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# RED WINE EXTRACT OBTAINED BY MEMBRANE-BASED SUPERCRITICAL FLUID EXTRACTION: PRELIMINARY CHARACTERIZATION OF CHEMICAL PROPERTIES.

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**Abstract –** This study aims to obtain an extract from red wine by using membrane-based supercritical fluid extraction. This technique involves the use of porous membranes as contactors during the dense gas extraction process from liquid matrices. In this work, a *Cabernet Sauvignon* wine extract was obtained from supercritical fluid extraction using pressurized carbon dioxide as solvent and a hollow fiber contactor as extraction setup. The process was continuously conducted at pressures between 12 and 18 MPa and temperatures ranged from 30 to 50ºC. Meanwhile, flow rates of feed wine and supercritical $CO_2$ varied from 0.1 to 0.5 mL $min^{-1}$ and from 60 to 80 mL $min^{-1}$ (NCPT), respectively. From extraction assays, the highest extraction percentage value obtained from the total amount of phenolic compounds was 14% in only one extraction step at 18MPa and 35ºC. A summarized chemical characterization of the obtained extract is reported in this work; one of the main compounds in this extract could be a low molecular weight organic acid with aromatic structure and methyl and carboxyl groups. Finally, this preliminary characterization of this extract shows a remarkable ORAC value equal to 101737 ± 5324 µmol Trolox equivalents (TE) per 100 g of extract.

*Keywords*: Supercritical fluid extraction, wine, membrane contactor, extract, chemical characterization.

## INTRODUCTION

In terms of food classification, some foods and beverages are not classified as functional, but these contain active substances of natural origin and may provide the same benefits offered by functional foods like green tea, chocolate and red wine (Sarmento et al., 2008).

Wines, specifically the red wines, are a rich source of different phenolic compounds, which contribute to its sensorial and antioxidant properties. Phenolic compounds have been identified as antioxidant, anti-inflammatory, neuro-sedative, anti-viral, anti-cancer and antimicrobial agents (Atanackovic et al., 2012; Colon and Nerin, 2012) and these are synthesized during plant growth in response to stress, representing the basic components of pigments, essences and flavors. These compounds are directly related to a huge variety of applications in food technology because of their contribution to oxidative stability and organoleptic characteristics (Sánchez et al., 2012). In humans, the consumption of these compounds has frequently demonstrated positive effects on health, due to their ability to modulate many homeostatic mechanisms, like lipid metabolism by controlling hepatic cholesterol

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absorption, triglyceride equilibrium and plasma lipoprotein processing. Furthermore, phenolic compounds may induce cardiovascular protective effects. Thanks to their antioxidant, vasodilator, anti-inflammatory, anti-fibrotic, antiapoptotic and metabolic properties, these biological properties are attributed mainly to their antioxidant and antiradical activity, which is related generally to the redox properties (Sánchez et al., 2012; Quintieri et al., 2012; Porgali and Büyüktuncel, 2011). At the same time, it is possible that all the properties described for red wines are due the effect of a mixture of compounds, rather than the effect of just one type of compound (Mendoza et al., 2011).

## MEMBRANE –BASEDSUPERCRITICAL FLUID EXTRACTION TO OBTAIN WINE EXTRACTS

### Chemical composition of wines

The phenolic compounds present in wines mainly derive from simple benzoic and cinnamic acids, stilbenes and flavonoids, which lead to more complex compounds formed by condensation, glycosylation and polymerization, where relevant contributions are made by tannic acids, anthocyanins, stilbene dimers, tartaric esters of cinnamic acids and proanthocyanidins and most generally could be divided into two major classes, based on their carbon structure: flavonoids and non-flavonoids. Flavonoids include anthocyanidins, flavanols, flavones and flavanones. The main non-flavonoid phenolic compounds include cinnamic acids, benzoic acids and stilbenes (Sánchez et al., 2012; Granato et al., 2011). Moreover, the phenolic acids are one of the most important quality parameters of wine, and they contribute to characteristics such as astringency and bitterness (Mendoza et al., 2011). Furthermore, some authors report direct and rapid chromatographic protocols for identification of specific active species in red wine, grape, and winemaking byproducts (Careri et al., 2003; Kolouchová-Hanzlíková et al., 2004).

On the other hand, through the supply chain of a product like red wine, we might find final products that do not meet the quality criteria, which may be a potential source for production of phenolic extracts, functional food components, healthy ingredients and additives (Boussetta et al., 2011). This extracts can be obtained by conventional and non-conventional processes.

The extraction process is a critical step in the isolation and recovery of high-added value compounds, in particular phenolic compounds (Aliakbarian et al., 2010). At the industrial scale, hydrodistillation or solvent extraction using methanol, ethanol, acetone or ethyl acetate, represent the traditional extraction methods for recovery of phenolic compounds from vegetable byproducts. These techniques require high volumes of organic solvents, which involve a high environmental impact (Li and Chase, 2010; García–Abarrio et al., 2012).

### Membrane–based supercritical fluid extraction

At present, membrane processes have been extensively applied to the recovery of valuable products (Li and Chase, 2010), which preserve the biological activity of the compounds contained in the raw materials, since these techniques reduce the consumption of chemicals, operating generally at low temperature.

On the other hand, supercritical fluids (SCFs) are good extracting solvents, with density and viscosity close to liquids and diffusivities of species like in gases (Bocquet et al., 2007; Oliveira et al., 2012). Carbon dioxide is a fluid widely used as supercritical solvent because of its relatively low critical point ($T_c$= 304.15 K and $P_c$ = 7.28 MPa) and non-toxic character. Furthermore, after extraction it is easy to separate it from the solute of interest by means of a depressurization step.

Membrane-based supercritical fluid extraction or Porocritical extraction is a commercial supercritical fluid extraction (SFC), which uses a hollow fiber membrane contactor (HFMC). In this process a macroporous membrane allows contact between two phases, where an aqueous liquid solution is circulated on one side and on the other side the extraction solvent in a SCF (Estay et al., 2007). However, supercritical $CO_2$ ($scCO_2$) shows affinity with lipophilic compounds and this characteristic becomes an inconvenience when polar compounds must be extracted, therefore, organic solvent (ethanol, methanol, ethyl acetate) mixtures – $CO_2$ under pressure have also been used to improve the extraction. Results reported by Santos and coworkers (Santos et al., 2012) showed a significant performance increase of phenolic extraction when ethanol was added as cosolvent. Meanwhile, the number of species detected by HPLC-MS increased from two to sixteen with this change. In the same way, Murga et al. (2000) reported that low molecular weight polyphenols were better extracted with $scCO_2$ and 15 % of methanol.

Previous studies by Yilmaz et al. (2011) on supercritical fluid extraction (SFE) show that the most important effect on the extraction of proanthocyanidins from dry grape seed in batch mode was the amount of ethanol added as cosolvent. Moreover, a large body of literature reports different experimental procedures for extraction of phenolic compounds using supercritical fluids and cosolvents (Satyajit and Lutfun, 2012).

Ruiz-Rodriguez et al. (2010) reported the production of a functional beverage prepared from wine, which was dealcoholized, obtaining a concentration of ethanol equal to 1% (v/v), by means of supercritical fluid extraction using a packed column of 2.8 m height with a pressure of 9.5 MPa and a temperature of 313K.

To our knowledge, there is no previous research on the continuous supercritical fluid extraction of specific compounds from wine using a membrane contactor device. In this work, an extract of red wine was obtained from supercritical fluid extraction in continuous mode where the

non-dispersive contact between the wine and dense carbon dioxide streams was achieved by a specially implemented hollow fiber contactor device.

This work aims to identify the main operating variables on the overall extraction performance, as well as to show a preliminary chemical characterization of the extract in terms of its potential applications.

## METHODOLOGY

### Materials and reagents

Chilean red wine *Cabernet Sauvignon* (Gran 120, Viña Santa Rita, L1309.2S) was obtained from a local market (Santiago, Chile). Meanwhile, liquid $CO_2$ (purity ≥ 99.0 %) was obtained from Praxair Chile. Furthermore, gallic acid $C_7H_6O_5$ (purity ≥ 99.0 %), sodium carbonate $Na_2CO_3$ (purity ≥ 99.9 %), Folin-Ciocalteu phenol reagent, deuterated chloroform (purity ≥ 99.8 %), glacial acetic acid (purity ≥ 99 %) and methanol (HPLC grade) were supplied by Merck. Acetonitrile (HPLC grade) and RMN tubes were obtained from Sigma Aldrich Chile.

### Membrane–based supercritical extraction assays

The supercritical fluid extraction setup was designed and built in the Laboratory of Membrane Separation Processes, LabProSeM, at the University of Santiago de Chile. This system contains a membrane contactor, which is formed by a single PTFE fiber (GoreTeX©; porosity=60 %; ID = 1.0 mm; OD = 1.8 mm), which is housed in a stainless steel module. This hollow fiber membrane contactor separates two independent circuits, one for the wine circulated into the lumen of the fiber by means of an isocratic pump (Jasco®PU-2080) and another one for the $scCO_2$ stream, which was circulated through the shell side by using a ISCO® 500D syringe pump. Red wine *Cabernet Sauvignon* was filtered before circulation through the extraction system.

This extraction system was operated in steady-state conditions and wine and $scCO_2$ streams were contacted countercurrently in the membrane contactor. Extract and raffinate samples were collected 15 min after the beginning of the operation in order to reach the steady state. Figure 1 shows the outline of the membrane-based extraction apparatus.

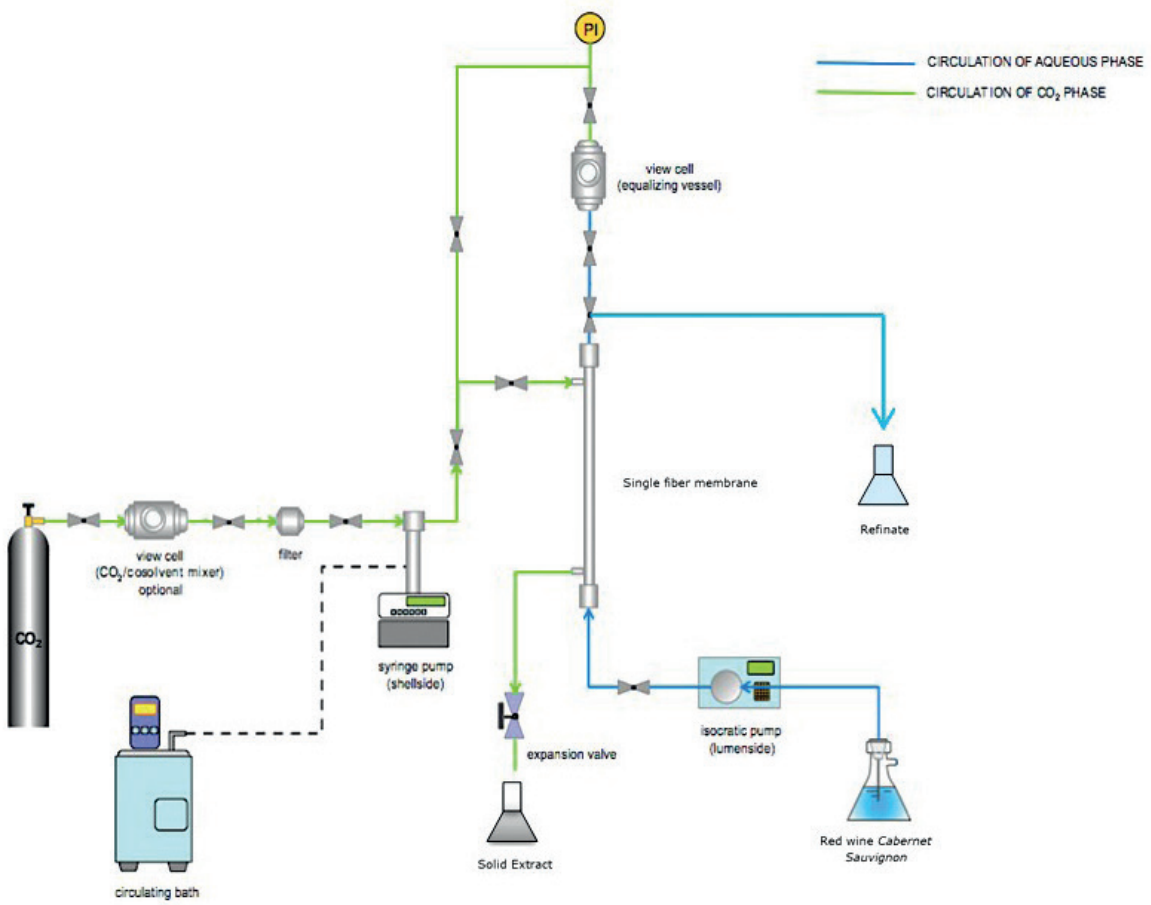



**Figure 1**. Setup of the continuous system for supercritical fluid extraction. (Adapted from Bocquet et al., 2007)

Preliminary experiments were carried out in order to evaluate the feasibility of phenolic extraction from Chilean red wine *Cabernet Sauvignon*. The operational conditions tested involved temperatures ranging from 303 to 323 K, pressure varying from 120 to 180 bar and $CO_2$ flow rate between 60 and 80 mL $min^{-1}$ (NCPT); meanwhile, wine flow rates ranged from 0.1 to 0.5 mL $min^{-1}$.

In order to assess the influence of the operational conditions on the extraction performance of this process, a full $3^4$ factorial design, which was distributed in 9 blocks, was previously defined.This experimental design considers the assessment of the effect of temperature, pressure, $CO_2$ flow rate and wine flow rate on the extraction percentage (%) and overall transmembrane phenolic flux (mol GAE $h^{-1}$ $m^{-2}$).

The coded and not-coded values of operating variables are reported in Table 1. The statistical analyses were carried out using the software Statgraphics® Centurion XV Version 15.2.05.

**Table 1.** Range of select levels for the variables in continuous supercritical extraction method.

| Variables | Low level (-1) | Medium level (0) | High level (+1) |
|---|---|---|---|
| **Liquid solution flow rate (mL $min^{-1}$)** | 0.1 | 0.3 | 0.5 |
| **$CO_2$ flow rate, NCPT (mL $min^{-1}$)** | 60 | 70 | 80 |
| **Temperature (°C)** | 30 | 40 | 50 |
| **Pressure (bar)** | 120 | 150 | 180 |

The experimental extraction percentage was estimated from the difference between the total concentration of phenolic compounds in the raw wine (*in*) and in the processed wine (*out*). This value is represented by equation 1:

$$Extraction\ yield\ (\%) = \frac{[Polyphenols]_{in}^{wine} - [Polyphenols]_{out}^{wine}}{[Polyphenols]_{in}^{wine}} \cdot 100 \quad (1)$$

Simultaneously, overall phenolic transmembrane flux transferred through the contactor was calculated from the extraction percentage value according to **equation 2**.

$$Transm.flux\ (mol\ GAE\ h^{-1}m^{-2}) = \frac{[Polyphenols]_{in}^{wine} - [Polyphenols]_{out}^{wine}}{A_{inner}^{mantle}} \cdot \frac{Q_{feed}^{wine}}{M.W_{G.Ac}} \quad (2)$$

where $Q_{feed}^{wine}$ represents the wine flow rate, $MW_{G.Ac}$ is the molecular weight of gallic acid and $A_{inner}^{mantle}$ is the contact surface available for mass transfer into the lumen side.

Probably, the extract is not composed exclusively of phenolic compounds, but these compounds represent a good basis to quantify the efficiency to obtain a dry extract from red wine.

### Chemical characterization of wine and extracts: analytical methods

#### Total phenolic content

The total polyphenols extraction was quantified using the Folin-Ciocalteu method (Sarmento et al., 2008) at 760 nm. The concentration of phenolic compounds in was calculated using Gallic acid standard solutions between 5 and 55 µg $mL^{-1}$ and the measurement were carried out in duplicate. The result was expressed as equivalent of gallic acid (mg GAE $mL^{-1}$ red wine).

#### Determination of ethanol content in raw wine and raffinate

The concentration of ethanol for raw wine and raffinate obtained after extraction were quantified by HPLC. This analysis was done with an Aminex HPX–87 H column, stainless steel 300 mm x 7.8 mm. The isocratic separation was done at 323K, using 0.004 M sulfuric acid solution as mobile phase with a volumetric flow rate of 0.5 mL $min^{-1}$ and running time of 30 min.

#### FTIR spectrum measurement

FTIR spectroscopy (Bruker, model ALPHA) was carried out on the dry extract obtained from the expansion valve of the membrane-based supercritical extractor device described in Figure 1. After expansion through the valve, freeze-drying at pressures between 0.01 and 0.10 mbar and – 40 ºC during 24 hours was applied to dry the collected extract sample.

On the other hand, the extract samples were analyzed by Attenuated Total Reflectance (ATR) spectroscopy, scanned from 4000 to 400 $cm^{-1}$ at 4 $cm^{-1}$ and 24 scans were generated per sample. Meanwhile, the processing of the obtained spectra was done with the Software Opus V.7.

### Analytical and semipreparative HPLC of extracts

Both analytical and semipreparative HPLC of extracts were carried out with a Waters 600 Chromatograph (Waters, Mildford, MA, USA), which was equipped with detector array diode Waters 2990. Analytical HPLC was achieved using a Symmetry C18 column (5 µm) 4.6 x 250 mm with 0.8 mL $min^{-1}$of mobile phase at room temperature by means of reverse phase with gradient (Table 2) at a wavelength of 300 nm. Moreover, semipreparative HPLC was implemented using a semipreparative column Waters Spherisorb S10 ODS2 10 x 250 mm isocratically operated (1400 psi) with mobile phase flow rates of 1.5 mL $min^{-1}$ and acetic acid (1%)/acetonitrile ratio equal to 40:60 at 300 nm.

The $^{1}H$ NMR spectra (400.13 Hz) and $^{13}C$ (100.62 Hz) were recorded in $CDCL_3$ solvent using a Bruker Advance DRX400 spectrometer with TMS as internal standard at 30 ºC.

### Antioxidant capacity ORAC assay

The procedure for ORAC assays was defined according to the method reported by Zhang et al. (2010). AAPH was used as a peroxyl radical generator, Trolox as a standard, and fluorescein as a fluorescent probe. The assays were carried out on a Victor Multilabel (Perkin Elmer, Germany) plate reader. All samples were analyzed in triplicate. The final ORAC value was calculated from the net area under the fluorescence decay curve and expressed in µmol Trolox equivalent (TE) per 100 g of sample.

**Table 2.** Mobile phase gradient utilized at analytic HPLC procedure.

| Time (min) | Volumetricflow ($mLmin^{-1}$) | A (Acetic acid 1%) | B (Acetonitrile) |
|---|---|---|---|
| 0 | 0.8 | 90 | 10 |
| 20 | 0.8 | 80 | 20 |
| 41 | 0.8 | 80 | 20 |
| 45 | 0.8 | 50 | 50 |
| 65 | 0.8 | 50 | 50 |

$^{1}H$ NMR and $^{13}C$ analysis of extracts

## RESULTS AND DISCUSSION

### Extraction performance

Extraction assays were done according to the procedure described above. The extraction performance was assessed through the experimental estimation of extraction percentage and transmembrane flux of total phenolic compounds, which were estimated from equations 1 and 2, respectively. Figure 2 shows the normal probability plot for extraction yield where it can be observed that extraction experiments were correctly done according to the random distribution of these tests.

Table 3 shows the summary of the extraction efficiency and transmembrane flow (expressed in GAE, Gallic Acid Equivalent) obtained from experiments as a function of pressure, temperature, wine and $scCO_2$ flow rates. This table shows that the most significant effect on the extraction percentage of total phenolic compounds is the wine flow rate, followed by pressure.

Figures 3 and 4 show that the phenolic extraction yield and the transmembrane flux of phenolic compounds increase for higher operating pressures and for higher wine flow rates in the membrane contactor. The combined effect of these operational variables allows obtaining transmembrane fluxes of phenolic extract ranging from

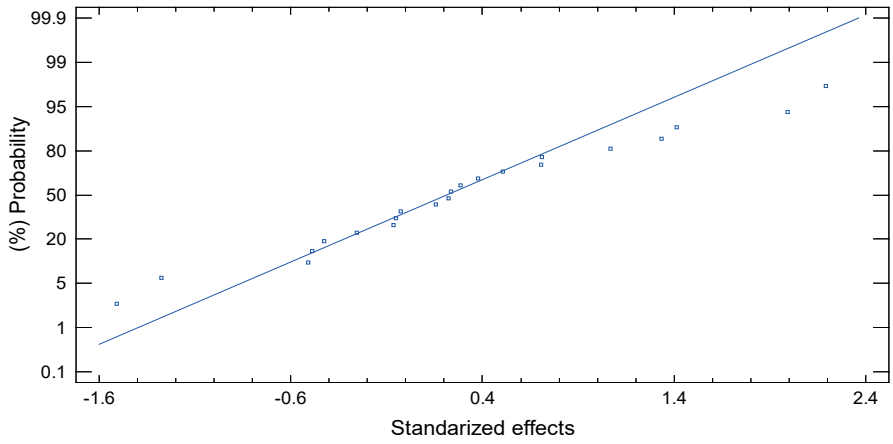


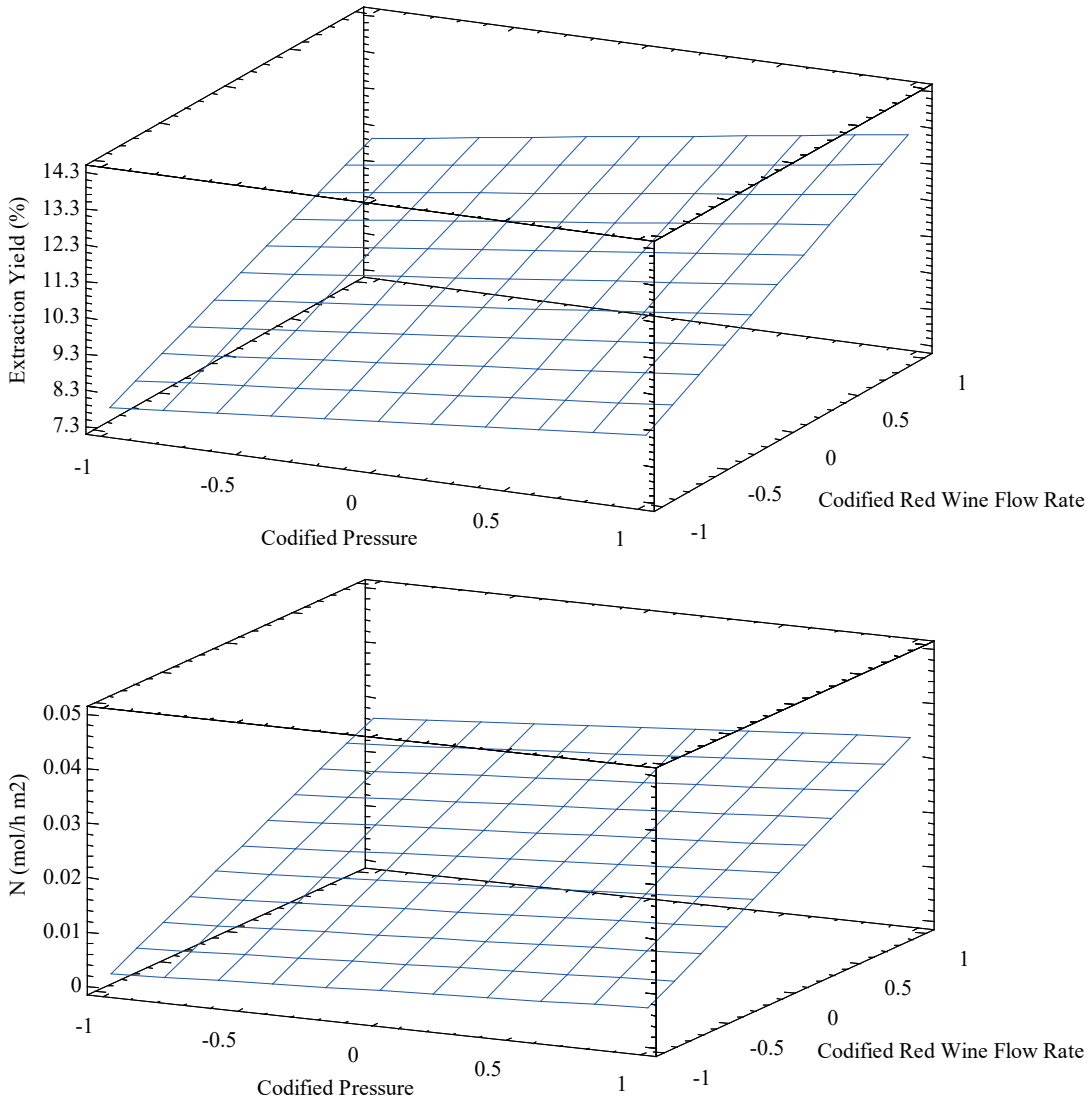

**Figure 2**. Normal probability effects plot for extraction yield evaluated according to the proposed experimental design.

**Table 3.** Values of significant effects on extraction percentage and transmembrane flux obtained under 95 % of confidence with Statgraphics Centurion XV.

| Effects | Extraction percentage | | | | Transmembrane flux (molGAE $h^{-1}$ $m^{-2}$) | | | |
|---|---|---|---|---|---|---|---|---|
| | Estimated (± 3.0596) | Optimum level | Magnitude | Value (%) | Estimated (± 0.0035518) | Optimum level | Magnitude | Value (mol $h^{-1}$ $m^{-2}$) |
| Temperature (ºC) | -0.4053 | -0.5 | 35 | 13.99 | -0.0004220 | 34.4 | 34.4 | 0.034 |
| Pressure(bar) | 1.7059 | 0.7 | 170 | | 0.0030090 | 178 | 178 | |
| Red wine flow ($mLmin^{-1}$) | 3.4962 | 1 | 0.5 | | 0.0242481 | 0.5 | 0.5 | |
| $CO_2$ SC flow ($mL\ min^{-1}$) | 3.1773 | 80 | 80 | | 0.0055239 | 75 | 75 | |

0.007 to 0.034 (mol GAE $h^{-1}$ $m^{-2}$) and extraction yields ranging between 8.3 to 14.0 %.

A large body of literature (Ruiz–Rodriguez et al., 2010; Moncada et al., 2013; Choi et al., 2010; Paviani et al., 2008) shows the effect of the pressure on extraction performance. However, this effect could be less important in our system because the mass transfer resistance in the feed solution boundary layer (red wine circulating in the lumen side) is




**Figures 3-4.** Estimated response surface of supercritical $CO_2$ extraction transmembrane flux from red wine *Cabernet Sauvignon.* T =30 ºC and $Q_{CO2}$ = 80 mL $min^{-1}$ NCPT.

significantly higher. The Reynolds number in the lumen side of capillary tubes or hollow fibers is extremely low (Re < 30). Thus, the feed solution flow rate represents a more important effect on the extraction performance for this system.

Table 4 summarizes the main results reported in the literature for extracts obtained from grape pomace, seeds, stem and skin using supercritical carbon dioxide and subcritical water. In these studies, the obtained extracts contain phenolic compounds, which could show degradation at high temperature or long times of processing. In the membrane extractor system of this study the residence time in the contactor varied between 5 and 32 seconds depending on the wine flow rates.

Chafer et al. (2007) report the increase of the solubility of gallic acid in $scCO_2$ when the pressure increases. The solubility of different types of compounds increases with pressure since the density of the supercritical fluid increases (Drake and Smith, 1990). Moreover, the natural presence of ethanol in red wine could improve the

**Table 4.** Extraction processes of phenolic compounds from grape products and byproducts.

| Matrix | Solvent | Operational conditions | Extraction Yield | Ref. |
|---|---|---|---|---|
| Grape pomace | Subcritical water | Batch system; 11.6 MPa – 140 °C. | Phenolic compounds/31.69 mg GAE $g^{-1}$ db. | Aliakbarian B. et al. 2012 |
| Grape seed | | Batch system; 1500 psig– 150 °C. | Catechins and Proanthocyanidins/ 70% db. | García-Marino et al. 2006 |
| Grape seed | Supercritical $CO_2$ | Batch system; 250-300 bar; 2 – 5% ethanol | Complex phenols and tannins/ less [EtOH] and pressure ➔ less [Polyphenols] | Murga et al. 2000 |
| Grape seed | | Batch system; 35 MPa – 313 K. | 13.42 % of extraction yield db at 430 min. | Meireles et al. 2012 |
| Grape seed | | Batch system; 250-300 bar; 30-50 °C; 15-20 % ethanol | Gallic acid 2.6 – 32.9 mg $kg^{-1}$ db. Catechins 5.8 – 78.83 mg $kg^{-1}$ db. Epicatechins 4.8 – 35.15 mg $kg^{-1}$ db. | Yilmaz et al. 2011 |
| Seed, stem, skin and grape pomace | | Batch system, 400 bar; 308 K; 5 % v/v ethanol | Resveratrol with extraction yield between 15 and 21 times greater than conventional methods. | Goli, Barzegar and Sahari 2005 |

extractability of phenolic compounds in this case. The concentration of ethanol was measured before and after the supercritical extraction assays in the raw wine and raffinate, respectively, observing a decrease from 13.2 ± 0.5 % v/v to 12.6 ± 0.12 % v/v because of this processing. This slight decrease could involve a modification in the extractability of different types of compounds present in wine at low concentrations.

On the other hand, the effect of the temperature on the extraction performance changes depending on the pressure or $scCO_2$ flow rate levels. Temperature can modify the density of pressurized $CO_2$ used as solvent as well as the transport properties through the membrane and boundary layers. This fact could explain the results reported in Figure 5 where the increase of temperature has a negative effect on the extraction yield at low $scCO_2$ flow rates and this effect is reversed at the highest level of $scCO_2$ flow rate.

The desirability function was calculated for optimization of the operating variables in the intervals studied in this work (Vera et al., 2014). Thus, the experimental responses -extraction percentage and transmembrane flux of phenolic compounds - are maximized in terms of the operating variables as a one objective function. Figure 6 shows the result obtained from this optimization where the optimum desirability value is equal to 0.76 for the lowest and highest values of temperature and pressure, respectively. Furthermore, the values of the factor levels that allow obtaining the maximum of the desirability function are summarized in Table 5.

Previously, the results reported in Table 3 showed that the highest value of extraction percentage reached 14 % when the wine flow rate, $CO_2$ flow rate, pressure and temperature were 0.5 mL $min^{-1}$, 80 mL $min^{-1}$ NCPT, 17 MPa and 35 ºC, respectively. The statistical analysis reported in Table 5 and Figure 6 validates this result.

Under the best operating condition tested in this work, 20.6 ± 1.13 mg of dry extract and 86.7 ± 2.1 mg GAE were collected per liter of processed red wine. This condition involves a residence time of wine in the extractor equal to 32 seconds with a consumption of 0.538 kg of $CO_2$, which can be considered low if compared with values reported by studies summarized in Table 4.

On the other hand, the highest transmembrane flux of total phenolic compounds obtained from a single extraction step was 0.034(mol GAE $h^{-1}$ $m^{-2}$) under the optimized conditions. This behavior is in agreement with the increase of the Reynolds number (Hoff et al., 2014) in the lumen side where the wine was circulated. Figure 7 shows the change of the dimensionless Sherwood number as a function of the Reynolds number (Re) in the lumen and in the shell

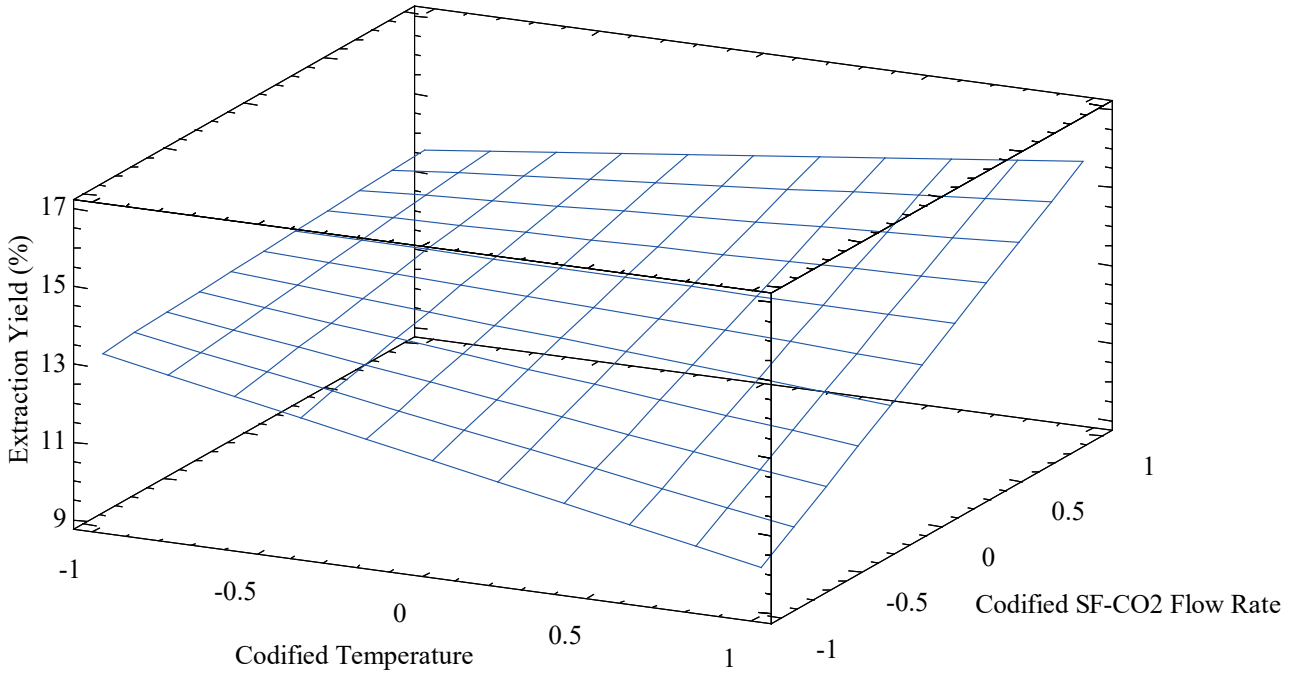



**Figure 5.** Estimate response surface of $scCO_2$ extraction yield from red wine *Cabernet Sauvignon.* P = 180 bar and $Q_{wine}$ = 0.5 mL $min^{-1}$.

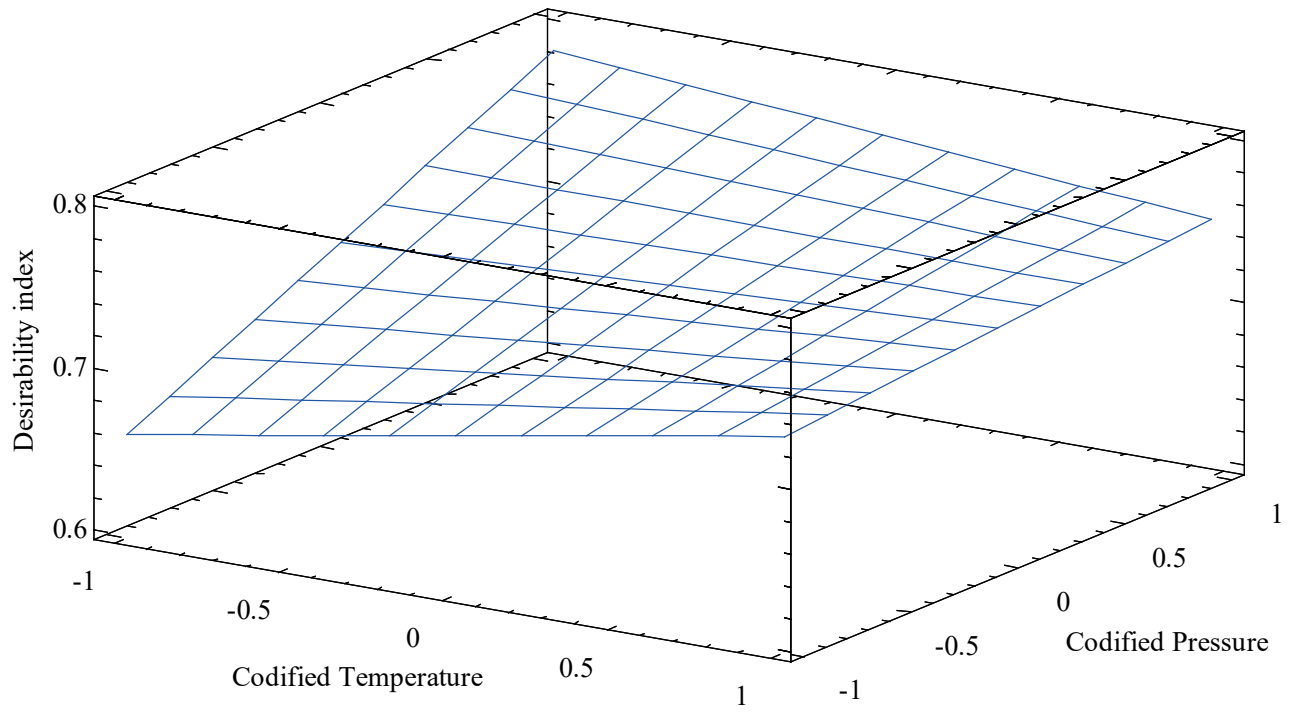



**Figure 6.** Estimated response surface of desirability for $scCO_2$ extraction of compounds from red wine *Cabernet Sauvignon*. $Q_{wine}$ = 0.5 mL $min^{-1}$ and $Q_{CO2}$ = 70 mL $min^{-1}$ NCPT.

**Table 5.** Values of factor levels to obtain the maximum of the desirability function.

| Factor | Value | Optimum level |
|---|---|---|
| Temperature | 32 ºC | -0.9 |
| Pressure | 180 bar | 1 |
| Red wine flow rate | 0.5 mL $min^{-1}$ | 1 |
| $scCO_2$ flow rate | 70 mL $min^{-1}$ NCPT | 0 |

side of the contactor. It is possible to observe a relatively significant change of the Sherwood number in function of the Re in both sides of the membrane contactor. Thus, the effect of the wine and $scCO_2$ flow rates on the extraction percentages can be understood.

**Chemical characterization of wine extracts**

Figure 8 shows the FTIR spectra, where it is possible to see the C–H stretching vibration for methyl and methylene groups, being the characteristic bands related to the main compounds in the dry extract with at least one aliphatic fragment (Coates, 2000) and potential presence of functional groups like alkanes, aromatics, ether, carboxylic acid, aldehydes or ketones.

Generally, bands of phenolic compounds found in grapes can be observed in the region between 900 and 1680 $cm^{-1}$. The bands in the range 1520 – 1600 $cm^{-1}$ might be attributed to vibrations of C=C bonds, typical of aromatic systems (Coates, 2000). The C–O bonds show peaks in the range 1060 – 1150 $cm^{-1}$, indicating the presence in the extract of an organic acid component (Edelman et al., 2001). The absorption band at 1750 $cm^{-1}$ is attributable to the stretching vibration of a C=O group in esterified carboxyl to methyl or protonated carboxylic acid O=C–O–H (Manrique and Lajolo, 2002). The presence of absorption bands in the interval 1420 – 1620 $cm^{-1}$ is attributable to absorbance of deprotonated $COO^-$ groups (Boulet et al., 2007). Peaks in the region 1407-1618 $cm^{-1}$ correspond to symmetric and asymmetric stretching for carboxyl ion ($COO^-$), indicating the presence of a carboxylic acid, ester or carbonyl group according to the information reported by other studies (Zhang et al., 2010). Thus, the change from carboxylic acid to salt could be a confirmation of acid structure, and logically the O–H stretching band disappears.

From the gradient HPLC analysis done on dry extract, it is possible to see only two peaks (Figure 9).

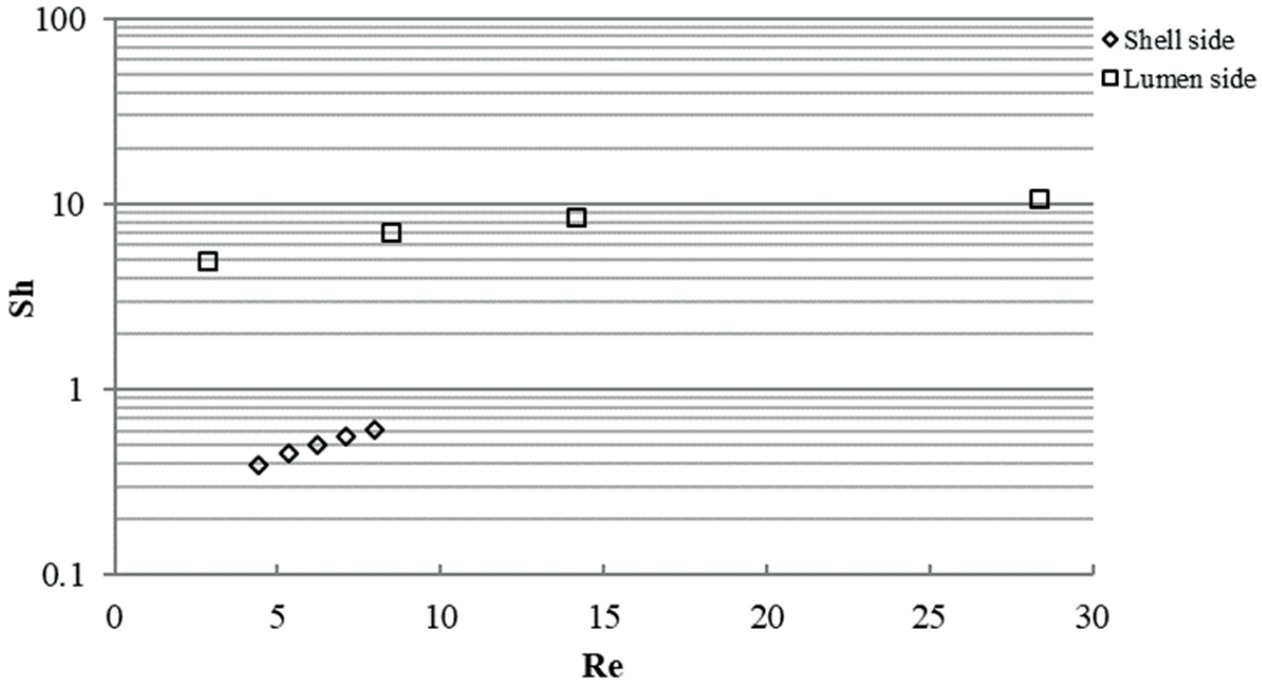



**Figure 7.** Dimensionless Sherwood number as a function of Reynolds number. T = 35 ºC; P = 180 bar. Correlation for shell and lumen sides (Bocquet et al., 2007):

$Sh_{shell} = 1.25 \cdot [(D_{eq}/L) \cdot Re_{shell}]^{0.93} \cdot Sc_{shell}^{1/3}$

$Sh_{lumen} = 0.5 \cdot Gz$ (Gz> 6.0)

$Sh_{lumen} = 1.62 \cdot Gz^{1/3}$ (Gz $\leq$ 6)

Visualizing the spectra obtained for the two separated compounds, it is seen that the component with the lowest retention time shows an absorption peak at 259 nm. However, the other compound showed two absorbance peaks at 228.4 and 311.1 nm. These values suggest the phenolic nature of one or both components according to the absorption range obtained (de Villiers et al., 2010).

The results of thermal analysis (DSC) show two endothermic peaks. Both peaks might represent one or two compounds present in the solid extract, showing that it does not represent a second order phase transition. Increasing the temperature, the first peak corresponds to the mean melting point temperature 124.42 ºC and it involves an absolute latent heat of 62.57 J $g^{-1}$, presenting a base width lower than the second peak. The width of the peak is associated with the change of size distribution of the structure from crystalline state to a disordered liquid state. The second peak presents a mean temperature of 248.4 ºC, which could denote a thermal resistance of the compound; this resistance might be attributed to the presence of aromatic rings. Masoud et al. (2012) obtained an identical result for gallic acid, which presented two endothermic peaks, at 124.5 ºC and 267.6 ºC in DTA analysis. Furthermore, benzoic acid shows two endothermic peaks, around 122.4 ºC and 250 ºC for melting point and boiling point, respectively, according to calibration standard DSC LGC2606.

The $^{13}C$ Nuclear Magnetic Resonance (NMR) spectrum of extract is reported in Figure 10 where it can be seen chemical shifts of δ = 172.63; 147.47; 140.91; 129.84; 118.23; 18.85 ppm, which could establish the presence of a carbonyl group with a displacement to lower field around δ = 172.63 ppm attributable to the presence of carbon in a O-$COCH_3$ group (Topcu and Ulubelen, 2007).

At higher field values, the carbon nuclei belong to aliphatic chains. Nevertheless, the peak at δ = 18.85 ppm could be related to $CH_2$ or $CH_3$ groups. The distribution of those types of carbon in the structure involves the presence of an aromatic ring structure because of the presence of the other 4 peaks above chemical displacement δ = 110 ppm and at least 1 type of carbon associated with a $CH_3$ group. Furthermore, at low field (δ < 40 ppm) in the level of the noise, there are peaks that could be associated with linear aliphatic structures of other components extracted from the red wine with supercritical $CO_2$. The compounds would represent a minor fraction of the whole extract.

The $^{1}H$ NMR spectrum reported in Figure 10 shows nuclei in aromatic, olefinic and alkyl regions. Moreover, its complexity makes it difficult to identify individual organic species in the absence of authenticated standard and/or reference NMR spectra. The spectrum obtained from this analysis showsaromatic regions with intensity lower than observed in the aliphatic region, showing chemical displacement values δ = 7.32 – 7.37 ppm for a doublet of doublets integrating as 4 hydrogens, which might correspond to an aromatic substituted compound bonded to an electron withdrawing aliphatic chain. Those peaks appearing at chemical shifts 6.23 ppm as a singlet and 6.21 ppm as a triplet with coupling constant J = 3.1 Hz are associated with hydrogens present in aromatic rings and/or carbons linked to double bonds. Furthermore, peaks around 5.75 – 5.79 ppm allow establishing the coupling constant J = 15.4 Hz for a doublet with configuration trans between neighboring hydrogens or the presence of negative elements in its composition.

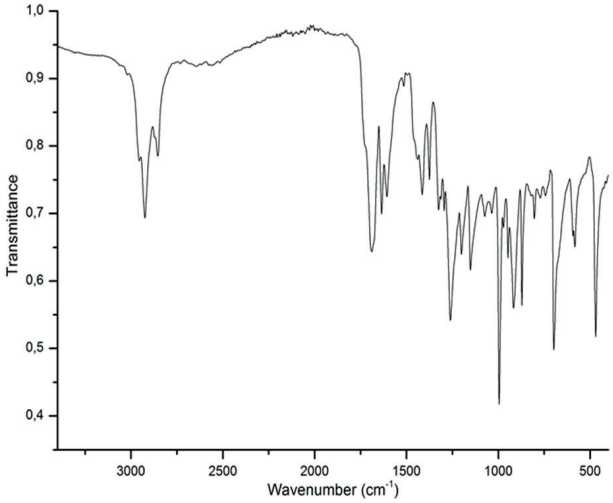



**Figure 8**. FTIR spectrum of the dry extract obtained from red wine *Cabernet Sauvignon* by means of membrane-based supercritical extraction under the best process conditions.

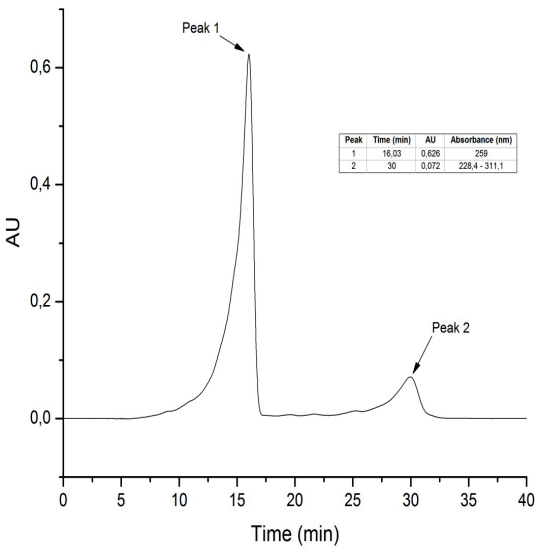



**Figure 9.** High performance liquid chromatography for dry solid extract obtained from red wine *Cabernet Sauvignon* using as mobile phase a solution of acetic acid 1 % in methanol under isocratic configuration 1.5 mL $min^{-1}$ and 300 nm.

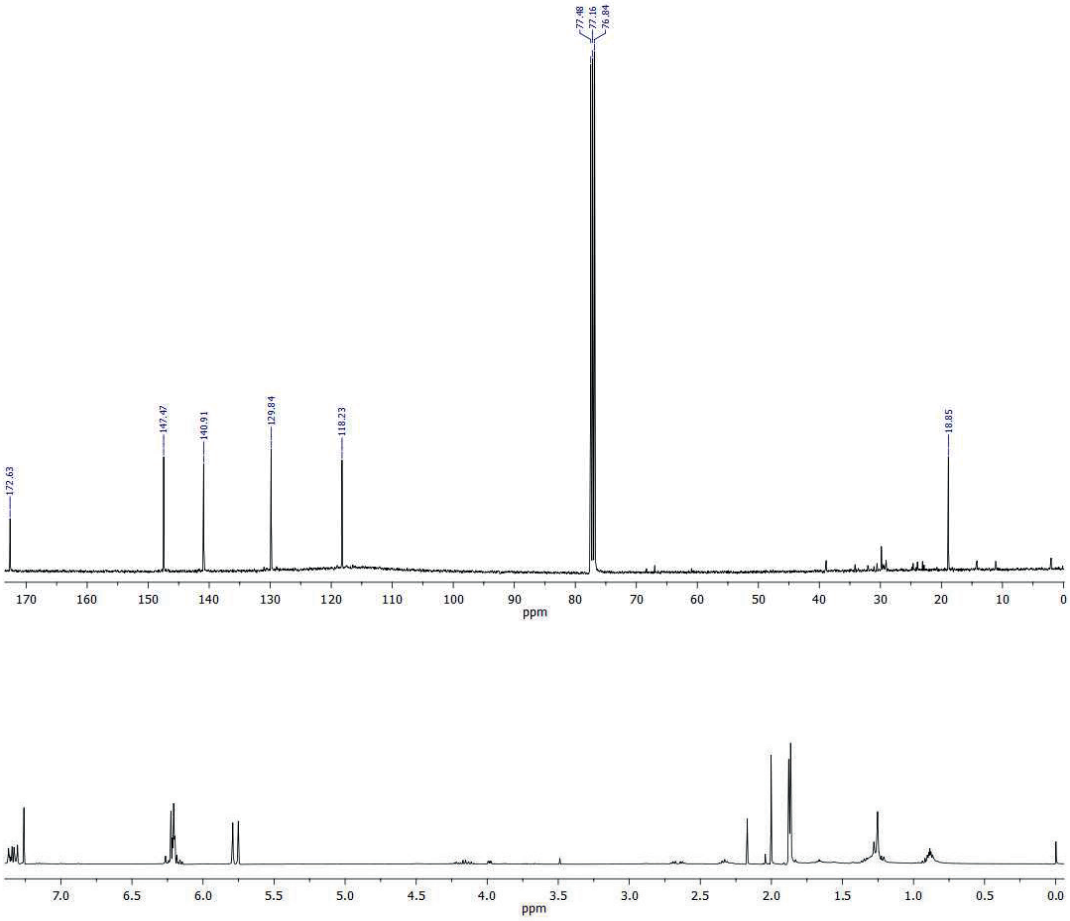



**Figure 10**. NMR spectrum $^{13}C$ (0 – 180 ppm) and $^{1}H$ (0 – 8 ppm) of the dry solid extract obtained from red wine Cabernet Sauvignon by means of membrane-based supercritical extraction under the best condition of extraction at 30 ºC using $CDCl_3$ as deuterated solvent

From the ensemble of these results, the most probable structure of the main molecules that constitutes the dry red wine extract obtained by membrane-based supercritical fluid extraction could be mainly two compounds of a intermediate polarities and low molecular weight. The principal compound could correspond to an acid with aromatic structure and the presence of methyl and carboxyl functional groups in the structure.

## Antioxidant Capacity ORAC

The antioxidant capacity quantification of the obtained red wine extract was by means of the ORAC method. The curve of ORAC assay reported in Figure 11 shows two curves of fluorescein decaying at dilution factors 100x and 60x for the solid extract in phosphate buffer, where the lag time decreased when the sample was diluted. It is possible to verify a significant antioxidant capacity and lag time of around 2000 seconds.

The antioxidant capacity of red wine extract is shown in Table 6 and equal to 101737 ± 5324 µmol TE per 100 g of lyophilized extract. This value is equivalent to the antioxidant activity of Açai fruit pulp/skin powder (ORAC value of 102700 µmol TE per 100 g) but is lower than

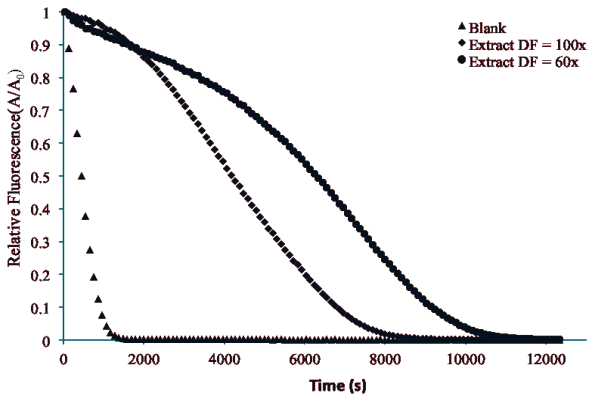



**Figure 11.** ORAC curves for antioxidant capacity of lyophilized extract obtained by means of membrane-based supercritical extraction from red wine *Cabernet Sauvignon* under the best operational conditions.

spices such as cinnamon, rosemary, oregano and cloves among others reported in Table 7, which shows a value range varying from 131420 to 290283 µmol TE per 100 g of sample (USDA, 2010). Thus, the extract obtained from red wine *Cabernet Sauvignon* by membrane-based supercritical $CO_2$ extraction could be among the 12 food products with higher antioxidant capacities reported in the ORAC database of the USDA (2010). Furthermore, the ORAC value of this extract is around ten times lower than BHA, which represents a commercial artificial antioxidant.

Furthermore, the red wine extract obtained in this work shows ORAC values between 24 - 40 times higher than wines and 1.3-7.0 times higher than the fruits mentioned above, respectively. Meanwhile, for pure substances such as Zeaxanthin, gallic acid and ascorbic acid, the ORAC values ranged from 9.8–272 times lower than the ORAC value obtained in this work.

**Table 6.** Equivalences for ORAC values of extract obtained from red wine *Cabernet Sauvignon* by means of membrane-based supercritical extraction.

| mmol TE $g^{-1}$ | µM Trolox | µmol TE $g^{-1}$ | µmol TE per 100 g |
|---|---|---|---|
| 1.017 | 10878.69 | 1017.37 | 101737.07 |

**Table 7.** Antioxidant capacity of substances and food products evaluated by the ORAC method.

| Substance/Food | ORAC value | Unit | Source |
|---|---|---|---|
| Ascorbic Acid | 40.4 ± 2.1 | µM TE | Zulueta et al. (2009) |
| Gallic Acid | 161 ± 4.8 | µM TE | |
| β - carotene | 582 ± 30.3 | µM TE | |
| Zeaxanthin | 1108 ± 5.9 | µM TE | |
| BHA (Butylated Hydroxy anisole) | 13500 ± 332 | µmol TE $g^{-1}$ | Dávalos et al. (2004) |
| Squalene | 304.3 | µmol TE $g^{-1}$ | Kraujalis and Venskutonis (2013) |
| Cinnamon, ground | 131420 ± 13867 | µmol TE/100$g^{-1}$ | Haytowitz and Bhagwat (2010) |
| Rosemary, dried | 165280 ± 1319 | µmol TE/100$g^{-1}$ | |
| Oregano, dried | 175295 ± 7683 | µmol TE/100$g^{-1}$ | |
| Cloves, ground | 290283 ± 3292 | µmol TE/100$g^{-1}$ | |
| Red wine *Cabernet Sauvignon* | | | |
| Santa Tierra (Chile) | 28.064 ± 2.073 | µmol TE $mL^{-1}$ | Van Leew et al. (2014) |
| Casillero del Diablo (Chile) | 42.669 ± 1,14 | µmol TE $mL^{-1}$ | |
| Sunrise (Chile) | 25.773 ± 1.493 | µmol TE $mL^{-1}$ | |
| Chocolate, Dark | | | |
| Black & Green´s Organic | 118.9 | µM TE$g^{-1}$ | Stockham et al. (2011) |
| Lindt, 70 % | 171.8 | µM TE $g^{-1}$ | |
| Lindt, 90 % | 290.0 | µM TE $g^{-1}$ | |
| Fruits | | | |
| Strawberry | 154 | µmol TE$g^{-1}$ | Yilmaz et al. (2006) |
| Plum | 80 | µmol TE $g^{-1}$ | |
| Red Grape | 36 | µmol TE $g^{-1}$ | |
| Orange | 52 | µmol TE $g^{-1}$ | |
| Blueberries | 63 – 282 | µmol TE $g^{-1}$ | |
| Blackberries | 8650 ± 200 | µM TE | Atala et al. (2009) |
| Raspberry | 2870 ± 700 | µM TE | |
| Tomato dry | 222.3 ± 285.61 | µmol TE $g^{-1}$ | Li et al. (2012) |

Chemical characterization of the extract described above involves the identification of a mixture with at least two main compounds. These compounds seem to show a synergic antioxidant action. Nevertheless, it is necessary to emphasize that the ORAC assay is a simplified technique that reduces the complex antioxidant mechanism to a reaction between the radicals produced by AAPH and the analyzed antioxidant. The combined effect of a mixture of antioxidants on different paths of the food oxidation processes effectively acts in a synergistic way, providing extra protection higher than the addition of the effects of the single antioxidant components (Bentayeb et al., 2014). However, red wine polyphenols have different physiological properties, which depend on the composition of the extracts (Zoechling et al., 2011). Thus, redox values become only a surrogate parameter and further investigation is necessary for the detailed characterization of the extract composition.

## CONCLUSIONS

In this work, the membrane-based supercritical fluid extraction process was used to obtain an extract directly from *Cabernet Sauvignon* wine. During the extraction runs implemented in a single PTFE fiber contactor, a permanent stability of the extraction system it was observed, as well as reproducible measurements.

The continuous extraction system with supercritical $CO_2$ coupled to a membrane contactor allowed the extraction of organic compounds from red wine *Cabernet Sauvignon*, as well as evidenced the great mechanical stability presented by membrane fiber.

The process of this extraction gave a maximum dry extract yield of 20.6 mg per L of red wine processed in only one extraction step in steady-state conditions when the wine flow rate, $CO_2$ flow rate, pressure and temperature were 0.5 mL $min^{-1}$, 80 mL $min^{-1}$ NCPT, 17 MPa and 35 ºC, respectively. Furthermore, the extraction yield as a function of supercritical $CO_2$ consumption was 40.9 mg per kg of $CO_2$.

From the chemical characterization of the dry extract, the most probable structure of the main molecules extracted by membrane-based supercritical fluid extraction could be associated with an acid with aromatic structure and the presence of methyl and carboxyl functional groups.

The extract obtained under the best operational condition showed an antioxidant activity equivalent to 101737± 5324 µmol TE per 100 g of lyophilized extract. This value was obtained by the ORAC method and is higher than those obtained for other raw food materials like fruits, chocolate, green tea leaves and red wine *Cabernet Sauvignon* itself. This ORAC – FL value of extracts allows positioning among food compounds with a significant antioxidant capacity for its use in food formulations or in mixtures with other compounds with synergic action. Nevertheless, further investigation is required to know the detailed chemical composition of these extracts.

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