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EVALUATION OF ONION OIL (SEED AND PULP) AS ANTIOXIDANT AND MICROBIAL GROWTH MATERIAL

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ABSTRACT*: Onion, (Allium cepa L.) seeds was used as an unexploited raw material to produce vegetable oil. The gross chemical composition of both seeds and pulp were studied. Physico- chemical properties, polyphenols, tocopherols, oxidative stability by Rancimat at 100 °C of onion seed oil and onion oil "fixed" were determined. Separation and identification of fatty acids and unsaponifible matters of onion seed oil and onion oil were carried out by gas liquid chromatograph (GLC) analysis. Antimicrobial activity of these oils was determined using agar diffusion method. It was active against (Gram positive and Gram negative) bacterial species and fungi namely Aspergillus flavus, Aspergillus niger and S.cereviasiae. Onion seed oil and onion oil were tested for their antioxidant properties using antioxidation of methyl linoleate. Results in this study suggest the potential use of the above onion seed and onion oil for the control of autoxidation and microbial effects.*

***Key words**: Allium cepa, onion seed, oil, antioxidant, antimicrobial.*

INTRODUCTION

Onion (*Allium Cepa* L.)is one of the oldest cultivated plants, and it is now used both as a food and for medical purposes. In fact, onion is a rich source of a number of phytonutrients, which make it an important element of the Mediterranean diet. It is also useful for the treatment or prevention of a number of diseases, including cancer, coronary heart disease, obesity, hypercholesterolemia scavenging of free radicals, type 2 diabetes, hypertension, cataract growth inhibition of tumor and microbial cells and disturbances of the gastrointestinal tract(e.g., colic pain, flatulent colic, and dyspepsia) (Corea, *et al*.,2005;Ly, *et al*.,2005 and Griffiths, *et al*.,2002).

The history of the onion is well documented and can be traced to its origin in a wide area from India to Israel, where its production started in 3000 B.C. and then it was introduced in Europe by the Phoenicians around 2000 years ago. In ancient Egypt, the onion was believed to be a sacred food, and it was also widely consumed by the Romans and Greeks, who liked its taste and knew of its curative properties (Block,1985). Epidemiological studies have consistently shown an inverse association between consumption of vegetables (onion included) and the risk of human diseases. Notably ,a population-based case-control study showed that the consumption of *Allium* vegetables was associated with a reduced risk of prostate cancer(Hsing,*et al*., 2002).Onion oil is a brownish yellow, occasionally pale yellow mobile liquid

with a very strong odor and distinct lachrymatory effect. Chemically , the oil consists of higher sulfides and traces of low boiling aliphatic aldhydes. The oil is distinguished by the complete absence of terpenes (Helen *et al*.,2000). The seed of onion do not remain viable for more than one season. In times of continued droughts, large quantities of unsown seed would be available for processing.Onion seed contain 22% crude oil (Reddy, *et al*.,1989). they analyzed onion seed oil found that in all the seed oil; linoleic acid (C18:2) represents 53.00% of total fatty acid while palmitic acid(C16:0) represents 7.00%.

The main aim of this work was to study the chemical composition of onion (seed and pulp), physico-chemical properties, fatty acid composition, and unsaponifiable matter of onion (seed and pulp) oils (crude and refined)) . evaluation of these oils as antioxidant and antimicrobial.

MATERIALS AND METHODS

a- Materials:

1-Sources of pulp and seeds: Onion (seeds and pulp) (local variety)were obtained from Horticulture Research Institute, Agriculture Research Center, Giza, Egypt.

2-Commercial antioxidants:Butylated Hydroxytoluene (BHT) and α-tocopherol were supplied by Estman chemical Co.

3-Solvents: All solvents used throughout the whole work were analytical grade and distilled before use.

b- Analytical methods:

1-Oil extraction: Onion (seeds and pulp) were crushed and pressed by hydraulics laboratory press. The extracted oil was dried over anhydrous sodium sulphate, filtered through Whatman No.1 filter paper ,and kept in brown bottles at 5°C until analysis.

2-Chemical analysis of seeds: Moisture, crude oil, crude protein , crude fiber, ash, and carbohydrate were determined according to A.O.A.C.(2000).

3-Physico-chemical properties of oils: Refractive index, color, acid value, peroxide value, iodine value, saponification number were determined according to A.O.A.C.(2000), induction period by Rancimat according to Mendez,*et al*.,(1996), tocopherol content was determined according to Wong et al.,(1988), total polyphenols of the oils were extracted with 60% aqueous methanol according to the method of Gutfinger (1981), identification of the fatty acid methyl esters by Gas-liquid chromatography (GLC) was determineed according to Farag *et al.,(*1984) and unsaponifiable mater was extracted from oils after saponification at room temperature according to method outlined by Mordert,(1986).

4- Antimicrobial screening : The antimicrobial screening as described by Tantawy,(1999) and Aboutable *et al*.,(2000). Microorganisms used were

obtained from stock collection of Microbiology Department Faculty of Agriculture Cairo university, subculture on the nutrient agar slants : B.*cerues*, S. *aureus*, S.*typhimurium*, E.*coli* while the A.*niger*, A.flavus and S.cereviasiae were sub cultured on the molt agar slants. A solution of each fresh onion seed oil and onion oil (100µL\disc) was used. The plateswere incubated at 37°C and 25°C for bacteria and fungi respectively, using Rifampicin, Amoxycillin + Flucloxacillin and Ciprofloxacin(1mg\ml for each,100µl\disc) as a control. The antimicrobial activity was recorded by measuring the diameter(in mm) of inhibition zone after 12-18 and 24-48 hrs for bacteria and fungi respectively. Three replicates were carried out and the average was determined.

The disc agar method used for antibacterial screening was performed as follows:

1-Bacteria: Nutrient agar media were inoculated with previously activated culture for each and were poured in plates, after the media were solidified, the discs were put in surface center and 100µl of the tested oils were used per disc, then the plates were incubated.

2-Fungi: All previous steps done for bacteria were repeated for fungi except using malt agar media inoculated with suspended fungi spores.

5-Antioxidantive activity: The antioxidative activity of oils (onion oil and onion seed oil) were measured by its inhibition against methyl linoleate autoxidation (Yamauchi *et al*., 1992). Methyl linoleate (294mg,1.0mmol) containing the oils (each 0.1 m mol; 0.01 mol % based on methyl linoleate or BHT and tocopherols (0.01 mol %, based on methyl linoleate) was placed in a test tube and incubated at 60°Cin the dark. After 36 hr incubation, each sample(25µl) was withdrawn and dissolved in 1.0 ml ethanol. The peroxide value in each sample solution was determined by the Iodometric method (A.O.A.C.2000).

6-Refining process of crude onion seed oil : crude onion seed oil was alkali refined using NaOH. Neutralization of acidity was done at 65°C , concentration of caustic soda (8%), the soap stock was separated from the refined oil by centrifugation , the oil was washed three times with water and kept in brown glass bottle(120ml) at 5°C till use.

7-Statistical analysis: The data were statistically analyzed by the least significant differences (L.S.D) at the 5% level of probability procedure according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Chemical composition of onion (seed and pulp):

The standard chemical methods outlined in A.O.A.C.(2000) were applied to determined the gross chemical composition of onion and onion seed and the results are shown in Table(1). Analysis of onion and onion seed (moisture content 55.00 and 8.00%) shows that the levels of crude oils, crude proteins,

Table (1):Chemical compassion of onion seed and pulp(*A.Cepa*)on dry weight

Component	Onion	
	Seeds	Pulp
Moisture	8.00	55.00
Oil	21.50	10.50
Protein	28.50	25.30
Fiber	17.50	22.50
Carbohydrate	26.50	30.20
Ash	6.00	11.50

total hydrolysable carbohydrates, crude fiber and ash were:10.50 and 17.90%,25.30 and 28.50%,22.50 and 15.50%,30.20 and 17.50%, and 11.50 and 5.40% respectively based on dry weight basis. Similar results for the chemical composition of onion seeds were also reported by Reddy, *et al*. , (1989).

Physico-chemical properties of oils:

Table(2) shows the physico-chemical properties of onion seed oil and fixed onion oil. The refractive index is considered one of the most important physical characteristics, as it is useful for estimating the degree of their saturation as well as for identification processing purposes, establishing their purity and observing the progress reaction such as catalytic hydrogenation. As shown in Table(2), the refractive index of onion seed oil and onion oil was found to be 1.4682 and 1.4671 respectively. The color of the edible oils is considered one of the most considerable commercial importance physical characteristics. From the obtained results(Table 2), it could be observed that onion seed oil and onion oil were superior in their color measurements which were found 35 Y, 3.1 R and 35 Y, 2.9 R. The intensity of the color of vegetable oils depends mainly upon the presence of various pigments such as chlorophylls and carotenoids. The obtained results of Table(2) showed that the acid value (% as oleic acid) of investigated oils were found to be 0.30 and 0.50, respectively. As given in Table(2), the peroxide value of the onion seed oil and onion oil were 1.5 and 1.7(meq./Kg oil), respectively. The iodine value is considered one of the most chemical constants for quality assurance of the edible oils and is a good index for the unsaturation extent of fatty acids in lipids. As shown in Table(2), the iodine values of onion seed oil and onion oil were found to be 125.00 and 127.00 gl2, respectively.

The obtained results(Table 2) illustrated that the saponification number of onion seed oil and onion oil were found to be 190.00 and 192.00 mg KOH/g oil, respectively.

It has been reported that the unsaponifiable mater content(%) should not exceed 1.5% in the edible oils(Egyptian Standard 2000). As illustrated in the obtained data (Table 2), the tested oils contained the unsaponifiable matter of the ratio of 1.70% and 1.40%, respectively. Results from Table(2) indicated that the oxidative stability of onion seed oil and onion oil on 100°C using Rancimat method was 18.30 and 13.20 hours, respectively. Total polyphenol (as caffeic acid) and tocopherols(as α -tocopherol) of investigated oils were found to be (311.00 and 290 ppm) and (300 and 255 ppm) of onion seed oil and onion oil, respectively. The higher oxidative stability of onion seed oil and onion oil may be due to their high contents of unsaponifiable matter, total polyphenols and α-tocopherols these latter materials are acted as natural antioxidants . The obtained data are also in agreement with those of Hellen *et al*., (2000).

Fatty acid composition:

The obtained results of Table (2) show that the total saturated fatty acids were 8.00% and 10.00% for the onion seed oil and onion oil , while the total unsaturated fatty acids were found to be 90.00% and 89.10% respectively. Palmitic acid (C16:0) was found to be the dominant saturated fatty acid(7.00% and 8.30%) of the onion seed oil and onion oil . Linoleic acid (C18:2) which was found to be the predominant unsaturated fatty acid in the onion seed oil and onion oil (59.10% and 53.20%), respectively.

Unsaponifiable matter composition :

From the obtained data Table(2), it could be revealed that the unsaponifiable matter extracted from the oils(onion seed and onion) composed of two groups; namely hydrocarbons and sterols. C28 and squalene were found to be the major dominant hydrocarbons in onion seed oil and onion oil , while the major sterols was found to be β -sitosterol which amounted to (29.50% and 25.00%) of the investigated oils.

Effect of refining on physical and chemical properties of onion seed oil:

The oils that finally undergoes refining is subjected to some or resinous , neutralization of free fatty acids, deodorization and decolorization . In the present investigation the refining process for brown color was made by using sodium hydroxide at concentration 8%. The effect of refining process on the physical and chemical properties of the investigated oils were studied and the results in Table(2). The acid value, peroxide value, color, phenolic compound ,tocopherol content, and unsaponifiable matter content were decreased after alkali treatment. Sodium hydroxide gave the lowest Rancimat stability. Slight variations between crude and refined oils in the fatty acid composition and unsaponifiable matter composition.

Table (2):Physico-chemical properties, polyphenols, α-tocopherol, and unsaponifiable matter of onion seed oil and onion oil (crude and refined).

Properties	Onion oil			
	Seed		Pulp	
	Crude	Refined	Crude	Refined
Physico-chemical properties				
Refractive index at 25°C	1.4682	1.4682	1.4671	1.4671
Color (yellow at 35)	3.10	2.30	2.90	2.10
Acid value (% as oleic acid)	0.30	0.10	0.50	0.20
Peroxide value (meq.Kgoil)	1.50	0.50	1.70	0.60
Iodine number (Hanus)	125.00	124.90	127.00	126.20
Saponification number	190.00	190.00	192.00	192.20
Unsaponifiable matter (%)	1.70	1.30	1.40	1.60
Total polyphenols (as caffeic acid ppm)	311.00	201.00	290.00	16.50
α-tocopherol (ppm)	300.00	150.00	255.00	110.20
Oxidative stability (hrs)	18.30	7.20	13.20	4.50
Fatty acid composition				
C14:0	0.40	0.33	0.20	0.42
C16:0	7.00	7.10	8.30	8.12
C18:0	1.00	1.10	1.30	1.40
C18:1	32.50	32.60	33.00	32.43
C18:2	58.74	58.67	57.10	57.50
C18:3	0.33	0.20	0.10	0.13
Unsaponifiable matter				
C12	2.10	1.50	2.90	2.00
C14	4.30	3.50	5.10	5.00
C16	0.11	0.10	0.10	0.10
C18	2.90	2.30	1.10	0.90
C20	4.50	4.00	3.50	2.90
C22	2.50	2.20	3.60	3.00
C24	8.90	8.20	10.50	8.50
C26	3.00	3.00	2.70	1.90
C28	6.38	5.60	7.30	6.40
Squalene	22.10	22.00	23.11	21.00
C30	0.11	0.10	0.09	0.07
Cholesterol	00	00	00	00
β-sitosterol	29.50	25.00	25.00	21.00
Stigmasterol	3.20	3.10	5.90	4.10
campasterol	5.90	4.20	9.10	8.50

Antimicrobial activity of onion seed oil and onion oil:

Antifungal activity:

The antimicrobial activity of onion seed oil and onion oil "fixed" at different concentrations were tested using some selected pathogenic microorganisms. The antifungal activity of onion seed oil and onion oil against two mycotoxic strains of fungi namely *Aspergillus flavus* and *aspergillus niger* and S.*cereviasiae* was evaluated and the data are shown in Table (3). There are no significant variations in the antifungal activity between onion seed oil and onion oil effected. From the Table (3) , the inhibitory effects of onion seed oil and onion oil significant increased with increasing the concentration of oil. Addition of 90 µL of both oils inhibited approximately *A. flavus* and *A. niger* and *S. cereviasiaeae* up to 98.25 and 98.00%, respectively. Clearly, the aforementioned results reveal that onion seed oil and onion oil "fixed" has more great affect as an antifungal agent.

Table (3) Effect of onion oil (seed and pulp) on growth of pathogenic fungi compared with antibiotics (Rifampicin, Amoxycillin, Flucloxacillin and Ciprofloxacin)

Pathogenic fungus / Concentration µL	A. *flavus* dry w.(gm)	Inhibition %	A. *niger* dry w.(gm)	Inhibition %	S. *cereviaisiae* dry w.(gm)	Inhibition %
Rifampicin	0.25a	34.30a	0.26a	33.90a	0.25a	34.30a
Amoxicillin + Flucloxacillin	0.20a	37.50a	0.21a	37.60a	0.21a	37.60a
Ciprofloxacin	0.14a	60.90b	0.16a	60.10b	0.13a	60.00b
Onion seed oil						
20	0.20a	37.50b	0.22a	36.50a	0.21a	37.60a
40	0.15a	60.20b	0.17a	58.30b	0.16a	60.10b
60	0.07a	81.30b	0.08a	80.70b	0.07a	81.30b
80	0.04b	95.20b	0.05b	94.20b	0.05a	95.20b
100	0.01b	99.13b	0.03b	97.70b	0.02b	98.90b
Onion oil						
20	0.23a	36.20a	0.22a	36.50a	0.21a	37.60a
40	0.17a	58.50b	0.18a	58.50b	0.17a	58.50b
60	0.09a	79.20b	0.09a	79.20b	0.10a	78.95b
80	0.07a	92.80b	0.06a	91.95b	0.07a	92.80b
100	0.03b	97.30b	0.04b	95.20b	0.03b	97.30b
L.S.D value >0.05	0.20	3.90	0.22	3.55	0.21	3.50

L.S.D demonstrates to least significant difference test.

Antibacterial activity:

Onion seed oil and onion oil "fixed" were tested for their antibacterial activity against two strains of gram positive bacteria (*Staph. aureus* and *Bacillus cerues*) and two strains of gram negative bacteria (*E. coli* and *Salmonella typhimurium*) and the obtained results are given in Table (4). The obtained data indicate that completely inhibition (100 %) was obtained when the lowest concentration of onion seed oil and onion oil was used against gram negative bacteria. The zones of growth inhibition significant increased gradually with raising both oils concentration and completely inhibition occurred at 70 µl of onion seed oil and onion oil . In contrast, both oils had the lowest inhibition effect against Gram positive bacteria compared with other tested pathogens. On contrary, onion seed oil and onion oil were more potent against all tested food borne pathogens and can be recommended as safe antibacterial agents to prevent the spoilage of food products.

Table (4): Antimicrobial activity of onion oil (seed and pulp) on growth of pathogenic bacteria compared with antibiotic (Rifampicin, Amoxycillin, Flucloxacillin and Ciprofloxacin)

Pathogenic bacteria Concentration µL	Zones of growth inhibition (mm)			
	B. cerues	*S. aureus*	*S. typhimurium*	*E. coli*
Rifampicin	63.20a	61.30a	64.50a	63.20a
Amoxicillin + Flucloxacillin	71.80b	70.20b	73.50b	72.10b
Ciprofloxacin	80.10b	80.09b	82.30b	81.50b
Onion seed oil				
20	37.80a	36.70a	38.50a	37.20a
40	77.80b	76.90b	78.20b	77.10b
60	88.20b	89.30b	88.50b	87.60b
80	92.30b	91.35b	93.00b	90.50b
100	96.20b	96.30b	96.90b	94.20b
Onion oil				
20	35.30a	37.00a	36.00a	35.00a
40	72.20b	76.11b	77.00b	74.11b
60	82.50b	83.40b	82.11b	83.20b
80	89.70b	90.10b	90.50b	88.90b
100	93.20b	92.90b	91.20b	92.10b
L.S.D value >0.05	3.55	2.98	3.22	3.13

L.S.D demonstrates to least significant difference test.

Subsequently, it could be concluded that it is practicable and economic to produce onion seed oil and onion oil "fixed" which could be utilized as antimicrobial agent.

Antioxidative activities of onion seed oil and onion oil:

Table (5) shows the inhibitory effect of both oils during the autoxidation of methyl linoleate. Onion seed oil and onion oil showed the highest antioxidative activity compared with α -tocopherol and BHT. The higher antioxidative activity of oils due to the high contents of polyphenolic compounds, α-tocopherol , squalene and β-sitosterol. These results show an antioxidant effect of onion seed oil and onion oil against methyl linoleate . So, it could be concluded that both oils had a highly antioxidant activity of lipids and provide protection against oxidation and rancidity. These results in agreement with that reported by Ly *et al.,* (2005).

In conclusion , the present study indicated that the onion might serve as an excellent source for a new oils (seed and pulp), polyphenol compound and tocopherol. This study also showed that the tested oils contain significant levels of natural antioxidants. Utilization of these oils in food and cosmetic products as natural antioxidants and antimicrobial well be beneficial.

Table(5): Inhibition of methyl linoleate oxidation by onion oils (seed and pulp).

Source	Inhibition of methyl linoleate
Control	0.00
Onion seed oil	95.00
Onion oil	94.00
α- tocopherol	95.50
BHT	94.30

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تقييم زيت البصل (البذرة والأوراق) كمادة مضادة للأكسدة والنمو الميكروبي

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الملخص العربي

يمكن استخدام بذور البصل كمصدر غير تقليدي للزيوت النباتية. في هذا البحث تم دراسة التركيب الكيماوي للبذور واللب. تم تقدير الخواص الطبيعية والكيماوية في كلآ من زيت بذور البصل وزيت البصل الثابت و أيضا تم تقدير كلآ من الفينولات الكلية و التوكوفيرولات والثبات الأكسيدى بواسطة جهاز الرانسيمات. تم فصل و التعرف على محتوى الأحماض الذهنية و المواد الغير المتصبنه في كلآ من زيت البصل وزيت بذور البصل بواسطة جهاز التحليل الكروماتوجرافى الغازي. تم اختبار كفاءة كلآ من زيت البصل (الثابت) و زيت بذور البصل من حيث تأثيرهما كمضادات للميكروبات و ذلك ضد أربعة سلالات من البكتيريا السالبة و الموجبة لجرام و أيضا ضد الفطريات المنتجة للسموم. بالإضافة إلى دراسة النشاط المضاد للأكسدة لزيت البصل و زيت بذور البصل باستخدام أللأكسدة الذاتية للميثيل لينوليات. أشارت النتائج في هذه الدراسة إلى أن كلآ من زيت البصل وزيت بذور البصل لهما تأثيرات قوية كمضادات للميكروبات ومضادات للأكسدة.