of PSR and GS

Antioxidant compounds (polyphenols, total carotenoids, and total flavonoids) and antioxidants activity are shown in Table (3). The results indicated that PSR contained significantly higher amounts of polyphenols and total flavonoids (412.60 and 91.41 mg/100g DM) than GS (100.59 and 56.23mg/100g DM). On the other hand, GS contained significantly higher amounts of total carotenoids (223.12 mg/ 100g DM) than PSR (107.08 mg/ 100g DM). The results indicated that antioxidants activity in PSR were significantly higher than that in GS. These results are nearly in accordance with those obtained by Gamal *et al.* (2011), Altunkaa lya (2014), Hea *et al.*(2014), Mekni *et al*.(2014) and Anahita *et al.* (2015).

Antinutritional factors of PSR and GS are shown in Table (3). The significantly higher amount of trypsin inhibitors, haemagglutinin activity, phytic acid and oxalate were found in GS (43.77 TIU /mg DM, 406.88 unit /100gDM, 70.35 mg/100gDM and 6.93 mg/100gDM, respectively.) compared with PSR (21.18, 286.66, 13.09 and 1.87, respectively). On the other hand, PSR contained significantly higher amount of Alkaloids (15.28 mg/ 100g DM) than GS (2.89 mg/ 100g DM). Protein digestibility in PSR were significantly higher (88.57%) than in GS (83.06%) as shown in Table (3). The obtained results for antinutritional factors and protein digestibility in the present study are less or more than those

Table 3: Antioxidant compounds, antioxidant activity, antinutritional factors and protein digestibility of pomegranate seed residues and guava seeds

Component	PSR*	GS**
A-Antioxidants compounds (mg/100g DM)		
Polyphenols	412.60 ± 2.31[a]	100.59 ± 1.23[b]
Total carotenoids	107.08 ± 1.24[b]	223.12 ± 1.08[a]
Total flavonoids	91.41 ± 1.49[a]	56.23 ± 1.78[b]
B- Antioxidant activity (%)	73.17 ± 2.50[a]	49.24 ± 1.64[b]
C- Antinutritional factors		
Trypsin inhibitors (TIU/ mg DM)	21.18 ± 1.81[b]	43.77 ± 1.63[a]
Haemagglutinin activity (unit/100g DM)	286.66 ± 4.25[b]	406.88 ± 5.80[a]
Phytic acid (mg/100gDM)	13.09 ± 1.31[b]	70.35 ± 2.07[a]
Alkaloids (mg / 100g DM)	15.28 ± 1.74[a]	2.89 ± 1.15[b]
Oxalate (mg/ 100g DM)	1.87 ± 0.22[b]	6.93 ± 0.30[a]
D- Protein digestibility (%)	88.57 ± 1.31[a]	83.06 ±1.09[b]

* (PSR) Pomegranate seeds residues **(GS) guava seeds. Values followed by the same letter in a row are not significantly different at $P \leq 0.05$.

mentioned by El-Safey *et al.* (2012) and Mekni *et al*.(2014).

Physicochemical properties of PSR and GS oils

The PSR and GS were found to contain 22.34 and 17.44% oil, respectively (Table 1). Table (4) shows the physicochemical properties of oils extracted from PSR and GS. The crude oil had a light yellow colour, for both sources. The results also indicated that oils of PSR and GS were liquid at ambient temperature and had low acid value and free fatty acids as well as peroxide value which indicate its high stability to deterioration. Oils of PSR and GS had iodine values quite close to that of semi-dry oils such as corn and soybean oil (Rossell, 1991). These results are in accordance with those reported by Opute (2008), EL-Safey *et al.* (2014), and Mekni *et al*.(2014). They found that PSR and GS oils are close in their characteristics to other commonly consumed vegetable oils. Also PSR and GS oils were found to contain phytochemicals such as tocopherols and pigments.

Table 4: Physicochemical properties of pomegranate seed residues and guava seeds oils

Properties		Oil	
		PSR*	GS**
Refractive index (25/25°C)		1.4330 ± 0.00[b]	1.4963 ± 0.00[a]
Specific gravity (25 °C)		0.9009 ± 0.00[b]	0.9803 ± 0.00[a]
Acid value (mg KOH/ g oil)		1.02 ± 0.11[b]	1.38 ± 0.17[a]
Free fatty acids (as% oleic acid)		1.7530 ± 0.15[b]	1.9246±0.10[a]
Peroxide value (meEqO_2/kg oil)		0.5317± 0.18[b]	0.8981 ±0.15[a]
Iodine value (g of I_2/ 100g oil)		120.64 ±1.10[b]	137.53 ±1.23[a]
Saponification value (mg KOH/ g oil)		163.80 ±1.42[b]	190.49±1.52[a]
Unsaponifiable matter (%)		1.27 ± 0.16[b]	1.75 ± 0.10[a]
Colour	Yellow	31.0 ± 0.0[b]	38.0 ±0.0[a]
	Red	4.3 ± 0.0[a]	2.5 ± 0.0[b]
	Blue	0.5 ± 0.0[a]	0.1 ± 0.0[b]

* (PSR) Pomegranate seeds residues **(GS) guava seeds. Values followed by the same letter in a row are not significantly different at $P \leq 0.05$.

Lipid classes and phospholipids of PSR and GS oils.

The fractionation of lipid classes of crude oils extracted from PSR and GS are shown in Fig. (1) and Table (5). The results showed that total lipids consisted of 8 classes of polar and non- polar lipids of which triacylglycerols represented 83.35 and 79.22% of total lipids of PSR and GS oils, respectively. The separated classes were, phospholipids, monoacylglycerols, 1,2 diacylglycerols, sterols, 1,3- diacylglycerols, and hydrocarbon plus sterols esters. The free fatty acids were slightly low indicated the stability of crude oils. No data in the literature regarding the lipid classes of PSR and GS oils were found for comparative purposes. The fractionation of phospholipids of PSR and GS oil are shown in Fig. (2) and Table (5). The dominant phospholipid sub-fractions of these oils were phosphatidyl choline followed by phosphatidyl ethanol amine. Table (5) demonstrates that phosphatidyl inositol was a major component of PSR and GS oils that contained phospholipids followed by lysophosphatidyl choline. The remaining phospholipid sub-fractions were found in small amounts in both phospholipids of both oils. GS oil contained significantly high amounts of phosphatidyl ethanol

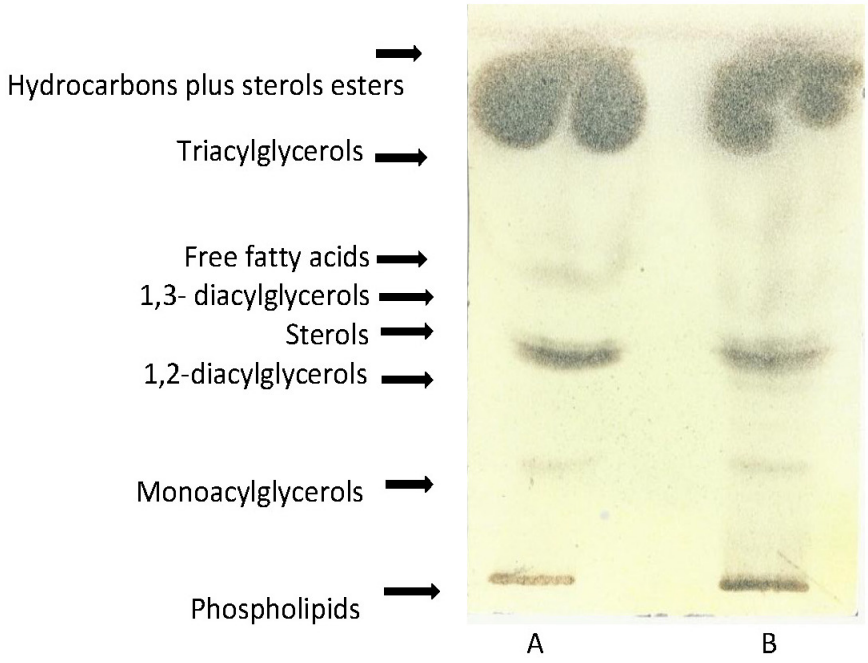

Fig. 1: Thin-Layer chromatogram of Lipid classes for pomegranate (A) and Guava (B) seeds oils

Table 5: Lipid classes and phospholipids of pomegranate seed residues and guava seeds oils

Lipid fractions	% of Total	
	PSR*	GS**
Lipid classes		
Phopholipids	3.64 ± 0.13^{b}	4.13 ± 0.16^{a}
Monoaclyglycerols	0.92 ± 0.18^{b}	1.87 ± 0.13^{a}
1,2- diacylglycerols	1.08 ± 0.10^{b}	2.35 ± 0.15^{a}
1,3-diacylglycerols	4.31 ± 0.14^{a}	3.40 ± 0.11^{b}
Sterols	4.12 ± 0.15^{b}	6.09 ± 0.11^{a}
Free fatty acids	0.74 ± 0.15^{a}	1.54 ± 0.17^{a}
Triacylglycerols	83.35± 0.54^{a}	79.22 ± 0.10^{b}
Hydrocarbons plus sterols esters	1.84 ±0.11^{a}	1.40 ± 0.10^{b}
Phospholipid sub-fractions		
Phosphatidyl serine	3.01 ± 0.37^{a}	1.74 ±0.22^{b}
Lysophosphatidyl choline	10.84 ± 0.58^{b}	13.09 ± 0.38^{a}
Phosphatidyl inositol	14.20 ± 1.68^{b}	17.83 ±1.18^{a}
Sphingomylein	5.84 ± 0.91^{a}	3.15 ± 0.90^{b}
Phosphatidyl choline	19.09 ± 1.95^{b}	25.65 ± 1.35^{a}
Phosphatidyl ethanol amine	31.45 ± 1.54^{b}	35.27 ± 2.24^{a}
Phosphatidyl glycerol	3.67 ± 0.83^{a}	1.98 ± 0.37^{b}
Phosphatidic acid	1.90 ± 0.13^{a}	1.29 ±0.18^{b}

* (PSR) Pomegranate seeds residues **(GS) guava seeds.
Values followed by the same letter in a row are not significantly different at $P \leq 0.05$.

amine, phosphatidyl choline, phosphatidyl inositol and lysophosphatidyl choline compared with PSR oil. No data in the literature on the phospholipid sub-fraction of PSR and GS oils were available for comparative purposes.

Fatty acid composition of PSR and GS oils

Fatty acid composition of oils extracted from PSR and GS are presented in Table (6). The fatty acid pattern revealed that total saturated content were 8.65% and 14.15% for PSR and GS oils, respectively, while the unsaturated fatty acids were 90.89 and 86.44%, respectively. Linolenic acid was found to be the dominant fatty acid (63.84%) in PSR oil, while linoleic acid was the major one (71.63%) in GS oil. Other minor fatty acids in PSR oil were linoleic acid and oleic acid represented 12.58% and 7.30%, respectively. On the other hand, the minor fatty acids in GS oil were linolenic acid (6.54%) and oleic acid (5.90%). The other saturated and unsaturated fatty acids in both PSR and GS oils were found in low or trace amounts. Mekni *et al.* (2014) indicated that pomegranate seed oil consist of 65-80% conjugated fatty acids, the most important of which is 9-trans, 11-cis, 13-trans, octadecatrienoic is the so-called punicic acid which isomers of linolenic acid. On the other hand, Opute (2008) indicated that linoleic acid was the major fatty acid represented about 51% of total fatty acids of GS oil. The presence of high amounts of linolenic acid and linoleic acid in PSR and GS oils suggested that these oils are of high nutritive value due to the ability of unsaturated oil to reduce serum cholesterol. These oils may be used as an edible cooking oil, as a salad oil or for the manufacture of shortening because their physicochemical characteristics and fatty acid composition are quite similar to corn oil and soybean oil (Rossell,1991).

Sensory evaluation of biscuit enriched with PSR and GS

The general appearance of biscuit enriched with PSR and GS are shown in Table (7). Generally all the attributes including taste, colour, texture, odour and overall acceptability of PSR and

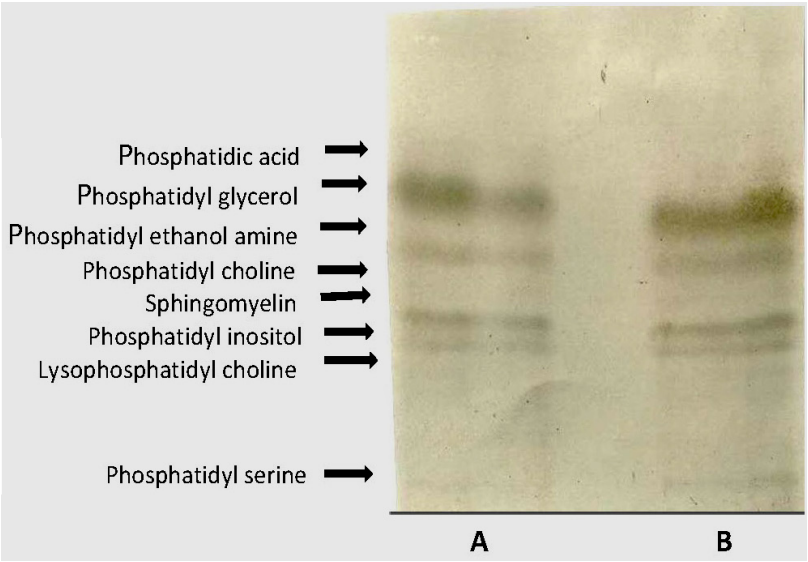

Fig. 2: Thin-Layer chromatogram of phospholipids for pomegranate (A) and guava (B) seeds oils

Table 6: Fatty acid composition of pomegranate seed residues and guava seeds oils

Fatty acids (%)	Oils	
	PSR*	GS**
Caproic	0.82 ± 0.08[a]	0.45±0.06[b]
Caprylic	1.02 ± 0.05	---
Capric	0.21 ± 0.06[b]	0.60 ± 0.04[a]
Hendecanoic	0.15 ± 0.03[b]	0.37 ± 0.02[a]
Lauric	0.06 ± 0.02[a]	0.08 ± 0.06[a]
Myrsitic	0.14 ± 0.05[a]	0.13 ± 0.07[a]
Pentadecanoic	0.33 ± 0.12[a]	0.08 ± 0.05[b]
Palmitic	3.44 ± 0.18[b]	5.12 ± 0.16[a]
Heptadecanoic	0.02 ± 0.03[a]	0.05 ± 0.03[a]
Stearic	2.10 ± 0.08[b]	6.29 ± 0.06[a]
Arachidic	0.23 ± 0.09[a]	0.10 ± 0.06[b]
Behenic	0.11 ± 0.02[a]	0.15 ± 0.04[a]
Total saturated fatty acids	8.65 ± 0.11[b]	14.15 ± 0.06[a]
Myristoleic	0.53 ± 0.10[a]	0.30 ± 0.09[b]
Pentadecenoic	0.14 ± 0.05[a]	0.20 ± 0.06[a]
Palmitoleic	2.85 ± 0.13[a]	1.08 ± 0.10[b]
Margaroleic	0.11 ± 0.06[b]	0.28 ± 0.05[a]
Oleic	7.30 ± 0.18[a]	5.90 ± 0.17[b]
Linoleic	12.58 ± 0.23[b]	71.63 ± 0.28[a]
Linolenic	63.84 ± 0.25[a]	6.54 ± 0.24[b]
Eicosenic	0.90 ± 0.17[a]	0.26 ± 0.14[b]
Docosadienoic	1.19 ± 0.09[a]	0.07 ± 0.06[b]
Docosatetradecenoic	1.10 ± 0.05	---
Docosahexaenoic	0.35 ± 0.05[a]	0.18 ± 0.08[b]
Total unsaturated fatty acids	90.89 ± 0.16[a]	86.44 ± 0.19[b]

* (PSR) Pomegranate seeds residues

**(GS) guava seeds.

Values followed by the same letter in a row are not significantly different at $P \leq 0.05$.

Table7: Sensory evaluation of biscuits enriched with pomegranate seed residues and guava seeds

Biscuit (%) of seeds enriched	Seeds	Taste	Colour	Texture	Odour	Overall acceptability
Control		8.85±o.50[a]	8.82±0.60[a]	8.88±0.67[a]	8.82±0.56[a]	8.84±0.49[a]
10	**PSR***	8.70±0.60[a]	8.79±o.50[a]	8.22±0.82[a]	8.40±0.73[a]	8.56±0.75[a]
	GS**	7.80±0.84[a]	8.60±0.49[a]	7.60±0.81[a]	8.12±0.63[a]	8.10±0.60[a]
15	PSR	8.66±0.96[b]	8.65±0.67[a]	7.84±0.62[a]	8.20±0.80[a]	8.36±0.80[a]
	GS	7.75±0.50[a]	7.50±0.84[b]	7.39±0.69[a]	7.81±0.73[a]	7.61±0.76[a]
25	PSR	7.81±0.67[a]	7.80±0.81[a]	7.21±0.50[a]	7.09±0.67[a]	7.48±0.65[a]
	GS	7.23±0.50[a]	7.15±0.80[a]	7.11±0.63[a]	7.80±0.95[a]	7.32±0.74[a]

* (PSR) Pomegranate seeds residues **(GS) guava seeds.
Values followed by the same letter in column are not significant different at $P \leq 0.05$.

GS biscuits were ranked between 7-8 score (like moderately and liked very much) at all enrichment levels (10,15 and 25%).

CONCLUSION

From the results presented in this work, it can be concluded that pomegranate seeds residues (PSR) and guava seeds (GS) contain phytochemicals, essential amino acids and essential unsaturated fatty acids which play an active role to improve the functional quality of biscuit, because they contain good amounts of phenolic compounds, pigments, tocopherols and crude fat. So, these seeds can be considered as a good source of oils which contain omega fatty acids (linoleic and linolenic acids). Therefore, further study is required to investigate the applicability of such seeds and their oils in several food products as fortifiers, such as meat products and bread.

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تقييم واستخدام بعض المركبات النشطة حيوياً فى متبقيات بذور الرمان وبذور الجوافة

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تعمل المركبات النشطة حيوياً على الحماية من الأمراض المتعلقة بالشريان التاجي للقلب وكذلك مرض السكرى. من ثم فأن تطوير الأغذية المحتوية على محتوى عال من المركبات النشطة حيوياً يكون امراً مرغوباً. تهدف هذه الدراسة الى التحقق من إمكانية استخدام متبقيات بذور الرمان (م ب ر) و بذور الجوافه (ب ج) كمصدر للمركبات النشطة حيويا.

أوضحت النتائج أن كلا من (م ب ر) و (ب ج) تحتوى على كمية عالية من المركبات النشطة حيوياً (تتمثل فى الألفا توكوفيرول وعديدات المواد الفينوليه و الكاروتينات و الفلافونويدات). ووجد أن (م ب ر) تحتوى على كميات أعلى من الألفا توكوفيرول والمواد عديدات الفينول والفلافونويدات (وتمثل ١,٨٣٩، ٤١٢,٦٠، ٩١,٤١ مجم/ ١٠٠ جم مادة جافة على الترتيب) وذلك بالمقارنة ب (ب ج) (١,٣٧٠، ١٠٠,٥٩، ٥٦,٢٣ مجم/ ١٠٠ جم مادة جافة على الترتيب). على الجانب الآخر وجد أن (ب ج) تحتوى على كمية أعلى من الكاروتينات (٢٢٣,١٢ مجم/ ١٠٠ جم ماده جافه) عن (م ب ر) (١٠٧,٠٨ مجم/ ١٠٠ جم ماده جافه). بالاضافة الي ذلك اشارت نتائج الدراسة إلى أن (ب ج) تحتوى على كمية عالية من مثبط التربسين و نشاط مسببات التجلط وحامض الفيتيك وحامض الاوكساليك (٤٣,٧٧ وحده مثبط تربسين / مجم مادة جافة ، ٤٠٦,٨٨ وحدة/١٠٠ جم مادة جافة، ٧٠,٣٥ مجم/١٠٠ جم مادة جافة، ٦,٩٣ مجم/١٠٠ جم مادة جافة على الترتيب) بالمقارنه ب (م ب ر) (٢١,١٨، ٢٨٦,٦٦، ١٣,٠٩، ١,٨٧ على الترتيب).

أشارت النتائج أيضاً إلى أن كلاً من (م ب ر) و (ب ج) تعتبر مصدراً جيداً للألياف الخام (٢١,١٨ ، ٦٥,٢٢ على الترتيب) بالاضافة الى ذلك فان كلا النوعين من البذور تحتوى على كمية مرتفعه من الزيوت الخام والاحماض الدهنية من النوع أوميجا. وكان الحامض الدهنى لينولينيك هو الحامض الدهنى غير المشبع السائد فى زيت (م ب ر) (يمثل ٦٣,٨٤ ٪) بينما كان الحامض الدهنى لينولينيك هو الحامض غير المشبع السائد فى (ب ج) ويمثل ٧١,٦٣ ٪.

تم دراسة استخدام كل من (م ب ر) و(ب ج) فى خلطات لإنتاج بسكويت وظيفي وأوضحت نتائج التقييم الحسى للمحكمين ان البسكويت الناتج لاقى قبولاً من قبل المحكمين.

تقييم واستخدام بعض المركبات النشطة حيوياً في متبقات بذور الرمان وبذور الجوافة

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تعمل المركبات النشطة حيوياً على الحماية من الأمراض المتعلقة بالشريان التاجي للقلب وكذلك مرض السكرى. من ثم فانه تطوير الأغذية المحتوية على محتوى عالياً من المركبات النشطة حيوياً يكون امراً مرغوباً. تهدف هذه الدراسة الى التحقق من إمكانية استخدام متبقيات بذور الرمان (م ب ر) و بذور الجوافه (ب ج) كمصدر للمركبات النشطة حيويا.

أوضحت النتائج ان كلا من (م ب ر) و (ب ج) تحتوى على كمية عالية من المركبات النشطة حيوياً (تتمثل فى الألفا توكوفيرول وعديدات المواد الفينوليه و الكاروتينات و الفلافونويدات). ووجد أن (م ب ر) تحتوى على كميات أعلى من الألفا توكوفيرول وعديدات المواد الفينوليه و الفلافونويدات (وتمثل ١,٨٣٩، ٤١٢,٦٠، ٩١,٤١ مجم/ ١٠٠ جم ماده جافه على الترتيب) وذلك بالمقارنه ب (ب ج) (١,٣٧٠، ١٠٠,٥٩، ٥٦,٢٣ مجم/ ١٠٠ جم ماده جافه على الترتيب). على الجانب الاخر وجد ان (ب ج) تحتوى على كمية أعلى من الكاروتينات (١٢، ٢٢٣ مجم/ ١٠٠ جم ماده جافه) عن (م ب ر) (١٠٧,٠٨ مجم/ ١٠٠ جم ماده جافه). بالاضافة الي ذلك اشارت نتائج الدراسة إلى أن (ب ج) تحتوى على كمية عالية من مثبط التربسين و نشاط مسببات التجلط وحامض الفيتك وحامض الاوكساليك (٧٧، ٤٣ وحده مثبط تربسين / مجم ماده جافه ، ٨٨، ٤٠٦ وحده/ ١٠٠ جم ماده جافه ، ٧٠,٣٥ مجم/ ١٠٠ جم ماده جافه، ٦,٩٣ مجم/ ١٠٠ جم ماده جافه على الترتيب) بالمقارنه ب (م ب ر) (١٨، ٢١، ٦٦، ٢٨٦، ١٣,٠٩، ١,٨٧ على الترتيب).

اشارت النتائج ايضا الى ان كلا من (م ب ر) و (ب ج) تعتبر مصدرا جيدا للالياف الخام (١٨، ٢١ ، ٢٢، ٦٥ على الترتيب) بالاضافة الى ذلك فان كلا النوعين من البذور تحتوى على كمية مرتفعه من الزيوت الخام والاحماض الدهنية من النوع أوميجا. وكان الحامض الدهنى لينولينك هو الحامض الدهنى غير المشبع السائد فى زيت (م ب ر) (يمثل ٦٣,٨٤ ٪) بينما كان الحامض الدهنى لينوليك هو الحامض غير المشبع السائد فى (ب ج) ويمثل ٧١,٦٣ ٪.

تم دراسه استخدام كلا من (م ب ر) و (ب ج) فى خلطات لانتاج بسكويت وظيفي واوضحت نتائج التقييم الحسى للمحكمين ان البسكويت الناتج لاقى قبولا من قبل المحكمين.