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Tentative application of compositional data analysis to the fatty acid profiles of green Spanish-style Gordal table olives

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ABSTRACT

Conventional multivariate and compositional data analyses were applied tentatively to investigate the effects of processing steps and fat extraction systems on the fatty acid profiles of green Spanish-style Gordal table olives. In the first case, the profiles (expressed in percentages) were subjected directly to a conventional statistical study. Secondly, the profiles were regarded as data in the Simplex space and subjected to its specific tools or transformed into coordinates in the Euclidean space to be analysed by conventional multivariate techniques. Exploratory classical and compositional data analyses showed that the main changes were due to fermentation. Tentative cluster and PCA analyses using the compositional coordinates in the Euclidean space led to a more realistic segregation among treatments than applying percentages. Overall, the results suggested that the fatty acid profiles of table olives should be considered as compositional data and analysed accordingly.

1. Introduction

According to the Commission Implementing Regulation (EU) 2015/1833 (12 October 2015), amending Regulation (ECC) No 2568/91, the content of a given component in olive oil, expressed as a percentage by mass of methyl esters, should be calculated by determining the percentage represented by the area of the corresponding peak relative to the sum of the areas of all peaks, using the formula:

$$w_i = (A_i/\sum A) \times 100$$

where A_i is the area under the peak of the individual fatty acid methyl ester i and ΣA is the sum of the areas under all the peaks of the individual fatty acid methyl esters. Furthermore, the characterization of the different categories of olive oils is based, among other parameters, on the percentages of myristic, linolenic, arachidic, eicosenoic, behenic, lignoceric, total transoleic isomers, and total translinoleic + translinolenic isomers (Commission Delegated Regulation (EU) 2015/1830). Chemometric analyses are frequently applied to the fatty acid composition of oils and fats with diverse aims. Diraman (2010) applied this methodology to the fatty acid composition of oils extracted from domestic and foreign olive cultivars grown in the National Olive Collection Orchard of Turkey for their separation into two and three groups, respectively. Kammoun and Zarrouk (2012) used exploratory chemometric characterization to differentiate oils from Tunisian olive cultivars, based on their lipid and sterol profiles. Portuguese varietal olive oils were correctly identified using their fatty acid, triacylglycerol, and phytosterol compositions (Amaral, Mafra, & Oliveira, 2010). Principal Component Analysis (PCA) was selected to compare the fat composition of *Pinus pinea* L. populations cultivated around the Mediterranean basin; the authors arrived at the conclusion that their fatty acid profiles were not influenced by production area (Nasri, Khaldi, Fady, & Triki, 2005). Giuffré (2013) found significant differences in the wax contents among diverse Italian oils. For most cultivars, there was a significant increase in wax (except Leccino and Pendolino) during olive maturation (Giuffrè, 2014). Chemometric methods, using traditional multivariate analysis, were also applied to study differences in the fatty acid compositions among several Spanish-cultivar table olive trade preparations (López, Montaño, García, & Garrido, 2006). Most of the fatty acids, triacylglycerols, diacylglycerols, and monoacylglycerols in Manzanilla and Hojiblanca fats underwent significant changes during their processing as ripe olives (López-López, Rodríguez-Gómez, Cortés-Delgado, Montaño, & Garrido-Fernández, 2009).

The standard statistical methods mentioned above are developed for data from the Euclidean space. However, the values expressing the fatty acid profiles of oils could be compositional data, characterised by being always positive and by summing together a constant value (Aitchison, 1986). The bases for compositional data analysis were established in the 1980s by Aitchison, who realised that compositions provide information about relative, not absolute, values of components. As a result, any statement about a composition should be established in terms of ratios of components (Aitchison, 1981, 1982, 1983, 1984). This new concept led to the development of several log-ratio transformations,

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which apparently solved the problems related to the statistical analysis of compositional data and, initially, enjoyed a wide acceptance. However, it was soon discovered that compositional data belong to the Simplex metric space (Pawlowsky-Glahn & Egozcue, 2001) and should be represented and interpreted in it. In any case, this possibility does not prevent the use of some transformations (Pawlowsky-Glahn, Egozcue, & Tolosana-Delgado, 2015), which allow converting the compositional data into coordinates in the Euclidean space, where they can be analysed with the habitual battery of current multivariate methods. Detailed information on the mathematical and statistical handling of compositional data may be found elsewhere (Aitchison, 1986; Pawlowsky-Glahn & Buccianti, 2011; Pawlowsky-Glahn et al., 2015). Therefore, according to the recently developed compositional concepts, fat and oil compositions are usually incorrectly studied, at least formally, by applying statistical tools developed for the Euclidean space to their fatty acid compositions (expressed in percentages).

Preservation of olive fat quality is essential due to the important role fatty acids play in human health. Clinical observations in Norway during World War II associated change in the diet of that country with a sharp decline in cardiovascular death and the decline of post-operative thrombosis, which returned to the pre-war levels when the usual diet of the population was restored (Malmros, 1950). The conclusion of the Seven Countries Study also showed that saturated fat and plasma cholesterol correlated with cardiovascular diseases (Keys et al., 1980). However, Knapp (1997) pointed out the importance of developing appropriate tools to examine haemostasis in vivo so that the mechanisms and eventual clinical utility of dietary fatty acids could be elucidated. Then, further studies moved from speculation to solid evidence that n-3 fatty acids were not only essential nutrients but also favourably modulate many diseases (Connor, 2000). Undoubtedly, olive oil and the Mediterranean diet (in which table olives are included) are closely associated with long life expectancy and low risk of chronic diseases (cancer, diabetes, and heart disease) (Huang & Sumpio, 2008).

The world table olive consumption was 2,480,000 tonnes in the 2014/2015 season (IOC, 2016). The best-known trade preparation is the so-called green Spanish-style table olive which accounts for ~60% of the total production. Because of its large size, appropriate fermentation behaviour, and multiplicity of stuffing alternatives, Gordal is one of the most appreciated cultivars for this process (Garrido-Fernández, Fernández-Díez, & Adams, 1997).

In this work, the effect of processing and extraction system on the fatty acid profiles of green Spanish-style Gordal table olives were studied by either the conventional statistics techniques using percentages or by the specific exploratory tools developed for compositional data. In addition, the standard multivariate techniques were applied directly to fatty acid percentages or coordinates in the Euclidean space (derived from compositions). Finally, the hypothesis of considering fatty acid profiles as compositional data was validated by comparing the results obtained by the classical studies and the new compositional statistical strategies.

2. Materials and methods

2.1. Cultivar

The fruits of this work, supplied by JOLCA S.L. (Huevar, Sevilla, Spain), were of the Gordal cultivar, harvested in the middle of September at the green maturation stage, which corresponds to a Maturity Index = 1 according to Ferreira (1979). The fruits were subjected to processing 24 h after their hand picking.

2.2. Processing

The olives were processed in duplicate, according to the green Spanish-style, in the pilot plant facilities at Instituto de la Grasa (Sevilla), using fibreglass fermenters (21 kg olives and 14 L solution). The debittering process was performed with an 18 g/L NaOH solution, which penetrated 2/3 of the flesh. The olives were then washed (~18 h) with tap water to remove excess alkali from the flesh and finally immersed in an 110 g NaCl/L brine solution. The physicochemical analysis of brines was carried out using the standard methods developed for table olives (Garrido-Fernández et al., 1997). After fermentation for eight months, 10 kg olives from each replicate were packaged in glass containers, using a fresh brine with characteristics estimated according to the procedures described in Garrido-Fernández et al. (1997); the rest of the fermented olives were used for fat extraction by Abencor and Soxhlet. The olive packages were stabilised by pasteurisation (15 min at 80 °C), and stored at room temperature (22 ± 2 °C). In this way, the processing and packaging conditions of these olives were similar to those followed at industrial scale while the storage mimicked their eventual commercial shelf-life. After storage for two months, the packaged olives were also subjected to fat extraction by Abencor and Soxhlet.

Samples (~5 kg olives) for fat analysis were withdrawn in duplicate from i) the fresh Gordal (RM), ii) each of the replicates of the fermented fruits extracted by Abencor (FO) and Soxhlet (FOS), and iii) packaged olives, also extracted by Abencor (PO) and Soxhlet (POS). The total number of samples was ten and each of them was composed of about 1250 olives. Therefore, these fruits should be considered as composite samples able to properly reflect the changes in fat composition produced by the diverse treatments.

2.3. Fat extraction

2.3.1. Extraction by Abencor

Oils from the fresh, fermented, and packaged olives were obtained by the procedure followed to estimate oil yield in olives intended for mills (Martínez, Muñoz, Alba, & Lanzón, 1975), although with manually pitted olives (to prevent mixing the oil from the pulp with the oil from the seed, which is not ingested by consumers). The pitted fruits were homogenised with an Ultra-Turrax T25 (IKA-Labortecnik, Staufen, Deutschland), the paste was mixed with hot water (mixture temperature ~30 °C), and the mixture subjected to malaxation for 40 min at room temperature (22 ± 2 °C). The suspension was centrifuged in the Abencor equipment (Abencor Suministros S.A., Sevilla) and the collected liquids were then transferred to a measuring cylinder to allow separation of the phases. Finally, the oils were recovered, filtered and subjected to fatty acid analysis.

2.3.2. Extraction by Soxhlet

Fermented and packaged olives were also extracted by Soxhlet to introduce a new source of variability among samples. For this method, three aliquots (25 g) of the homogenised paste were weighed (0.1 mg accuracy), freeze-dried (Laboratory Freeze-Dryer, Telstar Cryodos) until constant weight, and the fat was separated by extraction with hexane in Soxhlet for 6 h. Then, the solvent was removed in a rotary evaporator at 40 °C, and the residual oil was dried under vacuum in an oven at 60 °C until constant weight (AENOR, 2001).

2.4. Analysis of fatty acid methyl esters

Fatty acid profiles were obtained through analysis of their methyl esters (FAMEs) by GC. For methylation, 100 mg oil were mixed with 0.2 mL of 2 N methanolic potassium hydroxide, according to the procedures recommended in the Commission Implementing Regulation (EU) No 2015/1833. The fatty acid methyl esters were quantified in a Hewlett-Packard 5890 series II gas chromatograph, using a fused silica capillary column Select FAME (100 m × 0.25 mm, 0.25 µm film thickness) (Varian, Bellefonte), a flame ionization detector, and a reference standard of saturated and unsaturated fatty acid methyl esters (FAME Mix C4-24). Details of the procedure can be found elsewhere (López-López, Rodríguez-Gómez, Cortés-Delgado, García-