



Mitigate the Extreme Bitterness in Virgin Olive Oil Using Natural Aqueous Solutions

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Abstract

The extreme bitterness of virgin olive oil (VOO) is undesirable for many consumers. This study aimed to optimize the bitterness of VOO via using natural aqueous solutions. The efficacy of pure water and different concentrations from water solutions contained sodium chloride or citric acid or their mixtures (10 and 20% wt/v) was studied. The bitterness intensity was evaluated via determination of total phenol content (TPC), chloroplast pigments, radical scavenging activity, the bitterness compounds (K_{225}), the intensity of bitterness (IB), and oxidative stability. The co-relationship between TPC with each K_{225} , IB and induction period (IP) were studied. The results declared that the natural washing treatment of extreme bitterness in VOO by different water solutions optimized the TPC and IB to be acceptable by consumers. The pure water treatment kept the unique properties of VOO such as TPC, oxidative stability, chloroplast pigments and consequently preserving its virginity. This study reached to a simple and approved treatment, to mitigate the extreme bitterness in VOO using pure water. Also, used simple, quick and easy analytical methods to estimate the VOO bitterness, which can be applicable in olive oil factories.

Keywords: virgin olive oil; bitterness intensity; natural aqueous solutions; oxidative stability.

1. Introduction

In recent decades, large increases in the request for high-quality virgin olive oil (VOO). VOO obtained by squeezing olive fruits by mechanical or physical extraction methods without any treatment except washing, decanting, centrifuging and filtration [1]. The consumption of OO is increased by 76% between 1990 and 2016, this can be referred to its potential health benefits [2]. The nutritional value of VOO comes from high-levels of monounsaturated oleic fatty acid (omega-9) and natural phenolic compounds which have an important role in health-promoting abilities and shelf-life stability [3] [4]. In addition, Hassanein *et al.*, [2] found that the Egyptian mono-varietal coratina extra VOO is distinguished by the present high value of squalene. Squalene has unique properties, as an intermediate for the biosynthesis of phytosterol/cholesterol in plants, animals, and its various applications in many fields of human activity.

The European Food Safety Authority (EFSA) published that the TPC present in VOO had several benefits on human health. EFSA panel demonstrated that there is a strong correlation between the consumption of polyphenols and protection of humans from oxidative damage of LDL. Also, the panel specified that the condition of use to have the health benefit is 20g of VOO which contain 200 mg/kg of TPC [5] [6]. The polyphenols responsible for bitter taste is deeply cleansing to the body, it scrapes fat, toxins, kills germs, purify the blood, cleanse and support the liver and other many benefits health functions [6].

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Thus, it is important to make the awareness to the consumer about the link between phenolics, the bitterness of VOO and their positive health effect [5]. It is well known that the OO when bitter is better for human health [7] [8]. Although, the excessive levels of bitterness are undesirable for many consumers and are hardly marketable in many countries. Also, Gutiérrez-Rosales [9], reported that when the bitterness values increase, problems can appear for the VOO to direct consumption. Therefore, it is crucial to mitigate some the bitter taste from OO to be acceptable by consumer and increases marketability. Sometimes to overcome the problem, of VOO bitter it's preferred to blend it with refined OO. In this case according to legislation the product cannot be considered as a VOO. This leads to a problem in marketability.

Most previous studies were interested mainly to treat the olive fruits to remove or decrease the bitterness from it, which may affect the OO quality and stability during processing. Some commercial methods (Greek, Spanish, and Californian processing) depend on the acid, base, and/or enzymatic hydrolysis of bitter phenolic compounds naturally found in olives or olive oil into non-bitter hydrolysis products [4] [10]. Many scientists [11] [12] soaked the table olives in different acids, and they found that increasing the acid % leads to more removing of poly phenols. Yousfi *et al.*, [13], studied the effect of dipping three different olive fruits (in crop seasons, cultivation origins and fruit ripening levels) in hot water, on the oil characteristics. They found that the heat treatment of olives decrease the concentration of phenolic compounds, bitterness, yield and stability of oil.

A few studies have focused on the decrease of VOO bitterness by added sodium caseinate in olive oil/ water emulsion and other added lecithin [14]. However the addition of these compounds is not allowed to market VOO category [1]. On the other hand, the recommended method of evaluating the bitter taste of VOO is by sensory analysis using a panel of tasters [15]. However, an analytical panel is not likely to be available, since a permanent staff of trained tasters and a highly specialized panel chief is necessary. However, the difficulty of having a qualified and trained sensory evaluation team to be

available to perform the tasks of OO testing and determining its sensory characteristics. In addition, panel taste not suitable for industry. For this reason, methods for the mitigation and determination of the bitterness level would be natural, fast and simple to be useful and applicable for the industry. However, it is a matter of concern for the oil industry to conserve the VOO treatments without loss or deterioration of its main positive attributes or its quality. Beltran *et al.*, [16], reported that VOO bitterness may be classified based on the TPC into 4 categories: non-bitter oils; light bitterness; bitter oils and quite bitter or very bitter oils.

The present study aims to mitigate the extreme VOO bitter taste to suitable levels without any negative changes in the oil quality, its virginity as well as to increase its marketability by using natural treatments. At the same time, use simple and fast methods to estimate the VOO bitterness to be suitable as an applicable method in OO factories labs. This occurring by washing the VOO with pure water or water containing different concentrations from: sodium chloride or citric acid solutions or a mixture from them (10 and 20%).

Evaluate the bitterness intensity by measuring total phenol content, chloroplast pigments (chlorophyll & carotenoids), radical scavenging activity (R.S.A%), the compounds responsible for oil bitterness measured spectrophotometry at 225 nm (K_{225} value) and intensity of bitterness (IB), as bitterness predictors in different VOO samples as well as oxidative stability by Rancimat. Also, the relationship between TPC with each of K_{225} , IB and oxidative stability were done.

1. Materials and Methods

2.1 Materials

Extreme bitter virgin olive oil kindly supplied from Factory at 6th of October Industrial Zone, Giza, Egypt. Sodium chloride and citric acid were purchased from Merck.

Water solutions preparation:

VOO samples treated with different water solutions are illustrated in Table 1.

Table 1: The composition of different water solutions.

Number of treatment	Water solutions	Treatments (wt/v)
1	Pure water	Water
2	Water with 10% sodium chloride	NaCl (10%)
3	Water with 20% sodium chloride	NaCl (20%)
4	Water with 10% Citric acid	Citric acid (10%)
5	Water with 20% Citric acid	Citric acid (20%)
6	Mixture 1	NaCl + Citric acid 1:1 (10%)
7	Mixture 2	NaCl + Citric acid 1:1 (20%)

Concentration of NaCl and citric acid 10, 20% and their mixture wt/v

2.2 Methods

2.2.1 Liquid-liquid extraction of bitter taste from VOO

Olive oil samples were treated with different water solutions at room temperature (at ratio 90 oil: 10 water v/v), according to Abenoza *et al.*, [17] who found that the lower olive oil-to-water ratio (90/10) in the mixture were more efficient for phenol extraction. Then mixing by vortex for five minutes, after that, the separation of oil from different water solutions was done via centrifugation applying a force of 4000 xg for 10 min to separate the two phases.

2.2.2 Determination of total carotenoids and chlorophyll a & b

Analyses of samples were carried out using Shimadzu, UV-spectrophotometer UV-240. About 1 g of oil was dissolved in 10 ml acetone. The absorbance was measured at 662 nm, 645 nm and 470 nm according to Kotíková *et al.*, [18]. The total content of carotenoids and chlorophyll (a& b) were calculated from the equations mentioned below:

$$Ca = 11.75 A_{662} - 2.35 A_{645}$$

$$Cb = 18.61 A_{645} - 3.96 A_{662}$$

$$Cx+c = (1000 A_{470} - 2.27 Ca - 81.4 Cb)/227$$

Ca: content of chlorophyll a, Cb: content of chlorophyll b,

Cx+c: content of carotenoids.

All extraction procedures were carried out under subdued light in triplicate determinations.

2.2.3 Determination of total phenolic content

Determination of total phenolic content in oil was based on the procedure introduced by Gutfinger [19] using the Folin–Ciocalteu reagent. The detailed description of the experimental procedure was in accordance with our previous study [4] [20].

2.2.4 Evaluation of the bitter taste

a- Determination of bitterness index K_{225} : A sample of 1.0 g oil was dissolved in 4 mL hexane and passed over the C18 column [previously activated with methanol (6 mL) and washed with hexane (6 mL)]. After elution, 10mL hexane was passed to eliminate the fat, and then the retained compounds were eluted with methanol / water (1:1) to 25 mL in a beaker. The absorbance of the extract was measured at 225 nm against methanol: water (1:1) in a 1cm cuvette (Gutiérrez- Rosales, *et al.*, [21]).

b- Determination of the intensity of bitterness (IB) according to Gutiérrez-Rosales *et al.*, [21] and Mateos *et al.*, [22]:

IB was calculated by the following expression:

$$IB = 13.33 K_{225} - 0.837.$$

2.2.5 Oxidative stability

Oxidative stability was determined by the Rancimat according to AOCS methodology as the same described by Abdel-Razek *et al.*, [23]. The detailed description of the instrument and experimental procedure was in accordance with our previous study [4] [24] with modified temperature at 100°C.

2.2.6 Measurement of the radicals scavenging activity (R.S.A %)

DPPH[•] assay method was used to measure the antioxidant activity of oil samples as the same described by Abdel-Razek *et al.*, [4]. Toluene solution of DPPH[•] was prepared freshly at concentration of 10⁻⁴ M. Different amounts of oil was weighted in test tubes (10, 20, 30, 40 mg) and completed to 4 ml by toluenic DPPH[•] solution and vortexed for 20 seconds. The decrease in absorption was measured at 515 nm after 30 min using blank of toluene and DPPH[•] solution as control. The radicals scavenging activity (R.S.A %) was calculated from

the equation:

$$\text{R.S.A \%} = [(A \text{ control} - A \text{ sample}) / A \text{ control}] \times 100$$

Where A = Absorbance value at 515 nm

2.2.7 Statistical analysis

Results are presented as the mean \pm standard deviation from three replicates of each experiment. A P-value < 0.05 was used to denote significant differences between mean values determined by the analysis of variance (ANOVA) with the assistance of Statistica 7.0 (StatSoft Inc., Tulsa, OK) software.

3. Results and Discussion

3.1 Chloroplast pigments (chlorophylls and carotenoids):

Chloroplast pigments are responsible for the characteristic yellowish-green color of the oil, an important visual attribute which has a potent effect on the acceptance of the product to the consumer [25].

In recent years, a special attention has been paid to chloroplast pigments, to elucidate their influence on oxidation and the stability of VOO. The contents of chloroplast pigments were determined for different treatments of VOO and the results were presented in Table 2. Concerning the chlorophyll a & b, it was noticed that when the VOO was washed with water only or water that contained 20% citric acid nearly kept constant values comparing to original VOO. In case of carotenoid, VOO washed with water contained 20% NaCl or citric acid, no change in carotenoid values [22] was observed, comparing to control sample.

From the results in Table 2, it was found that the treatment of VOO samples by water solutions obviously had very little or no reduction of chlorophyll and carotenoids pigments content for all washed samples. This may be due to hydrophilic nature of water and the lipophilic nature of pigments (chlorophylls and carotenoids). The results in Table (2) agree with those of Gandul-Rojas *et al.*, [25].

Fakourelis *et al.*, [26] reported that an important role of chlorophylls and carotenoids in the oxidative activity of OO, is due to their antioxidant nature especially in the dark. On the other hand, they found that chlorophylls and their derivatives are present in

VOO, can act as photosensitizers. The chloroplast pigments have been postulated to act as protectors, capturing free radicals in a way similar to that of α -tocopherol [9]. International Olive Oil Council [1] reported that, carotenoids together with polyphenols and tocopherols support the oxidative stability of VOO.

3.2 Total phenolic content

The total phenolic content (TPC) and its reduction% (TPR %) of the VOO for the treated samples are reported in Table (3). It was noticed that the value of TPC 500 $\mu\text{g/g}$ for original VOO sample. Concerning the remaining TPC in case of VOO samples washed with different water solutions, the values ranged from 267 to 406 $\mu\text{g/g}$. Consequently, the TPR% ranged from 18.5 to 46.5%. The lowest TPR% about 18.5% was found in VOO washed with water contained 20% NaCl. However, the highest TPR% about 46.5% was observed in case of VOO washed with water containing 20% citric acid followed by TPR% 40% in sample washed with pure water (Table 3).

From the results (Table 3), it was found that the TPC of all VOO samples treated with water solutions (except oil treated by 20% NaCl) ranged from 267-340 $\mu\text{g/g}$, these findings agree with Beltran *et al.*, [16]. It was noticed that the effect of increasing NaCl concentration (from 10% to 20%) decreasing the solubility of phenolic compounds (Table 3). This phenomena may be due to salting-out effect relates to the strong tendency of ionic solutes to form shells of tightly bound water (hydration shells). This means that the decreases of availability of water and decreases the solubility of phenolic compounds in water solution. These results agree with Noubigh *et al.*, [27] [28] who reported that the solubility of phenolic compounds decreases as concentration of NaCl salt increases. The increases of citric acid concentration in water solution (20%) showed a higher reduction in TPC comparing to 10% citric acid and pure water. These results agree with Kiai and Hafidi [11] Abou-Zaid and Ibraheem [12]. Also, these results agreed with Kagan [29], who reported that the increase acidity to lower value (pH 2) enhanced the recovery of phenolic compounds from 2 to 7 fold higher than the higher pH value of solution (pH 6).

Table 2: Chloroplast pigments content of original and treated washed VOO

Treatment	content of chlorophyll a ($\mu\text{g/g}$)	Content of chlorophyll b ($\mu\text{g/g}$)	content of carotenoids ($\mu\text{g/g}$)
1	5.08	4.98	1.87
Original	± 0.050	± 0.049	± 0.018
2	5.03	4.93	1.63
Pure water	± 0.014	± 0.013	± 0.004
3	4.76	4.50	1.53
10% NaCl	± 0.024	± 0.023	± 0.007
4	4.76	4.71	1.7 ^a
20% NaCl	± 0.044	± 0.044	± 0.016
5	4.94	5.00	1.54
10% Citric	± 0.039	± 0.039	± 0.012
6	5.04	4.94	1.77
20% Citric	± 0.026	± 0.025	± 0.009
7	4.70	4.60	1.54
Mixture 1	± 0.066	± 0.064	± 0.021
8	4.76	4.71	1.47
Mixture 2	± 0.041	± 0.041	± 0.012

Mixture 1 and 2: a mixture from NaCl and citric acid 10 and 20% respectively.

Table 3: Chemical analysis of original and treated VOO

Treatment	TPC ($\mu\text{g/g}$)	TPC reduction %	K_{225}	IB	IB reduction %	IP (h)
Original	499.05 ± 5.05	-	0.480	5.56	-	79.49
Pure water	301.82 ± 11.72	39.52%	0.282 ± 0.03	2.93	47.3%	45.73
10% NaCl	312.45 ± 2.30	37.39%	0.286 ± 0.05	2.97	46.58%	51.95
20% NaCl	406.38 ± 9.95	18.56%	0.291 ± 0.01	3.04	45.32%	52.11
10% Citric acid	311.50 ± 0.58	37.58%	0.285 ± 0.03	2.96	46.76%	47.87
20% Citric acid	267.00 ± 9.89	46.49%	0.267 ± 0.02	2.72	51.07%	45.75
Mixture 1	309.39 ± 10.85	38.0%	0.282 ± 0.01	2.91	47.66%	50.1
Mixture 2	340.69 ± 10.80	31.73%	0.286 ± 0.03	2.97	46.58%	50.05

TPC: total phenolic compounds; IB: intensity of bitterness; IP: induction period;
Mixture 1 and 2: a mixture from NaCl and citric acid 10 and 20% respectively.

It is worthy to mention that the bitterness is related to the phenolic compounds, Beltran and coworkers [16] divided the TPC in VOO into four categories as follows: TPC equal or lower than 220 mg/kg known as non-bitter OO, TPC ranged from 220-340 mg/kg consider as light bitter; VOO bitter if TPC ranging from 340 to 410 mg/kg and the higher than 410 mg/kg corresponding to very bitter VOO. In addition Boskou, [30] classified VOOs bitter to mild, medium, or robust. Robust when a TPC above 300 mg/kg, and below 180 mg/kg known as mild VOO bitter. From the results (Table 3), it was noticed that the natural washing treatments (liquid-liquid extraction) decrease the values of TPC to suitable levels (as mentioned above) which not affect the VOO quality. This may be due to the VOO phenols are hydrophilic in nature and are more soluble in water than in the oil phase [31]. It's known that the nutritional benefits of VOO are due to its bioactive components, especially phenolics; they have antimicrobial, anti-inflammatory and antioxidant potency protect human against some diseases [3]. Moreover, phenolics play an important role to protect the OO against autoxidation and are responsible for the positive attributes (bitterness and pungency) of VOO [17] [32]. In addition EFSA [5] reported that, it is important to produce VOO having a phenol concentration higher than 200 mg/kg in order to meet nutritional requirement for human health claim.

Where the efficiency of pure water treatment of VOO sample reached 86% (phenolic reduction) comparing with the high efficient treatment (20% citric acid). So, for preserving the virginity of olive oil, the use of pure water treatment was selected as a simple and approved method to mitigate the extreme bitterness in virgin olive oil.

3.3 Bitterness index

The levels of bitterness in VOOs correlate with many factors such as conditions of cultivation, harvesting time, and extraction system. The bitterness evaluation is becoming a very important parameter in VOO research due to relation between bitter compounds and the oil oxidative stability as well as the bitterness and human health [30].

The values of bitterness index (K_{225}), intensity of bitterness (IB) and reduction % of IB in the VOO

samples were recorded in Table (3). Concerning the K_{225} value, the highest efficient treatment was found in VOO washed with water containing 20% citric acid, which minimized the K_{225} value from 0.48 (original sample) to 0.26. On the other hand, the lowest effect was found in VOO washed with aqueous solution containing 20% NaCl (0.29) but still low value than original sample. While, the other VOO samples treated with different water solutions nearly kept constant (0.28).

Regarding the IB, the highest and lowest efficient treatments were found in VOO samples treated with 20% from each NaCl and citric acid (3.04 and 2.72 respectively), compared to the control sample (5.56). Concerning the IB reduction %, it was found that the minimum reduction in case of VOO washed with water containing 20% NaCl (45.32%), while the highest reduction % found in VOO washed with water containing 20% citric acid reached to 51.07% (Table3).

The IB values of VOO treated with water solutions ranged from 2.70 to 2.97 (Table3). Beltran et al., [18] found that the VOO bitter taste was acceptable when IB values ranged between 2.50-2.99, while if IB value of VOO more than 3 then the oil became very bitter. Therefore, the measuring of TPC and VOO bitterness intensity can be providing an easy and fast tool for bitterness assessment without need to sensory evaluation, this view agreed with Beltran et al., [16] recommendations. According to Beltran et al., [16] finding, it can be depended on the estimation IB instead of sensory evaluation to determine the VOO bitterness. They found that a high correlation between sensory evaluation for 25 VOO varieties and estimated bitterness intensity reached to 92% and 100% for lowest and highest bitterness categories.

3.4 Oxidative stability (Rancimat method)

The susceptibility of the treated VOO with different water solutions to oxidation was determined by the Rancimat test. The end point of the Rancimat test can be estimated by IP to the inflection point in the oxidation curve. The length of the IP is considered a relative measure of the stability of oils.

From the results recorded in Table 3, it was noticed that the IP for original VOO was 79.49 h and ranged from 45 to 52 h at 100°C for other treated samples. These results agree with Krichene *et al.*, [33] who found that the IP of different varieties of VOO ranged from 21-43 h at 100°C. On the other hand, Abdel-Razek *et al.*, [4] found that the IP of VOO was 31 h at 110°C. The washing of VOO samples with natural water solutions for optimize or reduce the bitterness, has no negative effect on the IP and consequently on the oil stability.

3.5 Free radical scavenging activity RSA%

The use of DPPH[•] method provides an easy and rapid way to evaluate antioxidants activity. Variations in DPPH[•] scavenging activity were found in VOO treated with different water solutions as shown in Fig (1). From the figure (at concentrate 75µl oil), it was found that original VOO sample had the highest DPPH[•] scavenging activity with an estimated value of (94.49%). VOO treated with water contained 20% NaCl (86.12%) followed by mixture 2 (80.3%) whereas the VOO treated with 20% citric acid had the lowest DPPH[•] scavenging activity with an estimated value of 66.5%.

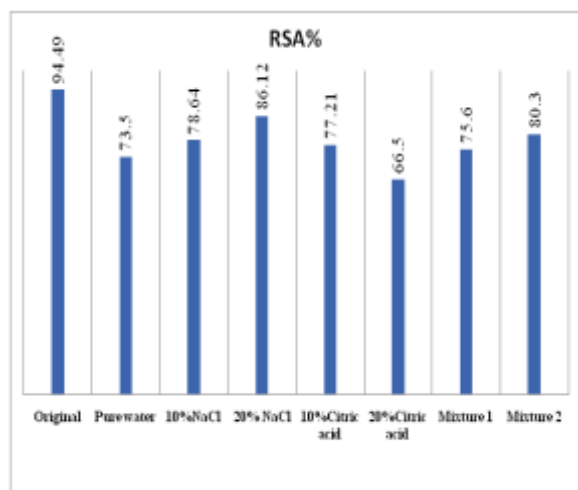


Fig. 1: Radical scavenging activity (RSA %) of original and treated VOO samples with different aqueous solutions on DPPH

The results in Fig. 1 agree with Abdel-Razek *et al.*, [4], they found that VOO had the highest DPPH[•] scavenging activity with an estimate value of (95.74%) comparing to the other studied oils. From the results, it was noticed that there are a relationship between TPC and RSA measured by DPPH[•]. Increases in TPC led to RSA% increases. These

results agree with Bouaziz *et al.*, [34], who reported that there is a correlation between the antioxidant activities and the phenol content in DPPH[•] assay.

3.6 Correlation results

Correlation between TPC and K_{225} , IB as well as IP were performed (Fig. 2 a, b & c). A correlation among TPC and K_{225} , IB & IP ($R^2 = 0.944$, 0.919 and 0.574 respectively) was found. The results gave a good relationship between TPC and K_{225} was associated with a high R^2 (0.944). Meanwhile, a high correlation between TPC ($\mu\text{g/g}$) and IB associated with high $R^2 = 0.919$ (Fig 2-b) was found. On the other side, the correlation between TPC ($\mu\text{g/g}$) and IP represent a correlation with $R^2 = 0.574$ (Fig 2-c). This result was in agreement with those of El Yamani *et al.*, [35], who reported that the contribution of total phenols content to the variability of oxidative stability was 51% ($R^2 = 0.51$).

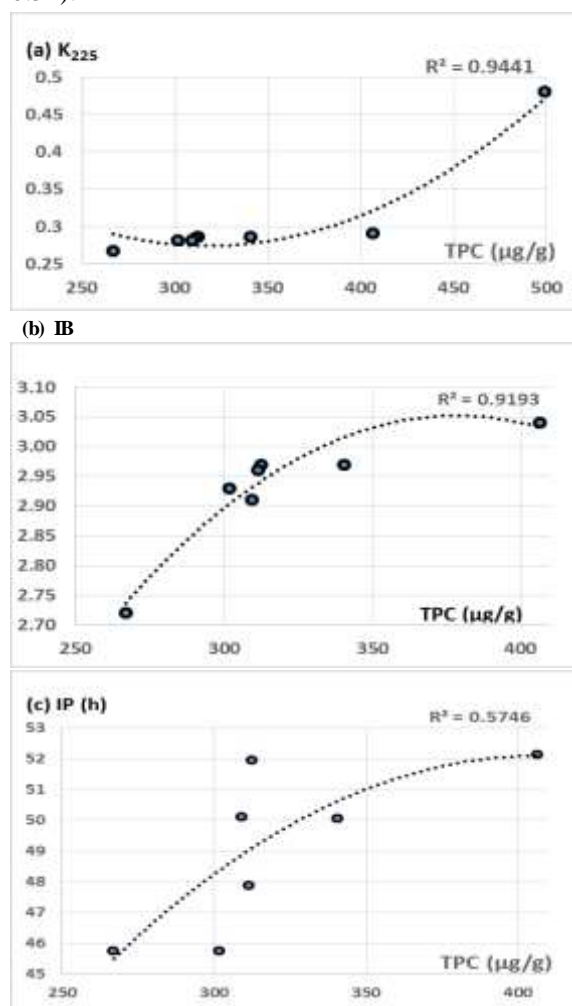


Fig. 2: Correlation between total phenol content (TPC) with; (a) K_{225} : bitterness index (b) IB: intensity bitterness (c) IP: induction period Rancimat

In this regards, several authors have found a strong direct relationship between TPC and bitterness intensity [16] [22]. Conversely, Favati *et al.*, [36] reported a poor correlation between TPC and IB but associated with a low R² (0.4225).

4. Conclusion

The natural aqueous solutions treatments (liquid-liquid extraction) of extreme bitter VOO mitigated the TPC and IB to desirable values recommended by EFSA and health authorities. Which leads to produce VOO has light bitter, acceptable by many consumers. The natural treatments of VOO kept the unique properties of VOO with no negative effect on the TPC, oxidative stability (IP and RSA %), chloroplast pigments and its virginity. Consequently, it may enhance the palatability, marketability and increases the consumption of VOO.

Where the efficiency of pure water treatment of VOO sample reached 86% (phenolic reduction) compared with the high efficient treatment (20% citric acid). So, for preserving the virginity of olive oil, it is recommended the use of pure water treatment as a simple and approved method to mitigate the extreme bitterness in virgin olive oil. Also, in this study, we used simple, quick and easy analytical methods to estimate the VOO bitterness, which can be applicable in olive oil factories.

4. Conflicts of interest

“There are no conflicts to declare”.

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التخفيف من حدة المرارة في زيت الزيتون البكر باستخدام المحاليل المائية الطبيعية

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تعتبر المرارة الشديدة في زيت الزيتون البكر أمر غير مرغوب فيه بالنسبة للعديد من المستهلكين. تهدف هذه الدراسة إلى تقليل حدة المرارة في زيت الزيتون البكر عن طريق استخدام محاليل مائية طبيعية، بهدف الحفاظ على المكونات الطبيعية المميزة لزيت الزيتون ولزيادة مدى قابليته واستساغته لدى المستهلك ولزيادة قدرته التسويقية. وقد تمت دراسة تأثير الماء النقي مع التركيزات المختلفة من المحاليل المائية المحتوية على كلوريد الصوديوم أو حامض الستريك أو مخاليطهما (١٠ و ٢٠٪ وزن / حجم). وقد تم تقييم شدة المرارة بدلالة تقدير المحتوى الكلي للفينولات وأصبغ الكلوروفيل والكاروتينات و كذلك قياس القدرة على اصطياد الشوارد الحرة والمركبات المسببة للمرارة (K_{225}) و شدة المرارة (IB) وثبات الأكسدة. كما تمت دراسة معامل الارتباط بين الفينولات الكلية مع كل من K_{225} و IB وقدرة الزيت على الثبات ضد الأكسدة (IP).

وقد توصلت هذه الدراسة إلى أن استخدام الماء النقي له القدرة العالية على تقليل شدة المرارة في زيت الزيتون إلى الحد المرغوب فيه للحفاظ عليه كزيت زيتون بكر طبقاً للإشترطات الدولية (من مجلس الزيتون العالمي وهيئة الكودكس). وأيضاً تم استخدام طرق تحليلية لتقدير مرارة زيت الزيتون البكر وهي طرق بسيطة وسريعة تصلح للإستخدام في معامل مصانع زيت الزيتون.