Extracts of white and red grape skin and rosehip fruit: phenolic compounds and their antioxidative activity

Maša Knez Hrnčič[a*], *Darija Cör*[a], *Petra Kotnik*[a,b], *Željko Knez*[a,b]

[a]*University of Maribor, Faculty of Chemistry and Chemical Engineering, Smetanova 17, SI-2000 Maribor, Slovenia,*

[b]*University of Maribor, Faculty of Medicine, Taborska ulica 8, , SI-2000 Maribor, Slovenia, phone: +386 2 2294477, fax:+38622527774*

e-mail: masa.knez@um.si*

Abstract: The current work concerns solid liquid extraction from red and white grapes skin and the rosehip (*Rosa canina*) to obtain extracts with a high phenolic content. Extracts have been acquired using conventional extraction techniques and extraction with supercritical fluids (SCFs). The effect of extraction method and experimental parameters (time, pressure, temperature and solvent mixture) mostly believed to affect the extraction process was undertaken. The quantitative parameters studied are: total phenolic compounds, proanthocyanin content, and the phenolic constituent profile. The qualitative parameter analyzed is antioxidant capacity. The results demonstrate that the contents of the major constituents significantly varied among the different types of materials. The highest content of total phenolics was determined in the extract from the white grape skin, macerated with MeOH (26.7 mg GA/g extract), and similar, 25.6 mg GA/g extract in the MeOH extract attained by Soxhlet. Ellagic acid (0.650 mg/100 g extract), catechin (0.164 mg/100 g extract), gallic acid (0.133 mg/100 g extract) as well as caffeic acid (0.038 mg/100 g extract) are the major compounds present in the rosehip extracts attained by maceration using MeOH as solvent. The presence of epictechin, hesperidin/neohesperidin, rutin, and chlorogenic acid was also confirmed. Aspects of each type of processing were correlated with the chemistry of the material. The obtained extracts could be used as natural bioactive compounds in several industrial applications.

Keywords: Phenolic compounds; Conventional extraction; Supercritical fluid extraction; LC-MS; DPPH,

1. Introduction

Increasing pressure on natural resources and the problems caused by unhealthy eating habits have brought along an enlarged incidence of different types of cancer. Besides, the worldwide population is aging and countries are facing ongoing challenges in caring for their elderly. Consequently, the demand for different types of high quality products is increasing. Substantial investigation has been concentrated on fruits and crops containing antioxidant bioactive compounds. A bioactive compound is known as a substance that beneficially influences the health of living organisms. These extra nutritional constituents are present in both plant and animal products, and typically occur in low quantities in foods. High ingestion of fruits has consequently been associated with low incidence of chronic-degenerative diseases, probably due to the presence of bioactive compounds, considered to enhance or boost the immune system.[1] Here, extraction is an important process to isolate the bioactive compounds. Biological activities of the extract highly depends on the extraction procedure and this releases a gateway for selection of appropriate extraction methods. A great deal of interest has been devoted to the extraction of active components from natural sources, aiming at satisfying the increasing request of natural products not only for therapeutic use but also as preventing and protecting agents.[2] Among the large number of active substances in the focus, polyphenols have received particular attention in the last decade.[3] The identification and development of phenolic compounds or extracts from different plants has become a major area of food, health- and medical-related research.[4] Divided into two major groups (nonflavonoid and flavonoids), phenolic compounds show antioxidant and radical scavenging activities possibly responsible for many health benefit effects[5] and for the yellow, orange and red pigments in a large variety of plants and animal kingdoms.[6] Extracts containing these natural ingredients are incorporated into different food, therapeutic and cosmetic products. Nutritional composition, colour and antioxidant activity of such products depends on the total composition of the extract. The development of such novel functional products emphasizes the bioactive and preservation potential of phenolic compounds. Most of the compounds were extracted using Soxhlet and conventional solvent extraction methods.[7] However, conventional solvent extraction has certain disadvantages such as application of large amount of solvents, long extraction times, and the presence of toxic organic solvents in the final products.[8] Those can lead to deterioration of the quality of the extracts and can cause thoughtful health difficulties.[9] Indeed, conventional techniques have been widely accepted, mainly because of the ease of procedure, effectiveness, and widespread applicability.[10,11]

Processes based on SCFs are an environment-friendly alternative to traditional solvent extraction techniques.[12] Supercritical carbon dioxide (SC CO_2) is the most prominent amongst various solvents used in this method because of the low critical temperature (31.18 °C) and pressure (7.4 MPa), inexpensiveness, nontoxicity, non-flammability, recyclability and environmental benignity.[13] The extraction with SC CO_2 was used to acquire extracts from over 300 plant species.[14,15] This technique has already been used to isolate health-promoting compounds from the pomace of a various plants, inter alia, grapes,[16] tomatoes,[17] olives,[18] sour cherries[19], and the guelder-rose[20] and the quality of the extracts has been evaluated. In view of the bio-refinery concept, the nutrient extraction from agro-food industry waste such as skins, stalks and seeds represent a recent challenge. Valorization of by-products for the recovery of

oil, phenolic compounds, and fibers by the means of sustainable extraction procedures has gained an increased interest.[21] Grape is one of the most popular and widely cultivated fruits in the world, but are usually discarded in regular dietary intake and the winery and grape juice industry. Grape seeds and skin are rich in polyphenols [22], traditionally, extracted by using organic solvents.[23] In recent years, according to the biorefinery concept sub- and supercritical fluid extraction has been utilized as an alternative extraction technique of both polar and non-polar compounds[24] for processing of a spectrum of marketable products. Extraction efficiency is predisposed by numerous factors such as polarity and concentration of solvent, material–solvent ratio, duration, temperature, pH, etc... The most studies the influence of a single factor has been discussed, while the relations between the factors on the extract yield and composition have not been studied comprehensively.[25]

Recent investigation has been oriented towards utilization of fruits and their specific parts with a high bioactive compounds content. The main aims of the present study were to maximize the recovery of phenolic compounds in the extracts by application of different extraction methods and variation of experimental parameters.[26,27] We have been focused mainly on materials that are available in the phytogeographical regions of Slovenia (eg *Rosa canina* L.) or even constitute waste in processing (grape skin) and have been relatively poorly studied so far.

Due to the low polarity of CO_2, EtOH-modified SC CO_2 extraction has been performed to obtain extracts with a high phenolic content. Alteration of operating pressure has been assumed to influence the extraction rate of phytochemicals. In the frame of the present research EtOH-modified SC CO_2 extraction has been carried out at 40°C and pressures of 150 bar and 250 bar by using EtOH as an entrainer. Conventional extraction methods, such as Soxhlet and maceration have been performed as the reference methods to compare success of the applied methodology. Soxhlet extraction and extraction with cold solvent have been carried out by using EtOH and MeOH as solvents since substantial number of scientific reports exist where non-conventional methods using EtOH and MeOH as extraction media contributed to the high phenolic recovery.[28] The effect of extraction technique, temperature, pressure, and solvent on the extraction yield, phenolic content and profile along with the estimation of the scavenging activity against the artificial radical DPPH* (2,2-diphenyl-1-picrylhydrazyl) has been considered. Phenolic profile varied significantly in the extracts obtained from different materials. The highest net content of identified phenolic compounds determined by LC-MS/MS analysis has been determined in the MeOH extracts of white grapes skin (1,55 mg/g extract), rosehip extract attained by cold extraction with MeOH (100 mg /100 g extract), whilst in red grape skin extract the content of phenolic compounds was only about 30 mg/100 g extract .

2. Experimental

2.1 Materials

All solvents/chemicals used were of analytical/HPLC grade and obtained from Merck, Germany. Folin–Ciocalteu reagent, DPPH, and chemical HPLC-grade standards (purity ≥ 95%) of ellagic acid, gallic acid, chlorogenic acid, (+)-catechin, (−)-epicatechin,

hesperidin/neohesperidin, myricetin, resveratrol, rutin and caffeic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Lyophilized material was milled and stored in a dry, dark place. Materials were further subjected to extraction experiments.

2.2 Methods

Extraction

Soxhlet extraction: Approximately 20 g of material was weighed in a filter bag, which was inserted into the cylindrical part of the apparatus. 180 mL of solvent was heated to reflux. After 240 min of extraction at a temperature above the solvent boiling point, the solvent was removed from the extract solution by means of a rotary evaporator, yielding the extracted compound, which was later dried and weighed. The samples were stored in a dark and cool place until analyzes.

Cold solvent extraction: Red and white grapes skin were purchased by the local suppliers, the rosehip fruits were donated by Frutarom Etol d.o.o.(Slovenia). The powdered materials (20 g) were extracted by stirring using a magnetic stirrer with 180 mL of MeOH at 25 °C for 4 h. The extract was filtered for removal of solid particles. The extracts were cooled to room temperature and concentrated under vacuum at 40 °C.

EtOH-modified SC extraction: SC extraction (SCE) experiments were performed on extraction unit previously described in the literature.[29] The high pressure vessel was loaded with 10 g of material and placed in a water bath heated to the desired temperature (40 °C). EtOH was pumped continuously using a high pressure pump with a flow rate of 2 mL / min. Pressurized CO_2 has been introduced in the autoclave from the gas cylinder using a HPLC pump and was kept constant during the entire experiment. The extract and the solvent were collected in the tubes. The total time of extraction was 100 minutes. Solution was transferred to evaporation flask and the solvent was evaporated using a rotary vacuum evaporator. The mass of the extract was determined gravimetrically and the extraction efficiency was calculated. The extract was stored in a freezer at -10 °C until the analyses.

2.3 Spectrophotometric analyses

Determination of total phenolic content, proanthocyanidin concentration in extracts and antioxidant activity was done using UV-visible spectrophotometer (CARY 50 UV-VIS).

Determination of total phenolic content

Total phenolic content in extracts was determined using Folin-Ciocalteu reagent as described in the literature.[30] Briefly, the Folin–Ciocalteu reagent solution has been prepared by diluting the basic Folin–Ciocalteu reagent solution with distilled water in a ratio of 1:10. Na_2CO_3

solution has been prepared by weighing approximately 3.75 g of Na_2CO_3 in a 50 mL volumetric flask, diluted with distilled water to the mark and sonicated until total dissolution of Na_2CO_3 was obtained. Approximately 50 mg of the extract was weighted in a 10 mL volumetric flask and diluted with MeOH. 2.5 mL of the Folin-Ciocalteu reagent solution and 2 ml of Na_2CO_3 were added to 5 mL of the prepared extract solution. The mixture was left for 30 min at room temperature (25 ± 2 °C), then the absorbance of the solution was measured at 765 nm using a UV–visible spectrophotometer. The total phenolic compounds was determined in triplicate for each sample. The calibration curve of gallic acid was used for quantification of total phenolic compounds and the amount of phenolic compounds in the samples was expressed as gallic acid equivalents, in mg of gallic acid / g of material.

Determination of proanthocyanidin content

The proanthocyanidins were determined by UV spectrophotometry method (Varian-UV–VIS Spectrophotometer) based on acid hydrolysis and colour formation.[31] The reagent was prepared by weighing 77 mg of $Fe(SO_4)\times 7\ H_2O$ and adding 500 mL of HCl: butanol solution (200 mL HCl and 300 mL of butanol). 50 mg of the extract was weighted into a 10 mL volumetric flask and filled with MeOH to the mark. After the entire extract is dissolved, 1.0 mL of the extract solution was mixed with 10 mL of iron sulphate solution. At the same time, a control sample was prepared; instead of the extract solution, the same amount of MeOH was added to iron sulphate solution. The samples were thermostated for 15 minutes at a water bath at 95 °C. The solutions were cooled and the absorbance was measured at 540 nm. The concentration of proanthocyanidins (PAC) in the extract solution is expressed as mg PAC /mL of extract solution.

DPPH radical scavenging assay

Radical scavenging activity of extracts was measured using the stable radical DPPH (2,2-diphenyl-picryl-hydrazil) reagent. 50 µL of extract solution in MeOH was added to 1.95 mL of the MeOH solution of DPPH (0.025 g/L). In parallel, a negative control was prepared by mixing 50 µL of MeOH with 1.95 mL of the MeOH solution of DPPH. After 15 min of incubation in the dark at room temperature, the absorbance was measured at 517 nm against a blank sample. An ascorbic acid was used as positive control of an antioxidant reference, measured at the same conditions as samples. For each concentration, the test is repeated 3 times. The absorbance is measured by the UV-Vis spectrophotometer.

2.4 Chromatographic analyses

LC-MS/MS analysis: For identification and quantification of detected phenolic compounds, the Agilent 1200 HPLC in tandem with Agilent 6460 QQQ with JetStream ionization was used. The HPLC apparatus was equipped by quaternary HPLC high pressure pump, automatic sampler and column thermostat. The chromatographic separation of the compounds was performed on analytical column Agilent Eclipse Plus, 150 mm x 4.6 mm i.d., 1.8 µm particle size. The column was maintained at 35°C. The elution gradient consisted of mobile phase A (water with addition of 0.1 vol.% of formic acid) and mobile phase B (acetonitrile with addition of 0.1 vol.% of formic acid). The flow rate was 0.5 mL/min using gradient program

as follows: 0 min 5% B, 5 min 18 % B, 10 min, 30 % B, 15 min 35 % B, 20 min 50 % B, 21 min 70 % B and at 25 min back to 5 % of B. Samples, subjected to the analyses, were prepared by weighing approximately 100 mg of the extract in a 10 mL volumetric flask and diluted with MeOH up to 10 mL. Prepared samples were filtered through 0.2 µm syringe filter and injected (volume of 5 µL) into the system. The multiple reaction monitoring (MRM) mode was used to quantify the analytes, where the assay of was performed following two transitions per compound, the first one for quantitative purposes and the second for confirmation. The optimum ESI conditions were determined: gas temperature 300 °C, gas flow 6 L/min, nebulizer 45 psi (nitrogen), sheath gas temperature 250°C, sheath gas flow 11 L/min, capillary 3500 V and nozzle voltage 500 V at delta EMV 200 in negative ionization.

3. Results and discussion

3.1. *Conventional and SC extraction*

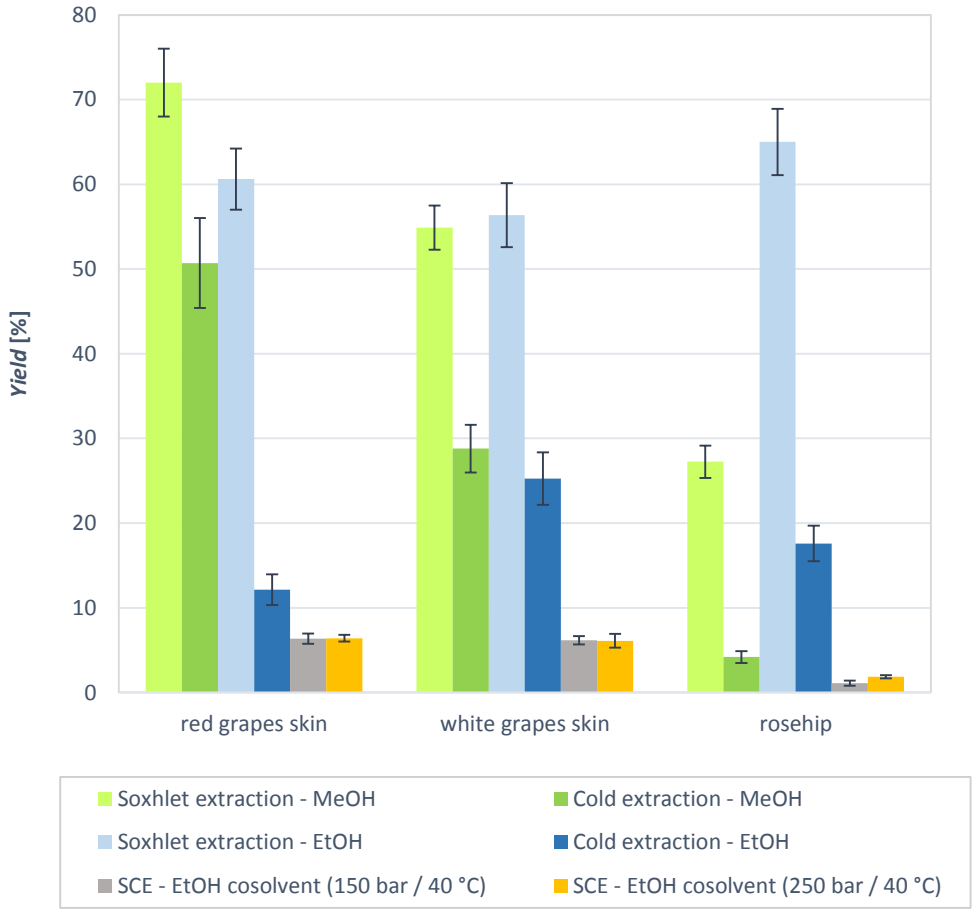


Figure 1: Comparison of extraction yield of red, white grapes skin and rosehip using different extraction methods.

Results in Figure 1 indicate that MeOH was an efficient solvent for extraction of red grapes skin using Soxhlet apparatus. Soxhlet extraction with MeOH gave the total mass yield of approx. 72 %, while cold solvent extraction with MeOH gave a yield of approx. 50 %. In general the extraction yield decreased with decreasing solvent temperature. Likewise, for the extraction of white grapes skin and rosehip, the MeOH was more efficient extraction media at elevated temperatures. For white grapes skin, the total extraction yield was 54.9 % by using Soxhlet extraction and only 28.8 % by cold extraction. Rosehip extraction by MeOH gave relatively low yields, about 27.5 % by Soxhlet and only 4.2 % by cold extraction. Higher solvent temperature apparently contributed to a higher extraction yield. EtOH was demonstrated as a less efficient solvent considering the total mass yield for both kinds of grapes skin. However, the yields were higher when utilizing hot solvent for all of the examined materials. Soxhlet extraction gave yields ranging from 56.4 % for white grape skin and up to 65 % for rosehip. Cold extraction of white grape skin gave a maximum yield approx. 25 %.

In addition, Figure 1 demonstrates that the extractions of grape skin (red and white) gave high yields with MeOH and EtOH. Consequently, it can be confirmed that MeOH and EtOH are a good choice for the solvent in the extraction of grapes, because they have high polarity and are therefore very good solvents for polyphenolic components, including resveratrol. The temperature has a significant effect in the extraction process. For instance, for red and white grape skin, it can be noticed that cold extraction yielded twice as low of extract as Soxhlet's extraction, regardless of solvent used. The same applies to rosehip, as this material also gets lower extraction efficiency than in extraction with higher temperatures. The relative polarity of the MeOH is higher than polarity of EtOH, due to this extraction efficiency of white and red grapes is higher when MeOH was used as a solvent media. In that case, the polarity of the solvent influences the extraction yields. Higher temperatures usually lead to higher yields of extraction. SC extraction gave lower mass yields compared to conventional extraction methods, despite the use of EtOH as entrainer. Beside the low solvent polarity, low extraction temperature may have also contributed to the low extraction yield. The highest yield, approx. 6.4 % has been attained by red grapes skin extraction at 250 bar, similar as for the white grape skin, where the yield was about 6.2 %. Supercritical extraction (SC extraction) of the rosehip gave yields only somewhat higher than 1.86 %. The effect of pressure has also been investigated and it was considered that the pressure does not have a tremendous effect on the extraction efficiency, since very similar yield have been attained at 150 and 250 bar for the same materials, except for the rosehip, where higher pressure gave higher yield.

3.2 Total phenolic content in extracts

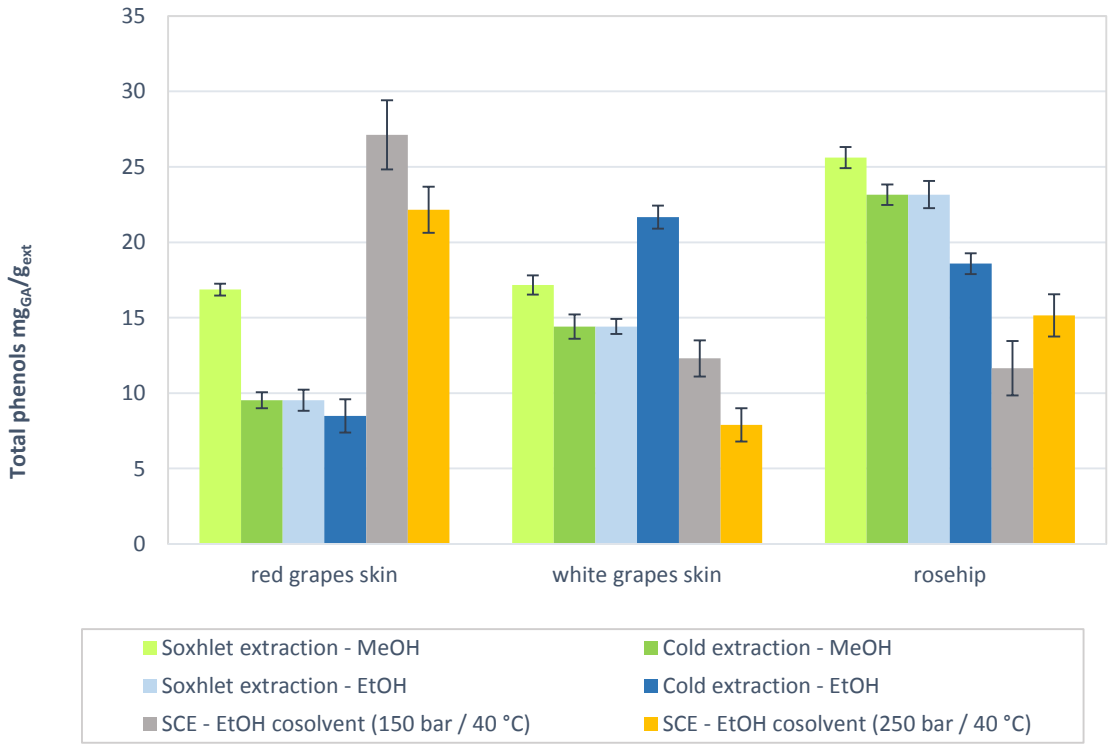
Comparison of total phenolic content, expressed as mg of gallic acid/g of extract (mgGA/gEXT) at different extraction procedures is given in Figure 2. The concentration of total phenolic compounds in extracts ranged from 8.49 mg of gallic acid per g of extract to 21.66 mg of gallic acid per g of extract for grape skin, depending on solvent selection and temperature during the extraction process. The highest concentration of phenolic compounds

for conventional extraction procedure was 25.61 mg of gallic acid per g of extract in red grape extract. In general, Soxhlet extraction with MeOH gave higher concentrations of phenolic compounds in comparison with EtOH.

In extracts obtained with SC extraction method, the concentration of total phenolic compounds ranged from 7.9 mg of gallic acid per g of extract to 27.12 mg of gallic acid per g of extract. The highest content was determined in extracts from red grape skin, attained at 150 bar and 40°C. The content varied depending on pressure during the extraction process; the content in the extract, attained at 250 bar and 40°C was 22.15 mg of gallic acid per g of extract.

The concentration of phenolic compounds in white grape skin extract was 8.83 mg of gallic acid per g of extract at 150 bar and 40°C, whilst at higher extraction pressure the concentration decreased to 6.77 mg of gallic acid per g of extract. In general, from both grape skin, higher concentrations of phenolic compounds have been attained at lower extraction pressure. On the contrary, in case of rosehip, the content increased with elevation of extraction pressure from 8.13 mg of gallic acid per g of extract at 150 bar and 40°C up to 9.01 mg of gallic acid per g of extract at 250 bar and 40°C.

Also, the influence of temperature during the extraction process on the total amount of phenols in extracts was noticed. Extraction of both grape skin and rosehip with MeOH at its boiling point resulted higher amount of phenolic compounds compared to the amount of phenolics in case of cold extraction with MeOH. In general, higher extraction temperature contributed to higher concentration of phenolic compounds except in case of cold extraction of white grape skin with EtOH, which was more efficient for extraction of phenolic compounds. White grape skin extract contains more phenolic compounds in comparison with red grapes skin.



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278 Figure 2: Comparison of total phenolic content, expressed as mg of gallic acid/g of extract
279 (mg_{GA}/g_{EXT}) at different extraction procedures.

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281 *3.3 Proanthocyanins in extracts*

282 The amount of proanthocyanins (PAC) in the extracts obtained by Soxhlet, cold and SC
283 extraction is presented in Figure 3. The highest concentration of proanthocyanins was
284 obtained in white grapes skin by cold extraction with MeOH (2.02 mg PAC /mL). It can be
285 observed that white grapes have higher amount of proanthocyanins in comparison with red
286 grapes skin, where the content of proanthocyanins was highest in the extract attained by
287 Soxhlet extraction with MeOH (1.34 mg PAC /mL). The concentration of proanthocyanins in
288 rosehip extracts was the lowest, up to 0.96 mg/mL PAC in the extract obtained by cold
289 extraction with MeOH.

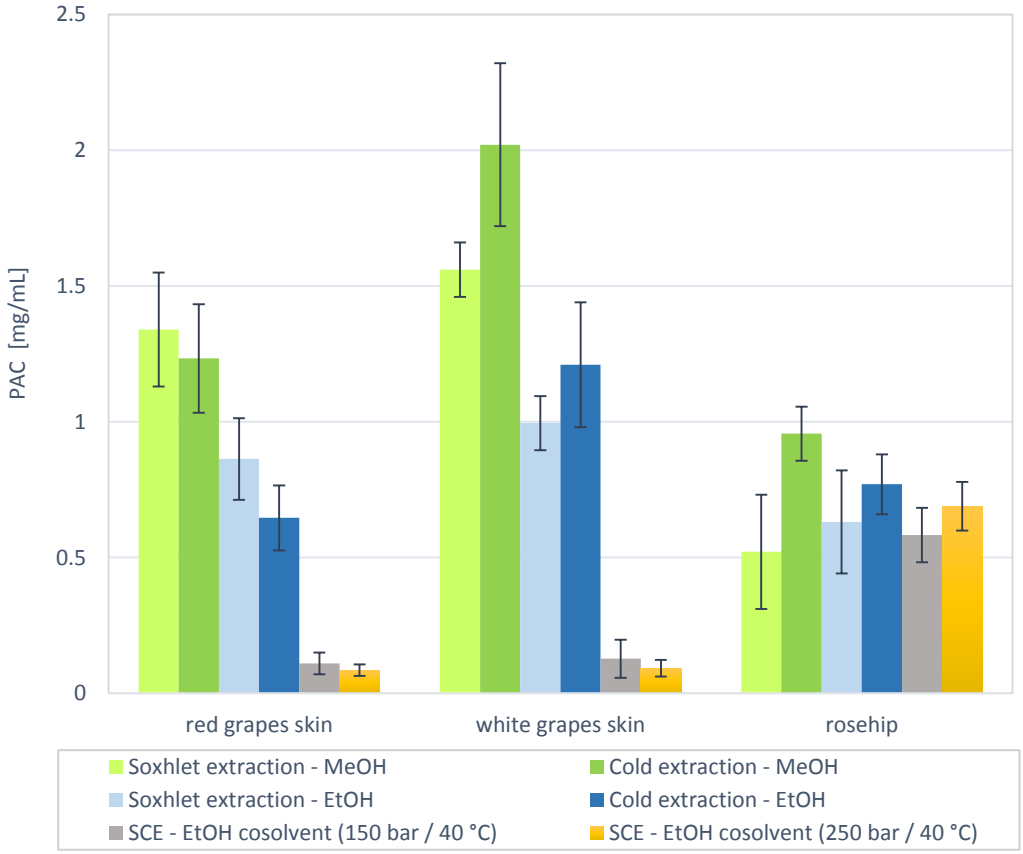

Figure 3: Comparison of proanthocyanins content in extracts obtained by Soxhlet, cold and SC extraction.

The highest concentration of proanthocyanins obtained by modified SC extraction was determined in rosehip extracts attained at 250 bar and 40°C; approximately 0.69 mg PAC /mL. The amount of extracted proanthocyanins in rosehip extracts decreased with decreasing extraction pressure to 0.583 mg PAC /mL).

It was found, that white grapes have slightly higher amount of proanthocyanins in comparison with red grapes skin. The content of proanthocyanins was highest in the white grape skin extract attained at 150 bar and 40°C; approximately 0.13 mg PAC /mL and about 0.09 mg PAC /mL in extract attained at 250 bar and 40°C. The concentration of proanthocyanins in red grapes skin extracts was the lowest, up to 0.11 mg PAC /mL at 150 bar and 40°C and only 0.085 mg PAC /mL at 250 bar and 40°C. Almost three times higher concentration of proanthocyanins was obtained in white grapes skin obtained by cold extraction with MeOH (2.02 mg PAC /mL).

3.4. LC/MS analysis

The phenolic compounds were analyzed using LC-MS/MS analytical method. The identification and quantification of individual phenolic compound was done using analytical

standards of each compound and the calculation was done using calibration curves, as described in section 2.4.

Table 1: Content of phenolic compounds in the extracts of white and red grape skin and rosehip at different extraction conditions.

Compound	caffeic acid	catechin	chlorogenic acid	ellagic acid	epicatechin	gallic acid	hesperidin/ neohesperidin	resveratrol	rutin	sum
sample	ug/mg	ug/mg	ug/mg	ug/mg	ug/mg	ug/mg	ug/mg	ug/mg	ug/mg	mg/100 g
White grapes skin, Cold - MeOH	0.013	0.838	0.001	0.043	0.340	0.074	0.015	0.030	0.015	1.370
White grape skin, Soxhlet - MeOH	0.016	0.980	0.007	0.044	0.346	0.096	0.012	0.027	0.023	1.550
White grape skin Soxhlet - EtOH	0.011	0.438	0.002	0.025	0.212	0.050	0.008	0.015	0.012	0.773
White grapes skin, Cold - EtOH	0.012	0.085	0.002	0.019	0.038	0.034	0.003	0.011	0.002	0.207
White grapes skin - 150 bar	0.014	0.028	0.006	0.027	0.015	0.071	0.009	0.020	0.009	0.199
White grapes skin - 250 bar	0.015	0.056	0.002	0.022	0.024	0.080	0.003	0.029	0.000	0.230
Red grapes skin, Cold - MeOH	0.025	0.009	0.001	0.050	0.009	0.016	0.033	0.007	0.100	0.249
Red grapes skin, Cold - EtOH	0.012	0.003	0.001	0.013	0.004	0.014	0.008	0.006	0.022	0.084
Red grapes skin, Soxhlet - MeOH	0.019	0.090	0.006	0.018	0.021	0.027	0.038	0.014	0.068	0.301
Red grapes skin, Soxhlet - EtOH	0.029	0.019	0.004	0.022	0.005	0.023	0.012	0.011	0.032	0.157
Red grapes skin, 250 bar	0.038	0.000	0.001	0.014	0.002	0.016	0.001	0.012	0.000	0.083
Rosehip, HT, MeOH	0.014	0.164	0.017	0.650	0.021	0.133	0.003	/	0.000	1.003
Rosehip, 150 bar	0.019	0.012	0.011	0.373	0.004	0.116	0.003	0.022	0.000	0.560
Rosehip, 250 bar	0.014	0.014	0.005	0.145	0.004	0.106	0.002	0.015	0.001	0.306

The content of phenolic compounds in extracts is shown in Table 1. The highest content of the identified phenolic compounds was present in MeOH extracts of white grape skin, whereas the major compound was catechin (0.980 μg/mg extract). EtOH extracts gave lower phenolics recovery, apparently by decreasing solvents polarity less phenolics were dissolved. EtOH white grape skin extract contained approximately a half of the identified compounds when utilizing Soxhlet procedure, again, catechin was the major compound (0.438 μg/mg extract), whilst the cold solvent extract contained only 0.085 μg/mg extract, which is ten times lower than in case of MeOH extracts. Gallic acid was the major compound in SC extracts of white grapes skin (0.080 μg/mg extract), higher pressure contributed to somewhat higher content. The major compound in red grape skin extract was ellagic acid (0.050 μg/mg extract), its recovery increased with increasing solvent polarity, whilst effect of temperature was not explicit. Caffeic acid was also identified, the highest content was present in the MeOH extract. Gallic, ellagic acid and resveratrol were identified in SC extracts. Likewise, rosehip extracts mainly contained caffeic, gallic, ellagic acid and resveratrol, although the highest recovery of

ellagic acid was analyzed in MeOH extract. Resveratrol was detected only in SC extracts. However, the highest recovery of resveratrol was observed in SC extract of white grape skin attained at 250 bar and the macerated white grape skin extracts (0.03 μg/mg extract). Rutin and hesperidin were present in lower concentration, however, 0.068 μg/mg extract of rutin was analyzed in MeOH extract of red grapes skin, whereas the content of hesperidin/neohesperidin was about 0.03 μg/mg extract in the same extract. All extraction procedures gave similar yield of epicatechin (up to 0.346 μg/mg). Chlorogenic acid was present in traces, up to 0.02 μg/mg extract in MeOH and SC extract of rosehip.

Among all the extracts, the highest content of caffeic acid (0.038 ug/$mg_{raw\ material}$) has been present in red grapes skin extract, attained at 250 bar and 40 °C. Rosehip extract attained at 150 bar contained up to 0.373 ug/$mg_{raw\ material}$ ellagic acid. The content of resveratrol was low in all samples, however, the highest contents were present in the MeOH extracts and white grapes skin extracts, attained by SC extraction at 250 bar, up to 0.030 ug/$mg_{raw\ material.}$

3.5 DPPH activity of extracts

The results of DPPH* radical scavenging activities of conventional extracts obtained using Soxhlet or cold extraction are presented on Figure 4 and vary between 2.75 % to 13.64 %, depending on solvent and method used. The highest DPPH* radical scavenging inhibitory activity 13.64% was observed for red grapes skin extract prepared by cold extraction with MeOH as solvent. DPPH* radical scavenging inhibitory activity for white grape skin were slightly lower, but trend is similar. In general, for grape skin solvent MeOH resulted higher DPPH* radical scavenging. Results obtained for grape skin show that extracts, attained by the cold extraction method exhibited higher DPPH* radical scavenging comparison to extracts attained by Soxhlet extraction. In case of rosehip extraction, Soxhlet extraction gave extracts with higher DPPH* radical scavenging activity regardless of the solvent used. Higher extraction yield does not necessarily mean higher biological activity of the extracts. The use of different solvents can result in the extraction of various types of metabolites from extracted material, with varying radical scavenging activities. Furthermore, increased temperatures during the extraction process may result in denaturation and a reduction of the loss of ability to act as an antioxidant.

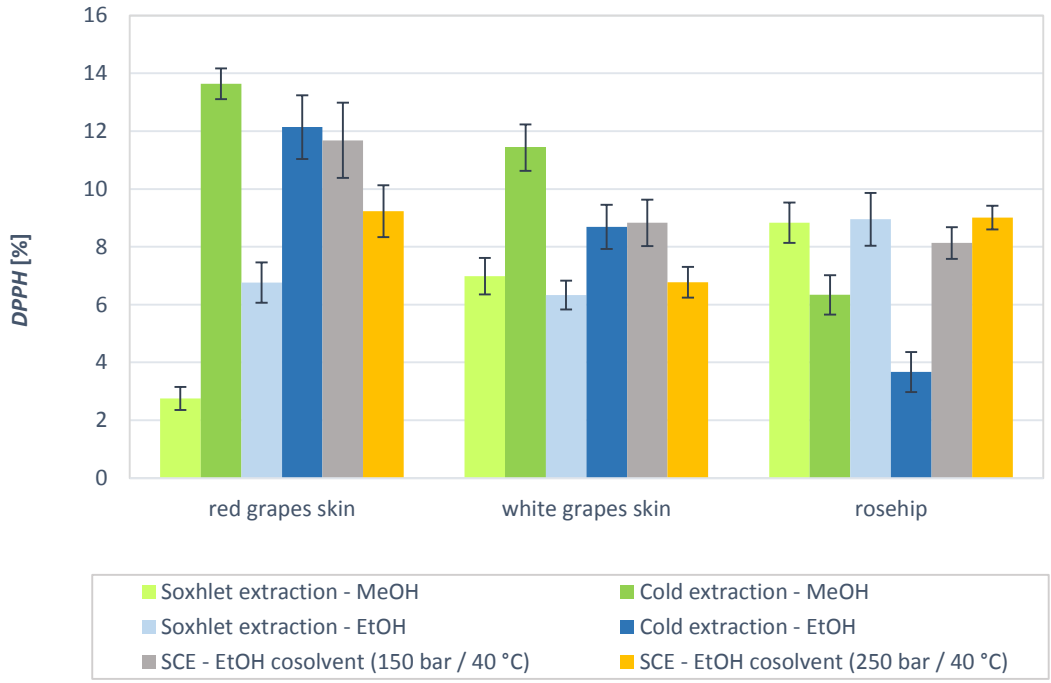

Figure 4: Comparison DPPH activity in % using different extraction procedures.

The results of DPPH* radical scavenging activities of extracts obtained using modified SC extraction vary between 6.67 % and 11.68 %, depending on the material. The highest DPPH* radical scavenging inhibitory activity was observed for red grapes skin extract, attained at 150 bar and 40°C. The activity is lower in the extract, attained at 250 bar and 40°C; 9.23 %.

DPPH* radical scavenging inhibitory activities for white grape skin were slightly lower in both extracts compared to the red grape skin extracts. Lower extraction pressure again resulted in somewhat higher scavenging activity of the extract; approximately 8.83 %, whilst the extract attained at 250 bar showed somewhat lower inhibition ability, approximately 6.67 %.

Rosehip extracts showed similar scavenging inhibitory activity as the extracts of white grape skin. However, in this case higher extraction pressure contributed to somewhat higher scavenging activity (9.01 %), whilst the extract attained at 150 bar showed lower inhibition ability, approximately 8.13 %. The use of different process conditions can result in the extraction of various types of metabolites from extracted material, with varying radical scavenging activities. In general, the amount of proanthocyanins and the total phenolic content is not directly related to the radical scavenging inhibitory activities. Red grapes skin extracts prepared by cold extraction and SC extraxtion exhibited similar scavenging inhibitory activities (ranging from 9 % up to nearly 14 %), whilst the contents of proanthocyanins and the total phenolics ranged from 0.085 mg PAC /mL in extract attained by SC extraction at 250 bar and 40°C up to 1.233 mg PAC /mL in cold MeOH extract, which exhibited highest inhibitory activity. Meanwhile, the concentration of total phenolic compounds in that extract was 9.53 mg of gallic acid per g of extract. Amongst all red grape skin extracts the lowest DPPH* was determined for Soxhlet extract prepared with MeOH, where the contents of proanthocyanins was the highest (1.34 PAC /mL).

Amongst all white grape skin extracts the highest DPPH* (11.43 %) was determined for MeOH extract prepared by cold extraction; the concentration of proanthocyanins was high, 2.02 mg PAC /mL, and the total phenolics content was about 14.41 mg of gallic acid per g of extract. Both Soxhlet extracts of rosehip exhibited similar scavenging activities (about 9 %). Similar value was measured for extract, attained at 250 bar and 40°C. The concentration of proanthocyanins was the highest for MeOH extract prepared by cold extraction; 0.956 PAC /mL, whilst total phenolics content was about 24 mg of gallic acid per g of extract.

4.Conclusion

The obtained results confirm earlier findings that rosehip, white and red grape skin are potentially good sources of natural antioxidants. Although, the content of total phenolics differs depending on the type of material, isolation method and applied solvent. Besides, anthocyanin content in grape skin can depends on the variety and the harvest year.

According to the previous research [32], the conventional extraction methods involving organic solvents and increased temperature yielded more total phenols. The present results show that the amount of anthocyanins depends on the plant material and extraction solvent. Regardless of the method and solvent used, the highest content of total phenolic compounds (27.12 mg_{GA}/g_{EXT}) has been determined in the extracts from red grape skin, obtained at 150 bar and 40°C. High content of total phenolic compounds was, according to their polar nature, determined also in extracts, obtained by conventional extraction with MeOH; up to 25.61 mg_{GA}/g of red grape skin extract. In general, Soxhlet extraction using MeOH gave higher concentrations of phenolic compounds in comparison to extractions using EtOH. In addition, the higher extraction temperatures contributed to higher concentration of phenolic compounds. The content of proanthocyanins in extracts obtained by modified SC extraction was the lowest in all extracts.

According to the analyses, the yield of phenolic compounds in extracts attained by SC extraction was lower than in the extracts attained by the conventional solvent extraction. This may be explained by the fact that CO_2 usually yields good recoveries for nonpolar compounds, but polar compounds may remain partially unextracted because of their lower solubility in this fluid. For this reason, EtOH as organic modifier has been added as cosolvent to the primary fluid to boost the extraction effectiveness. The extraction output considering mass yield was higher in case of conventional extraction methods, but the amount of anthocyanins and phenols successfully extracted with supercritical solvent is considerable. The results show, that generally SC CO_2 in combination with a polar entrainer, represents a good extraction media for isolation of total phenols, while the amount of extracted anthocyanins is low. LC-MS/MS analyses show that gallic, ellagic acid and resveratrol were identified in SC extracts. Therefore, we could consider that SC extraction with CO_2 therefore provides an alternative method to replace extractions with organic solvents for the recovery of phenolic compounds. In general, SC extraction was less efficient due to the limited solvent polarity. Despite the lower total mass yield and the proportion of total phenols in extracts

compared to the conventionally obtained extracts, the method was efficient for isolation of caffeic acid, galic and elagic acid.

DPPH* radical scavenging activities of extracts obtained using modified SC extraction and conventional extraction are similar and depend on the type of material. Highest DPPH* radical scavenging inhibitory activity was observed for red grapes skin using cold extraction and MeOH as solvent (about 13 %), where the content of total phenols was 9.53 mg of gallic acid per g of extract. The activity of supercritical extract attained at 150 bar and 40°C was only slightly lower, about 12 %, whilst the content of total phenols was the highest, 27.12 mg of gallic acid per g of extract.

The primary aim of this work was utilization of SC extraction for isolation of phenolic compounds as a sustainable method which involves lower consumption of organic solvents. Besides, SCFs are generally cheap, simple, and comparatively safe solvents which is of special attractiveness for industrial processes (especially in food and pharmaceutical applications). Toxic hazards from solvent manipulation are greatly reduced as well as disposal costs. Another motivation for developing processes involving SCFs as solvents SC extraction processes is the reduction or even complete elimination of residual solvents in the products, lower operating temperatures and prevention of oxidation during processing. In contrast to various organic solvents, SCFs can be more simply recycled which significantly reduces the cost of any analytical procedure.

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