

Quality losses in virgin olive oil due to washing and short-term storage before olive milling

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ABSTRACT

To identify critical points during olive mill pre-processing operations, the effect of the closed circuit washing stage on the olives microbiological contamination and the influence of the successive short-term storage on olives and VOO quality were evaluated. Microbiological, physical and chemical parameters were assessed in olives and oils at three mill pre-processing stages: reception, washing and short-term storage. Olive washing in closed loop systems was shown to be a critical control point at the olive mill due to microbiological cross-contamination and fruit physical damage. Moreover, when the olives were short-term stored before oil extraction positive VOO sensory attributes decreased by as much as one point of intensity, as justified by the changes observed in phenolic and lipoxygenase derived compounds. These results confirm the high risk of fruit cross-contamination due to the poor hygiene of the water used in olive mills to wash olive, and point out the effect of on VOO quality of a common practice such as short term silo storage of olives.

Keywords: virgin olive oil; olive washing; quality; sensory; microbiota

Running title: Olive mill critical pre-processing operations

1. INTRODUCTION

The concept of critical production steps has recently been applied to virgin olive oil (VOO) production as a tool to ensure the quality of the product [1]. Several critical points, which must be monitored to allow control of the sensory attributes of the olive oil, have been identified from harvesting to VOO storage. Among post-harvest operations prior to oil extraction, storage of the olives is the step that has been most considered. In the past years, several studies have been carried out to evaluate the effect of long time storage on olive oil quality on the quality of the olives and the oils extracted from them [2-7]. The storage periods evaluated range from three days to three weeks at temperatures from 4°C to 20°C. The conclusion to be drawn is that storage conditions are crucial for the quality of VOO. However, in most cases, storage for several days could not usually be considered an option; in order to preserve olive quality until processing for oil extraction, it is recommended that storage be short-term (<24h) [8], in keeping with the mill processing capacity. Although short-term silo storage is a common practice adopted to optimize the processing capacity of mills, little information is available on its effect on olive and oil quality.

In addition to the effects of storage conditions, recent reports indicate that there is a risk of microbiological cross-contamination at olive mills during washing in closed circuits [9-11] and that it is therefore important to control the microbiological quality of olives earmarked for VOO extraction. As recently reviewed by Clodoveo et al. [12], the sensory quality of the oil might be compromised by the effect of microbiological contamination of recycled water used in closed-loop systems. A decrease of bitter, pungent and fruity attributes has been observed by millers in oils from washed olives [13]. The cause of these sensory changes has not been clarified yet, and it requires further research. In a previous study, we report that lactic and enteric bacteria, fungi and *Pseudomonas* were much more prevalent on the surface of olives after washing in closed circuits, with increments in cfu/g of between 2 and 3 orders of

magnitude [11]. Such microbiological activity can affect VOO quality due to fermentation processes during olive storage [5,14] and also during the oil extraction process, where in some cases the effect of olive microbiota on oil characteristics can exceed that of malaxation time and temperature [11]. In view of these results, hygienic practices could be critical for VOO quality.

The present work is aimed to identify critical points or factors during pre-processing operations, in particular when they are carried out according to common practices generally accepted as suitable. To identify critical points during pre-processing operations, the effect of the closed circuit washing stage on the olives microbiological contamination and the influence of a short-term (<12h) storage on olives and VOO quality were evaluated at the scale of the olive mill, by analyzing five batches of Arbequina olives and oils, on different days of the harvesting period. With this aim, microbiological and physical parameters were assessed in olives at three mill pre-processing stages: reception, washing and short-term storage, and sensory, physical and chemical quality indices were determined in the corresponding oils.

2. MATERIALS AND METHODS

2.1. Reagents and materials.

The SPME fiber used as divinylbenzene/carboxen/polydimethylsiloxane 50/30 μ m, 2 cm long (DVB/CAR/PDMS) from Supelco (Bellefonte, PA, USA). Pentanal, 1-penten-3-one, 1-penten-3-ol, hexanal, 4-methyl-2-pentanol, limonene, 2-methylbutan-1-ol, (E)-2-hexenal, hexyl acetate, (Z)-3-hexenyl acetate, 1-hexanol, (Z)-3-hexen-1-ol, nonanal, (E)-2-hexen-1-ol, hexanoic acid were purchased by Sigma-Aldrich (S. Louis, MO, USA).

Chloroform, acetic acid, ethanol, diethyl ether, cyclooctane of spectrophotometric grade, potassium iodide, sodium thiosulfate and sodium hydroxide were from Panreac (Barcelona, Spain).

Mac Conkey agar, MRS agar, Cetrimide agar, yeast extract, casein peptone and Sharpe agar were supplied by Oxoid (Basingstoke, Hampshire, England). Sabouraud-chloramphenicol agar medium was from Sharlau (Barcelona, Spain). Sodium chloride, mannitol, cycloheximide and nisin were purchased by Sigma-Aldrich (St Louis, MO, USA).

2.2. Olives and oil samples.

Five different batches of olives (3000 kg each) of the Arbequina variety, grown in the same geographical area (DO "Siurana", Priorat, Tarragona, Spain), were handpicked and processed in the same industrial mill (Cabacés, Tarragona, Spain) in five distinct dates (**Table 1**). The experiment was carried out 5 times since mid-November to end of December. The olive maturity index at the reception of the above mentioned batches, determined according to the "Estación de Olivicultura de Jaén" [15], is reported in **Table 1**. In addition, it was computed the proportion of damaged fruits in each sample (**Table 1**).

Samples of olives were collected at three different stages before oil extraction: immediately after delivery to the mill (after weighing –step 1); after washing through a 2000 L water closed circuit 'Calero' machine (step 2); and after storing overnight (<12h) in a 4000 kg silo (step 3). Each olive batch was of 3000 kg, and representative samples of 5 kg of olives were obtained at each processing stage by collecting 200g of olives every 15 min during the unloading of the fruits at the reception, after washing, and after silo storage, respectively. VOO was extracted within 6 h after sampling of olives, by a pilot extraction plant, Abencor (Comercial Abengoa S.A., Seville, Spain), equipped with a hammer crusher, a paste beater and a pulp centrifuge. Malaxation was carried out at 30°C for 30 min. The VOOs obtained were then decanted, transferred into dark glass bottles and stored in the dark at 4°C until analysis.

2.3. Olive fruits microbiological profile

To determine the viable-culturable cell number in olives surface, a suspension of 50 g of olives was prepared in 100 mL of sterile water with 0.9% NaCl. After 5 min in the ultrasound bath,

the suspension was serially diluted in 0.9% NaCl, and 100 µL of appropriate dilutions were plated in triplicate. Fungi were evaluated on Sabouraud-chloramfenicol agar; lactic acid bacteria on MRS agar supplemented with 100 mg/L cycloheximide(MRS-C); enteric bacteria on Mac Conkey agar and *Pseudomonas* on Cetrimide agar supplemented with 100 mg/L cycloheximide (Cetrimide-C). The plates were incubated at 30 °C during 3-5 days and viable counts were expressed as log cfu/g olive. Analyses were performed in triplicate.

2.4. Virgin olive oils quality indices and sensory analysis

Free acidity, coefficients of specific extinction at 232 and 270 nm (K_{232} and K_{270}), and peroxide value (PV) of VOO samples obtained from the assay were determined in analytical duplicate according to regulation (EU) No 1348/2013 [16]. The sensory analysis of virgin olive oil samples was carried out according to regulation (EC) No 640/2008 [17] by the Official Tasting Panel of Virgin Olive Oils of Catalonia. This panel relies on ISO17025 accreditation and it is recognized by International Olive Council (IOC). Each oil sample was analyzed by eight tasters scoring the official sensory descriptors within a 10 cm open scale anchored on zero.

For the Rancimat experiments, 3 g of oil were placed in the reaction tube and heated at 120 °C under an air flow of 20 L/h. The oil stability was evaluated by measuring the oxidation induction time (h).

2.5. Virgin olive oil volatiles analysis.

2.5.1. Solid phase microextraction (SPME). Oils' volatile profile was determined according to Vichi et al. [18]. Briefly, 2 g of oil spiked with 4-methyl-2-pentanol (internal standard; final concentration 1.5 mg/kg), was weighed into a 10 mL vial fitted with a silicone septum. The vial was placed into a water or bath fixed at 40 °C, where the sample was maintained under magnetic stirring (700 rpm). After 10 minutes of sample conditioning, a DVB/CAR/PDMS fiber was exposed during 30 min to the oil headspace and immediately desorbed in the gas chromatograph injector. Each extraction was performed in duplicate.

2.5.2. GC-MS analysis. Identification of compounds was performed by gas chromatography coupled to quadrupolar mass selective spectrometry using an Agilent 5973 Network detector (Agilent Technologies, Palo Alto, CA, USA). Analytes were separated on a Supelcowax-10 (Supelco, Bellefonte, PA) 30 m x 0.25 mm ID, 0.25 μ m film thickness. For the analysis of the volatile profile, column temperature was held at 40 °C for 5 min and increased to 200 °C at 4°C/min. The injector temperature was 265 °C and the time of desorption of the fiber into the injection port was fixed at 5 min. Helium was the carrier gas, at a linear velocity of 38 cm/sec. The temperature of the ion source was 175 °C and the transfer line, 280 °C. Positive electron ionization mass spectra (EIMS) were recorded at 70 eV ionization energy, 2 scan/sec. GC-MS analysis was performed in the complete scanning mode (SCAN) in the 40 – 300 m/z range. The identification of compounds in olive and oil samples was carried out by comparison of their mass spectra and retention times with those of standard compounds. Response factors of volatile compounds were calculated by calibration curves constructed by reference substances in refined sunflower oil (range of concentration 0.01-5 mg/kg). When reference compounds were not available, concentrations were expressed as mg equivalents of IS/kg, as indicated in the legends of **Figures 1 and 2**.

2.6. Phenol analysis

Phenolic compounds were determined according to Mateos et al. [19] Briefly, 2.5 g of oil spiked with 0.025 mg of *p*-hydroxyphenylacetic acid and 0.005 mg of *o*-coumaric acid were dissolved in 6 mL of hexane and loaded on a diol-bonded phase cartridge previously conditioned with 6 mL of methanol and 6 mL of hexane. After washing with 6 mL of hexane and 4 mL of hexane:ethyl acetate 90:10 v/v, phenolic compounds were eluted with 10 mL of methanol. After evaporation at room temperature the residue was redissolved in 0.5 mL of methanol: water 1:1.

HPLC analysis was carried out on an Agilent 1200 liquid chromatographic system equipped with a diode array UV detector. A Luna C18(2) column (4.6 mm i.d. x 250 mm; particle size 5 μ m) (Phenomenex, Torrance, CA), coupled to a security guard C18 4 x 3.0 mm (Phenomenex) was used. Elution was performed at a flow rate of 1.0 mL/min, using as mobile phase a mixture of water/acetic acid (97:3, v/v) (solvent A) and methanol/acetonitrile (50:50 v/v) (solvent B). The solvent gradient changed according to the following conditions: from 95% (A)-5% (B) to 70% (A)-30% (B) in 25 min; 65% (A)-35% (B) in 10 min; 60% (A)-40% (B) in 5 min; 30% (A)-70% (B) in 10 min; and 100% (B) in 5 min, followed by 5 min of maintenance. Chromatograms were acquired at 240, 280, and 335 nm. Quantification was performed using the response factors calculated by Mateos et al. [19].

2.7. Statistics

Data were analyzed using the package "Statgraphics Plus 5.1". Differences between olive fruit samples and VOO samples obtained after each pre-processing step (1=reception; 2=washing; 3=storage <12h) were studied by analysis of variance (one-way-ANOVA). Significant results were considered at $p < 0.05$. Fisher's LSDs (least significant differences) were applied to establish the differences between each group of samples (Step 1 = reception ($n=5$); Step 2 = washing ($n=5$); Step 3 = storing <12h ($n=5$), with a confidence of 95 %.

RESULTS AND DISCUSSION

3.1. Olive quality parameters

Olive mill pre-processing operations had a remarkable influence on the physical and hygienic conditions of the olives. First, the integrity of the olives (**Table 2**) was assessed by visual examination ($n=100$ for each sampling) and computing bruised, squashed and fermented fruits. The initial incidence of injured fruit, corresponding to real conditions of handpicking and transport, is relatively high because it comprises also injuries of very low intensity. The variability of damaged fruits (SD values), which is quite constant throughout the process steps,

is given by the initial differences between olive batches, and it is in turn explained by the differences in the maturity of olives from the different batches. The incidence of damaged fruits progressively increased through the pre-processing steps from reception to silo exit, prior to milling. The loss of integrity due to blows during unloading and throughout the washing circuit is especially important if the olives are stored before milling, because rupture of the tissues provides a foothold for microbial growth. During silo storage, healthy olives undergo further damage caused by the weight of olives in the silo and fermentation processes. From the point of view of hygiene, microbiological assays showed that on delivery to the mill, fresh olives intended for oil production presented spontaneous microbiota composed by fungi, lactic bacteria, enterobacteria and *Pseudomonas* (**Table 2**), in agreement with previous reports [5,11,12]. At this point, considerable batch-to-batch variability was observed in contamination by *Pseudomonas*, enteric and acetic bacteria, as evidenced by the high standard deviation. Despite the heterogeneous microbiological profile of the olive batches on reception, the stage of passing through the olive mill washing tank resulted in a significant increase of microbiological contamination, also as previously reported [11]. This additional contamination was fairly similar for the different olive batches, and it remained after short-term silo storage. During this last step, a further increase of lactic acid bacteria was observed. It should be considered that these silos are usually not washed during the harvesting season, with heavy risks for the hygienic aspects of stored fruits. The surfaces of silo can be covered by molds, so the risk of cross-contamination with mycotoxins should be considered in future research. These results confirm the high risk of cross-contamination due to the use of recycled water to wash the olives [9-11], and the need to establish critical hygiene control points in olive oil production process.

Finally, no significant differences in the VOO yield have been found after the distinct treatments (**Table 2**), so we can conclude that possible losses of quality would not be compensated by an increase in the production of VOO.

3.2. Virgin olive oil quality parameters

Analysis of the VOOs obtained from olives collected at each pre-processing step did not produce any evidence that olive deterioration had substantial effects on the official VOO quality parameters (**Table 3**): all the oils corresponded to the EVOO category, according to EU regulations [16,17]. Indices of oxidative status such as K_{270} and PV were lower in the oils obtained after the olives were washed and stored in the silo; in the case of the stored olives, this could be explained by the reducing anaerobic conditions in the silo.

Although no sensory defects arose after any of the pre-processing steps, VOO sensory attributes were influenced by the different operations evaluated (**Table 2**). In particular, short-term silo storage negatively influenced VOO sensory quality by reducing the intensity of the positive attributes, as established in EU regulation [4,17]: fruity, bitter and pungent; as well as other secondary attributes, such as astringency and greenness (**Table 3**). In contrast, the ripe fruit (banana, kiwi, strawberry) note significantly increased after this stage. It is worth mentioning that pre-processing operations carried out according to overall accepted practices, caused a decrease of one point of fruity note intensity, which represents a remarkable loss of sensory quality. Although this loss did not determine the declassing the EVOO to lower categories, it would have commercial repercussions. In fact, according to the EU and the IOC Regulations [17,20], some samples of the study passed from a “intense fruity” (fruity>6) to a “medium fruity” (3<fruity<6) classification after olives short-term storage. As far as we know, this is the first report showing the effect of short term silo storage of olives on the quality of VOO. The global fruity attribute, which is the sum of all the fruity notes perceived by the panelists, not only became weaker after short-term storage, but also turned into a ripe fruit

note, as evidenced by the increase of this secondary attribute (**Table 2**). These results indicate that during fruit storage, at the very beginning of the olive fruit degradation, and before sensory defects or chemical alterations appear, the fruity note decrease and turns into a ripe fruit note. This modification could be induced by several factors including microbiological activity and the slight over-ripening caused by the storage conditions.

The reduction of VOO bitterness after olives storage had been previously described and proposed to increase the acceptability of oils with high bitter intensities [4,21]. In the present work, a slight but significant decrease of bitterness, as well as of pungency and astringency, was observed even storing olives during less than 12h (**Table 3**). In contrast to experimental findings at the laboratory scale [11], the intensity of the fruity but not of the bitter descriptor was reduced in oils obtained from olives contaminated during the washing step, due to the activity of olive microbiota during the oil extraction process. This could be explained by the fact that in the present study on reception at the mill the olive batches presented a higher microbiological charge than in the assay cited above, so modifications in the microbiological activity induced during the washing stage were less discernible in the extracted VOO.

3.3. Volatile and phenolic compounds in VOO

The alterations of the VOO sensory profile induced by the pre-processing steps can be explained by modification in the VOO volatile and phenolic fractions. **Figure 1** illustrates the modifications induced by the pre-processing steps on C6 compounds from the lipoxygenase pathway. It is worth mentioning that not only the short-term silo storage, but also the washing of olives with contaminated water had a significant effect on VOO C6 volatiles, confirming that the activity of olive microbiota influences VOO chemical composition even during the extraction process [11], and justifying the loss of fruity note reported in VOOs from washed olives [12,13]. In agreement with previous results [11], the C6 alcohols hexanol and (E)-2-hexenol were more abundant in the oils obtained after olive washing and silo storage,

respectively, while (Z)-3-hexenol progressively increased over both stages. C6 acetate esters showed behavior analogous to that of the corresponding C6 alcohols. In contrast, through the pre-processing steps considered in the present study, and in particular after short-term silo storage of the olives, C6 aldehydes hexanal, (Z)-3-hexenal and (E)-2-hexenal showed a progressive and significant decrease. C5 compounds and pentene dimers from the lipoxygenase pathway were also negatively affected both by microbiological contamination during washing and by microbiological activity during storage (**Figure 2**). Out of these LOX derivatives, 1-penten-3-one, (Z)-2-pentenol and all the C6 compounds were present at concentrations above their perception thresholds [21], excepting (E)-2-hexanol, which was always below the threshold of 5 mg/kg [22]. Interestingly, hexyl acetate, and (Z)-3-hexenol reached their perception threshold (1 mg/kg) [22] just after the olive washing and storage steps, respectively. On this basis, the changes in the proportion of C6 alcohols and esters versus C6 aldehydes and C5 compounds could explain the change of VOO sensory notes without the appearance of sensory defects. In fact, the green, herbaceous, leafy note has previously been reported to be positively related to some LOX C5 compounds and negatively related to LOX C6 alcohols such as (E)-2-hexenol [22]. Conversely, the ripe fruit note could be associated to the increase of LOX esters (**Figure 1**), although no previous references about this correlation are available.

Among the typical fermentative compounds (**Table S1, supplementary information**), acetoin and methylbutyl acetate were observed to increase slightly during the storage stage; however, the short duration of the storage meant that their concentrations did not reach those necessary to cause a defect [24].

Meanwhile, the changes in the phenolic fraction induced by the pre-processing operations explained the observed decrease of the related sensory attributes such as bitter, astringent and pungent notes (**Table 3**). In fact, major VOO secoiridoids containing both hydroxytyrosol

and tyrosol decreased in oils extracted after olive storage (**Table 4**). Other phenolic compounds were influenced by the pre-processing steps, including apigenin, the levels of which dropped after the olive washing stage; while the concentration of simple phenol tyrosol was observed to increase in oil after short-term olive storage, probably due to hydrolysis of ligstroside aglycon promoted by microbiological activity, in agreement with previous results [11]. Finally, the progressive decrease of VOO *o*-diphenols after each olive processing step could explain the observed reduction of the VOO oxidative stability, as measured by the rancimat test (**Table 2**).

In conclusion, of the post-harvest operations olive washing in closed loop systems, where the water is not renewed in a continuous process, and it is only periodically replaced, was shown to be a critical control point at the olive mill due to microbiological cross-contamination. At the olive mill scale, the volatile composition and the fruity attribute of VOOs were influenced by olive microbiota during oil extraction, while the relatively high initial microbiological charge of some batches on reception hindered the identification of further effects of contamination on VOO sensory and phenolic profiles. Moreover, the common practice of short-term silo storage of olives after washing was shown to influence VOO sensory quality. Although no sensory defects arose from this step, some positive VOO sensory attributes decreased by as much as one point of intensity. The reduction of the green and fruity attributes can be explained by the changes observed in lipoxygenase derived compounds, specifically the reduction in C6 aldehydes, pentene dimers and C5 compounds, and the increase in C6 alcohols. Short-term silo storage was also accompanied by the appraisal of a ripe fruit note. Moreover, bitter, pungent and astringent attributes were reduced in oils after olive silo storage, due to the decrease in phenolic compounds.

These results confirm the high risk of fruit cross-contamination due to the microbiologically contaminated water used in olive mills to wash olive and the need to establish critical hygiene

control points for olive oil production process. Moreover, the effect of short term (<12h) olives storage on VOO quality parameters was pointed out.

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Figure captions

Figure 1. Effect of pre-processing steps on C6 lipoxygenase compounds, as obtained by analysis of variance. Mean values and confidence intervals (95%) are shown. Differences between groups were assessed by one-way ANOVA. Different letters in the graphic indicate significant Fisher's LSDs (least significant differences) ($p < 0.05$). 1: olives reception; 2: washing; 3: short-term silo storage. (Z)-3-hexenal and (E)-2-hexenyl acetate are expressed as mg equivalents of IS/kg.

Figure 2. Effect of pre-processing steps on C5 lipoxygenase compounds and pentene dimers, as obtained by analysis of variance. Mean values and confidence intervals (95%) are shown. Differences between groups were assessed by one-way ANOVA. Different letters in the graphic indicate significant Fisher's LSDs (least significant differences) ($p < 0.05$). 1: olives reception; 2: washing; 3: short-term silo storage. Pentene dimers, (E)-2-pentenal and (Z)-2-pentenol are expressed as mg equivalents of IS/kg.

Tables

Table 1. Sampling date, maturity index (MI), fruit damage^a and microbiological profile^b of the five olive batches used in the experiments.

	sampling date				
	12-Nov	20-Nov	27-Nov	11-Dec	28-Dec
Maturity index	1.6	2.1	2.3	3.3	3.9
healthy fruits (%)	86	95	77	54	50
<i>Pseudomonas</i> (log cfu/g)	3.5	5.1	0.0	0.0	2.1
enteric bacteria (log cfu/g)	4.4	4.7	0.3	0.0	3.7
acetic bacteria (log cfu/g)	2.9	3.7	3.1	2.5	4.1
lactic bacteria (log cfu/g)	5.0	5.5	3.5	2.8	4.9
fungi (log cfu/g)	4.5	5.5	1.7	5.7	4.7

^a visual analysis on n=100 olives; ^b based on three replicates

Table 2. Microbiological profile^a, characteristics, and damage of olive fruits^b through the pre-processing steps. Differences between groups were assessed by one-way ANOVA. Different letters in the same row indicate significant Fisher's LSDs (least significant differences) ($p < 0.05$).

	Step ^c (n=5)		
	1	2	3
<i>Pseudomonas</i> (log cfu/g)	2.2±2.1 a	4.9±0.9 b	4.2±1.2 b
enteric bacteria (log cfu/g)	2.6±2.3 a	5.8±0.8 b	5.3±1.0 b
acetic bacteria (log cfu/g)	4.3±1.1 a	5.4±0.3 b	5.3±0.7 b
lactic bacteria (log cfu/g)	3.3±0.6 a	4.3±0.4 b	4.9±0.5 c
fungi (log cfu/g)	4.4±1.6 a	5.7±1.3 b	5.9±0.9 b
healthy fruits (%)	72±20 a	41±22 ^a b	22±19 b
Oil yield (% on dry matter)	53.5±4.9	55.3±4.5	53.4±2.8

^a based on three replicates; ^b visual analysis on n=100 olives; ^c 1: olives reception, 2: washing, 3:

short-term silo storage.

Table 3. Quality indices and sensory characteristics of olive oils extracted from fruits collected after each pre-processing step. Differences between groups were assessed by one-way ANOVA. Different letters in the same row indicate significant Fisher's LSDs (least significant differences) ($p < 0.05$).

	step ^a (n=5)		
	1	2	3
<i>Physical and chemical indices</i>			
K_{270}	0.13±0.01 a	0.09±0.01 b	0.09±0.01 b
K_{232}	1.60±0.11	1.56±0.15	1.63±0.15
free acidity (g of oleic acid/kg of oil)	0.2±0.05	0.1±0.04	0.2±0.05
peroxide value (mequiv O ₂ /kg)	7.3±2.9 a	5.8±1.2 b	5.8±1.3 b
rancimat (h)	20±2 a	16±2 b	17±2 b
<i>Sensory attributes^b</i>			
fruity	5.7±0.6 a	5.4±0.3 ab	4.7±0.4 b
bitter	5.1±0.3 a	4.9±0.7 a	4.4±0.5 b
pungent	5.5±0.3 a	5.2±0.3 a	4.8±0.4 b
<i>Secondary sensory attributes^c</i>			
green (grass, leaves)	4.1±0.6 a	4.0±0.2 a	3.3±0.5 b
ripe fruits (ripe banana, strawberry, kiwi)	0.2±0.3 a	0.1±0.0 a	0.7±0.6 b
astringent	2.9±0.3 a	2.8±0.4 a	2.4±0.2 b
<i>Defects</i>	-	-	-

^a 1: olives reception, 2: washing, 3: short-term silo storage; ^b: positive sensory attributes, according to EU regulation 796/2002 [16] (median of the intensity sensory attribute); ^c: secondary positive attributes (median of the intensity sensory attribute).

Table 4. Concentration^a (mg/kg) of phenols in virgin olive oils obtained from fruits collected after each pre-processing step. Differences between groups were assessed by one-way ANOVA. Different letters in the same row indicate significant Fisher's LSDs (least significant differences) ($p < 0.05$).

	Step ^b (n=5)		
	1	2	3
<i>p</i> -HPEA ^c	0.28±0.03 a	0.28±0.07 a	0.4±0.07 b
3,4-DHPA ^d	0.59±0.24 a	0.41±0.14 a	0.81±0.63 a
3,4-DHPA acetate	1.9±0.5 a	1.8±0.3 a	1.7±0.3 a
3,4-DHPEA-EDA ^e	319±82 a	286±39 a	192±77 b
<i>p</i> -HPEA-EDA ^f	33±8 a	26±4 ab	21±7 b
elenolic acid	44±14 ab	47±15 a	32±8 b
3,4-DHPEA-EA ^g	23±4 a	21±5 a	16±4 b
<i>p</i> -HPEA-EA ^h	3.4±0.3 ab	3.2±0.4 a	3.9±0.6 b
luteolin	0.06±0.01 a	0.07±0.02 a	0.07±0.03 a
apigenin	0.09±0.04 a	0.06±0.03 b	0.03±0.02 b
vanillic acid	1.7±0.4 a	1.8±0.2 a	1.9±0.1 a
<i>p</i> -coumaric acid	3.4±0.8 a	1.4±0.3 b	1.1±0.4 b
<i>Sum 3,4-DHPA secoiridoids</i>	342±86 a	307±43 a	209±80 b
<i>Sum p-HPEA secoiridoids</i>	36±8 a	29±4 ab	25±8 b

^a Quantification was carried out using the response factors determined by Mateos et al.¹⁷

^b 1: olives reception, 2: washing, 3: short-term silo storage; ^c *p*-HPEA, hydroxyphenylethanol (tyrosol); ^d 3,4-DHPEA, 3,4-dihydroxyphenylethanol (hydroxytyrosol); ^e 3,4-DHPEA-EDA, 3,4-dihydroxyphenylethanol-elenolic acid dialdehyde (dialdehydic form of oleuropein aglycon); ^f *p*-HPEA-EDA, hydroxyphenylethanol-elenolic acid dialdehyde (dialdehydic form of ligstroside aglycon); ^g 3,4-DHPEA-EA, 3,4-dihydroxyphenylethanol-elenolic acid (oleuropein aglycon); ^h *p*-HPEA-EA, hydroxyphenylethanol-elenolic acid (ligstroside aglycon).

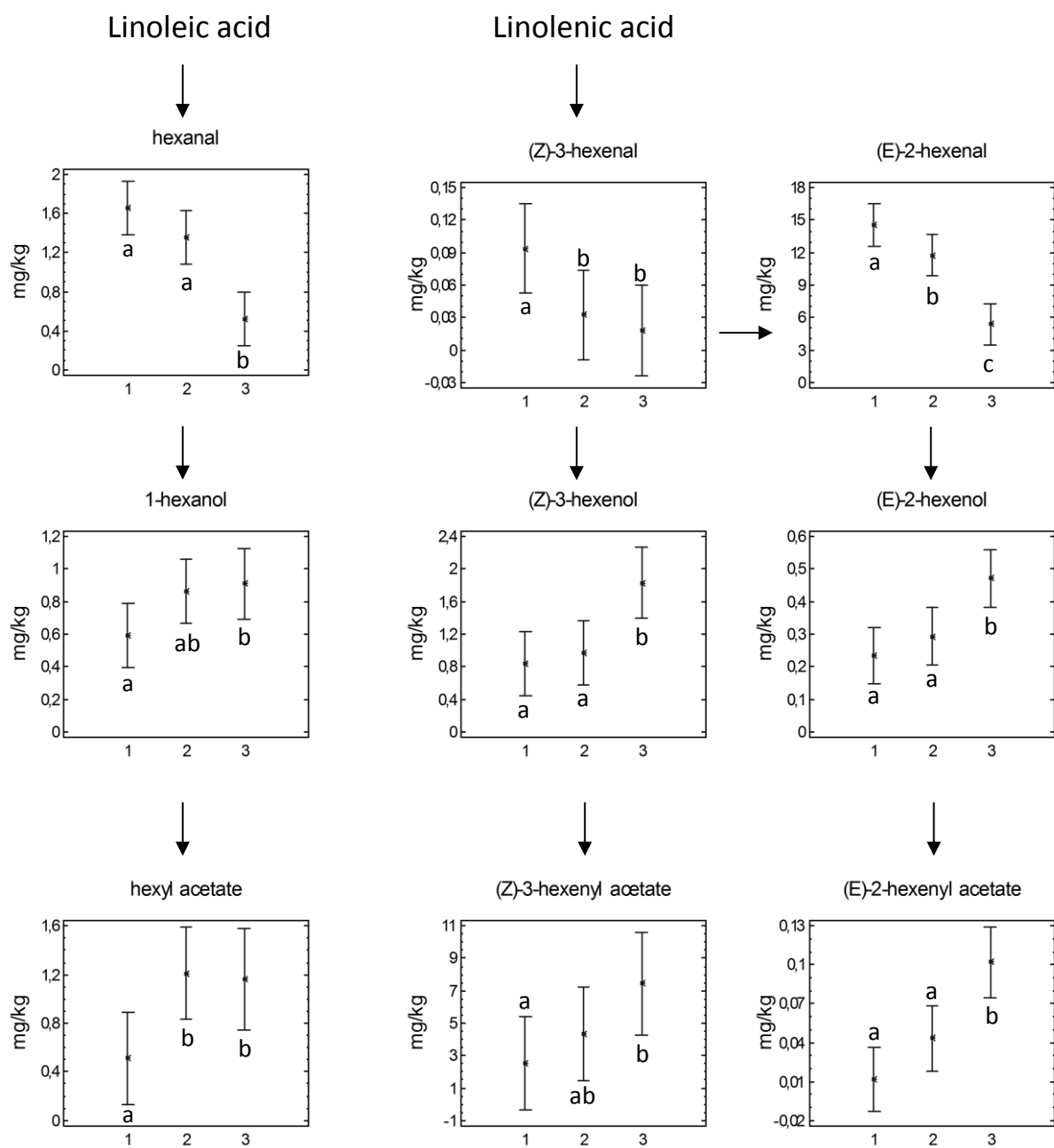


Figure 1

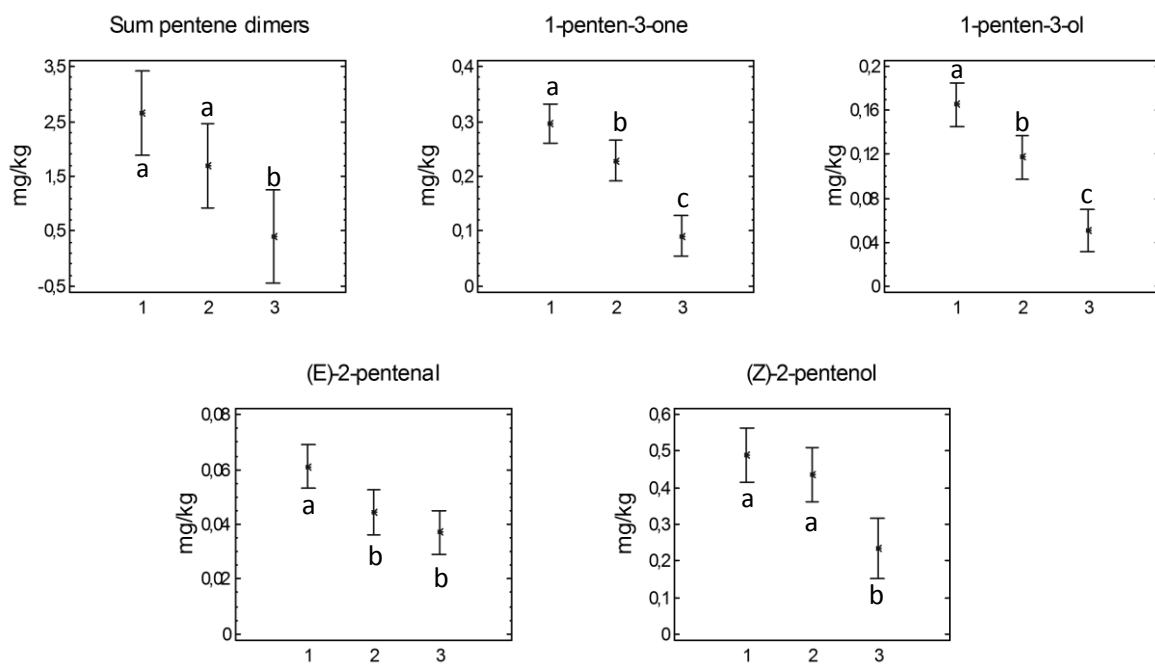


Figure 2