Protein and amino acid profiling of virgin olive oils during their industrial production

Araceli Sánchez-Ortiz*(1), Gabriel Beltrán-Maza (1), José Rubí-Villegas (2), Abraham Gila-Beltrán (1), Maria Paz Aguilera-Herrera(1) and Eduardo López-Huertas (2)

(1) Department of Agroindustry and Food Quality. Andalusian Institute of Agricultural and Fisheries Research and Training (IFAPA). Jaén, Spain.
(2) Food Quality, Safety and Security Division. Spanish National Research Council (CSIC), Estación Experimental Zaidín, Granada, Spain (www.eez.csic.es)

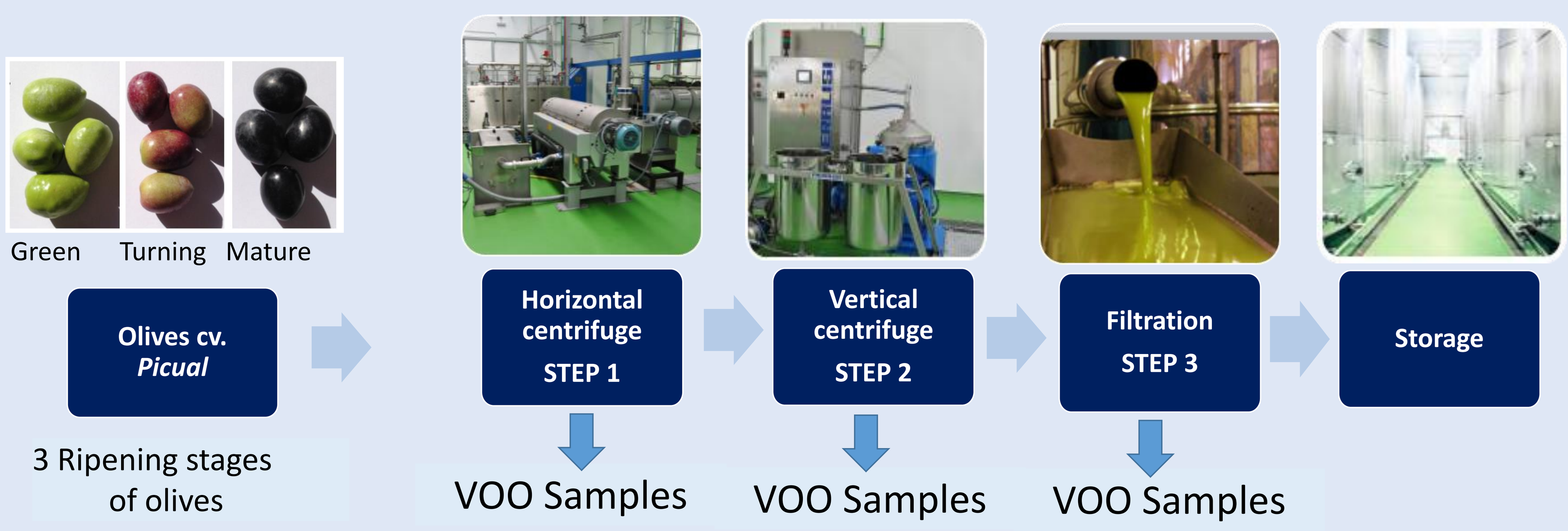
INTRODUCTION

The quality of virgin olive oils (VOOs) gradually deteriorates during the phase of storage after their production. Moisture, microorganisms, enzyme activities and proteins such as lipases, lipoxygenases and oxidases present in oils are in part responsible for the quality losses. A more efficient control of the clarification stage of production, either by optimizing the variables of the horizontal centrifuge, the vertical centrifuge or by using different types of filtration to eliminate solid particles, proteins, moisture, and microorganisms may originate olive oils of better quality.

The characterization of the protein and amino acid contents present virgin olive oils during their different production stages has not been investigated so far. We investigated the protein concentration of virgin olive oils obtained from green, turning and mature olives, after the horizontal centrifuge, vertical centrifuge and filtration phases of production.

MATERIALS AND METHODS

Olive oils were extracted from green, turning and mature olives, using an industrial olive oil extraction plant. After milling and mixing of the olives, VOOs were extracted with an horizontal centrifuge (step 1), followed by a vertical centrifuge (step 2) and a filtration by cellulose plates (step 3) were carried out before storage.
Five VOO samples extracted from green, turning and mature olives were obtained after steps 1- 3 of olive oil production (45 samples).



Proteins were extracted from VOO as described in reference (1) and they were quantified by LECO. The concentration of individual amino acids (partial) was determined by UPLC with fluorescence detection as in (2). Quality parameters of olive oils were determined as in Commission Implementing Regulation (EU) 2022/2105 for methods of analysis of the characteristics of olive oil according to Trade standard applying to olive oils and olive pomace oil (COI/T.15/NC No 3/Rev. 20, 2024). Minor compounds of VOOs: pigments and total polyphenols were determined as in (3) and (4), respectively. Turbidity was measured as in reference (5).

RESULTS AND DISCUSSION

All olive oils used in the study were of the highest quality, namely extra virgin olive oil. The polyphenols content were high in the green and turning VOO and moderate in the mature VOO. During olive oil extraction, the oils obtained after the horizontal centrifugation step possessed the highest turbidity and total polyphenol content, which were particularly prominent in the oils obtained from olives collected at the green and turning phase of ripening.

The vertical centrifugation step exerted a great impact in turbidity and in the content of total polyphenols. Indeed, the vertical centrifuge significantly (P<0.05) reduced the turbidity of the oils by 3-4 fold, and the polyphenol content by 3 fold, on average. However, it had little effect on the concentration of carotenes and other quality parameters of VOO.

As expected, industrial filtration by cellulose greatly reduced the turbidity of the olive oils of the study according to previous results (5).

Virigin Olive Oil (VOO) sample	Acidity (1)	Peroxide Index (2)	K232 (3)	K270 (4)	Carotenes mg/kg	Chlorophyll mg/kg	Total polyphenols (ppm)	Turbidity (5)
Green VOO after horizontal centrifugation step	0,177	2,76	1,326	0,079	7,35	6,99	1073 *	6266,5 *
Green VOO after vertical centrifugation step	0,176	3,91	1,425	0,092	7,7	7,49	362	2005,5
Green VOO after filtration step	0,176	3,89	1,419	0,106	7,27	6,93	302	5,1
Turning VOO after horizontal centrifugation step	0,177	3,89	1,369	0,104	6,09	5,26	790 *	7621,5 *
Turning VOO after vertical centrifugation step	0,176	4,42	1,435	0,102	8,29	9,41	283	2328,0
Turning VOO after filtration step	0,205	5,00	1,374	0,084	6,87	7,08	268	39,2
Mature VOO after horizontal centrifugation step	0,294	10,83	1,490	0,130	6,87	7,4	448 *	4619,0 *
Mature VOO after vertical centrifugation step	0,294	11,04	1,544	0,114	7,66	8,4	343	1109,0
Mature VOO after filtration step	0,294	11,04	1,525	0,111	7,19	7,66	245	0,6

(1): As % of oleic acid, <0.8 for VOO (3): <2.5 for VOO (5): in Nephelometric Turbidity Units
(2): in mEqO2/Kg, <20 for VOO (4): < 0.22 for VOO

Table 1. Analysis of regulated quality parameters of the VOO oils used in the study at the different stages of olive oil production

The VOOs obtained from olives collected at the turning phase ripening contained the highest concentration of proteins (55.8 mg/100g of olive oil), measured by LECO, followed by matured olives (25.7 mg/100g) and green olives (19.7 mg/100g). The vertical centrifugation step reduced the protein concentration of olive oils by approximately 90% compared with the horizontal phase of centrifugation (Table 2).

Negligible amounts of protein were detected in the VOO after the industrial filtration step (ND, not detected).

A positive correlation was observed between turbidity of the VOOs of the study and their protein content (P<0.05)

Virigin Olive Oil (VOO) sample	mg protein per 100 g of VOO
Green VOO after **horizontal** centrifugation step	19.7 ± 2.52
Green VOO after **vertical** centrifugation step	6.88 ± 0.27
Green VOO after **filtration** step	ND
Turning VOO after **horizontal** centrifugation step	55.8 ± 3.25
Turning VOO after **vertical** centrifugation step	3.98 ± 1.02
Turning VOO after **filtration** step	ND
Mature VOO after **horizontal** centrifugation step	25.7 ± 5.34
Mature VOO after **vertical** centrifugation step	2.45 ± 0.57
Mature VOO after **filtration** step	ND

Table 2. Quantification of protein content of the VOO oils used in the study at the different stages of olive oil production

Amino acids were detected and quantified in green, turning and mature VOOs after the first phase of horizontal centrifugation (Table 3). The highest content of amino acids was obtained in the samples of turning VOO. After the vertical centrifugation step, amino acids were only detectable in green VOO, but not in the rest of the oils. The samples were rich in antioxidant amino acids like histidine and tyrosine.

	Green VOO **horizontal** centrifugation	Green VOO **vertical** centrifugation	Turning VOO **horizontal** centrifugation	Mature VOO **horizontal** centrifugation
Amino acid	**mg/100g VOO**			
His	**0,055**	**0,016**	**0,206**	**0,084**
Ser	0,206	0,055	0,776	0,316
Arg	0,208	0,058	0,719	0,293
Gly	0,204	0,055	0,763	0,316
Asp + Asn	0,435	0,122	1,581	0,641
Glu +Gln	0,608	0,159	2,175	0,889
Thr	0,163	0,045	0,607	0,246
Ala	0,115	0,059	0,823	0,345
Pro	0,191	0,051	0,696	0,287
Lys	0,000	0,000	0,094	0,018
Tyr	**0,075**	**0,013**	**0,391**	**0,173**
Val	0,231	0,059	0,840	0,347
Ile	0,186	0,000	0,000	0,000
Leu	0,303	0,078	1,095	0,460
Phe	0,196	0,051	0,658	0,286
Totals	**3,18**	**0,82**	**11,43**	**4,70**

Table 3. Quantification of the amino acid (partial) detected in the VOO oils of the study.

CONCLUSIONS

- ✓ The clarification steps of production exerted a great impact on the protein concentration of olive oils and may influence olive oil quality.
- ✓ The vertical centrifugation step greatly reduced the concentration proteins and antioxidant polyphenols in the VOOs of the study.
- ✓ A positive correlation was observed between turbidity of the VOO and their protein content.

REFERENCES

(1) Alcaide-Hidalgo, et al., J.M.; Margalef, M.; Bravo, F.I.; Muguerza, B.; López-Huertas, E. Virgin olive oil (unfiltered) extract contains peptides and possesses ACE inhibitory and antihypertensive activity. Clin. Nutr. 2020, 39, 1242–1249.
(2) López-Huertas E, Rubí-Villegas J, Sánchez-Moreno L, Nieto R. Olive Pomace Extract Contains Low Molecular Weight Peptides and Possesses ACE Inhibitory Activity. Int J Mol Sci. 2024 Apr 2;25(7):3962. doi: 10.3390/ijms25073962.
(3) M. I. Mínguez-Mosquera, B. Gandul-Rojas, J. Garrido-Fernández, L.Gallardo-Guerrero. Pigments present in virgin olive oilJ. Am. Oil Chem. Soc. 1990, 67, 192.
(4) Vázquez-Roncero, A., Janer del Valle, C., Janer del Valle, M.L., 1973. Determinación de los polifenoles totales del aceite de oliva. Grasas Aceites 24, 350–357.
(5) Abraham Gila, M. Paz Aguilera, Araceli Sánchez-Ortíz, Antonio Jiménez, Gabriel Beltrán. Effect of centrifugal force (G) on stability of natural emulsions (water/oil) present in fresh virgin olive oils. J. Food Eng. 334 (2022) 111169.

Cofinanciado por la Unión Europea
MINISTERIO DE HACIENDA Y FUNCIÓN PÚBLICA
Fondos Europeos
Junta de Andalucía