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Invited review

Functional components of grape pomace: their composition, biological properties and potential applications

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Summary The roles of functional foods on human health have been realised by more and more researchers, food producers and consumers. Functional food ingredients from both plant and animal sources such as dietary fibre, soy protein isolate, whey protein isolate and omega 3 fatty acid have been widely used in functional food product development. Many fruit processing by-products such as grape, apple and orange peels are rich in bioactive phytochemicals, dietary fibre and unsaturated fatty acids, hence have potential to serve as functional food ingredients. In this review, we summarise recent advancement of research in grape pomace (GP), the residual of grapes after wine making. The polyphenol profile of GP and their biological, antioxidant and antimicrobial activities, the stability of GP polyphenols in food system, the interaction between GP polyphenol and other food ingredients, as well as the functionalities of grape seed oil and GP fibre are covered.

Keywords Biological properties, dietary fibre, grape pomace, grape seed oil, grape seed protein, polyphenol composition, thermal stability.

Introduction

Grape pomace (GP) is a by-product of wine industry. GP consists mainly of peels (skins), seeds and stems and accounts for about 20–25% of the weight of the grape crushed for wine production. Grape seed is rich in extractable phenolic antioxidants such as phenolic acid, flavonoids, procyanidins and resveratrol, while grape skins contain abundant anthocyanins. The health benefits of GP polyphenols have been the great interest of researchers, food industry and nutraceutical industry. In addition to phenolic antioxidants, GPs also contain significant amount of lipid, proteins, non-digestible fibre and minerals. Grape seeds contain 13–19% oil, which is rich in essential fatty acids, about 11% protein, 60–70% of non-digestible carbohydrates, and non-phenolic antioxidants such as tocopherols and beta-carotene (Rao, 1994; Baydar & Akkurt, 2001; Bravi *et al*., 2007; Llobera & Cañellas, 2007). This review summarises the recent studies on major components of GP, their important properties and their possible applications that are.

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Phenolic compounds of GP and their properties

Polyphenol composition of GP

Phenolics are the secondary metabolites of plants. Chemically, phenolics can be defined as substances possessing an aromatic ring bearing one or more hydroxyl groups, including their functional derivatives (Shahidi & Naczk, 2004). Polyphenols are compounds that have more than one phenolic hydroxyl group attached to one or more benzene rings (Vermerris & Nicholson, 2006). Most food phenolics have more than one hydroxyl group attached on the aromatic ring; therefore, in this review, phenolics and polyphenols are used interchangeably. In food science research, natural phenolics are generally classified into classes and sub-classes based on the similarity of their chemical structures, that is, the types of building blocks that appear as repeated units. Four major classes of polyphenols found in foods are phenolic acids, flavonoids, lignans and stilbenes (Spencer *et al*., 2008). Major stilbenoids found in foods of plant origin are resveratrol and it glycosides. Resveratrol is a phytoalexin produced in the plant in response to pathogen attack. It has a low toxicity in humans and is a naturally occurring fungicide.

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Phenolic acids are phenols that possess one carboxylic acid functional group and are divided into hydroxycinnamic acids and hydroxybenzoic acids. The hydroxycinnamic acids are more common than hydroxybenzoic acids, and they mainly include gallic acid, *p*-coumaric, caffeic, chlorogenic acid, ferulic and sinapic acids. These acids are rarely found in the free form, except in food that has undergone freezing, sterilisation or fermentation. The bound forms are glycosylated derivatives or esters of quinic acid, shikimic acid and tartaric acid (Vermerris & Nicholson, 2006).

The largest and best studied polyphenols are the flavonoids. Based on their molecular structures, flavonoids are divided into seven subclasses: flavones, flavanones, flavonols, isoflavones, anthocyanidins/anthocyanins, flavanols (or catechins and procyanidins) and chalcones (Karakaya, 2004). Another group of flavonoids, which are not included in this classification, are proanthocyanidins, also called, procyanidins, condensed tannins or oligomeric procyanidins (Prior & Gu, 2005).

Although phenolics are present virtually in all plant foods, some fruits such as grape, apple, blueberry and cranberry are extremely rich in these bioactive compounds. In grape berries, the phenolic compounds reside mainly in the skins, seeds and short stems (Rodriguez *et al*., 2006; Poudel *et al*., 2008). GP is rich in extractable phenolic antioxidants (10–11% of dry weight) (Makris *et al*., 2007). Anthocyanins, catechins, procyanidins, flavonol glycosides, phenolic acids and stilbenes are the principal phenolic constituents found in GP (Montealegre *et al*., 2006).

Polyphenol composition of GP is variety dependant. The red varieties are usually rich in anthocyaninins that are neglected in while white varieties. Within the same variety, different part has significantly different polyphenol composition. Cantos *et al*. (2002) analysed the polyphenol composition of four red and three white table grape varieties by HPLC-ADA-MS and found that anthocyanins were the main phenolics in red grapes ranging from 69 (Crimson Seedless) to 151 (Flame Seedless) mg kg^{-1} fresh weight of grapes, whereas flavan-3-ols were the most abundant phenolics in the white varieties ranging from 52 (Dominga) to 81 (Moscatel Italica) mg kg^{-1} fresh weight of grapes. Flavan-3-ols were also detected and were identified as gallocatechin, procyanidin B1, procyanidin B2, procyanidin B4, procyanidin C1, catechin and epigallocatechin. The study of the phenolic compounds content and antioxidant activity of pomace from the vinification of grape varieties widely produced in Brazil (Cabernet Sauvignon, Merlot, Bordeaux and Isabel) found that catechin was the most abundant non-anthocyanic compound identified in the GP (150.16 mg 100 g^{-1}) for all varieties; Cabernet Sauvignon pomace had the highest content of total phenolic compounds (75 mg g^{-1}), while Bordeaux variety showed the highest content of total anthocyanins (Rockenbach *et al*., 2011).

Phenolic compound distribution in different parts of grapes was reviewed by Xia *et al*. (2010). The grape skins, part of pomace, are proven to be rich sources of anthocyanins, hydroxycinnamic acids, flavanols and flavonol glycosides, whereas gallic acid and flavanols were mainly present in the seed portion (Kammerer *et al*., 2004; Xia *et al*., 2010). Thirty-seven anthocyanins have been separated by McCalluma *et al*. (2007) from Concord grape skin, and among them twenty-five were identified using LC-MS. Among the anthocyanins identified in GP, five of them are the 3-*O*-monoglucosides of delphinidin, cyaniding, petunidin, peonidin and mavidin; another five of them are the acetylglucosides of the five anthocyandins. Malvidin 3-*O*-glucoside was found to be the predominant anthocyanin. Grape seeds are rich in monomeric phenolic compounds, such as (+)-catechins, (−)-epicatechin and (−)-epicatechin-3-Ogallate, and dimeric, trimeric and tetrameric procyanidins. Anthocyanin content of GP varies with wine vinification method and contact time. The longer the contact time, the lower the anthocyanin content remains in the pomace (Gómez-Plaza *et al*., 2006).

The phenolic profile of grape skin and seeds of European variety *Vitis Vinifera* has been intensively studied by scientists all over the world. Montealegre *et al*. (2006) quantified many phenolic compounds in seeds and skins of ten *V. vinifera* grapes grown in the warm climate of Spain, including six white varieties (Chardonnay, Sauvignon blanc, Moscatel, Gewürztraminer, Riesling and Viogner) and four red grape varieties (Cencibel, Cabernet Sauvignon, Merlot and Shiraz). They found that the skin of Viogner had the least of total hydroxycinnamates, catechin and procyanidin dimmers, while that of Moscatel had the most of total hydroxycinnamates and flavonols. Chardonnay and Gewürztraminer skins were the richest in catechin and procyanidins among white grape varieties. Red grape skin contains higher amount of hydroxycinnamates than white grape skin (31–55 mg kg^{-1} fresh grape), but much lower amount of catechins (9.0–18 mg kg^{-1} fresh grape), procyanidin dimmers (8.0–18 mg kg^{-1} fresh grape) and total flavonols (12–19 mg kg^{-1} fresh grape).

The polyphenol composition of each part of the GP varies depending on the varieties of grapes and is influenced by the growing location, climate, maturity and the time of fermentation (Fuleki & Ricardo da Silva, 1997; Kennedy *et al*., 2000; Shi *et al*., 2003a; Montealegre *et al*., 2006). The per-berry extractable yield of all polyphenols decreased with maturity and followed second-order kinetics. The flavan-3-ol monomers decreased most rapidly, followed by the procyanidin extension units and finally the terminal units. The

relative proportion of procyanidin extension units did not vary with maturity (Kennedy *et al.*, 2000). The phenolic composition of white and red grape seeds is comparable, but catechin, epicatechin and procyanidin B1 are higher in white grape seeds. Overall, grape seeds contained lower amount of phenolic acid than grape skin, but rich in catechins and procyandins. Castillo-Muñoz *et al.* (2010) investigated the flavonol profiles of white GPs from twenty-one *V. vinifera* white grape cultivars in Spain and found that flavonol profiles of white grapes are dominated by quercetin-type flavonols, but some cultivars (e.g. Pedro Ximénez, Gewürztraminer, Verdejo, Albillo, and Riesling) were characterised by relatively high and significantly different proportions of isorhamnetin-type flavonols.

The phenolic compounds in grape seeds are essentially all flavonoids, particularly, flavan-3-ols (catechin, epicatechin and epicatechin-3-*O*-gallate monomers) and their polypmers. Flavan-3-ols easily condenses into oligomeric procyanidins and polymeric compounds (condensed tannins). The dimeric procyanidins are often referred as B-series, and the trimeric procyanidins as 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 (Shi *et al.*, 2003a). Analysis of procyanidins extracted from both white and red grape seeds by electrospray ionisation–mass spectrometry (ESI-MS and ESI-MS/MS) in the positive mode found protonated molecules of procyanidin species of nongalloylated and monogalloylated type-A and type-B oligomers, with degree of polymerisation 2–5, and digalloylated oligomers, with degree of polymerisation 2–3 (Passos *et al.*, 2007).

The native grape variety grown in the United States is Muscadine, which includes the popular cultivars of Carlos, Cowart, Jumbo, Magnolia, Sterling, Nesbitt, Scuppernong and Noble. These grapes have tougher skins and less seeds than *V. vinifera* grapes. The study conducted in our laboratory demonstrated that Muscadine GP had about 5% more skin, but 8% less seeds than Cabernet GP. The polyphenol composition of Muscadine GP and Cabernet GP was also different. The contents of total extractable polyphenol, total anthocyanin and total flavonoid were 36.42, 0.88 and 21.02 mg g^{-1} DM in Muscadine seeds, 19.39, 8.15 and 4.78 mg g^{-1} DM in Muscadine skin, respectively (Yu *et al.*, 2011). Ellagic acid, gallic acid, (−)-epicatechin, (−)-epigallocatechin, catechin, myricetin, quercetin, and kaempferol and some anthocyanidins including delphinidin, cyanidin, petunidin, peonidin and malvidin were identified in the extract of Muscadine pomace extract using the combination of retention time and spectral properties on a reverse-phase HPLC–PDA (Wang *et al.*, 2010). Approximately 90% of the total anthocyanins in Muscadine grapes were 3,5-diglucoside of delphinidin, cyanidin and petunidin; the remaining 10% were 3,5-diglucoside of peonidin and malvidin; purple-skinned muscadine grapes (Jumbo and Cowart) have significantly higher levels of anthocyanins than bronze-skinned muscadine grapes (Carlos and Higgin) (Huang *et al.*, 2009).

Resveratrol is another important polyphenol found in grape skins and seeds. Resveratrols are found largely in the skins of red grapes and in other foods such as mulberries and peanuts (Sanders *et al.*, 2000). The trans-resveratrol content was found to be 1.11–12.3 mg per 100 dry mass in grape skin, 8.64 ± 4.5 mg per 100 dry mass in white grape skin and 1.42 ± 0.18 mg per 100 g dry mass in white grape seeds (Kammerer *et al.*, 2004). The resveratrol content in grapes differs according to the variety of grape (Ector *et al.*, 1996) and the grape maturation (Moreno *et al.*, 2008). Muscadine grapes and their products were reported to contain more resveratrol than any other type of grape (Ector *et al.*, 1996). 'Carlos' and 'Magnolia' Muscadine cultivars had the greatest skin resveratrol concentration of all the Muscadine cultivars evaluated. Except for 'Sweet Jenny', bronze cultivars had greater skin resveratrol concentration than black skinned cultivars. 'Miss Blanc' *Vitis labrusca* grape had greater skin resveratrol concentration than all other cultivars (LeBlanc, 2006). Resveratrol contents in grape tissues can be modified by post-harvest technologies. Cold storage alone doubled skin stilbene concentration in 'Carlos' grape, but UV irradiation did not significantly change stilbene levels. In contrast, UV irradiation increased skin stilbene concentration by 50% in 'Noble' grape, but cold storage alone had no effect (LeBlanc, 2006). Although certain amount of resveratrols in grape transfers into wine during grape maceration, significant amount of resveratrols remains in the pomace (Feijóo *et al.*, 2008).

Like other plant materials, GP contains relatively higher amount of non-extractable polyphenols (NEP). Although the total polyphenol content in dry GP is about 4.8–5.4% (Makris *et al.*, 2007), only 2% of polyphenols in GP is extractable under mild conditions commonly used to develop polyphenol databases (Bravo & Saura-Calixto, 1998). The majority portion of GP polyphenols has been reported to be highly polymerised condensed tannin, and some polyphenols form complex with fibre and are non-extractable unless strong acidic treatments are applied (Arranz *et al.*, 2010). Monomeric and oligomeric proanthocyanidins are certainly soluble in the organic solvents usually used for polyphenol extraction, but a major proportion of high-molecular-weight proanthocyanidins and polyphenols complexed with protein or cell wall polysaccharides remain insoluble (Huemmer & Schereier, 2008). The quantification of NEP in GP needs hydrolysis of GP residual to release the bound phenolics from cell wall or protein after soluble polyphenols are

extracted (Ignat *et al.*, 2011). The NEP content of GP can be as high as 67 mg g^{-1} DM in red GP (var. Cencibel) and as low as 1.68 mg g^{-1} in white GP (var. Thompson, seedless) (Pérez-Jiménez *et al.*, 2009). Some food processing methods such as extrusion increased extractable and bioavailable polyphenols (catechin and epicatechin) and reduced the NEP by reducing the degree of polymerisation (Khanal *et al.*, 2009). Similar results were observed for the extractability of polyphenols in roasted peanut and almond skins (Yu *et al.*, 2005, 2006, 2007; Garrido *et al.* (2008). These studies suggest that thermal processing may increase the extractability and bioavailability of some polyphenols while destroying heat sensitive polyphenols, in grape skin and seeds.

Biological properties of GP polyphenols

Numerous studies have demonstrated that grape seed phenolics, particularly procyanidins, have many health benefits such as antimutagenic and anticarcinogenic activity (Joshi *et al.*, 2000; Carini *et al.*, 2000; Mantena & Katiyar, 2006; Yeh & Yen, 2006; Bagchi *et al.*, 2000; de Rezende, *et al.*, 2009), antioxidant and anti-inflammatory activities (Prior & Gu, 2005; Sartor *et al.*, 2002), prevention and delay of cardiovascular diseases (Brito *et al.*, 2002; Vigna *et al.*, 2003; Bagchi *et al.*, 2003; Karthikeyan *et al.*, 2009; Bradamante *et al.*, 2004; Sano *et al.*, 2005), increase in lifespan and retarded the onset of age-related markers (Valenzano *et al.*, 2006). Some recent studies have also shown that taking grape seed extract (GSE) reduced food intake in rats and energy intake in human (Vogels & Plantenga, 2004). The main bioactive compounds responsible for many reported health benefits of wine and wine by-products consist of antioxidant phenolics such as phenolic acids, anthocyanins, procyanidins and reveratrols (Shrikhande, 2000; Yilmaz & Toledo, 2004;).

Antimutagenic and anticarcinogenic properties

A study by Bagchi *et al.* (2000) demonstrated that grape seed procyanidin extract (GSPE) was highly bioavailable and provided significantly greater protection against free radicals and free radical induced lipid oxidation and DNA damage than vitamins C, E or β-carotene. Cytotoxicity of GSPE towards human breast, lung, gastric adenocarcinoma cells, while enhancing the growth and viability of gastric mucosal cells, was also observed in the same study. GSPE also exhibited protection against skin cancer by inhibiting UV-radiation-induced oxidative stress and activation of mitogen-activated protein kinase and NF-κB signalling in human epidermal keratinocytes (Bagchi *et al.*, 2000; Mantena & Katiyar, 2006; White *et al.*, 2006). The study of Kaur *et al.* (2008) found that irrespective of source, GSE strongly inhibits LoVo, HT29 and SW480 cell growth, with a G1 arrest in LoVo and HT29 cells, but an S and/or G2/M arrest in SW480 cell cycle progression. GSE also induced Cip/p21 levels in all three cell lines. Furthermore, an induction of apoptosis was observed in all three cell lines by GSE. These findings suggest that GSE could be an effective alternative and complementary medicine against colorectal cancer because of its strong growth inhibitory and apoptosis-inducing effects.

It also has been reported that phenolics from grape seeds and skin inhibit some matrix proteases, such as leucocyte elastase and gelatinases, associated with inflammation and cancer invasion (Sartor *et al.*, 2002). The cell cultural study of Mertens-Talcott *et al.* (2008) show that the polypenol extracts from both red Muscadine and Cabernet Sauvignon wine significantly inhibited the growth of MOLT-4 leukaemia cells. Wine extracts reduced cell viability up to 68% and cell numbers up to 50% after 48 h with muscadine extracts being more effective than cabernet sauvignon. These extracts also induced caspase-3 activity and cell cycle arrest in the G2/M phase. The roles of anthocyanins in cancer prevention were extensively reviewed by Wang & Stoner (2008) and will not be repeated in this review.

Grape seeds are rich in B type procyanidins. Many studies revealed that GSPE can serve as potential therapeutic agent for different cancers. An *in vitro* study found that grape seed procyanidins inhibited pancreatic carcinoma cells MIA PaCa-2 and BxPC-3 proliferation in a dose-dependent manner and induced G1-phase arrest of the cell cycle in BxPC-3 or mitochondria-mediated apoptosis in MIA PaCa-2. Grape seed procyanidin also inhibited the adhesion and invasion potential of both cell lines in a dose-dependent manner through downregulation of MMP-2 or MMP-9 in pancreatic carcinoma cells (Chung *et al.*, 2012). Grape seed proanthocyanidins also induced apoptosis of non-small cell lung cancer (NSCLC) cells, A549 and H1299, *in vitro* through increased expression of pro-apoptotic protein Bax, decreased expression of antiapoptotic proteins Bcl2 and Bcl-xl, disruption of mitochondrial membrane potential and activation of caspases 9, 3 and poly (ADP-ribose) polymerase (PARP). Further, administration of 50, 100 or 200 mg GSPs kg^{-1} body weight of mice by oral gavage (5 days $week^{-1}$) markedly inhibited the growth of s.c. A549 and H1299 lung tumour xenografts in athymic nude mice, which was associated with the induction of apoptotic cell death, increased expression of Bax, reduced expression of antiapoptotic proteins and activation of caspase-3 in tumour xenograft cells (Singh *et al.*, 2011). Grape seed catechin and procyanidin B_4 pretreatment was found to protect cardiomyocytes against doxorubicin-induced toxicity by decreasing reactive oxygen species generation as well as the number of apoptotic cells, preventing DNA fragmentation,

regulating the expression levels of the pro-apoptotic protein Bax-α and the antiapoptotic protein Bcl-2, and inhibiting apoptotic signalling pathways (Du & Lou, 2008).

There is growing evidence that resveratrol can prevent or delay the onset of various cancers, heart diseases, ischaemic and chemically induced injuries, pathological inflammation and viral infections. As a chemoprevention agent, resveratrol has been shown to inhibit tumour initiation, promotion and progression (Jang *et al*., 1997). The review of Shanker *et al*. (2007) summarises the molecular mechanisms of resveratrol and its clinical benefits for human diseases. Resveratrol induces apoptosis by upregulating the expression of Bax, Bak, PUMA, Noxa, Bim, p53, TRAIL, TRAIL-R1/DR4 and TRAIL-R2/DR5 and simultaneously downregulating the expression of Bcl-2, Bcl-XL, Mcl-1 and survivin. Resveratrol also potentiates the apoptotic effects of cytokines, chemotherapeutic agents and gamma-radiation. Pharmacokinetic and pharmacodynamic studies demonstrate that the main target organs of resveratrol are liver and kidney, and it is metabolised by hydroxylation, glucuronidation, sulfation and hydrogenation (Bishayee *et al*., 2010). Resveratrol (~25 μM) potentiated GSE (≤ 35 μg mL^{-1}) induced colon cancer cell apoptosis via the activation of p53-dependent pathways (Radhakrishnan *et al*., 2011). This discovery suggests the importance of understanding the potentiating effects of phytonutrients in combination as they would occur in nature rather than individually.

The cancer prevention mechanism of food polyphenols has been extensively studied. Many potential chemopreventive polyphenols may interrupt or reverse the carcinogenesis process by acting on intracellular signalling network molecules involved in the initiation and/or promotion of cancer (Manson, 2003; Surh, 2003). The programmed cell death is considered one of the important targets in a preventive approach against cancer. Reversing the conversion of a normal cell to a malignant one is a complex process that involves active participation of affected cells in a self-destruction cascade. In addition to signal transduction and regulation of proliferation and immune response, dietary polyphenols readily interact with reactive oxygen species or free radicals to form relatively stable compounds, thus prevent cells from oxidative damage and onset of cancer. The major chemoprevention and chemotherapy mechanisms of dietary polyphenols may include (i) alteration of phase-I and phase-II drug-metabolising enzymes, (ii) antioxidant properties, (iii) inhibition of protein kinases, (iv) blocking of receptor-mediated functions, (v) attenuation of protease activities, (vi) alteration of cell cycle checkpoint controls, transcription factor expression and apoptosis, (vii) inhibition of angiogenesis, invasion and metastasis, and (viii) epigenetic changes in promoter methylation and chromatin remodelling (Dashwood, 2007; Kundu & Surh, 2008).

Prevention of cardiovascular diseases

Epidemiological studies suggest that consumption of wine, grape products and other foods containing polyphenols is associated with decreased risk of cardiovascular disease. Cardiovascular disease is associated with modifications in fatty acid metabolism and excessive lipid peroxidation of LDL. These oxidation products are also implicated in the formation of thromboxane, which leads first to enhanced platelet aggregation, then to artery blockage and finally to thrombosis. The accumulation of lipid oxidation products from LDL can be attributed to the low levels of plasma antioxidants.

A rat study showed that a 15% GP in cholesterol diet (0.3%) produced a significant reduction in cholesterol and triacylglycerols in the liver and serum. The diet contains 15% GP reduced VLDL and LDL by 50 and 60–70%, respectively, while increase HDL level by 26% (Bobek, 1999). Grape seed polyphenols reduce the risk of heart disease by inhibiting the oxidation of LDL (Shi *et al*., 2003a,b). Intravenous and oral administration of grape seed procyanidins was found to significantly inhibit laser-induced thrombus formation in the carotid artery of mice (Sano *et al*., 2005). Protection against myocardial ischaemia-reperfusion and myocardial injury in rats was reported by Bagchi *et al*. (2000) and Karthikeyan *et al*. (2009). GSEs rich in polyphenols exhibited higher effectiveness in reduction in platelet adhesion, aggregation and generation of superoxide anion than pure resveratrol (Olas *et al*., 2008). Experimental studies indicate that grape polyphenols could reduce atherosclerosis by a number of mechanisms, including inhibition of oxidation of LDL and other favourable effects on cellular redox state, improvement in endothelial function, lowering blood pressure, inhibition of platelet aggregation, reducing inflammation and activating novel proteins that prevent cell senescence (Dohadwala & Vita, 2009).

Procyanidins from GP inhibit human endothelial NADPH oxidase, the enzyme responsible for the increased production of reactive oxygen species, regardless of their polymerisation degree and galloylation percentage. The procyanidin fractions even blocked NADPH oxidase activity in intact HUVEC, inhibiting ROS production at both extra- and intracellular levels. Therefore, grape procyanidins are suitable NADPH oxidase inhibitors, which could serve as models for therapeutic alternatives for cardiovascular diseases (Álvarez *et al*., 2012). Supplementation with the high dose of mixture of cathechin, caffeic acid and resveratrol significantly reduced the presence of

atherosclerotic plaque by 40 and 36% in the aortic sinus and in the ascending aorta, respectively Norata *et al*. (2007). Resveratrol alone also showed significant antiatherogenic and anti-inflammatory effects in an animal model of rabbits fed a hypercholesterolemic diet (1% cholesterol) Matos *et al*. (2012). CVD preventative activities of anthocyanins, including results from *in vitro* cell culture and *in vivo* animal model systems as related to their multiple proposed mechanisms of action, were reviewed by Wallace (2011) and will not be repeated in this review.

Antilipogenic properties

Vogels & Plantenga (2004) reported that GSE reduced food intake in rats and energy intake in humans. In an *in vitro* study by Moreno *et al*. (2003), GSE was found to significantly inhibit pancreatic lipase, lipoprotein lipase and hormone sensitive lipase that responsible for the fat digestion and metabolism in human body. A study with rats fed high-fat diet shows that GSPE rectifies dyslipidemia associated with a high-fat diet in rats and repress genes controlling lipogenesis and VLDL assembling in liver (Baiges *et al*., 2010). Low-dose (25 mg per kg body weight per day) GSPE treatment of high-fat-diet (HFD) fed rats significantly reduced the adiposity index and the weight of all the white adipose tissue depots and reversed the increase in plasma phospholipids induced by the HFD feeding (Caimari *et al*., 2012). Chronic consumption of grape phenolics has shown to reduce obesity development and related metabolic pathways including adipokine secretion and oxidative stress in a rat model (Décordé *et al*., 2009). These studies suggest that grape seed polyphenols may play a role in body-weight management.

Antiageing activities

Grape seed extract has been shown to have a modulatory role on age-related oxidative DNA damage and lipid peroxidation in central nervous system of rats (Feng *et al*., 2005; Balu *et al*., 2006). Aged rats given GSE showed improved memory performance, reduced reactive oxygen species production, decreased protein carbonyl level, increased thiol level and reduced hypoxic ischaemic brain injury in their central nervous systems. Recent studies show that resveratrol could exert neuroprotection against ischaemia, seizure and neurodegenerative diseases (Markus & Morris, 2008). In the study conducted by Zhang *et al*. (2010), rat primary midbrain neuron-glia cultures were used to elucidate the molecular mechanisms underlying resveratrol-mediated neuroprotection. The results clearly show that resveratrol protected dopamine neurons against lipopolysaccharide (LPS)-induced neurotoxicity in concentration- and time-dependent manners through the inhibition of microglial activation and the subsequent reduction in proinflammatory factor release. Mechanistically, resveratrol-mediated neuroprotection was attributed to the inhibition of NADPH oxidase.

Antioxidant activities of GP polyphenols in foods

Antioxidant activity is the most notable bioactivity of phenolic compounds from GP. The antioxidative characteristics have been widely studied, including scavenging of free radicals, inhibition of lipid oxidation, reduction in hydroperoxide formation and so on (Xia *et al*., 2010). Polyphenols are proven to be strong antioxidants against lipid oxidation in food system. Grape seed phenolics (GSE) were reported to inhibit lipid oxidation and warmed-over flavour development in meat products such as raw and cooked beef products (Ahn *et al*., 2002, 2007; Mielnik *et al*., 2006; Brannan & Mah, 2007). GSE has reduced rancid flavour development and associated primary and secondary lipid oxidation products in various meat products like raw and cooked beef, pork patties, turkey, fish oil, frozen fish and ground chicken breast and thigh meat (Ahn *et al*., 2002; Banon *et al*., 2007; Brannan & Mah, 2007; Brannan, 2009; Carpenter *et al*., 2007; Lau & King, 2003; Mielnik *et al*., 2006; Pazos *et al*., 2005) without altering the pH, water activity, binding strength or yield of the meat. The minimum concentration level of GSE required to produce an antioxidant effect was 400 $\mu g\ g^{-1}$ in cooked pork and 0.1% (w/w) in ground chicken (Lau & King, 2003). However, GSE did alter the colour of both raw and precooked chicken breast patties (Brannan, 2009). The degree of discoloration caused by adding GSE may depend on the variety of grape, type of meat and dosage. GSE may be more suitable for red meat than white meat. The GSE that contains less anthocyanins may be better choice as antioxidant in meat products.

Antimicrobial activities of polyphenols from GP

In addition to antioxidant activities and therapeutic functions, many plant phenolic extracts have been shown to have antimicrobial activity against specific strains of bacteria such as *Streptococcus Mutans*, *Staphylococcus aureus, Escherichia coli and Candida albicans* (O'Keefe & Wang 2006; Daglia *et al*., 2007), *S. aureus, E. coli and C. albicans* (*C. albicans*) (Papadopoulou *et al*., 2005). Ahn and others (Ahn *et al*., 2007) reported that 1.0% GSE in cooked ground beef reduced the growth of *E. coli* O157:H7, *L. monocytogenes, S. Typhimurim* and *A. Hydrophila* by 1.5, 2, 1 and 3 LogCFU, respectively, during a 9-day storage. By using scanning and transmission electron microscopy, these authors also found grape seed polyphenols functioned as bactericidal, which caused disruption of

the bacterial cell wall. It was reported that activities of GP extract against both spoilage and pathogenic bacteria including *Aeromonas hydrophila, Bacillus cereus, Enterobacter aerogenes, Enterococcus faecalis, E. coli, E. coli* O157:H7, *Mycobacterium smegmatis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas fluorescens, Salmonella enteritidis, Salmonella typhimurium*, *S. aureus* and *Yersinia enterocolitica* (Özkan *et al*., 2004; Sagdic *et al*., 2011). The crude extract of GP at 10% inhibited the growth of the foodborne pathogens including Enterobacteriaceae and coliform bacteria, *Salmonella, S. aureus* in beef patties during the storage periods. The spoilage microorganisms including yeasts and moulds and lipolytic bacteria were also inhibited by 5% of GP extracts in beef patties (Sagdic *et al*., 2011). These studies also indicate that the antimicrobial activity of crude GP extract in meat products is low. Adding 5–10% of crude GP extract may cause many undesirable effects to the beef patty, such as reduced sensory quality and reduced protein digestibility.

Resveratrol, which is rich in grape skin, has been reported to have strong antifungal and antibacterial activities. Fungi reported to be sensitive to resveratrol include human pathogens (*C. albicans, Saccharomyces cerevisiae* and *Trichosporon beigelii*) and plant pathogens (*Phytophthora palmivora, P. capisci*, *Aspergillus flavus, Fusarium* spp. and *Verticillium* spp. (Jung *et al*., 2005). The study of Paulo *et al*. (2010) verified the antibacterial activity of resveratrol against Gram-positive bacteria. This study also used microscopic analysis and flow cytometry techniques to reveal that the antibacterial effects of resveratrol were attributed to bacteriostatic action. The addition of resveratrol has allowed the identification of changes in cell morphology and DNA contents. This suggests that the cell cycle is affected by resveratrol.

Therefore, polyphenols extracted from GP have the potential to be used for food preservation and medicinal purpose to suppress the growth of pathogenic bacteria and prevent oxidation of lipids. However, higher polyphenol concentration is required to achieve desired antibacterial activity against pathogenic bacteria compared to conventional antibiotics/antimicrobials.

Thermal stability of polyphenols from GP

The bioactivity of polyphenols from different plant sources are usually determined using compounds extracted at room temperature or refrigeration temperature and dried using freeze-drying technology to preserve their activity. Food processing conditions such as baking, steaming and extrusion are usually very harsh. Under such conditions, many bioactive compounds may undergo chemical degradation, isomerisation or polymerisation and lose their activities. The most extensively studied stability of phenolics includes the enzymatic oxidation of fruit and vegetable polyphenols during harvest, storage, transportation and other handling. The thermal oxidation/degradation of polyphenols during food processing such juice making, nut roasting and raw material drying was also the subject of numerous studies. Both polyphenol content and antioxidant activity of foods decrease because of thermal processing and long-term storage (Klimczak *et al*., 2007; Hager *et al*., 2008). The study of Spanos *et al*. (1990) and van der Sluis *et al*. (2005) found that contents of polyphenols such as cinnamics, phloretin glycosides, procyanidins and quercetin derivatives in apple juice decreased during room temperature storage of apple juice. Drying of grape seeds at 100 and 140 °C resulted in 18.6 and 32.6% reduction in extractable total polyphenols, respectively, and reduced antioxidant activity of grape seeds compared to freeze-drying (Larrauri *et al*., 1997). The addition of lecithin in a solution containing tea catechins significantly reduced the oxidative degradation of the catechins at acidic pH and room temperature (Lin *et al*., 2007). Heating decreased procyanidin and anthocyanin concentrations in freeze-dried GP significantly ($P < 0.05$). Reduction in procyanidin occurred when heated at 60 °C or above for 8 h with no further reduction when heating temperature increased from 105 to 125 °C. Total anthocyanin loss was highest at 125 °C (70%). No significant loss of both procyanidin and anthocyaninin was observed when heated at 40 °C for up to 3 days (Khanal *et al*., 2010). When compared to freeze-drying, vacuum-drying of fresh GP at 60 °C for 24 h also caused significant loss of total polyphenol, flavonoid and anthocyanin (Yu *et al*., 2011).

Controversial results were reported by Kim *et al*. (2006) who found that phenolic extracts of thermal processed whole and powdered grape seeds had higher *in vitro* antioxidant activity than those of untreated grape seeds. They also found newly formed low-molecular-weight phenolics in the extracts of heat treated grape seeds. Whether the newly formed phenolics were responsible for the increased antioxidant activity of GSE needs to be investigated. The study of (Friedman & Jürgens, 2000) demonstrates that caffeic, chlorogenic and gallic acids are not stable to high pH and that the pH- and time-dependent spectral transformations are not reversible. By contrast, chlorogenic acid is stable to acid pH, to heat and to storage when added to apple juice. (−)-Catechin, (−)-epigallocatechin, ferulic acid, rutin and *trans*-cinnamic acid resisted major pH-induced degradation. Factors affecting the stability of anthocyanins are well known with pH being the most important factor for the colour of anthocyanins (Mazza & Brouillard, 1987;. More information about the effects of non-thermal and thermal processing on anthocyanin stability in foods can be found in the

reviews of Tiwari *et al.* (2009) and Patras *et al.* (2010), respectively, and will not be repeated in this article.

Polyphenol–protein interaction

In addition to thermal stability, the interaction between food ingredients plays a vital role in successful food product development. Grape seed procyanidins interact strongly with proteins leading to rapid the formation of protein–tannin aggregates, and the binding increases with the degree of polymerisation and molecular weight of procyanidins (de Freitas & Mateus, 2001). Present knowledge indicates that this interaction is affected by parameters of the protein (molecular size, hydrophobicity, structural flexibility), the polyphenol (degree of polymerisation, extent of galloylation, structural flexibility) and the environment (temperature, pH, ionic strength, presence of organic solvents and presence of carbohydrates) (Carvalho *et al.*, 2006). To date, the most intensively studied polyphenol-ingredient interaction is tannin–protein interaction using bovine serum protein (BSA) with a few studies using β-lactalbumin (Prigent *et al.*, 2009). This type of interaction is claimed to be responsible for the astringent taste of polyphenol-rich fruits and vegetables (Payne *et al.*, 2009), haze formation in beverages (Siebert, 1999) and reduced bioavailability of both food protein and polyphenols (Skrabanja *et al.*, 2000; Papadopoulou & Frazier, 2004). However, the interactions between polyphenols other than tannins and food ingredients have rarely been reported.

Furthermore, considering the protein nature of enzymes, the interaction of polyphenols with digestive enzymes may reduce enzyme activity, thus reducing the digestibility of other food components such as carbohydrates, proteins and lipids. The inhibition of digestive enzymes such as lipase (Moreno *et al.*, 2003), proteases (Gonçalves *et al.*, 2007), as well as glucosidases (McDougall *et al.*, 2005; Gonçalves *et al.*, 2011), because of interaction with grape seed procyanidins has been reported. The inhibitory effect increased with increasing degree of polymerisation of the procyanidin fractions. The inhibition is also accompanied by the formation of insoluble aggregates detected by dynamic light scattering and nephelometry (Gonçalves *et al.*, 2011).

However, research findings on the impacts of protein–polyphenol interaction on protein digestibility have been controversial. He *et al.* (2006) reported that tea polyphenols at concentration of 50 ppm inhibited the activities of α-amylase, pepsin, trypsin and lipase in buffer solutions by 61, 32, 38 and 54%, respectively. In contrast, other investigators reported that polyphenols, particularly procyanidins, have the ability to bind to dietary protein, thus protecting it from rumen degradation, and increase protein availability in the small intestine of the host (Mueller-Harvey & McAllan, 1992). A considerable interaction between polyphenols and proteins appeared during the hydrothermal treatment of buckwheat, and this interaction reduces the digestion of proteins through the small and large intestine. Microbial processes in the colon enhance the digestibility of protein, blocked by polyphenols in hydrothermally processed buckwheat (Skrabanja *et al.*, 2000). Adding carbohydrates into a tannin-BSA system induced a solubilisation of the protein/tannin complexes, with neutral and ionic polysaccharides displaying different behaviours in this process. Pectin, xanthan, polygalacturonic acid and gum arabic were much more effective in solubilising the protein–tannin aggregates than glucose, dextran, β-cyclodextrin or arabinogalactan (de Freitas *et al.*, 2003). Therefore, the presence of large amount of carbohydrate such as starch may reverse the enzyme–polyphenol interaction and protect enzyme activity.

Safety issue of polyphenol consumption

However, the pharmacological effects of these antioxidants are dose dependent and are affected by many factors including genotype of individuals. On the other hand, it has been reported that a number of antioxidants may have both anticarcinogenic and carcinogenic effects. Some of powerful antioxidants such as vitamin A, vitamin E, quecertin, catechins, procyanidin B2 were reported to cause oxidative damage to cellar and isolated DNA (Sakano *et al.*, 2005). It was also reported that feeding Swiss Webster mice with green tea extract epigalocatechin gallate at the dose of 50 mg kg^{-1} body weight caused liver necrosis and mortality in both male and female mice (Goodin *et al.*, 2006). The review by Mennen *et al.* (2005) pointed out that certain polyphenols might have carcinogenic/genotoxic effects or may interfere with thyroid hormone biosynthesis; consumption of polyphenols may also inhibit non-haem iron absorption and may lead to iron depletion in populations with marginal iron stores; finally, polyphenols may interact with certain pharmaceutical agents and enhance their biologic effects.

However, many adverse effects of polyphenols are dose related. Noticeable DNA damage was induced in mice spleen cells by incubating with higher concentration (150 μM) of catechin (Fan & Lou, 2008). Grape extracts were also found to promote mitomycin C (MMC) that induces sister chromatid exchange at concentration from 75 to 300 μg mL^{-1} in human peripheral blood lymphocytes (Stagos *et al.*, 2007). Among the phenolic compounds tested, caffeic acid, gallic acid and rutin hydrate enhanced

MMC-induced clastogenicity, whereas ferulic acid, protocatechuic acid, (+)-catechin, (−)-epicatechin and trans-resveratrol had no effect at concentrations between 5 and 100 μM. A considerable amount of evidence is accumulating, which supports the hypothesis that high-dose polyphenols can mechanistically cause adverse effects through pro-oxidative action (Martin & Appel, 2010). Feeding male lambs on diets containing 5 and 10% dry GP significantly improved their growth performance ($P < 0.01$) compared to the other treatments (Bahrami *et al*., 2010). The inclusion of GSE at levels 0.6, 1.8 and 3.6 g kg^{-1} body weight in broiler chicks of 1–42 days did not affect the performance and the relative liver and pancreas weights, but reduced relative intestinal length at 21 days of age, increase ileal digestibility of crude protein and increased relative spleen weight at 21 and 42 days of age, respectively (Brenes *et al*., 2010). Therefore, it is important to consider the doses at which these effects occur, in relation to the concentrations that naturally occur in the human diet.

Applications of GP polyphenols in food systems

Some polyphenols have long been used in food products. For example, anthocyanins from grapes and berries are used as food colourants (Shahidi & Naczk, 2004). Food products fortified with plant extracts containing polyphenols are beverages including water or tea-based drinks, dairy products such as yoghurt, and special formulations such as 'smoothies' (Buchwald-Werner *et al*., 2009). Significant effort has been made over past decade to explore the potential of using GP to produce functional food ingredients, such as natural antioxidants for nutrition fortification and food preservation, health promoting grape seed oil and dietary fibre. GSE and GP extract are granted Generally Recognized As Safe (GRAS) and can be used as colour additive in fruit juice and flavoured beverage as antioxidants (FDA, 2003). GP pomace has been reported to use in the baked food products such as bread to increase the antioxidant activity of the bread and inhibit lipid oxidation of raw and cooked chicken (Peng *et al*., 2009; Sáyago-Ayerdi *et al*., 2009). In the study of Peng *et al*. (2009), bread was fortified with GSE, and the *in vitro* antioxidant activities, texture and colour of breads incorporating different levels of grape see extract (300, 600 and 1000 mg per 500 g bread) were determined to evaluate the effects of adding GSE on the quality of bread. Feeding chickens (3–6 weeks old) GP extract at levels of 0, 30 and 60 mg kg^{-1} body weight for 3 weeks did not affect chicken's growth performance, but significantly inhibited lipid oxidation of raw and cooked breast chicken patties compared with samples obtained from birds fed the control diet at 20 days and long-term frozen storage (6 months) (Sáyago-Ayerdi *et al*., 2009). These results indicated that dietary GP polyphenols could be effective in inhibiting lipid oxidation of chilled and long-term frozen stored chicken patties.

However, the production of purified polyphenol extract is usually costly, and organic solvents such as ethanol, ethyl acetate and acetone are usually used (Masquelier, 1987; Karvela *et al*., 2009). The use of organic solvent not only has harmful health impact on workers, but also generates new environmental problems. Although some efforts have been made to avoid using organic solvents, the process for extraction, purification and concentration of polyphenols is very tedious and costly (Shrikhande *et al*., 2000; Ochiai & Ueda, 2007). Therefore, direct inclusion of GP in some food formulas, where GP function as polyphenol carrier, may be a better way for the polyphenol/antioxidant fortification of foods. Addition of 0, 5, 7.5 and 10% deseeded GP in cookie formula increased dietary fibre and ashes substantially in the cookies and did not affect the acceptability of cookies, although deseeded GP addition imparted a darker colour to the cookies. However, the higher the deseeded GP addition, the lower the net protein ratio, apparent digestibility and true digestibility of cookies (Canett Romero *et al*., 2004). This is most likely owing to the inhibiting effect of GP polyphenol on digestive enzymes. Incorporation of 0.5–5% of grape seed flour in frankfurters led to a decline in the oxidation level of the products. The increment of grape seed flour in the frankfurters enhanced the protein, total dietary fibre and water-holding capacity of the treatments ($P < 0.05$), but the colour values (L*, a* and b*) of frankfurters generally decreased ($P < 0.05$) with increasing amount of grape seed flour (Özvural & Vural, 2011).

The possibility of using GP as feed was also studied. Basalan *et al*. (2011) investigated the nutrient contents and *in vitro* digestibility of twenty-eight fresh GP samples from white and red wine grape varieties in Turkey. They found that *in vitro* disappearance of DM and NDF at 48 h determined using ruminal fluid was similar for pomace from both white and red grapes, but the *in vitro* disappearance of skin and seed DM was higher than that of stalk. This study suggests that fresh GP rich in skin and seed should be a suitable feed for ruminants and to non-ruminants with extensive cecal fermentation. However, inclusion of GP in the sheep diet to 55% reduced digestibility of protein significantly (Baumgärtel *et al*., 2007). GP may also be used to synthesise exo-polygalacturonase (exo-PG), pectinase, xylanase and cellulase by solid-state or submerged fermentation (Díaz *et al*., 2012).

Grape seed oil

The oil content of grape seeds was reported in range of 11.6–19.6% depending on the variety and maturity of grapes (Rao, 1994; Llobera & Cañellas, 2007). The fatty acid composition of grape seed oil also variety and maturity dependent. Major fatty acids of grape seed oil are linoleic (66.76–73.61%) acid, oleic acid (17.8–26.5), palmitic acid (6.35–7.93%) and stearic acid (3.64–5.26%), respectively (Beveridge *et al*., 2005; Rubio *et al*., 2009). It was found that polyunsaturated fatty acid (PUSFA) of oils from Cabernet Sauvignon and Royal Rouge pomace ranged from 60.9 to 64.4% with high ratios of PUSFA/SFA (ranging from 2.80 to 3.11) and high ratios of $n-6/n-3$ (20.8–36.9) (Yi *et al*., 2009). The total unsaturated fatty acid accounts for more than 86% of the oil, and they were all essential fatty acids (EFA) (Baydar & Akkurt, 2001). Dietary EFA was reported to determine the fluidity of neuronal membrane and control the physiological functions of the brain Yehuda *et al*. (2005). EFA deficiency during infancy delays brain development and in ageing accelerates the deterioration of brain functions. One study with human subjects found that high-monounsaturated fatty acid diets lowered total cholesterol by 10% and low-density lipoprotein (LDL) cholesterol by 14%, while the high-density lipoprotein (HDL) remained unchanged (Kris-Etherton *et al*., 1999). A study involving a large number of nurses revealed that the group with lowest intake of linoleic acid exhibited the highest incidence of breast cancer (Eynard & Lopez, 2003).

The antioxidant and fatty acid compositions of grape seed oil and thus its nutritional and cosmetic properties may be significantly affected by the grape variety, growing conditions, oil extraction methods and degree of refining. Many researchers investigated the possibility of using supercritical CO_2 fluid extraction method to produce high-quality grape seed oil (Passos *et al*., 2009). The study by dos Santos Freitas *et al*. (2008) found that the most proper solvent for grape seed oil extraction is propane because oil samples extracted with propane present a smaller amount of free fatty acids in the oil than samples extracted with carbon dioxide. However, owing to the high cost of supercritical fluid extraction, commercial grape seed oil is mainly produced by traditional oil extraction methods such as hydraulic press and solvent extraction.

In addition to phenolic antioxidants, grape seeds also contain non-phenolic antioxidants such as tocopherols and β-carotene, both vitamins are potent antioxidants and are critical to human health. Tocopherols and β-carotene are mainly concentrated in grape seed oil (Baydar & Akkurt, 2001; Bravi *et al*., 2007). The content of tocopherols in grape seed oil ranges from 265 to 454 mg kg^{-1} depending on the extraction method, grape variety, growing location and growing conditions (Baydar & Akkurt, 2001). α-Tocopherol was the most abundant tocopherol in the oil extracts, and γ- and δ-tocopherols were found with low concentrations, while β-tocopherol was not detected in the oil extracts (Baydar *et al*., 2007). The tocopherol and tocotrienol contents of grape seeds from 14 different varieties grown in Korea were determined by Wie *et al*. (2009) using saponification extraction followed by normal-phase liquid chromatography, and it was found that the total concentration of tocopherol (T) and tocotrienol (T3) was in the range of 4.8–9.9 mg 100 g^{-1} seeds (or 35.3–68.8 mg 100 g^{-1} oil basis). The Muscat Bailey A cultivar had the highest total tocopherol and tocotrienol contents, followed by Canner and Naples. γ-T3 ranged from 1.6 to 4.9 mg 100 g^{-1} seed (11.2–53.81 mg 100 g^{-1} oil basis) and was the main isomer, followed by α-T3 in most of the samples.

Grape seeds also contain certain amount of phytosterols. Phytosterols are well known to contribute antiarteriosclerotic activity. These sterols are concentrated in the grape seed oil. The total sterol content of grape seeds was reported to be 18 530 mg per kilogram oil. Among them, b-sitosterol is the most abundant (69.80–61.54%) followed by stigmasterol (11.87–16.03%), campesterol (10.79–9.28%), and sitostanol (3.47–3.97%) (Rubio *et al*., 2009). The concentration of each sterol changes with the variety and the maturity of grapes (Beveridge *et al*., 2005). The relatively high phytosterol content may make an important contribution to the health benefit of grape seed oil.

Grape seed oil is a preferred cosmetic ingredient for damaged and stressed skin tissues. The regenerative and restructuring qualities of grape seed oil are most likely owing to its high antioxidant and sterol contents that may make it an attractive product for direct food consumption and skin care.

Grape seed protein

Relatives fewer studies were found for grape seed protein. GPs/seeds are not considered as an important protein source as legumes and nuts, although grape seeds contain 11–13% proteins (Fantozzi, 1981; Rao, 1994; Goñi *et al*., 2005). The total protein content and the amino acid composition of grape seed protein may vary significantly depending on the variety of grape, location and fertilisation conditions. Amino acid analyses of grape seed protein revealed high levels of essential amino acids, but glycine, glutamic acid and aspartic acid were the most abundant amino acids found in GSP (Rao, 1994; Zhou *et al*., 2010). The major protein component in grape seed protein isolate was found to be a globulin-link protein with subunit

molecular weights varying from 25.5 to 40.0 kDa as determined by SDS-PAGE; the isoelectric pH of grape seed protein was found to be at the acidic pH of around 3.8; grape seed protein possesses better solubility, emulsifying capacity and emulsion stability than soy protein isolate, although its foaming capacity is poorer (Zhou *et al.*, 2011). However, grape seed protein was considered as non-digestible or resistant protein (Saura-Calixto *et al.*, 1991). Inclusion of GP in the sheep diet to 55% significantly reduced digestibility of protein (Baumgärtel *et al.*, 2007). This is most likely attributed to the strong interaction between protein and tannins. The complexation between protein and tannin limited the digestibility of grape seed protein because tannin is believed to be an inhibitor of digestive enzymes (Gonçalves *et al.*, 2007; Alipour & Rouzbehan, 2010).

The digestibility of purified grape seed protein has not been reported. More research is needed to investigate whether procyanidins bound to grape seed protein can be released and absorbed in the gastrointestinal tract after eaten.

Dietary fibre in GP

Dietary fibres (DF) are defined by the Association of Official Analytical Chemists as 'the polysaccharides and remnants of plant materials that are resistant to hydrolysis (digestion) by human digestive enzymes' (Cho *et al.*, 1997). DF includes many complex substances, each having unique chemical structure and physical properties. Health benefits of dietary fibre are well documented (Slavin, 2005; Anderson *et al.*, 2009; Mann & Cummings, 2009).

It is recognised that the physiological and physicochemical effects of dietary fibres depend on the relative amount of individual fibre components, especially as regards to the soluble and insoluble fractions (Elleuch *et al.*, 2011). Ideal fibre should include a balanced ratio of soluble and insoluble fractions (1:3) (Kunzek *et al.*, 2002). Soluble fibres are characterised by their capacity to increase viscosity and to reduce the glycemic response and plasma cholesterol, protects against inflammatory bowel diseases and to act as a prebiotic to improve host health (Anderson *et al.*, 2009; Chawla & Patil, 2010). Soluble fibre can be fermented in large intestine to produce short chain fatty acids that positively affect major regulatory systems, such as blood glucose and lipid levels, the colonic environment and intestinal immune functions (Roy *et al.*, 2006; Wong *et al.*, 2006; Scholz-Ahrens *et al.*, 2007). Insoluble fibres are characterised by their high porosity, low density and ability to increase faecal bulk and decrease intestinal transit (Isken *et al.*, 2010). The insoluble cereal fibre and whole grains were reported to be strongly associated with reduced diabetes risk in prospective cohort studies, indicating that other unknown mechanisms are likely to be involved (Isken *et al.*, 2010).

Dietary fibre is often intimately associated in the plant cell structure with other organic compounds, such as vitamins, phytochemicals, etc., displaying their own biological activity (FAO, 1998). GP is a fibre and polyphenol-rich by-product of wine making. GP accounts for about 20–25% of grapes crushed for wine making (Laufenberg *et al.*, 2003) and contains up to 75% of dietary fibre and over 60% of GP dry matter that was indigestible *in vitro* (Bravo & Saura-Calixto, 1998). The indigestible GP dietary fibre includes pectin, cellulose, Klason lignin and polyphenols (Llobera & Cañellas, 2007; González-Centeno *et al.*, 2010; Deng *et al.*, 2011).

The composition of GP dietary fibre also depends on the variety of grapes and the part of pomace. The white GP had lower fibre concentrations (crude fibre, neutral detergent fibre and acid detergent fibre) than the red wine pomace (Baumgärtel *et al.*, 2007). The dietary fibre of pomace and stem of Manto Negro red grape (*V. vinifera*) was studied by Llobera & Cañellas (2007). High percentage of soluble fibre (15%) in relation to the total dietary fibre was found in the pomace, while high content of Klason lignin was found in both by-products, especially in the stem (31.6%). The Klason lignin fraction had important amounts of condensed tannins and resistant protein. González-Centeno *et al.* (2010) investigated the dietary fibre compositions of pomace and stems from ten grape (*V. vinifera* L.) different varieties (six red and four white). Both presented considerable quantities of DF, ranging from 60 to 90% of total dry matter. The cell wall polysaccharides (CWP) composition of GPs and stems were very different, pectic substances being the main component of the cell walls (40–54% of total CWP), while cellulose being the predominant CWP for the stems (40–49% of total CWP). Klason lignin accounted for around 20–25% of DF in both GPs and stems. In addition, the pectin content and the degree of methyl-esterification of uronic acids of GPs varied depending on the varieties of grapes. The study of Deng *et al.* (2011) with GP from two white wine grape varieties and three red wine grape varieties found that insoluble DF composed of Klason lignin (7.9–36.1% DM), neutral sugars (4.9–14.6% DM) and uronic acid (3.6–8.5% DM) weighed more than 95.5% of total DF in all pomace samples from five grape varieties. White GP was significantly lower in DF (17.3–28.0% DM) than those of red GPs (51.1–56.3%). Although data about the soluble and insoluble fibre contents of GP vary from study to study, there is no doubt that GP fibre is low in solubility. Owing to the large quantity generated from worldwide wine and grape juice production every year, GP has potential to serve as an important source of insoluble fibre for functional food development.

The results from a randomised, controlled parallel-group trial with thirty-four non-smoking adults show that consuming 7.5 g day^{-1} grape antioxidant dietary fibre for 16 weeks significantly reduced total cholesterol (9%), low-density lipoprotein cholesterol (9%), and systolic and diastolic blood pressures (6 and 5%, respectively) (Pérez-Jiménez *et al*., 2008). The NEP bound to grape fibre are not bioaccessible in the small intestine but can be partially released from fibre matrix in large intestine by the action of gut bacteria (Selma *et al*., 2009; Saura-Calixto *et al*., 2010; Saura-Calixto, 2011). A study with female Sprague–Dawley rats found that NEPA were partially depolymerised during their transit along the intestinal tract, as evidenced by the presence of (epi)catechin (EC) monomers and dimers in faeces and phase-II conjugates of EC in urine 24 h after ingestion of NEPA-rich diet. Moreover, NEPA were further metabolised by the intestinal microbiota into smaller metabolites including phenolic acids (Mateos-Martín *et al*., 2011). Therefore, GP fibre could serve as a carrier for transportation of some polyphenols to the large intestine. Inclusion of GP in food products could result in the functional foods with beneficial effects of dietary fibre and grape polyphenols.

Conclusion

This review illustrates that the polyphenol composition of GP has been well characterised and their biological and functional properties are also intensively studied. The mechanisms of chemoprevention, anticardiovascular disease and other disease prevention activities of grape polyphenols have been graduate revealed by researchers of different disciplines all over the world. Therefore, GP has great potential to serve as a source of functional food ingredient. To optimise the health benefits and minimise possible negative health effects of GP, more studies are needed to set the proper dose of grape polyphenols, to characterise the properties of other GP components such as the oxidative stability of grape seed oil, solubility and weight management properties of GP fibre and to evaluate the sensory quality and consumer acceptance of food products developed from GP or components of GP.

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