# Effect of low-temperature storage under optimal conditions on olive oil quality and its nutritional parameters

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One of the causes of loss in olive oil quality is oxidation, which decreases its shelf life. Only few previous works have studied the effect of storage at cold and freezing temperatures on the quality of olive oil. The aim of this work was to follow the effect of freezing and cold temperatures during storage on the quality and nutritional parameters (fatty acids, α-tocopherol, total phenols and pigments) of olive oil in comparison with storage at 15°C. The physicochemical, nutritional and sensorial parameters of the olive oils were studied along storage in optimal conditions at different temperatures.
The research showed that freezing and cold storage temperatures maintained a higher quality in olive oils. Frozen storage of olive oil maintained higher levels of antioxidant compounds (α-tocopherol, total phenols), oxidative stability and fruitiness after 12 months of storage. For this reason, freezing could be considered as an appropriate system of storage to maintain the functional compounds of olive oil.
**Keywords:** Olive oil, quality, antioxidant compounds, cold storage, freezing

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**Effetto di basse temperature di conservazione in condizioni ottimali sui parametri di qualità e nutrizionali dell'olio di oliva**

Una delle cause della riduzione della qualità dell'olio di oliva è l'ossidazione che diminuisce la conservabilità. Solo pochi lavori in precedenza hanno studiato l'effetto della conservazione a freddo e del congelamento sulla qualità dell'olio di oliva.
Scopo di questo lavoro è di valutare l'effetto di queste condizioni di conservazione sulla qualità e parametri nutrizionali (acidi grassi, α-tocoferolo, fenoli totali e pigmenti) dell'olio in confronto con quello conservato a 15°C.
Parametri fisico-chimici, nutrizionali e sensoriali degli oli di oliva sono stati studiati durante la conservazione in condizioni ottimali alle diverse temperature.
Temperature di conservazione a freddo mantengono la qualità più elevata negli oli di oliva.
La conservazione dell'olio in congelamento mantiene i livelli elevati di composti antiossidanti (α-tocoferolo, fenoli totali), stabilità ossidativa e fruttato dopo 12 mesi di conservazione. Per questo motivo, il congelamento potrebbe rappresentare un adeguato sistema di conservazione per mantenere i composti funzionali nell'olio di oliva.
**Parole chiave:** olio di oliva, qualità, antiossidanti, celle frigorifere, congelamento.

## INTRODUCTION

Olive oil is a natural juice obtained from olives with a characteristic aroma, taste, colour, and nutritive properties. The fatty acid composition, very rich in the monounsaturated oleic acid, and the antioxidant compounds (phenols and α-tocopherol) give the product great importance in the context of the Mediterranean diet. The quality and chemical composition of olive oil depend of many factors, such as genetics, agronomic factors (production area, climatic conditions, maturity degree, and production system), extraction process, and storage conditions. One of the primary causes of loss of olive oil quality is oxidation, which decreases its shelf life and nutritional compounds. The oxidation is an inevitable process that starts after the virgin olive oil has been extracted, and leads to deterioration, which becomes more serious during storage. The two compositional factors of oils determining their susceptibility to oxidation are the fatty acid composition and the antioxidant compounds (tocopherols, phenols, and pigments). Light exposure, oxygen concentration, and temperature all influence the quality and freshness of virgin olive oil during storage. Various studies have been conducted on the effects of storage temperature on the quality of olive oil. Some of them centred on the effect of the olives storage [1 - 3], while others focused on the effect on the quality of storing the oil at room temperature only [4, 5]. Some works have studied the shelf life of olive oil stored at high temperatures [6 - 8], although, at these temperatures, the predictions do not have a good correlation with the shelf life of olive oils [9, 10, 5, 6]. Other studies included low-temperature storage of olive oil [11 - 15]. A decrease in temperature may cause a transition of the physical state of oil, and it can freeze. This transformation can affect the solubility of some compounds such as phenols [16]. Some of the aforementioned studies also dealt with the effect of freezing on the quality of extra virgin olive oil, but only described the phenolic composition and the aromatic profile [17]. In other cases, the storage by freezing was only performed over a three-month period [18, 16]. Li et al., [15] reported a study with many quality parameters during storage (including freezing), but this investigation only lasted 18 weeks. The aim of this paper was to observe the effect that freezing and cold temperatures during storage in optimal conditions may have on the quality of olive oil and its antioxidant compounds content, comparing it with the 15°C storage. We employed optimal storage conditions (such as darkness, etc) to verify whether the storage temperature could prolong the shelf life of olive oil. A physicochemical, nutritional and sensorial characterisation was carried out in this study for the 12 months duration of storage.



## MATERIAL AND METHODS

### OLIVE OIL

The Arbequina filtered olive oil sample was purchased from a hammer mill in Zaragoza (Spain). The analytical parameters of this olive oil are shown in the various tables and figures as the control (0 months storage).

### OLIVE OIL STORAGE CONDITIONS

Olive oil was transferred into amber glass bottles (24 × 500 ml) to protect it from the effects of light, adding nitrogen in the headspace. We used optimal storage conditions to check whether, even in this case, the storage temperature could prolong olive oil shelf life. After this, the samples (8 bottles for each storage condition) were stored at 15°C ± 1°C (in a temperature-controlled chamber), 4ºC ± 1°C (in a refrigerator), and -18ºC ± 1°C (in a freezer) in the dark. Before bottling, an olive oil sample was analysed. Bottles of this olive oil were kept in storage for 12 months. The samples were analysed after 3, 6, 9, and 12 months of storage; they were thawed at room temperature on the day of analysis and homogenized before sampling.

### ANALYTICAL DETERMINATIONS

Determinations of the physicochemical parameters (free acidity, peroxide value, and UV absorption coefficients $K_{270}$ and $K_{232}$) were made in compliance with the methods described in Regulation EEC/2568/91 of the European Union Commission [19]. The analytical methods and specification limits of this regulation have subsequently been amended a number of times.

### Determination of fatty acids

The fatty acid profile of samples was determined by gas chromatography, using a modified fatty-acid methyl-esters (FAMEs) method as described by Frega and Bocci [20]. The FAMEs were obtained by shaking a solution of each olive oil sample in n-hexane and 2N methanolic potassium hydroxide. Chromatographic analyses were performed using a Hewlett Packard 5890 gas chromatograph equipped with a flame-ionisation detector and a split/splitless injector. The experimental conditions were: DB-225 column (30 m × 0.25 mm i.d. × 0.15 mm film thickness) (J. & W. Scientific, Agilent). The injector and detector temperatures were maintained at 250°C. The oven temperature ranged from 190°C (1 min) to 210 at 4°C/min and was maintained for 5 min before being heated to 215 at 3°C/min. Finally, an isotherm was used for 18 min, and nitrogen was used as the carrier gas. Commercial mixtures of fatty-acid methyl esters were used as reference data for the relative retention times.

### α-tocopherol determination

A sample of oil in hexane was analysed by HPLC (high-pressure liquid chromatography) with a Zorbax SB-C18 phase-reverse column (Agilent), eluted with

acetonitrile/water (99:1 v/v) using a flow rate of 1 ml/min. A photodiode matrix detector was also used. Chromatograms were registered at 295 nm. The results were expressed as mg of α-tocopherol/kg oil.

### Total phenol content

For the extraction from the olive oil, we followed the method described by Favati et al., [21]. The phenols were extracted by solid phase extraction (SPE), using Isolute™ C18 cartridges. The extract was dried in a rotary evaporator, and the residue dissolved in 5 ml of methanol. For the colorimetric determination, 2.5 ml of extract was mixed with 1.25 ml of Folin-Ciocalteau reagent. Then, after 3 min, 2.5 ml of sodium carbonate was added. The solution absorption was measured at 725 nm. The results were expressed as mg gallic acid/ kg oil.

### Determination of chlorophyll and carotenoid compounds

Chlorophyll and carotenoid contents were obtained from the absorption spectra of the virgin olive oil for each sample (7.5 g) and were dissolved in cyclohexane (25 ml) according to the method of Minguez-Mosquera et al., [22]. The maximum absorption is related to the chlorophyll at 670 nm and to the carotenoid at 470 nm. The values of the coefficients of specific extinction applied were E0 = 613 for pheophytin, as a major component in the chlorophyll fraction, and E0 = 2000 for lutein, as a major component in the carotenoid fraction. The concentrations of chlorophyll and carotenoids were expressed as mg pheophytin and lutein/ kg of oil, respectively.

### Oxidative stability

It was expressed as the oxidation induction time (hours), measured with a Rancimat™ 743 instrument (Metrohm, Switzerland), using 3 g of oil, warmed to 120ºC with a 20 l $h^{-1}$ airflow. The induction time is the time it took to reach the break point of this curve. The break point is the point of the intersection of the two extrapolated straight parts of the curve [23].

### Sensory analysis

10 selected and trained panellists from Aragon's accredited panel and the Zaragoza Faculty of Veterinary Science carried out the sensory analysis of the samples, following the method described in Regulation EEC/640/2008 [24]. For each oil sample, the intensities of the positive (fruity, bitter, and pungent) and negative (fusty, winey, musty, muddy, rancid, metallic, and other) attributes were evaluated on a non-structured scale of 10 cm anchored by its origin.

## STATISTICAL ANALYSIS AND TREATMENT OF EXPERIMENTAL DATA

### Statistical analysis

Statistical analysis was performed using Statgraphics Plus 5.1. Results were expressed as mean ± standard deviation of three experiments and as least squares mean ± 95% confidence interval.

Significant differences among samples were determined by analysis of variance (one-way ANOVA) and a multiple range test. Different letters indicate statistically significant differences ($p \leq 0.05$).

## RESULTS AND DISCUSSION

Table I shows the evolution of the physicochemical parameters of the olive oils during storage at different temperatures. In all cases, the values were below the limits established by European regulations [19] for extra virgin olive oil during the 12-month storage. The acidity was increasing with the storage time regardless of the three different temperatures. The values, however, were lower for the olive oil stored at -18°C (freezing). At the end of the experiment, the acidity of the olive oil stored at 4°C (refrigeration) and that sored at -18ºC was similar. The olive oils stored at 15°C (am-



**Table I -** Evolution of the physico-chemical parameters of olive oils during storage time at different temperatures.

| Parameter | Temperature (°C) | Storage time (months) | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 3 | 6 | 9 | 12 |
| Acidity (% oleic acid) | 15 | 0.13±0.01$^{A}$ | 0.17±0.00$^{B;Z}$ | 0.17±0.00$^{B;Z}$ | 0.17±0.00$^{B;Z}$ | 0.23±0.00$^{C;Y}$ |
| | 4 | 0.13±0.01$^{A}$ | 0.16±0.00$^{B;Y}$ | 0.16±0.00$^{B;Y}$ | 0.16±0.00$^{B;Y}$ | 0.17±0.00$^{C;X}$ |
| | -18 | 0.13±0.01$^{A}$ | 0.13±0.01$^{A;X}$ | 0.13±0.00$^{A;X}$ | 0.14±0.00$^{B;X}$ | 0.17±0.00$^{C;X}$ |
| Peroxide value (meq $O_2$ active/Kg oil) | 15 | 5.64±0.02$^{A}$ | 6.33±0.00$^{B;Y}$ | 6.91±0.21$^{C;Y}$ | 7.36±0.03$^{D;Z}$ | 8.69±0.03$^{E;Z}$ |
| | 4 | 5.64±0.02$^{A}$ | 6.29±0.07$^{B;Y}$ | 6.55±0.20$^{C;X}$ | 7.20±0.03$^{D;Y}$ | 7.98±0.03$^{E;Y}$ |
| | -18 | 5.64±0.02$^{A}$ | 5.66±0.02$^{A;X}$ | 6.33±0.04$^{B;X}$ | 6.87±0.01$^{C;X}$ | 7.33±0.05$^{D;X}$ |
| $K_{232}$ (Abs 232 nm) | 15 | 1.48±0.01$^{A}$ | 1.62±0.01$^{B;Y}$ | 1.66±0.01$^{C;Z}$ | 1.68±0.01$^{CD;Y}$ | 1.69±0.02$^{D;Y}$ |
| | 4 | 1.48±0.01$^{A}$ | 1.61±0.00$^{B;Y}$ | 1.63±0.01$^{BC;Y}$ | 1.64±0.01$^{C;X}$ | 1.64±0.00$^{C;X}$ |
| | -18 | 1.48±0.01$^{A}$ | 1.51±0.02$^{B;X}$ | 1.53±0.02$^{B;X}$ | 1.62±0.02$^{C;X}$ | 1.62±0.01$^{C;X}$ |
| $K_{270}$ (Abs 270 nm) | 15 | 0.07±0.01$^{A}$ | 0.08±0.00$^{B;Y}$ | 0.08±0.00$^{BC;Y}$ | 0.09±0.00$^{D;Y}$ | 0.09±0.01$^{CD;Y}$ |
| | 4 | 0.07±0.01$^{A}$ | 0.07±0.00$^{AB;X}$ | 0.07±0.00$^{AB;Y}$ | 0.07±0.00$^{AB;X}$ | 0.08±0.01$^{B;XY}$ |
| | -18 | 0.07±0.01$^{A}$ | 0.07±0.01$^{A;X}$ | 0.07±0.00$^{A;X}$ | 0.07±0.00$^{A;X}$ | 0.07±0.01$^{A;X}$ |

Values reported are mean values and standard deviations of three replicates.

$^{A-E}$ For each parameter, different letters for the same temperature indicate statistically significant differences ($p \leq 0.05$) among storage months.

$^{X-Z}$ For each parameter, different letters for the same storage month indicate statistically significant differences ($p \leq 0.05$) among temperatures.

**Table II** - Slope and correlation coefficient of the linear regression of peroxide value and time required to reach the upper legal limit for the extra virgin olive oil during storage at different temperatures.

| Temperature (°C) | Slope | $R^2$ | Time (months)[a] |
|---|---|---|---|
| 15 | 0.2374 | 0.96 | 60.82 [b] |
| 4 | 0.1863 | 0.98 | 77.21 [b] |
| -18 | 0.1534 | 0.95 | 94.87 [b] |

[a] Maximun value of peroxide value for extra virgin olive oil (ECC 2568/1991 and later amendments): PV, 20 meq/Kg.
[b] Times calculated by extrapolation.

bient) showed a higher acidity level. Bonoli-Carbogni et al., [18] have also observed a higher acidity value in the oil stored at room temperature when compared to frozen oil, after 3 months. Also Li et al., [15] have described a slight acidity increase in olive oils stored at 4.5°C and -27°C. It is possible to say that the acidity reflected the oil's stability and its susceptibility to hydrolytic rancidity [1].

The degree of degradation during storage was determined in relation to the formation of hydroperoxides (expressed as peroxide values) and conjugated dienes. Peroxide value measures the primary oxidation products of lipids (hydroperoxides) while $K_{232}$ measures conjugated dienes and their oxidation products, which absorb at 232 nm. In turn, $K_{270}$ measures conjugated trienes and secondary products of oxidation (carbonyl compounds), which absorb at 270 nm.

The peroxide value (Table I and Table II) also increased with storage time. The highest values on the peroxide index were obtained for the samples stored at 15°C. The lowest value was ascribed to the olive oils stored by freezing. Significant differences were observed at the different dates of sampling. After 12 months of storage, increases of this value were observed to be 54.1% at 15°C, 41.5% at 4°C, and 30% at -18°C. Storage temperature improves the formation and decomposition rates of hydroperoxides and increases the oxidation reaction constant [9, 10, 25]. Other authors, such as Li et al., [15] found an increase in the peroxide index, which reached a maximum before decreasing. According to Vekiari et al., [26] this value increased during dark storage over the first 7 month, after which period a reduction was observed.

In most cases, $K_{232}$ and $K_{270}$ values (cf. Table I) increased along with storage time, a result in line with the findings of previous studies [14, 15]. The highest values for $K_{232}$ and $K_{270}$ at the end of the storage were found in olive oils stored at 15ºC, the lowest when the olive oils were stored at -18°C. The fact that only small changes were observed (after 12 months of storage, increases of 14.2% at 15°C, 10.8% at 4°C, and 9.5% at -18°C in the $K_{232}$ value were observed) may be due to the preservation conditions, possibly to the absence of light.

In order to qualify as extra virgin olive oil, the European Regulation [19] has fixed the maximum values for peroxide index, $K_{232}$ and $K_{270}$ at 20 meq/kg, 2.5 and 0.22, respectively. In our work, the limiting parameter would be the peroxide index. These results agree with those reported by Gómez-Alonso et al., [25]. According to other authors, however, the limiting shelf life parameter was $K_{232}$ [6]. Table II shows the slope and correlation coefficient of the linear regression of peroxide value and time required to reach the upper legal limit for the extra virgin olive oil during storage at different temperatures . The olive oil stored at -18°C would need more time to reach the upper



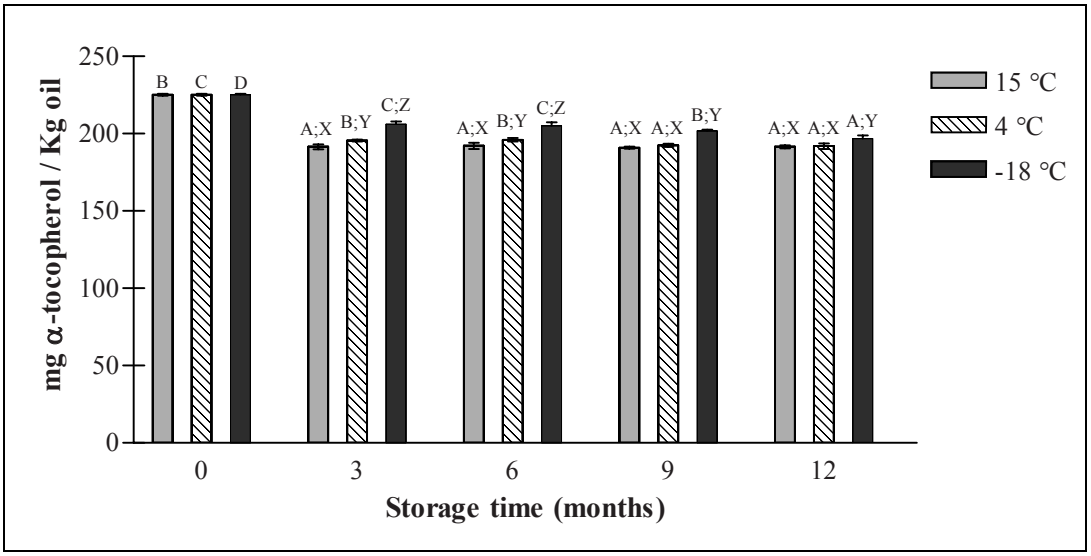



**Figure 1 -** Evolution of the content of α-tocopherol of olive oils during storage time at different temperatures
Values reported are mean values and standard deviations of three replicates.
[A-E] For each parameter, different letters for the same temperature indicate statistically significant differences ($p \leq 0.05$) among storage months.
[X-Z] For each parameter, different letters for the same storage month indicate statistically significant differences ($p \leq 0.05$) among temperatures.

**Table III -** Evolution of the fatty acid composition of olive oils during storage time at different temperatures.

| Parameter | Temperature (°C) | Storage time (months) | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 3 | 6 | 9 | 12 |
| SFA | 15 | 18.0±0.0$^{A}$ | 18.1±0.0$^{B;X}$ | 18.1±0.1$^{AB;X}$ | 18.0±0.1$^{AB;X}$ | 18.0±0.1$^{AB;X}$ |
| | 4 | 18.0±0.0$^{AB}$ | 18.1±0.1$^{B;X}$ | 18.0±0.0$^{AB;X}$ | 17.9±0.1$^{A;X}$ | 17.9±0.0$^{AB;X}$ |
| | -18 | 18.0±0.0$^{A}$ | 18.1±0.0$^{A;X}$ | 17.8±0.3$^{A;X}$ | 17.9±0.1$^{A;X}$ | 18.0±0.1$^{A;X}$ |
| MUFAS | 15 | 69.6±0.0$^{B}$ | 69.4±0.0$^{A;X}$ | 69.6±0.0$^{B;X}$ | 69.6±0.1$^{B;X}$ | 69.6±0.1$^{B;X}$ |
| | 4 | 69.6±0.0$^{A}$ | 69.5±0.1$^{A;X}$ | 69.6±0.0$^{A;X}$ | 69.6±0.1$^{A;X}$ | 69.7±0.1$^{A;X}$ |
| | -18 | 69.6±0.0$^{AB}$ | 69.5±0.1$^{A;X}$ | 69.7±0.1$^{B;X}$ | 69.7±0.0$^{B;X}$ | 69.7±0.1$^{B;X}$ |
| PUFAS | 15 | 12.4±0.0$^{A}$ | 12.4±0.0$^{A;X}$ | 12.4±0.0$^{A;X}$ | 12.4±0.1$^{A;X}$ | 12.4±0.0$^{A;X}$ |
| | 4 | 12.4±0.0$^{BC}$ | 12.4±0.0$^{B;X}$ | 12.4±0.0$^{A;X}$ | 12.5±0.0$^{C;X}$ | 12.4±0.0$^{AB;X}$ |
| | -18 | 12.4±0.0$^{A}$ | 12.4±0.0$^{A;X}$ | 12.4±0.0$^{A;X}$ | 12.4±0.1$^{A;X}$ | 12.4±0.0$^{A;X}$ |
| MUFAS/PUFAS | 15 | 5.6±0.0$^{A}$ | 5.6±0.0$^{AB;X}$ | 5.6±0.0$^{BC;X}$ | 5.6±0.0$^{A;X}$ | 5.6±0.0$^{C;X}$ |
| | 4 | 5.6±0.0$^{AB}$ | 5.6±0.0$^{AB;X}$ | 5.6±0.0$^{B;X}$ | 5.6±0.0$^{A;X}$ | 5.6±0.0$^{AB;X}$ |
| | -18 | 5.6±0.0$^{A}$ | 5.6±0.0$^{A;X}$ | 5.6±0.0$^{A;X}$ | 5.6±0.0$^{A;X}$ | 5.6±0.0$^{A;X}$ |

Values reported are mean values and standard deviations of three replicates.
SFA, saturated fatty acids; MUFAS, monounsaturated fatty acids; PUFAS, polyunsaturated fatty acids.
$^{A-E}$ For each parameter, different letters for the same temperature indicate statistically significant differences ($p \leq 0.05$) among storage months.
$^{X-Z}$ For each parameter, different letters for the same storage month indicate statistically significant differences ($p \leq 0.05$) among temperatures.

legal limit. Other authors have used the hexanal content as a quality index [27]. Since we used optimal storage conditions (darkness, no headspace, etc.) we didn´t observe peroxide index values above the limit established by regulations. This is also why the time required for reaching the limit is very high in all the observed cases.

As regards the nutritional parameters, the evolution of the α-tocopherol content of olive oils stored in each different condition is shown in Figure 1. This content decreased in all the cases along with storage time. The losses percentages were around 15%. These results agree with those obtained by Morelló et al., [4]. As these authors pointed out, when oxidation takes place under non-accelerated conditions, α-tocopherol is preferentially consumed to protect the oil from oxidation. Freezing allows for its preservation in the highest proportion (88%) in the olive oils after 12 months of storage. In the checks after 3 and 6 months of storage, significant differences were observed among the three storage conditions. In the checks after 9 and 12 months of storage, these differences were observed only between freezing and the other storage conditions. Since α-tocopherol is very important for the maintenance of the olive oil's antioxidant and nutritional properties, keeping the olive oils at -18°C becomes a very interesting possibility. Other authors [28] observed over a 9-month storage study a decrease in α–tocopherol of around 70-90%. For Li et al., [15] the loss percentage was between



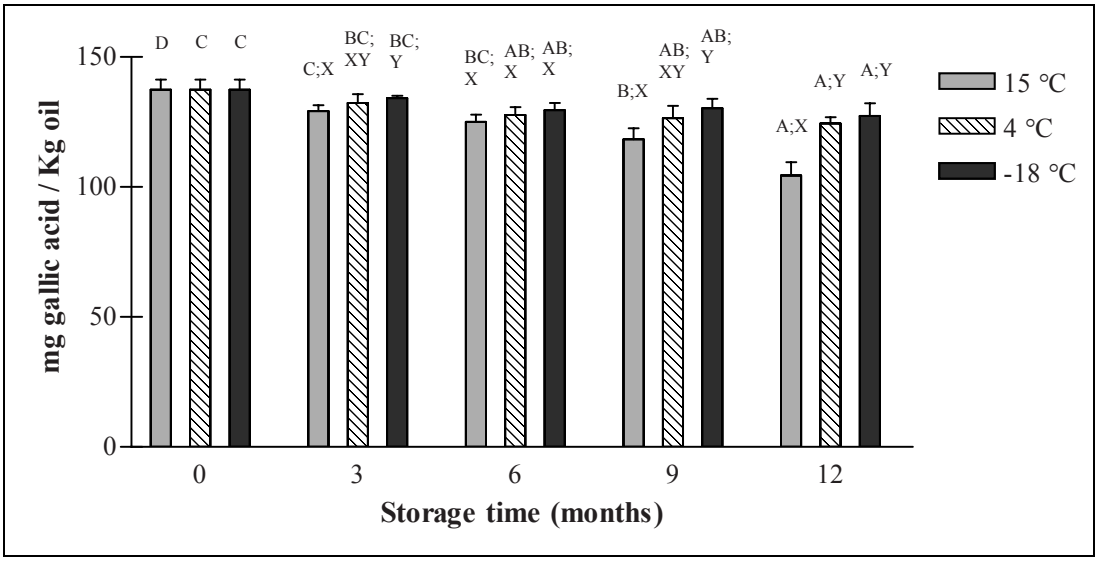
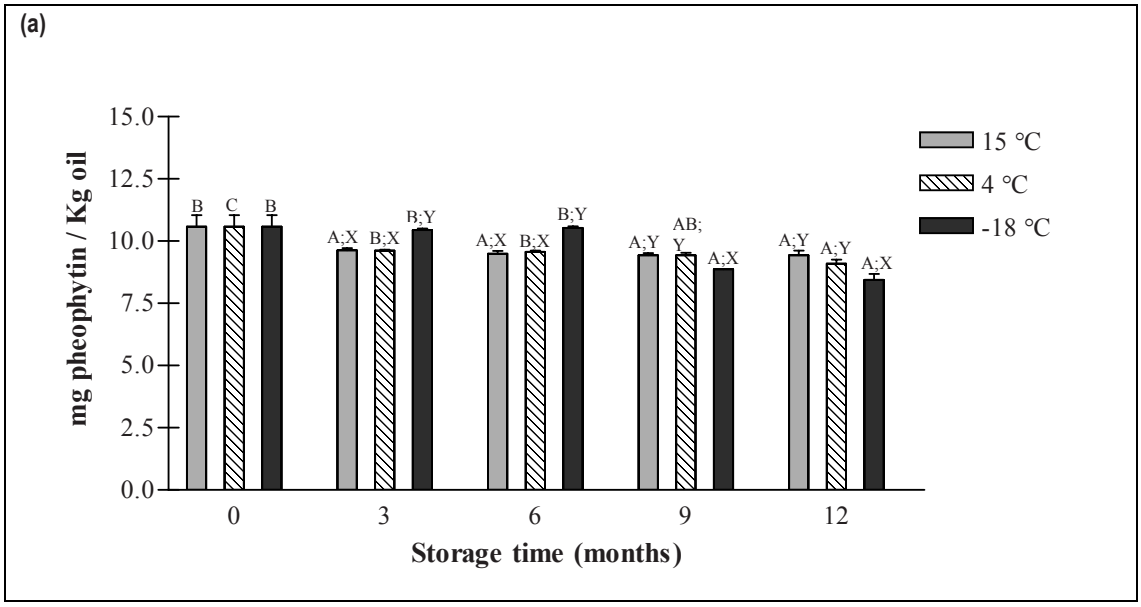
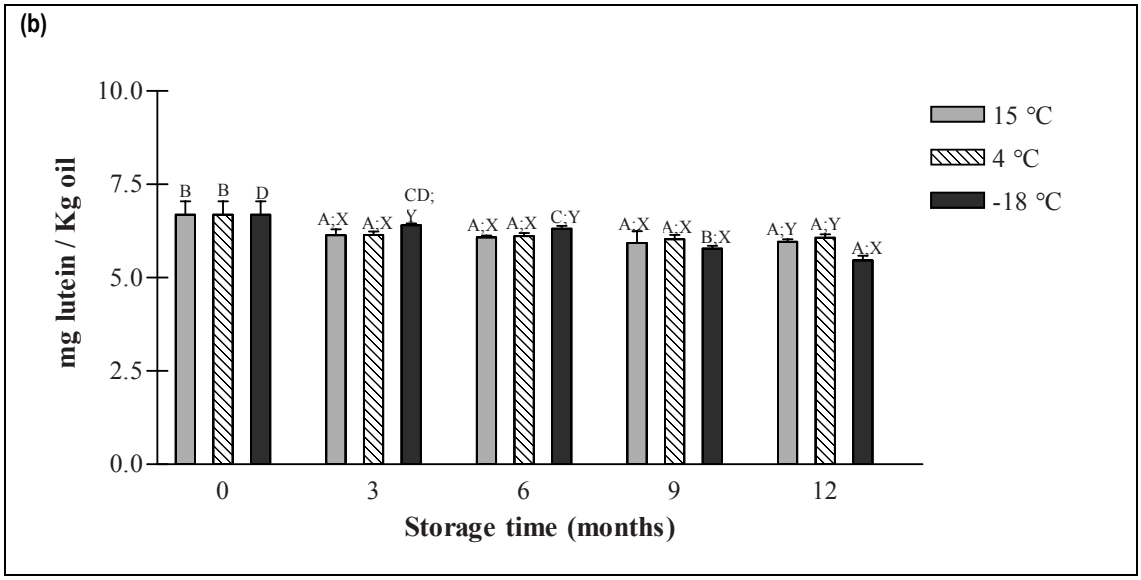



**Figure 2 -** Evolution of total phenols content of olive oils during storage time at different temperatures.
Values reported are mean values and standard deviations of three replicates.
$^{A-E}$ For each parameter, different letters for the same temperature indicate statistically significant differences ($p \leq 0.05$) among storage months.
$^{X-Z}$ For each parameter, different letters for the same storage month indicate statistically significant differences ($p \leq 0.05$) among temperatures.




Values reported are mean values and standard deviations of three replicates.
[A-E] For each parameter, different letters for the same temperature indicate statistically significant differences ($p \leq 0.05$) among storage months
[X-Z] For each parameter, different letters for the same storage month indicate statistically significant differences ($p \leq 0.05$) among temperatures.




Values reported are mean values and standard deviations of three replicates.
[A-E] For each parameter, different letters for the same temperature indicate statistically significant differences ($p \leq 0.05$) among storage months.
[X-Z] For each parameter, different letters for the same storage month indicate statistically significant differences ($p \leq 0.05$) among temperatures.

**Figure 3 -** Evolution of the content of chlorophylls (a) and carotenoids (b) of olive oils during storage time at different temperatures.



27-32.9% at 25°C over a period of 18 weeks. Even after a 12-month storage, we managed to keep a higher content in our stored olive oils.

Table III shows the fatty acid composition during the storage of the olive oils under the three different conditions. Unimportant changes were observed during the storage time at the different temperatures, in line with the studies of Li et al., [15] and Rastrelli et al., [29]. These changes were due to the existence of antioxidants such as α-tocopherol and polyphenols which protected fatty acids from oxidation. In the study by Rastrelli et al., [29] the concentration of poluyunsaturated fatty acids remained almost constant during 8 months, even in samples kept in dark glass bottles at -10ºC. Ayton et al., [30] obtained a similar result after 36 month storage at 15, 22 and 37ºC. Others authors have described changes in the fatty acid composition during storage at 25°C. Morelló et al., [4] described an increase of oleic acid after a 12-month storage, while Gómez-Alonso et al., [5] observed slight decreases in the percentage of polyunsaturated fatty acids after 21 months of storage.

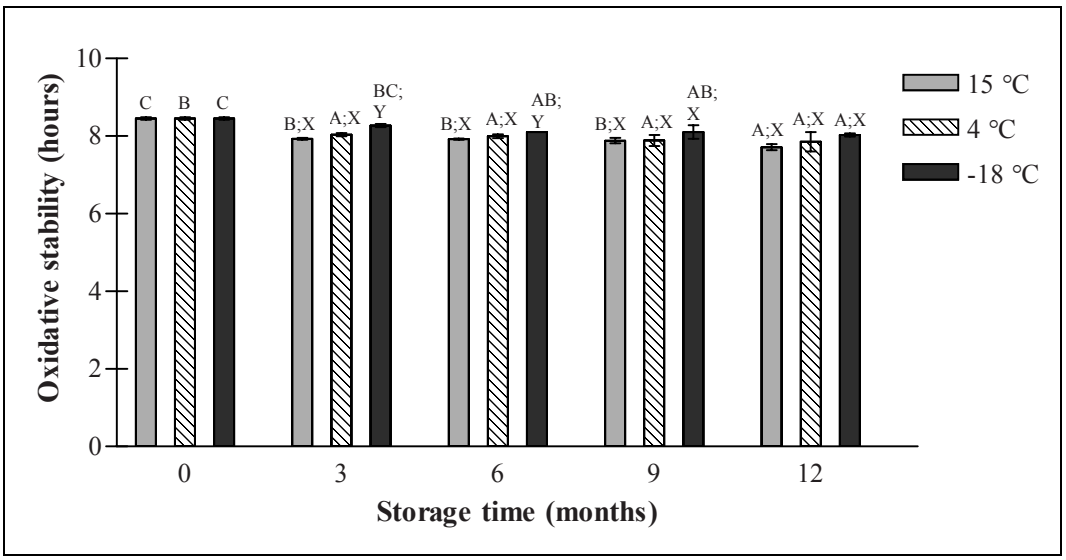



**Figure 4 - Evolution of the oxidative stability of olive oils during storage time at different temperatures.**
Values reported are mean values and standard deviations of three replicates.
[A-E] For each parameter, different letters for the same temperature indicate statistically significant differences ($p \leq 0.05$) among storage months.
[X-Z] For each parameter, different letters for the same storage month indicate statistically significant differences ($p \leq 0.05$) among temperatures.

The evolution of total phenols content during storage at the different temperatures is described in Figure 2. In all cases, the total phenols content decreased with the increase of storage time, especially when the olive oils were stored at 15°C, and mostly after 12 months of storage. In the latter case, significant differences were observed. After 12 months of storage, in fact, the loss percentages were 24% in ambient, 9.5% in refrigeration, and 7.3% in freezing. Phenols are very interesting antioxidants compounds in olive oil. Therefore, freezing at -18°C would provide a very convenient means of keeping a higher total phenols content. These results agree with the work of Mulinacci et al., [17] who stated that, as regards phenolic content, the olive oils maintained a superior quality upon freezing than when stored at room. Other authors [18, 16] explained that the variation of the oil's physical state during freezing and thawing modified the solubility of the phenols soluble in water. Although Cerretani et al., [16] quantified higher decreases in frozen oils after a 3-month storage than we did in our study, this is may be due to their higher initial phenol content. Oils with low amounts of phenols are more likely to enable their total redissolution after freezing and thawing.

Figure 3 shows the evolution of the chlorophylls (a) and carotenoids (b) pigments of the olive oils after storage. A decrease of the chlorophylls content was observed as storage time increased. At the end of the 12 months, the highest content was found in those olive oils that had been stored at 15ºC. The lowest content, on the other hand, was in the olive oils stored at -18ºC. In this latter case, the content decreased dramatically after 6 months of storage. A similar tendency was observed in the carotenoids content. Fatty acid accumulation during storage at low temperatures produces colour changes through pigments degradation. This decrease was statistically significant at -18°C, perhaps because lipoxygenases remain active at low temperatures and were therefore acting on these unsaturated pigments [31]. Furthermore, temperature variations occurring during the thawing of oil augment the reaction rate of the enzymes. It is perhaps for this reason that the frozen oil presented lower values of both pigments after being thawed for analysis.

The evolution of the oxidative stability during storage at different temperatures is shown in Figure 4. Usually, this stability is due to the contribution of the main antioxidant compounds in the olive oil, phenols and α-tocopherol. Since the control olive oils contained small amounts of these compounds, the oxidative stability for them was also small (8.4 hours). Fairly unimportant changes were observed during storage, one of these being a small decrease in stability, especially during the three first months. Also, the highest value was maintained in the olive oils stored at -18ºC (8.0 hours). These results differ from previous literature [18, 16] in that greater decreases were observed. These authors described a decrease in the oxidative stability of the frozen olive oils since certain parts of phenols precipitate during the freezing and thawing phases, consequently and proportionally reducing their oxidative resistance.

The change in sensorial parameters of the olive oils during storage is described in Table IV. A decrease in the 'fruitiness' was observed as following the 12-month storage in all of the storage conditions. At the end of the experiment, the frozen olive oils had maintained the higher value. A similar pattern was observed for the bitterness parameter. In this case, the lowest value was obtained by frozen olive oils. Pun-

**Table IV -** Sensory evolution of oils during storage time at different temperatures.

| Parameter | Temperature (°C) | Storage time (months) | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 3 | 6 | 9 | 12 |
| Fruitiness | 15 | $4.6^{E}$ | $4.2^{D;X}$ | $3.7^{B;X}$ | $3.8^{C;X}$ | $3.4^{A;Y}$ |
| | 4 | $4.6^{C}$ | $4.8^{D;Z}$ | $4.8^{D;Y}$ | $4.4^{B;Z}$ | $3.2^{A;X}$ |
| | -18 | $4.6^{D}$ | $4.3^{C;Y}$ | $3.7^{A;X}$ | $4.2^{B;Y}$ | $3.7^{A;Z}$ |
| Bitterness | 15 | $2.2^{E}$ | $2.1^{D;X}$ | $1.3^{B;X}$ | $0.7^{A;X}$ | $1.4^{C;Z}$ |
| | 4 | $2.2^{C}$ | $2.4^{D;Z}$ | $1.8^{B;Y}$ | $1.2^{A;Y}$ | $1.2^{A;Y}$ |
| | -18 | $2.2^{C}$ | $2.2^{C;Y}$ | $1.3^{B;X}$ | $1.3^{B;Z}$ | $1.1^{A;X}$ |
| Pungency | 15 | $2.8^{D}$ | $2.7^{C;X}$ | $3.2^{E;Y}$ | $2.0^{B;X}$ | $1.8^{A;X}$ |
| | 4 | $2.8^{B}$ | $3.2^{D;Y}$ | $3.3^{E;Z}$ | $2.9^{C;Y}$ | $1.9^{A;Y}$ |
| | -18 | $2.8^{C}$ | $3.2^{D;Y}$ | $2.7^{B;X}$ | $3.4^{E;Z}$ | $1.8^{A;X}$ |
| Defects (rancid) | 15 | - | - | - | - | 2.4 rancid |
| | 4 | - | - | - | - | - |
| | -18 | - | - | - | - | - |

Values reported are median values.
$^{A-E}$ For each parameter, different letters for the same temperature indicate statistically significant differences ($p \leq 0.05$) among storage months.
$^{X-Z}$ For each parameter, different letters for the same storage month indicate statistically significant differences ($p \leq 0.05$) among temperatures.

gency was also decreasing along with storage time, and showed small differences between the three storage conditions. Some authors, such as Bendini et al., [13] and Ben-Hassine et al., [14] reported that after storage the typical bitter taste and pungent note of fresh olive oil decreased in intensity. In these experimental conditions, negative attributes or defects were not detected in most of the olive oils after 12 months of storage. For this reason they could be classified as extra virgin olive oils. The only exception was provided by the olive oils stored for 12 months at 15°C. In this case, a small rancidity was detected; this olive oil would therefore be classified as virgin olive oil. Li et al., [15] have also detected this defect in olive oils following storage at 25ºC. This is due to the degradation of hydroperoxides in secondary oxidation products.



## CONCLUSION

The comparison of the quality of olive oils stored at 15ºC and those stored at 4ºC and -18ºC is a very interesting one. The characteristics of olive oil stored at low temperatures and in optimal conditions appear to be closer to those found in fresh oil. Additionally, the oxidation process is retarded. Frozen storage results in the best physicochemical parameters and antioxidant content (α-tocopherol and total phenol) and oxidative stability after 12 months of storage. As regards the organoleptic parameters, freezing preserved the best fruitiness and good levels of bitterness and pungency. For all these reasons, freezing would be an appropriate storage system, allowing us:

- To maintain those functional and antioxidant compounds found in the olive oil that are very important from a nutritional point of view.
- To extend the shelf life in the industry, especially in high quality and gourmet olive oils.
- And finally, at consumer level, to preserve olive oil at its best and for a longer period by storing it in the fridge.

Further research is required using longer storage time and/or different monovarietal olive oils.

## Acknowledgements

This work was made possible by a pre-doctoral fellowship awarded to María Abenoza by the Aragon government.

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*Received November 26, 2014*
*Accepted January 23, 2015*