SHORT COMMUNICATION

FATTY ACIDS METHYL AND ETHYL ESTERS BEHAVIOUR DURING OLIVES PROCESSING BY MEANS OF TECHNOLOGICAL COADJUVANTS

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ABSTRACT

Recently, the quantification of fatty acids alkyl esters has become mandatory for the extra virgin olive oil classification. However, the behaviour of such metabolites, during olives processing, is not yet well understood. Thus, the present paper aims to point out the influence of the use of calcium carbonate on the fatty acids alkyl esters content of the oils. The results showed that the content of fatty acids alkyl esters was significantly influenced by the technological coadjuvant. The use of calcium carbonate led to a general reduction of fatty acids alkyl esters compared to the untreated samples. Methyl esters of fatty acids were more susceptible to the use of processing aid than the ethyl esters.

Keywords: alkyl esters, extra virgin olive oil, olives processing

1. INTRODUCTION

According to the EC Regulation (OFFICIAL JOURNAL OF THE EUROPEAN COMMUNITIES, 2001), virgin olive oils are "oils obtained from the fruit of the olive tree solely by mechanical or other physical means under conditions that do not lead to alteration in the oil, which have not undergone any treatment other than washing, decantation, centrifugation or filtration, to the exclusion of oils obtained using solvents or using coadjuvants having a chemical or biochemical action, or by re-esterification process and any mixture with oils of other kinds". Such statement does not take into account the quality of the raw material that, instead, represents one of the main factors, together with the extraction process, affecting the final product quality.

To classify virgin olive oils, several parameters must be checked, some of which have very good correlation with the raw material features (SALVADOR *et al.*, 2001; KOPRIVNJAK *et al.*, 2010). The EU Regulation 61/2011 (OFFICIAL JOURNAL OF THE EUROPEAN UNION, 2011) added the determination of methyl and ethyl esters of fatty acids, generally recognised as fatty acids alkyl esters (FAAE), to the list of parameters to be checked for classifying the quality levels of virgin olive oils. Later, the EU Regulation 1348/2013 (OFFICIAL JOURNAL OF THE EUROPEAN UNION, 2013) stressed attention only on the fatty acids ethyl esters (FAEE). Alkyl esters originate from the esterification of fatty acids and low molecular weight alcohols, methanol and ethanol, respectively arisen from the progressive pectin degradation during the olive ripening and/or by the bad and/or prolonged storage of drupes (BIEDERMANN *et al.*, 2008; Pérez-Camino *et al.*, 2008). Thus, the presence of FAAE in oils became an established marker of the quality of the raw material employed.

The knowledge about the FAAE is continuously growing. Indeed, several researches have been carried out, focused on the correlation between FAAE and the raw material quality (CERT, 2006; MARIANI and BELLAN, 2008); the virgin oil sensory characteristics (BIEDERMANN *et al.*, 2008; GÓMEZ-COCA *et al.*, 2012); the olive pomace oil storage-related changes (RUIZ-MÉNDEZ and RAMOS-HINOJOSA, 2003); the olive storage conditions (JABEUR *et al.*, 2015) and, recently, on the oil storage temperature and substrate availability (GÓMEZ-COCA *et al.*, 2016) and on the influence of the washing process (ALCALÁ *et al.*, 2016).

Virgin olive oil producers try to reach the maximum yield during the extraction process while saving, at the same time, the nutritional, functional and organoleptic features that distinguish virgin olive oil from other oils. Several attempts have been done aiming to the improvement of the extraction yield, mostly on those cultivars, which give the so-called "difficult pastes" (DI GIOVACCHINO and MASCOLO, 1988). The most common strategies adopted regard: i) malaxation time and temperature increase (STEFANOUDAKI *et al.*, 2011); ii) use of technological coadjuvants able to break down the water-oil emulsions (CAPONIO *et al.*, 2016a; CAPONIO *et al.*, 2016b; SQUEO *et al.*, 2016).

As far as we know, no information are available about the evolution of the FAAE as a consequence of the use of processing aids, such as calcium carbonate, during the extraction process. Considering that the "extra" quality virgin olive oils have the highest price on the market and thus even the profit of the producers and farmers are linked to the compliance with the current legal limits, it is clear that such information could be very useful for both the operators as well as the lawmakers. Hence, the aim of this work was the assessment of the ethyl esters and methyl esters of fatty acids in extra virgin olive oils in regard to the use of calcium carbonate during the malaxation step.

2. MATERIALS AND METHODS

2.1. Sampling and experimental plans

All the experimental trials were carried out on olives (Coratina cultivar) milled within 24 hours after the harvest in industrial olive mills.
For studying the effect of the calcium carbonate, two olive lots, having 0.51 (lot A) and 1.40 (lot B) pigmentation index (*Pi*), calculated as reported in SQUEO *et al.* (2016), were divided in 14 homogeneous batches of about 300 kg. Two batches were processed without any treatment (controls) while the others (two batches per each treatment) were processed by using two different types of calcium carbonate (average particle size of 2.7 μm, Ca2, and 5.7 μm, Ca5, respectively) at three percentages of addition respect to the olives paste weight (1-2-4%). The full experimental plan was reported in Table 1.
Calcium carbonate was kindly furnished by Omya Spa (Milan, Italy). After being weighted, the coadjuvant was directly and gradually added into the malaxer at the begin of the malaxation stage without stopping the machine.

Table 1. Experimental plan for olive lots A and B.

Coadjuvant typology	Level of addition (%)	Trial name*
None	None	C
Calcipur®2	1	Ca2-1%
Calcipur®2	2	Ca2-2%
Calcipur®2	4	Ca2-4%
Calcipur®5	1	Ca5-1%
Calcipur®5	2	Ca5-2%
Calcipur®5	4	Ca5-4%

*Each trial was repeated twice.

2.2. Alkyl esters analysis

The analyses of the methyl and ethyl esters of fatty acids were carried out according to the official method (OFFICIAL JOURNAL OF THE EUROPEAN UNION, 2011). The gas chromatographic system was made up of a 7890B Agilent Technologies (Santa Clara, CA, USA) chromatograph equipped with a flame ionization detector (FID). The column used was a capillary fused silica DB-5HT (length 15 m, i.d. 0.32 mm, film thickness 0.10 μm). The operating conditions were as follows: oven temperature, 80 °C for 1 min and then increased at 20 °C min^{-1} to 140 °C, then increased at 5 °C min^{-1} to 335 °C and maintained for 20 min. The detector temperature was 350 °C. Helium was used as the carrier gas, with a flow through the column of 2 mL min^{-1} in splitless mode.

2.3. Statistical analysis

ANOVA and Tukey post-hoc test for multiple comparisons were carried out on the experimental data by means of Minitab 17 software (Minitab Inc., State College, PA, USA).

3. RESULTS AND DISCUSSIONS

All the virgin olive oils obtained in the industrial trials were classified as extra virgin olive oils according to the EU Regulation 1348/2013 (OFFICIAL JOURNAL OF THE EUROPEAN UNION, 2013) (data not shown).

Fig. 1 reports the average amounts of alkyl esters in the samples under study. The contents found were in accordance with those reported in literature (BIEDERMANN *et al.*, 2008; MARIANI and BELLAN, 2008; Pérez-Camino *et al.*, 2008) and lower than law limits. Overall, a gradual decrease of FAAE than controls (C) was observed as the percentage of calcium carbonate increased. A good discrimination was observed in particular between the samples of the 4% addition trial and those without (C) or with the lowest additions (1%). Moreover, the FAEE content was generally higher than the amount of fatty acids methyl esters (FAME).

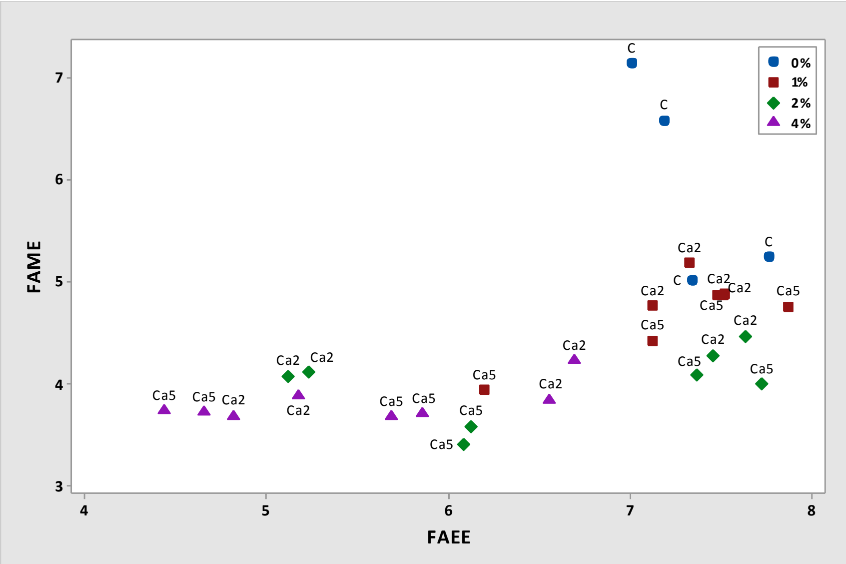

Figure 1. Fatty acids methyl esters (FAME) versus fatty acids ethyl esters (FAEE) (mg kg^{-1}) detected in the samples. Olive processed without calcium carbonate addition (C, control) and with the addition of 1%, 2%, and 4% of calcium carbonate. Ca2, Calcipur®2 with particle size of 2.7 μm; Ca5, Calcipur®5 with particle size of 5.7 μm (Squeo *et al.*).

The differences observed are clearly shown by Fig. 2, in which the differences of means, and the respective 95% confidence intervals obtained by the Tukey post-hoc test, are reported in regard to the variables *percentage of addition* and *type of coadjuvant*. Overall, the differences of means between the treatments and the control shown that the treated oils were poorer in FAEE (Fig. 2 A-D).

Considering the percentages of addition (Fig. 2 A-B), the differences between treatments and control were significant in all cases for the FAME, while only 4% of addition led to significantly lower amounts of FAEE compared to control. Among the treatments, significantly lower amounts of FAME were found using 2% and 4% of the coadjuvant respect to 1%. No significant differences have been highlighted between 2% and 4% addition (Fig. 2A). As regards the FAEE, a significant difference was reported for the 4%, as compared to 1% of addition (Fig. 2B).

The coadjuvant granulometry (Fig. 2 C-D) induced significant differences only for the FAME, with a more noticeable effect of the Ca5 than the Ca2 respect to the control. No statistical difference was highlighted between the different types of calcium carbonate adopted for both the FAME and the FAEE. Overall, our findings underline that the use of calcium carbonate in the olive oil mill causes a reduction of the amounts of FAAE, that seems to be proportional to the amount of processing aid employed, whereas the type of coadjuvant did not show any significant effect.

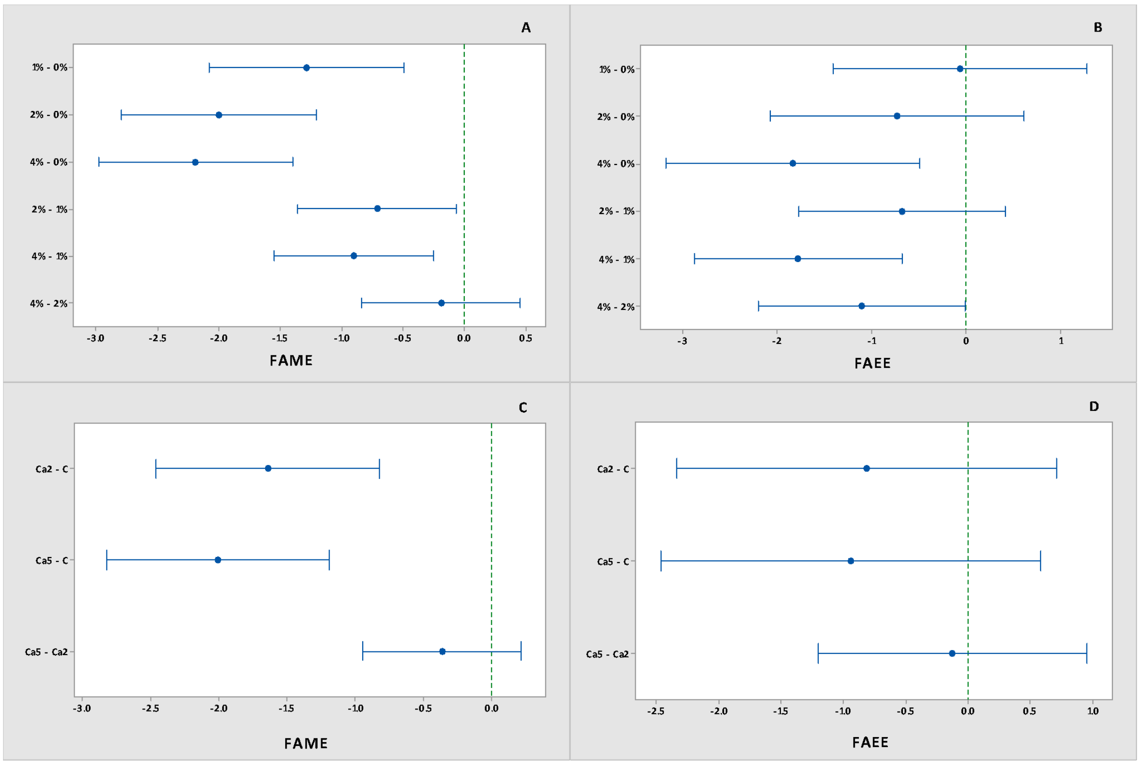

Figure 2. Tukey 95% Confidence Intervals for the differences of means for the calcium carbonate trials for both the olives lots. A) means differences in FAME as a function of the percentages of addition; B) means differences in FAEE as a function of the percentages of addition; C) means differences in FAME as a function of the type of coadjuvant; D) means differences in FAEE as a function of the type of coadjuvant (Squeo *et al.*).

It is known that the synthesis of the alkyl esters occurs in an acid environment and is catalysed by enzymes and temperature (PÉREZ-CAMINO *et al.*, 2003). In such conditions, methanol and ethanol react with fatty acids (mainly oleic acid) giving rise to the FAAE. The lowering in the content of these metabolites, observed in the treated oils, might be due to the interference on the enzymatic activity exerted by processing aids during the malaxation step or, more simply, by an absorption of the low weight alcohols, more evident for the methanol.

4. CONCLUSIONS

Our findings showed that the content of the fatty acids alkyl esters could be affected by the technological strategies adopted during olives processing into oil. In particular, the use of technological coadjuvants, aimed to increase the extraction yield, influences the amount of such compounds, bringing to a reduction. Anyway, this is more evident for the methyl esters, while a weaker influence has been observed on the ethyl ester amount. Further studies should be carried out in order to confirm these preliminary results and understanding deeply the FAAE behaviour in the olive oil matrix. As regards the economic point of view, the use of such processing aid will be easily faced by the producers considering a cost increase of about 0.40 € kg^{-1} of oil in the face of an extraction efficiency increase of about 4%.

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