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Extraction of antioxidants from winery wastes using subcritical water

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ABSTRACT

Subcritical water extraction of phenolic compounds from grape pomace was performed. The combined effects of extraction temperature (100, 120 and 140 °C) and pressure (8 MPa, 11.5 MPa and 15 MPa) were investigated using a 3^2 full factorial design and response surface methodology. Extractions with significantly higher polyphenols, flavonoids and antioxidant activity were achieved when using subcritical water extraction compared to conventional methods. The optimum extraction conditions and the desirability of model were at 140 °C and 11.6 MPa (0.9550). At this operating condition, 31.69 mg_{GAE}/g_{DP} and 15.28 mg_{CE}/g_{DP} of total polyphenols and flavonoids were recovered, respectively. The extracts showed antiradical power of 13.40 $\mu g_{DPPH}/\mu l_{extract}$. Subcritical water extraction was more efficient than using water and ethanol at atmospheric pressure for the extraction of these compounds.

1. Introduction

Polyphenols are compounds with many bioactivities such as antioxidant, neuro-sedative, anti-inflammatory, anti-viral and anti-cancer [1,2]. Fruits and vegetables are rich resources of antioxidants [3–7]. Valorization is the common method of using biomasses as raw material or as an energy carrier. In this method, there is a great emphasis on environmentally friendly processes to reduce emissions and environmental impacts [7]. In particular, grapes as one of the largest fruit's crops are the major dietary source of phytochemicals such as polyphenols which includes flavonoids and anthocyanins [8]. Each year, wine making industry (WMI) produces a substantial amount of grape by-products (pomace) including skins and seeds. These wastes may have a significant environmental impact due to high content of phenolic compounds and considerable chemical and biochemical oxygen demands (COD and BOD) [8]. The grape marc is mainly utilized by the distillery industry to produce alcohol and alcoholic drinks. The grape biomass is also used for seed oil extraction, and production of animal feed, compost, additives and natural color [9]. In addition, the grape biomass from WMI is a rich source of valuable compounds such as antibacterials, antifungals, antioxidants, phytopharmaceuticals, and nutraceutical products [10–15]. These can be extracted to use in food, cosmetic and pharmaceutical industries to enrich their final products [16,17].

Extraction is a critical step in isolation and recovery of high added valued compounds, in particular phenolic compounds [8]. Hydrodistillation or solvent extraction is the traditional extraction method for recovery of phenolic compounds from vegetable by-products [18]. These techniques are disadvantaged by the consumption of organic solvents which leads to unfavorable environmental impact. Alternatively, supercritical fluid (SCF), a fluid at above critical temperature and pressure, is used for the extraction of active compounds. Recent studies showed that in industrial scale, supercritical fluid extraction is an efficient and cost effective technique for recovery of phenolic compounds from biomass [12,19–22]. Carbon dioxide is the most common solvent for the supercritical fluid extraction process due to its inert, non-flammable, and non-toxic properties. Supercritical CO_2, however, exhibits low dielectric constant, ranged from 1.1 to 1.5 [23]. Very high pressure is therefore, required to dissolve highly polar compounds such as fatty acids, sterols, and terpenes in CO_2 [24]. Addition of small quantity of a cosolvent such as water and ethanol to supercritical CO_2, and replacing this fluid with solvents such as water and refrigerants are alternative approaches for improving the efficiency of the process for the extraction of polar compounds at lower pressures.

The dielectric constant of water at ambient conditions is 80, however, it can be decreased to 56 and 27 (similar to organic solvents) by increasing the pressure to 5 MPa and temperature to 100 °C and 250 °C, respectively [25]. Water is in subcritical condition at temperatures less than 374 °C and pressures below 22 MPa. Superior mass transfer properties of subcritical water lead to high diffusivity and hence higher extraction efficiency. The solubility and diffusivity of water at moderate pressure is, therefore, comparable with organic solvents. Unlike, organic solvents, there is no environmental impact associated with water. Different

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nutraceutical substances were extracted from WMI biomass, using subcritical water extraction technique [26–28].

García-Marino et al. have used subcritical water for the extraction of catechins and proanthocyanidins from grape seeds [27]. In their study sequential extractions were performed and subcritical water was used at 10.3 MPa and within the temperature range of 50–150 °C. The recovery of catechin and epicatechin was enhanced two-fold, compared to conventional methanol:water (75:25) extraction using ultrasonic bath for 15 min. Luque-Rodríguez et al. [28] employed superheated ethanol–water mixture for the extraction of anthocyanins and other phenolic compounds from grape pomace. In this study, neutral ethanol:water and acidified ethanol:water with 0.8% (v/v) HCl were used for extraction. The yield of extraction was increased 7 and 12 fold by increasing pressure and temperature from atmospheric conditions to 8 MPa and 120 °C when using neutral and acidified solvents, respectively. Monrad et al. [29] studied the effect of accelerated solvent extraction on recovery of anthocyanins from grape pomace at different temperatures (40–140 °C) and ethanol in water ratios (10–70%, v/v). The results of this study showed that the optimum ranges for temperature and ethanol concentration are 80–120 °C and 50–70% (v/v). It is concluded that despite lower extraction yield, running cost of the extraction process can be substantially decreased by using lower concentration of ethanol in hydroethanolic solvent.

The aim of this study was to investigate the feasibility of extracting antioxidants (phenolic compounds including flavonoids) using an environmentally friendly technique. Whole grape pomace was used to establish a cost effective methodology for the extraction of phenolic compounds.

2. Materials and methods

2.1. Samples and chemicals

Grape pomace (Croatina cultivar) was kindly provided by an Italian winery located in Tortona, Piemonte region. The grape pomace was collected after vinification and dried for 24 h at 40 °C, followed by grinding for 20 s to avoid heat generation and degradation of polyphenols and stored in a sealed container at −20 °C.

Analytical grades methanol, aluminium chloride, sodium nitrite, sodium hydroxide and sodium carbonate, Folin–Ciocalteu's phenol reagent (2 N), 2,2-diphenyl-1-picryl hydrazyl (DPPH°), gallic acid and catechin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Standard solutions of gallic acid and catechin were prepared with methanol, wrapped in aluminium foil and stored at −20 °C until use. Silicon oil was used in the oil bath for controlling the extraction temperature.

2.2. Extraction

The schematic diagram of apparatus used for the extraction of polyphenolic compounds using subcritical water is shown in Fig. 1. In the extraction system, a syringe pump (ISCO, Model 500D) was used for water delivery, pressurization and controlling the pressure of system. An oil bath was used to control the extraction temperature. A pressure transducer (Davidson, Druck) and thermocouple (Caveland Electric) were installed in the custom made high pressure vessel (100 ml volume) to monitor both pressure and temperature of system. Extract was collected, using a cold trap (150 ml volume) soaked inside an ice bath.

In each run pomace (2.00 ± 0.24 g) was loaded in a filter paper to avoid blockage of lines and also to facilitate its collection from the system for gravimetric analysis. The filter paper was then loaded into the high pressure vessel. The vessel was placed in the oil bath 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. After this step vessel was isolated for a period of 30 min (static extraction) followed by conducting the continuous mode of extraction for 100 min via opening both inlet and outlet valves and running the pump at a constant pressure mode. The water flow rate was adjusted at 1–2 ml/min using a metering valve (Swagelok). The system was then depressurized, the water solution and pomace were collected from the sampling and extraction vessels, respectively. The solution collected in sampling vessel and pomace were then dried using freeze-drier for 72 h to calculate the mass of extract. The extract was dried to calculate the yield of extraction process. Previous studies show that the antioxidants extracted in this study are stable at temperatures below 110 °C [8]. Methods such as rotary evaporator, spray drying and freeze drying can be used in this bench scale study for drying the sample; Freeze drying was used to produce dry product in this study. The preliminary results showed that this method was efficient to remove 99% of water after a period of 72 h and the relative standard deviation for at least three runs was less than 5%. Lyophilized extracts were dissolved in constant volume of methanol and filtered through 0.20 μm membranes (Sartorius Stedim Biotech GmbH, Goettingen, Germany) and kept at 4 °C for further analysis.

2.3. Conventional extraction procedure

Polyphenols and flavonoids were conventionally extracted using optimum conditions described elsewhere [8]. Briefly, 2 g of pomace was dissolved in 10 ml of pure ethanol or milli-Q water in test tubes with screw caps and placed on magnetic shaker (Heidolph Mr. 2002, Kelheim, Germany). The extraction was taken place

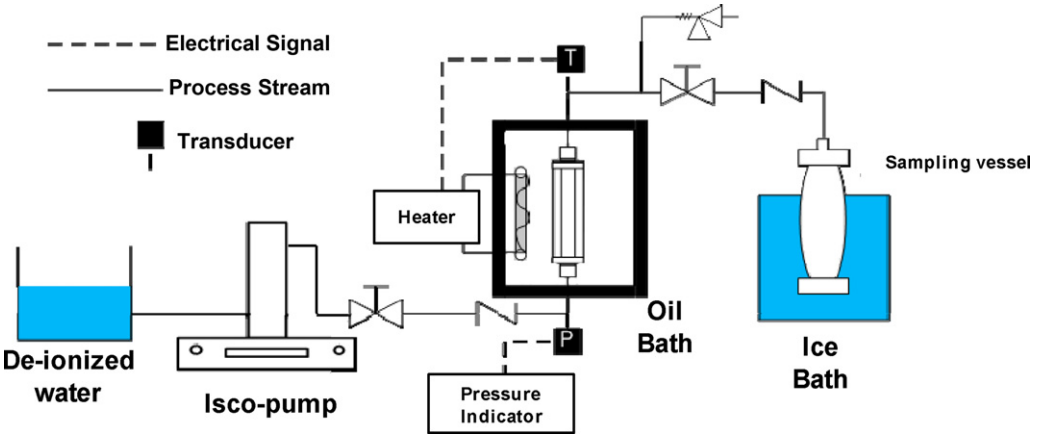

Fig. 1. Diagram of experimental setup of subcritical water extraction of phenolic compounds from grape pomace.

for 19 h at 25 °C. The liquid was separated from solid by centrifugation at 7500 rpm for 10 min (ALC PK131 Centrifuges, Alberta, Canada). The yield of this conventional method is used as the benchmark to evaluate the efficiency of subcritical water extraction. Samples were kept at −20 °C for further analysis. All experiments were repeated at least three times.

2.4. Folin–Ciocalteu assay

The concentrations of total phenolic compounds (TP) were measured using Folin–Ciocalteu assay [30]. Briefly, 4.80 ml of milli-Q water, 0.20 ml of extract, and 0.50 ml of Folin–Ciocalteu reagent were mixed in a 25 ml volumetric flask. Subsequently, 1 ml of 20% (w/w) sodium carbonate solution was added to the solution. The final volume of solution was adjusted to 10 ml by addition of milli-Q water and then kept in amber bottle to avoid degradation by light exposure. Sample aliquots were used for the determination of total polyphenols concentration, using a UV–vis spectrophotometer (Cary, CO, USA) at a wavelength of 725 nm. The calibration line for UV spectrophotometer was acquired using standard solution of gallic acid with known concentrations, varied in the range of 0.01–1.00 mg/ml. A linear equation with R^2 of 0.9994 was established and TP expressed as milligrams of gallic acid equivalents per gram of dried pomace (mg_{GAE}/g_{DP}).

2.5. Total flavonoids

The yield of total flavonoids (TF) extracted by subcritical water was estimated using the colorimetric method developed by Yang et al. [31] and expressed as milligrams of catechin equivalents per gram of dried pomace (mg_{CE}/g_{DP}). Briefly, 0.25 ml of extract was mixed with 1.25 ml of milli-Q water and 0.075 ml of 5% (w/w) sodium nitrite solution. After 5 min 0.15 ml of 10% (w/w) aluminium chloride was added to the solution, followed by addition of 0.5 ml of 1 M sodium hydroxide. The final volume of solution was increased to 3 ml, using milli-Q water. The UV spectrophotometer was used at wavelength of 510 nm to measure the absorption of total flavonoids. The calibration curve for this measurement was established from standard solution of catechin with predefined concentrations, varied in the range of 0.08–0.80 mg/ml.

2.6. Antiradical power of extracts

The antiradical power (ARP) of extracts was measured in terms of hydrogen-donating or radical scavenging ability, using the method developed by Brand-Williams et al. [32]. This method was also used in previous studies [14,22,33–35]. Seven different concentrations of extract were dissolved in methanol. To measure the absorbance with UV spectrophotometer, 0.10 ml of diluted extract was mixed with 3.90 ml of 9.15×10^{-5} M DPPH° methanolic solution and the ARP concentration was measured at 515 nm.

2.7. Quantitative analysis of color intensity

The color intensity was correlated to the amount of extracted material and extraction conditions. All extracts were dissolved in similar amount of methanol and image J software was used to determine the color intensity of solution [36]. The light intensity was measured for at least 750 points and the average values were reported to acquire statistically validated data for each sample.

2.8. Experimental design

Experimental design is broadly used for the optimization of process variables in extraction and many other processes [2,37–40]. The response surface methodology (RSM) combined with a 3^2 full factorial design was used to determine the optimum operating conditions for the extraction of phenolic compounds from grape pomace. A second order polynomial regression was used to assess the correlation between the mass of extract (response factor) and process variables, such as temperature and pressure (independent variables). The temperature (X_1) and pressure (X_2) are critical parameters, which affect the solubility of phenolic compounds in subcritical water [7,39,41]. The effects of temperature and pressure on dependent variables (i.e. TP, TF and ARP) were investigated by changing each of them in three levels. The temperature was varied between 100 °C and 140 °C to avoid oxidation of phenolic compounds during the extraction at high temperatures [7,42]. The effect of pressure on the extraction was investigated by changing the pressure from 8 MPa to 15 MPa to achieve an economically acceptable range below the critical pressure of water (Pc = 22 MPa).

The "Statistica" (trial version 6.0, StatSoft, Tulsa, OK, USA) and the "Design Expert" (trial version 6.0.10, Stat-Ease, Minneapolis, MN, USA) were used to conduct the regression and the numerical optimization analysis, respectively. The models of three responses were presented in forms of actual variables. For the modeling purposes, predefined ranges of dependent variables were considered, regardless of their statistical significance.

3. Results and discussion

3.1. Experimental design

The content of total polyphenols (TP), total flavonoids (TF) and antioxidant power (ARP) of extracts at different conditions are shown in Table 1. The experimental results were then fitted into the second-order polynomial equation (Eq. (1));

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 \quad (1)$$

where Y is the dependent variable (TP, TF, or ARP), β are the regression coefficients, and X_1, and X_2 are the coded levels of temperature and pressure, respectively. Three levels of variation for X_1 are 100 °C ($X_1 = -1$), 120 °C ($X_1 = 0$), and 140 °C ($X_1 = +1$) and for X_2 are 8 MPa ($X_2 = -1$), 11.5 MPa ($X_2 = 0$), and 15 MPa ($X_2 = +1$).

The regression coefficients for each three polynomial equation were calculated. The significance coefficients of models were determined by analysis of variance (ANOVA) using the F-test and p-value, shown in Table 2. The fitted quadratic models in terms of actual variables are given in Eqs. (2)–(4). In a model with significant p-value ($p < 0.05$), a high regression coefficient demonstrates substantial effect of independent variables on the corresponding responses [43]. The goodness of fittings for models was expressed by coefficients of determination (R^2).

$$\mathrm{TP} = -57.8731 + 0.3565X_1 + 0.6304X_2 + 0.0014X_1^2 - 0.0013X_2^2 - 0.0027X_1X_2 \quad (2)$$

$$\mathrm{TF} = -70.3523 + 0.8277X_1 + 0.4836X_2 - 0.0022X_1^2 - 0.0013X_2^2 - 0.0016X_1X_2 \quad (3)$$

$$\mathrm{ARP} = 32.4736 - 0.6374X_1 + 0.0842X_2 + 0.0033X_1^2 - 0.0004X_2^2 - 0.00002X_1X_2 \quad (4)$$

The coefficients of determination for these equations were close to 1, underlining that only 1.3%, 1.9% and 5.4% of experimental data were not predicted accurately for TP, TF and ARP, respectively. The F-value shown in Table 2 suggests that independent variables had paramount impacts on dependent variables. There was a linear correlation between TP ($p = 0.0049$), also ARP ($p = 0.00411$) and

Table 1
Experimental values and coded levels of the independent variables and the responses of the dependent variables utilized in the 3^2 full factorial design matrix and conventional extraction procedure.

Extraction procedure	Tests	Independent variable		Responses		
		X_1 [a] (°C)	X_2 [b] (MPa)	TP (mg_{GAE}/g_{DP})	TF (mg_{CE}/g_{DP})	ARP ($\mu g_{DPPH}/\mu l_{extract}$)
Subcritical water	1	100 (−1)	8 (−1)	12.78 ± 1.57	8.34 ± 0.43	5.90 ± 0.50
	2	100 (−1)	11.5 (0)	16.72 ± 1.35	9.99 ± 0.27	7.08 ± 0.54
	3	100 (−1)	15 (+1)	16.46 ± 1.23	10.02 ± 0.71	7.11 ± 0.62
	4	120 (0)	8 (−1)	20.15 ± 1.54	12.29 ± 0.58	9.10 ± 0.74
	5	120 (0)	11.5 (0)	23.86 ± 1.85	14.35 ± 1.41	9.31 ± 0.70
	6	120 (0)	15 (+1)	23.15 ± 2.12	11.52 ± 0.63	7.31 ± 0.55
	7	140 (+1)	8 (−1)	32.49 ± 2.63	15.11 ± 1.34	12.58 ± 1.10
	8	140 (+1)	11.5 (0)	30.80 ± 3.38	15.28 ± 1.02	12.87 ± 1.25
	9	140 (+1)	15 (+1)	28.50 ± 1.50	12.33 ± 1.26	13.85 ± 1.26
Conventional	10[c]	25	1	1.72 ± 0.05	1.25 ± 0.05	4.19 ± 0.50
	11[d]	25	1	7.87 ± 0.48	14.49 ± 2.17	22.57 ± 1.87

Values between brackets represent coded levels.
Values are means ± s.d. of three replicate analyses.
[a] Coded value of extraction temperature (T).
[b] Coded value of extraction pressure (P).
[c] Conventional extraction using water (19 h, 0.2 g_{DP}/ml).
[d] Conventional extraction using ethanol (19 h, 0.2 g_{DP}/ml).

Table 2
Results of ANOVA for the concentrations of total polyphenols (TP), total flavonoids (TF) and the antioxidant power (ARP) of the extracts.

Response	Source	Sum of squares	Degrees of freedom	Mean square	F-Value	p-Value
TP (mg_{GAE}/g_{DP})[a]	Model	371.48	5	74.30	46.04	0.0049
	Residual	4.84	3	1.61		
	Total	376.32	8			
TF (mg_{CE}/g_{DP})[b]	Model	46.62	5	9.32	30.65	0.0089
	Residual	0.91	3	0.30		
	Total	47.53	8			
ARP ($\mu g_{DPPH}/\mu l_{extract}$)[c]	Model	65.49	5	13.10	10.40	0.0411
	Residual	3.78	3	1.26		
	Total	69.27	8			

[a] The coefficient determination (R^2) of the model was 0.9871.
[b] The coefficient determination (R^2) of the model was 0.9808.
[c] The coefficient determination (R^2) of the model was 0.9455.

temperature (X_1). The effect of extraction pressure (X_2), however, was negligible ($p > 0.05$). For TF, X_1, $X_2{}^2$ and X_1X_2 were significant terms, $p = 0.0018$, 0.0260 and 0.0272, respectively. These results demonstrate that pressure had negligible effect on TP, TF, and ARP ($p < 0.05$).

3.2. Response surface analysis of total polyphenol (TP) yield

The results of response surface analysis for the effect of extraction temperature and pressure on TP yield are shown in Fig. 2. In this study increasing extraction temperature from 100 °C to 140 °C at 8 MPa, significantly enhanced the yield of TP from 12.78 mg_{GAE}/g_{DP} to 32.49 mg_{GAE}/g_{DP}. However, the pressure had negligible effect on TP extraction in the range of 8–11.5 MPa ($p > 0.05$). The TP extraction was slightly increased from 20.15 mg_{GAE}/g_{DP} to 23.86 mg_{GAE}/g_{DP} when the pressure was increased from 8 to 11.5 MPa. TP extraction approached a plateau by further increasing the pressure from 11.5 MPa to 15 MPa (TP extraction was changed from 23.86 mg_{GAE}/g_{DP} to 23.15 mg_{GAE}/g_{DP}, respectively). This result is in agreement with previous studies when subcritical water was used for the extraction [44–48]. In the extraction of pomegranate seed oil [44], essential oils from plants [45,46], phenolic acids from black cohosh [47], and phenolic compounds from parsley [48], temperature has more crucial effect on the extraction efficiency compared to pressure. This behavior might be due to minimal effect of pressure on the polarity of water (e.g. dielectric constant) at the examined range [44]. However, the dielectric constant of water was considerably decreased by increasing the temperature. For example when the temperature was increased from 80 to 220 °C the dielectric constant of water was reduced from 61 to 31. At 220 °C

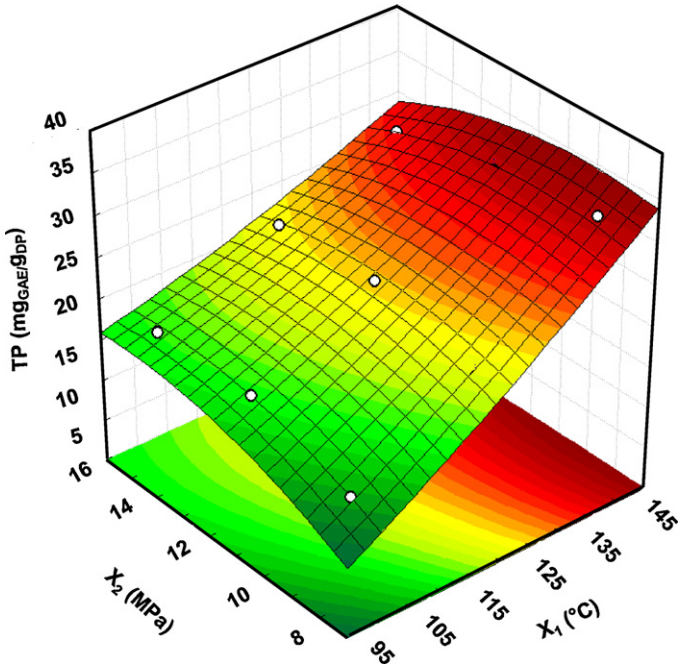

Fig. 2. Response surface of total polyphenols concentration (TP) as simultaneous functions of extraction temperature (X_1) and pressure (X_2) according to the 3^2 full-factorial design.

and 6 MPa, dielectric constant of water is close to methanol (ε = 33) [24]. The solubility of phenolic compounds in subcritical water at such temperature was similar to using organic solvents. Increasing temperature could also raise the vapor pressure of a solute, promote the mass transfer of phenolic compounds by enhancing the diffusivity and decreasing the viscosity [49]. Therefore, temperature had paramount effect on the yield of TP compared to pressure.

At 140 °C and 8 MPa, the highest yield of TP (32.49 mg_{GAE}/g_{DP}) was achieved. This result was comparable to the yield of extraction of TP when using acidic or organic solvents and showed a great improvement compared to previous aqueous based extractions [33,50]. For example, the yield of 1.53 $mg_{GAE}/100\ g_{DP}$ was achieved using aqueous extraction from grape pomace recovered from white vinification process [50]. A yield of 28 mg_{GAE}/g_{DP} was acquired using electrical assisted treatment for the extraction from red grape pomace, recovered from French cultivar (Pinot Meunier). Extraction of TP from Brazilian pomace by methanol and 0.1% (v/v) HCl solution at 50 °C and for a period of 1 h resulted in achieving TP yield within the range of 33.62 mg_{GAE}/g_{DP} and 74.75 mg_{GAE}/g_{DP} [33]. Besides, the extraction techniques used in previous studies, other factors including, vinification process, genetic and environmental characteristics of grape affect phenolic composition of biomass and hence the final TP yield of extraction. Therefore, it is important to consider other parameters that affect the phenolic composition of biomass to have better comparison over the efficiency of extraction techniques.

3.3. Response surface analysis of total flavonoids (TF)

Flavonoids represent a widespread and common group of aromatic compounds which play a significant physiological role on the function of vegetation tissues [8,18,34]. They are direct UV protectants, provide color to tissues and organs for attraction of pollinators and seed-dispersal agents and also act as general antioxidants against reactive oxygen species [51]. In grape, flavonoids are the major portion of soluble phenolics. The extraction of these crucial bio-active compounds from winery by-products was investigated in this work.

The 3D response surface of TF contents as a function of extraction temperature and pressure is presented in Fig. 3. Regression coefficient of X_1 (temperature) in Eq. (3) for TF was 0.8277 which is significantly higher than 0.3565 acquired for TP. This result demonstrated that the effect of temperature on the extraction of TF was more paramount compared to TP. In the present study, at 140 °C, increasing extraction pressure to 15 MPa resulted in 19.34% decrease of TF content. The highest amount of flavonoids (15.28 mg_{CE}/g_{DP}) was obtained at 140 °C and 11.5 MPa. Significantly lower content of flavonoids (1.01 mg_{CE}/g_{DP}) was recovered from grape skins of Pinot Noir cultivar, using ethanol at atmosphere conditions and 19 h of extraction time [18]. Ben Hamissa et al. reported a direct correlation between the extraction temperature (varied in the range of 25–150 °C) and the TF extraction from *Agave americana* leaves, using agitated high pressure and high temperature reactor and methanol as the solvent [6]. In this study after 15 min, the yield of TF was increased from 1.33 $mg_{Quercetin\ equivalent}/g_{Dry\ Biomass}$ to 2.60 $mg_{Quercetin\ equivalent}/g_{Dry\ Biomass}$ and to 4.90 $mg_{Quercetin\ equivalent}/g_{Dry\ Biomass}$ by raising the extraction temperature from 25 °C to 100 °C (P = 0.2 MPa) and 150 °C (P = 1 MPa), respectively.

3.4. Response surface analysis of ARP

There was a direct correlation between the extraction temperature and antiradical power, shown in Fig. 4. The effect of extraction pressure, however was not significant on the ARP. At 15 MPa by increasing temperature from 100 °C to 140 °C, the ARP was elevated

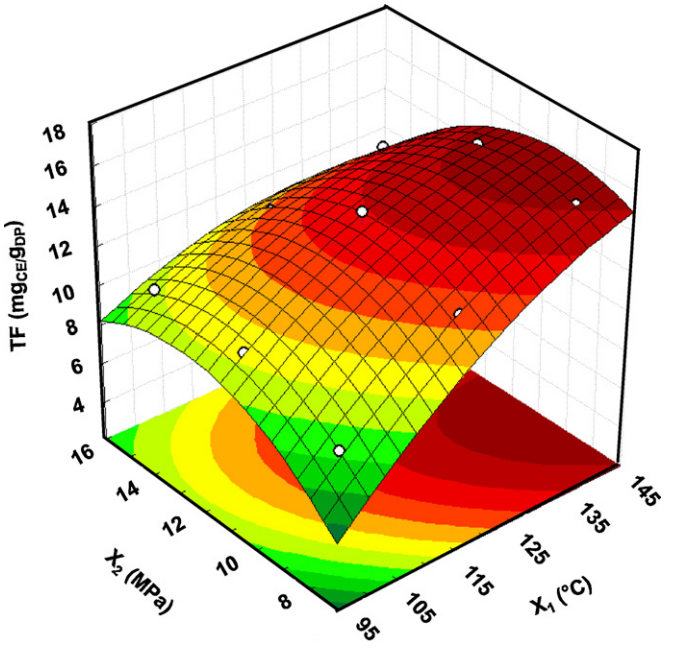

Fig. 3. Response surface of total flavonoids concentration (TF) as simultaneous functions of extraction temperature (X_1) and pressure (X_2) according to the 3^2 full-factorial design.

from 7.11 $\mu g_{DPPH}/\mu l_{extract}$ to 13.85 $\mu g_{DPPH}/\mu l_{extract}$. The ARP was significantly higher than the value acquired when using ethanol and conventional method for 19 h to extract phenolic compounds from grape skin of Pinot Noir cultivar (1.5 $\mu g_{DPPH}/\mu l_{extract}$) [18]. This effect might be due to reducing the polarity of subcritical water that allows acting as an organic solvent and dissolving less polar compound, which possesses higher antioxidant activity.

A good linear correlation (R^2 = 0.8334) was found between TP contents and their ARP. However, a poor coefficient of determination (R^2 = 0.6044) between TF content and ARP demonstrate that

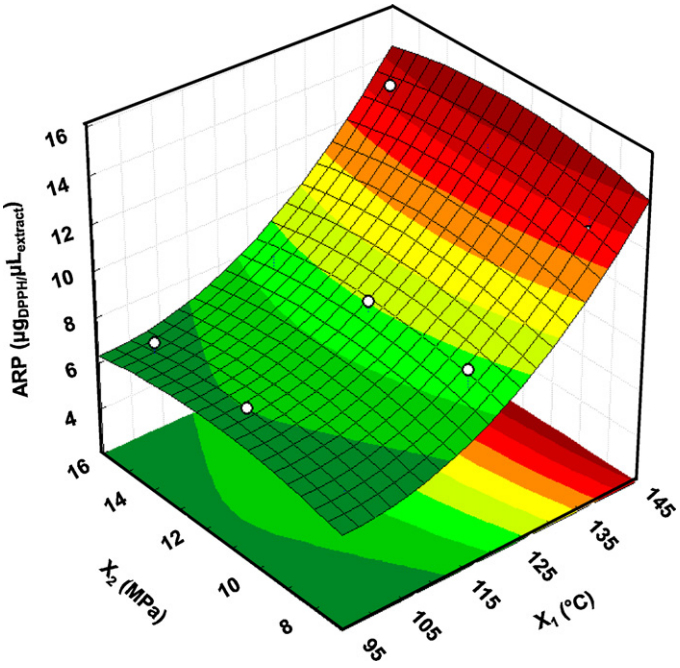

Fig. 4. Response surface of antiradical power of extracts (ARP) as simultaneous functions of extraction temperature (X_1) and pressure (X_2) according to the 3^2 full-factorial design.

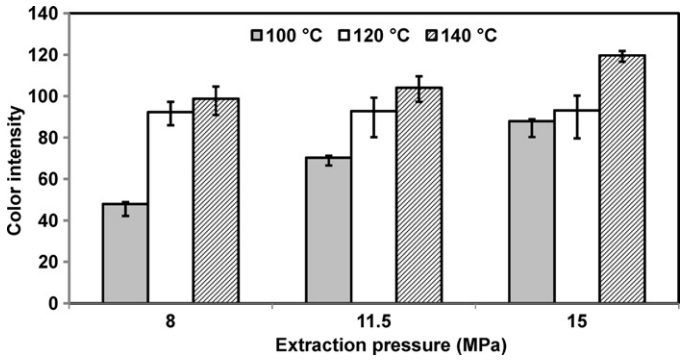

Fig. 5. Color intensity of aqueous phenolic extracts of grape pomace extracted by subcritical water as a function of extraction temperature and pressure measured using Image J software.

some non-flavonoid compounds with low antioxidant capacity were extracted. This result is in agreement with several studies, underlining significant correlation between DPPH° scavenging activity and the phenolic content of grape extracts from different cultivars [8,18,52]. For example, Casazza et al. showed a significant linear increase in the ARP when raising TP (R^2 = 0.9287) and TF (R^2 = 0.7262) contents of extracts from grape skins of Pinot Noir cultivar [18]. These correlations were achieved when phenolic compounds were recovered using ethanol for extraction and changing the processing period from 9 h to 29 h and solid to liquid ratio from 0.1 $g_{Dry\ Biomass}$/ml to 0.3 $g_{Dry\ Biomass}$/ml.

3.5. The effect of extraction temperature and pressure on the color intensity of extracts

It is common to use color intensity as a reliable data for determining the effect of extraction variables on phenolic compounds extracted from biomass [21,53]. Color intensity of extracted compounds produced at different temperatures and pressures is shown in Fig. 5.

At 100 °C and 8 MPa, a very light brown color of extract was observed (color intensity of 48). As pressure was increased from 8 to 15 MPa, the color intensity was enhanced to 88. At high temperatures (e.g. 120 °C and 140 °C) color of extract was found to be even darker than at lower extraction temperatures. At temperatures higher than 100 °C, the effect of extraction pressure was not correlated to color intensity of extracts. The highest color intensity (120) was observed at 140 °C and 15 MPa. The relationship between color intensity of extract and the yield of phenolic compounds was investigated to provide an insight for the observed trend. A poor linear correlation, R^2 of 0.4402 and 0.4934, was found between the color intensity of extract and the yield of TP and TF, respectively. The color change in the extracts can be explained as the reduction in the sugar content of solution due to the Maillard reaction at high temperature [54]. The results from the color intensity analysis are in agreement with results from previous analysis conducted by Singh et al. [21]. They reported that extraction temperature and the color extracts are directly correlated in the extraction of phenolic compounds from potato peel using subcritical water. They observed that the color of phenolic extracts at pressure of 6 MPa became darker as the yield of phenolic compounds was increased from 22.56 mg/100 $g_{Dry\ Potato}$ to 81.83 mg/100 $g_{Dry\ Potato}$ by increasing the temperature from 100 °C to 180 °C.

3.6. Comparison of subcritical water extraction (SWE) and conventional extraction

Conventional extraction from the same biomass was conducted, using ethanol and water as solvent. This part of the study was conducted to compare SWE with conventional technique regardless of the effect of biomass used for the extraction. The results of extraction when using ethanol and water are presented in Table 1. The content of TF, TP and ARP of extracts from SWE were 12, 19, and 3 fold, respectively, higher compared to that of those using water for extraction. This data corroborated that the subcritical water was superior for the extraction of phenolic compounds. There was no significant difference between the TF contents of extracts achieved from SWE and ethanol extraction. Extracts with higher TP and lower ARP were achieved when using SWE compared to ethanol extraction. In addition, the SWE processing time was remarkably shorter (130 min) compared to using ethanol for extraction (19 h).

In SWE achieves comparable level of extraction compared to conventional organic solvent extraction. It also eliminates the consumption of organic solvent, hence the recovery of extract was from aqueous system. SWE can, therefore, be considered as an alternative technique for the extraction of phenolic compounds.

3.7. Optimization

A numerical optimization was conducted to identify the overall optimal conditions for subcritical water extraction of antioxidants from grape pomace. The three responses (TP, TF and ARP) were analyzed by allocating same weight (1) and the same importance (+++) for each compound. In optimization process TP, as the major contributor of antioxidants properties, was selected as a target and the following constraints were imposed: 100 °C ≤ T ≤ 140 °C, 8 MPa ≤ P ≤ 15 MPa, 8.34 mg_{CE}/g_{DP} ≤ TF ≤ 15.28 mg_{CE}/g_{DP} and 5.90 $\mu g_{DPPH}/\mu l_{extract}$ ≤ ARP ≤ 13.85 $\mu g_{DPPH}/\mu l_{extract}$. The predicted optimum condition by model was at 140 °C and 11.6 MPa, which was close to 140 °C and 11.5 MPa that was examined in this study. At the optimum condition, extract consisted of 31.69 mg_{GAE}/g_{DP} TP, 15.28 mg_{CE}/g_{DP} TF and 13.40 $\mu g_{DPPH}/\mu l_{extract}$ ARP. The desirability of model (0.9550) indicates that only 4.50% of responses were beyond the acceptable region.

4. Conclusions

The results of this study demonstrate that subcritical water extraction was efficient for the recovery of phenolic compounds from grape pomace. Second-order polynomial equations were developed to predict the effects of pressure and temperature on the extraction of desired compounds within the range examined. The optimum conditions for the extraction of antioxidants from grape pomace were determined using numerical analytical method. Subcritical water extraction process was more efficient than using organic solvent and aqueous based system at atmospheric pressure for recovery of antioxidants. In this technique no organic solvent is used, therefore, the product is free of residual solvent. Elimination of organic solvent is desirable for the production of organic products of functional foods and nutraceuticals. Subcritical water extraction technique therefore, can be considered as a cost effective and benign process for the extraction of antioxidants from plants and biomass. Further research in pilot plant scale is required prior to scale up of this method.

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References

[1] M. Iriti, F. Faoro, Bioactive Chemicals and Health Benefits of Grapevine Products, Bioactive Foods in Promoting Health, Amsterdam, 2009, pp. 581–621.

[2] B. Aliakbarian, F. Dehghani, P. Perego, The effect of citric acid on the phenolic contents of olive oil, Food Chemistry 116 (2009) 617–623.
[3] J.L. Donovan, A.S. Meyer, A.L. Waterhouse, Phenolic composition and antioxidant activity of prunes and prune juice (*Prunus domestica*), Journal of Agricultural and Food Chemistry 46 (1998) 1247–1252.
[4] J. Zhishen, T. Mengcheng, W. Jianming, The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals, Food Chemistry 64 (1999) 555–559.
[5] T. Vatai, M. Skerget, Z. Knez, Extraction of phenolic compounds from elder berry and different grape marc varieties using organic solvents and/or supercritical carbon dioxide, Journal of Food Engineering 90 (2009) 246–254.
[6] A.M. Ben Hamissa, M. Seffen, B. Aliakbarian, A.A. Casazza, P. Perego, A. Converti, Phenolics extraction from *Agave americana* (L.) leaves using high-temperature, high-pressure reactor, Food and Bioproducts Processing 90 (2012) 17–21.
[7] B. Aliakbarian, A.A. Casazza, E.J.O. Montoya, A. Convert, Valorisation of olive oil solid wastes: valuable compounds recovery using high pressure–high temperature, Journal of Biotechnology 150 (2011) 332.
[8] A.A. Casazza, B. Aliakbarian, S. Mantegna, G. Cravotto, P. Perego, Extraction of phenolics from *Vitis vinifera* wastes using non-conventional techniques, Journal of Food Engineering 100 (2010) 50–55.
[9] L. Fiori, D. Basso, P. Costa, Supercritical extraction kinetics of seed oil: a new model bridging the 'broken and intact cells' and the 'shrinking-core' models, The Journal of Supercritical Fluids 48 (2009) 131–138.
[10] A. El-Din Bekhit, V.J. Cheng, M. McConnell, J.H. Zhao, R. Sedcole, R. Harrison, Antioxidant activities, sensory and anti-influenza activity of grape skin tea infusion, Food Chemistry 129 (2011) 837–845.
[11] Y. Choi, J. Lee, Antioxidant and antiproliferative properties of a tocotrienol-rich fraction from grape seeds, Food Chemistry 114 (2009) 1386–1390.
[12] E.E. Yilmaz, E.B. Özvural, H. Vural, Extraction and identification of proanthocyanidins from grape seed (*Vitis vinifera*) using supercritical carbon dioxide, The Journal of Supercritical Fluids 55 (2011) 924–928.
[13] G. Ruberto, A. Renda, C. Daquino, V. Amico, C. Spatafora, C. Tringali, N.D. Tommasi, Polyphenol constituents and antioxidant activity of grape pomace extracts from five Sicilian red grape cultivars, Food Chemistry 100 (2007) 203–210.
[14] G.K. Jayaprakasha, T. Selvi, K.K. Sakariah, Antibacterial and antioxidant activities of grape (*Vitis vinifera*) seed extracts, Food Research International 36 (2003) 117–122.
[15] D.P. Makris, G. Boskou, N.K. Andrikopoulos, Polyphenolic content and in vitro antioxidant characteristics of wine industry and other agri-food solid waste extracts, Journal of Food Composition and Analysis 20 (2007) 125–132.
[16] W. Andlauer, P. Fürst, Nutraceuticals: a piece of history, present status and outlook, Food Research International 35 (2002) 171–176.
[17] A.A. Adedapo, F.O. Jimoh, S. Koduru, P.J. Masika, A.J. Afolayan, Evaluation of medicinal potentials of methanol extracts of leaves and stems of *Halleria lucida*, Bioresource Technology 99 (2008) 4158–4163.
[18] A.A. Casazza, B. Aliakbarian, D. De Faveri, L. Fiori, P. Perego, Antioxidants from wine making wastes: a study on extraction parameters using response surface methodology, Journal of Food Biochemistry 36 (2012) 28–37.
[19] H.I. Castro-Vargas, L.I. Rodríguez-Varela, S.R.S. Ferreira, F. Parada-Alfonso, Extraction of phenolic fraction from guava seeds (*Psidium guajava* L.) using supercritical carbon dioxide and co-solvents, The Journal of Supercritical Fluids 51 (2010) 319–324.
[20] M.J. Ko, C.I. Cheigh, S.W. Cho, M.S. Chung, Subcritical water extraction of flavonol quercetin from onion skin, Journal of Food Engineering 102 (2011) 327–333.
[21] P.P. Singh, M.D.A. Saldaña, Subcritical water extraction of phenolic compounds from potato peel, Food Research International 44 (2011) 2452–2458.
[22] J.J. Wu, J.C. Lin, C.H. Wang, T.T. Jong, H.L. Yang, S.L. Hsu, C.M.J. Chang, Extraction of antioxidative compounds from wine lees using supercritical fluids and associated anti-tyrosinase activity, The Journal of Supercritical Fluids 50 (2009) 33–41.
[23] B. Eric J., Supercritical and near-critical CO_2 in green chemical synthesis and processing, The Journal of Supercritical Fluids 28 (2004) 121–191.
[24] L. He, X. Zhang, H. Xu, C. Xu, F. Yuan, Z. Knez, Z. Novak, Y. Gao, Subcritical water extraction of phenolic compounds from pomegranate (*Punica granatum* L.) seed residues and investigation into their antioxidant activities with HPLC-ABTS$^{\bullet+}$ assay, Food and Bioproducts Processing (2011), doi:10.1016/j.fbp.2011.03.003.
[25] Y. Yang, S. Bwadt, S.B. Hawthome, D.J. Miller, Subcritical water extraction of polychlorinated biphenyls from soil and sediment, Analytical Chemistry 67 (1995) 4571–4576.
[26] A. Mustafa, C. Turner, Pressurized liquid extraction as a green approach in food and herbal plants extraction: a review, Analytica Chimica Acta 703 (2011) 8–18.
[27] M. García-Marino, J.C. Rivas-Gonzalo, E. Ibáñez, C. García-Moreno, Recovery of catechins and proanthocyanidins from winery by-products using subcritical water extraction, Analytica Chimica Acta 563 (2006) 44–50.
[28] J.M. Luque-Rodríguez, M.D. Luque de Castro, P. Pérez-Juan, Dynamic superheated liquid extraction of anthocyanins and other phenolics from red grape skins of winemaking residues, Bioresource Technology 98 (2007) 2705–2713.
[29] J.K. Monrad, L.R. Howard, J.W. King, K. Srinivas, A. Mauromoustakos, Subcritical solvent extraction of anthocyanins from dried red grape pomace, Journal of Agricultural and Food Chemistry 58 (2010) 2862–2868.
[30] T. Swain, W.E. Hillis, The phenolic constituents of *Prunus domestica*. The quantitative analysis of phenolic constituents, Journal of Science of Food and Agriculture 10 (1959) 63–68.
[31] J. Yang, T.E. Martinson, R.H. Liu, Phytochemical profiles and antioxidant activities of wine grapes, Food Chemistry 116 (2009) 332–339.
[32] W. Brand-Williams, M.E. Cuvelier, C. Berset, Use of free radical method to evaluate antioxidant activity, LWT-Food Science and Technology 28 (1995) 25–30.
[33] V. Amico, R. Chillemi, S. Mangiafico, C. Spatafora, C. Tringali, Polyphenol-enriched fractions from Sicilian grape pomace: HPLC-DAD analysis and antioxidant activity, Bioresource Technology 99 (2008) 5960–5966.
[34] I.I. Rockenbach, E. Rodrigues, L.V. Gonzaga, V. Caliari, M.I. Genovese, A.E. de Souza Schmidt Gonçalves, R. Fett, Phenolic compounds content and antioxidant activity in pomace from selected red grapes (*Vitis vinifera* L. and *Vitis labrusca* L.) widely produced in Brazil, Food Chemistry 127 (2011) 174–179.
[35] C. Xu, Y. Zhang, L. Cao, J. Lu, Phenolic compounds and antioxidant properties of different grape cultivars grown in China, Food Chemistry 119 (2010) 1557–1565.
[36] N. Annabi, A. Fathi, S.M. Mithieux, P. Martens, A.S. Weiss, F. Dehghani, The effect of elastin on chondrocyte adhesion and proliferation on poly (ε-caprolactone/elastin) composites, Biomaterials 32 (2011) 1517–1525.
[37] B. Aliakbarian, D. De Faveri, A. Converti, P. Perego, Optimisation of olive oil extraction by means of enzyme processing aids using response surface methodology, Biochemical Engineering Journal 42 (2008) 34–40.
[38] D. De Faveri, P. Torre, B. Aliakbarian, J.M. Domínguez, P. Perego, A. Converti, Response surface modeling of vanillin production by *Escherichia coli* JM109pBB1, Biochemical Engineering Journal 36 (2007) 268–275.
[39] S. Liu, F. Yang, C. Zhang, H. Ji, P. Hong, C. Deng, Optimization of process parameters for supercritical carbon dioxide extraction of Passiflora seed oil by response surface methodology, The Journal of Supercritical Fluids 48 (2009) 9–14.
[40] O. Boutin, E. Badens, Extraction from oleaginous seeds using supercritical CO_2: experimental design and products quality, Journal of Food Engineering 92 (2009) 396–402.
[41] S.M. Ghoreishi, R. Gholami Shahrestani, S.H. Ghaziaskar, Subcritical water extraction of mannitol from olive leaves, World Academy of Science, Engineering and Technology 43 (2008) 114–124.
[42] M. Palma, Z. Piñeiro, C.G. Barroso, Stability of phenolic compounds during extraction with superheated solvents, Journal of Chromatography A 921 (2001) 169–174.
[43] H.S. Yim, F.Y. Chye, S.M. Koo, P. Matanjun, S.E. How, C.W. Ho, Optimization of extraction time and temperature for antioxidant activity of edible wild mushroom, *Pleurotus porrigens*, Food and Bioproducts Processing (2011), doi:10.1016/j.fbp.2011.04.001.
[44] H. Abbasi, K. Rezaei, Z. Emamdjomeh, S.M. Ebrahimzadeh Mousavi, Effect of various extraction conditions on the phenolic contents of pomegranate seed oil, European Journal of Lipid Science and Technology 110 (2008) 435–440.
[45] M.P.K. Choi, K.K.C. Chan, H.W. Leung, C.W. Huiea, Pressurized liquid extraction of active ingredients (ginsenosides) from medicinal plants using non-ionic surfactant solutions, Journal of Chromatography A 983 (2003) 153–162.
[46] C.H. Deng, N. Li, X.M. Zhangh, Rapid determination of essential oil in *Acorus tatarinowii* Schott. by pressurized hot water extraction followed by solid-phase microextraction and gas chromatography-mass spectrometry, Journal of Chromatography A 1059 (2004) 149–155.
[47] S. Mukhopadhyay, D.L. Luthria, R.J. Robbins, Optimization of extraction process for phenolic acids from black cohosh (*Cumicifuga racemosa*) by pressurized liquid extraction, Journal of Science of Food and Agriculture 86 (2006) 156–162.
[48] D.L. Luthria, Influence of experimental conditions on the extraction of phenolic compounds from parsley (*Petroselinum crispum*) flakes using a pressurized liquid extractor, Food Chemistry 107 (2008) 745–752.
[49] C.Y. Gan, A.A. Latiff, Optimisation of solvent extraction of bioactive compounds from *Parkia speciosa* pod using response surface methodology, Food Chemistry 124 (2010) 1277–1283.
[50] M.S. Guerrero, J.S. Torres, M.J. Nuñez, Extraction of polyphenols from white distilled grape pomace: optimization and modelling, Bioresource Technology 99 (2008) 1311–1318.
[51] E. Braidot, M. Zancani, E. Petrussa, C. Peresson, A. Bertolini, S. Patui, F. Macrì, A. Vianello, Transport and accumulation of flavonoids in grapevine (*Vitis vinifera* L.); review, Plant Signaling & Behavior 3 (2008) 626–632.
[52] R. Guendez, S. Kallithraka, D.P. Makris, P. Kefalas, Determination of low molecular weight polyphenolic constituents in grape (*Vitis vinifera* sp.) seed extracts: correlation with antiradical activity, Food Chemistry 89 (2005) 1–9.
[53] P. Thomas, Involvement of polyphenols in the after-cooking darkening of gamma irradiated potatoes, Journal of Food Science 46 (1981) 1620–1621.
[54] M. Pritchard, L. Adam, Relationships between fry color and sugar concentration in stored Russet Burbank and Shepody potatoes, American Journal of Potato Research 71 (1994) 59–68.