VALUE ADDED PRODUCTS FROM VINEYARD WASTES – A REVIEW

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1. ABSTRACT

Grape pomace is about 10% by weight of the grape input and consists of pressed skins, disrupted cells from grape pulp, seeds and stems. Grape pomace is mainly used as cattle feed or for soil conditioning or dumped in disposal sites. Cell walls of grape pomace are composed of cellulose, hemicelluloses, pectin and lignin arranged in a complex network. Lignin constitutes about 38-40% of the total grape pomace mass. Grape pomace is rich in polyphenols, fibres, tannins, tartaric acid, citric acid, anthocyanin and neutral sugars. Pomace can be separated in to marc (skin and pulp) and seeds in a breaker. Grape marc constituted mainly industrial source of anthocyanin based colorants. Grape seeds are a complex matrix containing approximately 40% fibre, 16% oil, 11% proteins and 7% complex phenols including tannins in addition to sugars and mineral salts. The seeds of the grapes contain about 8-22% edible oil and the seed oil has been identified as a potential product because of its low saturated fats content and its high concentration (70-75%) of linoleic acid. Grape pomace is rich in phenolic compounds and the interest in phenolic compounds are from their antioxidant properties and their ability to serve as free radical scavengers. Grape seed extracts are reported to possess anticancer, antiulcer, anticataract and antiarteriosclerosis effect. In conclusion grape pomace, the byproduct of wine industry has a huge potential for the isolation of compounds in food preservation as well as for nutraceuticals and therapeutic agents.

KEYWORDS: vineyard waste, grape, pomace, marc, seed, health benefits

2. INTRODUCTION

Juice pressed from grapes is used for wine making. The press residue remaining after the extraction is called grape pomace. Grape pomace consists of pressed skins, disrupted cells from grape pulp, seeds and stems. Grape pomace or winery pomace is about 10% by weight of the total grape input. A flow diagram on the process and products in wine making is presented in Fig 1. If the grapes are stripped from the stalks before processing, the residue consists of about 40% seeds and 60% skin and pulp. Winery waste pressed with the stalks is composed of about 30% stalks, 30% seeds and 40% skin and pulp. Pomace can be stored for a time by heaping and pressing, but dust formation may be a problem owing to disintegration of the pulp. The pomace may be separated into skin and seeds by loosening the pulp from the seeds in a breaker, after which a vibrating sieve separates the seed from the skin. Marc (skin and pulp) is one fraction and seeds the second fraction. It has been reported that the amount of dried skins in red grape pomace (54.9%) was higher than in white grape pomace (34.4%). Proportions of seeds in white grape pomace are reported to be between 43% and 52% (Larrauri *et al.*, 1996). Dried stem values of white grape pomace were 6.7 times higher than corresponding values of red grape pomace (Larrauri *et al.*, 1996).

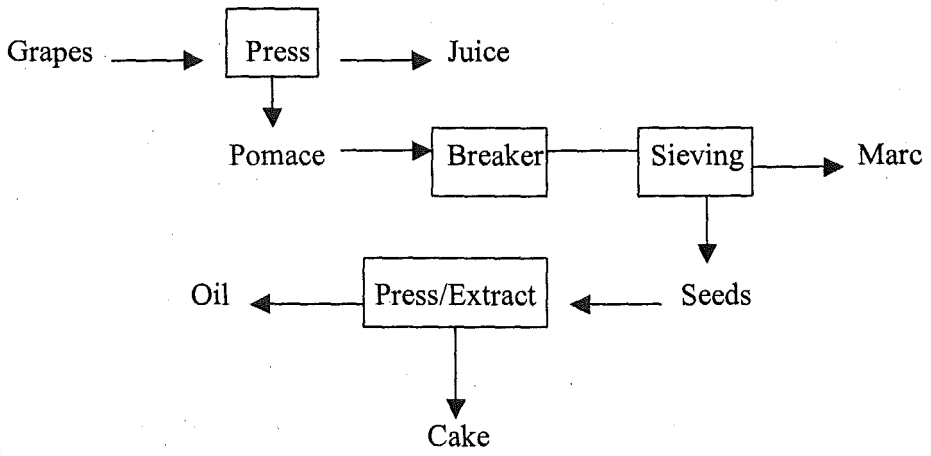

Fig 1. Flow diagram showing the processing of grapes and its wastes

Grape pomace is currently used as cattle feed or for soil conditioning or dumped in disposal sites (Mazza, 1995). It has also been reported that it is easy to make silage of winery pomace as the initial acidity is already high. Grapes have been reported to be one of the major sources of phenolic compounds (Macheix *et al.*, 1990) while grape seeds and skins are also reported to be rich in phenols (Sigleton and Esau, 1969). Research work is being carried out to seek industrial uses of grape pomace, including isolation of nutritive ingredients, dietary fibre, production of citric acid, tartaric acid, ethanol, grape seed oil, natural food colourants and compounds of therapeutical use.

Grape pomace is a rich source of polyphenols. Grape pomace contains both extractable and non extractable polyphenols. It has been reported that the almost all of the insoluble polyphenols were undigested and 32% of the ingested extractable polyphenols was recovered in faeces showing that major part of extractable polyphenols (68%) was digested in the test conducted in rats (Martin-Carron *et al.*, 1997). Extractable polyphenols in white grape pomace and seeds were higher than the corresponding value of red grape pomace. Catechin values are about 50% of extractable polyphenols for both white grape pomace and red grape pomace and their fractions (Larrauri *et al.*, 1996). Grape pomace constitutes a variety of polyphenols, which include phenolic acids (gallic acid, its 3- and 4-β-glucopyranosides, trans-caftaric acid, cis- and trans-coutaric acids), phenolic alcohol (2-hydroxy-5-(2-hydroxyethyl)phenyl-β-glucopyranoside), flavan-3-ols (catechin, epicatechin and procyanidin B1) and flavonoids (quercetin 3-glucoside and 3-glucoronide, kaempferol 3-glucoside and 3-galactoside, eucryphin, astilbin and engeletin) (Lu and Foo, 1999). The methanol extract (80%) of grape pomace is reported to be dominated by flavonoids and Table 1 represents its fractionation by column chromatography on a Diaion HP-20 column with different ratio of methanol (Lu and Foo, 1999).

Table 1. Composition of polyphenols in HP-20 chromatographic fraction

Fraction	Eluent	Main constituents
A	H_2O	Sugars
B	0-40% Methanol	Sugars and polyphenolic acids
C	40-60% Methanol	Flavan-3-ols
D	60-80% Methanol	Flavonoids
E	50-60% Dimethyl ketone	Oligomeric procyanidins

Tartaric acid is another potential product from grape pomace. Nurgel and Canbas (1998) reported that hot water extraction is simpler and more efficient compared to dilute acid extraction for tartaric acid from the grape pomace. The tartarate contents of pomace in dry base of Emir, Okuzgozu and Bogazkere were reported to be 4.5%, 8.07% and 13.2% respectively (Nurgel and Canbas, 1998). Amerine *et al.* (1967) reported that white wine pomace contained 4.2 to 11.1% and red wine pomace contained 11.1 to 16.1% tartarate.

Grape pomace presents a high content of dietary fiber with appreciable amounts of polyphenolic substances and resistant proteins associated with the polysaccharidic matrix of the fiber (Saura-Calixto *et al.*, 1991). Dietary fiber estimated using the AOAC enzymatic-gravimetric method (Prosky *et al.*, 1992). Martin-Carron *et al.* (1997) reported that grape pomace is a high dietary material containing both soluble and insoluble polyphenols in which the major part of the soluble polyphenol is bioavailable while insoluble condensed tannins are quantitatively recovered in faeces. The content of the chemical composition of the dietary fiber fractions of grape pomace was reported by Valiente *et al.* (1995) and presented in Table 2. 80% of dry matter was analysed as total dietary fiber (TDF) and insoluble fiber (IDF) was the main fraction. It has also reported that 90% of the neutral sugars were in IDF but the pectic substances were equally distributed in both IDF and soluble fiber fraction (SDF). Chemical composition and amount of dietary fiber fractions of deseeded grape pomace is presented in Table 3 (Valiente *et al.*, 1995). Valiente *et al.* (1995) also stated that the composition of dietary fiber fractions enables grape pomace to be considered as a useful fiber-rich food ingredient.

2.1 Grape Marc

The plant cell walls of grape skin are composed of cellulose, hemicelluloses, pectin and lignin arranged in a complex network. Lignin is the major phenolic constituent of cell walls (Tucker and Mitchell, 1993). Lignin constitutes about 38-40% of the total grape pomace mass (Saura-Calixto *et al.*, 1991; Valiente *et al.*, 1995). It has been reported that lignin is composed of hydrophobic polymers derived from p-coumaryl, coniferyl and sinapyl alcohols (Brett and Waldron, 1996). Lignin is deposited together with tannins (procyanidins), simpler flavonoids and hydroxycinnamic acids (mainly p-coumaric acid and ferulic acid) but due to the no enzymatic nature of the polymerization, the pattern is irregular (Brett and Waldron, 1996). Lignin has been covalently linked to polysaccharides via sugar residues or via phenolic acids esterified to polysaccharides (Macheix *et al.*, 1990; McDougall *et al.*, 1996). Lecas and Brillouet (1994) reported that the dominant structural cell wall polysaccharide in the grape pomace skin fraction is cellulose (approx 50%). Valiente *et al.* (1995) reported that neutral sugars, uronic acids, Kalson lignin, protein and ash were the constituents of marc and presented in Table 3.

Table 2. Chemical composition and amount of dietary fiber fractions of grape pomace (% dry matter)

Components	Dietary Fiber		
	IDF	SDF	TDF
Gravimetric values	68.36	9.53	77.89
Neutral sugars			
Rhamnose	0.28	0.20	0.48
Fucose	1.40	0.17	1.57
Arabinose	0.83	0.40	1.23
Xylose	1.03	0.07	1.10
Mannose	1.09	0.24	1.33
Glucose	11.34	0.19	11.53
Galactose	1.00	0.26	1.26
Total	16.97	1.53	18.50
Uronic acid	2.80	2.73	5.53
Kalson lignin	38.33	-	38.33
Total dietary fiber	58.10	4.26	62.36
Protein	6.93	0.50	7.43
Ash	5.77	3.07	8.84

Table 3. Chemical composition of marc

Components	% (Dry matter)
Neutral sugars	
Rhamnose	0.50
Fucose	1.24
Arabinose	2.07
Xylose	1.70
Mannose	1.52
Glucose	14.01
Galactose	1.60
Total	22.64
Uronic acids	5.45
Kalson lignin	53.64
Protein	10.72
Ash	8.77

It was estimated that about 90% of grape marc generated by the wine industry is collected for distillation processing into alcohol. Grape marc has a lower fibre content than pomace and has been used for feeding horses in proportions of up to 10% of the ration. It has also been reported that the digestibility can be increased by soaking the marc in hot (90°C) water for about 20 minutes to remove the tartarates.. Grape marc is also a major source of polyphenols, tannins and anthocyanins. Thiolysis of the total skin tannin extract released the same compounds as those from seeds plus an additional one corresponding to the benzylthioether of (-)-epigallocatechin (Labarbe *et al.*, 1999). Souquet *et al.* (1996) reported that skin tannins are proanthocyanidins containing both procyanidin and prodelphinidin units. It has been reported that the yield from the thiolysis degradation on these proanthocyanidins was 67% (Labarbe *et al.*, 1999). Until now, grape marc has constituted the main industrial source of

anthocyanin based colorants, commercially produced in Italy since 1879 (Girard and Mazza, 1998). With an annual world production of 65 million tonnes, grape marc constitutes very abundant and relatively inexpensive source of anthocyanins (Francis, 1989; Timberlake, 1986; Mazza, 1995). The colour stability of anthocyanins depends on a combination of factors such as structure and concentration of anthocyanins, pH, temperature and presence of complexing agents (phenols, metal ions) (Markakis, 1982).

2.2 Grape Seeds

Grape seeds are a complex matrix containing approximately 40% fiber, 16% oil, 11% proteins and 7% complex phenols including tannins in addition to sugars and mineral salts. Grape seeds are rich source of monomeric phenolic compounds such as (+)-catechins, (-)-epicatechin and (-)-epicatechin-3-*O*-gallate and dimeric, trimeric and tetrameric procyanidins (Saito *et al.*, 1998). Yamaguchi *et al.* (1999) reported that grape seed extracts extracted with aqueous ethanol are rich in polyphenols and the composition is presented in Table 4.

Table 4. Analytical data of grape seed extracts

Components	%
Proanthocyanidins	38.7
Monomeric flavanols	2.40
Moisture (by Karl Fischer's method)	2.10
Crude protein (total N x 6.25)	3.70
Fat (method with soxhelt extractor)	0
Fiber	0
Ash	5.00
Glucose	7.79
Fructose	8.85
Other sugars	2.66
Organic acid	11.60
Other acids	5.05
Other components from grape seeds	11.99

Studies have been reported of the procyanidin composition of grape seeds (Lee and Jaworski, 1987). The predominant compounds reported are hexamers, but only the structure of some dimer and trimer procyanidins and their acylated derivatives have been elucidated. Lee and Jaworski (1990) reported that all the acylated procyanidins found in grape seeds are esters of gallic acid. The major compounds reported from *V. Vinifera* grape seeds are (+)-catechin (11%), epicatechin-(4β→8)-epicatechin (dimer B2) (6%), (-)-epicatechin (10%), epicatechin 3-*O*-gallate-(4β→8)-catechin (B1-3-*O*-gallate) (7%) and (-)-epicatechin-3-*O*-gallate (9%). Fuleki and Ricardo da Silva (1997) have reported monomers of (+)-catechin, (-)-epicatechin and (-)-epicatechin-3-*O*-gallate, 14 dimeric, 11 trimeric procyanidins and one tetrameric procyanidin from grape seeds. Largest proportion of flava-3-ols is found in oligomeric or polymeric forms and not in monomeric forms (Waterhouse and Walzem, 1998). The yields obtained by using various extractants and their composition of total flavanols and monomeric flavanols are presented in Table 5 and 6 (Jayaprakasha *et al.*, 2001). Pekic *et al.* (1998) reported that ethyl acetate: water (90:10) selectively extracts proanthocyanidins and the use of this extractant can significantly simplify the separation of proanthocyanidins on preparative and industrial scales.

Supercritical fluid extraction (SFE) allows for the isolation of compounds without interference from air and light thereby guaranteeing conservation of their antioxidant properties. Tipsrisukond *et al.* (1998) reported that antioxidant activity was higher in extracts obtained by SFE as compared to extracts produced by conventional methods. This is because while using SFE, CO_2 is used, as the extracting fluid extraction conditions are gentler compared to other methods which avoids the degradation of labile compounds (Cheung *et al.*, 1998). Palma and Taylor (1999) reported that by increasing the polarity of the supercritical fluid using methanol as a modifier of CO_2 it was possible to fractionate the extracted compounds. With CO_2 only the fraction contained mainly fatty acids, aliphatic aldehydes and sterols while extraction using methanol modified CO_2 contained mainly phenolic compounds, catechin, epicatechin and gallic acid. Sterols have been isolated by SFE from aqueous samples (Jayasinghe *et al.*, 1998) and also reported in seed oils (Yildiz *et al.*, 1998; Tsankis, 1998) and in grape juice (Ng and Hupe, 1998).

Table 5. Yield of grape seed extracts

Solvents used for extraction	Extract yield (% dry grape seeds)
Acetone	6.7
Methanol	8.1
Ethyl acetate	4.5
Ethyl acetate: Water (9:1)	2.0
Ethyl acetate: Water (17:3)	2.5
Ethyl acetate:Water (4:1)	3.1

Table 6. Composition of grape seed extracts (%) (in catechin equivalents/100g extract)

Component	Acetone extract	Ethyl acetate extract	Methanol extract	Ethyl acetate:Water (9:1) extract	Ethyl acetate:Water (17:3) extract	Ethyl acetate: Water (4:1) extract
Monomeric flavanols	11.5	22.5	10.5	24.8	30.2	29.7
Procyanidins	3.5	12.5	5.5	18.2	23.8	20.8
Total Flavanols	15.0	35.0	16.0	43.0	54.0	50.5

Grape seed oil and tannin are the other two potential products from grape seeds. Grape seeds are considered to be a valuable by product for oil extraction (Kamel and Dawson, 1985) and seed hulls have been used for production of tannins (Pruthi, 1971). Seeds are reported to contain about 8-22% edible oil, which can be either pressed or extracted. Grape seed oil has been identified as a potential product, notable for its low saturated fats content and its high concentration (70-75%) of linoleic acid (Moret *et al.*, 2000). Its use as frying oil, combined with its value in low cholesterol diets means a potential market exists for the oil. Recovery of oil from grape seeds involves drying the pomace, separation of seeds and extraction of the oil by pressing the seeds or by grinding the seeds and then extracting the oil with solvents (Amerine *et al.*, 1980). Crude grape seed oil is then refined and dewaxed. Acetone is a selective solvent for oil and polycyclic hydrocarbons extraction and prevents wax, which is present in grape seed oil in high concentrations. It was reported that grape seed oil contains

high amounts of tannins, in levels 1000-fold higher than in other seed oils (Rao, 1994). Elshami *et al.* (1992) isolated campesterol, stigmasterol and sitosterol from grape seed oil by organic solvent extraction. Some research has been published on the supercritical extraction of oil from grape seeds (Sovova *et al.*, 1994; Molero-Gomez *et al.*, 1996). The seed oil cake has no feed value as it is not only too fibrous but also contains tannic acid. However it has been used a carrier for molasses in cattle feed.

2.3 Health Benefits

Major studies on the health benefits are reported in grape seeds. Grape seeds are rich source of monomeric phenolic compounds such as (+)-catechins, (-)- epicatechin and (-)-epicatechin-3-*O*-gallate and dimeric, trimeric and tetrameric procyanidins and these compounds possess antimutagenic properties (Saito *et al.*, 1998). Health benefits of catechins and procyanidins led to the use of grape seed extract as a dietary supplement (Laparra *et al.*, 1979). Jayaprakasha *et al.* (2001) reported that various grape seed extracts showed 65-90% antioxidant activity and a good reducing power, at 500 μg/ml concentration by potassium ferricyanide reduction method and stated that these properties of grape seed extracts may be exploitable for the preservation of food products as well as for health supplements and nutraceuticals.

Grape seed extracts have been reported to have a broad range of pharmacological activities among others including antiulcer properties (Saito *et al.*, 1998) and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) induced lipid peroxidation and DNA fragmentation in hepatic and brain tissues (Bagchi *et al.*, 1998). It has also been reported that grape seed extracts possess anticataract effect (Yamakoshi *et al.*, 1998), anticancer effect (Arii *et al.*, 1998) and antiarteriosclerosis effect (Yamakoshi *et al.*, 1998) *in vivo*. Meyer *et al.* (1997) reported that grape seed extracts exhibited properties against the oxidation of low density lipoproteins. Most of these properties are attributed mainly from the phenolic compounds. It has been reported that the interest in phenolic compounds are from their antioxidant properties (Vinson *et al.*, 1995) and their ability to serve as free radical scavengers (Maffei-Facino *et al.*, 1994). The most abundant phenolic compounds isolated from grape seed are catechins and their polymers (Fuleki and da Silva, 1997; Escribano-Bailon *et al.*, 1992).

Complex phenols including tannins (CPT), a high variety of natural phenolic compounds present in grape seeds proanthocyanidins (PA) form an important subgroup. The fundamental structural unit in PA subgroup is the phenolic flavan-3-ol (catechin) unit linked principally through the 4- and 8-positions. CPT is reported to be efficient free radical scavengers and could inhibit lipid peroxidation (Rice-Evans *et al.*, 1996; De Freitas *et al.*, 1998). The precise mechanism *in vivo* is still not completely explained but dietary intake of these natural antioxidants is inversely related to the risk of coronary heart disease and certain forms of cancer (Okuda, 1993; Haslam, 1996; Scott, 1997). Pataki *et al.* (2002) reported recently that grape seed proanthocyanidins have cardioprotective effects against reperfusion-induced injury via their ability to reduce or remove directly or indirectly, free radicals in myocardium that is reperfused after ischemia.

The protective abilities of a novel IH636 grape seed proanthocyanidin extract, ActiVin (InterHealth Nutraceuticals, Inc., Benica, California) against biochemically generated superoxide anion, hydroxyl radicals and peroxyl radicals (Bagchi *et al.*, 1997; Sato *et al.*, 1999). ActiVin is a natural extract of approximately 54% dimeric, 13% trimeric and 7%

tetrameric proanthocyanidins and 8% monomeric biflavonoids. It has also been reported that ActiVin possess greater free radical scavenging properties than Vitamin C and E alone or in combination (Bagchi *et al.*, 1997; Sato *et al.*, 1999; Bagchi *et al.*, 1999; Bagchi *et al.*, 1998; Joshi *et al.*, 1999). In an *in vivo* model ActiVin showed better protective action against oxidative stress than vitamins C, E and β-carotene (Bagchi *et al.*, 1999; Bagchi *et al.*, 1998; Ray *et al.*, 1999). Banerji and Bagchi (2001) suggested that ActiVin might also serve as a therapeutic agent in the management of chronic and relapsing pancreatitis.

3. CONCLUSIONS

In conclusion grape pomace is a rich source of polyphenols, natural pigments, dietary supplement and compounds of high therapeutic value. It appears that a huge potential for the development of high value products like edible oil, nutraceuticals and medicinals from grape pomace a byproduct of grape industry.

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