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Changes in polyphenol and polysaccharide content of grape seed extract and grape pomace after enzymatic treatment

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ABSTRACT

Grape seed extract and grape pomace are rich sources of polyphenols. The aim of this study was to evaluate the release of polyphenols, the solubilisation of carbohydrate, and the antioxidant capacity of these grape by-products after enzymatic reaction with carbohydrases (cellulolytic and pectinolytic activities) and tannase for 24 h. The use of tannase in these by-products, and pectinase in grape pomace changed the galloylated form of catechin to its free form, releasing gallic acid and increasing the antioxidant activity. In grape pomace, cellulase treatment was not efficient for phenolic release and antioxidant activity improvement. The addition of carbohydrases to grape pomace, either alone or in combination, degraded the cell wall polysaccharides, increasing the content of monosaccharides. These results provide relevant data about the potential of pectinase, tannase and combinations of enzymes on the release of polyphenols and monosaccharides from grape by-products, improving the antioxidant capacity and the nutritional value.

1. Introduction

Over the past few years, by-products of wine and grape juice processing have attracted considerable attention as a potential source of bioactive phenolic compounds, which have antioxidant properties and could be used in the pharmaceutical, cosmetic and food industries (Gonzalez-Paramás, Esteban-Ruano, Santos-Buelga, Pascual-Teresa, & Rivas-Gonzalo, 2004). After grapes are pressed and the juice is collected, the remaining material containing seeds, skins and stems is known as pomace. Grape seeds can be separated, extracted, and purified into grape seed extract (GSE). Catechins and their isomers and polymers are the main components in the seed. These phenolics have been reported to be linked to cell-wall polysaccharides. The grape pomace (GP) cell wall is a complex network composed of 30% of neutral polysaccharides (cellulose, xyloglucan, arabinan, galactan, xylan and mannan), 20% of acidic pectin substances, 15% of insoluble proanthocyanidins, lignin and structural proteins and phenols, these two latter cross-linked to the lignin–carbohydrate framework (Pinelo, Arnous, & Meyer, 2006). Cell wall polysaccharides contain hydrogen groups as well as aromatic and glycosidic oxygen atoms that have the ability to form hydrogen bonds and hydrophobic interactions with polyphenols (Le Bourvellec, Guyot, & Renard, 2004).

The growing interest in the exploitation of these bioactive compounds, especially flavonoids and phenolic acids from processed plant residues, has encouraged research on the application of cell-wall-hydrolysing enzymes to various wine by-products (Kammerer, Claus, Schieber, & Carle, 2005; Meyer, Jepsen, & Sorensen, 1998). The use of these enzymes, such as pectinase, cellulase, hemicellulase and glucanase, has been introduced to release cell wall complex polyphenols, facilitating the release of certain nutrients entrapped by the cell wall structure (Landbo & Meyer, 2004; Maier, Göppert, Kammerer, Schieber, & Carle, 2008). Enhancement of polyphenol bio-activities by enzyme reactions has also been reported by different authors (Kapasakalidis, Rastall, & Gordon, 2009; Zheng, Hwang, & Chung, 2009).

Another enzyme implicated in the release of polyphenols is tannase. Tannase or tannin acyl hydrolase (EC, 3.1.1.20) catalyses the hydrolysis of the ester and depside bonds present in hydrolysable tannins or gallic esters, such as epigallocatechin *O*-gallate (EGCG) or epicatechin *O*-gallate (ECG), releasing gallic acid (GA) or glucose (Lekha & Lonsane, 1997). Tannase is an enzyme produced by various filamentous fungi, mainly *Aspergillus* and *Penicillium* in the presence of tannic acid. The major commercial applications of tannase are the elaboration of instant tea and the production of GA. Tannase application in food and beverages (beer and wines) might contribute to the removal of the undesirable effects (turbidity) of tannins (Belmares, Contreras-Esquivel, Rodriguez-Herrera, Ramirez, & Aguilar, 2004). The release of GA by the action of tannase on galloylated tannins would be beneficial, since this compound is supposed to have great antioxidant power (Netzel, Shahrzad, Winter, & Bitsch, 2000).

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Studies on the effect of enzymatic treatment on grape polyphenols are of significant importance for the recovery of phenolics from grape by-products, in order to obtain an extract that may be used as a food supplement or as a novel functional ingredient. The hydrolysis of the complex polysaccharides and polyphenols into more simple sugars and phenols might increase the amount of bioactive substances available. Monomeric and some oligomeric polyphenols have been found to be absorbed (Shoji et al., 2006), while polymeric forms are poorly absorbed (Donovan et al., 2002; Gonthier et al., 2003). Thus, it is important to evaluate the effect of enzymatic treatment of grape by-products on the structure of phenolic compounds and on the antioxidant activity.

To our knowledge there has been little or no study on the effect of carbohydrases or tannase addition on the polyphenolic components and on cell wall carbohydrate degradation of grape seed by-products. The purpose of this work was to examine the release of phenolic compounds and the cell wall carbohydrate degradation of GP after treatment with tannase, cellulase and pectinase (individually or in combination) and its relationship with the antioxidant capacity. The release of phenolic compounds after the treatment of GSE with tannase was also evaluated.

2. Materials and methods

2.1. Materials

Grape pomace was obtained from Alcoholeras Reunidas, SA (Argamasilla de Alba, Ciudad Real, Spain). Grape powdered seed extract (GSE) was purchased from NOR-FEED Sud (Angers, France) and it was obtained with water extraction and spray dried. The pomace, consisting of stems, skins and seeds from red grapes, was dried in a convection oven at 60 °C and semi-finely pulverised using a milling machine (passing through a 1-mm mesh sieve).

2.2. Solvents and reagents

All solvents used for HPLC analysis were of liquid chromatography grade and were obtained from Sigma–Aldrich (St. Louis, MO), and all water was ultrapure. Catechin kit containing catechin (C), epicatechin (EC), epicatechin *O*-gallate (ECG), epigallocatechin (EGC) and epigallocatechin *O*-gallate (EGCG) was purchased from Extrasynthèse (Genay, France). Gallic acid (GA), gallocatechin (GC), gallocatechin *O*-gallate (GCG), procyanidin dimers B1 (PB1) and B2 (PB2), D-arabinose, and D-xylose were purchased from Sigma–Aldrich. D-Glucose, L-rhamnose and D-galactose were purchased from Merck, Steinheim, Germany. Acetone and methanol were obtained from Panreac (Castellar del Vallés, Barcelona, Spain).

2.3. Enzymes

Three different types of enzymes were selected on the basis of the structural composition of grape seeds. Pektozyme® (135 U/g; E.C. 4.2.2.10) is a pectolytic enzyme complex produced by fermentation with a selected strain of *Aspergillus niger* and Laminex® (3150 CMC-DNS U/g; E.C. 3.2.1.4) is a food enzyme complex hydrolysing β-glucans, pentosans and related carbohydrates produced by fermentation with a selected strain of *Penicillium funiculosum*. The enzymes were supplied by Danisco A/S (Denmark). Tannase (200 U/mg, E.C. 3.1.1.20.) from *Aspergillus ficum* was purchased from Sigma–Aldrich.

2.4. Enzymatic treatment

The selected hydrolysis conditions (pH 5.5 and 35 °C) were based on evaluations of temperature and pH activity curves for the enzymes, as given on the enzyme suppliers' data sheets, and in the work of Thomas and Murtagh (1985). Enzymatic hydrolysis of the samples was carried out in a thermostatically controlled shaking water bath with gentle agitation. To investigate the enzymatic release of polyphenols and monosaccharides of GP, one gram of sample was incubated with 6.75 and 13.5 U equivalent activity of Pektozyme® (pectinase) and 157.5 and 315 U of equivalent activity of Laminex® in a final volume of 10 mL 0.1 M Na acetate buffer (pH 5.5) for 24 h under agitation (35 °C, 100 rpm). Grape seed extract (0.250 g) and GP (1 g) were incubated with 500 and 1000 U equivalent tannase in a final volume of 10 mL of 0.1 M Na acetate buffer (pH 5.5) for 24 h under agitation (35 °C, 100 rpm). Combinations of Laminex®, Pektozyme® and tannase were also used in GP at similar concentrations. After 24 h of enzymatic hydrolysis, the samples were centrifuged (3500 rpm, 10 min), and the resulting supernatants were collected, filtered through a Technokroma filter (0.45 μm) and used to analyse the polyphenolic content and the monosaccharide contents.

For the GP samples, the residue remaining after the enzymatic hydrolysis was collected and subjected to a subsequent extraction by adding ten millilitres of acetone/water (70:30 v/v), shaking and repeating the centrifugation. This supernatant was collected, filtered through a 0.45-μm filter and used to analyse the polyphenolic content and the antioxidant activity. All supernatants were stored at −18 °C until analysed.

2.5. Analytical methods

2.5.1. Total polyphenols

Total polyphenols were determined in supernatants obtained after enzymatic treatments by Folin–Ciocalteau procedure (Montreau, 1972), using gallic acid as standard. A mixture of 0.5 mL of extract, 0.5 mL of Folin–Ciocalteau reagent and 10 mL of Na_2CO_3 1 M were introduced in a 25 mL volumetric flask. After reacting for 1 h, absorbance was measured at 750 nm using an ultraviolet–visible spectrophotometer (Hitachi U-2000; Hitachi, Ltd., Tokyo, Japan). The results were expressed as g of gallic acid equivalents per 100 g of GP and GSE dry matter (DM).

2.5.2. Polyphenolic content analysis

Analyses were performed using an Agilent 1100 series HPLC, comprising a quaternary pump with integrated degasser, autosampler, thermostated column compartment and diode array detector (DAD), coupled with an Agilent G1946D quadrupole mass spectrometer (Agilent Technologies, Waldbronn, Germany). Ten microlitres of filtered samples were separated on a Gemini C18 5 μm, 250 mm × 4.6 mm i.d. column, (Phenomenex, Torrance, CA), eluted with a mobile phase made up of a mixture of deionised water (solvent **A**) and acetonitrile (solvent **B**), both containing 0.1% formic acid, at a flow rate of 1 mL/min. The solvent gradient changed according to the following conditions: from 90% **A** to 70% in 30 min, to 65% in 5 min, to 55% in 5 min, and then back to initial conditions in 10 min.

Ionisation was achieved by atmospheric pressure electrospray ionisation (ESI) source, operated in negative ion mode, with the electrospray capillary voltage set to 3500 V, fragmentor 150 V, a nebulising gas flow rate of 12 L/min at 50 psi, and a drying temperature of 350 °C. Selected ion monitoring (SIM) was used for quantification with each standard: *m/z* 169 $[M-H]^-$ for GA, *m/z* 289 for C and EC, *m/z* 305 $[M-H]^-$ for GC and EGC, *m/z* 441 for ECG, *m/z* 457 for EGCG and GCG, and *m/z* 577 $[M-H]$ $[M-H]^-$ for PB1 and PB2. Data acquisition and analysis were carried out with Agilent ChemStation Software. Phenolics yields were expressed as mg per 100 g of GP and GSE dry matter.

2.5.3. Antioxidant assay procedure

The DPPH (2,2-diphenyl-1-picrylhydrazyl; Sigma–Aldrich) assay measuring the radical-scavenging activity of GSE and GP