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EFFECT OF OLIVE LEAVES ADDITION AND WASTEWATER RECYCLING ON THE PRODUCTIVITY AND QUALITY OF OLIVE OIL PRODUCED BY CENTERIFUGATION

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ABSTRACT

Two olive fruit cultivars (Picual and Chemlalli) were used to investigate the effect of olive leaves addition (2% w/w) during crushing process as well as wastewater recycling (60 L/100kg paste) during malaxation process on yield, physicochemical properties, fatty acid composition and sensory evaluation of oils produced by centrifugation. Produced oils after the addition of leaves were characterized (with respect to control oils) mostly by: (i) higher polyphenols content (4.2 –5.8%), (ii) similar values of acidity and peroxide, (iii) Similar contents of saturated and unsaturated fatty acids, (iv) more preferable taste and (v) categorized as extra virgin olive oil. Application of wastewater recycling led to increase oil yield by 2.5-3 % and reduce wastewater. The produced olive oils were characterized (with respect to control oils) by: (i) higher content of polyphenols (25%), (ii) similar values of acidity and peroxide, (iii) Similar contents of saturated and unsaturated fatty acids, (iv) similar organolyptic properties and (v) categorized as extra virgin olive oil. Therefor, addition of olive leaves during crushing process and recycling of wastewater during malaxation process could be recommended by production of extra virgin olive oil.

Keywords: Olive oil, olive leaves addition, wastewater recycling, quality parameters, fatty acids composition, sensory evaluation.

INTRODUCTION

Production of olive has been practiced in Egypt since time immemorial. Drawings on ancient pharonic temples indicated that ancient Egyptian cultivated olive and used its oil for food and medicine. The most ancient olive oil extraction machine in Egypt was dated back to the age of Alexander the Great in Siwa Oasis. In Egypt the cultivated area increased from 10,000 feddans in 1980 to 75,000 feddans in 1994. The most important olive production areas in Egypt are Matrouh, North Saini, Fayoum, El-Asmailia, the west part of El-Behaira and El-Wadi El-Gadid governorates (FAO, 1997). Picual and Chemlalli cultivars are considered the most distributed olive cultivars cultivated in North Sinai.

Virgin olive oil is an oil which is suitable for consumption in the natural state (Codex stand. No. 33-81, 1992). According to the Egyptian Standard specifications (2000), virgin oil must not subject to any treatments except washing, decanting, centrifugation and filtration. It classifies and evaluates the quality of olive oil mainly according to its acidity. Microbial and endogenous enzymes play an important role in the olive oil quality. Acidity of olive oil is progressively increased even when the fruits remain on the tree due to the activation of lipase enzymes. Moreover, acidity of the oil is also increased during the period of the fruits remain on the collection nets (Kiritsakis, 1990).

The malaxation of the olive paste is a very basic step for all extraction systems. It aids in coalescing of small oil drops into larger ones, Thereby, facilitating the separation of the oil (Kiritsakis, 1990). Montedoro *et al.* (1978) studied the effects of homogenization of the crushed olive leaves in water/oil mixture and oil only on the oil components. They found that volatile constituents increased 2 times over the control and total phenols increased from 109.6 to 144 mg/kg in the mixture of oil and leaves. Mixing of olive leaves with olive fruits at percentages ranged between 1-5% (w/w) caused no mechanical problems in the flow of olive paste through the pump when crushed with a fixed hammer metal crusher (Di-Giovacchino *et al.*, 1996). Their results showed that the addition of leaves even in amounts as low as 1-2 %, resulted in oil classified as "extra virgin" with an improvement in both taste and appearance. Addition of leaves led to noticeable increase in the trans 2-hexanal, hexanal content and some alcohols, such as cis-3-hexanol, trans-2-hexanol and 1-hexanol.

Furthermore, phenolic extracts of olive fruits as well as olive leaves prevent the oxidation of oils included olive oil and sunflower oil and showed more antioxidant effect compared with synthetic antioxidants such as butylhydroxyanisol and butylhydroxytoluene (Hartzallah and Kiritsakis, 1999). Piacquadio *et al.* (1998) studied new centrifugation system using the–two-phases decanter, which operates the separation of virgin olive oil by recycling part of vegetable water and found that total phenols and o-diphenols increased. Recycling of fresh vegetable water resulted in an approximate 30 % rise in the total polyphenol content of olive oil and a 35-40 % reduction of olive mill wastewater (Di-Giovacchino, 1996).

Our research was undertaken to study the effect of some treatments during extraction including recycling of wastewater and addition of olive leaves on yield and quality of olive oil produced by centrifugation from Picual and Chemlalli cultivars.

MATERIALS AND METHODS

1-Materials:

Olive fruits: The olive fruits (*Olea Europe*) of Picual and Chemlalli cultivars from 1999 crop were manually collected at the end of semi black-stage from El-Arish city in North Sinai governorate.

Olive oil: Commercial Extra virgin olive oil required for sensory evaluation was managed through the faculty of Environmental Agricultural Science, Arish, Suez-Canal University.

2- Methods:

Extraction of olive oil: olive oils were extracted from each of collected fresh olive cultivars with and without 2% (w/w) of olive leaves using centrifugation mill with a fixed hammer metal crusher (Pieralisi type, Spain). Malaxation process was achieved with and without particularly recycling of wastewater (60 L /100 kg). Oil samples were fully packed in dark glass bottles, stoppled and kept in dark for further analysis.

Physicochemical analysis of olive oil: -

Refractive index, acidity (% as oleic), peroxide and iodine values, Saponification number and unsaponifiable matter were determined according to the methods described by A.O.A.C. (1990)

Ultra-Violet absorption (K_{232} and K_{270}) were determined as the method described by A.O.C.S., (1991). 1% solution of the oil in cyclohexane was measured in 1 cm cell at 232 and 270 nm using Cecil spectrophotometer.

Total polyphenols were determined according to the method described by Gutfinger (1981).

The method of Sidwell *et al.*, (1954) was followed to determine the Thiobarbituric acid (TBA) value.

Gas-liquid chromatographic analysis of fatty acids was achieved according to the method recommended by A.O.A.C. (1990).

Sensory evaluation: All investigated olive oil samples were organolyptically evaluated according to Raganna (1977). Ten semi-trained members from staff of Agri- Industrialization Unit, Desert Research Center were asked to score color, odor and taste by giving a degree from ten comparing to the commercial extra virgin olive oil as a reference sample.

Statistical analysis: The data obtained from sensory evaluations were exposed to proper statistical analysis according to Waller and Duncan (1969) using Anova program for statistical analysis. The differences between means for all treatments were tested for significance at 5% level.

RESULTS AND DISCUSSION

Oil yield:

Fortunately, during the present investigation mixing of 2% (w/w) olive leaves with olive fruits during crushing process caused no technical problems during extraction particularly the flow of olive paste. Since the crusher crumbled the leaves to small pieces which were well amalgamated with the olive paste as previously proved by Di-Giovacchino *et al.* (1996). The addition of olive leaves showed no detectable effect on oil yield obtained from both investigated cultivars (Fig, 1). The determined oil yield was 12 and 15 (kg oil/100 kg olives) for Picual and Chemlalli cultivars, respectively.

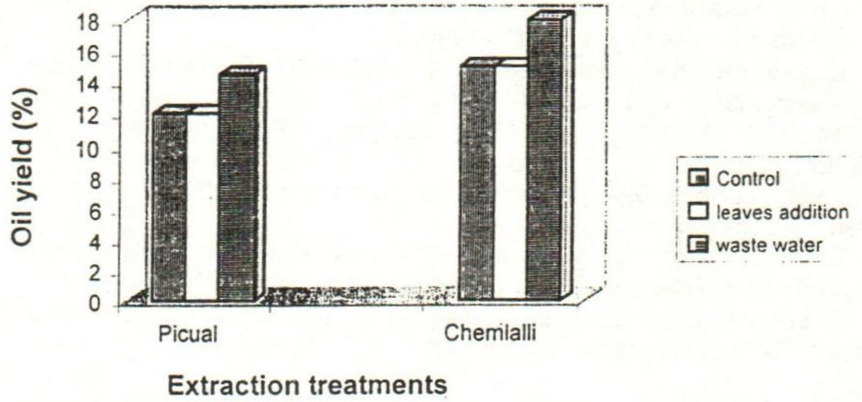

Fig. (1): Effect of leaves addition and wastewater recycling on oil yield obtained by centrifugation from Picual and Chemlalli cultivars.

On the other hand, the oil yield of both cultivars increased by approximately 2.5-3% due to recycling of wastewater (approximately 60 liter/100 kg fruits) during extraction with centrifugal system (Fig, 1). These data are in accordance with Ryan *et al.* (1998) who reported that wastewater can be recycled in the mechanical extraction of olive oil to increase oil yield and improve oil quality. The occurred increasing of oil yield could be explained as a result of disintegrating the intercellular connective tissue of the olive fruit by pectolytic enzymes presented in the wastewater. Moreover, the pectolytic enzymes break up not only the liquid-solid emulsion but also the liquid-liquid emulsions mainly caused by crushing and centrifuging the paste through its endopolygalacturonasic effect. Generally, Wastewater recycling process has a positive effect on the rheological characteristics of the paste. (Montedoro *et al.*, 1993, Ranalli and De Mattia, 1997 and Ranalli *et al.*, 1998 and 1999).

Physicochemical properties:

The effect of olive leaves addition (2% w/w) and wastewater recycling on some physicochemical properties of olive oils extracted from Picual and Chemlalli cultivars by centrifugal system was investigated and presented in table (1).

Results indicated that leaves addition had approximately no effect on measured refractive index, peroxide value and saponification number of extracted oils. While, there was limited increment in total acidity of produced oils related to addition of olive leaves. These results are in agreement with those of Di-Giovacchino *et al.* (1996). Generally, Chemlalli oil gave higher acidity (0.51 and 0.60 %) than Picual oil (0.38 and 0.41 %). Such differences could be more related to olive cultivar, since higher activity of lipase enzymes

Table (1): The effect of wastewater recycling and addition of 2 % olive leaves on some physicochemical properties of olive oils extracted from Picual and Chemlalli cultivars by centrifugal system.

Property	Picual oil			Chemlalli oil		
	Control	Wastewater	Leaves addition	Control	Wastewater	Leaves addition
Refractive Index	1.4679 ± 0.0	1.4680 ± 0.0	1.4679 ± 0.0	1.4689 ± 0.0	1.4689 ± 0.0	1.4688 ± 0.0
Acidity (% as oleic)	0.38 ± 0.02	0.43 ± 0.02	0.41± 0.02	0.51± 0.02	0.61 ± 0.02	0.60 ± 0.02
Peroxide value (meq. O_2/kg)	13.33± 0.47	14.86 ± 0.19	13.70 ± 0.17	17.06 ± 0.09	17.66 ± 0.19	17.93 ± 0.09
T.B.A. O.D. at 538 nm	0.152 ± 0.002	0.159 ± 0.001	0.171 ± 0.000	0.248 ± 0.002	0.256 ± 0.002	0.267 ± 0.002
Polyphenols (ppm as Vanillic)	240 ± 4.0	300 ± 4.0	251 ± 5.9	261 ± 2.9	325 ± 3.3	274 ± 3.3
K232	1.098 ± 0.001	1.105 ± 0.004	1.121 ± 0.002	1.971 ± 0.002	2.110 ± 0.008	2.091 ± 0.002
K270	0.099 ± 0.000	0.099 ± 0.000	0.143 ± 0.000	0.063 ± 0.000	0.074 ± 0.000	0.091 ± 0.000
Saponification number	191.60 ± 0.66	189.80 ± 2.48	189.80 ± 2.48	193.10 ± 1.69	191.10 ± 3.33	193.70 ± 0.37
Unsaponifable matter (%)	1.16 ± 0.02	1.20 ± 0.01	1.23 ± 0.01	1.01 ± 0.02	1.11 ± 0.02	1.11 ± 0.01

Data are means (± SD of three replicate analysis)

in Chemlalli olives was proved compared to their activity in Picual olives (Basuny, 1996).

The determined TBA values were also slightly increased due to addition of olive leaves (Table, 1). Such increment could be attributed in part to the damaging of the leaf cells by crushing thereby their chlorophyll and pheophytin content could be released and acted as pro-oxidants in the presence of light (Kiritsakis, 1990). Moreover, some oxidized substances could be transferred from olive leaves to olive paste and eventually to the produced oil.

The tabulated data showed also that refractive index and saponification numbers were not approximately affected by wastewater recycling. Whereas, slight increase in total acidity, peroxide value and TBA test related to wastewater recycling was occurred. Such limited increasing could be due to activities of lipolytic and oxidative enzymes eventually found in wastewater (Kiritsakis, 1990).

Concerning, the absorption in UV region (K232 and K270), which reflect the oxidation state of the oil (Ranalli *et al.*, 2000), was negligible affected by addition of leaves as well as recycling of wastewater (Table, 1). The obtained data are in accordance with those of Di-Giovacchino *et al.* (1996). Such data indicated that oil content of primary and secondary oxidized substances was not remarkably changed due to application of the aforementioned treatments.

Furthermore, addition of 2% olive leaves caused remarkable increase in polyphenols content of extracted oil comparing with control samples. It was increased by 4.2 and 5.8%, respectively for oils extracted from Picual and Chemlalli cultivars (Table, 1). The obtained polyphenol results are agreed with those reported by Montedoro *et al.* (1978) and Di-Giovacchino *et al.* (1996). They found that addition of leaves increased polyphenol content by 4.9-12.5 %.

On the other hand, polyphenol content of oils extracted from both investigated olive cultivars after recycling of wastewater was increased by 25% comparing with control samples (Table, 1). These results agreed with those obtained by Di-Giovacchino (1996) who reported that polyphenol content showed approximately 30% increasing due to recycling of wastewater.

The unsaponifiable matter was slightly increased due to mixing of leaves during crushing process as well as after recycling of wastewater. Such increment of unsaponifiable matter could be related to the increment in polyphenols which, considered as unsaponifiable matter (Table, 1).

It could be concluded that all determined physicochemical properties included total acidity and peroxide values of oils obtained after addition of olive leaves or wastewater recycling indicated that such oils could be categorized as extra virgin olive oil according to Egyptian Standard specifications of olive oil (2000).

Fatty acid composition:

Fatty acids composition of olive oils obtained after addition of olive leaves and wastewater recycling by centrifugal extraction of both investigated cultivars was determined and presented in table (2).

Table (2): The effect of wastewater recycling and addition of 2 % olive leaves on fatty acids composition (%) of olive oils extracted from Picual and Chemlalli cultivars by centrifugal system.

Fatty acid (%)	Picual oil			Chemlalli oil		
	Control	Waste water	Leaves addition	Control	Wastewater	Leaves addition
Lauric (C12: 0)	0.90	0.80	0.62	1.30	1.40	0.80
Myristic (C14 :0)	0.01	0.01	0.01	0.07	0.06	0.05
Palmitic (C16: 0)	15.60	15.70	15.52	13.30	13.25	13.32
Palmitoleic (C16:1)	2.00	2.00	2.10	1.60	1.60	1.63
Stearic (C18:0)	2.60	2.60	2.60	2.20	2.30	2.30
Oleic (C18:1)	68.40	68.40	68.40	71.00	71.10	71.01
Linoleic (C18:2)	8.70	8.60	8.60	8.00	8.00	8.13
Linolenic (C18:3)	1.30	1.40	1.30	1.40	1.40	1.50
Arachidic (C20:0)	0.02	0.02	-	-	-	-
Total unsaturated	80.40	80.40	80.40	82.00	82.10	82.20
Total saturated.	19.60	19.60	19.60	18.00	17.90	17.80

The most predominated fatty acids in the investigated oil samples were the monounsaturated acids oleic followed by palmitic, linoleic, stearic and palmitoleic. The obtained data are in agreement with many investigators (Kiritsakis, 1990, Santinelli *et al.*, 1992 and Koutsaftakis and Stefanoudaki, 1995).

The results indicated generally that fatty acid profile was not affected by the addition of olive leaves. While, Lauric acid content in Picual and Chemlalli oils was decreased by 0.28% and 0.50%, respectively due to the addition of olive leaves compared with control oil sample (Table, 2).

The tabulated results indicated also that there was no changes in fatty acid composition of both investigated oil samples associated with recycling of wastewater (Table, 2). Accordingly, the recycling of wastewater during malaxation process didn't influence the oil content of monounsaturated fatty acids which are considered as one of the effective quality parameters used in evaluation of olive oil.

The fatty acid profile of oil samples obtained after mixing of olive leaves or wastewater recycling was in accordance with fatty acid profile of virgin olive oil as suggested by Codex (1992).

Sensory evaluation:

The sensory characteristics of the produced oils were evaluated and the results are presented in table (3)

Table (3): Effect of olive leaves addition and wastewater recycling on sensory properties of olive oil produced from Picual and Chemlalli cultivars.

Organoleptic parameter	Picual oil			Chemlalli oil		
	Control	Leaves addition	Waste water	Control	leaves addition	Waste Water
Color	9.0_b*	8.5_c	9.0_b	9.5_a	9.0_b	9.5_a
Odor	9.0_a	9.0_a	9.0_a	8.5_b	8.5_b	8.5_b
Taste	8.5_c	9.5_b	8.5_c	8.5_c	10.0_a	8.5_c

*** Values within each row followed by the same letter are not statistically differ from each other (Duncan's Multiple Range Test 5%).**

The results of sensory evaluation showed clearly that leaves addition caused slight darkening of the produced oils. Development of such colors may be due to the transmission of pigments from the leaves to the extracted oils. Besides, leaves addition didn't affect the odor of the oils obtained from both examined cultivars.

Concerning, the effect of leaves addition on the taste of produced oils, it could be remarkably noticed that the taste of olive oils of investigated cultivars was improved due to addition of leaves. The obtained results are in accordance with Di-Giovacchino *et al.* (1996), who reported that such improvement in taste could be related to the transmission of some effective constituents such as trans-2-hexanal from leaves to oils. Such components showed desirable effects on the olive oil flavor. It could be noticed also that due to leaves addition more improvement of Chemlalli oil taste was occurred comparing with Picual oil.

The tabulated results indicated also that wastewater recycling showed no detectable effect on all examined organolyptic parameters of the oils obtained from either Picual or Chemlalli cultivars.

Interestingly enough, leaves addition caused remarkable improvement of oil taste and increased polyphenol content by approximately 4.2 – 5.8 % without any undesirable changes in the evaluated quality parameters of produced olive oil.

Depending on the aforementioned results it could be concluded that recycling of wastewater during malaxation process could be recommended to increase the oil yield by about 2.5-3% and rise the oil content of polyphenols by approximately 25%. Furthermore, application of such treatment led to reduction of wastewater and its associated environmental problems as well as reduction in water consumption.

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تأثير إضافة أوراق الزيتون وتدوير المخلف المائى على إنتاجية وجودة زيت الزيتون المنتج باستخدام الطرد المركزى

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تم استخدام صنفى البيكوال والشملالى لدراسة تأثير إضافة أوراق الزيتون (٢ % وزنية/وزنية) خلال عملية الجرش وكذا تأثير تدوير المخلف المائى (بمعدل ٦٠لتر/١٠٠ كجم من عجينة الزيتون) أثناء عملية الخضخضة على إنتاجية الزيت الناتج باستخدام الطرد المركزى. وقد وجد أن الزيت الناتج بعد معاملة خلط الأوراق يمكن توصيفه بعد أخذ عينة الزيت غير المعاملة فى الاعتبار على النحو التالى: ١) محتواه من الفينولات الكلية أعلى بنسبة تصل إلى ٤,٢- ٥,٨ % ، ٢) تشابه من حيث قيم الحموضة والبيروكسيد ، ٣) تقارب محتواه من الأحماض الدهنية المشبعة وغير المشبعة ، ٤) أكثر أفضلية من حيث الطعم ، ٥) يصنف كزيت زيتون بكر ممتاز. أما تطبيق تدوير المخلف المائى فقد أدى إلى زيادة إنتاجية الزيت بنسبة ٢,٥- ٣% وإلى اختزال كمية الماء المتخلف ، كما تم توصيف الزيت الناتج مقارنة بالعينة غير المعاملة على النحو التالى : ١) محتواه من الفينولات الكلية أكبر بنسبة ٢٥% ، ٢) تشابه قيم الحموضة والبيروكسيد ، ٣) تشابه المحتوى من الأحماض الدهنية المشبعة وغير المشبعة ، ٤) تشابه الصفات الحسية ، ٥) يصنف كزيت زيتون بكر ممتاز. ومن ثم يمكن التوصية بإضافة أوراق الزيتون أثناء عملية الجرش وكذا تدوير المخلف المائى خلال عملية الخضخضة على النحو المشار إليه وذلك عند إنتاج زيت زيتون بكر ممتاز.