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Extraction of polyphenols from grape seeds and concentration by ultrafiltration

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Abstract

A solvent extraction method utilizing 50% ethanol and 50% water as solvent was used for the extraction of polyphenols from grape seeds. An additional ultrafiltlration step was also included to determine its beneficial effect. Various experimental conditions, such as solid to liquid ratio (0.1–0.25 g/ml), number of extraction stages (single, double and triple) and membrane pore size (0.22 and 0.45 μm) were investigated to optimize the extraction. When compared to a gallic acid standard, the extraction of grape seed polyphenols with a 0.2 g/ml solid to liquid ratio, double stage extraction and 0.22 μm membrane pore size were the optimal conditions. Under these conditions, the maximum amounts of polyphenols (11.4% of the total seeds weight) were recovered from grape seeds. From the standpoint of solvent toxicity, extraction efficiency and percentage of grape seed polyphenols recovered, this method proved to be adequate on all three counts.

Keywords: Polyphenols; Ultrafiltration; Solvent extraction; Grape seed; Neutraceutical

1. Introduction

The importance of polyphenols is due to their positive contribution to cellular processes within the body. In terms of pharmacological activity, they protect against the oxidation of high-density lipids (HDLs) [1]. Hence, they help the body to retain important HDLs while helping to remove problematic low-density lipids (LDLs). Additionally, polyphenols also have anti-ulcer [2], anti-carcinogenic [3] and anti-mutagenic activities [4]. The reason for these activities is the strong antioxidant nature of polyphenols, which is based on their ability to absorb free radicals. Polyphenols are broadly distributed in the plant kingdom and are the most abundant secondary metabolites found in plants [5]. These polyphenolic substances or polyphenols include many classes of compounds ranging from phenolic acids, colored anthocyanins, simple flavonoids and complex flavonoids. Polyphenols represent the third most abundant constituent in grapes and wines after carbohydrates and fruit acids [6]. The most common polyphenolic acids in grapes include cinnamic acids (coumaric, caffeic, ferulic, chlorogenic and neochlorogenic acids) and flavonoids include colorless flavan-3-ol (such as catechin, epicatechin, their polymers and their ester forms with galactic acid or glucose) [5] (Fig. 1).

Oligomeric proanthocyanidins (OPCs) are a class of polyphenolic biflavanoids that are found in fruits and vegetables. The highest concentration of these is in the seeds of grapes. OPCs are made up of two or more monomers, which are chemically bonded. For example, dimers are two monomers, trimers are three monomers and tetramers are four monomers bonded together. The dimeric procyanidins are often referred to as the B-series and the trimeric procyanidins as the C-series. Five different dimers (procyanidin B1, B2, B3, B4 and B5) and two trimers (C1 and C2) were identified from grape skin and seeds [7]. Catechin and epicatechin are the two proanthocyanidin monomers. Dimers, trimers, tetramers, etc. are created when each of these two monomers bind at the α or β position on their molecular structures. Additionally, catechin and epicatechin can combine to create esters, such as catechin/epicatechin gallate. They can also bond with sugars and proteins to create glycosides and peptides. About 162 dimers, such as gallic acid

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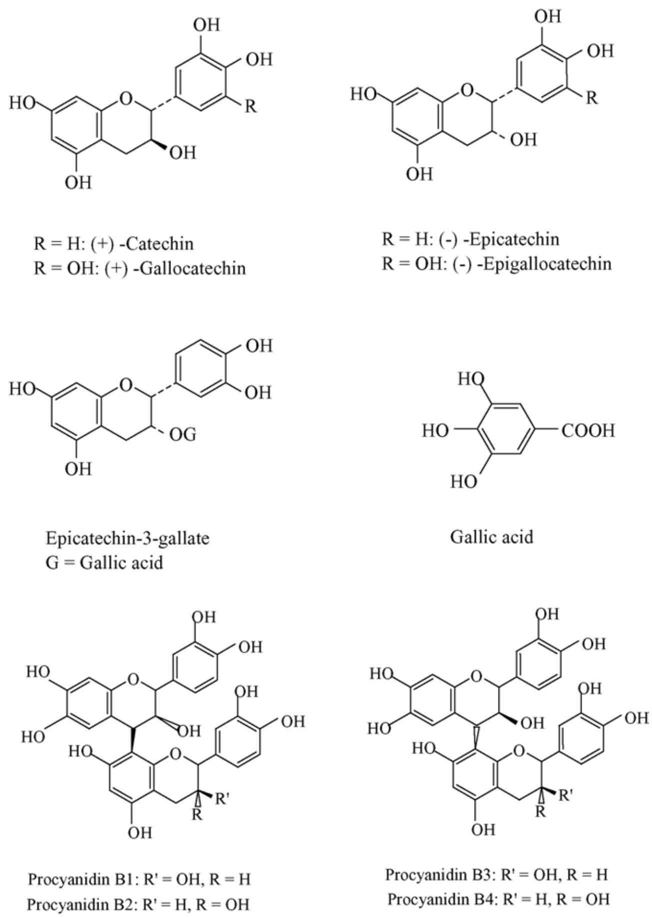

Fig. 1. Structures of major polyphenols identified in grape seed extract [5].

and glucose esters, can be created [8]. The polyphenolic compounds are broadly distributed inside grapes. The composition of polyphenols depends upon whether the extraction is performed on whole grape pulp, skin or seeds. The percentage of the total extractable polyphenols in grape tissues are: 10% or less in the pulp, 60–70% in the seeds and 28–35% in the skin. The polyphenol content of seeds may range from 5 to 8 wt% [9].

The extraction of polyphenols is dependant upon two actions, the dissolution of each polyphenolic compound at the cellular level in the plant material matrix, and their diffusion in the external solvent medium [10]. Previously, all extractions were performed with organic solvents. These extraction procedures were efficient, but the extracts were not safe for human consumption due to potential toxic effects from the residual solvent. At the present time, there is no reported grape seed extraction procedure utilizing ethanol as the sole solvent. In the past, solvents, such as hexane and methanol combinations [11], ethanol–benzene combinations [12], ethyl acetate [13] and sulfur dioxide have been used [14]. All these solvents are toxic to humans if consumed in large doses. These health concerns have sparked research into methods that would reduce the use of organic solvents in the extraction procedure. Membrane processing is one method

Table 1
Molecular weight of various polyphenols [15]

Polyphenol type	Molecular weight (MW)
Catechin	290.3
Epi-catechin	290.3
Epi-catechin gallate	442.4
Procyanidin dimer	578.5
Procyanidin trimer	870
Procyanidin tetramer	1160
Kaempferol	286.2
Gallic acid	170.1
Quercetin	448.4
Caffeic acid	180.2
Coumaric acid	164.2
Chlorogenic acid	354.3

that reduces the use of toxic organic solvents and concentrates the final product. Solvents are still used with this method, but not to the same extent as with organic solvent extraction. Membranes are used towards the end of the process, when the polyphenols are to be separated and concentrated from the solvent–solute complex.

Ultrafiltration (UF) is the most commonly used procedure to separate desirable components from a mixture. UF is dependent on the particle size, and typical rejected species include sugars, bio-molecules, polymers and colloidal particles. The driving force for transport across the membrane is the pressure differential. UF operate at 2–10 bar, although in some cases up to 25–30 bar have been used. UF performs feed clarification, concentration of rejected solutes and fractionation of solutes. UF membranes can reject molecules within the molecular weight ranges of more than 1000 MW, a reason why they are successful in separating polyphenols [15]. The molecular weight of various polyphenols are in Table 1.

There are numerous advantages associated with membrane separation processes. Compared to mechanical separations, membrane separation involves high purity, low energy requirement, no additive, mild operating conditions, greater separation efficiency and easy scaling up. UF has been used in the past for the removal of polyphenols from grape musts or wines [16]. Therefore, the objective of this study was to extract polyphenols from grape seeds using an ethanol–water mixture, optimize the extraction procedure and then concentrate the polyphenols by UF.

2. Materials and methods

2.1. Plant materials and chemicals

Whole grape seeds were collected from Joseph's Estate Wines (Niagara, Ont., Canada) and milled to a fine powder. Ethanol was of analytical reagent grade (Commercial Alcohols Inc., Brampton, Canada). Folin–Ciocalteau reagent, sodium bicarbonate powder and gallic acid (Sigma Chemical, St. Louis, USA) were used for the analysis of total polyphenols.