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An examination into the effects of frozen storage of olive fruit on extracted olive oils

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Abstract: This study was achieved to examine the effects of freezing olive fruits of the Arbequina, Koroneiki and Mission cultivars (the most common olive oil producing cultivars in Iran) on the standard indices used for assessing virgin olive oil quality. Oil was obtained from olive fruits stored at -4˚C for 1 week and 3 weeks, and compared with oil obtained immediately after harvest (control). The quality indices of oils obtained from frozen fruit showed no significant degradation in quality compared with the control samples. In fact the peroxide value of the frozen fruits decreased compared to the control, which is considered to have a positive effect on oil quality. In addition, compositions of the main fatty acids are not altered by freezing which demonstrate frozen storage as a viable option. Oil derived from frozen olive fruit is not of inferior quality to non-frozen fruit in the production of olive oil.

1. Introduction

Conservation of foods prior to processing by means of cold and frozen storage has been a relatively recent technique coming to prominence over the last 50 years (Poerio *et al.,* 2008). Olive oil consumption is increasing throughout the world, even in countries that traditionally have not used olive oil. This trend has been promoted due mainly to the nutritional value of the Mediterranean diet (Patumi *et al.,* 2002). Olive fruits (*Olea europaea* L.) undergo some mechanical procedures (milling, malaxation and centrifugation) to extract extra virgin olive oil. The quality of virgin olive oil is intimately related to the characteristics and composition of the olive fruit at the time of its processing (Inarejos-Garcîa *et al.,* 2010). Highest quality extra virgin oils require optimum harvest stage, reduction in the time between harvest and milling, high quality oil extraction procedures and optimum storage conditions. The storage time between harvest and processing is one of the most important postharvest factors in oil quality. This becomes a significant problem when the volume of olive fruit exceeds the capacity of the mill plants. Olive fruits are often stacked

into large heaps at ambient temperature for several weeks prior to milling, which exceeds the storage limits (~48 hr) for the highest quality oil (Garcia *et al.,* 1996 a; Ranali *et al.,* 2000; Angerosa, 2002). During this period of time fermentation may also occur, and pressure and heat within the piles provide a medium for fungi and bacteria growth (Olias and Garcia, 1997). Anaerobic and aerobic processes take place inside and outside the piles of olive fruits, respectively, which causes deterioration of the fruit. This deterioration increases the acidity and reduces stability of the recovered oils (Garcia *et al.,* 1996 a). Increase in volatile acids (acetic and butyric acid) during decomposition results in an unpleasant musty smell (Olias and Garcia, 1997). Pigment content also decreases during this period, and additional refining needs to be done to clear all these unfavorable characteristics, increasing production cost and lowering market value (Gutierrez-Rosales *et al*., 1992). It is important to increase storage duration before milling the olive fruits to permit higher yields of better quality oil (Petruccioli and Parlati, 1987).

Studies have shown that cold storage can increase storage time without significantly affecting oil quality. Clovodeo *et al*. (2007) stored 'Coratina' olives used for oil production for 30 days at different temperatures and under different atmospheric conditions. They found that storage at 5°C, both under a flow of humidified air and a flow of 3% O_2 + 5% CO_2, produced oils that maintained their initial chemical qualities until the end of the experimentation. However, the olives stored at room temperature deteriorated after 15 days of storage and the extracted oil had significantly reduced quality.

Poerio *et al*. (2008) frozed olives at -18˚C for 24 hours and extracted the oil with and without thawing the fruits. Results were significantly different regarding peroxide value (PV), free fatty acids and polyphenols compared to control samples. Oils from frozen olives had lower free fatty acids and PVs and freezing reduced the oxidative stability of the oil.

The Iranian Government supports and subsidizes the expansion of olive cultivation to see about a six-fold increase in olive cultivated lands, i.e. from approximately 103,000 in 2014 to 600,000 ha by 2025, starting at 4,800 ha in 1993 at the launch of The Expansion of Olive Cultivation Plan (Asheri *et al.,* 2016). This has resulted in the spread of olive cultivation to areas where olive is not traditionally cultivated. Therefore, many of the olive groves produce small amounts of fruit and have no olive processing and oil extraction facilities, and the fruit must be transported to an extraction facility. The ability to reduce deterioration during the storage time between harvest and processing is important to maintain quality. Hence, it is worthwhile to discover ways to preserve olive fruits during this critical period.

The aim of this study is therefore, to find methods that allow olive fruits to be stored for longer periods of time, without compromising the quality of extracted oils. This study investigated the effect of freezing olive fruits at moderate temperatures (-4°C) on quality indices of extracted oil, to understand if it was possible to extract high quality oil from fruits frozen for extended periods of time. The study is aimed to examine how susceptible the oil quality of different cultivars is to freezing, and to establish if the fruit of a particular cultivar responds better to freezing. The study considered 3 freezing treatments for 3 cultivars: fresh samples (control), freezing for 1 week and 3 weeks.

2. Materials and Methods

The olive cultivars Mission, Arbequina, and Koroneiki were selected for study as they are commonly used for the production of olive oil in Iran. Fruits were hand-picked from Fadak Grove located near Qom, Iran (34° 30′ N, 51° 00′ E). The grove is located about 15 Km south of Qom, a city at 150 Km south of Tehran, in the central hyper-arid to arid parts of Iran. The fruits selected were at similar stages of ripening according to their ripening index which were 3.93, 3.84 and 4.23 for 'Arbequina', 'Koroneki' and 'Mission', respectively. The fruits were immediately transferred to the university lab, washed and de-leafed. Olives used to produce control samples were processed immediately, and the remaining olives stored in a freezer at -4°C.

Oil extraction

For oil extraction, only sound and undamaged fruits were used. Olives in the control samples were crushed using a hammer mill to form a paste. The paste was malaxed at 30˚C, then centrifuged at 4000 rpm for 10 minutes.

Olives used for treatment 1 were kept in freezer at -4˚C for one week and treatment 2 for 3 weeks at the same temperature. Frozen samples were crushed and malaxed until they reached 25˚C (due to not thawing the samples), and then centrifuged at 4000 rpm for 10 minutes. Extracted oil was collected using

a pipette and stored in dark glass jars in a refrigerator until analysis.

Determining oil content

Fifteen olive fruits from each replicate were placed in a Petri dish and then placed in an oven at 105°C for 48 h. The dried olives were used to determine oil content in three replicates. Olives were ground to a paste by mortar and pestle. The amount of 10 g of paste was placed in a Soxhlet cartridge and oil extracted with 150 ml hexane at 70°C. After 6 h of extraction, hexane was collected and oil content was calculated on dry mass basis (Agar *et al.,* 1998).

Peroxide value

The peroxide value of the oils was measured using the methods of Garcia *et al*. (1996 b). A 5 g sample of the extracted olive oil was placed in a 250 ml Erlenmeyer flask. The sample was shaken and then dissolved in 25 ml solution of acetic acid and chloroform (2:1, v/v). One milliliter of saturated potassium iodide (KI) solution was added. The mixture was placed in darkness for 5 min and then 75 ml of distilled water was added to stop the reaction. Half of one milliliter of freshly prepared starch indicator solution (0.5%) was added to each sample. Finally, the mixture was titrated with 0.01 N sodium thiosulfate until the blue indicator color disappeared. The peroxide value was expressed as milliequivalents of active oxygen per kilogram of oil (meq O_2 kg^{-1}) (Agar *et al.,* 1998).

Coefficients of specific extinction values (K232 and K270)

Coefficients of specific extinction at 232 and 270 nm were measured by the methods reported in Regulation EEC/2568/91 of the European Union Commission (EEC, 1991). The amount of 1 g of oil sample was diluted in 100 ml isooctane. The sample was then homogenized using a vortex and the solution was transferred to a 10 mm cuvette. Absorbance at 232 and 270 nm was measured in a spectrophotometer using pure isooctane as a blank.

Fatty acid composition

Fatty acid composition in the oil was determined by the AOCS Official Method (1997). Methyl esters were prepared by vigorous shaking of a solution of oil dissolved in hexane (0.5 g in 7 ml) with 2 ml of 2N methalonic potash, and analyzed by gas chromatography. Chromatographic analysis was performed on a Trace GC (gas chromatograph), equipped with a flame ionization detector and split/splitless injector (Trace GC, ThermoFinnigan, Italy), using a silica capillary column, BPX-70 (30 m × 0.25 mm i.d. × 0.25 µm film thickness). The injector temperature was set at 250˚C and samples were injected manually (1 µL) with a split ratio of 1:80. The oven temperature was maintained at 175˚C for two min, and increased gradually to 230˚C at 3 ˚C/min and maintained for 10 min. Nitrogen was used as carrier gas at a flow rate of 0.8 ml/min. The detector temperature was maintained at 270˚C. Fatty acids were identified by comparing retention times with those of standard compounds. Oxidizability (Cox value) was calculated based on the fatty acid content of three unsaturated fatty acids [oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3)] using following relation (Fatemi and Hammond, 1980).

$$\text{Oxidizability} = [1 \times (C18{:}1\%) + 10.3 \times (C18{:}2\%) + 21.6 \times (C18{:}3\%)]$$

Determining oil chlorophyll and carotenoid content

Pigment contents were assayed using the spectrophotometric method of Minguez-Masquera *et al*. (1991). One gram of oil was dissolved in 10 ml of isooctane and the resulting solution transferred to a cuvette. Absorbance at 470 and 670 nm (for carotenoid and chlorophyll, respectively) was measured in a spectrophotometer (unico/2800 uv/VIS) using pure isooctane as a blank. The results are expressed as milligram of carotenoid or chlorophyll per kilogram of oil.

Statistical analysis

Experimental layout was factorial with three levels of freezing treatments and three olive cultivars. Data were analyzed by SPSS software. Comparison of means is performed using Duncan's multiple range test at a 95% confidence level and tables for analysis of variance (ANOVA) are provided.

3. Results and Discussion

Oil content

In fruit from the Koroneiki and Arbequina cultivars, there is no statistically significant difference in oil content between the control and the frozen samples (Table 1). However, the Mission cultivar fruit frozen for 1 and 3 weeks showed a significant decrease in mean oil content compared with the control under Duncan's multiple range test at 5%. Since the independent variables (i.e. cultivar and freezing) act independently, freezing had no significant overall effect on the content of extracted olive oil (Table 2). However in interpreting the results, it needs to be

considered that oil extraction of frozen treatments was performed at a different temperature (25°C) in respect to the control (30°C).

Peroxide value

The peroxide value (PV) is a measure of primary oxidation. Table 1 shows the measured mean PV (meq O_2 /kg) of the oils obtained from olives stored for the different time periods. Our data showed that freezing reduces the peroxide value. The reduction was significant when olive fruits were frozen for three weeks, but not significant when frozen for only 1 week. Cultivar's effect in reduction of PV was significant at a 95% statistical level, while freezing had a very significant effect at a 99% level (Table 2). No oil had peroxide values above the limit for extra virgin olive oil (20 meq O_2/kg).

Table 1 - Means of chemical characteristics of olive oils derived from fresh and frozen olive fruits and their comparison with Duncan's multiple range test at 5%

Samples	Oil content (% of dry matter)	PV (meq O_2 kg^{-1})	K232 nm	K270 nm	Chlorophyll (mg kg^{-1})	Carotenoid (mg kg^{-1})
M C	47.21 a	7.33 a	1.13 bc	0.08 c	2.22 a	1.84 a
M T1	43.08 b	6.63 ab	1.06 c	0.08 c	2.14 a	1.86 a
M T2	42.73 b	5.47 bcd	1.05 c	0.08 c	2.06 a	1.79 ab
K C	43.34 b	6.97 a	0.82 d	0.13 ab	2.20 a	1.53 bc
K T1	41.80 b	6.53 ab	0.80 d	0.12 b	2.03 a	1.50 bc
K T2	41.65 b	5.2 cd	0.76 d	0.12 b	2.02 a	1.43 c
A C	43.57 b	6.53 ab	1.33 a	0.15 a	2.12 a	1.52 bc
A T1	45.00 ab	5.63 bc	1.24 ab	0.14 ab	2.01 a	1.48 c
A T2	42.82 b	4.47 d	1.22 ab	0.14 ab	1.98 a	1.48 c

M= Mission; K= Koroneiki; A= Arbequina; C= control sample;
T1= 1 week freezing sample;
T2= 3 week freezing sample;
PV= peroxide value.
Means within a column with the same lowercase letters are not significantly different.

Poerio *et al*. (2008) and Gomez and Escoda (2010) also found reduced peroxide values in oil from olive paste and fruits that had been frozen. The lower peroxide value of oil from frozen fruit could be due to reduced enzymatic activity during crushing-malaxation due to the initial low temperature.

Specific extinction coefficients at 232 nm and 270 nm

The K_{232} and K_{270} values for all treatments were below the maximum permitted values for extra virgin olive oil [2.50 and 0.20 respectively, according to Regulation EEC (1991)]. Across all cultivars there was a net decrease in the K_{232} values due to freezing, but the difference was statistically insignificant. No change was detected in the K_{270} values between treatments (Table 2). Between cultivars however, the difference in the K_{232} and K_{270} values was significant with the Arbequina cultivar showing the highest values, 'Koroneiki' showing the lowest K_{232} and intermediate K_{270}, and 'Mission' showing intermediate K_{232} and the lowest K_{270} (Table 1). These differences were consistent with the variations between olive cultivars shown by Asheri *et al*. (2016). Gomez and Escoda (2010) also showed freezing to have no effect on the K_{270} and K_{232} values independent of cultivar.

Pigment content (chlorophyll and carotenoid)

The slight decrease in the pigment content observed between the frozen treatments and the control was not statistically significant (Tables 1, 2). Also different cultivars did not demonstrate different levels of chlorophyll content. Carotenoid content, on the other hand, was significantly different among the cultivars at 99% statistical level. Chlorophylls and carotenoids play crucial roles in health and also in the oxidative activity of processed food stuff, due to their antioxidant nature in the dark and pro-oxidant activi-

Table 2 - Results of ANOVA for chemical characteristics of olive oils derived from fresh and frozen olive fruits

Source	Mean square					
	Oil content	PV	K232	K270	Chlorophyll	Carotenoid
Treatments	8.83 NS	2.63 **	0.129 **	0.002 **	0.023 NS	0.091 *
Cultivar (A)	10.46 NS	2.11 *	0.50 **	0.009 **	0.024 NS	0.352 **
Freezing (B)	12.14 NS	8.35 **	0.16 NS	0.00004 NS	0.066 NS	0.008 NS
AB interaction (cv *fr)	6.37 NS	0.04 NS	0.001 NS	0.000004 NS	0.002 NS	0.002 NS
Error	3.56	0.39	0.004	0.00009	0.058	0.026

PV= Peroxide value.
NS= not significant; * significant at 5% level; ** significant at 1% level.

ty in the light (Fakourelis *et al.,* 1987). The higher the amount of these pigments, the higher the resistance to oil oxidation. Our results indicated that freezing did not change olive oil resistance to oxidation based on pigment contents.

Kiritsakis *et al*. (1998) found that storage at lower temperatures reduced the pigment content of the extracted oils and Morello *et al*. (2003) found slight decreases in chlorophyll and carotenoid concentration of oils obtained from frosted olives. These authors suggested that this could be due to the involvement of chlorophyllase and lypoxygenase. Amongst cultivars, the Mission cultivar shows significantly higher carotenoid content than 'Koroneiki' and 'Arbequina', as observed by Asheri *et al.* (2016).

Fatty acid composition

This paper reports 12 fatty acids detected in GC analysis including myristic acid (C14:0), palmitic acid (C 16:0), palmitoleic acid (C 16:1), heptadecanoic acid (C 17:0), heptadecenoic acid (C 17:1), stearic acid (C 18:0), oleic acid (C 18:1), linoleic acid (C 18:2), linolenic acid (C 18:3), arachidonic acid (C 20:4), arachidic acid (C 20:0), and erucic acid (C 22:1) (Table 3).

Analysis of variance (ANOVA) shows that freezing olive fruits did not have significant effects on the fatty acid composition of the oils, while cultivars had very significant influence on all the fatty acids except for myristic acid. In addition, freezing and cultivar variables acted independently on fatty acids level, except for myristic and palmitic acids in which the interaction of variables were very significant (at 99%) and significant (at 95%), respectively (Tables 4, 6).

Comparison of the means demonstrate that 'Koroneiki' and 'Mission' had significantly higher levels of oleic acid (74.85 and 72.96% in fresh state, respectively) than 'Arbequina' (60.76%). Oleic acid is the most prominent fatty acid in olive oil and is a monounsaturated fatty acid (MUFA) with demonstrated qualities in the stability of the oil. Oleic acid levels did not change significantly during the storage except for the 1 week freezing of 'Arbequina' (Table 5). The other predominant fatty acids are palmitic acid [a saturated fatty acid (SFA)] and linoleic acid [a polyunsaturated fatty acid (PUFA)]. 'Arbequina' had the highest amounts of palmitic and linoleic fatty acids. This indicates lower quality of its oil, especially when it is considered together with its lower oleic

Table 3 - Fatty acid (Myristic, Palmitoleic, Heptadecanoic, heptadecenoic, Stearic, Linolenic, Arachidic, arachidonic and Erucic acids) content (%) of olive oils derived from fresh and frozen olive fruits and comparison of the means with Duncan's multiple range test at 5%

Samples	Myristic acid	Palmitoleic acid	Heptadecanoic acid	Heptadecenoic acid	Stearic acid	Linolenic acid	Arachidic acid	Arachidonic acid	Erucic acid
M C	0.010 d	0.730 bc	0.0255 b	0.044 b	2.55 b	1.05 a	0.290 cd	0.409 a	0.068 c
M T1	0.014 d	0.701 bc	0.024 b	0.042 b	2.44 b	1.00 ab	0.281 d	0.387 ab	0.065 c
M T2	0.051 a	0.682 c	0.022 b	0.038 b	2.38 b	0.95 b	0.271 d	0.382 abc	0.063 c
K C	0.030 b	0.798 bc	0.031 b	0.050 b	3.26 a	0.76 c	0.465 a	0.379 abc	0.137 a
K T1	0.018 bcd	0.828 b	0.035 b	0.051 b	3.20 a	0.80 c	0.459 a	0.356 c	0.132 a
K T2	0.011 d	0.817 b	0.032 b	0.050 b	3.16 a	0.77 c	0.448 a	0.359 bc	0.136 a
A C	0.028 b	2.512 a	0.070 a	0.169 a	1.43 c	0.68 d	0.324 b	0.159 d	0.114 b
A T1	0.027 bc	2.515 a	0.070 a	0.170 a	1.45 c	0.68 d	0.319 bc	0.151 d	0.103 b
A T2	0.016 cd	2.579 a	0.072 a	0.169 a	1.44 c	0.69 d	0.319 bc	0.167 d	0.107 b

M= Mission; K= Koroneiki; A= Arbequina; C= control sample.
T1= 1 week freezing sample; T2= 3 week freezing sample.
Means within a column with the same lowercase letters are not significantly different.

Table 4 - Results of ANOVA for fatty acids content (reported in table 3) of olive oils derived from fresh and frozen olive fruits

Source	Mean square					
	Oil content	PV	K232	K270	Chlorophyll	Carotenoid
Treatments	8.83 NS	2.63 **	0.129 **	0.002 **	0.023 NS	0.091 *
Cultivar (A)	10.46 NS	2.11 *	0.50 **	0.009 **	0.024 NS	0.352 **
Freezing (B)	12.14 NS	8.35 **	0.16 NS	0.00004 NS	0.066 NS	0.008 NS
AB interaction (cv *fr)	6.37 NS	0.04 NS	0.001 NS	0.000004 NS	0.002 NS	0.002 NS
Error	3.56	0.39	0.004	0.00009	0.058	0.026

PV= Peroxide value.
NS= not significant; * significant at 5% level; ** significant at 1% level.

acid content. Palmitic and linoleic acids amounts in the Mission cultivar and linoleic acid in 'Koroneiki' show significant changes after three weeks of frozen storage (Table 5). Among the fatty acids of lower content, only linolenic and myristic acids demonstrate significant changes in their mean values (Table 3). Linolenic and myristic acid contents of the Mission cultivar show significant increases after three weeks of frozen storage. Myristic acid levels of 'Koroneiki' and 'Arbequina' decreased due to freezing.

Total SFAs, MUFAs and PUFAs did not demonstrate any statistically significant change under frozen storage, and all the change is due to cultivar (Table 6). Cultivar had very significant effect on the ratios of MUFA/PUFA and oleic/linoleic acids, and the effect of freezing on these ratios was significant at 95% confidence level. Also, the analysis indicated that cultivar and freezing have significant interaction during the storage of olive fruit on these ratios. Cox value, however was not influenced significantly by freezing (Table 6).

Mean total SFA levels for 'Koroneiki' and 'Arbequina' did not change, while 'Mission' showed a significant reduction in total SFA after 3 weeks of freezing. This change could be due to the reduction of palmitic acid. Means comparison of total MUFA content did not detect any significant changes. 'Koroneiki' shows a significant increase in the total PUFA content after 3 weeks of frozen storage, which leads to a reduction of mean MUFA/PUFA and oleic/linoleic acids ratios. Similar to MUFA/PUFA and oleic/linoleic acids ratios, Cox values did not change significantly for 'Mission' and 'Arbequina', while 'Koroneiki' experienced a significant decrease for 3 weeks freezing treatment. The higher the MUFA/PUFA and oleic/linoleic acids ratios and the lower the Cox values, the higher the oxidative stability of the oil. The implication of the reduction in MUFA/PUFA and oleic/linoleic acids ratios and increase in Cox values after 3 weeks freezing for 'Koroneiki' is that longer periods of freezing storage could reduce oxidative stability of its oil. However, it is important to note that despite the changes after 3 weeks of storage, 'Koroneiki' oil still possessed signif-

Table 5 - Fatty acid (Palmitic acid, Oleic acid, Linoleic acid, ∑SFA, ∑PUFA, MUFA/PUFA, Oleic/Linoleic and cox value) compositions and comparison of the means with Duncan's multiple range test at 5%

Samples	Palmitic acid	Oleic acid	Linoleic acid	ΣSFA	ΣMUFA	ΣPUFA	MUFA/PUFA	Oleic/linoleic acid	Cox value
M C	11.11 c	72.96 c	10.47 c	14.25 c	73.80 c	11.93 b	6.19 c	6.97 c	2.03 b
M T1	10.73 c	73.49 bc	10.53 c	13.76	74.30 c	11.91 b	6.24 c	6.98 c	2.03 b
M T2	10.23 d	73.60 bc	11.00 b	13.24 d	74.39 bc	12.34 b	6.03 c	6.69 c	2.07 b
K C	12.48 b	74.85 a	6.51 e	16.51 b	75.83 a	7.65 d	9.92 a	11.52 a	1.58 d
K T1	12.48 b	74.74 a	6.62 e	16.43 b	75.75 a	7.78 cd	9.74 a	11.29 a	1.60 cd
K T2	12.47 b	74.15 ab	7.09 d	16.37 b	75.15 ab	8.22 c	9.14 b	10.46 b	1.64 c
A C	16.63 a	60.76 e	15.96 a	18.73 a	63.56 d	16.80 a	3.78 d	3.81 d	2.40 a
A T1	16.54 a	61.76 d	15.94 a	18.65 a	64.55 d	16.77 a	3.85 d	3.87 d	2.41 a
A T2	16.96 a	61.53 de	15.68 a	19.05 a	64.39 d	16.54 a	3.89 d	3.92 d	2.38 a

M= Mission; K= Koroneiki; A= Arbequina; C= control sample.
T1= 1 week freezing sample; T2= 3 week freezing sample.
SFA= saturated fatty acids; MUFA= monounsaturated fatty acids; PUFA= polyunsaturated fatty acid; Cox value: calculated oxidizability.
Means within a column with the same lowercase letters are not significantly different.

Table 6 - Results of ANOVA for fatty acids (reported in table 5) composition of olive oils derived from fresh and frozen olive fruits

Source	Mean square								
	Palmitic acid	Oleic acid	Linoleic acid	ΣSFA	ΣMUFA	ΣPUFA	MUFA/PUFA	Oleic/linoleic acid	Cox value
Treatments	14.46**	80.35 **	31.51 **	9.76 **	58.30 **	29.31 **	12.70 **	19.90 **	0.23 **
Cultivar (A)	57.37 **	320.35 **	126.63 **	38.45 **	232.15 **	116.90 **	50.43 **	78.93 **	0.93 **
Freezing (B)	0.06 NS	0.338 NS	0.13 NS	0.13 NS	0.33 NS	0.10 NS	0.14 *	0.30 *	0.001 NS
AB interaction (cv*fr)	0.21 *	0.365 NS	0.14 NS	0.24 *	0.36 NS	0.12 NS	0.11 *	0.19 *	0.001 NS
Error	0.03	0.142	0.04	0.05	0.12	0.05	0.03	0.04	0.0004

SFA= saturated fatty acids; MUFA= monounsaturated fatty acids; PUFA= polyunsaturated fatty acid.
Cox value: calculated oxidizability;
NS= not significant; * significant at 5% level; ** significant at 1% level.

icantly higher MUFA/PUFA and oleic/linoleic acid ratios and lower Cox values than the other cultivars, and therefore indicated the highest resistance to oxidative stability among these three cultivars.

4. Conclusions

The oils obtained from olive fruits stored at -4˚C are demonstrated to maintain the same characteristics of the control.

Among the biochemical indices, the decrease in PV was the only factor that varied significantly from the control samples. This decrease is received positively, as less oxidation occurs. Pigment content remained stable during freezing period which is considered to be good to maintain quality oil. No differences in characteristics evaluated in this study were observed between cultivars, suggesting that freezing the olive fruits of all three cultivars in this research did not have any negative effect on the studied characteristics of the extracted oil. Based on this study, freezing could be a suitable method of preserving olive fruits in the waiting period between harvesting and processing, which could assist in the maintenance of characteristics evaluated in this study during olive oil production. However, it may be worthwhile extending this experiment with different cultivars and to test characteristics such as sensory quality evaluation and oxidative stability. Nonetheless, our results suggest that olive fruit may be stored frozen before processing into oil. So the products could be harvested at the optimal stage of ripening and preserved frozen while shipping them to the mill plant stations. This preservation system could be extended to the pre-processing waiting period at the mill plant, so that the processing of olives could be optimized.

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