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Shelf Life of Monovarietal Extra Virgin Olive Oils Cv. Arbequina and Coratina from Uruguay

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Abstract

Two monovarietal extra virgin olive oils (Arbequina and Coratina), produced in Uruguay, were studied over a period of 12 months storage in amber bottles at 30 ℃. Peroxide index, K_{232}, K_{270}, ΔK, OSI induction time did not exceed the maximum limits set by the International Olive Council (IOC) during the entire period. However, the content of ethyl esters increased with storage time and it is in relation with the shelf life. The International Olive Council (IOC) establishes the limits in the content of ethyl esters ≤ 35 ppm. The Coratina oil ceased to be extra virgin quality after 6 months of storage due to its content of ethyl esters (46 ppm), and the Arbequina oil after 9 months (52 ppm).

Taking into consideration their content of ethyl esters, both the Arbequina and Coratina oils showed a very short shelf life less than six months. The determination of ethyl ester content proved to be a sensitive method for determining the loss of quality of extra virgin olive oils.

Keywords: Extra virgin olive oil, shelf life, ethyl esters

1. Introduction

Morelló *et al.* (2004) studied the changes undergone extra virgin Arbequina oil during storage for 12 months. Although this paper included different analysis for determining the variation of certain minor components, the only stability analysis conducted consisted in determining the OSI times utilizing a Rancimat equipment set at 120 ℃. Gómez-Alonso *et al*. (2007) studied the evolution of these minor components and the oxidation indexes in seven samples of virgin olive oil stored during 21 months at room temperature and in the dark. It was found that the peroxide index, K232 and K270 increased linearly over time (although never surpassing the limits set by the IOC). Del Caro *et al*. (2006) studied the influence of storage (in the light and in the dark), during 16 months, on the components of extra virgin olive oil (Bosana *cv*) and on the peroxide index, K232 and K270. During this period, the oxidation indexes did not exceed the limits set by the IOC.

Generally, extra virgin olive oil has a relatively long shelf life of 12-18 months of storage in bottles at room temperature, that means that oil produced in a given year may still be commercialized up until the next olive harvest. From a physicochemical viewpoint, the study of shelf life is generally based on determining acidity, peroxide index, and ultraviolet absorption.

Fatty acids methyl esters and ethyl esters are components of edible vegetable oils extracted form raw materials in bad conditions, therefore, should not be present in extra virgin olive oils (Gomez-Coca et al., 2012; Jabeur et al., 2015). Methanol and ethanol should first be formed in the system, possible through fermentation of the carbohydrates in the olives. Then, a transesterification of the triglycerides or esterification of the free fatty acids with these alcohols takes place. As a consequence, the presence of these esters in extra virgin olive oil indicates a poor treatment of the olives, which led to their fermentation.

If upon their elaboration, the virgin olive oils contain free methanol and ethanol, these will gradually esterify with the free fatty acids leading to the corresponding esters. As this process is relatively slow, an increase of the alkyl ester content and decrease of the free alcohols should take place during storage.

Among the quality requirements for extra virgin olive oils, the norm of the International Olive Council (IOC) 2015, establishes the limits in the content of ethyl esters: ≤ 35 ppm for the 2014/2015 and 2015/2016 crop years.

Waxes have a fundamental role in protecting the fruit from microorganisms attack and also from the point of view of the regulation of moisture in the fruit. This content in the fruit is determined by factors including agro-climatic as well as the variety of olives in others. However, the total wax content and its oil profile is affected by the extraction method used. The olive pomace oils, which are extracted with solvent, have a higher content compared to olive oils extracted by thermo-mixing. Moreover, the wax content increases during storage, although the reason for their formation has not yet been fully explained. The IOC establishes a limit for the content of waxes, so it is possible to consider it as a quality parameter as a tool for distinguishing between olive oil and olive-pomace oil and as a quality parameter for extra virgin olive oils enabling the detection of fraudulent mixtures of extra virgin olive oils with lower quality oils.

Olive oil has antioxidants such as tocopherols and polyphenols which protect it from oxidation. The α-tocopherol represented more than 90% of total tocopherols. However, α-tocopherol is the least protects oils from thermo-oxidation (Barrera-Arellano et al., 2002). Thus, the polyphenol content is extremely important because it largely polyphenols slow oil oxidation phenomena. Antioxidants in contact with air oxygen are degraded and therefore is of interest to study its evolution over time. To compare the oxidative stability of rancidity accelerated methods are the OSI and Rancimat method is used. Therefore, if there is a variation of the antioxidants present in the oil induction times determined by these methods should change.

The purpose of this study was to determine the shelf life based on different chemical parameters of extra virgin olive oils cv. Arbequina and Coratina, made in Uruguay and selected by their differences in antioxidant content.

2. Materials and Methods

2.1 Samples

Extra virgin olive oils of the Arbequina and Coratina varieties, were extracted used an Abencor laboratory oil mill (Comercial Abengoa S. A., Sevilla, Spain) kneading the olive paste at 30 ℃ during 30 minutes. The analyzer consisted of three basic elements: a hammer mill a thermomixer and a pulp centrifuge.

2.2 Shelf Life Tests

Samples were stored in 100 mL capped amber glass bottles, in an oven at 30 ±1 ℃ (usual summer temperature in Uruguay). Every month or every 3 months (as indicated) one bottle was removed from the oven and the oil analyzed.

2.3 Analytical Techniques

Peroxide Index: AOCS method Cd 8b-90. The weighed samples were dissolved in acetic acid-isooctane (3:2). Then saturated potassium iodide solution is added and left in the dark for 1 minute. Once this time elapsed was added 30 mL water and titrated with 0.1 N sodium thiosulphate using starch indicator as.

Ultraviolet Absorption (K232 and K270): AOCS method CH 5-91. The previously weighed samples were dissolved in isooctane. Subsequently, its absorbance was determined at wavelengths of 232 nm and 270 nm in a Shimadzu, Mini-UV 1240 model.

Polyphenol Content: method IOC/T.20/Doc. nº. 29, "Determining olive oil biophenols through HPLC". Polyphenols present in the sample were extracted in a mixture of MeOH-water (80:20) with added syringic acid as internal standard. Polyphenols were determined on a Shimadzu HPLC, Model 20A, using a Phenomenex C18 column with a particle size of 5 μm, 250 mm long and 4.6 mm diameter. For elution of polyphenols the following solvents were used: methanol, acetonitrile and phosphoric acid 0.2% -water. Quantification of polyphenols was performed at 280 nm.

Tocopherol content: according to published results by Andrikopoulus et al. (1991) determined through HPLC.

Tocopherols were determined in a Shimadzu model 20A HPLC equipped with Phenomenex C18 column with a particle size of 5 μm, 250 mm long and 4.6 mm diameter. For this purpose, the following solvents were used: acetonitrile, methanol, 5% acetic acid in water and isopropanol. Detection of tocopherols was performed by a fluorescence detector at the following wavelengths: 290 nm excitation and 330 nm emission.

Induction time (OSI): method AOCS Cd 12b-92. The oxidative stability was determined by OSI analysis in a Omnion OSI-8 instrument. The temperature of the heating block was maintained at 110 °C.

Ethyl esters content: method IOC/T.20/Doc. nº. 28 "Determining the wax and methyl ester and ethyl esters of the fatty acids through a gas chromatography with capillary column". Methyl and ethyl esters were separated together

with waxes by solid phase chromatography (SPE) using hexane-diethylether (99:1) as elution solvent. The extracts were analyzed in a gas chromatograph Shimadzu, Model 2010, using a column Restek, RTX-5MS. The methyl esters were quantified by addition of a solution of methyl ester heptadecanoic as internal standard and waxes by adding a solution palmityl palmitate.

All determinations were performed in duplicate.

3. Results and Discussion

Figure 1 shows the peroxide index evolution of the extra virgin olive oils of the Arbequina and Coratina varieties, over the 12 months of storage. The evolution of both oils was similar, although Arbequina oil showed greater to form peroxides than Coratina oil. This is justified by its polyphenol content (Figure 3). The maximum value allowed by the IOC 2015 Norm is 20. After 12 months of storage these oils were far below that value. Therefore, according to this parameter, both oils were far from losing its quality extra virgin storage for one year. A similar behavior is found in the literature. (Gomez-Alonso *et al*., 2005; Del Caro *et al*., 2006; Vekiari *et al*., 2007; Lerma-Garcia *et al*., 2009; Krichene *et al.*, 2010; Fadda *et al*., 2012)

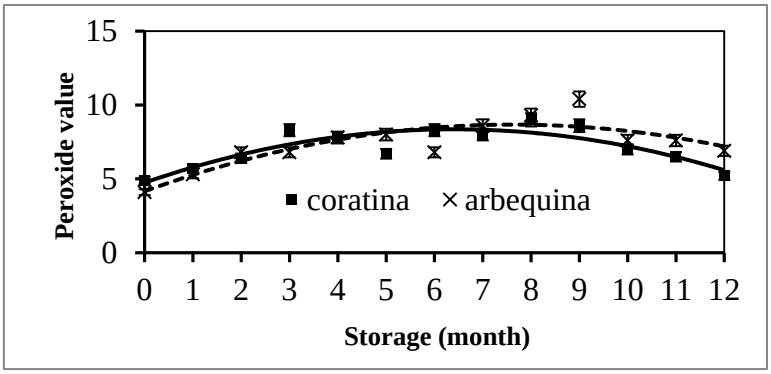

Figure 1. Variation of the Peroxide index of the Arbequina and Coratina oils over the 12 months of storage.

Figure 2 (where A corresponds to Arbequina and C to Coratina) shows the variation of the K232 and K270 indexes for both oils. During the 12 months period, the Coratina oil did not surpass the limits set by the IOC 2015 Norm: 2.5 for K232 and 0.22 for K270. Therefore, according to these parameters, this oil did not lose its quality extra virgin storage for one year. The Arbequina oil, however, surpassed both limits on the 12th month. During the entire year of storage, the Arbequina oil always showed values slightly higher than those of the Coratina. A similar behavior is found in the literature. (Gomez-Alonso *et al*., 2005; Del Caro *et al*., 2006; Vekiari *et al*., 2007; Lerma-Garcia *et al*., 2009; Krichene *et al.*, 2010; Fadda *et al*., 2012).

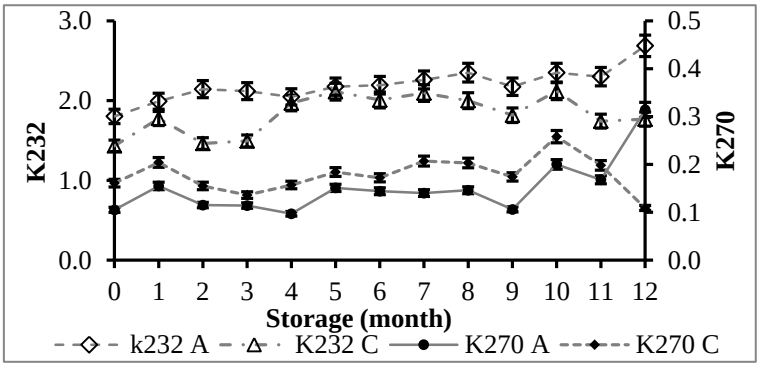

Figure 2. Variation of K232 and K270 for the Arbequina and Coratina oils over the 12 months of storage.

Wax esters are formed by the esterification of high molecular mass alcohols with fatty acids. The waxes most frequently found in olive oil are C40, C42, C44 and C46 (Samaniego-Sánchez *et al*., 2010). The wax ester content is used as a quality parameter and has also been used to detect adulteration. The 2015 IOC Norm established that the waxes content (not including the C40) of extra virgin and virgin olive oil must be ≤ 150 ppm but if C40 is included, the oil will be considered ordinary virgin if that content is ≤ 250 ppm. The composition of waxes has also been used to differentiate varieties (Aragon *et al*., 2011).

Table 1 shows a variation in the wax content (as the addition of C40, C42, C44, C46 and not including C40) over

the storage months for the Arbequina and Coratina oils.

The wax content of both oils increased during storage. The explanation for this increase during storage of the oil is not known in the literature since no references to it found. While the content of wax (C42 + C44 + C46) is a test of purity established by 2015 IOC Norm, because this increase could also be considered a quality test, because the IOC itself establishes a maximum of 150 ppm for Extra virgin and virgin oils. However, neither of the oils lost their extra virgin quality, in spite of the increase in wax content over the storage period.

Table 1. Variation of the content of waxes (ppm) in the Arbequina and Coratina oils during the 12 months of storage.

	Arbequina waxes (ppm)		**Coratina waxes (ppm)**	
time (months)	**With C40**	**Without C40**	**With C40**	**Without C40**
0	78±5	14±5	0	0
3	95±5	0	0	0
6	49±5	0	5±5	0
9	152±5	43±5	12±5	12±5
12	198±5	96±8	44±5	44±5

The total polyphenol and tocopherols content variation (ppm) for both oils is shown in Figure 3.

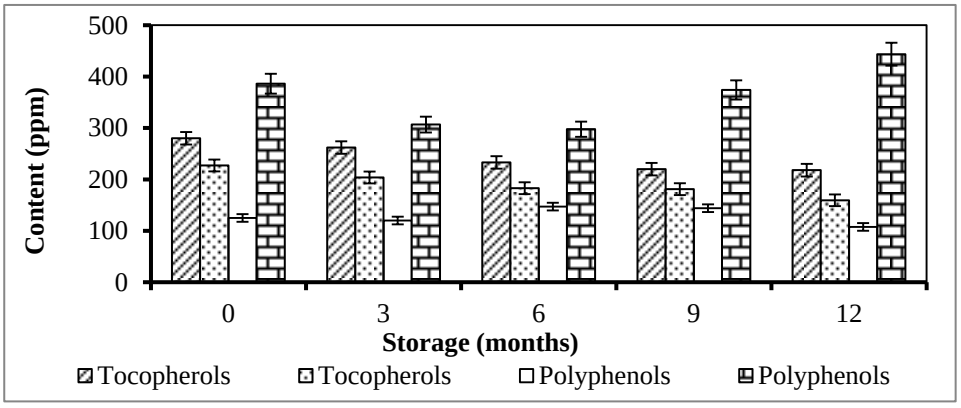

Figure 3. Variation of the content (ppm) of tocopherols and polyphenols in the Arbequina (A) and Coratina (C) oils during the 12 months of storage.

Over the 12 months storage period loss of tocopherols in both oils was statistically significant. Regarding decreased tocopherols during storage a similar behavior was found in the literature. (Morello *et al*., 2004; Gomez-Alonso *et al*., 2005; Del Caro *et al*., 2006; Lerma-Garcia *et al*., 2009; Fadda *et al*., 2012)

The polyphenols content of Arbequina oil was much lower than the Coratina, although its tocophenol content was slightly higher. From the point of view of their oxidative stability, polyphenols are the most important antioxidants of virgin olive oil. (Gutierrez *et al*., 2001; Carrasco-Pancorbo *et al*., 2005; Franco *et al*., 2014), Thus, Arbequina oil was less protected against oxidation than Coratina oil and was more prone to oxidation.

The polyphenol content Coratina oil showed a decrease and then an increase during storage; in oil Arbequina, that content first increased and then decreased. These variations are not easy to explain because modifications phenol content during storage of extra-virgin oil are complex. The main effects tested in the phenolic fraction during oil storage have been hydrolysis of secoiridoids and oxidation of some phenolic molecules.

During the storage of virgin olive oil hydrolitic mechanisms may be involved in the release of simple phenolics such as hydroxytyrosol and tyrosol from the more complex secoiridoids (Gutierrez Gonzalez Quijano *et al*, 1977). According to Krichene *et al*, (2010) the content of the simple phenols hydroxytyrosol and tyrosol increased in the initial stage of storage (ap. 16-20 weeks) as a result of phenolic aglycon hydrolysis. After this initial increase a clear decrease of hydroxytyrosol was observed probably due to its decomposition acting as an antioxidant. The concentration of the hydroxytyrosol measured at a particular time would therefore be the sum of its formation from the decomposition of its secoiridoid derivatives and its decomposition due to its role as antioxidant. The decrease in the complex phenols must be largely due to a lack of stability of these secoiridoid compounds. A similar behavior found Lozano-Sánchez et al. (2013).

Figure 4 shows the evolution of the induction times (OSI) for both oils during storage. OSI times for the Arbequina oil were considerably lower than for the Coratina. This is a consequence of their respective

polyphenols content, according to Franco *et al*. (2014) that found positive correlation between total phenolic compounds and antioxidant capacity. Gutierrez *et al*. (2001) also demonstrate the contribution of polyphenols to virgin olive oil stability (measured as Rancimat induction tieme). OSI time did not evolve during storage because neither significant changes were observed in content of antioxidants. OSI time decrease with storage times are also reported in the literature. (Del Caro *et al*., 2006; Lerma-Garcia *et al*., 2009)

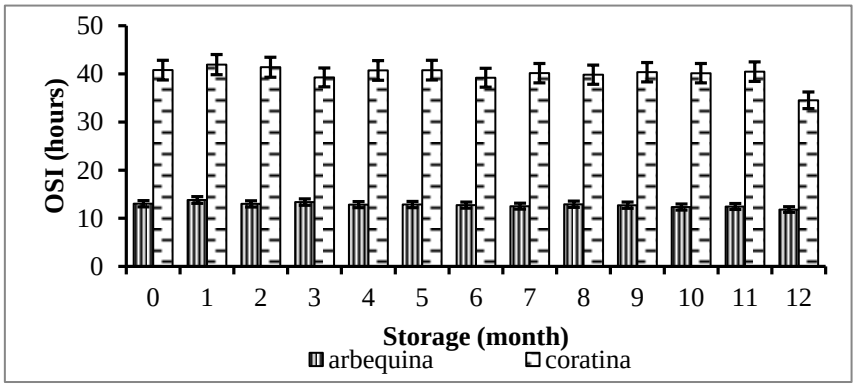

Figure 4. Variation of the OSI times (in hours) for the Arbequina and Coratina oils over the 12 months of storage.

The formation of ethyl and methyl esters in the oil has its origin in the esterification of free methanol and ethanol (possibly formed by fermentations of the olives, prior to oil extraction) with the free fatty acids (acidity). Bendini *et al*. (2009) related the ethyl fatty esters content of a number of samples with its sensory classification, together with the content of water and ethanol. When ethyl fatty esters are present at a certain concentration, the use of olive fruits with fermentative alteration becomes evident (Pérez-Camino *et al*., 2002). Gomez-Coca *et al*. (2012) studied the presence of fatty acid alkyl esters (FAAEs) of Spanish varietal extra virgin olive oils (bought in local markets). Ethyl esters content reached to 42 ppm values. They also concluded that the content of ethyl esters and organoleptic data are complementary criteria when classifying olive oils and must be utilized as such. Fermentative defects are reflected in very high concentrations of FAAEs, whereas other kinds of defects, such as oxidative ones and frozen olives, do not produce FAAEs. Both kinds of attributes are detectable during the organoleptic assessment. In contrast, non-fermentative defects, do not affect the FAAEs classification criterion, but they are evidenced during the organoleptic evaluation. Figure 5 shows the variation of ethyl ester content (ppm) over the months of storage. The maximum allowed by the IOC 2015 Norm is 35 ppm. As a consequence, the Coratina oil ceased to be extra virgin quality at 6 months of storage, and the Arbequina at 9 months. As a consequence, the shelf life of both oils lasted about half a year (labeling usually indicates it must be consumed "before one year"). On the other hand, after 9 months of storage there was a significant acceleration in the formation of ethyl esters in both oils; the reason for this is unknown.

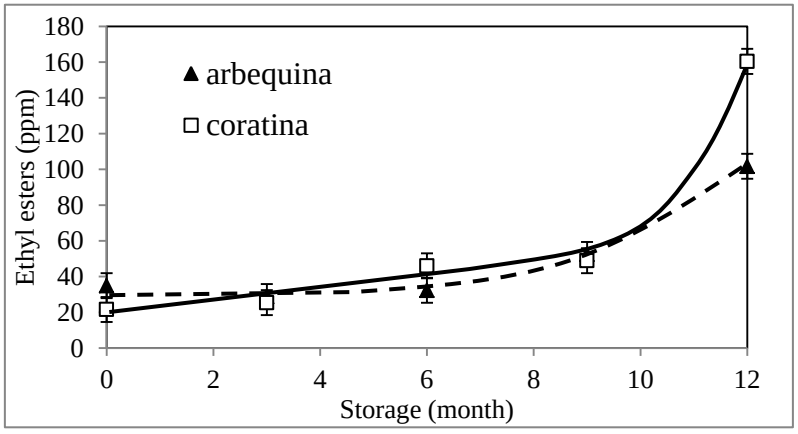

Figure 5. Variation in the content of ethyl esters (ppm) of the Arbequina and Coratina oils during the 12 months of storage.

At 6 months of storage, for both oils, all the legal quality parameters met the limits set by the 2015 IOC Norm,

except the ethyl ester content, as it exceeded the legal limits. In the period of one year, the other quality parameters determined (peroxides index and ultraviolet absorbance) did not exceed the limits set by the IOC. Therefore, according to its FAEEs content both oils lost their quality extra virgin within 6 months. As a consequence, the ethyl esters content behaves as a sensitive parameter with regards to the shelf life of extra virgin olive oils.

4. Conclusions

In general, all parameters related to quality deterioration requires the IOC (peroxides index, ultraviolet absorbance to, ethyl esters content, phenol content) underwent significant variations during storage. However, according to the index values of peroxides, both oils were far from losing its quality extra virgin for one year storage. According to the values of ultraviolet absorbance, oil Coratina not lost its quality extra virgin for a year storage although oil Arbequina exceeded the limits set by the IOC at 12 months. Instead, the content of ethyl esters of both oils exceeded the limit set by the IOC, at about 6 months of storage. The content of ethyl esters is a sensitive parameter for determining the quality of extra virgin olive oil and for determining its shelf life. Using this parameter, both the Arbequina and Coratina olive oils showed a very short shelf life: under 6 months. However, they should carry out studies with other varieties of olives to draw more general conclusions about it.

Moreover, it would be important to relate the content of ethyl esters with the possible occurrence of defects in sensory oil.

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