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Effects of enzymatic treatment on anthocyanic pigments from grapes skin from chilean wine

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Abstract

The preparation of pectic enzymes are used for a more efficient extraction of desirable red grape pigments and other compounds which are bound in plant cells and can be faster released by the action of pectic enzymes; shorten the time of maceration, setting, and filtration. The main objective of the present study was to investigate the enzymatic extraction of anthocyanic pigments from the residue remaining after the vinification process of three varieties of *Vitis vinifera* from Central Chile. The best results of extraction of anthocyanins can be obtained with Vinozym EC using skin grape Ribier after 2 h of treatment.

Keywords: Grape pomace; Enzymatic treatment; Anthocyanins

1. Introduction

Anthocyanins are a widespread source of natural pigments in foods. They are responsible for the red, blue, and purple colors in many fruits and berries and food products derived from them (Eiro & Heinonen, 2002). However, their use as an added color to food additives and drinks has been limited, due to a number of drawbacks; such as sensitivity to bleaching by sulfur dioxide and limited coloring capability at pH values over 3.5. Overcoming such problems would greatly enhance the possibility of using anthocyanins as food additives, especially since there is a current interest in natural colors with potential added health benefits (Bridle & Timberlake, 1997; Kotamballi, Chidambara, Ravendra, & Gudd Adaram Garvanahally, 2002). As grape and red wine phenolics were found inhibit the oxidation of human low-density lipoproteins (Frankel, Waterhouse, & Teissedre, 1995), a large number of investigations on the recovery of phenolic compounds from grape pomace has been published (Schieber, Kammerer, & Carle, 2002).

Grape is the single most abundant fruit harvested in the world from, which a natural dye is commercially obtained. Grapes are highly pigmented with anthocyanin, a pool of colorants responsible for the purple, violet, blue red and orange color. The traditional and most common means of anthocyanin extraction involves maceration or soaking of plant material in a low boiling point alcohol containing a small amount of mineral acid (e.g., $\leqslant 1\%$ HCL) acidified ethanol is most often used in food sources, though it is a less effective extractant and more difficult to concentrate due to its higher boiling point.

Established grape skin extraction methods involve maceration or soaking in a low boiling point alcohol (e.g., ethanol) containing a small amount of mineral acid (e.g., 1% hydrochloric acid or sulfurous acid, Harborne, 1973), thus maintaining a low pH to keep the anthocyanins in their stable flavylium form. The extract is made up of various anthocyanins, sugars, acids and salts. It is usually concentrated to a viscous thick liquid of about 20–30° Brix, containing about 0.5–1.5% anthocyanins content. However, the use of hydrochloric acid may alter the native form of the anthocyanin: when extracts are concentrated by vacuum rotary evaporation (40 °C), hydrolysis of the anthocyanin may take place (Van

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Sumere, Van de Casteele, De Loose, & Heursel, 1985); furthermore, deacylation of anthocyanins acylated with acids can occur, even at room temperature (Harborne, 1986). Therefore, in order to obtain anthocyanins closer to theirs natural state, several solvent have been suggested for the initial pigment extraction (Garcìa-Viguera, Zaprilla, & Tomas-Barberan, 1998) such as acetone, or acetone: sulfur dioxide, (Timberlake & Bridle, 1971) tetrahydrofuran, alcohol (Lea, 1988) and others (Jackman & Smith, 1996). However, a choice of an adequate method for extracting anthocyanins will depend of the purpose of the extraction and also on the nature of the anthocyanins. It is important to ensure that the extraction and clean-up procedure is not to complex, time-consuming or costly (Timberlake & Bridle, 1980).

The preparation of pectin enzymes are used for more efficient extraction of desirable red grape pigments and other phenol compounds, which are bound in plant cells and can be faster released by the action of pectic enzymes. Moreover, the short time of maceration, settling, and filtration. The released of red grape pigments and aroma compounds can thus be quicker. (Capounova & Drdak, 2002; Meyer, Jepsen, & ol Sorensen, 1998; Schieber, Stintzing, & Carle, 2001).

In this paper we describe quick, clean, economic and efficient methods for obtaining anthocyanins-rich extracts using active pectolitic enzyme preparation in water aqueous medium.

2. Materials and methods

2.1. Sample preparation

The samples used in this work was obtained from commercial concentrate juice (Ribier) and crushed grapes (Cabernet Sauvignon of Central Valley of Chile) recovered after fermentation. For each treatment and variety, replicate trials were used. Table 1 shows the results of the analysis performed of the raw materials used

2.2. Enzymatic treatment

Commercial pectic enzyme preparations were used in the experiments. The preparation were obtained from Novo Nordisk to unclarified juice that was obtained from skin of red grade (*V. vinifera*). The enzymatic complexes used were Pectinex BE3-L (pectinesterase, pectinlyase, polygalacturonase, hemicellulase and cellulase from *Aspergillus niger*), Vinozym EC (pectinase and cellulase of *Aspergillus niger* and *Trichoderma longibrachiatum*) and Vinozym G (pectinlyase, polygalacturonase, hemicellulase and cellulase of *Aspergillus niger*).

The effect of concentration on the color density was determined using 1% solutions of three commercial enzymatic complexes. 10, 20 and 30 mL were dosed according to treatment. The experiments were carried out at 37 °C for 6 h, using the ratio skin:water = 1:5. Samples taken after 1 h were centrifuged and filtered before reading in the spectrophotometer. The color density (Mazza, Fukumoto, Delaquis, Girard, & Ewert, 1997; Bordeu & Scarpa, 2000) was calculated as:

$$\text{Color density} = (A_{520\,nm} - A_{700\,nm}) + (A_{420\,nm} - A_{700\,nm})$$

2.3. Extraction and fractionation

Grape skins (skins and seeds) of red grape (*V. vinifera*) of each varieties (4 kg) were submitted extraction with water and enzyme solution (1%) (20 L) and keep at 37–40 °C for 6 h with stirring. After this, the liquid was decanted, crushed and filtered (extract 1) and discarding the residue 1. The extract 1 (20, 5 L; 0, 6° Brix) was used for the obtention of fresh skin extract number two, after 6 h at 37 °C, it was pressed to obtain extract number two. A fresh charge of enzyme was use for obtain extract number 3, removing extract skin 2, gathering these two fraction, signed as extracts 2 and 3, respectively; both were clarified with pectolytic enzymes (Ultrazym 100) and concentrate at reduced pressure until 25° Brix, at 35 °C (Fig. 1).

2.4. Statistical analysis

A factorial design of 3×3 thoroughly randomized with four repetitions was used. The results were analyzed with ANOVA and the differences by the Duncan's Test of multiple comparisons. General linear models procedure were used to the determine treatment effects.

3. Results

3.1. Dependence of color density on enzyme concentration

As can be observed in Table 2, after 6 h of extraction, a greater color index is obtained at the highest concen-

Table 1
Characterization of the raw materials

Variety	Moisture (g/100 g)	Total solids (g/100 g)	Soluble solids (°Brix)	Seeds (g/100 g)
Cabernet Sauvignon	67.03	32.77	0.10	21
Carménère	72.33	27.67	0.20	17
Ribier	48.42	51.58	0.15	65

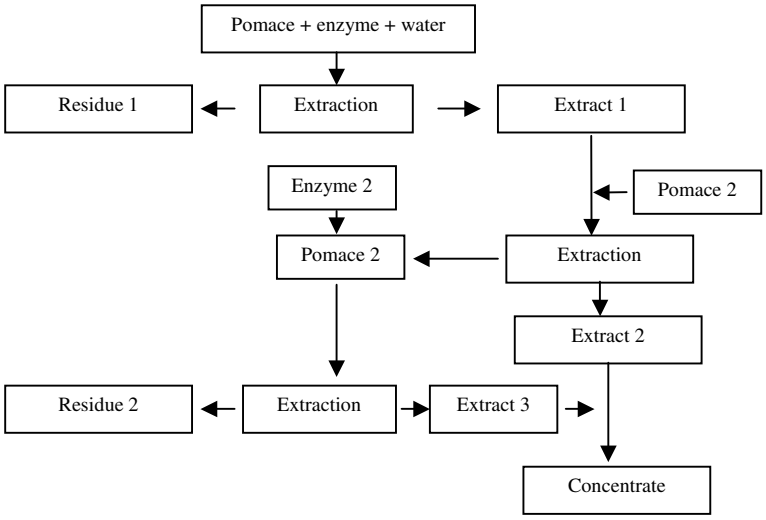

Fig. 1. Extraction and fractionation methods.

Table 2
Color index by variety and enzyme, after 6 h of extraction

Variety	Pectinex			Vinozym EC			Vinozym G			Water
	10 mL	20 mL	30 mL	10 mL	20 mL	30 mL	10 mL	20 mL	30 mL	
Cabernet Sauvignon	806	813	1039	743	1011	1354	846	961	1365	621
Ribier	1137	1346	1447	1260	1625	1684	1258	1347	1431	715
Carménère	1080	1396	1642	1334	1441	1775	1150	1333	1701	458

tration (greater dosing) ($p < 0.05$). In addition, it is possible to appreciate that the varieties Ribier and Carménère are those that give better values for color indexes, showing meaningful differences with respect to Cabernet Sauvignon. To understand better the quality of the method a control with water and without enzyme was included in all experiments and data.

Fig. 2 shows variation of color density values for Carménère vs. the extraction time. It can be observed that initially there are no meaningful differences with respect to the type of enzyme used, however after 3 h, Vinozym G is more effective than Pectinex and Vinozym EC, even though they were used at equal dose. However, after 6 h of extraction Vinozym EC reaches the highest color index value (1775, $p > 0.05$) obtained in the nine treatments.

With respect to Ribier, results obtained with Vinozym EC are significantly different to the other two enzymes ($p < 0.05$), displaying the highest values during all the processes (Fig. 2). Thus, the maximum color indexes after 6 h were 1431, 1447 and 1684 using Vinozym G, Pectinex and Vinozym EC, respectively.

Therefore, a comparison of Figs. 2 and 3 show the best results of extraction of anthocyanins can be obtained with Vinozym EC using skin grapes Ribier after 2 h of treatment.

The use of pectolytic enzymes Vinozym EC gives high yields of anthocyans from the skin grapes residues obtained after the elaboration of red wines from grapes cultivated in Chile. These anthocyanic extracts could have wide applicability as natural pigments in foods and

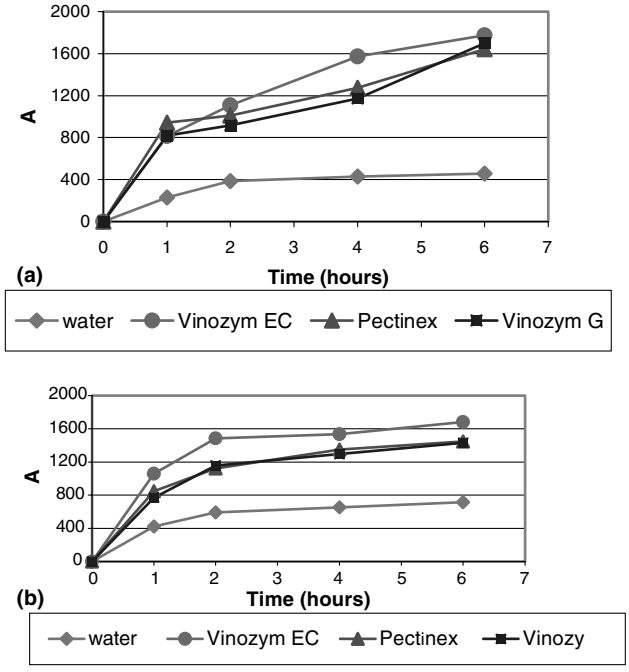

Fig. 2. (a) Color density variety Carménère, with 30 mL of enzymatic solution. (b) Color density variety Ribier, with 30 mL of enzymatic solution.

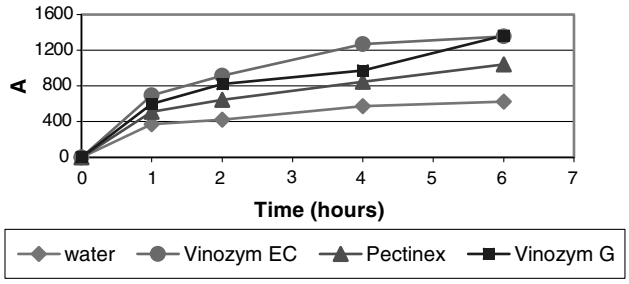

Fig. 3. Color density variety Cabernet Sauvignon, with 30 mL of enzymatic solution.

beverages. Additional advantages are their "biodegradability" and their efficient yield from industrial wastes.

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