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Chemical investigation of commercial grape seed derived products to assess quality and detect adulteration

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ABSTRACT

Fundamental concerns in quality control arise due to increasing use of grape seed extract (GSE) and the complex chemical composition of GSE. Proanthocyanidin monomers and oligomers are the major bioactive compounds in GSE. Given no standardized criteria for quality, large variation exists in the composition of commercial GSE supplements. Using HPLC/UV/MS, 21 commercial GSE containing products were purchased and chemically profiled, major compounds quantitated, and compared against authenticated grape seed extract, peanut skin extract, and pine bark extract. The antioxidant capacity and total polyphenol content for each sample was also determined and compared using standard techniques. Nine products were adulterated, found to contain peanut skin extract. A wide degree of variability in chemical composition was detected in commercial products, demonstrating the need for development of quality control standards for GSE. A TLC method was developed to allow for rapid and inexpensive detection of adulteration in GSE by peanut skin.

1. Introduction

Grapes and grape-derived products are rich in numerous bioactive dietary polyphenols which have been attributed to improving health and nutrition (Castaneda-Ovando, Pacheco-Hernández, Páez-Hernández, Rodríguez, & Galán-Vidal, 2009; Welch, Wu, & Simon, 2008). Polyphenols from grape-derived products have been associated with the prevention of numerous diseases including cardiovascular diseases, neurodegenerative diseases such as Alzheimer's disease, as well as several forms of cancers (Aziz, Kumar, & Ahmad, 2003; Bertelli & Das, 2009; Ho et al., 2013; Pasinetti & Ho, 2010; Renaud & de Lorgeril, 1992). Epidemiological and experimental evidence supports the hypothesis that specific grape polyphenols may serve as disease preventative agents (Aziz et al., 2003; Bertelli & Das, 2009; Jang et al., 1997; Pasinetti & Eberstein, 2008; Renaud & de Lorgeril, 1992). These compounds include proanthocyanidin monomers and oligomers such as catechin, epicatechin, and proanthocyanidin dimers, which are the major constituents in extracts of grape seed (Bertelli & Das, 2009; Fuleki & da Silva, 1997; Ho, Yemul, Wang, & Pasinetti, 2009; Wang et al., 2008).

Proanthocyanidins (PACs) are oligomeric conjugates of any combination of the four isomers (±)-catechin and (±)-epicatechin (Fuleki & da Silva, 1997). Two distinct classes of PACs can be defined based on chemical structure, known as A-type (Lou et al., 1999) and B-type PACs (Manach, Williamson, Morand, Scalbert, & Rémésy, 2005). A-type PACs (not found in GSE) contain a 4β → 8 C–C bond and a 2β → O → 7 C–O bond between the two monomer units, whereas the B-type PACs contain only the 4β → 8 bond, and occasionally the 4β → 6 bond (Passos et al., 2007) (Fig. 1). Proanthocyanidin oligomers containing (−)-epicatechin also occur with differing degrees of galloylation (Rasmussen, Frederiksen, Struntze Krogholm, & Poulsen, 2005). While proanthocyanidins come with a wide range of degree of polymerization (DP), interest is focused on the proanthocyanidin monomers and dimers (DP < 3) because researchers have found that only the monomers and dimers are absorbed into human intestinal tissue and into

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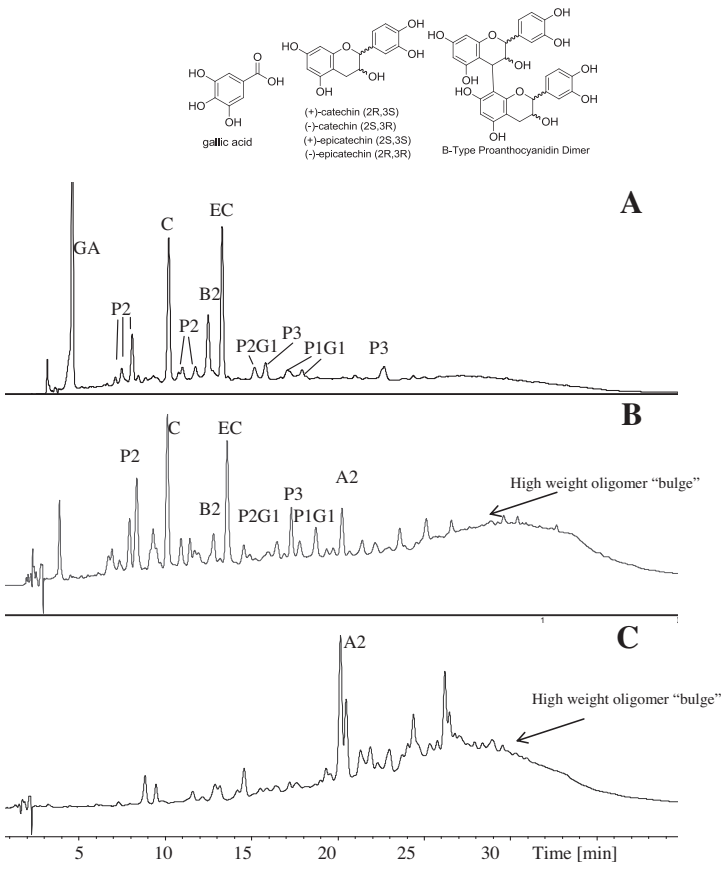

Fig. 1. Structures of major compounds found in grape seed extract, GSE (top); representative UV chromatograms at 280 nm of (A) authentic GSE; (B) authentic pine bark extract; (C) authentic peanut skin extract. *Abbreviations:* GA, gallic acid; C, catechin; EC, epicatechin; P, proanthocyanidin unit; G, galloyl unit; B2, proanthocyanidin B2 [(−)-epicatechin-(4β → 8)-(−)-epicatechin]; A2, proanthocyanidin A-type dimer.

circulation in significant quantities (Ferruzzi et al., 2009; Manach et al., 2005; Passos et al., 2007; Wang et al., 2012).

A wide range of studies have evaluated the bioactivity of GSE derived products. Protection against gastric mucosal damage induced by ethanolic HCl was shown in rats administered GSE (Saito, Hosoyama, Ariga, Kataoka & Yamaji, 1998). Antibacterial activity of GSE has been demonstrated in a number of studies, both in gram positive and gram negative bacterial strains (Jayaprakasha, Selvi & Sakariah, 2003; Furiga, Lonvaud-Funel & Badet, 2009; Baydar, Özkan, & Sağdiç, 2004) including methicillin resistant *Staphylococcus aureus* (Al-Habib, Al-Saleh, Safer, & Afzal, 2010). GSE has also been shown to inhibit pancreatic lipase and lipoprotein lipase, as well as inhibition of lipolysis in 3T3-L1 adipocytes (Moreno, Ilic, Pouley, Brasaemle, Fried & Raskin, 2003).

In grape seed extract, typically the B-type PACs have been reported, with highest concentrations of catechin and epicatechin (Wu, Wang, & Simon, 2003), as well as proanthocyanidin B2 and its isomers (Xu et al., 2011). A number of studies have evaluated the proanthocyanidin constituents in grape seed extract. While some variability exists due to source and process used in preparation, reviewed in detail by Liu and White (2012), grape seed extract is typically composed of primarily of PAC monomer and low weight oligomers, with a much smaller concentration of tetramers or greater polymers. Other natural sources of PACs have been reported. Peanut skin extract, for example, is composed principally of A-type PACs with negligible quantities of the monomers, catechin and epicatechin (Sarnoski, Johnson, Reed, Tanko, & O'Keefe, 2012). Pine bark extract has been reported to contain both A and B-type PACs, as well as the monomers (Karonen, Loponen, Ossipov, & Pihlaja, 2004), reported to contain 55 mg/g total PACs (Hellström & Mattila, 2008).

Given the increasing use and myriad commercial products containing grape seed extracts, our research team focused on examining the quality of such plant-based dietary supplements. The Office of Dietary Supplements (ODS) and Food and Drug Administration (FDA) have long raised serious questions as to the safety, efficacy and quality of dietary supplements, especially those derived from plants, citing concerns relating to adulteration, contamination, lack of bioactive compounds, and a lack of compositional standardization which is the principle source of batch to batch inconsistency (Betz, 2006; Ho, Simon, Shahidi, & Shao, 2006; Wang, Liang, Wu, Simon, & Ho, 2006). This study was conducted as part of a larger program which has been examining the uses of grape seed extracts and specific compounds found within grape seed in the treatment of Alzheimer's disease and mitigation against cognitive memory loss (Ferruzzi et al., 2009; Wang et al., 2012). A wide degree of variability was found during routine vetting of GSE vendors—leading us to question the overall quality and product consistency available to consumers when purchasing this dietary supplement. The objective of this study was to evaluate commercially available GSE derived products by chemical profiling, identify whether adulteration of GSE by other common PAC containing material such as peanut-skin extract or pine-bark extract, and develop a TLC technique to identify adulteration.

2. Materials and methods

2.1. Materials and reagents

Twenty-one commercially available products containing grape seed extract were obtained and stored at room temperature in