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Olive oil extraction optimization of 'Galega Vulgar' fruits by response surface methodology

Fátima Peres[1,3], Conceição Vitorino[1], Cecília Gouveia[1] & Suzana Ferreira-Dias[2,3]

[1] Instituto Politécnico de Castelo Branco, Escola Superior Agrária, Castelo Branco, Portugal, fperes@ipcb.pt

[2] Universidade de Lisboa, Instituto Superior de Agronomia, Lisboa, Portugal, suzanafdias@mail.telepac.pt

[3] LEAF, Linking Landscape, Environment, Agriculture and Food, Instituto Superior Agronomia, Universidade de Lisboa, Lisboa, Portugal

ABSTRACT

New strategies to attain better yields, without compromising olive oil quality, are necessary in olive oil extraction. To improve virgin olive oil yield, several processing aids (talc, calcium carbonate, salt, enzymes) have already been tested. 'Galega Vulgar', one of the most abundant Portuguese cultivar for olive oil, usually gives difficult pastes in the malaxation step, which represents a loss of income. The present work aimed to optimize olive oil extraction, at laboratory-scale, from fruits of 'Galega Vulgar' cultivar in 2016 (ripening index=4; moisture content=62 % and fat content (FW) =15.8 %) with the addition of processing aids (ProZym® and natural microtalc FC8-AW), using response surface methodologies (RSM). The combined effects of the concentrations of natural microtalc (*MT*) and of the enzyme preparation (*E*) on the oil extraction yield (Y), extractability index (IE), the concentration of total phenols (TPH), chlorophyll pigments (CP), as well as on chemical quality criteria parameters of the extracted olive oil, were investigated by RSM. A Central Composite Rotatable Design (CCRD) was followed, as a function of the contents of enzyme preparation (0-0.12 %, v/w) and of natural microtalc (0-0.46 %, w/w). For 'Galega Vulgar' olives, the highest extractability was obtained with simultaneous addition of enzymes between 0.02 and 0.08 % (v/w) and of microtalc (0.45-0.50 %, w/w).

Keywords: *enzymes; 'Galega vulgar'; microtalc; virgin olive oil; yield*

INTRODUCTION

For some researchers, processing aids should always be added in olive oil technology, especially in pastes without adequate rheological properties in order to facilitate phase separation in malaxation. With processing aids, higher oil yield with environmental benefits, as less CO_2 is emitted, less water is consumed and all the olive oil process is more profitable, are attained without modification of olive oil characteristics (Cert et al., 1996; Ranalli et al., 2004; Cruz et al., 2007; Fernandez-Valdivia et al., 2008).

Natural microtalc is the most important processing aid used by the olive oil industry, due to its hydrophobic surface and a platy particle shape that adsorbs the natural emulsifiers from the surface of the olive oil droplets. Microtalc does not react with oils and it is easily removed by centrifugation together with olive pomace due to its high density (2.72 g cm^{-3}) and water affinity (Espínola et al., 2009). Enzyme preparations are used as processing aids in several food industries. Although not allowed in Europe, in some countries, enzyme preparations have also been used in olive oil extraction (Canamasas & Ravetti, 2011). Most of the studies have been performed with the addition of blends of hemicellulases, cellulases and pectinases (Chiacchierini et al., 2007). These blends of commercial enzyme preparations are obtained from vegetable extracts or from microorganisms. Also, these enzymes are of the same type of those naturally present inside the olive fruit, which are strongly deactivated during olive oil extraction, probably due to the formation of oxidized phenols responsible for bonding the enzyme prosthetic group (Vierhuis et al., 2001).

The effects of the simultaneous addition of microtalc and enzymes on extraction yield and on olive oil quality was previously studied for "Cobrançosa" and "Galega Vulgar" Portuguese cultivars with a ripening index of 3.6 (Peres et al., 2014). In the present work, a similar study was carried out at lab-scale with olives from "Galega Vulgar" cultivar harvested in 2016, in the same orchard, using the same microtalc but a different enzyme preparation, to evaluate the effect of the enzyme preparation on the extraction yield and olive oil quality.

MATERIALS & METHODS

The fruits of 'Galega Vulgar' cultivar from a rain fed orchard located in Beira Baixa region (39º 49' N, 7º 27'W), Portugal, were harvested manually in November 2016. The ripening index, the moisture and the fat content of the fruits were evaluated following the indications of the International Olive Council (IOC, 2011). The virgin olive oils were extracted at lab-scale (Abencor analyser; MC2 Ingenieria y Sistemas S.L., Seville, Spain) under optimized conditions, as previously described (Peres et al., 2014). Malaxation of the pastes was performed at 28-30 ºC, during 30 min. Processing aids were added in the beginning of the malaxation. The processing aids used and respective characteristics were:

- (*MT*) Microtalc FC8-AW (Mondo Minerals), mean particle size 2.2 μm, largest particle size 9.5 μm and specific surface area of 12 m^2g^{-1}.
- (*E*) enzyme preparation ProZym® (PROENOL), obtained from *Aspergillus,* consists of a pectolitic standard enzyme (>10000 U/mL) with complementary polygalacturonase activity.

The combined effects of the concentrations of natural microtalc (*MT*) and of the enzyme preparation (*E*) on the oil extraction yield, extractability index, total phenols, chlorophyll pigments of the extracted olive oil, were investigated by response surface methodology (RSM). A Central Composite Rotatable Design (CCRD) was followed, as a function of the contents of enzyme preparation and of natural microtalc, using the Abencor extraction system under the previously optimized conditions. In this design, the five levels tested for microtalc concentration and for the concentration of the enzyme preparation were between 0.0 and 0.46 % (w/w) and between 0.0 and 0.12 % (v/w), respectively (Table 1). In the experiment 5 of this design, no microtalc was added and the enzyme concentration corresponds to the central value; in the experiment 7, the enzyme is absent while microtalc is added in the amount corresponding to the central value.

The variation ranges for microtalc and enzyme preparation concentrations were chosen taking into account the recommendations of the respective manufacturers: 0.3 % (w/w) for microtalc FC8-AW and 0.02-0.06 % (v/w), for ProZym®. The Abencor yield (Y, %) and the extractability index (EI) were calculated in accordance with Beltran et al. (2003). The evaluation of European Union parameters for olive oil, namely acidity, UV absorbances and triacylglycerols was carried out following the analytical methods described in EEC/2568/91 EU Regulation. The determination of chlorophyll pigments was performed in accordance to IUPAC method proposed by Pokorny et al. (1995). Total phenols were evaluated in accordance with the liquid-liquid microextraction (LLME) method of Pizzarro et al. (2013) by VIS spectroscopy and quantification in mg GAE/kg.

The obtained results of central composite rotatable designs were analyzed using the software Statistica, version 10, from *Statsoft*, Tulsa, OK, USA. The linear effects of each factor (variable) as well as the quadratic effects of each factor tested (microtalc and enzyme preparation concentrations), and of their linear interactions, on each response (extraction yield, extractability index, total phenols and chlorophyll pigments contents in olive oil) were calculated. The significance of each effect was evaluated by analysis of variance. A response surface, described by a first or a second order polynomial equation, was fitted to each set of experimental results obtained for each CCRD. First and second order coefficients of these equations were generated by regression analysis. The goodness of the fit of the polynomial models was evaluated by the coefficient of determination (R^2) and adjusted R^2.

RESULTS AND DISCUSSION

The fruits of 'Galega Vulgar' cultivar used in the present work had a ripening index of 4.0. The moisture content of the fruits was 62 % and the fat content (FW) was 15.8 %. Acidity of the blend of olives was 0.22 %. Triacylglycerol composition of Galega vulgar olive oil obtained without processing aids addition was as follows: ECN 42 = 0.37%; ECN 44= 2.83%; ECN 46= 16.83%; ECN 48= 72.27% and ECN 50= 7.69%.

The obtained results concerning Abencor yield, extractability index, chlorophyll pigments and total phenols contents are shown in Table 1.

The extractability index (EI) was 0.27 without processing aids, much lower than for Hojiblanca (0.73), a cultivar that gives rise to the so called "difficult" pastes (Beltran et al., 2003). In all the experiments of the CCRD, extraction yield was higher than that observed in the trial without processing aids (4.3 %) (Table 1). When only one of the processing aid was added (experiments 5 and 7), Y and EI were 5.86 % and 0.37, respectively. Concerning both Abencor yield and EI, higher values were in general observed for all the experiments (except for the experiment 1) where combined addition of microtalc and enzymes was performed. Microtalc and enzyme addition had significant positive linear effects on Abencor yield and EI. The quadratic negative effect of enzyme concentration and the linear negative effect of the interaction talc x enzyme have p values higher than 0.05 but they are important enough to be considered (data not shown).

Response surfaces, described by polynomial equations as a function of the significant effects and of those important enough not to be neglected, were fitted to the experimental results to visualize the dependence of the responses on the significant variables. The extraction yield (Y, %) and extractability index (EI) can be described by the following second-order polynomial models:

$$Y = 4.92 + 4.06\, MT + 19.48\, E - 76.76\, E2 - 26.73\, MT \times E \quad \text{(Eq. 1)}$$

$$EI = 0.312 + 0.26\, MT + 1.24\, E - 4.87\, E2 - 1.70\, MT \times E \quad \text{(Eq. 2)}$$

where MT and E are the amounts of microtalc (%, w/w) and enzyme preparation (%, v/w) in decoded values, respectively. Both models present a R^2 of 0.85 and R^2_{adj} of 0.78, showing a good fit to the experimental data points.

Figure 1 shows the response surface and respective contour plot fitted to the Abencor yield. The surface describing the EI (not shown) is similar to the yield response surface. For 'Galegar Vulgar' olives, the highest extractability is observed under the presence of enzyme between 0.02 and 0.08 % (v/w) and microtalc amounts of 0.4 to 0.5 %. Under these selected conditions, a 64 % increase in extraction yield was observed for 'Galega Vulgar' olives, compared to the values obtained in the absence of processing aids.

The acidity of all the olive oils obtained with the addition of microtalc and/or enzymes was similar to that of the olive oil obtained without adjuvants (0.22 %). Concerning oxidation parameters (K_{232} and K_{270}), the values were not significantly different from those of the olive oil obtained without processing aids (K_{232}= 0.11; K_{270} = 1.23) and all were within the limits for extra virgin olive oil. Also, no significant effects of the addition of microtalc and/or enzyme were found on chlorophyll pigments or total phenols in the obtained olive oils. Therefore, the presence of these processing aids did not affect these characteristics of the olive oils (Table 1). This was also observed in previous trials with another enzyme preparation (Peres et al., 2014). Conversely, several authors observed an increase in phenol content when enzymes were applied (De Favieri et al., 2008; Garcia et al., 2001) or when talc was used (Caponio et al., 2014). However, in those studies, higher doses of enzymes and/or different commercial formulations were used, namely 0.5-1.5 % of mixtures of cellulases, pectinases and hemicellulases (De Faveri et al, 2008), 0.05 % of Olivex/Novoferm 12, Olivex/Glucanex (Garcia et al., 2001) or the addition of 1 or 2 % of talc (Imerys Talc, Lusenac) (Caponio et al, 2014). Moreover, the variations in total phenols are quite dependent on the cultivar. Canamasas and Ravetti (2011) did not found any increase in phenol contents for 'Barnea' cultivar when four different enzyme preparations were added at processing plant level, and lower phenol contents were achieved when Pectinex Ultra SP-L was added to Arbequina cultivar (Abencor trials).

The simultaneous addition of microtalc and enzymes showed to have great benefits in terms of olive oil extraction yield from 'Galega Vulgar' fruits without any negative effects on quality parameters.

Higher extraction yields were also observed in our previous work, when simultaneous addition of microtalc and enzymes was carried out (Peres et al., 2014). Also, for each cultivar, the amounts of microtalc and enzymes preparation to be used in olive oil extraction must be previously optimized (Peres et al., 2014). In addition to the cultivar effect, the moisture content, the ripening stage and the content of endogenous enzymes may all influence the final yield. In the center of Portugal, where 'Galega Vulgar' is abundant, the mean yield is 13% vs. 15.2% in Alentejo (the Portugal region that produces about 76 % of the olive oil in the country) (GPP, 2017). So, improving yields of this cultivar is an important aim for olive oil producers.

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REFERENCES

- Beltran, G., Uceda, M., Jiménez, A. & Aguilera, M.P. 2003.Olive oil extractability index as parameter for olive cultivar characterisation. Journal Science Food Agriculture 85, 503-506.
- Canamasas, P. & Ravetti, L. 2011.Evaluation of processing aids for olive oil extraction and quality improvement. Rural Industries Research and Development Corporation.
- Caponio, F., Monteleone, J. I., Martellini, G., Summo, C., Paradiso, V. M., & Pasqualone, A.2014. Effect of Talc Addition on the Extraction Yield and Quality of Extra Virgin Olive Oils from Coratina Cultivar after Production and during Storage. Journal of Oleo Science,63 (11), 1125-1132.
- Cert, A., Alba, J., Leon-Camacho, M., Moreda W. & Pérez-Camino, M. C.1996. Effects of talc and operating mode on the quality and oxidative stability of virgin olive oils obtained by centrifugation. J. Agric. Food Chem. 44: 3930 - 3934.
- IOC. 2011. Guide for the determination of the characteristics of oil-olives. COI/OH/Doc. Nº.1 . November
- Cruz, S., Yousfi, K., Pérez, A. G., Mariscal, C. & Garcia, J. M. 2007. Salt improves physical extraction of olive oil. Eur. Food. Res. Technol. 225: 359-365.
- Chiacchierini, E., Mele, G., Restuccia, D., & Vinci, G. 2007. Impact evaluation of innovative and sustainable extraction technologies on olive oil quality Trends in Food Science & Technology, 18(6), 299-305.
- De Faveri, D., Aliakbarian, B., Avogadro, M., Perego, P., & Converti, A. 2008. Improvement of olive oil phenolics content by means of enzyme formulations: Effect of different enzyme activities and levels. Biochemical Engineering Journal 41(2), 149-156.
- Espínola, F., Moya, M., Fernández, D. G., Castro, E..2009. Improved extraction of virgin olive oil using calcium carbonate

as coadjuvant extractant. Journal of Food Engineering. 92, 112-118.

- Fernández-Valdivia, D., Espínola-Lozano, F. & Moya Vilar, M.2008. Influencia de diferentes coadyuvantes tecnológicos en la calidad y rendimiento del aceite de oliva virgen utilizando la metodologia de superfícies de respuesta. Grasas y Aceites 59, 1: 39 – 44.
- García, A., Brenes, M., Moyano, M. J., Alba, J., García, P., & Garrido, A. 2001. Improvement of phenolic compound content in virgin olive oils by using enzymes during malaxation Journal of Food Engineering, 48 (3), 189-194.
- GPP.2017. Siaz- Sistema de Informação sobre o Azeite e Azeitona de Mesa. Gabinete de Planeamento Políticas e Administração Geral.

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- Peres, F., Martins, L. L., & Ferreira-Dias, S. 2014. Laboratory-scale optimization of olive oil extraction: Simultaneous addition of enzymes and microtalc improves the yield. European Journal of Lipid Science and Technology, 116 (8), 1054-1062.
- Pizarro, M. L., Becerra, M., Sayago, A., Beltrán, M., & Beltrán, R. 2013.Comparison of Different Extraction Methods to Determine Phenolic Compounds in Virgin Olive Oil. Food Analytical Methods, 6(1), 123-132
- Pokorny, J., Kalinova, L. & Dysseler, P. 1995. Determination of Chlorophyll Pigments in Crude Vegetable Oils. Pure Appl. Chem. 67: 1781-1787.
- Ranalli, A.; Lucera, L.; Contento, S.; Simone, N. & Del Re, P. 2004. Bioactive constituents, flavors and aromas of virgin oils obtained by processing olives with a natural enzyme extract. Eur. J. Lipid Sci. Technol. 106, 187-197.

Table 1. CCRD followed in the experiments for optimization of 'Galega Vulgar' fruits extraction using processing aids (microtalc, MT, %-w/w and enzyme concentration, E, %-v/w,), the results obtained in each experiment: abencor yield (%), extractability Index (EI), chlorophyll pigments (CP, mg pheophytin kg^{-1}), total phenols (TPH; mg GAE kg^{-1})

Experiment	Microtalc (%)	Enzyme (%)	Abencor yield (%)	EI	CP	TPH
1	0.067	0.018	5.49	0.35	5.76	171.14
2	0.067	0.102	6.22	0.39	6.55	171.28
3	0.393	0.018	6.65	0.42	5.67	199.58
4	0.393	0.102	6.65	0.42	7.24	208.60
5	0	0.06	5.86	0.37	6.74	171.28
6	0.46	0.06	6.99	0.44	5.07	169.87
7	0.23	0	5.86	0.37	5.49	157.12
8	0.23	0.12	6.32	0.40	6.04	204.54
9	0.23	0.06	6.59	0.42	6.08	203.89
10	0.23	0.06	6.22	0.39	5.34	169.87
11	0.23	0.06	6.32	0.40	4.93	184.91
12	0.23	0.06	5.99	0.38	4.97	175.89
13	0.23	0.06	6.65	0.42	4.68	177.37

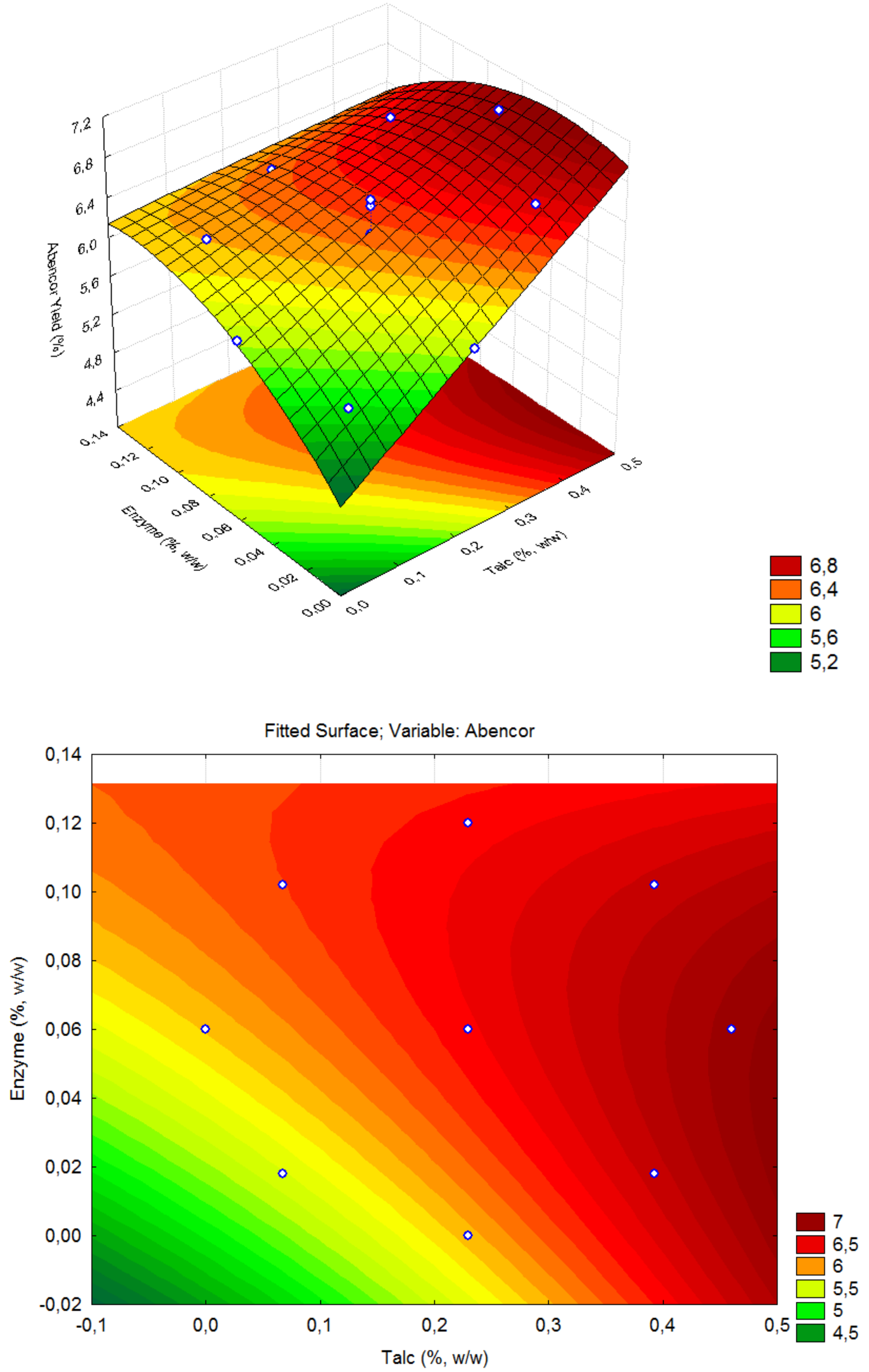

Figure 1. RSM and respective contour plot for Abencor yield (%) as a function of microtalc (FC8-AW) and enzyme (ProZym®) concentration, added to 'Galega Vulgar' fruits during malaxation.