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UTILIZATION OF SOME VEGETABLES WASTES AND BY-PRODUCTS AS NATURAL SOURCES OF ANTIOXIDANTS

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ABSTRACT

Nowadays, Natural antioxidants are concern a great demand for consumer preference and health . This study was carried out to investigate the possibility of using vegetables wastes and by products as natural antioxidants . Five commercial waste and by-products namely, tomato pomace , potato peels , eggplant peels and olive leaves were evaluated . Phenolic compounds for each ethanolic extracts were identified using HPLC and different compounds also, were identified namely, chlorogenic , vanillic , ferrullic , Salicylic and caffeic acids … etc . Results of total phenolic contents (TPC) of the investigated extracts ranged from 29 to 144 mg /g. gallic acid. Olive leaves extracts showed the highest antioxidant activities (DPPH) in compare with other extracts (92.1 %) . Results of rancimat test indicated also, that addition of olive leaves extract to palm oil exhibited the highest induction period (I.P) (11.3) hours comparing with the other extracts .

From the economical point of view, vegetable wastes and by- products can used as natural source of antioxidants and play an important role in food industry .

Keywords:Vegetable wastes , agro-industrial ,by-products, phenolic compounds. oil stability .

INTRODUCTION

Antioxidants are both natural and synthetic compounds, able to scavenge free radicals and to inhibit oxidation processes. Currently, the growing interest to find cheap, renewable and abundant sources of antioxidant compounds has fostered research on vegetable sources of residual origin from agricultural industries (Moure *et al*., 2001). The antioxidants from natural sources, with potential nutritional and therapeutic value, can be used for increasing the stability of foods by preventing lipid oxidation (Zia-ur-Rehman,2006).

There is a great interest in natural antioxidants as bioactive components of foods. The protective effects of diets rich in fruit and vegetables against cardiovascular diseases and certain cancers have been attributed partly to the antioxidants contained therein, particularly to phenolic compounds Flavonoids are a widely distributed group of polyphenolic compounds, identified in recent years as antioxidants in various biological systems .

Processing of fruits, vegetables, and oil seeds results in high amounts of waste materials such as peels, seeds, stones, and oilseed meals. Disposal of these materials usually represents a problem that is further aggravated by legal restrictions. Plant waste is prone to microbial spoilage; therefore, drying is necessary before further exploitation. The cost of drying, storage, and transport poses additional economical limitations to waste utilization. Therefore, agro industrial waste is often utilized as feed or fertilizer.

However, demand for feed or fertilizer varies and depends on agricultural production. Moreover, valuable nutrients contained in agro industrial wastes are lost. Thus new aspects concerning the use of these wastes as by-products for further exploitation on the production of food additives or supplements with high nutritional value have gained increasing interest because these are high-value products and their recovery may be economically attractive. (Tzia *et al* ., 1998 Thomas, 1994).

It is well known that agro industrial by-products are rich in dietary fibers, some of which contain appreciable amounts of colorants, antioxidant compounds or other substances with positive health effects, while some of them, like the oilseed meals,are rich in proteins. Dietary fibers consist of polysaccharides and lignin that are neither digested nor absorbed in the human small intestine. The beneficial role of dietary fibers in reducing risk of coronary heart disease and certain types of cancer has been pointed out by several epidemiological studies.

Pomace is the residue remaining when fruits are processed for juice, wine, or other products. Many studies have reported that fruit pomaces are rich sources of phenolic compounds and these by-products, obtained from the juice and wine industry, might be useful raw materials for creating new value-added products (Zhou *et al*., 2009). Citrus is an important crop, mainly used in food industries for fresh juice production, and peel and pomace are the main by-products during its processing Citrus pomace has been used as a source for molasses, pectin, and limonene. Citrus pomace has also been widely studied, because it contains numerous biologically active compounds . (Li *et al*. , 2006a, 2006b).

Eggplant (*Solanum melongena* L.) is a plant native in India, and many cultivars exhibiting different size, shape, and color are cultivated in tropical, subtropical, and temperate zones. Its fruit, commonly known as aubergine, melanzana, garden egg, brinjal, or patlican, also has same name and widely used as a vegetable in cooking. The most widely cultivated varieties are elongated ovoid or slender type in a dark purple skin. Eggplant is ranked as one of the top ten vegetables in terms of oxygen radical scavenging capacity due to the fruit's phenolic constituents (Cao *et al*., 1996). Anthocyanins, an important group of naturally occurring pigments of red and/or purple colored fruits, are the main phenolic compounds in eggplant peel (Mazza *et al*., 2004).

Potato (*Solunum tuberosum L*) is the largest vegetable crop worldwide, amounting to approximately 320 million metric tons annually (FAO, 2005). Processing of potatoes (mainly for the production of chips, French fries, and dehydrated products) has presented a steady. Solid waste generated during processing consists mostly of potato peels but also contains green, immature, and cull potatoes and amounts to 15–45% depending on the procedure applied (Schieber *et al*., 2001). It is used as animal feed, though fermentation for the production of single cell or alcohol has been considered. (Natu *et al*., 1991).

Olive tree (*Olea europaea L.)* is one of the most important fruit trees in mediterranean countries, where they cover _8 million ha, accounting for almost 98% of the world crop. This demonstrates the great economic and

social importance of this crop and the possible benefits to be derived from utilization of any of its by products

Olive leaves are one of the by products of farming of the olive grove; they can be found in high amounts in the olive oil industries (10% of the total weight of the olives) and they accumulate during pruning of the olive trees (Tabera *et al*., 2004).

Phenolic acids have attracted increasing attention for their antioxidant behavior and beneficial health-promoting effects and they account for about one-third of the phenolic compounds in plant foods. It is assumed that many antioxidative phenolic compounds in plants are usually presented in a covalently-bound form. Therefore, reliable and practical methods for liberation of natural antioxidants from plant materials are of considerable interest. (Xu, *et al* ., 2007)

Therefore, the aim of the present work was to study the possibility of using some vegetable wastes and by-products as an economic, cheap and effective source of natural antioxidants.

MATERIALS AND METHODS

Materials :

Palm oil obtained from Misr Oil and Soap Company, El- Mansoura city , Egypt. Tomato pomace(*Lycopersicon enculentum* l.) was provided by Egyptian canning company BEST- El- Dakahleia -Egypt , Potato peels (*Solunum tuberosum)* was obtained from International Company For Agricultural Development(Farm Frites) , 10 Ramadan city, Egypt ,Eggplant peels *(Solanum melongena* L): was obtained from local restaurants - El-Mansoura-Egypt., Olive leaves (*Olea europaea L*.) : were obtained from olive farm- El-Salhiea- Egypt .

Chemicals : tertiary butyl hydroquinone (TBHQ) was obtained from Misr Oil and Soap Company, El- Mansoura city , Egypt , DPPH (1.1 Diphenyl 1-2-picrylhydrazl) was obtained from Sigma/ Aldrich Company .Cairo ,and other chemical used in the experiments were purchased from El-Gomhoria Company. for trading Medicines, Chemicals and Medical Supplies, El-Mansoura city , Egypt.

Methods:

Preparing of vegetable wastes and by-products :

Fresh olive leaves, Egg plant and potato peels were washed with distilled water, and dried at 40 ± 50 ^{O}C for 6 hrs., then ground using demotic grinder , and packaged in dark glass bottle until using

While tomato pomace was dried at 40^{o}C for 8 hours with air circulation dryer (Officine specializzate, GARBUIO, Essiccatioi, TREVISO, ITALY) The dried materials were ground in a domestic mill (Braun ,German). Then sieved and packaged in plastic air tight polyethylene .The samples were stored in domestic refrigerator at 3 ±2^{o}C until the extractions were performed.

Determination and identification of phenolic compounds :

Extraction of phenolic compounds of waste and by-products were carried out according to the method described by (Wojdylo *et al*., 2007).

- Total phenolic compounds of all extracts under investigation were determined according to the method described by Waskmundzka *et al.,(2007)*. Phenolic compounds were calculated as mg gallic acid /100g of dry weight material.
- Phenolic compounds of all wastes and by- products ethanolic extracts were identified using High Performance liquid Chromatography (HPLC) ,"HP1050" . at Food Technology Research Institute , Giza, Egypt .

Radical scavenging assay DPPH:

DPPH radical was determined by the method reported Mau *et al*., (2004) with minor modifications . The extracts 100,200, 250,500, 1000µg) in methanol (1 mL) was mixed with 4 mL of 0.004% methanolic solution of DPPH . The mixture was shaken vigorously and left to stand for 30 min in dark at 30° C , and the absorbance was then measured at 517 nm. Using Spekol 11, Carl Zeiss Jena, Germany

The percent of DPPH discoloration of the samples was calculated according to the equation:-

Antiradical Activity% = (Ac- Ab / Ac) X100

Ac = Absorbance of control . Ab = absorbance of sample

Oxidative stability of palm oil: :

The oxidative stability of palm oil treated with different waste and by product extracts was evaluated by the rancimat method using metrohm 679 as described by Deman and Deman 1984 . Palm oil without any addition was used as control. The induction period (I.P.) was calculated at 100°C and calculated at 25° C as described by (Pardun and Kroll 1972) . Experiment was conducted at Food Technology Research Institute , Giza, Egypt .

Stabilization Factor (S.F.) = I.P of sample / I.P of control

Increasing Index %= (I.P of sample - I.P of control / I.P of control) x 100

I.P. = Induction Period (hours) .

RESULTS AND DISCUSSION

Total Phenolic Compounds Content (TPC) in different vegetables wastes and by-products using HPLC :

Plant phenolics constitute one of the major groups of the compounds acting as primary antioxidants or free radicals terminators; it was reasonable to determine their total phenolics content (Zeyada *et al* ., 2007) . TPC were determined and expressed as Gallic acid equivalents (mg/100g) and results in Table (1)showed the percentage of each phenolic compound in different extracts namely, olive leaves, potato peels , egg-plant peels and tomato pomace .

It was noted that olive leaves had the highest phenolic content compared with the other extracts was 144 mg /g. gallic acid , followed by potato peels extracts was 55 mg /g. gallic acid . While tomato pomace extract was the lowest one compare to other extracts . This variation in total

phenolic content could be attributed to the specific nature of the plant type .Kim *et al* ., (1994) reported that the antioxidant activity extracts produced from herbs was dependent on the type of plant than the solvent .

Table (1): Total Phenolic Content (TPC) in ethanolic extracts from different vegetable wastes and by-products as mg/g Gallic acid .

Phenolic Extract	Mg/g Gallic acid
Olive leaves extract	**144**
Potato peels extract	**55**
Eggplant peels extract	**40**
Tomato pomace extract	**29**

Separation and identification of some Phenolic compounds ethanolic extracts.

The amount of phenolic compounds is an important factor when evaluating the quality of different extracts, it involved for their resistance to oxidation and the properties attributed to these antioxidants (Moure *et al* ., 2001) .

Results in Table (2) indicated that there was a great variation among the components identified in the ethanolic extracts of each material.

Data presented in Table (2) showed that olive leaves extract contained different types of phenolic compounds , the most abundant one being oleuropien, comprised about 71.91% of the total phenolics . Concerning to the derivatives with the hydrocinnamic acid chlorogenic and caffeic acids being 13.74 and 1.90% .

Data in the same Table showed that potato peels extract contained different phenolic compounds which could be mentioned as follows: Non anthocyanien flavonids derivatives , Salicylic acid was the predominant phenolic compound and represented 4.32 and Catechien was 3.17 .

Results of eggplant peels extract showed that this extract have different amount of phenolic constitutes , among hydrocinammic acid compounds, chlorogenic acid represented the highest content in compare with the other compounds (920.79%) followed by caffeic acid (37.50%) and cinammic acid (3.56%) , while p- coumaric acid was disappeared.

Seven phenolic compounds were identified in tomato pomace namely , epicathien, chlorgenic acid, caffeic acid, P-OH benzoic , vanillic acid, catechien and caffeine with amounts ranged from 7.50 to 0.61 % .

The variation of phenolic compounds could be due to the concentration of these compounds which varied between the examined extracts and the effect of different agronomic and technological condition of different vegetables plants namely riping stage, geographic origin and cultivator method (Esti *et al* ., 2009 , Franconi *et al* ., 2006 and Noda *et al* ., 2000)

Table (2) :Separation and Identification of phenolic compounds in different ethanolic extracts of vegetable wastes and by-products by HPLC

Phenolic compounds	Ethanolic extracts			
	Olive leaves	Potato peels	Egg-plant peel	Tomato pomace
A-Hydrocinammic acid				
1-Chlorgenic acid	13.74	2.11	920.79	1.89
2- caffeic acid	1.90	-----	37.50	1.30
3- P-coumaric acid	0.14	1.17	-----	-----
4- Ferrullic acid	---	-----	1.44	-----
5- Cinnamic acid	-----	-----	3.56	-----
B - Hydro benzoic acid	-----			
1-P-OH benzoic		-----	-----	1.80
2- Vanillic acid	0.73	1.31	11.45	1.01
3-Salicylic acid	-----	8.55	-----	-----
4- Protocatchoic acid	-----	-----	340.65	-----
4- Synergic acid	-----	1.34	78.31	-----
C- Non anthocyanien flavonids				
1- Catechien	-----	4.32	86.69	3.17
2- Epicathien	-----	-----	-----	7.50
3- Quercetin	-----	-----	-----	-----
1- chrisin	0.12	-----	11.59	-----
2- Oleuropien	71.91	-----	-----	-----
3- Caffene	3.06	-----	-----	0.61

Assessment of antioxidant extracts activity:

Radical Scavenging activity of some vegetable wastes and by-products:

The radical scavenging assay was evaluated by DPPH technique which depend on donate hydrogen to free radical and inhibiting the propagation stage in lipids oxidation pathway (Picerno *et al* ., 2003)

The radical scavenging activity of natural extracts depends greatly on the concentration as resulted in Table (3). The radical scavenging ability increased with the increasing amount of different extracts which ranged from (20.1 to 92.1). All extracts under investigation exhibited low DPPH values in compare with synthetic one TBHQ, while, The highest scavenging activity was detected at the concentration of 25µl for olive leaf extract , which reached to 92.1%, followed by potato leaf extract which was 85.6 at the same concentration.

Egg-plant extract showed a moderate DPPH effect in compare with the other extracts, this may be due to multi hydroxy flavonids, especially OH group in the B-ring which increased production of hydroxyl radicals Picerno *et al* ., 2003 stated that quinine derivatives could inhibit the free radical induced peroxidation , but also play a peroxidation role in the vesicle of dipalmitoyl phosphatidylcholine and this could be due to the electron attracting group at the ortho position to hydroxyl group in the phenoxy radical of quinone derivatives which initiation lipid peroxidation .

Results in the same table indicated that the lowest scavenging effect detected in tomato pomace extract (50.2%). This degradation of phenolic compounds in tomato pomace extract could be due to the thermal treatment

which somewhat resemblance with the behavior of the components responsible for antioxidants activity in the tested vegetable (Kim *et al* ., 1994)

The antiradical scavenging activity would be related to the substitution of hydroxyl groups in the aromatic rings of phenolic, thus contributing to their hydrogen donating ability, and their content of phenolic compounds and hydroxyl groups contribute markedly to the antioxidant activity (Wojdylo *et al* ., 2007).

So, it could be concluded from the abovementioned data that all extracts almost have identical ability to scavenge DPPH radicals.

Table (3): Free radical of scavenging activity DPPH on ethanolic extracts from different vegetable wastes and by-products .

Antioxidants	Extracts concentrates				
	5µ l	10µl	15 µl	20 µl	25 µl
TBHQ	88.26	94.72	95.07	96.71	98.11%
Olive leaves extract	35.5	76.5	79.2	86.5	92.1
Potato peels extract	31.2	68.2	70.1	79.5	85.6
Eggplant peels extract	20.5	40.3	58.3	60.5	66.5
Tomato pomace extract	20.1	40.2	45.2	49.2	50.2

Stability of palm oil :

The antioxidant activities of various ethanolic extracts from different vegetable wastes and by-products were assessed by the rancimat method. This method assigned the induction period for the onset of oxidative rancidity in palm oil at 100^{O} C .Results in Table (4) indicated that the induction period in all treated palm oil with different antioxidants extracts at the level of 0.2 ml were greater than those of the control palm oil samples except palm oil treated with pomace extracts . An induction period up to 11.3 hours was recorded in palm oil treated with olive leaves extract, while the data for palm oil treated with potato and egg plant peels extracts were nearly the same (11.2) hours. These results were in accordance with those given (Farag *et al*., 2003) who stated that polyphenols extracted from olive leaves could extend the induction period up to 11.5 hours . Several authors Khalifa *et al* ., 2009, Franconi *et al* ., 2006 and Farag *et al* ., 2004 suggested that various phenolic compounds extracted from different plant sources possessed an ability to increase the shelf-life of vegetable oils.

Table (4): Oxidative stability of palm oil treated with different ethanolic extracts from vegetable wastes and by-products .

Treated oil	I.P at 100° C (hrs)	Shelf Life (months)	Antioxidant activity	Increasing Index %
Control	10.00	16.02	--	---
Oil + TBHQ	11.3	15.7	1.13	13
Olive leaves extract	11.2	15.2	1.12	12
Potato peels extract	11.2	10.2	1.12	12
Eggplant peels extract	11.1	10.2	1.11	11
Tomato pomace extract	8.5	9.5	0.85	-15

I.P= induction period

From the above mentioned data in Table (4) it could be observed that olive leaves extract was the best antioxidant in compare with the other extracts. So, this extract was selected to be added to palm oil at the levels of 0.01,0.03 ,0.05,0.10 and 0.15 ml for the stability test (Rancimat) . Data in Table (5) showed that addition of olive leaves extract increased the stability of treated palm oil in compare with control sample. Moreover, increasing the concentration of olive leaves extract from 0.01 to 0.15 ml showed highest antioxidant activity for treated palm oil and increase the stability of oil to one time and half in compare with untreated one . This could be due to the high amount of oleuropien and other phenolic compounds which protect oil from oxidation deterioration Farag *et al* .,(2004) .

Table (5): Oxidative stability of palm oil treated with different concentration Of olive leaves ethanolic extracts .

Treated oil	I.P at 100° C (hrs)	Shelf Life (months)	Antioxidant activity	Increasing Index %
Control	10.00	16.02	--	---
Palm oil + TBHQ	11.30	15.7	1.13	13
Palm oil +0.02 Oex	11.20	15.2	1.12	12
Palm oil +0.03 Oex	12.30	11.5	1.23	23
Palm oil +0.05 O ex	12.40	11.6	1.24	24
Palm oil +0.1 O ex	13.50	12.3	1.35	35
Palm oil +0.15 O ex	15.55	13.1	1.55	55.5

O ex =olive leaves extract

Generally, treated palm oil with olive leaves extract increased, the induction period at all tested concentration, also, revealing an increase in oxidative in the stability of the treated oil and in induction period .

In conclusion, our obtained results suggested that different extracts from vegetable wastes and by-products could be effective in scavenging radicals and protecting lipid-oxidation when assessed by DPPH assay and thermal oxidation assay. The ability of ethanolic extracts to scavenge free radicals could significantly change total phenolic contents, and compositions, and potency of antioxidant capacity. Considering high consumer demand due to the beneficial health effects.

So, vegetable wastes and by-products can be utilized as functional food, health-promoting and pharmaceutical agents. Moreover, ethanol extraction could be a step for obtaining by-products extract with acceptable yields of important phenolic compounds although some further optimization of extraction procedures is still required.

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الاستفادة من بعض مخلفات الخضروات و المنتجات الثانوية كمصدر طبيعي لمضادات الاكسدة

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في هذة الايام تعتبر مضادات الاكسدة الطبيعية مطلب هام للمستهلك بسبب فوائدها الصحية . اجريت هذة الدراسة بغرض دراسة امكانية استخدام مخلفات بعض انواع الخضر ومنتجاتها الثانوية كمصدر طبيعي وآمن لمضادات الاكسدة . تم استخدام خمسة من انواع المخلفات الصناعية وهي تفل الطماطم و قشر البطاطس وقشر الباذنجان الاسمر وورق اشجار الزيتون . وتم تقدير وفصل المركبات الفينولية للمستخلص الايثانولي باستخدام (جهاز الفصل عالي الاداء HPLC) وكانت اهم المركبات كالتالي , Chlorgenic vanillic , Ferrullic , Salicylic and caffeic acids … ومن التنائج المتحصل تراوحت المركبات الفينولية الكلية مقدرة كحمض الجاليك من (١٤٤: ٢٩ ملجم/جم) و اظهرت نتائج اختبار DPPH ان مستخلص اوراق الزيتون اعطت اعلي نشاطا كمضاد اكسدة مقارنة بالمستخلصات الاخري ٩٢,١ % واعطت ايضا نتائج اختبار الرانسيمات ان اضافة مستخلص اوراق الزيتون بتركيز ٠,٠٢ الي زيت النخيل اعطي اعلي فترة ثبات وقدرت ب١١,٣ ساعة مقارنة بالمستخلصات الاخري .

قام بتحكيم البحث

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