BIOACTIVE COMPOUNDS AND ANTIOXIDANT PROPERTIES OF FRUIT AND VEGETABLES BY-PRODUCTS

Hussein, A. Abdel Aal[1], Mohamed, A. Sorour[2], Mohamad, A. Kenawy[1], Sanaa, A. M. Saleh[1] and Amna, A. A. Osman*[2]

[1]Food Sci., Dept., Fac. of Agric, Minia Univ. [2]Food and Nutr Sci., Dept., Fac. of Agric, Sohag Univ.

Keywords: Fruit and vegetable antioxidants, total phenols, flavonoids, tannins and antioxidant activity, DPPH.

ABSTRACT

Fruits and vegetables play an important and vital role in human's life by its essential and significant amounts of nutrients and bioactive compounds which distributed in whole fruit and particularly concentrated in peels, seeds and kernels, known as "by-products". In this study 13 samples of fruit and vegetable by-products were investigated "orange, mandarin peels and seeds, pomegranate peels, mango peels and kernels, guava seeds, carrot, potato, green pea, onion peels and okra by-products", which are commonly used in domestic and food processing industries. The gross chemical composition were investigated, carbohydrates were abundant elements in peel, its content were related in pomegranate, mandarin, orange peels and mango peels and kernels samples ranged from (78.72 to 71.74%), crude fats and fiber were prevalent elements in seed samples, orange and mandarin seeds had highest content of crude fat (40.94 and 40.65 %), respectively, guava seed had highest content of crude fiber (60.24%). Bioactive compounds such as ascorbic acid, total phenol, and total flavonoids and tannins contents were investigated. The results indicated that the bioactive components were detected in sample extracts but its contents were varied in different samples. In general, total phenols were the highest concentration in both fresh and dried samples; pomegranate, mango peels and mango kernels had the highest content of total phenols (8.88, 7.21 and 8.99 mg/g, respectively) in fresh samples and (37.66, 27.62 and 24.75 mg/g, respectively) in dried samples. The antioxidants activities of methanolic extracts were measured using DPPH as free radical and the ability of by-product antioxidant extract to scavenging it. The results exhibited considerable degrees of activities towards scavenging of DPPH free radical, as well as, mango kernels, pomegranate and mango peels exhibited the highest degree of activity with IC50 (21.62, 31.09 and 27.19µg/ml of extract) from fresh by-product samples and (18.79, 20.99 and 5.88 µg/ml of extract) from dried by-product samples for pomegranate, mango peels and mango kernels, respectively.

INTRODUCTION

Fruit and vegetables production, trade and consumption have been increased significantly all over the world, on the domestic and international markets due to their attractive sensory properties and a growing the recognition of its nutritional and therapeutic value. As well as; raising the voices warning against the danger of using synthetic ingredients in food industries and decreasing the exposure to free radicals induced by pollution, cigarette smoke, drugs, illness, stress, and even exercise. Free radicals have been implicated in over a hundred disease conditions in human, damaging cells causing aging process, progressing and suffering from many chronic and degenerative diseases, including cancer, cardiovascular disease, cataract formation, malaria, and rheumatoid arthritis....ect. **(Bagchi, *et al.*, 2000; Fang, *et al.*, 2002 and Dauchet, *et. al.*, 2006).** Antioxidants where being "in vitro" or "in vivo" acts as a free radical scavenger, reducing agent, chelator, and/or singlet oxygen scavenger. There are two kinds of antioxidants, natural and synthetic antioxidants, natural antioxidants are healthier and safer than synthetic ones. Since about 1980, natural development of safer natural antioxidants extracted from plant materials could be replace synthetic antioxidants.**(Halladay, *et. al.*, 1980 and Shahidi, 2000).** Natural antioxidants such as amino acids, peptides, proteins, vitamins A, E and C and phenolic compounds, including flavonoids, tannins and lignins, have benefits due to health implications and functionality such as solubility in both oil and water, of interest for emulsions in food systems. Fruits and vegetables are rich alternative sources of natural antioxidants which could be complemented with antioxidants produced by human body, most of those components with antioxidant activities accumulated and concentrated in vascular plants, mainly are found in peels, leaves, buds, seeds and roots, to protect them

against predation from herbivorous animals and pathogenic attack from bacteria and fungi, by increasing the resistance of plants to fungal moulds, and could be toxic to insects **(Hernes, *et al*., 2001; Xie, *et al*., 2003 and Nothlings, *et al.*, 2008**). Fruits and vegetables consumption and processing generated large quantities of by-products as pomace, peels, seeds or kernels, outer leaves and unsuitable pulp for processing or eating, containing great amounts of substances with great biological, physiological and pathological importance as mentioned above. **Ayala-Zavala *et al*., (2011),** mentioned that the plant food processing industry produces large amounts of wastes and residues, estimated to be around 800 000 tons per year of fresh fruit and vegetable matter globally, might reach around 60% of harvested plants, without considering the wastage during processing, these residues are very perishable products and difficult to manage because of environmental problems in the industries. **Fylaktakidou, *et al.* (2004**) reported that coumarins are lactones belonging to phenolics, occurs in fruits, olive oil, vegetables, wine, and beverages like tea and coffee, and have been shown to have antioxidant and anticancer effects in cells and animal models. **Gonzalez-Aguilar, *et al.*, (2010)** reported that fruit and vegetable by-products are sources of a great variety of antioxidants, useful in maintaining food quality avoiding enzymatic browning in fruits.

In Egypt, the total annual production of the fruits and vegetables were increased slowly and rapidely for fruits and vegetables, respectively during the last five years (2009-2014), according to the last updated data of FAO organization statistics in 2016. It was 490≈ 500 thousand tons and 699 ≈ 700 thousand tons, respectively and the annual amounts of agricultural residues were about 30 million tons, **(FAOSTAT, 2016).** This study was carried out to evaluate the chemical composition, extraction yield, bioactive compounds content and its ability to scavenge free radical or

antioxidant activity of samples under investigation such as, orange and mandarin, peels and seeds, mango peels and kernels, guava seeds, peels of pomegranate, carrot, green pea, potato and onion and okra by-product.

MATERIALS AND METHODS

Materials:

Ten kinds of fruit and vegetable were purchased from local markets at Sohag City, Egypt.

2,2'-diphenyl-1-picrylhydrazyl (DPPH), 2.6 dichlorophenol indophenols, gallic acid, quercetin, vanillin, catechin, ascorbic acid, 2, 6-Di-tert-butyl-4-methylphenol (BHT),Trolox, were purchased from Sigma-Aldrich (St Louis, MO, USA). Folin-Ciocalteau was sourced from Appli Chem (GER). All other chemicals were either of analytical grade or of the highest quality.

Methods:

Preparation of by-products for analysis and extraction:

Fruit and vegetable samples were weighted, carefully washed by tap water followed by distilled water, trimmed and peeled manually by kitchen knife to obtain its by-products as samples "orange, mandarin peels and seeds, pomegranate peels, mango peels and kernels, guava seeds, carrot, potato, green pea, onion peels and okra by-products". By-product samples were divided into two portions, the first portion analyzed at fresh state and the second was dried in an electric oven at 50°C, grinded to a fine powder (32 meshes), samples were stored at -20°C in a deep freezer until further analysis.

Extraction of antioxidants:

Three grams (3 g) of each fresh and dried by-product samples were extracted with 30 ml of 80% aqueous methanol, stirred for 1 hr by a magnetic stirrer, and then centrifuged for 10 min at 3500 rpm. The extracts were filtered using Whatman NO.1 filter paper, the extraction of the residue was repeated under the same conditions, two filtrates were combined and transferred into an evaporating flask with an additional

25 mL 80% (v/v) aqueous methanol. The solvent was evaporated using a rotary evaporator (Stone-Staffordshire. ST15 0SA. UK) at 40°C till obtained dried extract, dried extract were weighted to calculate its percentage of extraction yield as shown in following equation. Constant weight of dried extracts were adjusted to constant volume by aqueous methanol and stored at –22°C. Each sample was prepared and analyzed for each assay in triplicate. (**Balasundram, *et al.*, 2006).**

$$\text{Extraction yield (\%)} = \frac{\text{Weight of the residue}}{\text{Total weight of the peel powder}} \times 100$$

Analytical methods:

Determination of gross chemical composition and ascorbic acid:

Moisture, crude protein, crude fat, ash, crude fiber contents (after lipid extraction, by using acid and alkali digestion method) and ascorbic acid (using 2.6 dichlorophenol indophenol) were determined by standard and official methods (**A.O.A.C, 2000).**

Determination of Total Phenolics Content (TPC):

Total phenolics content was determined by the Folin–Ciocalteu method using Folin–Ciocalteu's Reagent (FCR). Gallic acid was used as standard and total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per gram of by-product samples (mg GAE/100g) **(Vinson, *et al.*, 2001**).

Determination of Total Flavonoids Content (TFC):

Aluminum chloride colorimetric method was used for Total Flavonoids Content (TFC) determination, the concentration was expressed in terms of mg of quercetin/100g.

(Prasad, *et al.*, 2010)

Determination of tannin compounds:

Quantitative estimation of tannins was carried out using modified vanillin–HCl in methanol method, tannin contents were expressed as milligrams of catechin equivalents (CE) per gram by-product samples (mg CE/100g) **(Price, *et al.*, 1978)**.

Determination of antioxidant activity:

Free Radical Scavenging Activity (FRSA) assay by using DPPH:

The electron donation ability of the obtained extract was measured by bleaching of the purple colored methanolic solution of DPPH, according to the method described by **Molyneux, (2004**). The FRSA of the sample extracts were calculated as a percentage of DPPH decolouration using the following equation:

% FRSA or % Inhibition = (1 – Abs of sample /Abs of control) × 100

Determination the IC50 of sample extracts:

IC50 means concentration of sample extract providing fifty percent inhibition of DPPH, IC50 was calculated by plotting inhibition percentages against concentrations of the sample. Standards with ascorbic acid, 2, 6-Di-tert-butyl-4-methylphenol (BHT) and Trolox at various concentrations were used for comparison.

Statistical Analysis: The data were analyzed in order to determine significant differences between samples (ANOVA). Comparison of means was performed by the least-squares difference (LSD) method. Differences were considered significant at level $p < 0.05$ **(SAS, 1993).**

RESULTS AND DISCUSSION

***Moisture, dry matter content and extraction yield of by-product samples*:**

Moisture and dry matter and the percentage of extraction yields for fresh and dried samples were shown in Table (1).The percentages of all by-product samples were obviously decreased after drying due to the highly contents of moisture. In general fruit and vegetable by-products contained high amount of water and it are known as a perishable substance **(Ajila, *et al.*, 2010).**

Table (1); Moisture, dry matter content and extraction yield of by-product samples.

Samples	Moisture (%)	Dry matter (%)	Extraction yield (g/100 g of F.s*)	Extraction yield (g/100 g of D.s**)	Extraction yield ratio Wet : Dry
Orange peels	75.80 ±0.15^{F}	24.20±0.15^{E}	11.66 ± 0.15 ^{BC}	45.28±0.94 ^{D}	1 :3.88
Orange seeds	53.99 ±0.23 ^{I}	46.01±0.23 ^{B}	11.69 ± 0.77 ^{CD}	16.43±0.80 ^{H}	1 :1.39
Mandarin peels	77.85 ±0.13^{EF}	22.15±0.13^{FG}	8.39 ± 0.75 ^{D}	50.34±1.15 ^{B}	1 :6.00
Mandarin seeds	49.53 ±2.76 ^{J}	50.47±2.76 ^{A}	8.22 ± 1.94 ^{D}	13.80±0.26 ^{I}	1 :1.68
Pomegranate peels	60.40±2.1^{G}	39.60±2.1 ^{D}	13.12 ± 0.70 ^{B}	56.52±3.47 ^{A}	1 :4.29
Mango peels	78.05±0.27 ^{D}	21.95±0.27 ^{G}	17.65 ± 0.28 ^{A}	43.94±4.82 ^{D}	1 :2.49
Mango kernels	60.06±2.99 ^{G}	39.94±2.99 ^{D}	11.80 ± 0.45 ^{B}	24.88±1.06 ^{G}	1 :2.11
Guava seeds	80.55±1.21 ^{CD}	19.45±1.21^{GH}	4.33 ± 0.34 ^{F}	2.31±0.24 ^{J}	1:0.533
Carrot peels	87.83±0.08 ^{A}	12.17±0.08 ^{J}	6.96 ± 0.34 ^{EF}	48.17±1.61^{C}	1 :7.31
Potato peels	85.43±0.44 ^{B}	14.57±0.44 ^{I}	3.47 ± 0.21 ^{F}	14.98±0.17 ^{I}	1 :4.32
Green pea peels	58.10±1.39 ^{H}	41.90±1.39 ^{C}	5.18 ± 0.16 ^{F}	32.10±2.45 ^{F}	1 :6.37
Onion peels	79.73±0.24 ^{ED}	20.27±0.24^{FG}	7.50 ± 0.57 ^{DE}	31.70±2.08 ^{F}	1 :4.51
Okra by-product	82.37±0.11 ^{C}	17.63±0.11^{H}	4.49 ± 0.12 ^{F}	38.99±0.28 ^{E}	1 :8.41
LSD*	2.16	2.16	2.33	1.34	

Values are expressed as mean ± S.D standard deviation, triplicate determination
Values within the same column followed by same letters are not significantly different ($p< 0.05$).
* Least Significant Difference ($p < 0.05$). * Fresh samples, ** Dried samples at 50°C.

Moisture levels of food products have a bearing on their dry matter content. The higher moisture content the lower dry matter yield on drying. Therefore information on moisture content would help the food processors to make decisions on the economics of thermal processing of the foods **Fedha, *et al.*, (2010).**

Moisture content were varied between samples where some of them had high levels of moisture (carrot, potato peels and okra by-products about 87.83%, 85.43%, and 82.37%, respectively) others had low contents (green pea peel, orange and mandarin seed about 58.10 %, 53.99% and 49.53%, respectively. **Asquer, *et al.*, (2013)** reported that by-product had high moisture content (80%), and is difficult to be handled, this by-product contains plenty of dietary fiber and bioactive compounds which can be extracted and used as value-added materials by incorporate it into food, utilization of this could not only eliminate a possible source of pollution but also add economic value.

Methanolic extraction were applied to extract natural antioxidants from by-product samples and to

evaluated extraction yields, aqueous methanol solution (80:20 v:v) was used. **Rehman, (2006**) reported that methanol was more efficient solvent in extraction of bioactive component. Fresh samples as mango and pomegranate peels produced had highest extraction yield 17.65 and13.12%, respectively, orange peels and seeds and mango kernels came after with nearest percentages and potato peels presented lowest extraction yield (3.47%) but in general, there were no great differences between fresh samples. Dried samples exhibit great differences in extraction yield, especially between peel and seed samples, it were remarkable low values for seed's which could be discussed by low moisture content of seeds. Pomegranate peels came in first rank with extraction yield about 56.52%, and then mandarin and carrot peels about 50.34 and 48.17%, respectively, while guava seed came in last rank by percentage about 2.31%. **Jayaprakasha, *et al.*, (2001),** reported that variation in extraction yields of different extracts might be attributed to differences in polarity of compounds found in plants. **Kwok, *et al.*, (2004)** mentioned that drying process is increasingly used to extend the shelf life of raw materials with high moisture content like fruits, vegetables and its by-products. Drying allows longer periods of storage, minimizes packing requirement, transport, handling, and distribution, soft drying conditions should be realized immediately and as close to the production location as possible to prevent the deterioration of nutrient and biological active of food components by heating, enzymatic and microbial changes. Existing industrial production lines facilitate procurement, reduce transport costs and avoid long-term storage of voluminous wet material. **(Yusof, *et al.*, 1990 and Van der Sluis, *et al.*, 2001)** reported that most of the antioxidants in fresh apples were retained in the solid matter rather than being transferred into the juice during pressing, and mentioned that peels were much richer on its

content of bioactive compounds than seeds but, the composition of seeds and peels is not always the same for a determined species.

Chemical composition of fruit by-product samples: The gross chemical compositions of by-product samples are shown in Table (2). There were significant differences between samples on its content of different constituents, in general Carbohydrates were abundant constituents but its contents were varied between samples. Mango kernels had highest amounts (78.72 %) and mandarin seeds had lowest contents (16.42%), but it had highest contents of crude fiber (60.24%), as well as most samples were poor on its contents of crude fat except orange and mandarin seeds had considerable amounts of crude fat 40.94 and 40.65%, respectively. Crude Protein and ash content were varied in by-product samples and Okra by-product had highest contents 16.24 and 10.40%, respectively and carrot peels and guava seeds had lowest contents (2.32 and 0.84%) of protein and ash, respectively.

Table (2); Chemical composition of by-product samples.

Constituents / Samples	Crude protein (%)	Crude fiber (%)	Crude fat (%)	Carbohydrate* (%)	Ash (%)
Orange peels	6.49±0.11 ^{G}	9.65±1.00 ^{FG}	4.59±0.36^{EF}	75.71±0.38 ^{C}	3.56±0.04 ^{G}
Orange seeds	13.50±0.22^{D}	14.02±0.07 ^{D}	40.94±1.35 ^{A}	28.72±0.43 ^{H}	2.82±0.06 ^{H}
Mandarin peels	8.09±0.21 ^{F}	6.11±0.91^{I}	6.30±0.28 ^{D}	76.82±0.36 ^{B}	2.68±0.03 ^{HI}
Mandarin seeds	14.04±0.16 ^{C}	26.47±0.09 ^{B}	40.65±0.67 ^{A}	16.42±0.39 ^{J}	2.42±0.62 ^{I}
Pomegranate peels	5.30±0.22^{I}	9.99±0.18^{G}	4.09±0.32 ^{F}	76.76±0.20 ^{B}	3.86±0.09^{FG}
Mango peels	3.72±0.01^{J}	12.41±0.43 ^{E}	8.02±0.11 ^{C}	71.74±0.15 ^{D}	4.11±0.05 ^{F}
Mango kernels	5.76±0.13^{HI}	4.37±0.94^{J}	8.60±0.23 ^{C}	78.72±0.33 ^{A}	2.55±0.02 ^{HI}
Guava seeds	6.01±0.20 ^{GH}	60.24±0.41 ^{A}	13.24±1.03 ^{B}	19.67±0.44 ^{I}	0.84±0.12 ^{J}
Carrot peels	2.32±0.24^{K}	10.46±1.35 ^{F}	3.99±1.37 ^{F}	68.82±0.78 ^{E}	14.41±0.16^{A}
Potato peels	13.83±0.97 ^{CD}	8.86±0.26^{H}	4.12±0.35^{F}	65.76±0.42 ^{F}	7.43±0.10 ^{C}
Green pea peels	15.10±0.16 ^{B}	20.94±1.55 ^{C}	5.23±2.59 ^{DEF}	53.30±1.08^{G}	5.43±0.02 ^{E}
Onion peels	10.13±0.18 ^{E}	11.99±0.18 ^{EF}	5.98±0.72 ^{DE}	65.90±0.28^{F}	6.00±0.04 ^{D}
Okra by-product	16.24±0.29 ^{A}	13.91±0.42 ^{D}	5.82±0.26 ^{DE}	53.63±0.25^{G}	10.40±0.02^{B}
LSD**	0.524	0.312	1.633	1.10	0.312

* Carbohydrates were evaluated by difference.
Values are expressed as mean ± standard deviation, triplicate determination
Values within the same column followed by same letters are not significantly different ($p < 0.05$).
** Least Significant Difference ($p < 0.05$).

Antioxidant compounds content of fresh and dried by-product samples: Antioxidant compounds and antioxidant activity were studied in many researches which revealed that these compounds are concentrated and accumulated in peels and seeds of some fruits and vegetables by-products **Ajila, *et al.*, (2007)**. Ascorbic acid, total phenols, total flavonoids and tannins (as antioxidant compounds) of different by-product samples were determined; its contents in fresh and dried samples were illustrated in Tables (3 and 4).

Ascorbic acid is considered as a major, naturally occurring nutrient and antioxidant in our daily diet. It has an anti-carcinogenic effect, **Kim, *et al.*, (2002).** Statistical analysis revealed significant differences (P <0.05) between samples. As shown in Tables (3 and 4), fresh or dried mandarin and orange peels are excellent sources of ascorbic acid and presented a higher content of ascorbic acid in comparison with the other peel samples studied (0.963 and 0.718 mg of ascorbic acid/g of f. w., respectively) mean while guava seeds presented a lowest contents of ascorbic acid (0.034 mg of ascorbic acid/ g f.w). Ascorbic acid content after drying were highest in mandarin and orange peels samples too, (5.17 and 4.33 mg of ascorbic acid/g of d.w., respectively), guava and orange seeds presented a lowest contents of ascorbic acid (0.010 and 0.094 mg of ascorbic acid/g d.w., respectively). So that fruit and vegetable by-products especially citruses could be used in food and pharmaceutical industries as a source of ascorbic acid or natural antioxidant compound **Yusof, *et al.*, 1990). Ramful, *et al.*, 2011** classified fruits according to the ascorbic acid content into three categories: low (< 0.3 mg/g), medium (0.3– 0.5 mg/g) and high (> 0.5 mg/g). The phenolic compounds found in fruits and vegetables by-products have attracted much interest due to their potential as antioxidants. Total phenols were highest concentration in both fresh and dried samples but its content were

obviously increased after drying process as illustrated in Tables (3 and 4) pomegranate, mango peels and mango kernels had the highest content of total phenolics (8.88, 7.21 and 8.99 mg/g, respectively) in fresh samples and 37.66, 27.62 and 24.75 mg/g, respectively in dried samples.

Table (3); Antioxidant compounds content of fresh by-product samples.

Samples	Conc mg/g of Fresh by-product			
	Ascorbic acid	Total phenolics	Total flavonodies	Tannins
Orange peels	0.718±0.02 ^{B}	1.78±0.07 ^{D}	0.15±0.01 ^{EF}	0.172±0.13 ^{B}
Orange seeds	0.09±0.01 ^{ED}	0.433±0.03 ^{FGH}	0.082±0.02 ^{GH}	0.0833±0.01^{E}
Mandarin peels	0.963±0.02 ^{A}	1.17±0.15 ^{E}	0.165±0.01 ^{E}	0.106±0.02 ^{C}
Mandarin seeds	0.091±0.03 ^{D}	0.645±0.01 ^{FG}	0.147±0.01 ^{EF}	0.006±0.01 ^{I}
Pomegranate peels	0.087±0.01 ^{ED}	8.88±0.46 ^{A}	0.901±0.04 ^{B}	0.423±0.35 ^{A}
Mango peels	0.086±0.01 ^{ED}	7.21±0.17 ^{B}	0.391±0.01 ^{D}	0.0528±0.05 ^{F}
Mango kernels	0.085±0.01 ^{ED}	8.99±0.39 ^{A}	0.67±0.03 ^{C}	0.0889±0.01^{D}
Guava seeds	0.034±0.03 ^{F}	0.71±0.11 ^{F}	0.055±0.06 ^{H}	0.0111±0.02^{H}
Carrot peels	0.040±0.01 ^{F}	0.29±0.08 ^{H}	0.121±0.03 ^{FG}	Nd
Potato peels	0.041±0.03 ^{F}	0.36±0.02 ^{GH}	0.046±0.01 ^{H}	0.0167±0.04^{GH}
Green pea peels	0.073±0.03 ^{E}	0.53±0.09 ^{FGH}	0.065±0.01 ^{H}	0.0028±0.01 ^{J}
Onion peels	0.283±0.01 ^{C}	4.45±0.27 ^{C}	1.17±0.03 ^{A}	0.031±0.02 ^{G}
Okra by-product	0.231±0.01 ^{F}	0.36±0.04 ^{FGH}	0.086±0.01 ^{GH}	0.011±0.02^{H}
LSD*	0.0184	0.345	0.043	0.0056

Values are expressed as mean ± S.D standard deviation, triplicate determination
Values within the same column followed by same letters are not significantly different ($p < 0.05$).
* Least Significant Difference ($p < 0.05$).Nd: not detected.

Flavonoids are a widely distributed group of polyphenolic compounds with health-related properties, which are based on their antioxidant activity. Epidemiological studies suggest dietary intake of flavonoids may reduce the risk of many chronic diseases **(Romagnolo and Selmin, 2012)**.

In this study, the contents of total flavonoids obtained from fresh and dried samples were lowest than ascorbic acid and total phenol content, onion, pomegranate, mango peels and mango kernels had highest contents (1.17, 0.901, 0.39 and 0.67 mg of quercetin/ g f.w., respectively). Green pea and potato peels had the lowest content of flavonoids (0.065and 0.046 mg of quercetin/ g f.w., respectively), total flavonoids contents were increased after drying process for onion, pomegranate peels with highest contents (2.35, 1.359 mg of quercetin/ g d.w., respectively), but decreased for mango peels and kernels (0.346

and 0.510 mg of quercetin/ g d.w., respectively).

Table (4); Antioxidant compounds content of dried by-product samples.

Samples	Conc mg/g of Dried by-product		
	Ascorbic acid	**Total phenolics**	**Total flavonodies**
Orange peels	4.33±0.04 ^{B}	5.46±0.36 ^{D}	0.114±0.01^{GH}
Orange seeds	0.094±0.01 ^{J}	6.01±0.14 ^{D}	0.163±0.01 ^{FG}
Mandarin peels	5.17±0.08 ^{A}	4.52±0.27 ^{DEF}	0.142±0.02^{FGH}
Mandarin seeds	0.19±0.01 ^{I}	1.22±0.35 ^{G}	0.172±0.03^{F}
Pomegranate peels	0.70±0.03 ^{E}	37.66±3.7 ^{A}	1.359±0.07 ^{B}
Mango peels	1.29±0.02 ^{D}	27.62±3.4 ^{B}	0.346±0.02 ^{D}
Mango kernels	0.39±0.02 ^{G}	24.75±0.99 ^{C}	0.510±0.02 ^{C}
Guava seeds	0.10±0.02 ^{J}	1.53±0.22 ^{G}	0.243±0.03^{E}
Carrot peels	0.543±0.03 ^{F}	2.79±0. 56 ^{EFG}	0.113±0.01 ^{GH}
Potato peels	0.311±0.02 ^{H}	2.29±0.29^{FG}	0.092±0.01 ^{H}
Green pea peels	0.584±0.02 ^{F}	2.14±0.14^{G}	0.129±0.01^{FGH}
Onion peels	0.379±0.02 ^{G}	5.07±0.65^{DE}	2.35±0.05 ^{A}
Okra by-product	2.46±0.07 ^{C}	1.93±0.23 ^{G}	0.172±0.03^{F}
LSD*	**0.059**	**2.37**	**0.051**

Values are expressed as mean ± S.D standard deviation, triplicate determination
Values within the same column followed by same letters are not significantly different ($p < 0.05$).
* Least Significant Difference ($p < 0.05$).Nd: not detected.

The contents of tannins in fresh samples were obviously law compared with other antioxidant compounds and evaluated in mg per gram of samples. Pomegranate, orange mandarin peels had highest content (0.423, 0.172 and 0.106 mg of catechin/ g f.w., respectively).

Prior and Cao (2000) mentioned that there were large variations in total antioxidant capacity, total polyphenols, flavonol and carotenoids content of the samples and documented that some factors such as cultivars, maturity and other environmental factors such as sun light exposure, may influence the antioxidant capacity of fruits and vegetables.

Dorta, *et al*., (2012) mentioned that drying treatment may cause an enhancement of the extractability of different compounds.

DPPH radical-scavenging activity of sample extracts:

The DPPH radical scavenging capacity estimation is simple, it has been routinely applied in aqueous-organic extracts **(Pérez-Jiménez, *et al*., 2008**) suggested that the antioxidant capacity values, ought to be compared when the measurements have been made by the same method and with the same solvent. So that this method had been used in screening the antioxidant properties of pure compounds (standard antioxidants like ascorbic acid, BHT and Trolox) and by-products sample extracts. **Cheng, *et al*. (2006)** reported that the major advantage of this method over other assays is its broad solvent compatibility with aqueous and polar and nonpolar organic solvents, allowing it to evaluate both hydrophilic and lipophilic antioxidant compounds for their DPPH$^{\bullet}$ scavenging capacities under same experimental conditions without the use of stabilizing agents. Figure (1) showed extracts screening process of by-product samples by its antioxidant activity or its ability to scavenging and inhibiting DPPH free radical and the percentage of inhibition.

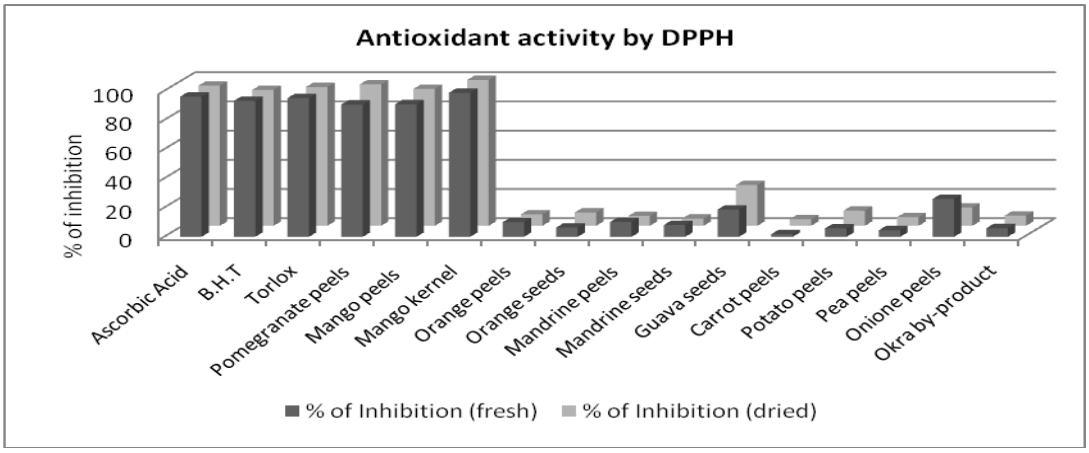

Figure (1) percentage of DPPH inhibition of extract (100 µg/ml) of fresh and dried by-product samples.

Presented data demonstrated that there were significant differences between the studied fruit and vegetable by-products in their abilities

to inhibit DPPH free radical. However fresh and dried mango kernels, pomegranate and mango peels exhibited the highest degree of activity almost equal the activities of standard antioxidants such ascorbic acid, BHT and Trolox which used for compare as shown in Figure (1). The IC50 (concentration of sample extracts inhibiting fifty percent of DPPH) were determined for sample extracts. The relationship between percentage of inhibition and concentrations of extract of fresh and dried mango kernels, pomegranate and mango peels compared with standard antioxidants were illustrated in Figures (2 and 3). As shown IC50 were about 21.62, 31.09 and 27.19µg/ml of extract from fresh by-product samples and 18.79, 20.99 and 5.88 µg/ml of extract from dried by-product samples for pomegranate, mango peels and mango kernels, respectively. Since the antioxidant capacity of food is determined by a mixture of different antioxidants with different action mechanisms, among which synergistic interactions, it is necessary to combine more than one method in order to determine in vitro, the antioxidant capacity of foodstuffs **(Frankel & Meyer, 2000** and **Mohdaly, *et al.* 2010).**

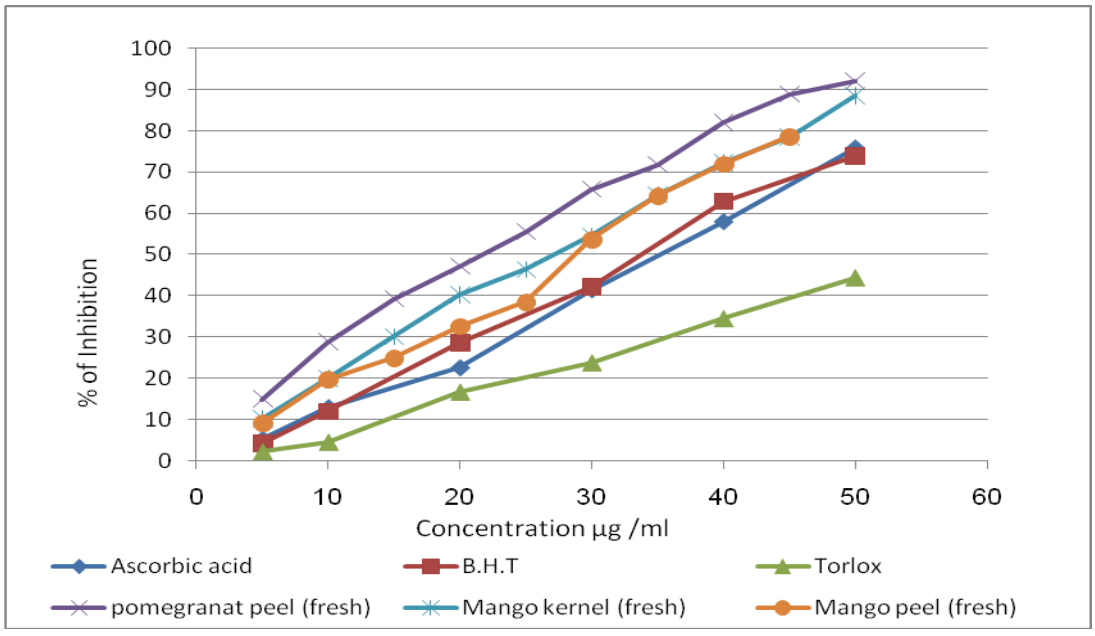

Figure (2) percentages of DPPH inhibition at different concentrations of extract (µg/ml) of screened fresh by-product samples compared with standard antioxidants.

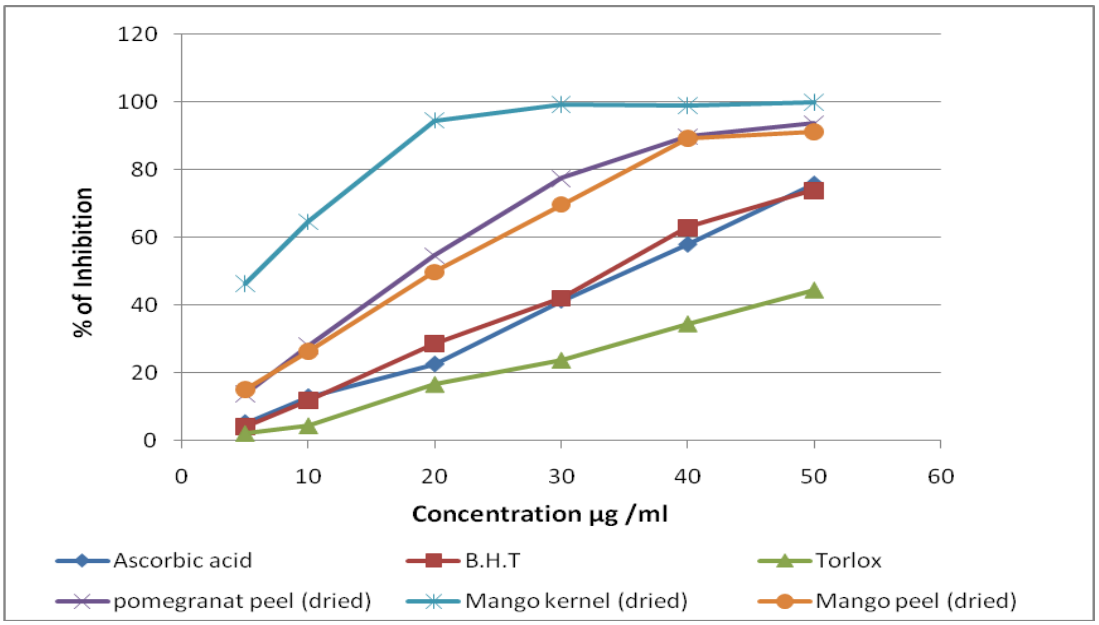

Figure (3) percentages of DPPH inhibition at different concentrations of extract (µg/ml) of screened dried by-product samples compared with standard antioxidants.

CONCLUSION

Most extracts of fruit and vegetable by-products exhibited potent antioxidant activities in DPPH free radicals. It was indicated that the highest level of antioxidants activity was detected in the extracts of mango kernels, which exhibited efficient antioxidant activity and lowest value of IC50 with DPPH free radicals. A correlation was observed between antioxidant activities and phenolics contents of extracts. Thus, fruit and vegetable by-products are potential source of natural antioxidants. The exploitation of these abundant and low-cost renewable resources could be anticipated for food industries as food additives or ingredients especially; Fats, oils and lipid-based foods, and for the pharmaceutical and cosmetic industries.

REFERENCES

Ajila CM, Naidu KA, Bhat SG, and Prasada-Rao UJS, Bioactive compounds and antioxidant potential of mango peel extract. *Food Chemistry*, **105(3), 982-988(2007)**.

Ajila CM, Aalami M, Leelavathi K., and PrasadaRao UJS, Mango peel powder: A potential source of antioxidant and dietary fiber in macaroni preparations. *Innovative Food Science and*

Emerging Technologies **11: 219–224 (2010).**

A.O.A.C. Official Method of the Association of Official Analytical Chemists 17th Ed**.** Published by the Association of Official Analytical Chemists.ArlingtonVirginia. U.S.A **(2000).**

Asquer C, Pistis A, and Scano EA, Characterisation of fruit and vegetable wastes as a single substrate for the anaerobic digestion. *Environmental Engineering and Management Journal*, **12, 11, 89-92 (2013).**

Ayala-Zavala JF, Vega-Vega V, Rosas-Dominguez C, Palafox-Carlos H, VillaRodriguez JA, Siddiqui M, *et al*, Agro-industrial potential of exotic fruit byproducts as a source of food additives. *Food Research International*, **44, 1866–1874 (2011).**

Bagchi D, Bagchi M, Stohs SJ, Das DK, Ray SD, Kuszynski C A, Joshi SS and Pruess HG, Free radicals and grape seed proanthocyanidin extract: importance in human health and disease prevention. *Toxicology*, **148(2-3): 187-197 (2000).**

Balasundram N, Sundaram K, and Samman S, Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses.*Food Chemistry* **99:191-203 (2006).**

Charles DJ, Antioxidant Properties of Spices, Herbs and Other Sources. Springer, New York Heidelberg Dordrecht *London*, **p.50 (2013)**.

Cheng Z, Moore J, and Yu L, A high-throughput relative DPPH radical scavenging capacity (RDSC) assay. *J Sci Food Agric* **54:7429–7436 (2006).**

Dauchet L, Amouyet P, Hercberg S, **and Dallongeville J, (2006).** Fruit and vegetable consumption and risk of coronary heart diseases. A meta-analysis of cohort studies. JN. *The J. of Nutrition, Nutritional Epidemiology*.**P:2588- 2593**

Dorta E, Lobo MG, and Gonzále M, **(2012).** Using drying treatments to stabilize mango peel and seed: Effect on antioxidant. *LWT - Food Science and Technology*, **45. 261-268**

Fang YZ, Yang S, and Wu G, (2002). Free radicals, antioxidants, and nutrition. *Nutrition*, **18(10): 872-879**

FAOSTAT (Food and Agriculture Organization Statistics), (2016). http://faostat.fao.org/

Frankel EN, and Meyer AS, Review. The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. *Journal of the Science of Food and Agriculture*, **80, 1925–1941 (2000**).

Fedha MS, Mwasaru MA, Njoroge CK, Ojijo NO and Ouma GO, (2010). Effect of drying on selected proximate composition of fresh and processed fruits and seeds of two pumpkin

species. *Agriculture and Biology Journal of North America*. **1(6): 1299-1302**

Fylaktakidou KC, Hadjipavlou-Litina DJ, Litinas KE, and Nicolaides DN, (2013). Natural and synthetic coumarin derivatives with anti-inflammatory antioxidant activities. *Curr Pharm Design*, **10:3813–3833 (2004),** (**C.F, Charles, 2013**).

Gonzalez-Aguilar GA, Villa-Rodriguez JA, Ayala-Zavala JF, and Yahia EM, (2010). Improvement of the antioxidant status of tropical fruits as a secondary response to some postharvest treatments. *Trends Food. Sci. Technol*. **21, 475–482.**

Halladay SC, Ryerson BA, Smith CR, Brown JP, and Parkinson TM, (1980). Comparison of effects of dietary administration of butylatedhydroxytoluene or a polymeric antioxidant on the hepatic and intestinal cytochrome P-450 mixed-function oxygenase system of rats.
Food Cosm Toxicol, **18(6):569-574**

Hernes PJ, Benner R, Cowie GL, Goni MA, Bergamaschi BA, and Hedges JI, (2001). Tannin diagnosis in mangrove leaves from a tropical estuary: a novel molecular approach. *Geochimica et Cosmochimica Acta*. **65: 3109-3122**

Kim DO, Lee KW, Lee HJ, and Lee CY, (2002). Vitamin C equivalent antioxidant capacity of phenolic phytochemicals. *Journal of Agricultural and Food Chemistry*, **50(13), 3713–3717**

Kwok B, Hu C, Durance T, and Kitts DD, (2004). Dehydration techniques affect phytochemical contents and free radical scavenging activities of saskatoon berries (Amelanchieralnifolia Nutt.). *Journal of Food Science*, **69, 122–125.**

Mohdaly AA, Sarhan MA, Smetanska I, and Mahmoud A, (2010). Antioxidant properties of various solvent extracts of potato peel, sugar beet pulp and sesame cake. PhD. Thesis, Inst of Technical Univ of Berlin .

Molyneux P, (2004). The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin *J. Sci. Technol* **26(2) : 211-219.**

Nothlings U, Schulze MB, Weikert C, Boeing H, van der Schouw YT, and Bamia C, (2008). Intake of vegetables, legumes, and fruit, and risk for all-cause, cardiovascular, and cancer mortality in a European diabetic population. *The Journal of Nutrition,* **138, 775−781**

Jayaprakasha JGK, Singh RP, and Sakariah KK, (2001). Antioxidant activity of grape seed (Vitis vinifera L.) extracts on peroxidation models in vitro. *Food Chem*. **73, 285–290**.

Prasad KN, Chew LY, Khoo HE, Kong KW, Azlan A, and Ismail A, (2010). Antioxidant capacities of peel, pulp, and seed fractions of Canariumodontophyllum Miq. Fruit. *J Biomed Biotechnol*. **pii:871379**

Pérez-Jiménez J, Arranz S, Tabernero M, Díaz-Rubio E, Serrano J and Goñi I (2008). Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: Extraction, measurement and expression of results. *Food Research International*, **41(3), 274–285 .**

Price ML, Vansoyoc S, and Butler LG, (1978). A critical evaluation of the vanillin reaction-An assay for tannins and sorghum grain. *Journal of Agricultural and Food Chemistry*, **26, 1214−1218.**

Prior RL, and Cao G, Antioxidant Phytochemicals in Fruits and Vegetables: Diet and Health Implications. *Hort Science* **35(4), 588–592 (2000).**

Ramful D, Tarnus E, Aruoma OI, Bourdan E, and Bahorun T, Polyphenol composition, vitamin C content and antioxidant capacity of Mauritian citrus fruit pulps. *Food Research International*, **44, 2088–2099 (2011).**

Rehman ZU, (2006). Citrus peel extract – A natural source of antioxidant. *Food Chemistry* **99, 450 – 454**

Romagnolo DF, and Selmin OI, (2012). Flavonoids and cancer prevention: A review of the evidence. *Journal of Nutrition in Gerontology and Geriatrics*, **31, 206–238**

SAS, Statistical Analysis System. User's guide: Statistics.SAS Institute Inc, Cary, Nc.USA **(1993).**

Shahidi F, (2000). Antioxidants in food and food antioxidants. *Nahrung* **44, 158–163**

Van der Sluis AA, Dekker M, de Jager A, and Jongen WMF, (2001). Activity and concentration of polyphenolic antioxidants in apple: effect of cultivar, harvest year, and storage conditions. *J. Agric. Food Chem* **49, 3606-3613**

Vinson JA, Su X, Zubik L, and Bose P, (2001). Phenol antioxidation quantity and quality in foods: fruit. *Journal of Agricultural and Food Chemistry* **49(11), 5315–5321**

Xie DY, Sharma SB, Paiva NL, Ferreira D, and Dixon RA, Role of anthocyanidin reductase, encoded by BANYULS in plant flavonoid biosynthesis. *Science* **299:396-399 (2003).**

Yusof S, Ghazali HM, and King GS, (1990). Naringin content in local citrus fruits. *Food Chem* **37, 113-121**