Extraction by subcritical water of polyphenols from Dunkelfelder and Cabernet Franc grape pomace coupled with membrane filtration

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ABSTRACT

The residuals of winemaking, 5-8 million tons of potentially utilizable matter, are extremely rich in compounds that could be exploited for industrial purposes, due to their relevant biological properties, with applications in the nutritional, medical and cosmetic fields . The potential economic importance of these materials has been highlighted by several research studies. The extraction of polyphenols from residuals, which can be achieved using non toxic processes (e.g. Pulsed electric field, High voltage electric discharge, Subcritcal water extraction) coupled with membrane separation processes with low environmental impact, may yield high amount of raw material to be marketed in this form or further refined in order to obtain more valuable substances. Subcritical water extraction of polyphenols from the red grape pomace of Dunkelfelder and Cabernet Franc varieties was performed during two years. The combined effects of extraction temperature (100, 125, 150 and 200 °C) and pressure (25, 50, 75 and 100 bar) were investigated and compared to pulsed electric field and traditional solvent extraction. Subcritical water extraction (SWEX) process was more efficient than using organic solvent and aqueous based system at atmospheric pressure for recovery of antioxidants, leading to double the extraction quantity. Several analytical tests such as ORAC test, Folin-Ciocalteau test showed similar results using the different grape varieties. Optimum extraction conditions were shown to be 175°C irrelevant of the pressure used. Quantification of tannins from subcritical water extracts by HPLC showed that the higher the extraction temperature, more tannins are extracted. While the quantification of anthocyanins by HPLC of the same samples gave differing results, by increasing the temperature the amount of extracted anthocyanins decreases. Membrane processes offered a powerful alternative for the concentration of natural grape extracts, due to their flexibility and mild operating conditions. Three molecular weight cut off 0.33 µm, 30kDa, 3kDa were chosen, that gave interesting differential results. The fractions obtained will be tested in several food industry domains.

INTRODUCTION

Bioactive phenolic compounds have antioxidant, neuro-sedative, anti-inflammatory, antiviral and anticancer properties [1,2]. The extraction is a key step in the isolation and recovery of these compounds [3]. Traditional methods of recovery are usually based on solid-liquid solvent extraction [4]. These techniques have disadvantages due to their high consumption of organic solvents, which leads to an adverse impact on the environment. Alternatively, subcritical water extraction has been proposed as an efficient and effective recovery method for the recovery of phenolic compounds [5]. The overall objective of our work was to define the best parameters for extraction with subcritical water (SWEX). More specifically, we studied the extraction of polyphenols from red grape marc (Dunkelfelder and Cabernet Franc Varieties) at several temperatures and pressures.

MATERIALS AND METHODS

Grape pomace (Dunkelfelder and Cabernet Franc) provided by the engineering school of Changins, Switzerland was stored at 4 °C until the further.
The schematic diagram of the apparatus used for the extraction of polyphenolic compounds using subcritical water is shown below. In the extraction system, an HPLC pump was used for water delivery, pressurization and controlling the pressure of the system. A pressure transducer and thermocouple were installed in the custom-made high-pressure vessel to monitor both pressure and temperature of the system. After passing in an ice bath the extract was collected in vessels containing inert gas (200 ml volume).

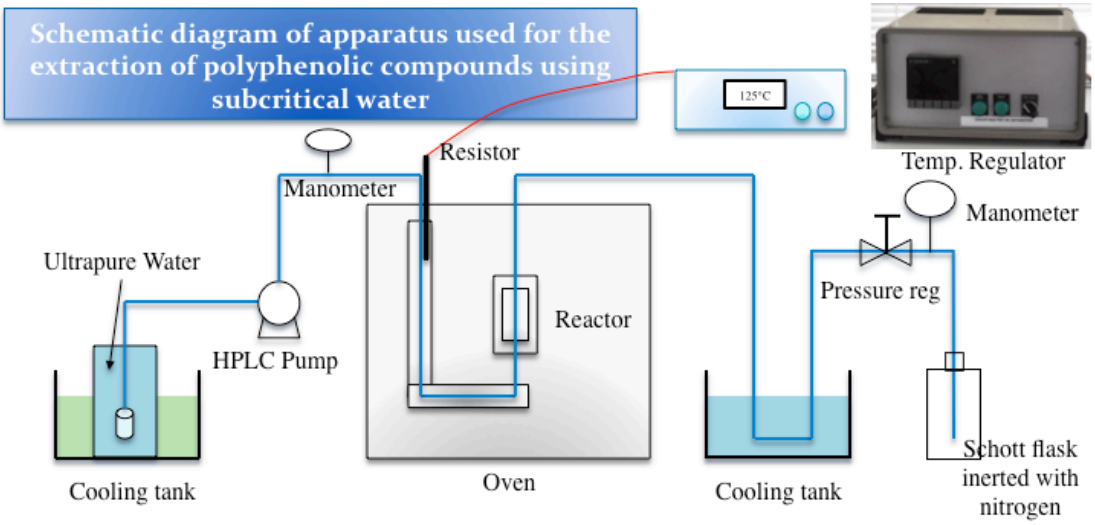

Figure 1. Schematic diagram of the apparatus used subcritcal water extraction.

In each run pomace (13.00 g) was loaded into the high-pressure vessel. The vessel was placed in an oven at a predetermined temperature. The outlet valve of extraction vessel was then closed and the system was pressurized to a desired pressure at a constant flow rate.
The water flow rate was adjusted at 6 ml/min using a metering valve on the HPLC pump.
Conventional extraction procedure: 20 g of pomace was added to 50 ml of pure ethanol and 50 ml of milli-Q water in 125 ml bottles with screw caps and placed on magnetic shakers. The extraction took place for 7 h, at, 20 °C and at 160 rpm. The liquid was separated from solid by

centrifugation at 5000 rpm for 10 min. The yield of this conventional method is used as the benchmark to evaluate the efficiency of subcritical water extraction. Samples were kept at 4°C for further analysis.
The concentrations of total phenolic compounds (TP) were measured using Folin–Ciocalteu assay [6]. Colouring Intensity (CI) and Total Polyphenol Index (TPI) were calculated according to the handbook of oenology. The major anthocyanins and tannins were quantified by HPLC [7]. Some samples were analysed for their volatile compound content by GC-MS. The antioxidant however, activity was measured by the Oxygen Radical Absorbance Capacity (ORAC) assay [8].

RESULTS

The subcritical water extraction process was more efficient than using an organic solvent and aqueous based system at atmospheric pressure for recovery of antioxidants, leading to double the extraction quantity.

Several analytical tests such ORAC test and Folin-Ciocalteau test with a higher polyphenol extraction from Dunkelfelder varieties in comparison to Cabernet Franc and the optimum extraction for Dunkelfelder variety is at 175°C irrelevant of the pressure used.

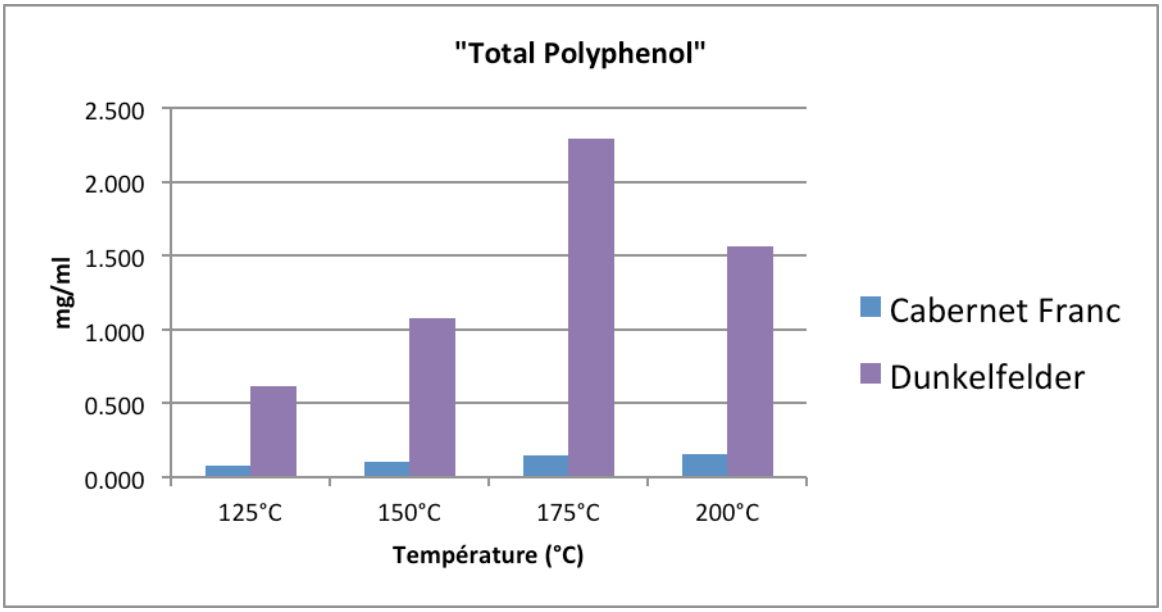

Figure 2. Total polyphenol content In Dunkeldet and Cabernet Franc varities at several extraction temperature (100, 125, 150 and 200 °C)

Coloring intensity results of SWEX samples revealed higher values in comparison to traditional extraction methods (30% higher).

Chromatograms of the tannins and the anthocyanins indicated some differential molecules, which were only extracted using SWEX.

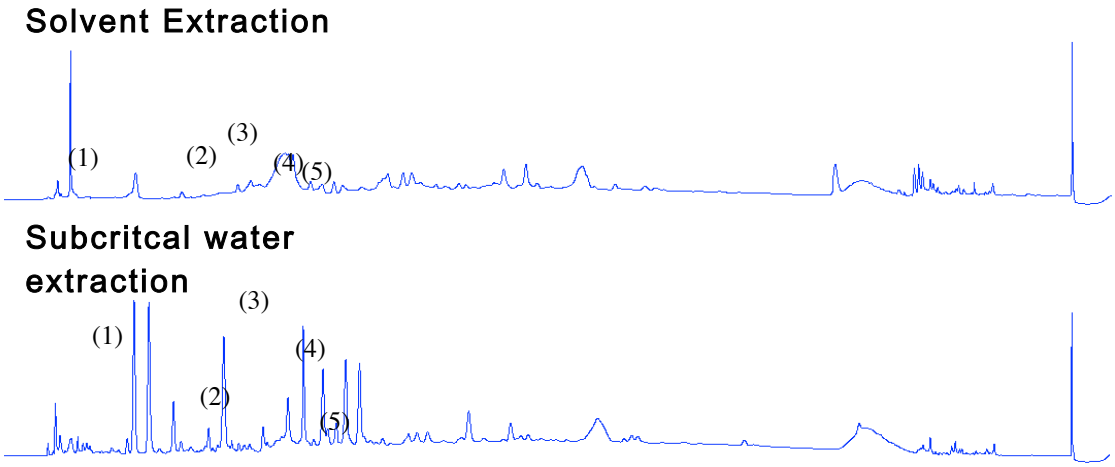

Figure 3. Tannin chromatogrammes by HPLC of the of tannins extracted by hydroalcoholic extraction or Subcritical (gallic acid (1), dimer B1 (2), catechin (3), epicatechin (4) C1 trimer (5)).

Quantification of tannins from subcritical water extracts by HPLC showed that the higher the extraction temperature, more tannins are extracted. While the quantification of anthocyanins by HPLC of the same samples gave differing results, by increasing the temperature the amount of extracted anthocyanins decreases.

Comparing GC-MS results between SWEX and traditional method of extraction showed that during extraction furfural compounds are produced and volatile esters are degraded.

Membrane processes offered a powerful alternative for the concentration of natural grape extracts, due to their flexibility and mild operating conditions. Three molecular weight cut off 0.33 μm, 30kDa, 3kDa were chosen, that gave interesting differential results.

CONCLUSION:

Subcritical water extraction process showed a higher efficiency than organic solvent.175 °C appears to be the optimum extraction parameter for Dunkelfelder variety. Pressure doesn't seem to have a significant difference on the extraction yield. An increase in temperature increases the tannin and decreases the anthocyanin extraction. Filtration analysis should be further studied.

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