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USING OF SOME DIFFERENT ACIDS IN DE-BITTERING OF GREEN OLIVES

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ABSTRACT

The efficacy of different debittering treatments on table picual olive characteristics was evaluated. The treatments included : soaking in acetic acid solutions (1, 0.5 and 0.25 N), soaking in hydrochloric acid solutions (1, 0.5 and 0.25 N) and soaking in lactic acid solutions (1, 0.5 and 0.25 N), in comparison with lye and traditional treatments. The obtained results demonstrated obviously that, all acid treatments (acetic, hydrochloric and lactic) had noticeable effects on polyphenol removing when compared with traditional and lye treatments. The highest effect was recorded for 1N hydrochloric acid treatment, which removed about 69.9 % of total polyphenols in end product, leading that to improve the sensory parameters of end product. On the other hand, using of these acids in debittering of table olive does not need any washing process like lye treatment, leading that to safe large quantities of clean water.
Keywords: Table green olive ; De-bittering; Acid treatments ; water saving.

INTRODUCTION

Table olives are one of the main pickled products prepared throughout the world. Spain is the first producing and exporting country. In general, any processing method aims to remove the natural bitterness of this fruit, caused by the glucoside oleuropein. The olive fruit can be pickled at any stage from the beginning of ripening, when it is green, until it is black and fully mature. To render the fruit edible it is important to remove some or all of the bitter phenolic glucoside oleuropein, which is found in all olives in varying amounts. The fruit is then preserved by one of a number of possible processes. Methods of oleuropein removal and preservation are dependent on many factors, including the olive cultivar, fruit maturity, growth conditions, and cultural preference (*Antonio et al., 2006*).

There are three main types of preparations widely used worldwide to produce edible olives: green or Spanish-style olives, black ripe or Californian-style olives and Greek-style or natural black olives in brine (*IOOC, 2004*). More than fifty percent of the table olives are prepared according to the Spanish-style green olives *(Ruiz Barba & Jiménez Díaz, 2012)*. The procedure consists of treating olive fruits with lye (20-50 g/l) to hydrolyze oleuropein and consequently eliminate partially the bitterness and increase skin permeability (*Garrido,et al., 1992*). The olives are left in sodium hydroxide solution until the lye has penetrated two-thirds to three-fourths of the distance from the olive surface to pit. Then, olives are washed once, twice or three times with tap water in order to eliminate the excess of alkali. Finally, washed fruits are stored in brine (100-120 g/l NaCl), where lactic fermentation occurs (*Hurtado, et al., 2012; Sánchez-Gómez, et al., 2006; Vergara,et al.,* *2013*). The initial treatments (lye treatment and washing) are olive variety dependent, due to the different physical properties (texture, size) of each cultivar (*Montaño, et al.,, 2003*) .

During fermentation, important physico-chemical changes occur. Water-soluble compounds such as carbohydrates, phenolic compounds mainly oleuropein and hydroxytyrosol glucoside and other nutrient, diffuse from olives to the brine, while salt goes from the brine into the flesh until it reaches a steady state by the end of brining process. The fermentable substrates (glucose, fructose, mannitol, sucrose, etc) are the main energy source of fermentative microorganisms, which will provide organic acids (mainly lactic acid) essential for the stability and preservation of table olives during fermentation and storage. However, the phenolic compounds undergo quantitative and qualitative transformations during olive processing, mainly, the alkalin hydrolysis and/or the microbial degradation of oleuropein into hydroxytyrosol and elenolic acid glucoside during debittering and brining processes (*Brenes and de Castro, 1998; Servili et al.,2006*) .

Shasha and Leibowitz (1959) reported that oleuropein had been isolated from fruits, leaves, stems and roots of olive tree and they identified oleuropein as a colorless substance, soluble in acetone, water, methanol and ethanol. They added also that, oleuropein could be hydrolyzed either by alkali or acid.

Throughout all stages of treatment , large quantities of clean water are used and wastewaters of about 3.9-7.5 m3/t of olives, depending on the olive variety, are produced (kopsidas, 1992)

So, the aim of this investigation was replace the lye treatment and its harmful effects on public health by different concentrations of some acids found already in foods or stomach (which do not need further washing process), leading that to safe large quantities of clean water.

MATERIALS AND METHODS

Materials

- Olives: Picual olive fruits (season 2014) have been selected of cultivated green olives in El-Maghara Research Station - Desert Research Center. In North Sinai.
- Sodium chloride was purchased from Al- Nasr Salines Co. - El Arish- North Sinai.
- Citric acid E330 manufacture : TTCA OC., Ltd China
- Lactic ,hydrochloric and acetic acid were purchased from Al-Gomhoria Co. – Cairo – Egypt.

Methods

Traditional treatment

Two kg of picual olive variety were soaked in fresh water for 15h as a control debittering treatment and then, placed in plastic jars and pickling brine solution (12% Sodium chloride + 0.5% Citric acid) was added then the jars were closed tightly and left for 90 days until the end of the pickling process. (*Ibrahim, 2002 and Ross et al., 2002*).

Lye treatment

Olives were placed into tanks and soaked in a lye solution (1% w/v, sodiumhydroxide) for about 15h for de-bittering. During this stage hydrolysis of oleuropein, which is labile under alkaline conditions, takes place. Lye is allowed to penetrate through three-quarters of the flesh, leaving a small volume around the stone unaffected. This part of the flesh provides the necessary sugars for subsequent fermentation and confers to the olives a slight bitter taste. Olives are washed with water twice in order to remove excess alkaline. Then, two kg of picual olives were placed in plastic jars and pickling brine solution (12% sodium chloride + 0.5% citric acid) was added, then the jars were closed tightly and left for 90 days until the end of the pickling process. (*Hurtado et al., 2012*) .

Different acid treatments

Three concentrations of each acid were prepared separately as follow: 1 N, 0.5 N and 0.25 N.

Picual Olive fruits of were divided to 9 parts (2 kg for each) as follow:

- The first part was soaked in 1N acetic acid solution for 15 hr.
- The second part was soaked in 0.5 N acetic acid solution for 15 hr.
- The third part was soaked in 0.25 N acetic acid solution for 15 hr.
- The same procedure was repeated for the hydrochloric acid with olive parts from 4 to 6 and so on for lactic acid with olive parts from 7 to 9.

After finishing acids soaking treatments, 2 kg of each soaking treatment were placed in plastic jars and pickling brine solution (12% Sodium chloride + 0.5% Citric acid) was added then the jars were closed tightly and left for 90 days until the end of the pickling process.

Chemical analysis:

Olive fruits:

Total acidity, moisture, total lipids, chlorophyll (a) and (b), carotenoids and polyphenols were determined in olive fruits (fresh and pickled separately) of each treatment as follow:

- Total acidity: Total acidity was determined according to the *AOAC (1970)* and expressed as citric acid.
- Polyphenols: bitter substances were estimated as total polyphenols using the method described in the *AOAC(1970)*.
- Moisture content: were determined according to the *AOAC (1990)* .
- Chlorophyll (a) and (b), carotenoides were determined by using the method of *Wettestein (1957)*.
- Total lipids: were determined according to the *AOAC (1990)*
- Sensory evaluation: Sensory properties were evaluated as described by *Balatsouras and Doutsias, (1983)*, where the final products from all treatments were presented to 10 untrained member panelists for organoleptic evaluation. The panelists were requested to assess the samples for taste, color, texture and over all acceptability by 10 points in scale levels of quality.

Treatment Solutions:

Total acidity and total polyphenols were determined in treatment solutions (different acids ,lye, and traditional treatments)as described previous.

Statistical analysis:

All determinations were carried out in triplicate and data is reported as mean. Significant differences ($p<0.05$) were calculated using Duncan's multiple range test, followed the method reported by *Steel and Torrie, (1980)*.

RESULTS AND DISCUSSION

Olives

Moisture content

The Moisture content of the final product considered an important parameter related to firmness and other sensory properties. The moisture content of the fresh picual olives, after treatments by different debittering treatments and after pickling were determined and the results are presented in figure (1)

The moisture content of pickled picual olives after lye treatment recoded the highest moisture content compared with other treatments .The results also showed that, pickled olives treated by 1N of different acids recorded higher contents of moisture followed by pickled olives treated by 0.5N then 0.25N compared with fresh and traditional treatment , The presented results demonstrated that also pickled olives treated by different concentration of lactic acid were the lowest moisture contents compared with other acids treatments. These results are in agreement with those of Ibrahim ,2002, who found that pickling picual olives by traditional or Spanish style (lye treatment) led to increase the moisture content comparing with fresh olives.

The increasing in moisture contents could be attributed to the decrease in total soluble solids during the fermentation period (Yassa *et al.,* 1990).

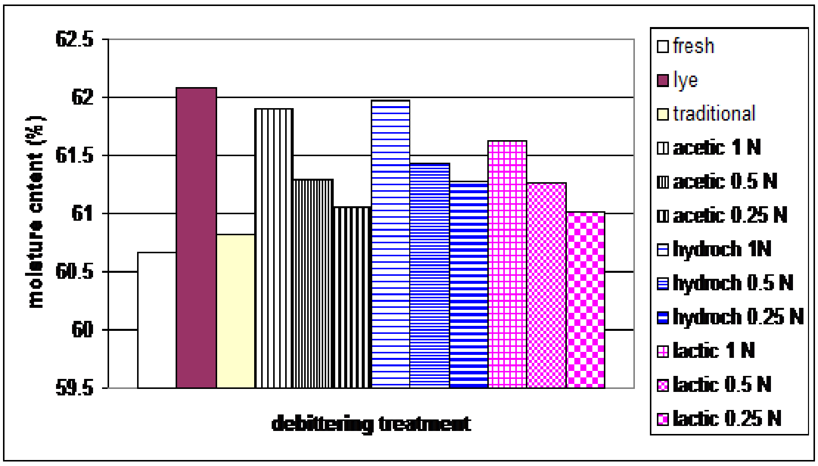

Figure (1) The effect of different debittering treatments on moisture content of picual green olive end products.

Total acidity and Total lipids:

Total acidity and total lipids of picual olives were determined after different debittering treatments and after pickling, and the obtained results were tabulated in table (1).

Regarding to total acidity, from the presented data in Table (1), it could be noticed that, hydrochloric acid treatments were more effective than the other treatments in increasing the total acidity of picual olives either after treatment or after pickling process, where the treatments of 1N hydrochloric acid recorded the highest raise in total acidity for both picual olives after treatment and after pickling process comparing with the other studied treatments. From the same table , it could be observed that, lye treatment led to neutralize the total acidity, which not detected after lye treatment. Also, the previous results showed that, the higher the acid concentration the higher the total acidity of picual olives either after treatment or after pickling process, .these findings are in agreement with those of *Ibraheem and Abou-Zaid, (2015*), who found that using of 1,2 and 3% citric acid as debittering treatments led to increase total acidity of table olive end products of four different varieties. This may be due to the residual traces of the used acid.

Concerning to the results of total lipids in the same table, the results showed obviously that, all studied treatments led to decrease the total lipids after treatment (except traditional one) and after pickling, where the lower the acid concentration the lower the lipid loss. From the same table, it could be noticed that, the highest loss of total lipids was recorded for lye treatment followed by hydrochloric treatments (1N and 0.5 N), this may be due to the effect of different studied treatments on the permeability of cell wall of olive fruits leading that to lipid loss in soaking and brine solutions. On the other hand, lye treatment could be reacted with lipids, in addition to its effect on permeability.

Chlorophyll a, b and Carotenoids:

Chlorophyll a, b and carotenoid contents were measured for picual olives fruits after different studied debittering treatments and after pickling process and the results were presented in Table (2).

Regarding to chlorophyll a, it could be noticed that, all studied treatments led to decrease chlorophyll a content of olive fruits either after treatment or after pickling comparing to fresh sample.

The highest effect on chlorophyll a reduction was observed for 1 N hydrochloric acid treatment followed by 0.5 N hydrochloric acid treatment for olive fruits either after debittering treatment or after pickling process. While the lowest effect on chlorophyll a reduction was recorded for traditional treatment.

Concerning to chlorophyll b, it could be observed that, slight degradation of chlorophyll b was occurred after different debittering treatments, except lye treatment, which recorded the highest degradation rate. On the other hand, all studied treatments had obviously effect on degradation of chlorophyll b for end product, where the highest degradation rate was observed for 1N hydrochloric acid treatment, while the lowest degradation rate was recorded for traditional treatment. This may be due to, most common color changes that take place during green olive processing are as a result of conversion of chlorophyll a and chlorophyll b to their respective pheophytins (*Forni, et al., 1988 and Baadseth and Hvan, 1989*).

1-2

In relation to carotenoid results in the same Table (2), which indicated that all studied debittering treatments had slight effect on changing carotenoid contents after treatments. On the other side, all studied treatments led to increase the carotenoid contents of the end products, where the maximum increase was recorded for 1N hydrochloric acid followed by 1N acetic acid treatment, while the minimum increase was recorded for lye treatment followed by traditional treatment. These findings are in agreement with those of *Ibraheem and Abou-Zaid, (2015)* , who found that, carotenoids content was increased in table olive end product of different varieties comparing to fresh sample.

polyphenols of olive fruits:

Polyphenol contents of olive fruits after different debittering treatments and after pickling process (for end product) were determined and the obtained results were presented in Table (3).

Table (3): the effect of different debittering treatments on total polyphenols content (ppm) of picual green olive fruits after treatment and end products

Treatments	Conce.	After treatment	% residual	Final product	% residual
Acetic acid	1 N	2279^{g}	90.2	910^{i}	36.0
	0.5 N	2317^{d}	91.7	1040^{f}	41.1
	0.25 N	2332^{b}	92.3	1215^{d}	48.1
Hydrochloric acid	1 N	2231^{k}	88.3	760^{j}	30.1
	0.5 N	2264^{i}	89.6	1019^{g}	40.3
	0.25 N	2282^{f}	90.3	1235^{c}	48.9
Lactic acid	1 N	2276^{h}	90.1	950^{h}	37.6
	0.5 N	2295^{e}	90.9	1090^{e}	43.1
	0.25 N	2321^{c}	91.9	1285^{b}	50.9
Traditional		2487^{a}	98.5	1475^{a}	58.4
Lye		2252^{j}	89.1	1034^{f}	40.1
Fresh		2527			

Values bearing the same superscript within the same column are not significantly different (P> 0.05)

The mentioned data in Table (3) indicated obviously that, all studied treatments had a positive effect on polyphenol removing comparing to the traditional treatment either for olive fruits after treatment or after pickling, where the maximum removing rate was recorded for 1N hydrochloric acid , while the minimum removing rate was recoded for traditional treatment.

These results in harmony with those of Hajar and Hafidi, (2014), who found that the loss of total phenolic contents in the olive flesh by the end of fermentation process was up to 79% in “Languedoc Picholine”, “Sevillana” and “Moroccan Picholine”.

Sensory Evaluation:

Sensory evaluation is the most important factor that determines the consumer acceptance. Where, he always interests in good appearance, attractive color, firm texture and good organoleptic qualities of a foodstuff many more than the nutritive and biological value.

Table (4) Sensory evaluation of final picual green olive fruits after pickling process.

Treatments / Characteristics	Acetic acid			Hydrochloric acid			Lactic acid			Lye	Traditional
	1 N	0.5 N	0.25 N	1 N	0.5 N	0.25 N	1 N	0.5 N	0.25 N		
Taste	7.5^{ab}	7^{c}	6.5^{cd}	8^{a}	7^{bc}	6de	7.5^{ab}	7^{bc}	6.5^{cd}	6de	5.5^{e}
Color	8^{a}	7.5^{b}	7^{c}	8^{a}	8^{a}	7^{c}	7.5^{b}	7^{c}	6.5^{d}	6.5^{d}	6^{e}
Textures	7.5^{b}	7.5^{b}	7^{c}	8^{a}	7^{c}	7^{c}	7.5^{b}	7^{c}	6^{d}	7^{c}	7^{c}
Acceptance	7.5^{b}	7^{c}	6.5^{d}	8.5^{a}	7.5^{b}	6.5^{d}	7.5^{b}	7^{c}	6.5^{d}	7^{c}	6.5^{d}

Values bearing the same superscript within the same row are not significantly different (P> 0.0)

Sensory properties of pickled picual olive fruits by different acids concentration treatments were tested and the results were presented in Table(4).

Presented data in Table (4) showed that, picual olive fruits pickled by hydrochloric acid 1N had the highest sensory attributes comparing to all other studied treatments.

In relation to both taste and color it could be noticed that, all different acid concentration treatments recorded higher score comparing to both traditional and lye treatments (except 0.25% hydrochloric acid for taste and 0.25% lactic acid for color), these results are in harmony with those of Vergara et al., (2013), who found that pickling Conservolea green olives in acidified brine led to produce pickled olives with minimum bitter taste.

Pickled olive texture was affected by different debittering treatments, where lye and traditional treatments had more preferable texture compared with 0.25% lactic acid only, while all acid treatments of 1N concentration had more desirable effect on texture comparing with the other treatments. These are in harmony with Vergara et al., (2013), who reported that , acidifying of brine led to pickled olives more hardness and fibrousness.

On the other hand, acceptance results showed obviously that 0.25 N of all acids and traditional treatment recorded the lowest value, while 1N of all acids recorded the highest values.

Treatment solutions:

Brine Acidity:

Brines titratable acidity (% citric acid) after pickling process for all studied debittering treatments was determined and the results are presented in figure (2)

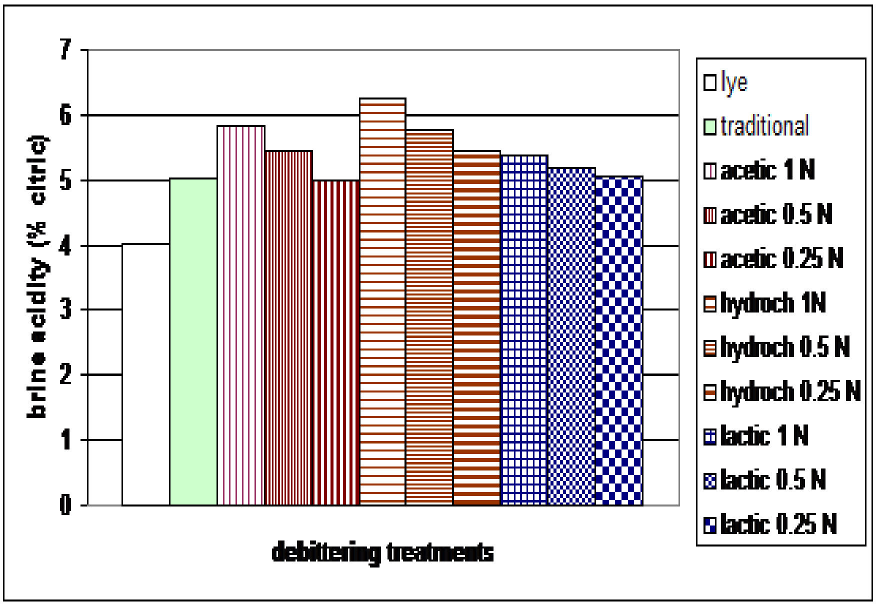

Figure (2) The effect of different debittering treatments on total acidity of brine for picual green olive fruits after different pickling treatments.

pickled olives fruits after lye treatment recorded the lowest value of brine total acidity compared with other treatments, while the total acidity of brine for pickled olives fruits treated by 1N of hydrochloric acid recorded the highest value compared with other treatments , this may be due to the effect of alkali residual traces of lye treated olives which, could neutralize part of producing acidity. These results are in harmony with those of Chammem et al., (2005), who reported that increasing the lye concentration from 1.5% to 2.5% led to decrease the free acidity from 0.636 % to 0.483 % after pickling process. On the other hand, the higher recorded acidity for 1N hydrochloric acid treatment, may be as a result of acid concentration and strong.

The results, also showed that the total acidity of brine for pickled olives treated by 1N of different acids recorded higher values followed by the total acidity of brine for pickled olives fruits treated by 0.5N then 0.25N compared with the total acidity of brine for lye and traditional treatments, The presented data in Table (5) demonstrated that also the total acidity of brine for pickled olives treated by different concentration of lactic acid were the lowest value of acidity compared with other acid treatments . these findings are in agreement with those of Vergara et al., (2013) , who found that, brine acidification with 0.1% lactic acid folded the combined acidity of the brine from the initial acidity (0.043 %) to final acidity (0.087%)

polyphenols of solutions

Polyphenol contents of different debittering solutions (after olive fruits treating) and final brine solutions (after pickling) were determined and the results were tabulated in Table (5).

From the tabulated data in Table (5), it could be noticed that, the higher the acid concentration the higher the polyphenol content of either treatment solutions or brine solution for each acid separately, but the most effective one was hydrochloric acid comparing to the other acids.

Table (5): the effect of different debittering treatments on total polyphenols content (ppm) of treatment solution and end brine solution

Treatments	Conce.	Treatment solution	% removing	Brine	% removing
Acetic acid	1 N	248^{d}	9.8	1369^{b}	54.1
	0.5 N	210^{f}	8.3	1277^{d}	50.5
	0.25 N	195^{g}	7.7	1117^{g}	44.2
Hydrochloric acid	1 N	296^{a}	11.7	1471^{a}	58.2
	0.5 N	263^{c}	10.4	1245^{e}	49.2
	0.25 N	245^{d}	9.7	1047^{h}	41.4
Lactic acid	1 N	251^{d}	9.9	1326^{c}	52.4
	0.5 N	232^{e}	9.1	1205^{f}	47.6
	0.25 N	206^{f}	8.1	1036^{i}	41.0
Traditional		40^{h}	1.5	1012^{k}	40.0
Lye		275^{b}	10.9	1018^{j}	40.3
Fresh		2527			

Values bearing the same superscript within the same column are not significantly different (P> 0.05)

From the same table, it could be observed that, traditional treatment recorded the minimum polyphenol contents either for treatment solution or brine solution, while 1N hydrochloric acid treatment recorded the maximum polyphenol contents for both treatments solution and final brine solution comparing to the other studied treatments. These findings are in acceptance with those of Ibraheem and Abou-Zaid, (2015), who reported that, soaking of picual olive in 1, 2 and 3% citric acid solutions led to removing about 7.2, 15.55 and 22.69 % of olive polyphenols in soaking solutions.

CONCLUSION

As indicated by the obtained results, the current practice of debittering of table olives by using different acids, led to successful debittering and fermentation processes without any washing process, so the lye treatment which needs two to three washing processes could be substituted by them. In the same time, the residual traces of these acids which naturally found in foods (acetic and lactic acids) or in the stomach had no concerning effect to health and may be enhance fermentation process.

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استخدام بعض الأحماض المختلفة فى نزع مرارة الزيتون الأخضر

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تم دراسة كفاءة استخدام بعض المعاملات المختلفة فى ازالة المرارة من زيتون المائدة الأخضر صنف بيكوال وتأثير ذلك على خصائص الزيتون الناتج. وقد شملت هذه المعاملات : النقع فى محاليل حمض الاستيك (تركيز ١ ، ٠.٥ ، ٠.٢٥ ع) ، النقع فى محاليل حمض الهيدروكلوريك (تركيز ١ ، ٠.٥ ، ٠.٢٥ ع) ، النقع فى محاليل حمض اللاكتيك (تركيز ١ ، ٠.٥ ، ٠.٢٥ ع) ثم مقارنتها بالمعاملة التقليدية وكذلك المعاملة بالقلوى . وكانت اهم النتائج المتحصل عليها أن كل المعاملات المختلفة للأحماض كان لها تأثير ايجابى ملحوظ على نزع المرارة وازالة البولى فينولات مقارنة بكل من المعاملة التقليدية والمعاملة بالقلوى. ولكن كانت المعاملة بحمض الهيدروكلوريك ١ ع هى الأكثر تأثيرا حيث ادت إلى إزالة حوالى ٦٩.٩ % من البولى فينولات فى المنتج النهائى مقارنة بالزيتون الطازج مما أدى إلى تحسين الصفات الحسية. ومن ناحية اخرى فإن استخدام مثل هذه الأحماض فى ازالة المرارة من الزيتون الأخضر لا يتطلب أى معاملة غسيل كما يحدث فى المعاملة بالقلوى مما يؤدى لتوفير كميات كبيرة من الماء النظيف .

Table (1): The effect of different debittering treatments on total acidity (% citric) and total lipids (%) of Picual olives after treatments and pickling process.

Treatments	Acetic acid			Hydrochloric acid			Lactic acid			Lye	Traditional	Fresh
	1 N	0.5 N	0.25 N	1 N	0.5 N	0.25 N	1 N	0.5 N	0.25 N			
Total acidity												
After treatment	1.74^{b}	1.58^{c}	1.46^{e}	1.79^{a}	1.60^{c}	1.52^{d}	1.61^{c}	1.52^{d}	1.48^{de}	-	1.02^{g}	1.40^{f}
End product	2.30^{b}	2.00^{de}	1.98^{ef}	2.38^{a}	2.09^{c}	1.98^{ef}	2.10^{c}	2.03^{d}	1.94^{fg}	1.57^{h}	1.90^{g}	1.40^{i}
Total lipids												
After treatment	35.75^{bc}	35.86^{abc}	35.91^{abc}	35.25^{d}	35.52^{cd}	35.71^{c}	35.58^{cd}	35.64^{cd}	35.75^{bc}	33.08^{e}	36.14^{ab}	36.24^{a}
End product	34.32^{ef}	34.53^{cde}	34.70^{c}	33.86^{h}	34.08^{g}	34.21^{fg}	34.23^{fg}	$34.40d^{ef}$	34.61^{cd}	30.27^{i}	35.79^{b}	36.24^{a}

Values bearing the same superscript within the same row are not significantly different (P> 0.05)

Table (2): The effect of different debittering treatments on chlorophyll a, b and carotenoids (mg/kg) of Picual olive fruits after treatments and pickling process.

Treatments	Acetic acid			Hydrochloric acid			Lactic acid			Lye	Traditional	Fresh
	1 N	0.5 N	0.25 N	1 N	0.5 N	0.25 N	1 N	0.5 N	0.25 N			
Chlorophyll a												
After treatment	7.856^{g}	7.991^{f}	8.286^{c}	7.153^{k}	7.560^{i}	7.621^{h}	8.100^{e}	8.190^{d}	8.280^{c}	7.284^{j}	8.422^{b}	8.964^{a}
End product	0.838^{i}	0.933^{g}	0.972^{f}	0.778^{j}	0.795^{j}	0.864^{h}	0.855^{hi}	1.081^{d}	1.160^{c}	1.007^{e}	2.041^{b}	8.964^{a}
Chlorophyll b												
After treatment	4.178^{d}	4.337^{bcd}	4.505^{b}	4.086^{cd}	4.180^{d}	4.371^{bc}	4.800^{a}	4.820^{a}	4.850^{a}	2.427^{e}	4.737^{a}	4.885^{a}
End product	1.609^{i}	1.835^{f}	1.957^{d}	1.151^{k}	1.324^{j}	1.619^{i}	1.765^{h}	1.853^{e}	2.014^{c}	1.805^{g}	2.451^{b}	4.885^{a}
Carotenoids												
After treatment	1.850^{c}	1.850^{c}	1.850^{c}	1.900^{b}	1.850^{c}	1.900^{b}	1.950^{a}	1.900^{b}	1.950^{a}	1.850^{c}	1.900^{b}	1.900^{b}
End product	2.947^{b}	2.760^{d}	2.549^{f}	3.162^{a}	2.942^{b}	2.772^{d}	2.884^{c}	2.686^{e}	2.436^{g}	2.118^{i}	2.255^{h}	1.900^{j}

Values bearing the same superscript within the same row are not significantly different (P> 0.05)