Review article

Potential uses and applications of treated wine waste: a review

Ioannis S. Arvanitoyannis,[1]* Demetrios Ladas[2] & Athanasios Mavromatis[2]

1 Department of Agriculture, School of Agricultural Sciences, Animal Production and Aquatic Environment, University of Thessaly, Fytokou Street, 38446 Nea Ionia Magnesias, Volos, Hellas, Greece
2 Department of Agriculture, School of Agricultural Sciences, Crop Production and Agricultural Environment, University of Thessaly, Fytokou Street, 38446 Nea Ionia Magnesias, Volos, Hellas, Greece

(Received 17 March 2005; Accepted in revised form 19 September 2005)

Summary Recently, there has been an upsurge in the exploitation of the waste materials generated by the wine industry. Wine waste is characterised by the presence of natural antioxidants much safer than synthetic antioxidants. Wine waste-derived antioxidants have been recently used in the food industry. Moreover, wine waste can be potentially used as soil conditioner, as adsorbent for heavy metals, for fertiliser and for pullulan production. This review aims at presenting the most important and economically viable applications of treated wine waste.

Keywords Adsorbents, fertiliser production, natural antioxidants, wine waste.

Introduction

Nowadays, there is a growing interest in the exploitation of the residues generated by the wine industry. In particular, winery wastes could be an alternative source for obtaining natural antioxidants, which are considered completely safe in comparison with synthetic antioxidants. Moreover, wine waste can be potentially used as soil conditioner or for fertiliser production. Furthermore, in this review, their utilisation as adsorbent and for pullulan production is described. Table 1 summarises both uses and end by-products of winery wastes.

Grape pomace represents a rich source of various high-value products such as ethanol, tartrates and malates, citric acid, grape seed oil, hydrocolloids and dietary fibre. Moreover, grape pomace is characterised by high-phenolic contents because of poor extraction during winemaking, making their utilisation worthwhile and thus supporting sustainable agricultural production. (Kammerer *et al.*, 2004).

In recent years, the use of grape seed extracts (GSE) has gained ground as a nutritional supplement in view of its antioxidant activity (Gonzalez-Paramas *et al.*, 2004). The by-products obtained after winery exploitation, either seeds or pomaces, constitute a very cheap source for the extraction of antioxidant flavanols, which can be used as dietary supplements, or in the production of phytochemicals, thus providing an important economic advantage (Alonso *et al.*, 2002; Negro *et al.*, 2003; Gonzalez-Paramas *et al.*, 2004).

Extensive research has demonstrated that many biodegradable organic wastes can be composted in a convenient and economical way. Composting of organic matter is a simple and efficient manner of transforming agro-industrial wastes into the products suitable for use as soil conditioners (Ferrer *et al.*, 2001). Different substrates such as tomatoes waste, cork residues, olive husks and tannery sludge for composting resulted in end-products adequate as organic fertilisers in terms of their physical–chemical characteristics (Vallini *et al.*, 1983).

*Correspondent: Fax: +30 24210 93157;
e-mail: parmenion@uth.gr

doi:10.1111/j.1365-2621.2005.01111.x

Table 1 Treatment of grape wastes, physicochemical properties and their use

No	Final products	Treatment	Physicochemical characteristics	Uses	References
1	Grape waste	Composting of grape waste and hen droppings	Organic matter content	Fertiliser for corn seed	Ferrer *et al.* (2001)
2	Grape seed and skin extracts	Fractionation of grape seed and skin extracts from grape waste	Phenol content	Dietary supplements for disease prevention	Shrikhande (2000)
3	Grape waste	Gasification of waste products from grape	Concentrations of unused residues	Gas production for heating purpose	Blasi *et al.* (1997)
5	Pressed grape skin	Composting of solid waste and wastewater	Organic matter content	Fertiliser	Manios (2004)
6	Wine pomace and grape seeds	Lyophilisation and extraction of flavanols	Flavanol content	Dietary supplements, production of phytochemicals	Gonzalez-Paramas *et al.* (2004)
7	Grape marcs, stalks and dregs	Lyophilisation and extraction of polyphenols	Polyphenolic content	Dietary supplements	Alonso *et al.* (2002)
8	Grape skins, seeds and stems	Acidolysis of a polymeric proanthocyanidic fraction of grape pomace in the presence of cysteamine	Flavanol content	Source of flavanols	Torres & Bobet (2001)
9	Grape seed extract (GSE)	Pre- and post-mortem use of grape seed in feeding experiment	Phenol content	Feedstuff for dark poultry meat	Lau & King (2003)
10	Grape skin pulp	Fermentation by *Aureobasidium pullulan*	Ethanol precipitate	Pullulan production	Israilides *et al.* (1998)
11	Grape seeds	Solid-state cultivation by *Trametes hirsuta*	Lignocellulosic content	Laccase production	Moldes *et al.* (2003)
12	Grape pomace	Solid-state cultivation by *Pleurotus* sp.	Pruning content, high phenolic components and total sugars	Feedstuff for animals	Sanchez *et al.* (2002)
13	Wastewater	Electrodialysis	Tartaric acid content	Additive in medicines and cosmetics, acidulant compound in soft drinks	Andres *et al.* (1997)
14	Wastewater	Electrodialysis at 60 °C	Tartaric acid and malic acid content	Food and pharmaceutical industries	Smagge *et al.* (1992)

Exclusive addition of chemical fertilisers is no longer considered the best method to feed the plant and keep the plant pathogens under control. Growers understand that they must add some type of organic material to soil, whether it is compost or another type of organic amendment. This organic matter increases microbial biomass and helps maintain these beneficial bacterial and fungi populations (http://www.jgpress.com/BCArticles/2000/070030.html). Moreover, there are economic benefits, as the use of residues means lower costs than those pertaining to conventional materials, with consequent improved competition between the user companies (Abad, 1991).

In the case of winery wastes, Diaz *et al.* (2002) report that the grape marc, a primary waste of wine production, could be recycled as a soil conditioner in view of its organic and nutrient contents. Moreover, a comparison of the best compost obtained from winery wastes with those from other organic wastes showed that its chemical values fell within the same range in most cases, with the exception of a high-calcium value owing to the nature of wine-making process (Soliva & Felipo, 2002; Bertran *et al.*, 2004).

Winery waste sludge was shown to be an effective adsorbent for the adsorption of heavy metals from aqueous solutions. Metal sorption consists of several mechanisms that quantitatively and qualitatively differ according to the metal species in solution and the origin and processing of the sorbent (Villaescusa *et al.*, 2004; Yuan-shen *et al.*, 2004). It is noteworthy the fact that the properties of winery waste are similar to those of other adsorbents, providing it with the ability to adsorb heavy metals (Yuan-shen *et al.*, 2004).

Grape skin pulp should be considered as the best substrate for pullulan production (Israilides *et al.*, 1998). Hot water extracts of the pulp can serve as a good substrate for fermentation with *Aerobasidium pullulans* for the production of pullulan (Arapoglou *et al.*, 2002). Moreover, it was shown that the pullulan produced from winery waste was of high-molecular weight (4.22×10^6) and rather pure as determined by its gel elution profile, glucose content and the number of residues in repeating units (Israilides *et al.*, 1994).

Main applications/constituents to be exploited

Polyphenol content

The phenolic compounds of wine, and particularly the flavanols (e.g. catechins, proanthocyanidins), have been the centre of attention of recent studies as their relation to the beneficial effects attributed to a moderate consumption of wine was observed. (Renaud & De Lorgeril, 1992; Shrikhande, 2000). These compounds have their origin in grape, and only a part of them is transferred to the must. Their extractability mainly depends on the employed technological conditions during vinification (Kammerer *et al.*, 2004). For this reason, important quantities of phenolic compounds still remain in the wine by-products and there is great interest in the exploitation of this type of grape by-products to obtain potentially bio-active phenolic compounds (Santos-Buelga & Scalbert, 2000; Moure *et al.*, 2001; Ray *et al.*, 2001).

The 'French paradox' initiated numerous studies focusing on the antioxidative and health-promoting effects of plant secondary metabolites in grapes and wine and revealing the inhibition of human low-density lipoprotein oxidation by grape and wine phenolics (Frankel *et al.*, 1995; Teissedre *et al.*, 1996; Kammerer *et al.*, 2004). Furthermore, free radicals have been implicated in over a hundred diseases in humans, including arthritis, atherosclerosis, advancing age, Alzheimer and Parkinson's diseases, gastrointestinal disfunctions, tumour promotion and carcinogenesis and AIDS among others. Antioxidants are potent scavengers of free radicals and serve as inhibitors of neoplastic processes (Alonso *et al.*, 2002). Moreover, antioxidants have an intense anti-inflammatory activity (Miyake *et al.*, 1999).

Natural flavonoids can donate hydrogen to and/or react with superoxide anions, hydroxyl radicals and lipid peroxyl radicals, all of which can cause lipid oxidation *in vitro*, leading to LDL oxidation implicated in the development of atherosclerosis (Shrikhande, 2000).

It is noteworthy that the winery residues could be an alternative source for obtaining natural antioxidants, and are considered completely safe in comparison with synthetic antioxidants such as butylated-hydroxyanisole (BHA) and butylated-hydroxytoluene (BHT), compounds now largely used in the food industry with undesirable effects on the enzymes of human organs (Nakatani, 1997).

Because of these beneficial properties, winery by-products are now being sold to the rapidly growing dietary supplement industry. Table 2 provides an idea of the various grape-based products of different origin currently available in the US market. Table 2 shows that the grape seeds stand for the most popular by-products of winery wastes in the most representative market of the world. Grape seed oil derives from the grape seeds that are left in abundance from the wine-making process . In a large survey published in 1993, Nash showed that in a sample group of 56 men and women consuming up to 3.81 cm day^{-1}, an amount that one can cook with, grape seed oil had the ability to raise high density lipoproteins (HDL) levels by 13% and reduce LDL levels by 7% in 3 weeks. The total cholesterol/HDL ratio was reduced 15.6% and the total LDL/HDL ratio was reduced by 15.3%, which could be significant for those at risk of heart attack.

Grape seed oil contains vitamin E (80–120 mg per 100 g), vitamin C, beta-carotene, 0.8–1.5%

Table 2 Number of grape-based by-products of health relevance in the USA (Adapted from Shrikhande, 2000)

Grape seed	22
Grape extract	5
Red wine powder	7

unsaponifiables rich in tocopherols, steroids (campesterol, beta-sitosterol and stigmasterol) and several fatty acids, which are reported in Table 3 (http://en.wikipedia.org/wiki/Grape.seed.oil).

Furthermore, it is noteworthy that grape seeds contain flavan-3-ols and its repeat unit including dimers, trimers, etc., and much larger polymers, perhaps with a degree of polymerisation from seven to sixteen (Haslam, 1980). The structure of seed extract is very complex, but reverse phase HPLC provides separation of major groups such as monomers, procyanidin oligomers and polymers (Prieur *et al.*, 1994). Moreover, Aaby *et al.* (2004) reported that flavanoids are UV absorptive and have traditionally been analysed by HPLC with UV/visible detectors. Figure 1 represents the relative phenolics in a GSE.

However, concentration of phenols in commercial preparations is adequately represented by total phenols by the Folin-Ciocolteau procedure and expressed as gallic acid equivalent (Singleton & Rossi, 1965). The concentration of total phenolic substances, total flavanoids and their different classes are illustrated in Table 4.

As can be seen from Table 4, the quantity of total phenolic substances and total flavonoids contained in the GSE was higher than that obtained from the peel and the marc as such. Moreover, Shrikhande (2000) reported that the total phenol ranges from the mid-40s to mid-90s which clearly indicates that higher numbers are the result of greater purification of phenols from other seed substances, possibly requiring multiple solvent extractions and precipitations. Such processes are expensive, less flexible and provide lower yields.

Alonso *et al.* (2002); Gonzalez-Paramas *et al.* (2004) and Negro *et al.* (2003) have published specific studies, regarding the antioxidant activity of winery wastes. A general diagram based on experimental data obtained by Gonzalez-Paramas *et al.* (2004) regarding to the antioxidant activity of GSE (winery wastes) is shown in Fig. 2. Figure 2 illustrates the antioxidant capacity of the extracts at different concentrations obtained from grape seed. All the extracts had an antioxidant capacity and this rose with an increase in concentration of phenolic substances until it reached the highest concentration determined (160 ppm) (Negro *et al.*, 2003).

Table 3 Average composition of grape seed oil fatty acids (http://en.wikipedia.org/wiki/grape.seed.oil)

Common name	Acid name	Average percentage range
Omega-6	Linoleic acid	69–78
Omega-9	Oleic acid	15–20
Palmitic acid	Hexadecanoic acid	5–11
Stearic acid	Octadecanoic acid	3–6
Omega-3	A-Linolenic acid	0.3–1
Palmitoleic acid	9-Hexadecenoic acid	0.5–0.7

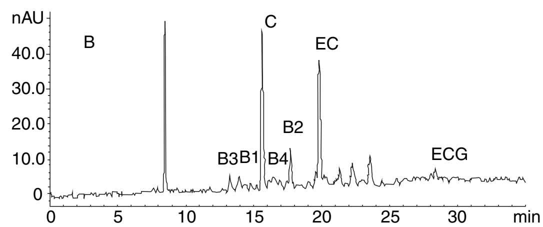

Figure 1 Relative phenolics in a grape seed extract (GSE) by HPLC. C, catechin; EC, epicatechin; ECG, epicatechin-3-*O*-gallate; and proanthocyanidin dimers B1 (EC-C), B2 (EC-EC), B3 (C-C) and B4 (C-EC) (Adapted from Gonzalez-Paramas *et al.*, 2004).

Table 4 Quantity of total phenolic substances, total flavanoids and proanthocyanidins reported as g L^{-1} of grape extract and gram per 100 g d.m. in grape seeds (Adapted from Negro *et al.*, 2003)

Total phenols (GAE)
2.86 ± 0.01 g L^{-1}
8.58 ± 0.03 g per 100 g d.m.
Total flavanoids (CE)
2.79 ± 0.01 g L^{-1}
8.36 ± 0.04 g per 100 g d.m.
Proanthocyanidins (CyE)
1.38 ± 0.06 g L^{-1}
5.95 ± 0.17 g per 100 g d.m.

GAE, gallic acid equivalent; CE, catechin equivalent; ME, cyanidin equivalent.

Furthermore, it is noteworthy that GSE is reputed to have antioxidant activity when fed to animals (Tebib *et al.*, 1997). Grape seed tannins or proanthocyanidins were shown to have a hypocholesterolemic, antiatherosclerotic, and antioxidant effect *in vivo* when fed to rats receiving diets with cholesterol (Tebib *et al.*, 1994, 1997;

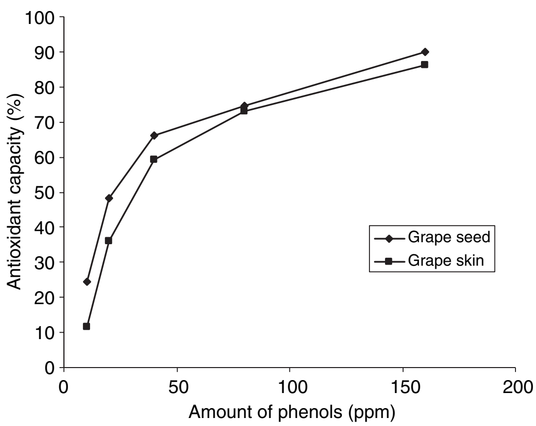

Figure 2 Antioxidant capacity (%) of the extracts from grape seeds and grape skin extracts (Gonzalez-Paramas *et al.*, 2004: Alonso *et al.*, 2002: Negro *et al.*, 2003).

Yamakoshi *et al.*, 1999). Also, addition of grape seed proanthocyanidins (GSPC) to a system containing polyunsaturated fatty acids and mice liver or brain microsomes inhibited oxidation by UV light peroxidation (Bouhamidi *et al.*, 1998).

Apart from GSEs, the grape skin extracts are also being marketed because of their complex structure consisting of anthocyanins and procyanidins (Shrikhande, 2000). Yamakoshi *et al.* (1999) suggested that procyanidins might react with reactive species in plasma and interstitial fluid of the arterial wall, thereby inhibiting oxidation of LDL and displaying an antiatherosclerotic activity.

Anthocyanins are a group of phenolic compounds that belong to the flavanoid family. They are responsible for the colouration (orange, rose, red, violet and blue) of the petals of flowers and fruit of a great variety of plants (Strack & Wray, 1989). There are numerous sources of anthocyanins but the main raw material is the pomace from the red wine vinification process. After the first isolation of enocyanin, dating from 1879, many authors have developed patents for the production of concentrated aqueous solutions of anthocyanins for food use (Marakakis, 1982). Nowadays, the European Union allows the use of the anthocyanins as food dyes in drinks, marmalades, candies, ice creams and pharmaceutical products (EU, 1994). The contents of individual anthocyanins in the grape seeds from several varieties of *Vitis vinifera* are summarised in Table 4.

Table 5 shows that malvidin 3-*O*-glucoside is the predominant compound, mostly followed by peonidin 3-*O*-glucoside. On the contrary, malvidin 3-*O*-glucoside, delphinidin 3-*O*-acetglucoside, petunidin 3-*O*-acetglucoside, peonidin 3-*O*-acetglucoside and peonidin 3-*O*-acetglucoside are represented in lower values.

It is evident from Fig. 2 that an increase in the amount of phenols leads to an increase of the antioxidant capacity. Moreover, Negro *et al.* (2003) found by testing the grape skin extracts at 160 ppm that it is possible to obtain high antioxidant capacity ranging from 11.7–86.3%.

It can be concluded that wine and other products derived from the grape have a high antioxidant capability and as consequence of this, they are endowed with potential health benefits. Both grape skin and seed extracts stand for a valuable solution for the recovery of antioxidant compounds and a significant advance in maintaining the environmental equilibrium, because in grape- and wine-producing zones large quantities of residues are generated, and this presents problems of storage, conversion or elimination, in both ecological and economic terms.

Compost-fertiliser

The production of healthy, uniform plants is a basic requirement of modern greenhouse agriculture. Container media must be homogeneous,

Table 5 Anthocyanin content (mg kg^{-1} d.m.) of grape skins. (Adapted from Kammerer *et al.*, 2004)

Compound	Value (mg kg^{-1} d.m.)
Delphinidin 3-*O*-glucoside	68–5552
Cyanidin 3-*O*-glucoside	37–1903
Petunidin 3-*O*-glucoside	65–6680
Peonidin 3-*O*-glucoside	515–12 450
Malvidin 3-*O*-glucoside	1117–50 981
Delphinidin 3-*O*-acetglucoside	392–956
Petunidin 3-*O*-acetglucoside	545–1375
Peonidin 3-*O*-acetglucoside	1371–1484
Peonidin 3-*O*-acetglucoside	45–8688
Cyanidin 3-*O*-coumaroylglucoside	374–1071
Petunidin 3-*O*-coumaroylglucoside	974–2458
Peonidin 3-*O*-coumaroylglucoside	68–6828

aerated, reproducible and pathogen-free. Growers, in an attempt to meet the need for a substrate with proper air and water capacity, use many types of organic and inorganic materials. Increasing demand and rising costs for peat, used as a substrate in horticulture, have led to a search for high quality and low-cost composts derived from organic wastes such as winery wastes (Inbar *et al.*, 1986, 1988).

The effort to decrease agricultural income may come from the expansion of integrated production systems and organic farming. This approach will provide certified agricultural products, which will be both beneficial to consumer's health, and attract higher financial premiums. However, this will require the gradual replacement of chemical fertilisers, currently extensively used in agriculture production, with organic soil amendments (Bazzoffi *et al.*, 1998; Navas *et al.*, 1998; Edwards *et al.*, 2000; Querejeta *et al.*, 2001).

The use of compost, derived from winery wastes, in vineyards is of growing interest because of the general poverty of soils, typified by low levels of humus and their exposure to erosion (Balanya *et al.*, 1994). Graefe (1980) proposed the use of composted winery wastes as a high-grade organic fertiliser, while recovering heat and CO_2, which are produced during the composting process. The application of compost from winery wastes increases the percentages of organic matter, nutrient levels (providing a slow fertilisation action over a long-period time), microbial biomass and improves the soils physical properties (aeration, water-holding capacity, etc.) (Ribererau-Gayon & Peybaud, 1982).

Moreover, grape waste was subjected to composting studies in several countries: France (Faure & Deschamps, 1990; Faure, 1991), Germany (Dittmer *et al.*, 1990), Spain (Costa *et al.*, 1989; Garcia *et al.*, 1990, 1992), Yugoslavia (Stojanovic *et al.*, 1989), Israel (Mandelbaum *et al.*, 1988) and Venezuela (Ferrer *et al.*, 1993; Sanchez & Ferrer, 1994).

Regarding combinations of winery wastes with other materials, Ingelmo *et al.* (1998) investigated the feasibility of using composted municipal solid wastes (MSW), sewage sludge and other organic wastes to produce alternative substrates for ornamental plants and to improve the re-vegetation of a closed landfill with satisfactory results. Ferrer *et al.* (2001) reported the combinations of recently compressed grape waste and hen droppings used as an organic fertiliser for 20-day corn. In this study, satisfactory results were observed when these materials were applied at several doses as a soil conditioner for corn seed germination in greenhouses.

Table 6 Chemical composition of compost derived from winery waste

Element	Values
n (%)	2.14–3.74
P (%)	0.18–0.52
Ca (%)	3.17–14.3
Mg (%)	0.3–0.61
Fe (%)	0.5
Zn (mg kg^{-1})	77–109
Cu (mg kg^{-1})	30–46
Ni (mg kg^{-1})	9.1–17.6
Cr (mg kg^{-1})	23.4–147
Pb (mg kg^{-1})	8–19
Cd (mg kg^{-1})	0.2–0.4

In addition, Raviv *et al.* (1986) reported that combinations of sewage sludge and MSW compost with other residual materials such as pine bark, grape marc or rice hull were worth investigation because negative properties of single materials, such as heterogeneity, high salinity, low content of organic matter, low cation exchange capacity or high content of contaminants can be minimised thus obtaining a sound and cheap substrate (Ingelmo *et al.*, 1998).

The chemical composition of compost derived from winery waste was shown in Table 6. The physicochemical and chemical characteristics of compost derived from winery wastes were summarised in Table 7.

As can be seen from Tables 6 and 7, the compost derived from winery wastes is of good quality with good physicochemical characteristics and a sufficient amount of nutrients. In addition, Lasaridi *et al.* (2000) has reported that the compost, derived from pressed grape skin, produced one of the best qualities composts both in terms of its physicochemical characteristics and agronomic value.

Particularly, the compost obtained is recommended for application to the vineyards because: (i) the humified nature of the organic matter would facilitate its incorporation and improve the

Table 7 Physicochemical and chemical characteristics of compost derived from winery wastes

Parameter	Values
pH	6.5–8.5
EC (MS cm^{-1})	1.57–4.1
Volatile solids (%)	46.8–67.5
C/N ratio	11.9–19.5
Moisture (%)	47–66
CEC (Cmol kg^{-1})	108.65
OM (%)	84.15–89.1
C (%)	40.5–51.5

water-holding capacity of the soil, an important factor for the quality and specificity of wine production, (ii) nitrogen is released only gradually which is particularly appropriate for the vineyards that suffer from high nitrogen levels and (iii) it reports high-to-moderate values of potassium considered a quality factor in wines (Ribererau-Gayon & Peybaud, 1982; Vez, 1993; Delas, 2000).

Manios (2004) has investigated the potential use of pressed grape skins as a substrate component for the cultivation of strawberries. A mixture containing 25% (v/v) pressed grape skin compost and 75% (v/v) pumice was used and produced an average fruit yield of 306 g per plant and biomass yield of 97 g per plant. These results were the highest in comparison with others, where various organic residues derived composts used and were shown in Table 8.

Furthermore, Ingelmo *et al.* (1998) have reported the results derived from the use as a soil substrate: (i) a mixture of 50% grape marc and 50% peat and (ii) a mixture of 50% grape marc, 25% sphagnum moss peat and 25% anaerobically treated sewage sludge regarding in the final height of ornamental plants cultivation (*Nerium oleander*, *Cupressus sempervirens* and *Rosemarinus officinalis*). The duration of the experiment was 10 months and the results were summarised in Table 9.

Table 9 shows that the combination of grape marc with other materials gives satisfactory results in ornamental plants cultivation comparatively with other substrates. Ferrer *et al.* (2001) reported that all the treatments, in a 20-day corn experiment, with winery wastes are superior to that with a chemical industrial fertiliser composed of urea, K_2O and P_2O_5, 0.45 g d.m. per pot. This superiority is because of the existence of other minerals

Table 8 Fruits and biomass yield (wet weight) of strawberries plants, growing in substrates containing different composts (Adapted from Manios, 2004)

Substrate composition	Fruits yield (gram per plant)	Biomass yield (gram per plant)
PGSC (25%) and pumice (75%)	306	97
OTLC (25%) and pumice (75%)	256	85

PGSP, press grape skin compost; OTLC, olive tree leaves compost.

Table 9 Results regarding the substrate suitability and final height (cm) of three different plants (Adapted from Ingelmo *et al.*, 1998)

Substrate	*N. oleander*	*C. sempervirens*	*R. officinalis*
50% grape marc and 50% peat	32.8	55.5	41–42.2
50% grape marc, 25% sphagnum moss peat and 25% anaerobically treated sewage sludge	42	59.6	39.3–44.7

and micronutrients in the composted organic fertiliser, which contribute to a better development of the corn plant, as well as to humic compounds that provide the porous capacity to the soil (Ferrer *et al.*, 1993; Sanchez & Ferrer, 1994; Sastre *et al.*, 1996). Finally, Ferrer *et al.* (2001) recommended that a 3000 kg ha^{-1} of grape waste compost should be supplemented with phosphorus.

In spite of its advantages, the application of agricultural wastes in soil can lead to problems pertaining to their heavy metal content. The continuous application of waste enriched in heavy metals results in heavy metal accumulation in soil. Land disposal of organic waste materials may directly or indirectly alter the heavy metals status of the soil by affecting metal solubility or dissociation kinetics. (Del Castilho *et al.*, 1993; Karaka, 2004). In addition, Pinamonti *et al.* (1997) reported that the heavy metal content is a crucial factor leading to restricted agricultural use of compost.

Table 10 Concentrations of DTPA extractable Cd, Cu, Ni and Zn (mg kg^{-1}) in the soil at increasing rates of grape marc applied (Adapted from Karaka, 2004)

Rate of applied grape marc (%)	Cd	Cu	Ni	Zn
0	0.054–0.057	2.10–2.20	2.50–2.90	1.20–1.50
2	0.040–0.043	1.80–1.90	1–1.10	1.90–2.30
4	0.039–0.041	1.60–1.70	0.80–0.90	2.10–2.40
8	0.027–0.031	1.76–2.10	0.65–0.83	2.70–2.90

The presence of heavy metals is undesirable because it can cause adverse effects both to the environment and to a variety of living species including humans. Metals can be distinguished from other toxic pollutants, as they are not biodegradable and can been accumulated in living tissues, causing various diseases and disorders (Villaescusa *et al*., 2004).

The total heavy metal content of the soil is commonly used to indicate the degree of contamination, but the heavy metal concentration in solution mostly determines the actual environmental exposure or risk. Distribution of heavy metals between soils and solute is the key to evaluate the environmental impact of the metals (Sposito, 1989; Karaka, 2004).

Karaka (2004) investigated the influence of grape marc on the DTPA extractable Cd, Cu, Ni and Zn in soil (typic xerofluvent). As a result of this investigation, Table 10 gives the concentrations of DTPA extractable Cd, Cu, Ni and Zn (mg kg^{-1}) in the soil at the increasing rates of grape marc applied.

As can been seen from Table 9, significant negative correlation was found between extractable Cd, Cu, Ni and rate of applied grape marc. On the contrary, DTPA-extractable Zn increased with increasing rate of grape marc.

Finally, Karaka (2004) concluded that the effect of organic waste application on the extractability of Cd, Cu, Ni and Zn in the soil depended on pH, organic matter content of the organic wastes, the metals studied and the time elapsed after their application.

Pullulan

Grape skin pulp is a by-product of the wine industry amounting to thousands of tones annually. In Greece, the average annual production of grape skin pulp for the years 1996–2000 was 14 200 ton (Arapoglou *et al*., 2002). Hot water extracts of grape skin pulp can serve as a good substrate for the production of pullulan, an industrially important polysaccharide (Le Duy & Boa, 1982). Pullulan is an exocellular homopolysaccharide of economic importance produced by the yeast-like fungus *Aureobasidium pullulans*. It consisted of maltotriosyl units linked through a1 $\rightarrow$ 6 glycosidic bonds (Saha & Zeikus, 1989).

Moreover, pullulan is used as a low-calorie ingredient in foods, as a viscosity imparter and binder and, because of its low oxygen permeability, as a packaging agent. It is used in the pharmaceutical industry as a bulking agent and binder and as an oxidation–prevention agent for tablets. Other applications include films and adhesives, microencapsulating agents for flavour and spices and as a water-solubility enhancer in fertilisers (Israilides *et al*., 1998).

The industrial applications of pullulan have been thoroughly reviewed by Deshpande *et al*. (1992). The market price of food and pharmaceutical grade pullulan is about 88 € kg^{-1} and the current world supply (c. 1000 ton) is produced almost exclusively by the Japanese company Hayashibara, Co. Ltd, Okayama, Japan (Arapoglou *et al*., 2002).

Pullulan is usually recovered from the fermentation broth by ethanol or methanol precipitation after the removal of cells. It has been shown, however, that the purity of pullulan in the ethanol-precipitated substances (EPS) may vary according to the substrate used for the fermentation (Israilides *et al*., 1994). The determination of pullulan in mixtures is usually carried out by hydrolysis with pullulanase followed by estimation of the resulting maltotriose with chromatography, radiometry or other methods (Catley, 1972; Finkelman & Vardanis, 1982).

Arapoglou *et al*. (2002) and Israilides *et al*. (1998) have investigated the solid state fermentation of grape skin pulp extracts by *A. pullulans* with the aim to obtain pullulan. Figure 3 shows the biomass and EPS as a function of fermentation time using the grape skin pulp extracts. From Fig. 3, it is evident that the biomass and EPS increase with the increase of the fermentations days. The above-mentioned parameters reached their highest values on the 7th day of fermentation.

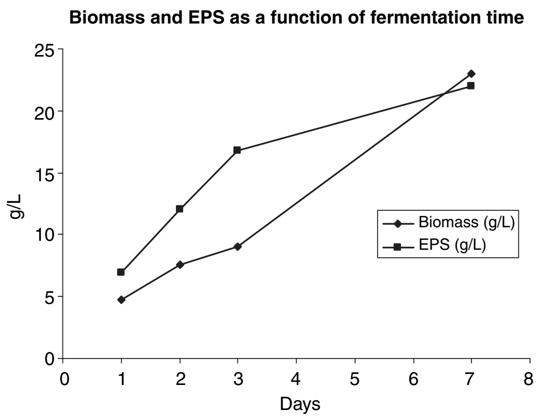

Figure 3 The biomass and ethanol-precipitated substances (EPS) as a function of fermentation time using grape skin pulp extracts (Israilides *et al.*, 1994; Arapoglou *et al.*, 2002).

Table 11 gives the pullulan content of EPS as estimated by coupled-enzyme assay and by high-pressure anion-exchange chromatography pulsed amperometric detection (*HPAEC-PAD*) for grape skin pulp extracts supplemented with NH_4NO_3 and K_2HPO_3 after 7 days fermentation. In this table, pullulan is expressed as a percentage of the EPS (Israilides *et al.*, 1998).

Table 11 shows the percentage pullulan present in the EPS, on the basis of both the coupled-enzyme assay and on the maltotriose and maltotetraose produced by pullulanase hydrolysis (HPAEC-PAD). Israilides *et al.* (1998) reported that the grape skin pulp extracts were the richest in pullulan (97.4%) in comparison with other materials.

In lieu of conclusion, it can be said that the grape skin pulp can be recycled and used as a substrate for the production of a high-added value product, effectively Pullulan a polysaccharide with many industrial uses and applications.

Adsorbent

Effluent industrial wastewater containing heavy metals usually causes serious problems. Because of the toxicity and assimilation of heavy metal to organisms, safe and effective treatment of heavy-metal-containing wastewater becomes a challenging task for industry because, in part, of the fact that the cost effective methods are not available (Sistrava *et al.*, 1997; Yuan-shen *et al.*, 2004). Conventional methods for removing metals from industrial effluents include chemical precipitation, coagulation, solvent extraction, electrolysis, membrane separation, ion exchange and adsorption (Patterson, 1997).

The high capital and regeneration costs of activated carbon and different types of ion exchange resins, materials which were often used in adsorption processes, limit their large scale use for the removal of heavy metals (Kratochvil & Volensky, 1998; Bailey *et al.*, 1999; Villaescusa *et al.*, 2004). The presence of complex ligands in industrial wastewater can hinder metal hydroxide precipitation and may result in residual metal concentration so that meeting the increasing stringent effluent regulations may be difficult (Weng, 2002).

For these reasons, absorption, as an alternative method, has been shown to be feasible for removing heavy metals from industrial wastewater. Several natural and synthetic hydrous solids have been investigated as absorbents of heavy metals (Yuan-shen *et al.*, 2004). Among these, waste products from agricultural operations, like winery wastes, were considered as low-cost sorbents in the removal of toxic heavy metal. These sorbents, compared with others, have several advantages; they are cheap and these materials that are considered as waste products can be

Table 11 Pullulan content of EPS as estimated by coupled-enzyme assay and by high-pressure anion-exchange chromatography pulsed amperometric detection (*HPAEC-PAD*) for grape skin pulp extracts (Adapted from Israilides *et al.*, 1998)

Substrate	Concentration of EPS (% w/v)	Coupled-enzyme assay *A* (ΔA min^{-1})	*B* (ΔA min^{-1})	*A* – B (ΔA min^{-1})	*A/B*	Pullulan % d.m.	Pullulan by (HPAE-PAD) % w/w
Grape skin pulp extract	0.25	0.1084	0.001	0.1074	108	97.4	128.5

A, the rate observed for the coupled-enzyme assay; *B*, the rate observed as for A, less pullunase.

reused for effluents decontamination. In addition, these sorbents appear to have an application as preconcentration agents (Villaescusa *et al.*, 2004). The utility of winery wastes as an adsorbent to remove chromium, nickel and copper from aqueous solutions was investigated (Villaescusa *et al.*, 2004). The sorption of metals by winery wastes might be attributed to their proteins, carbohydrates and phenolics compounds that have carboxyl, hydroxyl, sulphate, phosphate and amino groups that can bind metal ions.

In order to evaluate the utility of winery wastes as an adsorbent, these wastes were washed with deionised water and dried in an oven at 105–110 °C. Then these were cut, sieved into several particle sizes and desiccated. Metal solutions were prepared by dissolving appropriate amounts of $CuCl_2{\cdot}2H_2O_{(S)}$, $NiCl_2{\cdot}2H_2O_{(S)}$ and $Cr(NO_3)_3$ in distilled water. Finally, batch adsorption experiments were performed to determine the adsorption characteristics of Cu, Cr and Ni on the winery wastes. In this case, isotherm adsorption tests were conducted in a series of glass tubes, which were filled with Cu, Cr and Ni ion solutions of varying concentrations and adjusted to the desired temperature and pH with either HCl – H_2SO_4 or NaOH solution. (Villaescusa *et al.*, 2004; Yuan-shen *et al.*, 2004).

Furthermore, it is noteworthy that Langmuir isotherms were used to describe sorption data. In particular, Langmuir isotherms have been useful tools for the description and comparison of heavy metal sorption by different sorbents (Chong & Volesky, 1995; Kaewsarn & Yu, 1999). Particular details about Langmuir isotherms were reported by Villaescusa *et al.* (2004); Yuan-shen *et al.* (2004).

Figure 4 displayed the Cu, Cr and Ni removal using winery wastes as a function of pH. Particularly, the pH of metal solutions was identified as the most important variable sorption on hydrous solids. This is partly because of the fact that the hydrogen ions themselves are strong competing sorbates and partly that the solution pH influences the chemical speciation of metal ion (Kratochvil & Volensky, 1998; Villaescusa *et al.*, 2004).

As can be seen from Fig. 4, the removal for all metals increases with the increase in pH value. Particularly, the uptake increased from practically zero at equilibrium pH 1.0 to 78–80% at pH 5.5 for all metals. The absence of sorption at low pH values can be explained by the fact that at these pH values the H^+ concentration is high, which can compete with Cu, Cr and Ni cations for surface sites (Villaescusa *et al.*, 2004). The results showed that winery wastes can be used as an adsorbent for the effective decontamination of metal-containing effluents.

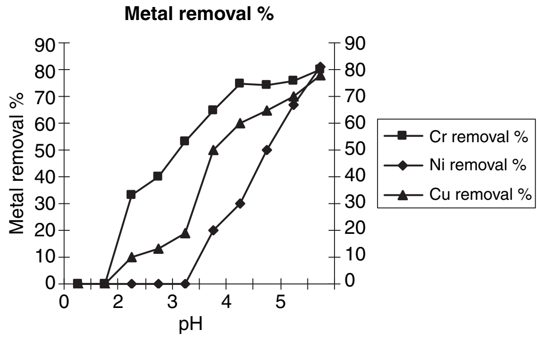

Figure 4 Cu, Cr and Ni removal % using winery wastes as a function of equilibrium pH.

Conclusions

The results presented in this study demonstrate that winery wastes have generally very high-polyphenolic contents, making their utilisation worthwhile and thus supporting sustainable agricultural production. In particular, the by-products from vinification are suitable as dietary supplements or as ingredients in functional foods. Moreover, the compost from winery wastes confers adequate characteristics for its use as a soil conditioner. Furthermore, wine-processing sludge shows to be an effective adsorbent for the adsorption of pollutant heavy metals. Finally, winery wastes can be recycled and used as a substrate for the production of a high-added value product, Pullulan.

References

Aaby, K., Hvattum, E. & Skrede, G. (2004). Analysis of flavonoids and other phenolic compounds using high-performance liquid chromatography with coulometric array detection: relationship to antioxidant activity. *Journal of Agricultural Food and Chemistry*, **52**, 4595–4603.

Abad, M. (1991). Los sustratos horticolas y tecnicas de cultivo sin suelo. In: *La horticultura Espanola* (edited by L. Rallo & F. Nuez). Pp. 271–280. Tarragona, Spain, Reus.

Alonso, A., Guillean, D., Barroso, C., Puertas, B. & Garcia, A. (2002). Determination of antioxidant activity of wine byproducts and its correlation with polyphenolic content. *Journal of Agricultural Food and Chemistry*, **50**, 5832–5836.

Andres, L., Riera, F. & Alvarez, R. (1997). Recovery and concentration by electrodialysis of tartaric acid from fruit juice industries waste waters. *Journal of Chemical Technology and Biotechnology*, **70**, 247–252.

Arapoglou, D., Israilides, C.J., Bocari, M., Scanlon, B. & Smith, A. (2002). A novel approach to grape waste treatment. In: *Proceedings of the International Conference. Protection and Restoration of the Environment VI* (edited by University of Thessaly and Aristotle University of Thessaloniki). Pp. 469. Greece: Skiathos.

Bailey, S., Olin, T., Bricka, R. & Adrian, D. (1999). A review of potentially low-cost sorbents for heavy metals. *Water Research*, **33**, 2469–2479.

Balanya, T., Sana, J., Gonzalez, M. & De la Pena, M. (1994). Utilizacion de compost de residuos solidos urbanos en un vinedo del Penedes. *Viticultura/Enologia Profesional*, **31**, 20–25.

Bazzoffi, P., Pellegrini, S., Rocchini, A., Mmorandi, M. & Grasselli, O. (1998). The effect of urban refuse compost and different tractor tyres on soil physical properties, soil erosion and maize yield. *Soil and Tillage Research*, **48**, 275–286.

Bertran, E., Sort, X., Soliva, M. & Trillas, I. (2004). Composting winery waste: sludges and grape stalks. *Bioresource Technology*, **95**, 203–208.

Blasi, C.D., Tanzi, V. & Lanzette, M. (1997). A study on the production of agricultural residues in Italy. *Biomass and Bioenergy*, **12**, 321–333.

Bouhamidi, R., Prevost, V. & Nouvelot, A. (1998). High protection by grape seed proanthocyanidins (GSPC) of polyunsaturated fatty acids against UV-C induced peroxidation. *Comptes Rendus de l'Académie des Sciences – Series III – Sciences de la Vie. III*, **321**, 31–38.

Catley, B. (1972). Pullulan elaboration, an inducible system of *Pullararia pullulans. FEBS Letters*, **20**, 174–176.

Chong, K.H. & Volesky, B. (1995). Description of two-metal biosorption equilibria by Langmuir-type models. *Biotechnology and Bioengineering*, **47**, 51–60

Costa, F., Moreno, J.L., Hernandez, T., Lax, A., Cegarra, J. & Roig, A. (1989). Mineralization of organic materials in a calcareous soil. *Biological Wastes*, **28**, 189–201.

Del Castilho, P., Chardon, W. & Salomons, W. (1993). Influence of cattle-manure slurry application on the solubility of Cd, Cu, and Zn in a manured acidic, loamy sand soil. *Journal of Environmental Quality*, **22**, 689–697.

Delas, J. (2000). La fertilisation de la vinge. Pp. 159. Bordeaux: Editions Feret.

Deshpande, M., Rale, B. & Lynch, J. (1992). *Aureobasidium pullulans* in applied microbiology: a status report. *Enzyme and Microbial Technology*, **14**, 514–523.

Diaz, M., Madejon, E., Lopez, F., Lopez, R. & Cabrera, F. (2002). Optimization of the rate vinasse/grape marc for co-composting process. *Process Biochemistry*, **37**, 1143–1150.

Dittmer, U., Budde, K., Stindt, A. & Weltzien, H. (1990). The influence of the compost extracts on different plants pathogens. *Gesunde-Pflanzen*, **42**, 219–235.

Edwards, L., Burney, J., Richter, G. & MacRae, A. (2000). Evaluation of compost and straw on soil-loss characteristics in erosion plots of potatoes. *Agriculture Ecosystems and Environment*, **81**, 217–222.

EU (1994). *Eu 94/39 EC: Colours for use in foodstuffs*. Brussels, Eu.

Faure, D. (1991). The effect of bacterial inoculation on the initiation of composting of grape pulps. *Bioresource Technology*, **37**, 235–238.

Faure, D. & Deschamps, A. (1990). Physical-chemical and microbiological aspects in composting of grape pulps. *Biological Wastes*, **34**, 251–258.

Ferrer, J., Mujica, D. & Paez, G. (1993). Composting from grape pomace. *Revista Técnica de la Facultad de Ingenieria*, **16**, 191–198.

Ferrer, J., Paez, G., Marmol, Z. *et al*. (2001). Agronomic use of biotechnologically processed grape wastes. *Bioresource Technology*, **76**, 39–44.

Finkelman, M. & Vardanis, A. (1982). Simplifed microassay for pullulan synthesis. *Applied and Environmental Microbiology*, **43**, 483–485.

Frankel, E., Waterhouse, A. & Teissedre, P. (1995). Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. *Journal of Agricultural and Food Chemistry*, **43**, 890–894.

Garcia, C., Hernandez, T. & Costa, F. (1990). The influence of composting and maturation processes on the heavy-metal extractability from some organic wastes. *Biological Wastes*, **31**, 291–301.

Garcia, C., Hernandez, T. & Costa, F. (1992). Characterization of humic acids from uncomposted sewage sludge by degradative and nondegradative techniques. *Bioresource Technology*, **41**, 53–57.

Gonzalez-Paramas, A., Esteban-Ruano, S., Santos-Buelga, C., Pascual-Teresa, S. & Rivas-Gonzalo, J. (2004). Flavanol content and antioxidant activity in winery byproducts. *Journal of Agricultural Food and Chemistry*, **52**, 234–238.

Graefe, G. (1980). Methods and apparatus for preparing high grade fertilizer. *United States Patent*, **4**, 211–545.

Haslam, E. (1980). Oligomeric procyanidins and aging of red wines. *Phytochemistry*, **19**, 2577–2582.

Inbar, Y., Chen, Y. & Hadar, Y. (1986). The use of composted separated cattle manure and grape marc as a peat substitute in horticulture. *Acta Horticultura*, **178**, 147–154.

Inbar, Y., Chen, Y., Hadar, Y. & Verdonck, O. (1988). Composting of agricultural wastes for their use as container media: simulation of the composting process. *Biological Wastes*, **26**, 247–259.

Ingelmo, F., Canet, R., Ibafiez, M., Pomares, F. & Garcfa, J. (1998). Use of MSW compost, dried sewage sludge and other wastes as partial substitutes for peat and soil. *Bioresource Technology*, **63**, 123–129.

Israilides, C., Scanlon, B., Smith, A., Jumel, K. & Harding, S. (1994). Characterisation of pullulans produced from agro-industrial wastes. *Carbohydrate Polymers*, **25**, 203–209.

Israilides, C., Smith, A., Harthill, J., Barnett, C., Bambalov, G. & Scanlon, B. (1998). Pullulan content of the ethanol precipitate from fermented agro-industrial wastes. *Applied Microbiology and Biotechnology*, **49**, 613–617.

Kaewsarn, P. & Yu, Q.M. (1999). Binary adsorption of copper (II) and cadmium (II) from aqueous solutions by biomass of marine alga durvillaea potarum. *Separation Science and Technology*, **34**, 1595–1605.

Kammerer, D., Claus, A., Carle, R. & Schieber, A. (2004). Polyphenol screening of pomace from red and white grape varieties (*Vitis vinifera* L.) by HPLC-DAD-MS/MS. *Journal of Agricultural Food and Chemistry*, **52**, 4360–4367.

Karaka, A. (2004). Effect of organic wastes o the extractability of cadmium, copper, nickel, and zinc in soil. *Geoderma*, **122**, 297–303.

Kratochvil, D. & Volensky, B. (1998). Advances in biosorption of heavy metals. *Trends Biotechnology*, **16**, 291–300.

Lasaridi, K., Stentiford, E. & Evans, T. (2000). Windrow composting of wastewater biosolids: process performance and product stability assessment. *Water Science and Technology*, **42**, 217–226.

Lau, D. & King, A. (2003). Pre- and post-mortem use of grape seed extract in dark poultry meat to inhibit development of thiobarbituric acid reactive substances. *Journal of Agricultural Food and Chemistry*, **51**, 1602–1607.

Le Duy, A. & Boa, J. (1982). Pullulan production from peat hydrolysate. *Canadian Journal of Microbiology*, **2**, 143–146.

Mandelbaum, R., Hadar, Y. & Chen, Y. (1988). Composting of agricultural waste for their use as container media: effect of heat treatments on suppression of *Pythiun aphanidermatum* and microbial activities in substrates containing compost. *Biological Wastes*, **26**, 261–274.

Manios, T. (2004). The composting potential of different organic solid wastes: experience from the island of Crete. *Environment International*, **29**, 1079–1089.

Marakakis, P. (1982). *Anthocyaninsas Food Colors*. New York: Academic Press.

Miyake, Y., Murakami, A., Sugiyama, Y., Isobe, M., Koshimizu, K. & Ohigashi, K. (1999). Identification of coumarins from lemon fruit citrus limon as inhibitors of *in vitro* tumor promotion and superoxide and nitric oxide generation. *Journal of Agricultural and Food Chemistry*, **47**, 3151–3157.

Moldes, D., Gallego, P., Rodrıguez-Couto, S. & Sanroman, A. (2003). Grape seeds: the best lignocellulosic waste to produce laccase by solid state cultures of *Trametes hirsuta*. *Biotechnology Letters*, **25**, 491–495.

Moure, A., Cruz, J., Franco, D. *et al.* (2001). Natural antioxidant from residual sources (review). *Food Chemistry*, **72**, 145–171.

Nakatani, N. (1997). Antioxidants from spices and herbs. In: *Natural Antioxidants: Chemistry, Health Effects and Applications* (edited by F. Shaihidi). Pp. 64–75. Illinois: AOCS Press.

Navas, A., Bermudz, F. & Machin, J. (1998). Influence of sewage sludge application on physical and chemical properties of gypsisols. *Geoderma*, **87**, 123–135.

Negro, C., Tommasi, L. & Miceli, A. (2003). Phenolic compounds and antioxidant activity from red grape marc extracts. *Bioresource Technology*, **87**, 41–44.

Patterson, J.W. (1997). *Industrial Waste Water Technology*. New York: Science Publishers .

Pinamonti, F., Stringari, G., Gasperi, F. & Zorzi, G. (1997). The use of compost: its effect on heavy metal levels in soil and plants. *Resources, Conversation and Recycling*, **21**, 129–143.

Prieur, C., Rigaud, J., Cheynier, V. & Moutounet, M. (1994). Oligomeric and polymeric procyanidins from grape seeds. *Phytochemistry*, **26**, 781–784.

Querejeta, J., Roldan, A., Albaladejo, J. & Castillo, V. (2001). Soil water availability improved by site preparation in a *Pinus halepensis* afforestation under semiarid climate. *Forest Ecology and Management*, **149**, 115–128.

Raviv, M., Chen, Y. & Inbar, Y. (1986). Peat and peat substitutes as growth media for container-grown plants. In *Developments in Plant and Soil Sciences: The Role of Organic Matter in Modern Agriculture* (edited by Y. Chert & Y. Avnimelech). Pp. 257–287. Dordrecht: Martinus Nijhoff Publish.

Ray, S., Bagchi, D., Lim, P. *et al*. (2001). Acute and long-term safety evaluation of a novel IH636 grape seed proanthocyanidin extract. *Research Communications in Molecular Pathology and Pharmacology*, **109**, 165–197.

Renaud, S. & De Lorgeril, M. (1992). Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet*, **339**, 1523–1526.

Ribererau-Gayon, J. & Peybaud, E. (1982). *Tomo I: Biologia de la vina. Suelos de Vinedo*. Pp. 671. Argentina: Editorial Hemisferio Sud S.A.

Saha, B. & Zeikus, B. (1989). Novel highly thermostable pullulanase from thermophiles. *Trends Biotechnology*, **7**, 308–316.

Sanchez, E. & Ferrer, J. (1994). Nutritive solution to hydroponic culture from grape pomace. *RevistaTécnica de la Facultad de Ingenieria, University of Zulia*, **17**, 197–206.

Sanchez, A., Ysunza, F., Beltran-Garcia, M. & Esqueda, M. (2002). Biodegradation of viticulture wastes by *Pleurotus*: a source of microbial and human food and its potential use in animal feeding. *Journal of Agricultural Food and Chemistry*, **50**, 2537–2542.

Santos-Buelga, C. & Scalbert, A. (2000). Review: proanthocyanidins and tannin-like compounds-nature, occurrence, dietary intake, and effects on nutrition and health. *Journal of the Science of Food and Agriculture*, **80**, 1094–1117.

Sastre, I., Vicente, M. & Lobo, C. (1996). Influence of the application of sewage sludges on soil microbial activity. *Bioresource Technology*, **57**, 19–23.

Shrikhande, A. (2000). Wine byproducts with health benefits. *Food Research International*, **33**, 469–474.

Singleton, V. & Rossi, J. (1965). Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, **16**, 144–158.

Sistrava, S., Gupta, V.K. & Mohan, D. (1997). Removal of lead and chromium by activated slag – a blast-furnace

waste. *Journal of Environmental Engineering*, **123**, 461–468.
Smagge, F., Mourgues, J., Escudier, J., Conte, T., Molinier, J. & Malmary, C. (1992). Recovery of calcium tartrate and calcium malate in effluents from grape sugar production by electrodialysis. *Bioresource Technology*, **39**, 85–189.
Soliva, M. & Felipo, M.T. (2002). Organic wastes as a resource for Mediterranean soils. In: *Biological Treatment of Biodegradable Wastes-Technical Aspects* (edited by Eu). Pp. 19. Brussels: DG Environment and the JRC.
Sposito, G. (1989). *The Chemistry of Soils*. USA: Oxford University Press.
Stojanovic, S., Stojsavljervic, T., Vunurevic, N., Vikicvranjes, M. & Mandic, A. (1989). Nutritive and feeding value of dried grape pomace in feeding fattening cattle. *Stocarstvo*, **43**, 313–319.
Strack, D. & Wray, V. (1989). Anthocyanins. In: *Methods in Plant Biochemistry, Vol. 1 – Plant phenolics*. (edited by P. Dey & J. Harborne). Pp. 325–356. London: Academic Press.
Tebib, K., Bitri, L., Besancon, P. & Rouanet, J. (1994). Polymeric grape seed tannins prevent plasma cholesterol changes in high cholesterol fed rats. *Food Chemistry*, **49**, 403–406.
Tebib, K., Rouanet, J.M. & Besancon, P. (1997). Antioxidant effects of dietary polymeric grape seed tannins in tissues of rats fed a high cholesterol-vitamin E-deficient diet. *Food Chemistry*, **59**, 135–141.
Teissedre, P., Frankel, E., Waterhouse, A., Peleg, H. & German, J. (1996). Inhibition of in vitro human LDL oxidation by phenolic antioxidants from grapes and wines. *Journal of the Science of Food and Agriculture*, **70**, 55–61.
Torres, J. & Bobet, R. (2001). New flavanol derivatives from grape (*Vitis vinifera*) byproducts. Antioxidant aminoethylthio-flavan-3-ol conjugates from a polymeric waste fraction used as a source of flavanols. *Journal of Agricultural Food and Chemistry*, **49**, 4627–4634.
Vallini, G., Bianchin, M.L., Pera, A. & De Bertoldi, M. (1983). Composting agro-industrial byproducts. *Byocycle*, **24**, 43–47.
Vez, A. (1993). La fumure de la vigne. Station federale de recherchers agronomiques de Changins. *Revue Suisse de Viticulture d'* Arboriculture Et d' Horticulture, **25**, 57–64.
Villaescusa, I., Fiol, N., Martinez, M., Mirrales, N., Poch, J. & Seralocs, J. (2004). Removal of copper and nickel ions from aqueous solutions by grape stalks wastes. *Water Research*, **38**, 992–1002.
Weng, C.H. (2002). Removal of nickel (II) from dilute aqueous solution by sludge-ash. *Journal of Environmental Engineering*, **128**, 716–722.
Yamakoshi, J., Kataoka, S., Koga, T. & Ariga, T. (1999). Proanthocyanidin rich extract from grape seeds attenuates the development of aortic atherosclerosis in cholesterol-fed rabbits. *Atherosclerosis*, **142**, 139–149.
Yuan-shen, L., Cheng-chung, L. & Chyow-san, C. (2004). Adsorption of Cr(III) from wastewater by wine processing waste sludge. *Journal of Colloid and Interface Science*, **273**, 95–101.

Electronic references

1. http://www.allspiritfitness.com/library/gs/gs_grapeseedoil.shtml
2. http://www.bodybuildingforyou.com/health-supplements/grape-seed-extract.htm
3. http://www.fact-index.com/g/gr/grape_seed_oil.html
4. http://www.grapeseedoilcorp.com/everything.html
5. http://www.hort.purdue.edu/newcrop/Crops/Grape_seed_oil.html
6. http://www.jgpress.com/IBArticles/2000/JF_12.htm
7. http://www.jgpress.com/BCArticles/2000/070030.html
8. http://www.nf-2000.org/secure/FP5/F1738.htm
9. http://p2library.nfesc.navy.mil/P2_Opportunity_Handbook/9-IV-5.htm
10. http://www.wholehealthmd.com/refshelf/substances_view/1,1525,793,00.htm
11. http://www.woodsend.org/aaa/inst.html
12. http://en.wikipedia.org/wiki/Grape.seed.oil

