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Influence of O_2 on Extra Virgin Olive Oil Fatty Acids Composition during Malaxation

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Many variables are related to extra virgin olive oil (EVOO) quality and, therefore, to its polyphenols content and fatty acids composition including the steps that lead to EVOO extraction, considering that it is mainly contained in the vacuoles of the fruit mesocarp. The main steps of EVOO production are: harvest, crushing, malaxation, centrifugation, storage and filtration. Considering the olive oil extraction procedures, many studies have been conducted in recent years on oil mill plant and processes for improving EVOO quality. Malaxer is the most studied machine among all; it is responsible for malaxation, which represents a very important and critical step in the EVOO extraction process. Many studies focus on the control of oxygen in the malaxer headspace, with the aim of determining its influence on EVOO quality evaluating volatile and phenolic components. Considering these factors, the main objective of the present study was to evaluate the influence of the malaxer headspace oxygen concentration on EVOO fatty acids composition from cv. Nocellara del Belice olives. The results show that oxygen content in the malaxer headspace, in different time-points and concentrations during the process, influences EVOO fatty acids composition.

1. Introduction

Recent studies have confirmed that EVOO interacts on the prevention of the metabolic syndrome as hypercolesterolemia, hypertriglyceridemia, high blood pressure, obesity, fatty liver and insulin resistance, all closely linked to diabetes and coronary heart disease (Esposito et al., 2010; Pérez-Martinenz et al., 2011). It is generally considered to be a major contributor to human health in the Mediterranean area (Carluccio et al., 2007; Covas, 2008; Covas et al., 2009). It has been confirmed that some chemical components of EVOO, polyphenols and oleic acid, are inhibitors of the vascular endothelial growth factor signalling pathway (Lamy et al., 2014). These results underscore the chemopreventive properties of EVOO and highlight the importance of nutrition in cancer prevention. Many variables are related to extra virgin olive oil (EVOO) quality and, therefore, to its polyphenols content and fatty acids composition including the steps that lead to EVOO extraction, considering that it is mainly contained in the vacuoles of the fruit mesocarp. The main steps of EVOO production are: harvest, crushing, malaxation, centrifugation, storage and filtration. Malaxer is the most studied machine among all; it is responsible for malaxation, which represents a very important and critical step in the EVOO extraction process (Selvaggini et al., 2014). The main parameters studied concerning the malaxation process are temperature, time and oxygen in the headspace of the machine. Time and temperature have been exhaustively studied, while oxygen, defined as the third important process parameter, needs further investigation as recently stated many authors (Aiello et al., 2012; Leone et al., 2014; Jimènez et al., 2014; Selvaggini et al., 2014; Servili et al., 2008). Moreover, process monitoring and control are fundamental requirements in the modern EVOO processing industry (Carrara et al., 2008; Catania et al., 2013b; Tamborrino et al., 2014). In a previous study (Catania et al., 2013a) the authors developed an innovative monitoring system aimed at continuously measuring oxygen concentration during the malaxation process of a very important Italian cultivar, Nocellara del Belice (Catania et al., 2014). Malaxation carried out in an oxygen free atmosphere for the first 25 min followed by the presence of oxygen until the end of the process enhanced volatile compounds in EVOOs, without compromising the phenolic composition.
Therefore, it was decided to conduct further research studying aspects related to the fatty acid composition of the EVOO obtained (Catania et al., 2015 and 2016). In fact, fatty acids have important implications from the

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nutraceutical point of view as anti-cancer and cholesterol-lowering, stimulate the immune system and prevent the onset of diabetes and chronic non-communicable diseases.
EVOO is mainly composed of triglycerides (98-99%), made up of glycerol and three molecules of fatty acids, and from minor components. The average composition of the most important fatty acids found in EVOO is formed by 9-14 % saturated fatty acids (palmitic acid, stearic acid), 66-80 % monounsaturated fatty acids (palmitoleic acid and oleic acid) and 6-10 % polyunsaturated fatty acids (linoleic acid and linolenic acid). An excess of saturated fatty acids in the blood of the human organism (above 10%) reduces efficiency and number of membrane receptors which are responsible for recognizing the specific proteins of low-density lipoprotein (LDL); LDL have the function of carrying 50% of blood-cholesterol (Viola and Viola, 2014). An excess of polyunsaturated fatty acids in the human organism start peroxidative processes with production of free radicals that oxidize LDL via chain reactions. Therefore, saturated, monounsaturated and polyunsaturated fatty acids play important structural and functional roles in the human organism.
The aim of this work was to evaluate the influence of oxygen in the malaxation machine headspace on Nocellara del Belice EVOO fatty acids.

2. Materials and methods

2.1 Experimental olive oil mill plant

The experimental tests were performed in 2013 employing an Alfa Laval oil mill plant on a typical Sicilian olive variety, “Nocellara del Belice”, manually harvested and processed within 24 hours from the harvesting. The oil mill plant was operated in continuous mode and it was equipped with an olive washing machine, a disk crusher, a single-stage malaxation machine, a horizontal decanter, and a vertical centrifuge. After washing, olives were processed with a disk crusher; then the malaxation was performed in a close system for 45 minutes at a temperature of 27°C. The extraction was performed with a triphasic centrifugal extractor with no water addition. Oil samples were collected after each test, put in 100 mL dark glass bottles, stored at 12 °C and transported to the laboratory where analyses were performed. The malaxation machine used in the tests was the Alfa Laval Atmosphera 650 with a capacity of 650 L, featuring a stainless steel and airtight cylinder. The machine was equipped with a pair of inlet valves for gas in order to achieve a controlled or modified malaxation atmosphere by blowing nitrogen or oxygen in the headspace of the machine and a probe for olive paste temperature control. The machine has a gap over the entire inner surface of the tank in which hot water is circulated in order to control olive paste temperature. A rotary double bladed reel with spiral inside the machine realizes the olive paste mixing and removes it from the walls avoiding overheating. Paste loading and unloading operations are carried out by means of automatic valves.

2.2 Real time monitoring system

The measurement system has been described in Catania et al. (2013a). The oxygen concentration inside the malaxation machine is sampled by means of a gas extraction system that continuously circulates the gas inside the malaxation machine through a closed loop pipe where the oxygen sensor is located. Thus, the oxygen monitoring circuit consists of a pipeline, a gas pump, a filter and an oxygen sensor.

2.3 Experimental tests

The experimental tests consisted of performing the malaxation process in four different configurations. The atmosphere inside the malaxation machine was modified by blowing nitrogen or oxygen (pure gases) by cylinders in the mixing chamber at specific stages of the process. In all the tests except T_C (control), the malaxation machine was filled with nitrogen before the entry of the olive paste. The following test configurations were thus performed (Table 1).
The drupes were completely healthy and had the same degree of ripeness. The variable applied in the different tests was atmosphere composition in the malaxation chamber headspace, which was altered by blowing nitrogen and/or oxygen at different times during the process.
Test T_C, the control, was conducted without changing the gaseous component in the headspace of the machine. Tests $T_{5\text{-}15}$, $T_{5\text{-}25}$ and $T_{5\text{-}35}$ were carried out by blowing 5 L of oxygen at different timepoints of the process and precisely after 15, 25 and 35 minutes, respectively, from the beginning and keeping constant the percentage of oxygen until the end. Tests $T_{30\text{-}15}$, $T_{30\text{-}25}$ and $T_{30\text{-}35}$ were carried out by blowing 30 L of oxygen at the same time of the previous tests.
Moreover, in these tests, nitrogen for food was introduced immediately after filling and before the start of mixing, thus eliminating the low amount of oxygen present in the head space of the malaxation chamber. This was done to evaluate the sole effect of oxygen insufflation at different times of malaxation on EVOO fatty acids.

Table 1: Tests used in the experimentation. Pure oxygen was inflated by cylinders at different stage during malaxation

Test	Description
T_C (control)	Malaxation in un-modified atmosphere
$T_{5\text{-}15}$	5 L of oxygen introduced 15 min after malaxation start
$T_{5\text{-}25}$	5 L of oxygen introduced 25 min after malaxation start
$T_{5\text{-}35}$	5 L of oxygen introduced 35 min after malaxation start
$T_{30\text{-}15}$	30 L of oxygen introduced 15 min after malaxation start
$T_{30\text{-}25}$	30 L of oxygen introduced 25 min after malaxation start
$T_{30\text{-}35}$	30 L of oxygen introduced 35 min after malaxation start

In all the tests, the filling of the malaxation machine lasted 10 min. The dissolved oxygen measurements in the malaxation machine were performed every 30 seconds. Each test configuration was replicated three times.

2.4 FAME (Fatty Acid Methyl Esters) analytical determinations in EVOO

Fatty acids in olive oil samples (100 mL) were directly methylated with 2 mL of 0.5 M NaOCH3 at 30 °C for 15 min, followed by 1 mL of 5 % HCl in methanol at 50 °C for 15 min. Fatty acid methyl esters (FAME) were recovered in hexane (1.5 mL). One microliter of each sample was injected by autosampler into an HP 6890 gas chromatography system equipped with a flame-ionization detector (Agilent Technologies Inc., Santa Clara, CA). Fatty acid methyl esters from all samples were separated using a 100 m length, 0.25 mm i.d., 0.25 µm capillary column (CP-Sil 88; Chrompack, Middelburg, the Netherlands). The injector and the detector temperature were kept at 255 °C and 250 °C respectively, with an H_2 flow of 40 mL min^{-1}, air flow of 400 mL min^{-1}, and a constant He flow of 45 mL min^{-1}. The initial oven temperature was held at 70 °C for 1 min, increased at 5 °C min^{-1} to 100 °C, held for 2 min, increased at 10 °C min^{-1} to 175 °C, held for 40 min, and then finally increased at 5 °C min^{-1} to a final temperature of 225 °C and held for 45 min. Helium, with a head pressure of 158.6 kPa and a flow rate of 0.7 mL min^{-1} (linear velocity of 14 cm s^{-1}), was used as the carrier gas. Fatty acid methyl ester standard solution in hexane mix solution was used to identify each FA. To quantify total FA, C23:0 (Sigma-Aldrich) was added to each sample (4 mg g^{-1} of oil) as the internal standard.

2.5 Statistical analysis

The EVOO analyses were performed on three EVOO samples for each test within one week from extraction. The data were subjected to the Student "t" test for mean comparison at the 95% confidence level (Statgraphics Centurion, Statpoint Inc., USA, 2005). Principal component analyses (PCA) were applied to investigate the relationships between the considered experimental variable and the amount of SFA, MUFA and PUFA. The amount of each compound was considered as the dependent variable of the considered experimental parameters. The obtained principal components were considered as significant if their Eigen values were >1. PCA was carried out using Statistica 6.0 for Windows (Stat Soft Italia).

3. Results and discussion

Table 2 shows the percentages values of fatty acids obtained in the different tests.
In the tests where oxygen concentration in the headspace of the malaxer was monitored and controlled, SFA values were lower than those obtained in the control (T_C). In particular, in the tests where 5 litres of oxygen were inserted inside the machine, we obtained hexadecanoic acid (palmitic acid) values 16% lower than T_C. Benoit et al. (2009) showed that palmitic acid would cause the man obesity as a result of resistance to insulin and leptin production, which are responsible for satiety. In the tests performed inflating 30 litres of oxygen, hexadecanoic acid values are similar to T_C. Ocatdecanoic acid (stearic acid) had a per cent decrease in all the tests compared to T_C. Docosanoic acid (behenic acid) also shows a 20% decrease in $T_{30\text{-}25}$ compared to T_C. Cater and Denke (2001) studied if docosanoic acid was a cholesterol-raising saturated fatty acid in humans; results of this metabolic ward investigation indicate that behenic acid raises cholesterol in humans, and to a greater extent than does hexadecanoic acid (palmitic acid).
As a part of the MUFA 8-Octadecenoic acid (Oleic acid) shows a 7% increase in $T_{30\text{-}25}$ compared to T_C. That was a fundamental standpoint and a high 8-Octadecenoic acid content improved EVOO oxidation resistance that making it more stable during the storage period (Youssef et al., 2010). Therefore, the same values were found with the addition of oxygen during 25 or 35 minutes. Youssef et al. (2013) studied the effect of malaxation time on fatty acid composition, announcing that the highest 8-Octadecenoic acid values were obtained in malaxation time of 15 min (67.57) and 30 min (67.89). Longer malaxation time, equal to 45 and 60 min, causes a decrease of 8-Octadecenoic acid content with values of 65.05 and 65.20 respectively.

Table 2: Fatty acids composition SFA (saturated fatty acid), MUFA (monounsaturated fatty acid), PUFA (polyunsaturated fatty acid). Data are the mean values of three independent experiments ± standard deviation

	Tc		T_{5-15}		T_{5-25}		T_{5-35}		T_{30-15}		T_{30-25}		T_{30-35}	
	mean	std	mean	std	mean	std	mean	std	mean	std	mean	std	mean	std
SFA														
Hexadecanoic acid	16.88	8	13.90	5	13.90	6	14.85	42	17.15	54	16.63	35	16.20	45
Heptadecanoic acid	0.00	11	0.00	1	0.00	7	0.00	18	0.09	25	0.07	16	0.00	42
Octadecanoic acid	7.69	5	5.16	12	5.47	12	6.18	14	7.55	21	6.96	11	6.69	13
Eicosanoic acid	1.41	9	0.79	24	0.91	23	1.01	25	1.41	8	1.18	16	1.15	8
Docosanoic acid	0.30	15	0.19	13	0.28	11	0.26	12	0.31	9	0.24	7	0.24	12
Tetracosanoic acid	0.14	18	0.00	21	0.13	7	0.16	15	0.15	32	0.10	52	0.13	3
MUFA														
9-Hexadecenoic acid, (Z)	1.78	1	0.99	1	0.99	7	1.16	1	1.79	1	1.66	4	1.55	4
9-Octadecenoic acid,(Z)	0.15	6	0.00	4	0.00	6	0.11	13	0.15	5	0.13	11	0.13	12
8-Octadecenoic acid	67.95	1	62.86	1	61.93	2	68.54	0	67.62	0	72.49	1	71.24	1
PUFA														
11-14 Eicosenoic acid,	0.90	4	0.93	6	0.90	1	0.87	0	0.89	1	0.75	6	0.79	1
9,12-Octadecadienoic acid (Z,Z)	-		-		6.58	3	-		-		-		-	

Comparing these values with those obtained in our study, although of different olive varieties, it could be confirmed that oxygen monitoring in the malaxation machine headspace allows to obtain higher 8-Octadecenoic acid values, 72.49 and 71.24 in T_{30-25} and T_{30-35} respectively with malaxation time of 45 min. However, the absence of oxygen at the beginning of malaxation improves the EVOO 8-Octadecenoic acid content. Therefore, the system application allows to prolong the malaxation time without compromising the EVOO 8-Octadecenoic acid.

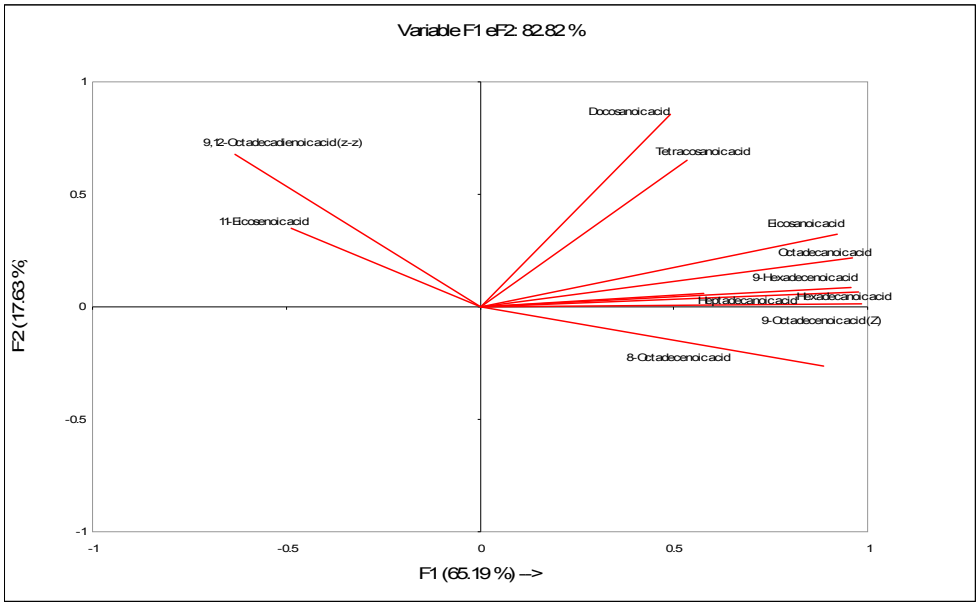

Figure 1: Principal component analyses of fatty acids composition (continue)

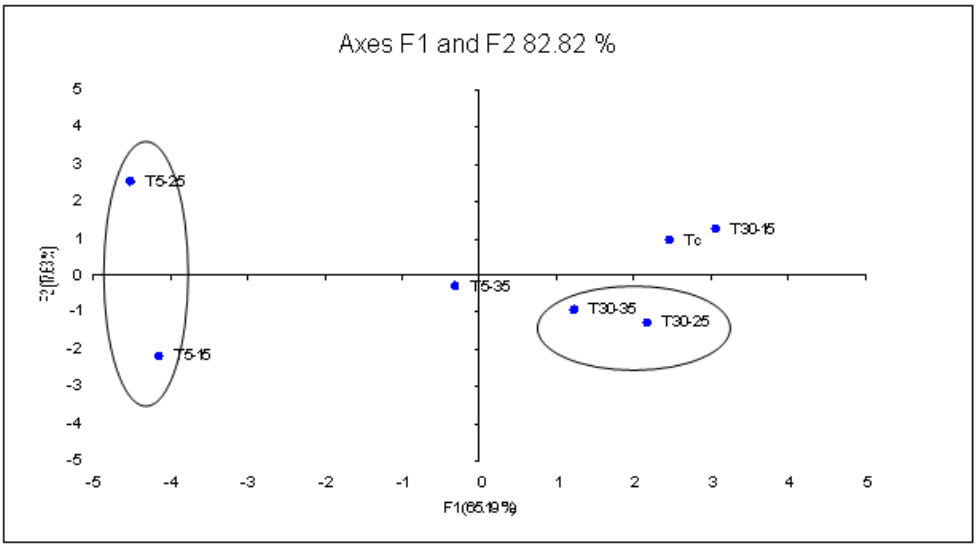

Figure 1: Principal component analyses of fatty acids composition

PUFA values decrease in all the tests were the oxygen concentration was monitored and controlled respect to T_C. PUFA reduce both cholesterol and LDL, but unlike oleic acid, also resulted in a reduction of HDL, the "good cholesterol", which favor its disposal. Furthermore, being more unstable, they quickly oxidize and form dangerous free radicals in the human body. Even experimental studies showed that high levels of polyunsaturated fatty acids adversely affect the antioxidant capacity of plasma, damage DNA of peripheral blood lymphocytes and the serum lipids metabolism (Quiles, et. Al. 2004).
The PCA reduces the number of total variables to only few retaining the major part of the information. The amount of variables was reduced to only two (PC1 and PC2) which retained 82.8% of the total variance (Figure 1).
The EVOO with the highest amount of oxygen treatments retained positive score on PC1. The lowest oxygen treatments were placed in the directly opposite position with respect to the 8-Octadecenoic acid loading. The highest oxygen concentration ($T_{30\text{-}25}$ and $T_{30\text{-}35}$) revealed a positive correlation with nutraceutical properties.

4. Conclusions

The oxygen content in the malaxer headspace in different time-points and it concentrations during the process influences the EVOO fatty acids composition. The results have demonstrated a significant influence of oxygen on fatty acid composition through the implementation of a system which improves an electronic management and control of the oxygen in the headspace of the malaxer. When 30 L of oxygen were inflated SFA, i.e. palmitic and stearic acids significantly decreased compared to the control. The system allowed to control the atmosphere inside the malaxation machine with particular reference both to the amount and to the time-point in which oxygen is blown. The oxygen monitoring and control in the malaxer headspace improve the concentration of fatty acid content of Nocellara del Belice EVOO. The best results were obtained when 30 L of oxygen were blown after 25 minutes from the beginning of the malaxer procedure. The application of the new system during malaxation allow to obtain very interesting results in terms of nutraceutical properties.

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