Optimization of Supercritical Fluid Consecutive Extractions of Fatty Acids and Polyphenols from *Vitis Vinifera* Grape Wastes

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Abstract: In this study, supercritical fluid extraction has been successfully applied to a sequential fractionation of fatty acids and polyphenols from wine wastes (2 different *vitis vinifera* grapes). To this aim, in a 1st step just fatty acids were extracted and in a 2nd one the polyphenols. The variables that affected to the extraction efficiency were separately optimized in both steps following an experimental design approach. The effect of extraction temperature flow, pressure, and time were thoroughly evaluated for the extraction of fatty acids, whereas the addition of methanol was also considered in the case of the polyphenols extraction. A quantitative extraction with high efficiency was achieved at a very short time and low temperatures. Concerning quantification, fatty acids were determined by means of gas chromatography coupled to mass spectrometry after a derivatization step, whereas the polyphenols were analyzed by means of high performance liquid chromatography coupled to tandem mass spectrometry and the Folin–Ciocalteu method.

Keywords: fatty acids, optimization, polyphenols, supercritical fluid extraction, wine wastes

Practical Application: A sequential supercritical fluid extraction of fatty acids and polyphenols from wine wastes was achieved with very good yields at very short time and low temperatures comparing with previous published works. In this sense, we think our work could be an interesting and important contribution to this field in order to achieve a full revalorization of wine wastes before giving them any other application.

Introduction

Because of the hectic pace of life where we live in, during last decades, there have been changes in food consumption habits as well as in food itself. Hence, industrially processed food has turned indispensable into our daily life. As a consequence of the loss of nutritional value of many processed foods, the addition of nutrients that compensate this loss such as antioxidants and a large variety of dietary supplements is gaining interest. Among others, polyphenols and the polyunsaturated fatty acids are included in those compounds with high nutritional interest (Shahidi 2012).

Polyphenols has a high antioxidant capacity, because they can neutralize free radicals turning them less dangerous and thereby, stopping side reactions (Dugas and others 2000; Tikhonov and others 2009; Leopoldini and others 2011). Therefore, this property makes them interesting for many different applications such as the treatment of inflammations (Kim and others 2014) or human degenerative diseases as cancer (Hertog 1994; Cordova and Watson 2014), anti-ageing aims in cosmetics (Jean-Gilles and others 2013; Menaa and Menaa 2014), or nutraceutical purposes (Gollücke and others 2013). Moreover, polyphenols are known to be nootropics, owing to be helpful in several brain functions, such as learning, memory, attention, or motivation (Papandreou and others 2009).

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However, fatty acids are commonly classified in 3 different families: polyunsaturated fatty acids (PUFAs), monounsaturated fatty acids (MUFAs), and saturated fatty acids (SAFAs). PUFAs, such as linoleic acid, have anti-inflammatory, antiarrhythmic, lipid lowering, and antithrombotic effects and thus, they may play an important role in cardiovascular prevention (Fedačko and others 2007; Tousoulis and others 2014). The main MUFA in vegetal oils is the oleic acid; it reduces blood total triacylglycerol and cholesterol levels and it is essential for cancer prevention (Stein and others 2008; Gnoni and others 2010). Furthermore, it presents positive effects on coronary heart disease and hypertension prevention (Gnoni and others 2010). On the contrary, in general, SAFAs increase serum cholesterol levels (Mensink 2013). Among them, lauric and myristic acids have the strongest effects and stearic acid the weakest (Mensink 2013).

Agro-industrial wastes have gained the interest of several industrial areas because many of them may become an interesting source of by-products of high added-value, including antioxidants and fatty acids. In this sense, wine production is one of the most outstanding industrial activity, due to the high concentration of polyphenols and PUFAs present in wine products and the high volume of wastes produced yearly (according to the Intl. Organization of Vine and Wine, the worldwide production of wine requires 7575000 ha). Currently, however, only a small percentage of the wine wastes are reused in Europe. The production of biofuel and bioenergy, and the preparation of manure or feed for animals are the most used alternatives to avoid costs overrun of waste management. Owing to these drawbacks, grape pomace may become a promising and economically profitable source of polyphenols and

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PUFAs. However, their quantitative extraction with suitable analytical techniques is still a challenge (Arvanitoyannis and Varzakas 2007).

Carbon dioxide–based supercritical fluid extraction (SFE) offers remarkable advantages over traditional methods in the extraction of bioactive compounds and in food processing (Raventós and others 2002; Sahena and others 2009; Mendiola and others 2013). On the one side, CO_2 is an inert, nontoxic, environmentally safe solvent, and allows extraction at low temperatures and pressures, avoiding thereby the degradation of thermosensitive compounds. On the other side, these high-purity extracts are recognized as safe to be used in food products. In addition, SFE technique is scalable up to industrial scale. Moreover, in spite of the fact that supercritical CO_2 is not a polar solvent, the addition of a cosolvent such as ethanol or methanol improves the extraction of polar compounds and may allow the fractionation of the extracts that contain compounds of different polarities (Chaudot and others 2000; Ashraf-Khorassani and Taylor 2004; Omar and others 2013).

Taking into account the rise in value of agroindustrial wastes, the aim of this study is a complete optimization of SFE procedure in order to obtain 2 different extracts: one rich in fatty acids and the other one rich in polyphenols, using the same grape pomace. The enhanced value of performing a full optimization of each step is getting extracts with high concentration of bioactive compounds with the least reagent cost and time wasting. The polarity of the solvent mixture used during the SFE is the key parameter to separate these 2 groups of compounds. In the case of the fatty acids, the extraction based on pure CO_2 is foreseen, and in the case of polyphenols, the addition of methanol is required.

Materials and Methods

Chemicals and materials

A standard solution mixture containing 19 fatty acid methyl esters (Grain FAME mix 10 mg/mL in dichlorometane; methyl arachidate, methyl behenate, methyl caprylate, methyl decanoate, methyl *cis*-11-eicosenoate, methyl elaidate, methyl erucate, methyl heptadecanoate, methyl laurate, methyl linoleate, methyl linolenate, methyl myristate, methyl myristoleate, methyl oleate, methyl palmitate, methyl palmitoleate, methyl pentadecanoate, methyl stearate, and methyl tridecanoate), sodium hydroxide (98%), Folin–Ciocalteu reagent, gallic acid (97.5% to 102.5%), catechin (≥96%), epicatechin (≥90%), and coumaric acid (≥98%) were purchased from Sigma-Aldrich (Steinheim, Germany) and propyl gallate from Supelco (Bellefonte, Pennsylvania, USA).

High-performance liquid chromatography (HPLC) quality methanol (MeOH) used as cosolvent in SFE extraction and mobile phase in HPLC coupled to tandem mass spectrometry (HPLC-MS/MS) analysis and isopropanol used for collecting SFE extracts, ethanol (EtOH, 96%) used for Folin–Ciocalteu analysis and sodium chloride (99%) were obtained from Panreac (Barcelona, Spain). HPLC quality isooctane used in fatty acids derivatization was purchased from Lab-Scan (Gliwice, Poland); sodium carbonate (99.5%) and sulfuric acid (97%) were obtained from Merck (Madrid, Spain); and formic acid (Liquid chromatography mass spectrometry [LC/MS] quality) was purchased from Thermo Fisher Scientific (Erembodegem, Belgium).

Ultrapure water (<0.057 S/cm) was generated with a Milli-Q water purification system Element 10 from Millipore (Billerica, Massachusetts, USA). The CO_2 (99. 5%) used as mobile phase in SFE system was obtained from Air Liquide (Madrid, Spain) and washed thin sea sand from Scharlab (Barcelona, Spain).

Sample preparation

Wine wastes were selected from 2 different Basque designations of origin (D.O.): D.O. Rioja and D.O. Txakoli. Regarding the first one, the wastes were collected from Samaniego (Araba, *Ostatu* winery and *Tempranillo* red grapes) and the 2nd wastes were collected from Getaria (Getaria, *Mokoroaga* winery and *Hondarribi zuri* white grapes), both in the Basque Country (North of Spain).

First, the wine wastes were air dried for 2 d. A representative fraction of seeds per each type of wine wastes was separated and used for the optimization of the extraction of fatty acids. The optimization of the extraction of polyphenols was performed using a mixture of seeds and skins. These fractions were further analyzed under optimized conditions. Separated sample fractions were ground in a cryogenic mill (SPEX SamplePrep 6770 Freezer/Mill, Stanmore, U.K.) under liquid nitrogen for 10 min at 11 cps, and stored in the refrigerator at 4 °C until extraction.

Supercritical fluid extraction

SFE was performed on a Thar SFC (Waters Co., Saint-Quentin, France) Method Station SFC system, consisting of a fluid delivery module (CO_2 pump and solvent pump), an automated back pressure regulator (ABPR), and a high-pressure extraction vessel of 1 mL (EV-1 Jasco).

Under optimized conditions, approximately 0.25 g of sample and 0.75 g of sea sand were accurately placed in a "sandwich" way (sand–sample–sand) into an extraction vessel with frits placed at the ends to maintain the sample and prevent particulate matter entering the fluid transfer lines. Samples were extracted in dynamic mode with supercritical CO_2 for 5 min. Supercritical fluid pressure was maintained at 250 bar at a constant flow of 2 mL/min for the extraction of fatty acids and the extraction temperature was fixed at 35 °C. Regarding the 2nd fraction where polyphenol compounds were obtained, the supercritical fluid pressure was set at 103 bar, at a constant flow of 2 mL/min and at 35 °C, and in this case, the use of 40% of MeOH as cosolvent was required. The use of MeOH is justified because the supercritical conditions of CO_2-MeOH are milder than those obtained with CO_2-EtOH, which ease to test the performance of the extraction and then, it can be modified if necessary for industrial applications. Finally, the extracts were collected in glass vials containing 4 mL of isopropanol as solvent for fatty acids and 3 mL of MeOH for polyphenols. The sample extracts were kept at −20 °C in complete darkness until the analysis.

Derivatization of fatty acids and analysis by gas chromatography mass spectrometry

All the extracts were evaporated by a gentle stream of N_2 (Caliper Life Sciences TurboVap® LV) and the fatty acids were transformed to methyl ester (FAME) derivatives in order to be determined by gas chromatography mass spectrometry (GC-MS). For this purpose, a derivatization process described by Dayhuff and Wells (2005) was carried out with minor modifications. Briefly, 1.5 mL of sodium hydroxide (0.5 M) in MeOH were added to evaporated fatty acid extracts and maintained in an oven at 100 °C for 5 min. Afterward, 2 mL of 15% sulfuric acid in MeOH were added and maintained at 100 °C for 1 h. Subsequently, 5 mL of saturated sodium chloride solution and 1 mL of isooctane were added. The 2 phases were thoroughly mixed and the organic phase was collected. Finally, 1 mL of isooctane was added to ensure a quantitative recovery of the compounds and the organic phase was collected again. The 2 organic fractions were mixed and diluted for quantification.

GC-MS analysis was performed in a 7890A gas chromatograph coupled to an Agilent 5975C inert triple-axis mass detector and an Agilent autosampler 7693 (Agilent Technologies, Avondale, Pa., U.S.A.). The analysis was performed in the splitless mode for 35 min at 275 °C. A capillary column DB-Wax (30 m × 0.25 mm, 0.25 μm; Agilent Technologies) was used for the separation of FAMEs. The temperature program used was as follows: started at 80 to 250 °C (at 8 °C/min) where it was held for 13.75 min. Helium (99.999%, Air Liquide) was used as carrier gas at a constant pressure of 9.38 psi. The transfer line temperature was maintained at 300 °C, and the ion source and quadrupole at 230 and 150 °C, respectively. The measurements were performed both in SCAN (30–550 amu) and selected ion monitoring modes. Peak identification of the FAMEs was carried out by the comparison with retention times and mass spectra of Grain FAME mix standards. In real samples only 4 FAMEs were identified among all standards; methyl palmitate (t_R: 14.77 min), methyl stearate (t_R: 17.02 min), methyl oleate (t_R: 17.25 min), and methyl linoleate (t_R: 17.76 min).

Folin–Ciocalteu method for total phenolic content

The total amount of phenolic compounds was determined by using the Folin–Ciocalteu colorimetric method according to Singleton and Rossi (1965). Briefly, all the extracts were evaporated by a gentle stream of N_2, dissolved in 1 mL of EtOH and diluted 10 times. One milliliter of diluted samples was mixed with 5 mL 1:10 (v/v) of diluted Folin–Ciocalteu reagent and 4 mL of sodium carbonate (0.7 M). Absorbance was measured at 765 nm in a spectrophotometer Multispec-1501 Shimadtzu UV/VIS after 2 h of incubation in darkness at room temperature. Total phenolics were expressed as mg of GAE (gallic acid equivalents)/g by comparison to the gallic acid standard calibration curve.

Liquid chromatography tandem mass spectrometry (LC-MS/MS) method for polyphenol analysis

Liquid chromatographic system was an Agilent Technologies (Santa Clara, Calif., U.S.A.) Infinity Liquid Chromatograph 1260, consisted of an autosampler, a column thermostat, and a binary solvent management system coupled to a triple quadrupole mass spectrometer equipped with an electrospray ionization ion source (ESI; Agilent Technologies 6430).

The chromatographic separation was achieved using a Kinetex C_{18} column (2.6 μm, 150 mm × 3 mm i.d.) with a guard column (0.5 μm depth filter × 0.1 mm) both purchased from Phenomenex (Aschaffenburg, Germany) and a binary A/B gradient (solvent A was water with 0.5% formic acid and solvent B was methanol with 0.5% formic acid). The gradient program was as follows: 0 min: 20% B; 0 to 4 min: 20% to 50% B, 4 to 10 min: 50% to 100% B, 10 to 14 min: 100% B, 14 to 16 min: 95% to 20% B, 16 to 33 min: 20% B. A flow rate of 0.20 mL/min was used, the column temperature was maintained at 35 °C and the injection volume was 10 μL.

MS acquisition was carried out in the ESI positive and negative ionization mode using the following conditions: capillary voltage of 4000 V, a nebulizer gas to spray the sample of 15 psi, and heated nitrogen flow of 6 L/min at 300 °C. High purity nitrogen gas (99.999%, Air Liquide) was used as nebulizer, drying, and collision gas. Under these conditions, the quantitative analysis of the target compounds was performed in multiple reaction monitoring (MRM) mode. System variations were corrected with an internal standard (propyl gallate). The method included quantitation and confirmation transitions for each analyte and for each transition, the fragmentor potential and the collision energy were optimized. Table 1 summarizes the LC-MS/MS parameters used for the 4 main target analytes (available standards) including quantitation and confirmation transitions, optimized energies, and retention times.

Table 1–MRM transitions, optimized potentials, and applied voltage polarity and retention times of the analytes and the internal standard for HPLC-MS/MS analysis. Mass/charge ratio of the precursor ion (Q1), mass/charge ratio of the fragment ion (Q2), fragmentation potential (FP), collision energy (CE), retention time (t_R).

Analyte	Q1 (amu)	Q2 (amu)	FP (V)	CE (eV)	t_R (min)	Polarity
Gallic acid	169	125	100	12	1.8	Negative
		79	100	21		
Catechin	291	139	100	10	3.3	Positive
		123	100	5		
Epicatechin	291	139	100	10	5.4	Positive
		123	100	20		
Coumaric acid	163	119	100	10	8.8	Negative
		93	100	35		
Propyl gallate	213	153	60	10	10.1	Positive
		127	60	10		

Fatty acids and polyphenols SFE optimization by response surface methodology

To extract fatty acid compounds, the effect of temperature (°C), CO_2 flow (mL/min), pressure (bar), and the extraction time by supercritical fluid was assessed by means of a central composite design (CCD). The design matrix consisted of 27 randomized assays allowing the study of the effects of the variables within the ranges described in Table 2. Those ranges were established taking into account the equipment restrictions and the limits of CO_2 to be a supercritical fluid. Because fatty acids are essentially nonpolar compounds, the extraction was carried out with pure CO_2 (that is, the addition of modifier was not considered). All the experiments were performed with a representative mixture of the 2 types of seeds.

All the extracts were analyzed by means of GC-MS after the derivatization step and the responses were defined as the chromatographic peak area of the main 4 FAMEs (methyl palmitate, methyl linoleate, methyl oleate, and methyl stereate). The optimum conditions were established by the analysis of the response surfaces, which were build by multiple linear regression (MLR) analysis including linear and squared terms and the interactions of any 2 variables. The precision was estimated from the 3 replicates of the central point and the data were treated with the statistics software The Unscrambler® (9.7 Camo Asa, Oslo, Norway). Model fitness was assured by the analysis of variance of the residuals (lack of fit significantly lower than pure experimental uncertainty) and the uncertainties of each parameter.

The same strategy was performed in order to find the optimum extraction conditions of polyphenol compounds by means of SFE. In this case, the studied variables were extraction temperature, pressure, and CO_2 flow; and due to the chemical properties of the target compounds, the addition of a polar cosolvent (MeOH) was also considered. All these parameters were simultaneously studied by means of a CCD in the ranges summarized in Table 2. The extractions were accomplished using a mixture of both ground seeds and skins of grapes. The extraction time was initially fixed at 10 min and once the optimum conditions were attained this

Table 2–Studied variable ranges in FAMEs and polyphenol extraction optimizations and optimum values.

Variable	Studied range in FAMEs	Optimum values	Studied range in polyphenols	Optimum values
Temperature (°C)	35–55	35	35–55	35
Flow (mL/min)	1–2	2	1–2	2
Pressure (bar)	103–253	253	103–253	103
Extraction time (min)	5–79	5	–	–
Methanol as a modifier (%)	–	–	0–40	40

variable was fine-tuned (5, 10, 15, and 20 min). CCD responses were obtained by means of Folin–Ciocalteu method and the results were defined as total phenolic content (mg GAE/g).

Results and Discussion

Fatty acid SFE optimization

MLR data obtained with the experimental design assays revealed that the significant variables affecting the extraction yield of fatty acids were the pressure and the flow (P-level < 0.10, no factors were significant with a higher confidence level, that is a P-level < 0.05, probably due to the high deviations introduced by the derivatization process). As it is illustrated for linoleic acid in Figure 1, the response surface was flat and a local maximum was found at the highest flow and pressure values within the factor space. The same result was observed for the rest of the analytes for all the evaluated variables. Hence, high pressure and flow values were established as optimum conditions, whereas temperature and extraction time were set at the minimum values because they were not significant and the method throughput was guaranteed with short extraction times. In this way, the fatty acids optimal extraction conditions were established as follows: 35 °C of extraction temperature, 2 mL/min of CO_2 flow, 253 bar of pressure, and 5 min of extraction (Table 2).

Polyphenol SFE optimization

Regarding the 2nd fraction, in which more polar compounds must be recovered, besides the parameters studied for the first extraction step the effect of the addition of MeOH as cosolvent was evaluated. In this case, MLR results showed that besides the flow of CO_2 used during the extraction, the percentage of MeOH was also significant parameter at 95% of confidence level (P-level < 0.05) to obtain the best extraction yields. Figure 2 illustrates the response surface in which both significant parameters are varied; a local maximum was found when both the CO_2 flow and the MeOH% were fixed at the highest values. As it occurred for the isolation of fatty acids extraction temperature and pressure were not significant parameters. Consequently, optimal extraction conditions for polyphenols were established as follows: low extraction temperature (35 °C), high flow (2 mL/min), low pressure (103) bar, and high presence of cosolvent (40% of MeOH; Table 2).

Regarding the extraction time, it was fine-tuned between 5 and 20 min once the other parameters affecting the extraction procedure were fixed. According to the results, it was concluded that the extraction time was nonsignificant (P-level > 0.10) in the studied range and, therefore, the minimum extraction time (5 min) was chosen. In fact, 3 successive extractions of 5 min were carried out in order to check the effectiveness of the extracted polyphenols and the results were as follows: 88.2 ± 0.9%, 7 ± 1%, and 4.4 ± 0.4%. According to these results, extraction time was established in 5 min, because it was considered that it is not worth the expense of reagent that would be realized in case of doing longer extractions in order to improve a small per cent.

Consecutive extraction of fatty acids and polyphenols using SFE

Once the SFE variables were optimized for the extraction of fatty acids and polyphenols, consecutive extraction from the same sample were carried out in order to isolate fatty acids and polyphenols, and hence, estimate the efficiency of the fractionation. For this purpose, each fraction was independently collected (that is, first step: isolation of fatty acids and 2nd step: isolation of

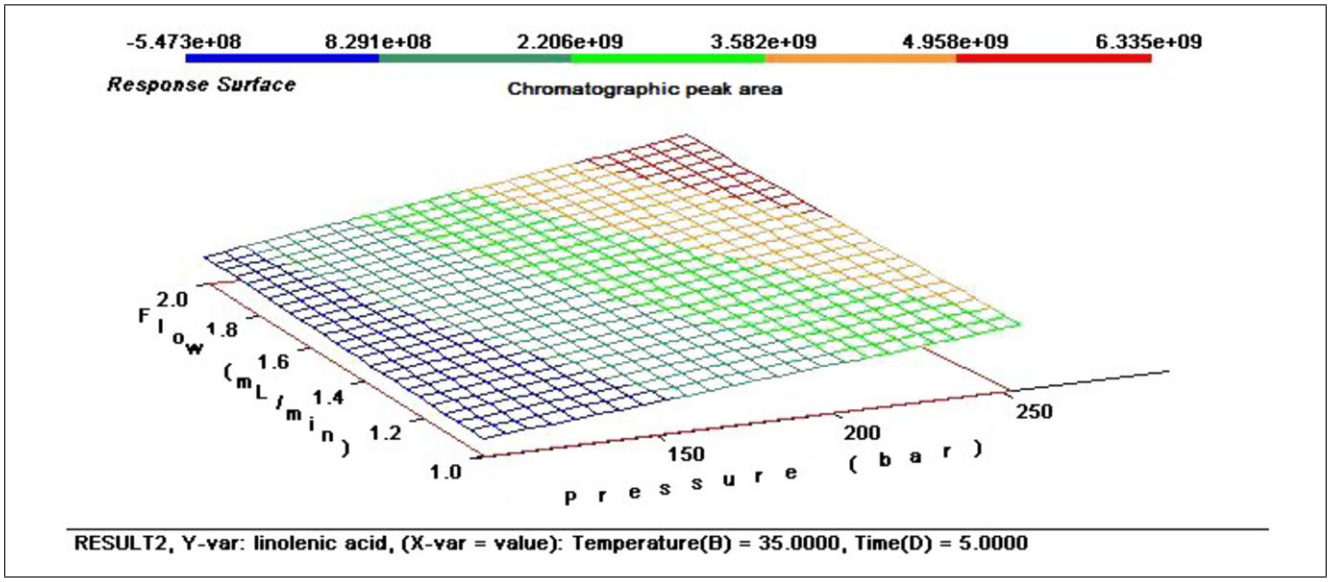

Figure 1–Response surface of the pressure (bar) and flow (mL/min) factors for the extraction of linoleic acid.

polyphenols) at previously established conditions and further analyzed. In addition, in order to check the percentage of fatty acids remaining in the 2nd fraction (that is, fatty acids co-eluting with polyphenols) and to assess if there is any loss during consecutive extraction, samples were extracted directly using the experimental conditions set up for each fraction. In this case, the analysis of fatty acid and polyphenols were performed by GC-MS and HPLC-MS/MS, respectively.

As it is illustrated in Figure 3, only traces of the fatty acids were extracted in the polyphenol fraction and no polyphenols were observed in the fatty acid fraction so the differentiation of both fractions can be easily conducted in the same extraction run at the established conditions. Moreover, the extraction yields carried out sequentially or directly did not show significant differences (P-level > 0.05), so no losses occur during the consecutive extraction.

Figures of merit

The reproducibility of the optimized SFE extraction and analysis was checked performing the optimized fractionate extractions of fatty acids and polyphenols in different days. A mixture of both grounded seeds was used as sample. As it can be observed in Table 3, there were no statistical differences between results obtained in different days (P-level > 0.05). The repeatability of the analytes in terms of relative standard deviations (RSD%) was calculated carrying out 5 extractions from the same sample in the same day. The RSD values were in the 5% and 20% range for fatty acids and 3% and 10% range for polyphenols.

Regarding the quantification, good linearity was found out over the wide range of the tested concentrations (1 and 200 μg/mL for fatty acids and 20 and 500 ng/mL for polyphenols) being the coefficients of determination higher than 0.998 for FAMEs and polyphenols. The instrumental limits of detection (LODs) were set at the lowest concentration where the signal-to-noise ratio of the analytes were >3; the LODs were between 0.1 and 0.5 μg/mL for fatty acids and between 1 and 10 ng/mL for polyphenols.

Application to real samples

The optimized extraction method was applied to 2 different samples to observe its potential for recovering bioactive compounds from wine wastes. In this sense, red wine and white wine ground wastes were extracted consecutively with the optimized conditions in order to isolate fatty acids and polyphenols.

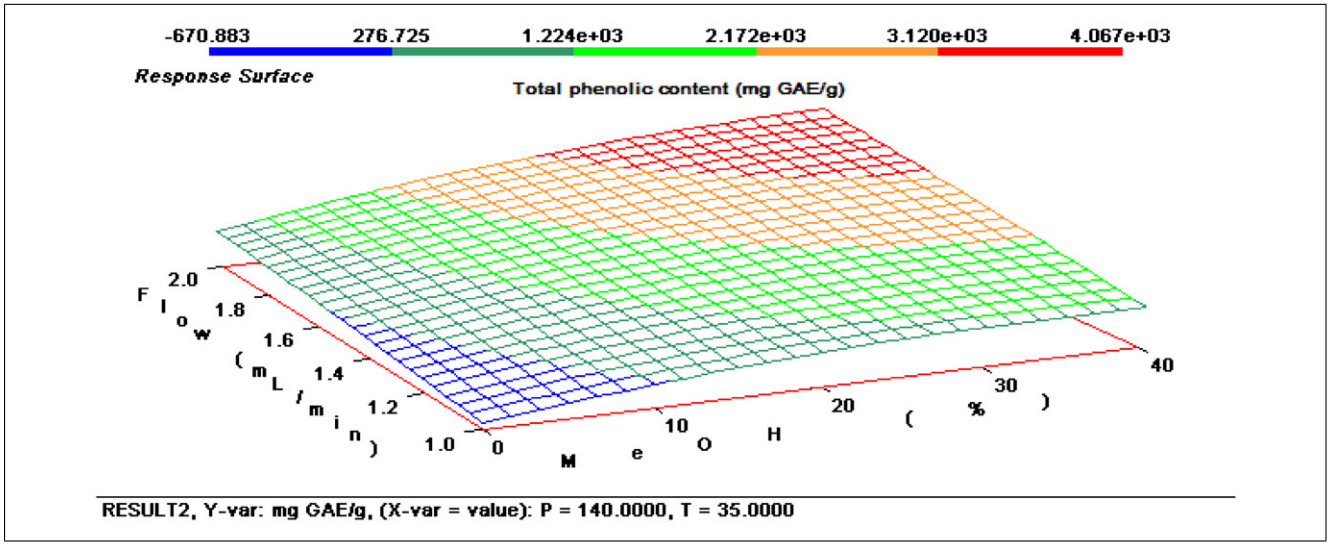

Figure 2–Response surface of flow (mL/min) and percentage of methanol (%) as modifier factors for the extraction of total content of polyphenols.

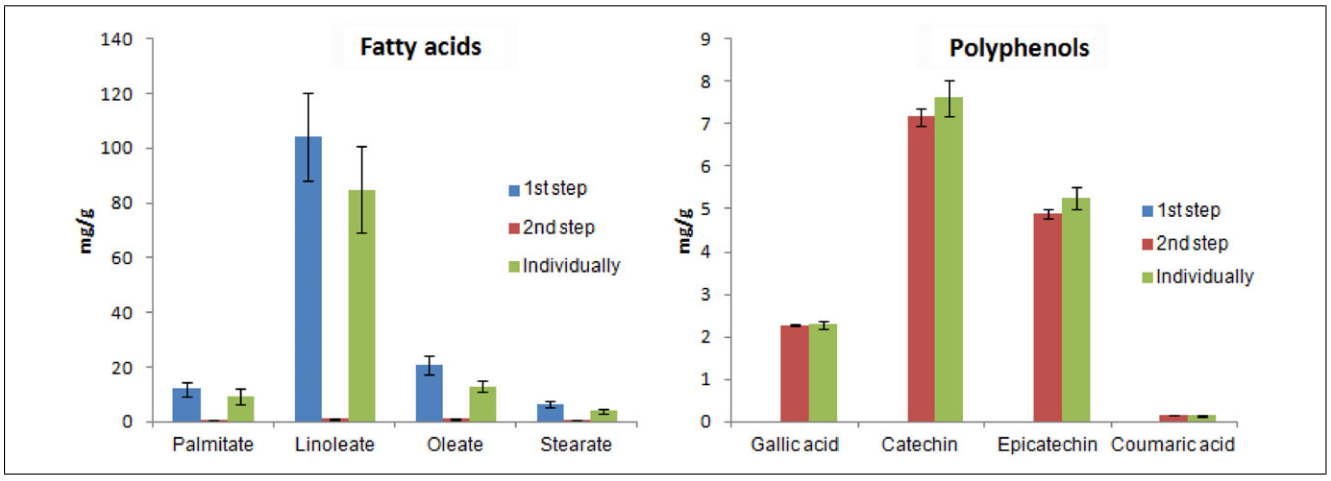

Figure 3–Fatty acid and polyphenol content in the 1st fraction (fatty acid step), the 2nd fraction (polyphenol step) and the individual extractions (extractions performed directly) with the corresponding standard deviations ($n = 3$).

Table 3–Fatty acid and polyphenol content of consecutive extractions ($n = 3$) in different days.

	Fatty acid (mg/g)					Polyphenols (mg/g)			
Extraction	Oil Yield (%)	Palmitic	Linoleic	Oleic	Stearic	Gallic acid	Catechin	Epicatequin	Coumaric acid
1st day	14 ± 2	12 ± 2	100 ± 20	21 ± 3	7 ± 1	2.28 ± 0.01	7.2 ± 0.2	4.9 ± 0.1	0.15 ± 0.01
2nd day	15 ± 2	13 ± 2	110 ± 10	23 ± 2	7 ± 1	2.1 ± 0.2	7.9 ± 0.6	4.5 ± 0.3	0.13 ± 0.02

Table 4–Fatty acid and polyphenol content of samples ($n = 5$).

	Fatty acid (mg/g)					Polyphenols (mg/g)			
Extraction	Oil yield (%)	Palmitic	Linoleic	Oleic	Stearic	Gallic acid	Catechin	Epicatequin	Coumaric acid
Red wine wastes	5.9 ± 0.4	5.6 ± 0.5	42 ± 2	9 ± 1	2.9 ± 0.5	2.1 ± 0.1	3.6 ± 0.5	3.1 ± 0.1	0.23 ± 0.01
White wine wastes	1.7 ± 0.1	1.5 ± 0.1	12.6 ± 0.7	2.3 ± 0.1	0.4 ± 0.1	3.4 ± 0.2	56 ± 2	100 ± 4	0.046 ± 0.003

Table 4 summarizes the concentration of fatty acids and polyphenols found in the analyzed white and red wines. On the one hand, it can be observed that in both varieties of grapes the proportion between the 4 fatty acids was the same, being the most concentrated the linoleic acid and then the oleic acid. This is a very promising result for the revalorization of wine industry wastes because the linoleic acid and the oleic acid are highly valued MUFA and PUFA, respectively. Moreover, being the proportion between fatty acids, the same in different varieties may be helpful for an industrial revalorization of different wastes all together. Furthermore, the obtained yields (6% oil yield in red wine wastes and 2% in white wine wastes) were high in comparison with previous works, where yields were from 6% to 15% (in this case in a seed mixture the yield was 14%) by means of SFE and from grape seeds fatty acids (Gómez and others 1996; Cao and Ito 2003; Agostini and others 2012).

On the other hand, it can be observed that white wine wastes have less fatty acids but more polyphenols than red wine wastes (Table 4). However, literature reviews that red wine grapes have more polyphenols than white wine grapes (Alonso Borbalán and others 2003; Makris and others 2007). This may be due to *Ostatu winery* red wine grape wastes are below the average in polyphenol content and on the contrary, *Mokoroaga Txakoli winery* above the average in comparison with other authors.

Conclusions

To provide a quantitative method to isolate bioactive compounds simultaneously from grape wastes, a SFE method has been fully optimized. The developed method allows the consecutive extraction of fatty acids and polyphenols from the same wine wastes with very good yields, at very short time (that is, 10 min taking into account all the procedure) and at low temperatures (that is, 35 °C) comparing with literature, where extraction time higher than 100 min was required (Ashraf-Khorassani and Taylor 2004). The proposed method is a promising alternative for a full revalorization of wine wastes before giving them any other application. The optimized method was successfully applied to 2 different wine wastes obtaining very good yields and the results obtained from grape residues were particularly promising.

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