

Antioxidant Property and Health Benefits of Grape Byproducts

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Abstract

Grape skin and seeds, produced by the winemaking industry, are increasingly used to obtain functional food ingredients. Grape seed is a better source of antioxidative constituents than skins of grape/wine byproducts. Functional ingredients of grape seed include several flavonoids with a phenolic nature such as monomeric flavanols, dimeric, trimeric, and polymeric procyanidins, and phenolic acids. The antioxidant activity of grape seed phenolic compounds is closely associated with activity against various types of cancer as well as cardiovascular and other dermal disorders. Grape seed has antioxidant, anti-allergenic, antihistamine, anti-inflammatory and immune boosting properties and it helps the body against allergens and carcinogens. Grape seed extract is frequently recommended to combat macular degeneration, cataracts, and eyestrain. The present review article discusses the functional characteristics of grape pomace and their health benefits against several human diseases and disorders.

Keywords

Grape
Antioxidant
Phenolic compound
Seed
Pomace
Byproducts

INTRODUCTION

Grape (*Vitis vinifera*) is a sub-tropical crop. However, in India, grapes are cultivated for their excellence under tropical conditions. Grapes are grown in an area of 64.3 thousand ha with a total production 1,630.7 thousand tons and productivity of 25.4 tons/ha. Because of special arbour training systems provided for grape cultivation in India, productivity is the highest among the grape growing countries of the world. India's high productivity in grape has made it to reach 12th position in the world as far as production (total world production 67,696 thousand tons) is concerned.

Grapes, the edible fruit of the grapevine, are a prime example of a true berry. A berry in botanical terms is a class of fleshy fruit lacking a stony layer, with the fruit wall being fleshy or pulpy (Robinson, 2006). Grapes grow in bunches which vary in size

and shape depending on the grape variety (Galet, 2002). Grape color varies from green to yellow, pink, crimson, dark blue, and black, with the majority of grapes being yellow or very dark purple (Robinson, 2006).

The significant parts of the berry are the flesh, skin, and seeds (Robinson, 2006; Ayala-Zavala et al., 2011). The flesh or pulp is the bulk of the berry. The pulp contains the juice in vacuoles of pericarp cells (Mullins et al., 1992). A central core of vascular strands connects to a mesh of veins that encircles the outer edge of the flesh like a "chicken-wire" cage. The veins contain the xylem, which transports water and minerals from the roots and phloem, the pathway for sugar from the leaves (Robinson, 2006). The grape skin is a tough enveloping layer around the grape that holds it together. The outside layer, or bloom, consists of waxy plates and cutin,

which resist water, fungal spore growth, and other biological infections. Below the bloom are the cell layers that form the skin which contain concentrated carotenoids, xanthophylls, and anthocyanins (Mullins et al., 1992). Tannins, along with a significant amount of the grape flavor compounds, are also located in the skin. Seeds contain tannins, mostly proanthocyanidins, which if crushed, confer a bitter taste (Robinson, 2006).

Environmental hazards

Disposal of grape pomace, the waste generated during winemaking, has posed a major challenge for wineries (Ayala-Zavala et al., 2011). During wine production, wine grapes are harvested and pressed to extract juice for fermentation. Because of pressing, the skins, stems, and seeds are left behind as waste. Removal of this pomace is costly and if the pomace is not treated effectively, it can initiate a number of environmental hazards, ranging from surface and ground water contamination to foul odours (Bonilla et al., 1999).

Winery waste can also have an environmental impact through the increase of the chemical oxygen demand (COD) and biochemical oxygen demand (BOD) within wastewater streams. The high COD and BOD levels of the grape pomace originate from their high pollution loads and high content of lipids and other organic substances such as sugars, tannins, polyphenols, polyalcohols and pectins (Schieber et al., 2001). Due to the environmental problems that these high COD and BOD cause, it is beneficial for wineries to find other applications for their grape pomace waste other than animal feeds or fertilizers (Inbar et al., 1988). To help alleviate the issues associated with grape pomace, its use in alternative applications has been explored. Applications have included the production of value-added products such as dietary supplements for disease prevention, grappa (grape pomace

alcohol) production, laccase production and pullulan production (Israilides et al., 1998). In addition to finding a productive use for a waste product, these products have been produced in response to a changing consumer demand for naturally processed, additive-free, and safe products. Consumers tend to prefer safe, traditional products, which are promoted as “natural” and without other additives (Bianco and Uccella, 2000; Siddiqui and Dhua, 2010). Thus, the substitution of currently used synthetic food antioxidants by ones perceived as “natural” by consumers interests the research community. The market demand for natural antioxidants rather than chemical antioxidants added to baked products has directly increased the demand for novel polyphenolic containing ingredients. As part of this trend, the formation of antioxidant rich flours milled from dried grape waste and the subsequent incorporation of these flours into baked foods is a promising option (Ayala-Zavala et al., 2011; Vega-Vega et al., 2013).

Various concerns have caused consumers to monitor their dietary requirements closely. The rising incidence of health conditions such as obesity, diabetes and cardiac problems, concerns over physical appearance and the increasing price of health care have all contributed to the demand for healthier bakery products (Lempert, 2008). One way to create a healthier bakery product with an enhanced nutritional profile is through the addition of functional ingredients such as phytosterols, multigrain, prebiotics, multivitamins, and polyphenolics. Polyphenolic compounds are also known to have beneficial health effects related to their antioxidative capabilities (Siddiqui et al., 2013a).

PHENOLIC COMPOUNDS

Phenolic compounds in grape seed flour

Grape seeds have been proven to be rich in phenolic compounds, particularly flavonoids [(+)-catechin and

proanthocyanidins], which have demonstrated a wide range of biochemical and pharmacological effects pertaining to their antioxidant activities. The available information seems to suggest that regular consumption of fruits and vegetables should have long-term health benefits, related to their antioxidant activity (Siddiqui and Dhua, 2010). Thus, it is reasonable to conclude that consumption of grape seed flour, with potent antioxidant activity, will also result in long-term health benefits. However, for increased availability of health beneficial compounds new food products such as breads rich in these phenolic compounds need to be researched and developed. There is also a need for better understanding of the chemistry of grape seed phenolics not only to determine the antioxidant activities of those compounds, but also to determine grape seed phenolic stability under thermal processing and frozen storage, and the effect of grape seed replacement on bread sensory acceptance and instrumental analyses. Therefore, it is of importance to explore the potential benefit of the use of grape seed flour in a food system such as bread.

Grape seed flour has been shown to be rich in polyphenolic compounds and because of this profile; a growing demand exists for the inclusion of this flour into processed foods. Proanthocyanidins are the major polyphenols found in red wine and grape seeds with grape seeds being rich sources of monomeric phenolic compounds such as (+)-catechin, (-)-epicatechin, (-)-epicatechin-3-o-gallate, and dimeric, trimeric, and oligomeric procyanidins (Saito et al., 1998). However, there is concern that food preparation processes such as baking cause a loss of some of the health benefits through phytochemical loss (Wang and Zhou, 2004). Thus, it is critical to consider the chemical changes which occur during food preparation.

Within the grape, proanthocyanidins are the major polyphenols in the skins, stems, and seeds. Procyanidins are the predominant proanthocyanidins in grape seeds, while procyanidins and prodelfinidins are dominant in grape skins and stems (Souquet et al., 1996).

Grape seeds are considered good sources of polyphenolic tannins responsible for the astringent mouthfeel in wine. Grape seeds are concentrated sources of gallic acid, monomeric phenolic compounds, such as (+)-catechin (0.16 mg/mL GSE), (-)-epicatechin (0.66 mg/mL GSE), (-)-epicatechin-3-o-gallate (0.20 mg/mL GSE), and dimeric (0.329 mg/g DW grape seed), trimeric (0.384 mg/g DW grape seed) and polymeric (0.905 mg/g DW grape seed) proanthocyanidins (Khanal et al., 2009). (+)-Catechin shows antioxidant activity in human blood plasma by delaying the degradation of α -tocopherol and β -carotene and by inhibiting the oxidation of plasma lipids. (+)-Catechin has hydroxyl, peroxy (Scott et al., 1993), superoxide and DPPH radical scavenging activities. (-)-Epicatechin is capable of scavenging hydroxyl radicals, peroxy radicals, superoxide and DPPH radicals (Fukumoto and Mazza, 2000). Gallic acid is a phenolic acid that can scavenge peroxy radicals and DPPH radicals and has also shown activity at stomach pH (Gunckel et al., 1998).

Proanthocyanidins are defined as oligomers and polymers of polyhydroxy flavan-3-ol units such as catechin, gallic acid, and their epimers. Proanthocyanidins range in size from dimers through very large polymers and exhibit a wide range of biological activities. Proanthocyanidins are considered to be superior antioxidants compared to their corresponding monomers (catechin or gallic acid) (Ursini et al., 2001). The unique polyhydroxy phenolic nature of proanthocyanidins and the stability of reduction products (semiquinones and quinones) results in an electron configuration that allows for an

easy release of protons and thus substantial antioxidant activity.

Antioxidant activities of grape phenolics

Normal human metabolism is dependent on oxygen, a radical, as the terminal electron acceptor. As the two unpaired electrons of oxygen spin in the same direction, oxygen is a bi-radical; however comparatively, it is not a dangerous radical. Other oxygen-derived free radical species, such as superoxide or hydroxyl radicals, formed during metabolism or by ionizing radiation are much stronger oxidants and thus more of a concern (Bao and Fenwick, 2004; Siddiqui et al., 2013b).

Grape seeds contain mainly phenols such as proanthocyanidins (oligomeric proanthocyanidins). Scientific studies have shown that the antioxidant power of proanthocyanidins is 20 times greater than vitamin E and 50 times greater than vitamin C. Research suggests that grape seed oil helps protect the body from sun damage, improve vision, improve flexibility in joints, improve blood circulation, and reduce LDL oxidation and the occurrence of coronary heart disease.

Three different stages of radical-mediated oxidation of membrane lipids have been proposed: (1) Initiation during which free radicals remove hydrogen from an unsaturated fatty acid to form a lipid radical, (2) Propagation during which the lipid radical plus molecular oxygen forms a lipid peroxy radical, which further breaks down into more radicals, and (3) Termination during which time the new radicals react together or with antioxidants to eliminate free radicals (Cook and Samman, 1996).

There is evidence to suggest that the consumption of flavonoid-rich foods, in particular fruits and vegetables, is associated with a lower incidence of cancer, cardiovascular disorders, and other degenerative and chronic diseases caused

by oxidative stress (Sharangi et al., 2014). Consumption of foods containing flavonoids has resulted in six conclusions (Wildman, 2001): (1) inverse correlations between flavonoid consumption and the incidence of diseases thought to involve oxidative stress, (2) depression of the concentrations of oxidant products such as lipid peroxides; (3) elevation of concentrations of endogenous antioxidants, or prevention of their depletion during oxidant stress; (4) elevated measures of plasma or serum antioxidant capacities determined, (5) inhibition of exercise-induced muscle tissue breakdown and inflammation; and (6) depression of lipoprotein oxidation rates assessed.

Two primary conditions exist that characterize a polyphenol as an antioxidant. First, polyphenols delay or inhibit the oxidation of the substrate when present in low concentration compared to the oxidizable substrate. Second, polyphenols are present as stable intermediates, which act as potential terminators of the propagation step by reacting with other free radicals (Rice-Evans et al., 1997).

Health benefits of polyphenolics

Delay or inhibition of cancer growth

Diet can play an important role in either promoting or preventing diseases. The study performed by Hertog et al. (1996) reported a significantly reduced risk of cancer mortality for men with a high fruit intake. A review of over 200 studies found a significant protective effect of fruit and vegetable intake against lung, colon, breast, cervix, esophageal, oral cavity, stomach, bladder, pancreas and ovarian cancer (Block et al., 1992). It has also been shown that a diet high in fruits reduced oxidative damage to DNA, which may be one critical step in the onset of some types of cancers (Siddiqui et al., 2014).

Jang et al. (1996) studied the cancer chemopreventive activity of resveratrol, a natural product derived from Grapes. Resveratrol was found to act as an antioxidant and antimutagen and to induce phase II drug-metabolizing enzymes (anti-initiation activity); it mediated anti-inflammatory effects and inhibited cyclooxygenase and hydroperoxidase functions (antipromotion activity); and it induced human promyelocytic leukemia cell differentiation (antiprogession activity).

Animal studies and cell models suggest that the polyphenolics contained in grape seed act as anti-carcinogens by influencing molecular events at the initiation, promotion, and progression stages of cancer (Lin and Weng, 2006). Flavonoids can act as anti-carcinogens by scavenging of free radicals, regulation of signal transduction pathways of cell growth and proliferation, suppression of oncogenes and tumor formation, induction of apoptosis, governance of enzyme activity related to detoxification, oxidation, and reduction, stimulation of the immune system and DNA repair, and the regulation of hormone metabolism (Liu, 2002). The most intriguing of these properties is the role of polyphenols in apoptosis, programmed cell death. Apoptosis minimizes leakage of potentially toxic cellular constituents from dying cells. Apoptosis is a key pathway because a damaged or blocked pathway results in uncontrolled cell division that ultimately leads to tumor formation and propagation. Oxidative stress, cancer, viral infections, and other degenerative diseases all are correlated to improper regulation of apoptosis (Liu, 2002).

Cardiovascular disease

Cardiovascular disease (CVD), and in particular atherosclerosis, remains the leading cause of death in both men and women. Endothelial injury is one of the first events in this process, which is followed by

a large number of reactions and molecular responses. All of these events may lead to the formation of atherosclerotic plaques, resulting in constriction of blood vessels and a reduced capacity to dilate. Grape seed proanthocyanidins appear to remedy several of the steps in this complex process. Much epidemiologic evidence indicates that consumption of polyphenolics results in a reduction of CVD risk factors and decreased mortality (Ross, 1999). A few of the mechanisms thought to play a key role in the development of atherosclerosis are low density lipoprotein (LDL) oxidation, platelet aggregation, and nitric oxide (NO) dependent dilation (Pataki et al, 2002).

LDLs that transport lipids throughout our bodies are targets for oxidation. The oxidative susceptibility of LDL and the subsequent oxidation of arterial walls are thought to be critical steps in the development of atherosclerosis (Ross, 1999). This oxidation occurs through a number of highly reactive oxygen species such as singlet oxygen, O₂, OH, NO, and alkyl peroxy free radicals. Recent investigations of proanthocyanidins from grape seeds suggest that they are effective at protecting against LDL oxidation via free radical scavenging activities (Arteel and Sies, 1999).

Daily intake of platelet inhibitors such as aspirin has been shown to slow the progression of heart disease in animal models (Anderson et al., 2001). Similarly, grape products, especially grape seeds, are effective platelet inhibitors, at a minimum concentration of 50 mg/L, and appear to reduce the development of atherosclerosis through this pathway (Vitseva et al., 2005). Procyanidins have recently been shown to possess endothelium dependent relaxing (EDR) activity in blood vessels. The enzyme, nitric oxide synthase (NOS), uses L-arginine and oxygen as substrates to produce NO, which interacts with smooth muscle cells to cause vasorelaxation.

Furthermore, increased levels of cyclic GMP, the vascular smooth muscle cell messenger through which NO acts accompanied EDR activity. It has been recently shown that vasodilating compounds tend to be of the proanthocyanidin type (Fitzpatrick et al., 2002).

In blood vessels, NO not only resists vasoconstrictor influences, but also decreases platelet aggregation and adherence of platelets to the endothelium, inhibits oxidation of LDL, and diminishes vascular smooth muscle cell proliferation. In order to determine the respective contributions of grape seed proanthocyanidins on platelet adhesion and intravascular coagulation of blood (thrombosis), male C57BL/6 mice were given either 2 or 20 mg grape seed proanthocyanidin extract/kg body weight (Sano et al., 2005). In these mice, injecting them with 20 mg grape seed proanthocyanidin extract/kg body weight increased bleeding time, reduced the thrombus weight and reduced the apparent platelet adhesion to collagen in arterial walls. No effect was observed with the injection of 2 mg grape seed proanthocyanidin extract/kg body weight (Sano et al., 2005). The data collected from animal models suggested that grape seed proanthocyanidins inhibit platelet aggregation when taken orally. Thus, it would appear that consumption of NO-stimulating compounds, such as grape seed proanthocyanidins, in the diet could contribute to the prevention or slowing of atherosclerosis.

Grape seed proanthocyanidins are potent accelerators of NOS activity, quenchers of *in vitro* oxidation of LDL, and reducers of platelet aggregation. A combination of the reduction of these three risk factors suggests the potential for dietary grape seed proanthocyanidins in preventing diseases. Thus, incorporation of grape seed flour into

the daily diet through commercial products, such as bread, needs to be studied.

Leifert and Abeywardena (2008) suggested that grape extracts and purified grape polyphenols possess a diverse array of biological actions and may be beneficial in the prevention of some inflammatory mediated diseases including cardiovascular disease. The active components from grape extracts, which include the grape seed, grape skin, and grape juice, that have been identified thus far include polyphenols such as resveratrol, phenolic acids, anthocyanins, and flavonoids. All possess potent antioxidant properties and have been shown to decrease low-density lipoprotein cholesterol oxidation and platelet aggregation. These compounds also possess a range of additional cardioprotective and vasoprotective properties including antiatherosclerotic, antiarrhythmic, and vasorelaxation actions. Consumption of grape and grape extracts and/or grape products such as red wine may be beneficial in preventing the development of chronic degenerative diseases such as cardiovascular disease.

Xia et al. (2010) studied the biological activities of polyphenols from grapes and found that the dietary consumption of grape and its products is associated with a lower incidence of degenerative diseases such as cardiovascular disease and certain types of cancers. Anthocyanins, flavanols, flavonols and resveratrol are the most important grape polyphenols because they possess many biological activities, such as antioxidant, cardioprotective, anticancer, anti-inflammation, antiaging and antimicrobial properties. They concluded that the polyphenols from grape could widely be employed to prevent and treat diseases in association with reactive oxygen species, such as atherosclerosis, coronary heart diseases, and cancer

Diabetes prevention

Pycnogenol treatment effectively improved capillary resistance, reduced blood leakages into the retina, and was as effective as the drug, calcium dobesilate (Schonlau and Rohdewald, 2001). Grape seed flour and pycnogenol have a similar polyphenolic make-up, and the inclusion of grape seed flour into novel products geared toward the diabetic community, should be researched.

Digestibility and Absorption

Upon ingestion, proanthocyanidins first react with proline rich proteins in the mouth to yield an astringent sensation. Those compounds that cross the intestinal barrier travel to the liver via the portal vein, where they further degrade into metabolites. Within hours of consumption, these metabolites may reach all tissues as seen in radio labeling experiments with live rats. Confirmation of the presence of low-molecular weight metabolites in the urine and feces of these rats, as well as in chickens and sheep, indicates that polymeric proanthocyanidins may not be absorbed through the intestinal barrier without first being degraded into low-molecular weight metabolites by gut microflora (Gonthier et al., 2003). Urine analysis of 69 human subjects after consumption of grape seed extract supplement throughout six weeks (1000 mg/day total polyphenols) supported these results. Results indicated three phenolic acids as breakdown products of proanthocyanidin metabolism: 3-hydroxyphenylpropionic acid, 4-O-methylgallic acid and 3-hydroxyphenylacetic acid (Ward et al., 2004). Of all the classes of flavonoids, proanthocyanidins appear to be the least well absorbed specifically 10 to 100 fold less than their monomeric constituents (Tsang et al., 2005). Polymers with a mean degree of polymerization equal to seven are not as well absorbed by the human intestine due to their lower permeability through paracellular absorption (movement of ions through intercellular spaces between

epithelial cells), and their likely complexation with luminal and mucosa proteins (Manach and Donovan, 2004).

007

Effect of thermal processing on phenolics and antioxidant activity

Processing can alter and often damage fruit and vegetable antioxidants, particularly in the case of vitamin C and phenolic antioxidants. Heating, maceration, and separation steps can cause oxidation, thermal degradation, and/or antioxidant leaching, leading to lower levels of antioxidants in processed foods compared to fresh. However, as was found in rice hulls, processing can lead to a dissociation of antioxidants from the plant matrix components, increasing the phenolic content and improving digestive absorption (Kim et al., 2006).

Thermal processing is among the most popular ways of food processing. Through thermal processing, a complex set of chemical reactions takes place that dictate many of the final quality attributes of processed foods. In phenolic compounds, antioxidant activity may experience significant degradation during processing. In a study evaluating the effect of blanching and long term frozen storage, researchers found a 20-30% reduction in vitamin C, β -carotene, and α -carotene, in potatoes, carrots, and spinach due to blanching, while frozen storage only slightly reduced these compounds (Puupponen-Pimia et al., 2003). Total phenolic content was positively correlated with antioxidant activity, suggesting that among the compounds tested, phenolic acids contributed to the antioxidant activity (Siddiqui et al., 2013a; Khue et al., 2013). In blueberry juice processing, involving heating, substantial losses of phenolics were observed. Specifically, reduction of anthocyanins, procyanidins, and chlorogenic acid, between 47 and 68%, were observed when comparing a fresh product to the final juice (Kalt, 2005). A more recent

study of grape seed extracts in bread showed a 30-40% reduction in antioxidant activity, compared to a pure extract, at the completion of processing (Peng et al., 2009). The authors attributed the loss in antioxidant activity to either proanthocyanidins complexation with proteins, or the thermal degradation of the proanthocyanidins.

However, one study has demonstrated that processed fruits and vegetables may retain or increase their antioxidant activity throughout heating. These researchers found that the heat treatment of grape seeds liberated phenolic compounds, and subsequently increased the amount of active compounds by up to 46% in the extracts. The authors also suggested that because of the increase in total phenolics, simple heating could be used as a tool to increase antioxidant activity of commercially available grape seed extracts (Kim et al, 2006). Thus, it would seem that with respect to the thermal stability of grape seed antioxidants, published studies vary in their conclusions.

CONCLUSIONS

Functional ingredients of grape seeds include several flavonoids with a phenolic nature such as monomeric flavanols (catechin and epicatechin), dimeric, trimeric and polymeric procyanidins, and phenolic acids (gallic acid and ellagic acid). These flavonoids have been reported to exhibit antioxidant activity. The antioxidant activity of flavonoids is closely associated with activity against various cancer types, cardiovascular diseases and several dermal disorders. Functional components of grape seeds or skins can be used in the human diet. Since a major class of phenols in red wine is procyanidins, mostly extracted from the skins and seeds of red grape, a very opportunistic and viable business has emerged within the wine industry. This industry produces grape seed and grape skin extracts from winery waste as nutritional adjuncts for the rapidly growing nutraceutical industry. Such products from seed, skin, and wine are finding increasing

applications as dietary supplements for disease prevention and for claims involving structure/function relationships.

008

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