GRASAS Y ACEITES 72 (2)
April-June 2021, e413
ISSN-L: 0017-3495
http://grasasyaceites.revistas.csic.es

INFORMATIVE NOTE

Captive fatty acids of fresh olive oils?

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Submitted: 04 May 2020; Accepted: 06 October 2020; Published online: 10 June 2021

SUMMARY: Olive oil is mainly made up of triglycerides. It is well known that olive oil contains free fatty acids, the proportion of which is variable, depending on the extent of the hydrolysis of triglycerides. Besides, globular structures have been reported in cloudy virgin olive oils. The pseudo-walls of these globules would be constituted by amphiphilic molecules, while fatty acids are amphiphilic. This brief review aims to inform on the importance of the possible interaction of 'free' fatty acids of veiled virgin olive oils, as structural units in the pseudo-wall of the colloidal globules, already reported. The binding of fatty acids to the colloidal globules can mean they are not free in the olive oil. They could be 'captive' in said pseudo-walls, thus exerting less influence on the perception of acidity by the consumer or taster of the olive oils. The official method of analysis of olive oil acidity cannot detect this effect. This may suppose that functional acidity is lower than the acidity values determined by chemical analysis in cloudy virgin olive oils.

KEYWORDS: *Captive fatty acids; Colloid; Colloidal globules; Free fatty acids; Veiled virgin olive oil.*

RESUMEN: *¿Ácidos grasos "cautivos" de los aceites de oliva frescos?* El aceite de oliva está compuesto principalmente de triglicéridos. Es bien sabido que contiene ácidos grasos libres, cuya proporción es variable, dependiendo del grado de hidrólisis de los triglicéridos. Se han informado la existencia de estructuras globulares en el aceite de oliva virgen coloidal. Las pseudo-paredes de estos glóbulos estarían constituidas por moléculas anfifílicas, siendo anfifílicos los ácidos grasos. El objetivo de esta breve revisión es informar de la importancia de la posible interacción de los ácidos grasos 'libres' de los aceites de oliva virgen coloidales, como unidades estructurales en la pseudo-pared de los glóbulos coloidales referidos. La unión de los ácidos grasos a los glóbulos coloidales puede significar que no están libres en el aceite de oliva. Podrían estar "cautivos" en dichas pseudo-paredes ejerciendo menos influencia en la percepción de acidez por parte del consumidor o catador de los aceites de oliva. El método oficial de análisis de acidez del aceite de oliva no puede detectar este efecto. Esto puede suponer que la acidez funcional es inferior a los valores de acidez determinados por análisis químico en aceites de oliva virgen coloidales.

PALABRAS CLAVE: *Aceite de oliva sin filtar; Ácidos grasos cautivos; Ácidos grasos libres; Coloide en rama; Glóbulos coloidales.*

Citation/Cómo citar este artículo: Cayuela-Sánchez JA. 2021. Captive fatty acids of fresh olive oils?. *Grasas Aceites* **72** (2), e413. http://grasasyaceites.revistas.csic.es

1. INTRODUCTION

Olive oils (OO), as other vegetable oils, are mainly made up of triglycerides (between 98% and 99%). Their content in free fatty acids is well known (Barjol, 2013). The proportion of free fatty acids of OO is variable, depending on the extent of the hydrolysis of triglycerides. Thus, the free fatty acids in vegetable oils are generally considered as those that do not form part of the triglycerides. Olive oil free acidity is one of the major quality features of virgin olive oils. In fact, according to the standards in force of the European Commission (1991), the classification into Extra Virgin Olive Oil class (EVOO) requires the free acidity to be lower than 0.8%. The class EVOO also requires the fruity sensory attribute to be perceptible, and for it to be free of organoleptic defects. The product without these features but with free acidity up to 2%, belongs to the class VOO. An acidity value higher than 2% implies that the product is to be classified as lampante olive oil, which should be refined. The official method of analysis of olive oil's free acidity (European Commission, 1991) is a volumetric titration. Briefly, 4 to 6 g of olive oil are weighed into 250 mL wide-mouth Erlenmeyer flasks, and 50-mL ethyl alcohol: ethyl ether at 1:1 and a few drops phenolphthalein are added, and it is then neutralized with NaOH 0.1 N until it turns a pink color, and expressed as a percentage of oleic acid. Thus, olive oil acidity is considered to be caused by the fatty acids liberated from triglycerides by hydrolysis processes, and expressed as the analyzed value according to the method explained above.

It is interesting to highlight that the physical state of cloudy virgin olive oil (VOO) is due to a phase formed by microscopic droplets of water in meta-stable dispersion (Lercker *et al*., 1994). Moisture spreads homogeneously in the major mass of triglyceride liquid, making it an emulsion colloid. Besides, VOO has another phase formed by microscopic solid particles, thus it has been described as a sol-emulsion colloid (†Gómez-Herrera, 2007). In this article, we explain how the fatty acids in veiled olive oils could not be free in the triglyceride mass, and that the functional free acidity of these olive oils could be lower than the acidity determined through official analysis.

2. CAPTIVE FATTY ACIDS IN VEILED OLIVE OILS?

A study on the influence of the scattered matter in veiled olive oils on their shelf-life reported that the free fatty acids precipitated with the residue or sediment in the oil (Lercker *et al*., 1994). The cited study related the precipitate of the solid particles contained in the olive oils to the attraction of opposite electrical charges. The same study pointed out that the scattered matter dampened the acidity, at the same time exerting an antioxidant effect.

Together with this, there are several studies on the conservation of alternatively cloudy or filtered olive oils. The literature agrees that changes in the acidity during the period of olive oil storage are greater in veiled olive oils than in the filtered ones (Tsdimidou *et al*., 2005; Fregapane *et al*., 2006; Stefanoudaki *et al*., 2010). The literature establishes that free fatty acids have a prooxidant effect (Frega *et al*., 1999), and that high acidity exerts a negative influence on the stability of olive oil (Ciafardini, *et al.,* 2006; Frega *et al*., 1999). The referred studies concur that a slightly better oxidative stability index (OSI) is determined for cloudy olive oils. Frega *et al*. (1999) reported that the suspended-dispersed material of cloudy virgin oil exerted a positive effect on oxidative stability. Moreover, the same authors found a higher antioxidant effect of the dispersed particles compared to the suspended particles. This last study proved the olive oil quality decay was significantly delayed by removing the scattered matter. The authors pointed out the possibility of an effect from the higher surface to dimension ratio of the dispersed particles compared to the suspended particles. It is possible to think the authors referred to the 'suspended' particles as those with little stability among all participating in the dispersion. Nevertheless, the study cited lacks a clear description of what particles are referred to by the concepts 'suspended' and 'dispersed'. It is also interesting to consider that, according to Chaiyasit *et al*., (2007), the interface of association colloids is a likely site of oxidation reactions since many prooxidants and antioxidants are also surface active and thus would concentrate at the water-lipid interface.

A recent microscopy study (Cayuela-Sánchez and Caballero-Guerrero, 2019) revealed that the major part of the spread matter in cloudy virgin olive oil is formed by microscopic droplets of water, while solid particles seem to be contained within an aqueous medium inside colloidal globules (Figure 1). The name of reverse micelles does not seem appropriate for these globules, because their size, even larger than 15 µm, is too large for it. The diameter of reverse micelles ranges from $7\ 10^{-4}$ µm to $25\ 10^{-4}$ µm (Amararene *et al*., 1997), thus quite smaller. The referred solid particles were reported to show a wide diversity both in shape and size. Besides, they were observed in motion. The peculiar movement of said solid particles inside the colloidal globules was recorded in various video sequences (Cayuela-Sánchez and Caballero-Guerrero, 2019). The authors first related the observed movement to the possible physical influence of electrostatic charges. However, the observed particles' agitation clearly matches the Brownian movement according to the literature (Einstein, 1905; Smoluchowski, 1906; Einstein, 1956). This physiochemical effect is observed particularly in emulsions. The major interest of this movement of the solid particles in olive oil colloidal globules is that it confirms the hypothesis the internal content of the colloidal globules is aqueous. This movement of particles is not observed in the mass of veiled olive oil, formed largely by triglycerides, outside the globules. Albert Einstein explained in detail (Einstein, 1905) how the movement Brown had observed in pollen was the result of the particles being pushed by individual water molecules. Briefly explained, the notorious scientist attributed the movement to the atomic bombing force, whose direction is constantly changing. Therefore, the observed movement could exist independently from the microscopy illumination. However, artificial light should provide additional excitation because this physical phenomenon is temperature-dependent (Seddig, 1908). Thus, the heat provided by the light used for microscopy observation could be responsible for most of the observed movement.

The suspended matter identified by Frega *et al*., (1999) probably relates to these colloidal globules, despite such globular structures containing water was not described. Moreover, Frega *et al*., (1999) related the amino groups of lignin, derived from the olive nut, as primarily accountable for the cloudy material. However, the study did not consider the possibility that microscopic droplets of water are directly responsible for the opalescence of veiled olive oils. The referred authors hypothesized that after reacting with FFA, the amino groups may liberate the phenolic groups that were previously bonded to hydrogen, a state in which they were not able to display any antioxidant activity. Frega *et al*., (1999) related this to the observation that FFA bonded to the disperse particles and precipitated with them as a brown colored residue, as reported in a previous study (Lercker *et al*., 1994). However, these studies did not consider that FFA may participate in the colloidal globules reported (Cayuela-Sánchez and Caballero-Guerrero, 2019), among the structural units forming their pseudo-wall (perceptible in Figure 1),

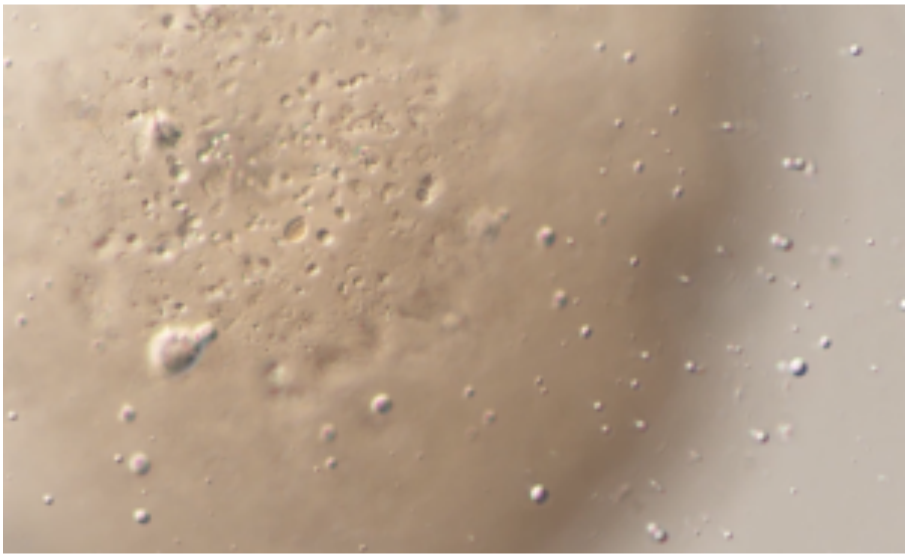

FIGURE 1. Microscopy focus inside a colloidal globule of fresh virgin olive oil.

as the present study proposes. This last hypothesis agrees that FFA precipitate with the solid residue noted by Lerker *et al*., (1994); although a different scenario for the modulation of the acidity of veiled virgin olive oils can be shown. Frega *et al*., (1999) inferred that the effects from spread matter are so positive that avoidance of oil filtration is desirable in order to extend the olive oil's shelf-life. However, this conclusion should be assessed considering that olive oil filtration implies a drastic removal of moisture, and the elimination of water soluble phenolics along with it. (Bakhouche *et al*., 2014). The phenolic compounds which provide the highest antioxidant activity in olive oil, such as hydroxytyrosol and tyrosol, are the most water-soluble ones (Capasso *et al*., 1992; Bakhouche *et al*., 2014). Therefore, the filtration of virgin olive oil implies a drastic loss in its antioxidant power, with negative influence both on its oxidative stability and nutritional benefits. It is advisable to consider all of the above to decide the most suitable handling of olive oil according to its destination.

The structure and dynamics of colloidal dispersions in veiled virgin olive oil (VOO) have been the subject of a study (Papadimitriou *et al.,* 2013). The authors used confocal microscopy and three different dispersive techniques for characterizing samples of turbid VOO, among which the best was the "Small Angle Light Scattering Apparatus" (SALSA). The SALSA data showed a colloid diameter range from 1.5 to 14 μm. The authors indicated that the resolution limit for the method used was 15 μm, with higher colloids not being detected. Some new details concerning colloidal globules found in olive oil have been reported recently (Cayuela and Caballero-Guerrero, 2019). These colloidal globules are formed by amphiphilic molecules, among which proteins, sugar glycosides, and glycoproteins are found, according to the contents reported for fresh olive oils (Koidis and Boskou, 2006; Lercker *et al*., 1994), and to organic fragments of diverse complexity. It is important to note that free fatty acids, which determine olive oil acidity, are also amphiphilic. The shorter their chain and the greater their unsaturation, the greater their affinity for water (Nelson and Cox, 2017). Total unsaturated fatty acids in olive oils range from 77% to 88% and monounsaturated fatty acids averaging around 75% (García-González *et al*., 2013). However, up to now, the relation of fatty acids to the colloidal globules of olive oil has not been characterized. Here is a proposal on the hypothesis to a link of free fatty acids to the pseudo-wall of the colloidal globules, suggesting that adsorption of free fatty acids in the interface of water globules may result in a decrease in their chemical reactivity.

3. EVOLUTION OF THE ACIDITY OF VEILED OLIVE OILS

The literature on the evolution of acidity in the conservation of veiled and filtered olive oils (Tsdimidou *et al*., 2005; Fregapane *et al*., 2006; Stefanoudaki *et al*., 2010) is consistent with the possible link of 'free' fatty acids to the globules as amphiphilic molecules, as explained below. Their binding to the pseudo-walls of such globules may involve some restraint of their chemical reactivity. This hypothesis is consistent with a brief report on the idea that the scattered matter of veiled oil drops its acidity level (Lercker *et al*., 1994), although these referred authors did not give reasons.

The segregation of the molecules framing the pseudo-wall of the colloidal globules may explain the subsequent decantation of water and sedimentation of solids. This is consistent with the slight increase in acidity noted during the storage of cloudy olive oils (Tsdimidou *et al*., 2005; Fregapane *et al*., 2006; Stefanoudaki *et al*., 2010). Also, according to our microscopy study, the solid particles seem to lodge inside the colloidal globules, but not spread into the triglyceride matrix. Under microscopy observation, the repulsion exerted by the triglyceride mass seems to force the droplets of water through the amphiphilic compounds of the pseudo-wall, towards the inside of the globules. Therefore, the colloidal globules collapse, due to the coalescence of microscopic droplets of water is the logical necessary outcome. Thus, the globules collapse implies the disassembly of their amphiphilic compounds. The solid particles previously suspended in a watery medium inside the colloidal globules must also be released during the proves. Consequently, the solid matter may begin the process of precipitation. From these results, a logical hypothesis is that the free fatty acids released from the pseudo-walls from the collapse of the globules, then begin exerting their characteristic chemical activities.

4. CONCLUSIONS

Colloidal globules have been reported in forming part of the sol-emulsion colloid characteristic of unfiltered virgin olive oils, as well as in the product before a rest period in tanks. In this study it is hypothesized that the adsorption of free fatty acids on the pseudo-wall of these globules may reduce the functional olive oil acidity. Therefore, the fatty acids resulting from the triglyceride hydrolysis, are not free in the veiled olive oil but 'captive' in the pseudo-walls. It is reasonable that this fact can reduce its chemical activity, as may be the case with its prooxidant activity. Thusly, it is logical to believe that when the fatty acids adhere to the globules pseudo-walls, they may have less influence on the perception of acidity by the consumer of these cloudy virgin olive oils. The official analysis method of olive oil acidity can't detect this last effect since it is a titration test. If this is true, veiled fresh virgin olive oils can offer natural modulation of their acidity, while preserving said physical state. To the best of our knowledge, these ideas have yet to be considered in scientific literature.

ACKNOWLEDGMENTS

This work has been produced without specific funding. The author would like to thank the Spanish Council for Scientific Research for supporting their activity as staff. The author sincerely thanks Mrs. Belén Caballero-Guerrero, responsible for the Unity of Microbiology at the Instituto de la Grasa (CSIC), for the microscopy study. And finally, the author would like to thank Dr. Eddy Plasquy, producer of the virgin olive oil brand 'Del Cetino', for his commentary on the Brownian movement characteristic of emulsions.

5. REFERENCES

Amvararene A, Gindre M, Le Huérou JY, Nicot C, Urbach W, Waks M. 1997. Water Confined in Reverse Micelles: Acoustic and Densimetric Studies. *J. Phys. Chem. B* **101**, 10751-10756. https://doi.org/10.1021/jp972718f

Bakhouche A, Lozano-Sánchez J, Ballus CA, Martínez-García M, González Velasco, M, Olavarría-Govantes A. 2014. Monitoring the moisture reduction and status of bioactive compounds in extra-virgin olive oil over the industrial filtration process. *Food Control* **40**, 292-299. https://doi.org/10.1016/j.foodcont.2013.12.012

Barjol JL. 2013. Introduction, in Aparicio R, Harwood J (Eds.) *Handbook of Olive Oil. Analysis and Properties, second ed.*, Springer, New York, pp. 1-17. https://doi.org/10.1007/978-1-4614-7777-8_1

Capasso R, Evidente A, Scognamiglio F. 1992. A simple thin layer chromatographic method to detect the main polyphenols occurring in olive oil vegetation waters. *Phytochem. Analysis* **3** (6), 270 –275. https://doi.org/10.1002/pca.2800030607

Cayuela-Sánchez JA, Caballero-Guerrero B. 2019. Fresh extra virgin olive oil, with or without veil. *Trends Food Science Tech.* **83**, 78–85. https://doi.org/10.1016/j.tifs.2018.11.014

Ciafardini G, Zullo BA, Iride A. 2006. Lipase production by yeasts from extra virgin olive oil. *Food Microbiol.* **23** (1), 60–67. https://doi.org/10.1016/j.fm.2005.01.009Chaiyasit W, Elias RJ, McClements DJ, Decker EA. 2007. Role of physical structures in bulk oils on lipid oxidation. *Crit Rev Food Sci Nutr.* **47**, 299-317. https://doi.org/10.1080/10408390600754248

Einstein A. 1905. Über die von der molekularkinetischen Theorie der Wärme geforderte Bewegung von in ruhenden Flüssigkeiten suspendierten Teilchen. *Ann. Phys.* **17**, 549. https://doi.org/10.1002/andp.19053220806

Einstein A. 1956. Investigations of the Theory of Brownian Movement. Dover Publications, 119 pp.

European Commission, 1991. Commission Regulation (EEC) Ner 2568/91 of 11 July 1991, on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. 128 pp.

Frega N, Mozzon M, Lercker G. 1999. Effects of free fatty acids on oxidative stability of vegetable oil. *J. Am. Oil Chem. Soc.* **76**, 325. https://doi.org/10.1007/s11746-999-0239-4

Fregapane G, Lavelli V, Leon S, Kapuralin J, Salvador MD. (2006). Effect of filtration on virgin olive oil stability during storage. *Europ. J. Lipid Sci. Tech.* **108**, 134–142. https://doi.org/10.1002/ejlt.200501175

García-González DL, Infante-Domínguez C, Aparicio R. 2013. Tables of olive oil chemical data, in Aparicio R, and Harwood J (Eds.) *Handbook of olive oil: Analysis and properties*, Springer, New York, pp. 739–768.

†Gómez- Herrera C. 2007. Matter transfer during virgin olive oil elaboration. *Grasas Aceites* **58** (2), 194–205. https://doi.org/10.3989/gya.2007.v58.i2.85

Koidis A, Boskou D. 2006. The contents of proteins and phospholipids in cloudy (veiled) virgin olive oils. *Europ. J. Lipid Sci. Tech.* **108**, 323–328. https://doi.org/10.1002/ejlt.200500319

Lercker G, Frega N, Bocci F, Servidio G. 1994. “Veiled” extra-virgin olive oils: Dispersion response related to oil quality. *J. Am. Oil Chem. Soc.* **71**, 657–658. https://doi.org/10.1007/s11746-999-0239-4

Nelson DL, Cox MM. 2017. Lehninger principles of biochemistry. 7th. ed. W. H. Freeman and Co. New York. 1340 pp.

Papadimitriou V, Dulle M, Wachter W, Sotiroudis TG, Glater O, Xenakis A. 2013. Structure and dynamics of veiled virgin olive oil: Influence of production conditions and relation to its antioxidant capacity. *Food Biophys*. **8** (2), 112–121. https://doi.org/10.1007/s11483-013-9286-3

Seddig M. 1908. The measurement of temperature dependency of Brown’s molecular movement. *Physik. Zeitschrift* **9**, 465-468.

Smoluchowski M. 1906. Zur kinetischen Theorie der Brownschen Molekularbewegung und der Suspensionen. *Ann. Phys.* **21** (14), 756-780. https://doi.org/10.1002/andp.19063261405

Stefanoudaki E, Williams M, Harwood J. 2010. Changes in virgin olive oil characteristics during different storage conditions. *Europ. J. Lipid Sci. Tech*. **112**, 906–914. https://doi.org/10.1002/ejlt.201000066

Tsdimidou MZ, Georgiou A, Koidis A, Boskou D. 2005. Loss of stability of ‘‘veiled’’ (cloudy) virgin olive oils in storage. *Food Chem.* **93**, 377–383. https://doi.org/10.1016/j.foodchem.2004.09.033