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ORIGINAL PAPER

Optimization of a process for enzyme-assisted pigment extraction from grape (*Vitis vinifera* L.) pomace

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Abstract A process for enzyme-assisted extraction of polyphenols from grape pomace was developed on laboratory and pilot-plant scale. After resuspending grape pomace in water, the skins were ground and the resulting mash was pasteurized to inactivate the deteriorative enzymes responsible for polyphenol degradation, and then pre-extracted with hot water. Subsequently, cell wall polysaccharides were hydrolyzed. The extract was separated from the solid residue by pressing, and finally spray dried. Before scaling-up, enzymatic hydrolysis was optimized on laboratory scale using a D-optimal design and analyzed by response surface methodology. A mixture of pectinolytic and cellulolytic enzyme preparations (ratio 2:1) yielded the highest amounts of phenolic compounds after 2 h of treatment, applying a dosage of 4,500 mg/kg (based on dry matter) at $T = 40$ °C and pH 4.0. Aqueous pre-extraction of the pomace followed by enzymatic treatment resulted in significantly improved extraction yields reaching 91.9, 92.4, and 63.6% for phenolic acids, non-anthocyanin flavonoids and anthocyanins, respectively. As the yields obtained were comparable to those from sulfite-assisted extraction, this process can be considered a suitable alternative to the application of sulfite.

Keywords Enzyme-assisted extraction · Anthocyanins · Phenolic compounds · Grape pomace · Natural colorants · Spray drying

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Introduction

Grapes (*Vitis vinifera* L.) belong to the world largest fruit crops with an annual production of 66 million tons in 2005 [1]. Taking into account that about 80% is used in wine-making, some 10 million tons of grape pomace arise within a few weeks during harvest. Besides its use as a soil conditioner or feed [2], grape pomace can be utilized for the recovery of numerous high-value compounds such as ethanol [3], consumable acids such as tartaric, malic and citric acids [4–7], grape seed oil [8], and dietary fiber [9–11]. Furthermore, phenolic constituents present in grapes have experienced intense interest due to their antioxidant properties and putative health-promoting effects [12]. Therefore, considerable effort has been devoted to the extraction of phenolic compounds from winery by-products in order to use the extracts as dietary supplements or as food colorants [13–15]. Besides acidified alcohols, sulfited water or alcohols are commonly used for anthocyanin extraction [16–18]. However, sulfite cannot be removed quantitatively from the extracts and pseudoallergic reactions have been reported after the ingestion of sulfited foods. Intolerance reactions are mostly manifested by bronchospasm, urticaria, and bronchoconstriction [19, 20]. Furthermore, reactions of sulfite with a number of food constituents, such as anthocyanins, ascorbic acid, thiamin, and phenolic compounds have been described. The toxicological relevance of the products formed remains to be elucidated [21].

As an alternative to SO_2-based processes, cell wall degrading enzymes have been used for an enhanced release of phenolic compounds from grape pomace [22, 23]. In a recent study [24] a process for the recovery of polyphenols from grape pomace using a combination of pectinolytic and cellulolytic enzymes has been reported. However, the enzyme concentrations required for a sufficient degradation

of cell wall polysaccharides were comparatively high, which limits the economic feasibility. Therefore, the aim of the present study was to optimize the enzyme-assisted liquefaction of grape pomace with respect to enzyme dosage, temperature and pH value, for the production of an extract rich in polyphenols and its application to pilot-plant scale. For this purpose, a D-optimal design (DOD) was performed in order to assess the effects of the above-mentioned parameters on the yields of anthocyanins and phenolic acids on laboratory scale.

Experimental

Materials and reagents

All reagents and solvents were of analytical or HPLC grade and were purchased from VWR, Darmstadt, Germany. C18 reversed-phase cartridges (Chromabond®, 1,000 mg) were from Macherey-Nagel, Düren, Germany.

Standards used for identification and quantification purposes with HPLC-mass spectrometry (MS) and HPLC-diode array detection (DAD) were as follows: cyanidin-3-*O*-glucoside, delphinidin-3-*O*-glucoside, malvidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, petunidin-3-*O*-glucoside (Polyphenols, Sandnes, Norway); (+)-catechin, *p*-coumaric acid (−)-epicatechin, ferulic acid, gallic acid, caffeic acid, protocatechuic acid, quercetin (Q) (Roth, Karlsruhe, Germany); Q-3-*O*-galactoside, Q-3-*O*-glucoside, procyanidin B1, procyanidin B2 (Extrasynthèse, Lyon, France); epicatechin gallate, *trans*-resveratrol (Sigma, St. Louis, MO, USA); syringic acid (Fluka, Buchs, Switzerland); *trans*-resveratrol-3-*O*-glucoside (*trans*-polydatin) (Sequoia Research Products, Oxford, UK). The enzyme preparations Novoferm 106 (pectinolytic activity) and Cellubrix ®L(cellulolytic activity) were obtained from Novo Nordisk Ferment, Dittingen, Switzerland. All enzyme dosages mentioned in this paper are expressed on a dry matter basis of the grape pomace used.

Grape pomace samples from red wine (*V. vinifera* L. cv. 'Lemberger') of the 2005 vintage were obtained from Felsengartenkellerei Besigheim, Hessigheim, Germany. Samples originated from a process based on high-temperature short-time treatment of the mash followed by enzymatic degradation of soluble grape pectins. Musts were obtained using a screw extrusion press. Pomace samples were sealed in polyethylene bags and kept at $T = -20$ °C.

Enzyme-assisted digestion of grape pomace

The enzymatic treatment of grape pomace was based on a process described by Kammerer et al. [24] and optimized as described below.

Optimization of enzymatic hydrolysis on laboratory scale

Frozen grape pomace was manually separated into seeds and skins using a sieve (mesh size 5.6 mm). The skins were minced with a UM 12 cutter (type K 20, Seydelmann, Aalen, Germany) for $t = 1$ min. Subsequently, the residual seeds were completely removed by a second sieving step (mesh size 5.6 mm). The skins were suspended in water (1:3, w : w) and finally comminuted for $t = 19$ min using the cutter mentioned above. Aliquots of 500 g of the skin suspension obtained were stored under nitrogen at $T = 30$ °C for all optimization trials on laboratory scale. Aliquots of 200 g of the skin suspension were used for the laboratory experiments. All determinations were performed in duplicate. After adjusting the pH value and temperature, enzyme preparations (Novoferm 106 and Cellubrix L) were added (Table 1) and the homogenous mash was stirred under nitrogen in a water bath for $t = 120$ min. Subsequently, enzymes were inactivated at $T = 90$ °C for $t = 1$ min in a water bath. Immediately after enzyme inactivation the samples were cooled in an ice bath to avoid thermal degradation of phenolic compounds. Liquid–solid separation was performed using a Büchner funnel. Individual phenolic compounds of the extracts were quantified as described in Sample preparation section. For the evaluation of the efficiency of a pre-extraction process the comminuted grape skins were extracted with deionized water ($T = 50$ °C, $t = 2$ h, 1:3, w : w) and the extract was subsequently filtered using a Büchner funnel. The solid residue was resuspended in water (1:3, w : w) and enzyme-assisted extraction was performed as described previously.

Table 1 Uncoded level combinations for the D-optimal designs

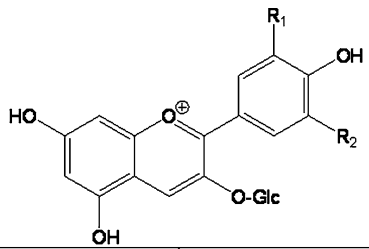
Numbers	pH value	Temperature [°C]	Enzyme dosage [ppm]
1	6	40	1,500
2	6	45	1,500
3	5	35	4,500
4	4	45	7,500
5	6	50	3,000
6	3	35	6,000
7	4	55	0
8	3	50	0
9	3	45	7,500
10	5	40	0
11	3	35	0
12	5	50	1,500
13	6	55	7,500
14	6	45	7,500
15	3	40	6,000
16	5	40	4,500
17	3	50	6,000
18	6	35	3,000
19	4	55	3,000
20	4	55	4,500

Experimental design and statistical analysis

The effects of the individual factors, i.e., pH (3–6), temperature (T = 35–55 °C) and enzyme concentration (c = 1,500–7,500 mg/kg), and their interactions on the recovery of phenolic compounds from grape pomace were investigated using a DOD. The extractability was examined by response surface methodology. The factor combinations of the 20 design experiments are listed in Table 1 with the resulting amounts of the 3-*O*-glucosides of delphinidin, cyanidin, peonidin, petundin, and malvidin (Fig. 1a) as the response (Y, dependent variable) for the first series of design experiments and those of caftaric, coutaric, and fertaric acids (Fig. 1b) as the response for the second series. All laboratory experiments were performed in duplicate. Results of the DOD trials were analyzed by non-linear multiple regression with backward elimination to fit the following second-order equation to the dependent Y variables:

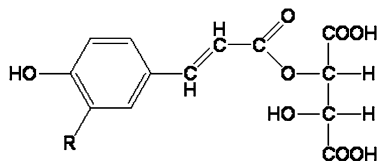
$$Y = B_0 + \sum_{i=1}^{3} B_i X_i + \sum_{i=1}^{3} B_i X_i^2 + \sum_{i=1}^{3} B_{ij} X_i X_j. \quad (1)$$

B_0, B_i, and B_{ij} are constant regression coefficients of the model and X_i and X_j are independent variables in coded values. For each experimental factor the variance was partitioned into the components linear, quadratic, and interaction in order to assess the adequacy of the second-order polynomial function and the relative importance of these components. The significance of the equation parameters for each response variable was assessed by F-test (α = 0.15). The regression procedure (PROG REG) of the Statistical Analysis System (SAS Institute, Caly, NC, USA, Software Version 9.1) was used to calculate regression coefficients, analysis of variance, correlation coefficients for the model, and to eliminate insignificant terms.



A

Anthocyanins	R_1	R_2
delphinidin-3-*O*-glucoside	OH	OH
cyanidin-3-*O*-glucoside	OH	H
peonidin-3-*O*-glucoside	OCH_3	H
petunidin-3-*O*-glucoside	OCH_3	OH
malvidin-3-*O*-glucoside	OCH_3	OCH_3



B

Phenolic acids	R
trans-caftaric acid	OH
trans-coutaric acid	H
trans-fertaric acid	OCH_3

Fig. 1 Target compounds of the first (**a**) and second (**b**) experimental design. Total amounts of each target compound were used as response

Enzymatic hydrolysis of grape skin cell walls on pilot-plant scale

For the pilot-plant experiments without pre-extraction, 5 kg of frozen grape pomace were treated as described in Optimization of enzymatic hydrolysis on laboratory scale section. After mincing, pH value and temperature were adjusted to 4.0 and T = 40 °C, respectively. Enzymes (Novoferm 106, c = 3,000 mg/kg; Cellubrix L, c = 1,500 mg/kg) were added, and the homogenous mash was stirred in a laboratory mixer (Typ MP 694, Exco-Labor, Riehen, Switzerland) for 2 h under nitrogen. The mash was heated at T = 90 °C for t = 1 min and subsequently cooled in a water bath. Liquid–solid separation was performed with a tincture press HP 20 (Tinkturenpressen Schwanke, Neuss, Germany). The liquid extract was collected, and individual polyphenolics were quantified as described in Sample preparation section.

Pre-extraction process on pilot-plant scale

For the evaluation of a pre-extraction step on pilot-plant scale, two different processes were performed. In both cases, 5 kg of frozen grape skins were minced for t = 1 min using a UM 12 cutter (type K 20, Seydelmann).

Pre-extraction process I Pre-extraction process I followed general steps of the laboratory scale extraction procedure (Optimization of enzymatic hydrolysis on laboratory scale section). The skins were suspended in water (1:3, w : w) and finally comminuted for t = 19 min using the cutter mentioned above. The minced pomace was extracted with water (1:3, w : w; T = 50 °C; t = 2 h; pH 4.0) under nitrogen. After liquid–solid separation using the tincture press, the pomace was resuspended in water (1:3, w : w, T = 50 °C). Enzyme-assisted extraction was performed as described previously.

Pre-extraction process II The minced skins were resuspended in water (1:3, w : w, T = 80 °C). The mash was heated at T = 90 °C for t = 1 min and cooled to T = 50 °C. Subsequently, the mash was comminuted using a type 60 colloid mill (Probst & Class, Rastatt, Germany) and extracted with water (1:3, w : w; T = 50 °C; t = 2 h; pH 4.0). Liquid–solid separation was performed using a rack and cloth press MH-20 (Wahler, Stuttgart, Germany). The liquid fraction was collected, whereas the solid fraction was resuspended in water (1:3, w : w, T = 40 °C). Enzymatic extraction was accomplished as described for the laboratory-scale experiments except for the liquid–solid separation which was performed using the rack and cloth press MH-20 (Wahler). The pilot-plant scale extraction process employing the latter pre-extraction technique is illustrated in Fig. 2.

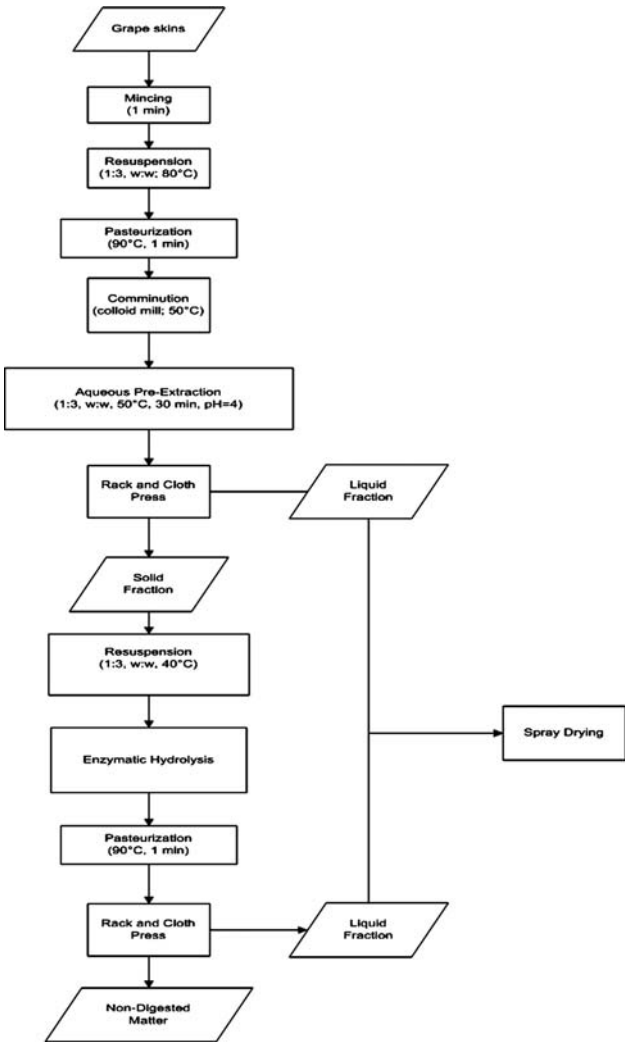


Fig. 2 Scheme of enzyme-assisted extraction of polyphenols from grape pomace

Spray drying

The combined extracts obtained from the two-step extraction process were spray dried to obtain a powder. A 5% soluble starch solution (Merck, Darmstadt, Germany) was used as a carrier. All components were added under stirring at room temperature. After complete dissolution, the resulting liquid was spray dried using a Büchi 190 mini spray dryer (Büchi Labortechnik, Flawil, Switzerland) at temperatures of 165 °C (inlet) and 113 °C (outlet). The powder was filled in amber glass screw cap bottles and stored in a desiccator until analysis of phenolic compounds.

Identification and quantification of individual phenolic compounds

Sample preparation

Anthocyanins were analyzed by direct injection of the extracts obtained from laboratory and pilot-plant experiments. The powder obtained after spray drying was dissolved in water. Non-anthocyanin phenolic compounds were analyzed according to a previously published method [25].

HPLC-DAD system

Phenolic compound analysis was performed with an Agilent HPLC series 1100 (Agilent, Waldbronn, Germany) equipped with Chemstation software, a model G1322A degasser, a model G1312A binary gradient pump, a model G1329/G1330A thermoautosampler, a model G1316A column oven, and a model G1315A diode array detector. The separation was carried out with a Phenomenex, Torrance, CA, USA, Aqua C18 column (250 × 4.6 mm i.d.; d = 5 μm particle size) with a C18 ODS guard column (4.0 × 3.0 mm i.d.) operated at T = 25 °C. UV-Vis spectra were recorded from 200 to 600 nm at a spectral acquisition rate of 1.25 scans/s (peak width 0.2 min). Mobile phases consisting of water, formic acid, and acetonitrile were used for the separation of anthocyanins, whereas water, acetic acid, and acetonitrile were employed for the determination of phenolic acids, non-anthocyanin flavonoids, as previously described [25]. For all samples the injection volume was 10 μL. Simultaneous monitoring was performed at 280 nm (hydroxybenzoic acids, flavanols), 320 nm (hydroxycinnamic acids), 370 nm (flavonols), and 520 nm (anthocyanins) at a flow rate of 0.8 mL/min (anthocyanins), and of 1.0 mL/min (other polyphenols), respectively.

Mass spectrometry

For peak assignment, polyphenols were analyzed with the HPLC system described in the previous section, coupled to a Bruker, Bremen, Germany, model Esquire 3000+ ion trap mass spectrometer fitted with an electrospray ionization (ESI) source. Data acquisition and processing were performed using Esquire Control software. The mass spectra of phenolic acids and non-anthocyanin flavonoids were recorded in the negative mode, those of anthocyanins in the positive mode. Mass spectrometric conditions were applied as previously reported [25].

Quantification of individual phenolic compounds

Individual polyphenolics were quantified using a calibration curve of the corresponding standard compound. In case of lacking reference compounds, the calibration of structurally related substances was used including a molecular weight correction factor [26]. The yields of the target compounds were calculated based on total amounts of the respective compounds in grape pomace, which were determined after extraction with methanol 0.1% HCl (v/v) [25].

Results and discussion

Polyphenol contents of grape pomace have been shown to differ significantly, depending mainly on the cultivar and vintage. Furthermore, the ripening stage, phytosanitary conditions of the grapes, and the vinification technology may also be responsible for varying contents [25]. Because of its complex profile in each phenolic subclass, a pomace obtained from the cultivar 'Lemberger' was chosen for all experiments.

Enzyme-assisted digestion of grape pomace

Optimization of enzymatic hydrolysis on laboratory scale

Previous studies have revealed synergistic effects of pectinase and cellulase activities on cell wall degradation [27]. Grape pomace liquefaction using pectinolytic and cellulolytic enzyme preparations to enhance polyphenol extraction has also been investigated [23, 24]. Since in the latter study, Novoferm 106 and Cellubrix L (ratio 2:1) were shown to be most effective, this combination was also used in the present work. Because intact grape seeds were not affected by the enzyme preparations [28] they were removed before comminution. Accessibility of the substrate is one of the most important factors affecting enzymatic digestion. Liquefaction can also be enhanced using an aqueous pre-extraction step, which removes part of the phenolics that are known to inhibit enzyme activity. To prove the efficacy of this step, enzymatic digestion was performed with and without pre-extraction. Extractability of polyphenols is mainly affected by enzymatic degradation of grape skin polysaccharides. Furthermore, extraction yields also depend on the chemical nature of the target compounds and thus on their hydrophilicity. Additionally, the solubility was also affected by the glucosidase side activity of the enzyme used, since the aglycones formed exhibit lower hydrophilicity and stability. Therefore, two different DOD were used to determine the optimum conditions for enzyme-assisted extraction of anthocyanins (DOD 1) and phenolic acids (DOD 2).

Optimization of enzyme-assisted extraction on the recovery of anthocyanins (DOD 1) In the first series of experiments the effects of the three factors pH value (a), enzyme dosage (b) and temperature (c) on the recovery of total anthocyanins ($\hat{y}$), calculated as the sum of individually quantified compounds (Fig. 1a), were evaluated. After successive rejection of insignificant terms by backward elimination, the following mathematical model ($R^2 = 0.85$) was obtained:

$$\hat{y} = -4{,}093.1280 - 198.3210a + 281.2674b + 0.2455c - 3.3183b^2 - 0.00002476c^2. \quad (2)$$

This mathematical model was used to generate three-dimensional response surface plots with one constant factor. Regression analysis revealed these factors to be significant linear variables ($p < 0.0001$, $p = 0.0071$ and 0.0006, respectively) affecting the extraction yield. Variation of the enzyme dosage (b) was found to exert the most significant effect of all parameters studied. As can be seen from Fig. 3a, pigment yields increased with higher enzyme dosages up to $c = 4{,}500$ mg/kg. With higher concentrations, a slight decline of total anthocyanin yields was observed. From Eq. 2 it becomes evident that the influence of temperature (c) within the range tested in this study is very low. Maximum yields were obtained at $T = 40$ °C. A further rise in temperature resulted in lowered amounts of the target compounds. In contrast to the temperature, the pH value (a) had a significantly higher impact (Fig. 4a) on the pigment yields. At a constant enzyme dosage of $c = 4{,}500$ mg/kg and a temperature of $T = 40$ °C, maximal pigment yields were obtained at pH 3, while increasing pH values resulted in considerable regression of anthocyanin yields. Since anthocyanins represent the most abundant phenolic constituents of

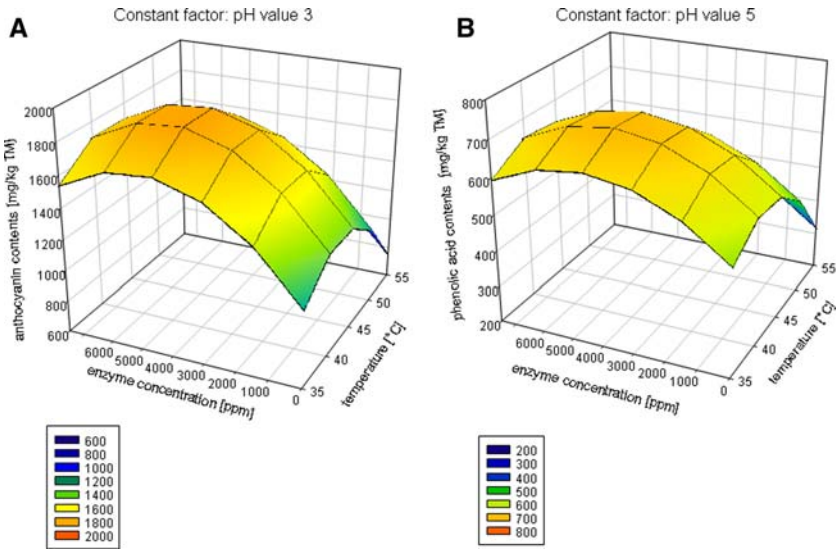


Fig. 3 Response surface plots of anthocyanin (**a**) and phenolic acid (**b**) contents in grape pomace extracts depending on the enzyme concentration and temperature [°C] (mg/kg based on dry matter)

grape pomace, any deviation from optimal extraction conditions resulted in lowered yields, which is of particular importance since a previous study had shown that anthocyanin yields employing enzymatic extraction were rather poor [24].

Optimization of enzyme-assisted extraction on the recovery of selected phenolic acids (DOD 2) The second series of experiments aimed at optimizing the recoveries of the hydroxycinnamoyl tartrates, caftaric, coutaric, and fertaric acids, as the most prominent compounds among the phenolic acids in grape pomace. After backward elimination of insignificant terms Eq. 3 was obtained (R^2 = 0.80):

$$\hat{y} = -2{,}684.2980 + 429.3968a + 111.1016b + 0.0143ac - 4{,}848.9121a^2 - 1.3315b^2 - 0.00001c^2. \quad (3)$$

The linear, quadratic, and cross-product terms in the second-order polynomial function were used to generate response surface plots with one constant factor. As described for DOD 1, the effects of pH value (a), enzyme dosage (b), and temperature (c) on the yields of the three phenolic acids ($\hat{y}$) were assessed. In contrast to DOD 1, only pH value and enzyme dosage (p = 0.0124 and 0.0145, respectively) were significant linear variables influencing the extraction yield. Variation of the pH value had the most prominent effect on the response, particularly the quadratic term (p = 0.0096) significantly affected extraction yields. As opposed to the results of DOD 1, the impact of enzyme dosage on the recovery of phenolic acids (Fig. 3b) was less pronounced. The highest extraction yield was obtained at an enzyme dosage of c = 4,500 mg/kg. The temperature showed a significant effect only as an interaction term with the pH value. Maximum yield was observed at T = 40 °C and pH 5, similar to the conditions determined in DOD 1 (Fig. 4b). Thus, the optimal conditions of the enzyme-assisted extraction of anthocyanins and phenolic acids differed only in the pH values. Although the pH value was the most decisive factor for phenolic acid extraction with an optimum around pH 5, all further experiments on laboratory and pilot-plant scale were performed at pH 4 as a compromise between the respective optimum for anthocyanin and phenolic acid extraction.

Laboratory scale extraction experiments Based on the optimum conditions determined with DOD 1 and DOD 2, enzyme-assisted extractions on laboratory scale were performed at pH 4.0 and T = 40 °C, applying an enzyme dosage of c = 4,500 mg/kg of the pectinolytic and cellulolytic enzyme preparations. Yields are given relative to the contents of individual compounds in a 'Lemberger' pomace, which were determined in a pomace screening based on a method published by Kammerer et al. [25]. The quantification of individual compounds by HPLC was performed with the extracts obtained after a 2-h enzymatic digestion of the comminuted grape pomace. Extraction yields for anthocyanins and phenolic acids were 16.0% (Fig. 5a) and 40.9% (Fig. 5b), respectively. Aqueous pre-extraction of the pomace prior to enzymatic treatment significantly improved extraction yields. Total contents of phenolics in this two-stage extraction process amounted to 27.1% (anthocyanins) and 74.1% (phenolic acids; Fig. 5). Anthocyanin recovery was still comparatively low, even after the pre-extraction step. However, compared with a former study using 'Lemberger' pomace [24], anthocyanin recovery was increased threefold (from 8.6 to 27.1%) by the systematic optimization of the extraction parameters using DOD.

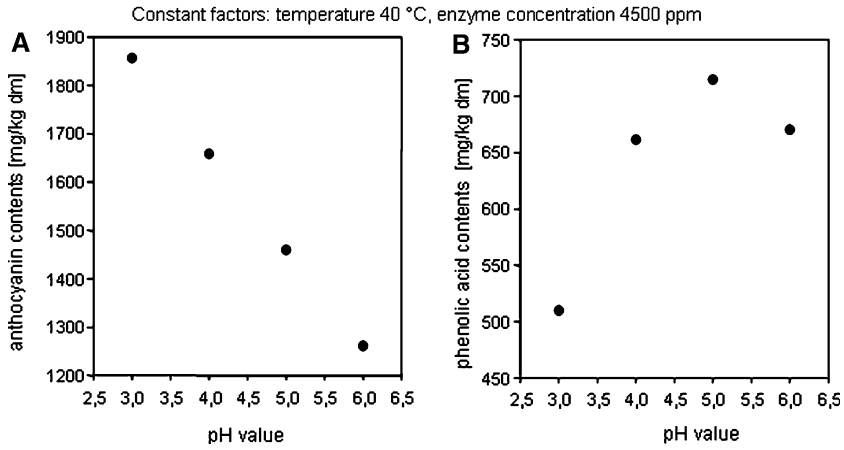


Fig. 4 Anthocyanin (**a**) and phenolic acid content (**b**) of the first and second DOD in subject to different pH values. Experiments were performed at $T = 40$ °C and pectinolytic and cellulolytic enzymes were added at a dosage of $c = 3{,}000$ and 1,500 mg/kg, respectively

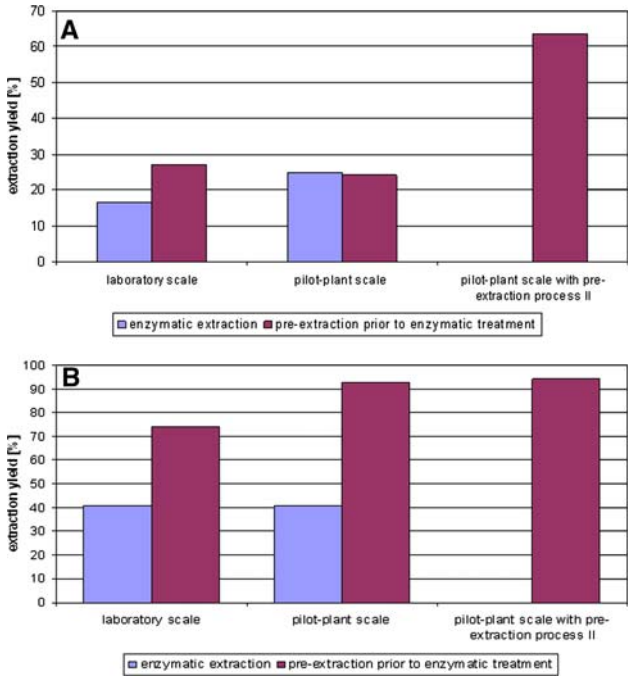


Fig. 5 Yields of anthocyanins (**a**) and phenolic acids (**b**) of laboratory and pilot plant-scale experiments (based on DOD 1 and 2)

Scale-up experiments

Based on the results of the laboratory scale experiments, grape pomace liquefaction and extraction were carried out on pilot-plant scale applying the above-mentioned conditions. Furthermore, a second pilot-plant experiment was performed using a modified pre-extraction step. The main modification of this step consisted in the inclusion of a pasteurization step of the mash immediately after grinding, in combination with the application of the colloid mill and the rack and cloth press, which seemed to be a very feasible means to support the extraction of polyphenols from grape pomace. Since the above-mentioned pasteurization affected the extraction yield of the pigments, anthocyanins were determined after the heat-treatment (data not shown). However, significant pigments losses were not observed. The yields of phenolic acids and anthocyanins obtained in the different experiments applied on laboratory and pilot-plant scale are shown in Fig. 5a and b. As can be seen from Fig. 5, the pre-extraction step I (without pasteurization after grinding) increased extraction yields of phenolic acids from 74.1% (laboratory-scale) to 92.8%. Without the pre-extraction step, no difference between laboratory and pilot-plant scale was observed in terms of phenolic acid extraction, which can be explained by the well-known enzyme inhibitory effect of some phenolics. As mentioned previously, recovery of anthocyanins on laboratory scale was poor (below 27%), and similar results were obtained on pilot-plant scale (24.7%). Our preliminary investigations have shown that the low recovery of anthocyanins may be ascribed to high losses occurring during the first mincing step, which may be due to the endogenous enzymes, such as polyphenoloxidases and peroxidases. Consequently, thermal enzyme inactivation of the grape pomace immediately after mincing should result in significantly improved extraction yields after enzyme-assisted liquefaction. Accordingly, recoveries increased to 91.9% (phenolic acids), 92.4% (non-anthocyanin flavonoids) and 63.6% (anthocyanins) after enzymatic hydrolysis of grape skin cell wall polysaccharides for 120 min. It is assumed that native enzymes of the grapes were not completely inactivated by the high-temperature short-time treatment of the mash during vinification. Therefore, increased extraction yields are obviously due to the immediate inactivation of deteriorating enzymes. The contents of individual phenolic compounds recovered from extraction including pre-extraction step II are summarized in Fig. 6. The phenolic composition of the press residue obtained after this two-stage extraction process was determined based on a method published by Kammerer et al. [25]. Polyphenol contents of

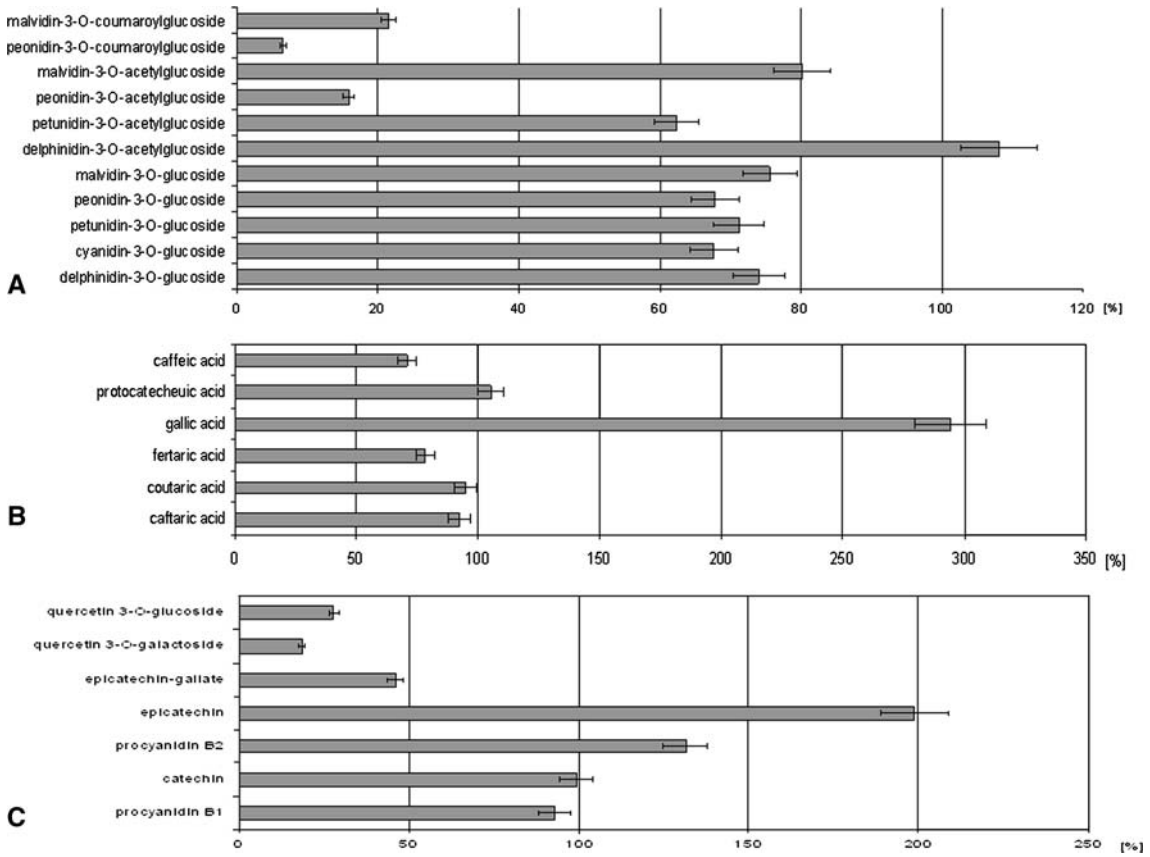


Fig. 6 Extraction yields [%] of individual anthocyanins (**a**), phenolic acids (**b**), non-anthocyanin flavonoids (**c**), using enzymatic extraction combined with pre-extraction step II on pilot-plant scale

the press residue amounted to 7.8% (phenolic acids), 9.2% (non-anthocyanin flavonoids) and 19.9% (anthocyanins). As can be seen from Fig. 6c, catechin was completely extracted. HPLC analysis revealed recovery rates of 93 and 212% for procyanidin B1 and B2.

Since grape pomace contains matrix bound procyanidins, the high amounts of procyanidin B2 might result from enzymatic hydrolysis of these compounds during liquefaction. Epicatechin was recovered in high yields (198%), which is in accordance with the lower amounts of epicatechin gallate and the considerably higher yield of gallic acid (Fig. 6b). Therefore, it is concluded that, among other compounds, epicatechin gallate was hydrolyzed by an esterase side activity of the technical enzyme preparations, resulting in the release of epicatechin and gallic acid. These findings are in accordance with a previous study [24] revealing Novoferm 106 to contain an esterase side activity. Furthermore, the low extraction yields of quercetin 3-*O*-galactoside and quercetin-3-*O*-glucoside can be attributed to a partial degradation caused by the presence of a flavonoid glycosidase side activity in the enzyme preparations [8]. Since the quercetin aglycone levels did not correspond to the losses of quercetin glycosides, further degradation reactions may be assumed. For other compounds, such as delphinidin-3-*O*-acetylglucoside, extraction yields exceeding 100% were observed. However, these were present in the native pomace samples in very low amounts, and even small increments after extraction resulted in a comparatively high relative increase.

Spray drying

The extracts were spray dried to protect phenolic compounds from oxidative and hydrolytic degradation and to obtain a free-flowing powder which can be used as a food additive, e.g., for coloring purposes. The spray drying experiments were performed using the extract obtained after enzyme-assisted liquefaction and addition of starch as the carrier material. The spray drying process resulted in a storable powder of an intensely red color, which showed excellent rehydratization properties when dissolved in water. The total contents of phenolics in the spray dried powder were 1,506.6 mg/kg of anthocyanins, 158.2 mg/kg of phenolic acids, and 408.8 mg/kg of non-anthocyanin flavonoids, respectively. Since this powder is free from

added sulfite, pseudoallergic reactions caused by sulfite residues can be excluded.

Conclusions

An enzyme-assisted process for the liquefaction of grape pomace was developed on laboratory and pilot-plant scale. Due to its considerably lower enzyme dosage compared to our previous study and the high extraction yields, the economic feasibility of the process was significantly enhanced. Additionally, this process may serve as an alternative to conventional pigment extraction procedures applying sulfite. The extraction procedure described in this study was optimized using grape pomace originating from a high-temperature short-time process. It is expected that enzyme-assisted extraction can also be applied to recover phenolics from by-products of traditional fermentation and maceration processes. Further studies will be directed towards the application of the spray dried products as food supplements with additional benefit, in particular their incorporation in various matrices.

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