Integrated Enology- Utilization of winery by-products into high added value products

Elias T. Nerantzis and Panagiotis Tataridis
Laboratory of Biotechnology & Industrial Fermentation, Dept. of Enology & Beverage Technology, TEI of Athens, Ag. Spyridona Street, 12210 Aegaleo, Athens, Greece. (p_tataridis@yahoo.gr).

ABSTRACT

Grape growing and wine making generate a number of wastes and by-products. These materials include vine prunings, grape stalks, grape pomace and grape seeds, yeast lees, tartrate, carbon dioxide and wastewater. Only a very small portion of these materials is used world wide. This article presents a number of value adding technologies for the valorization of those products

Keywords: Integrated enology, winery wastes, by-products, grape stalks, vine prunings, grape pomace, grape seed, yeast lees.

1. INTRODUCTION

In Greece, grape pomace is used for alcoholic beverage production while yeast lees and tartrate are used for ethanol and tartaric acid recovery. In the past farmers had a number of agricultural activities permitting them to "recycle" wastes and byproducts from grape and wine production. Tradition, experience and common "rural" sense had taught farmers that there is nothing to waste. Every byproduct would become fertilizer, animal feed, or fuel.

Today's grape growers and winemakers specialize in just one sector. Industrialized production demanded higher production volumes and the use of traditional byproducts were replaced by commercial products of low cost and high efficiency. The new production methods along with the increase in the number of wineries and vineyards as well as the increase in production volume resulted in an exponential increase of wastes. Most of the possible traditional uses of wastes and byproducts in other agricultural sectors slowly eclipsed, transforming wine and vine byproducts to waste. The "rural wisdom" was also forgotten.

As legal, environmental and economic issues are being reconsidered in the past two decades (Hazell, 2000; Bisson et al. 2002), it becomes more and more obvious that disposal and landfill of those wastes present environmental and social drawbacks. In the same time advances in modern chemistry and biotechnology, academic awareness and industrial interest permitted the study of these "wastes". New technologies were proposed not only for their re-use in agriculture, but also for the production of common and novel products for other sectors. Today they can be used for compost, vermicompost, animal feed and supplements, food and nutritional supplements, (functional foods / nutraceuticals), alcoholic drinks, color and tannin extracts, inks and pigments, antibacterial agents, skin, hair and healthcare products, soaps and spa products, filtration and structural material, wood and leather preservatives and gifts, biofuel and fuel additives, as well as, for the production of other forms of bioenergy.

Environmental concerns of the past decades, together with results of water resources pollution, gave rise to legislation regarding waste minimization and disposal in many countries like Australia, USA and France. The environmental policy of the European Union (EU) is based on the notion that "he who pollutes must pay cost of remediation". This started from "taxes" regarding packaging material and continued to other consumer products like, cars and car fluids, tires, electrical appliances, e.t.c. It is not unreasonable to assume that future legislation regarding industrial waste including those of wineries will become even more demanding, thus increasing the cost of waste management. The recovery of added value products can help in that direction. These recovery processes are part of a new philosophy of sustainable agriculture which is closer to the old "rural" practices (Fig. 1, Einion 1999).

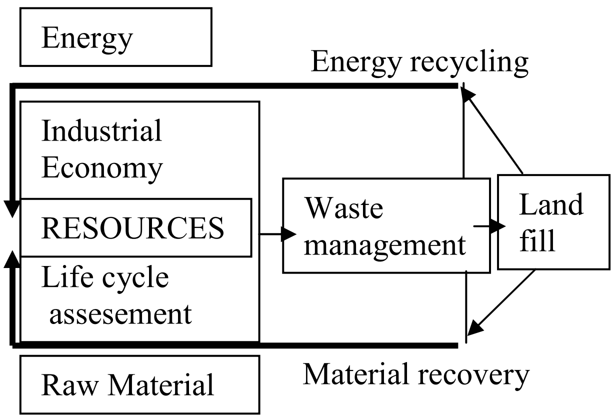

Figure 1. Sustainable resource management.

The interest on developing product and processes for winery residues is increasing and this is evident from the number of scientific publications, as well as, from the number of deposited patents (European Patent Office, http://ep.espacenet.com/).

2. WINE AND VINE BY-PRODUCTS

Wine and vine by-products present an important percentage of the produced grapes. Table 1 presents the average quantities of these products.

Table 1. Winerywaste and by-products concentrations

	% of grapes
• **Grape stalks:**	~2,5% to 7,5%
• **Grape pomace:**	~15% dry (wet up to 25-45% f=(pressing))
– ***Sugars:***	~(up to 150 g/Kg)
– ***Phenolics/Pigments:***	~9 kg/t (red grape pomace)

  - *Tartrate:* ~50 to 75 kg/t
  - *Fibers:* ~ 30 % to 40 %
- **Grape seeds:** ~ 3 % to 6 %
  - *Grape seed oil:* ~Oils 12-17% (76% linoleic (omega-6 fatty acid)
  - *Phenolics:* ~4-6 %
- **Yeast lees:** ~ 3,5-8,5 % (compared to initial grape quantity)
  - *Pigments/colorants:* ~ 12 kg/t (red wine lees)
  - *Tartrate:* >100-150 Kg/t
  - *Ethanol:* ~ 50 % of 10-12 % vol wine
  - *Beta-1,3-glucans:* ~ 6-12 % of dry weight
- **Vine prunings:** ~5 tons/ha/year

2.1 Lignocellulosic substrates- Grape stalks and vine prunings

The main by-products of vineyard are the grape stalks Grape stalks have a high degree of fibers (lignin and cellulose) and a high percentage of nutritive mineral elements, especially nitrogen and potassium. Several different techniques are used for their valorisation.

Grape stalks can be used for producing compost (a high-quality fertilizer and soil amendment) by mixing them with winery sludge digested aerobically and centrifuged (Bertan et al., 2004). Grape stalk compost has a high agronomic value and is particularly suitable for the soils of the vineyards which have very low organic matter content.

The use of grape stalks in the form of SCP, as ruminant feed or feeding component has also been proposed (Nicolini et al., 1993) after solid state fermentation. Results indicate that, after biological lignin removal, the cellulose is better accessible to rumen micro-organisms, due to its good protein value and low lignin content, has a similar value of digestibility as forages (54-60%).

They have also been proven useful in the removal of metal ions from aqueous solutions (Villaescusa et al., 2004).

Vine prunings have been studied as a wood substitute for particleboard production. The results prove that even though it is not the best material, the final product conforms to EU standards (EN 312, Part 3, 1996) (Ntalos and Grigoriou, 2002). Vine pruning production is 5 tons per hectare per year, which is higher than that of forests in temperate zones.

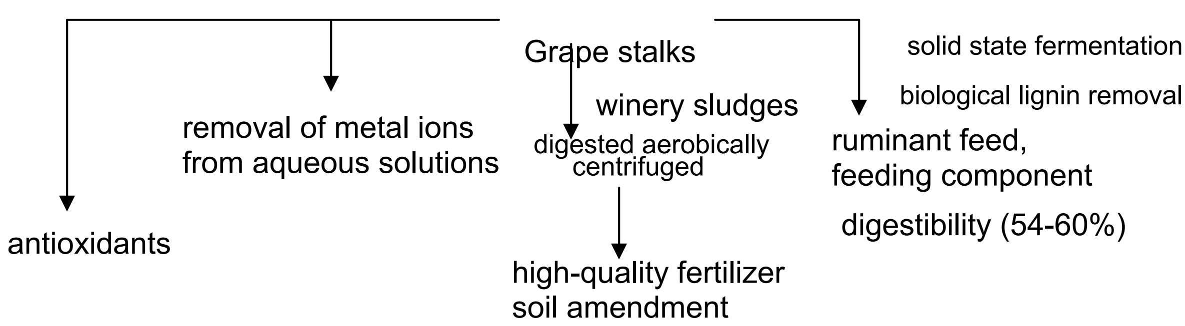

Figure 2. Grape stalks uses.

2.2 Grape pomace

Grape pomace has a humidity of about 70 % and makes up for 11-15 % of grapes crushed. One ton of pomace is composed of 249 kg of "stalks",. *225* kg of grape seeds and 425 kg of grape pellicles. All the major products deriving from grape pomace are depicted in Figure 3 and table 2.

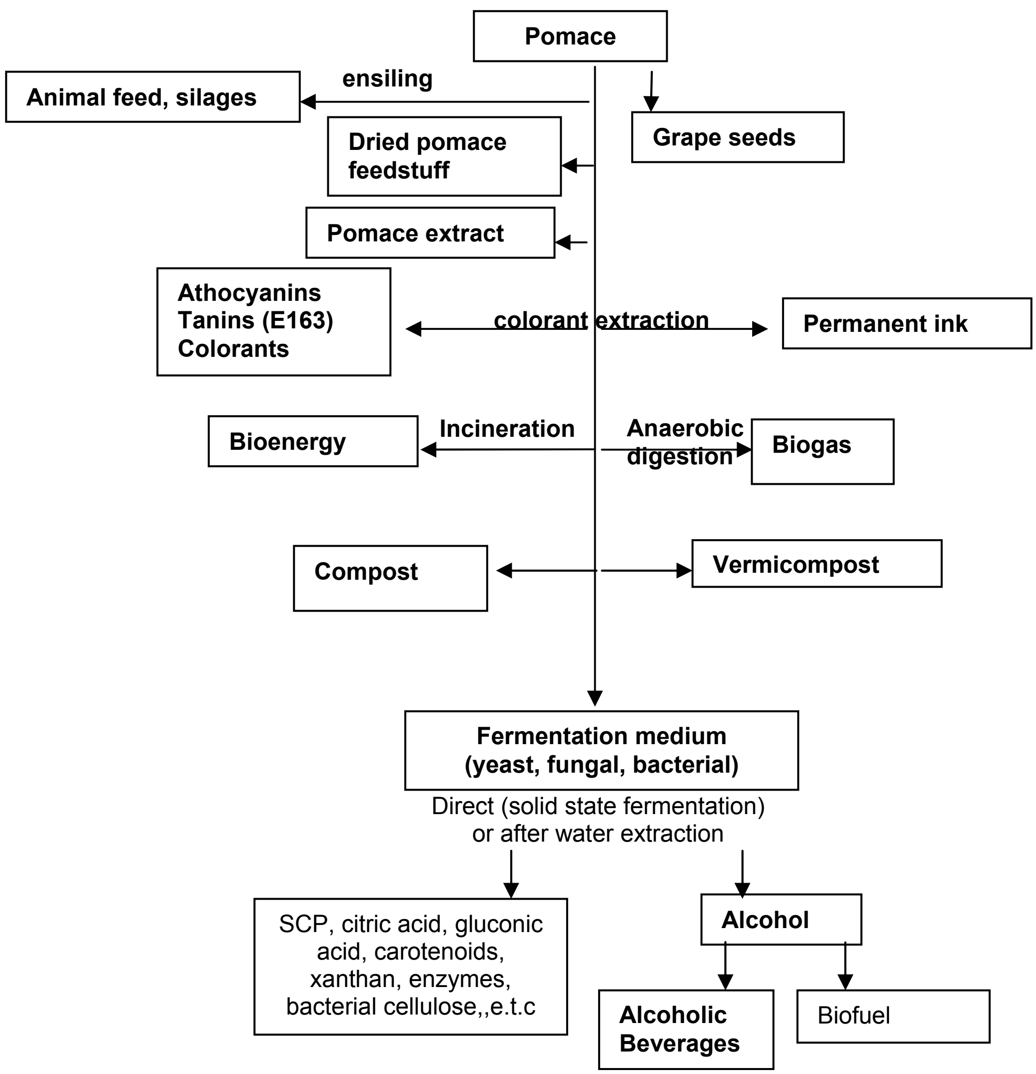

Figure 3. Products deriving from grape pomace.

Table 2. Grape pomace Composistion

Quantity: 11-15% of grapes
Composition: 70% humidity

- Grape seeds (~30% of wet pomace)
- Fibers (cellulose), pectins, mineral (K), organic acid
- Sugars (up to 150 g/Kg)
- Phenolics (tannins, anthocyans)
    - Pigments ~9 kg/t (red grape pomace)
- Tartrate ~50 to 75 kg/t

After water extraction grape pomace can be been used as fermentation medium to produce single cell protein (SCP) (Lo Curto and Tripodo, 2001) and bacterial cellulose (Tataridis et al., 2006) as well as other valuable metabolites.

Solid state fermentation (SSF) is another way to produce a variety of compounds like ethanol (Tataridis et al., 2005), citric acid, gluconic acid, carotenoids, xanthan, e.t.c. Also the possibility of enzyme production by solid state fermentation (cellulases, xylanases and pectinases) has been studied (Botella et al., 2005). Solid state fermentation essentially involves the growth of microorganisms on wet solid supports with limiting free water. This technology is interesting because is considered an appropriate approach for processes including the bioremediation of agricultural wastes and the biotransformation of crops. Moreover, SSF has been successfully applied in the preparation of new high value products, such as secondary metabolites, organic acids, pesticides, aromatic compounds, fuels and enzymes. The advantages of SSF in comparison to traditional submerged fermentation are better yields, easier recovery of products, foam absence and smaller reactor volumes. Moreover, contamination risks are significantly reduced due to the low water contents and, consequently, the volume of effluents decreases. It has been shown that for some specific processes, particularly enzyme production, the cost of these techniques is lower and the production higher than that in submerged cultures.

Direct incorporation of grape pomace into agricultural land, a common practice, has caused serious problems since degradation products can inhibit root growth. An alternative to overcome such disadvantages and to recycle wastes is composting (Diaz et al., 2002).

Another suitable soil amendment is the wastewater (winery-sludge) derived from the aerobic treatment of wastewaters of the winery.

Vermincompost has even more beneficial effect than normal compost (increased nitrogen, humic materials, hormones and auxins, and pH) (*Dominguez et al., 1997)*. As an aerobic process, composting leads to a nitrogen mineralization and the use of earthworms in vermicomposting increases and accelerates this nitrogen mineralization rate. Winery wastes are already being used for commercial production of vermicompost together with other material (Nogales et al., 2005; Xenopoulou, 2001).

Grape by-products have been used for animal nutrition (Vaccarino et al., 1992). Ensiling of grape pomace in earthen pits either alone or in combination with poultry litter gave good quality silages, which can be consumed by cattle.

Grape skin pigment is used in wine making. The average figures for the grape pigment vary from 12 kg/t (red wine lees) to 9 kg/t (red grape pomace), the final product being in the form of a liquid concentrate with 30 g of pigments per kilogram of solution. Another proposed use is making them into a very strong permanent ink (Leber, 2004).

2.3 Feed and food supplements

Grape pomace and other solid winery waste has been used for he recovery of food ingredients, nutraceuticals and functional foods (Hang, 1988), that provide demonstrated physiological benefits or reduce the risk of chronic disease (grapeseed oil, β-glucans, antioxidants, e.t.c.).

However antioxidant value depends on the recovery process. In most cases treatment procedures tend to decrease that value thus negating their potential uses as food supplements. If a cascade process is used for the recovery of more than one substances then depending on the order of recovery we can get higher quality for some products and lower for others (figure 4).

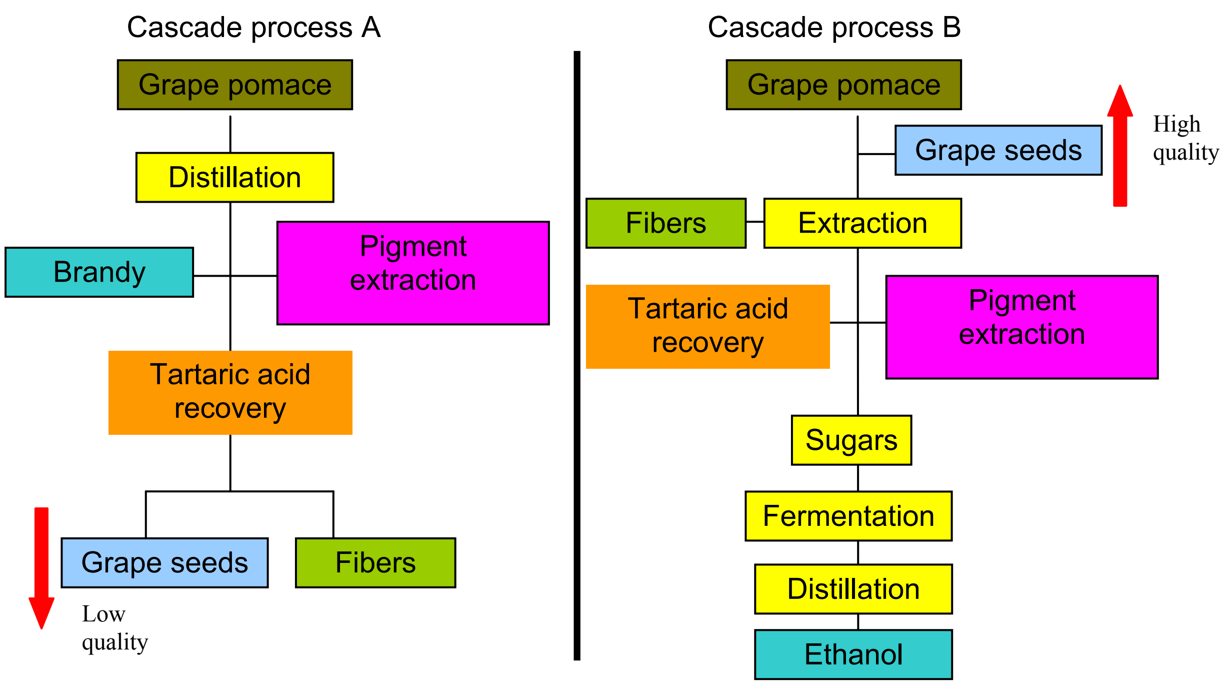

Figure 4. Quality differences due to cascade process

By-products like grape pomace, seeds and stalks are known sources of antioxidants (phenolic acids, quercetin, flavonoids, phytoelexins and pterostylbenes, resveratrol, e.t.c.), counteracting the effects of saturated fat and reducing the incidence of coronary heart disease mortality. These substances also have anti-inflammatory activity, anti-carcinogenic and anti-mutagenic effects (Shrikhande 2000). There is considerable amount of scientific articles regarding recovery of these products from grape by-products and of new methods which deal with the improvement of techniques for the recovery and antioxidant activity of the isolated compounds. (Palenzuela et al. 2004; Yilmaz and Toledo, 2006).

These substances have an important added value. According to Leber (2004) grapeseed flour has “3,000- to 5,000-fold more in antioxidant value than regular flour”.

Grape pomace, seeds, skin and stems extracts have effective anti-bacterial results when tested on bacteria species at a concentration of five per cent. The extracts can be used in food formulations to protect food against spoilage bacteria. (Ozkan et al., 2004).

2.4 Grape seed, Grape seed oil

Cardioprotective effects of grape seed proanthocyanidin are well known. Grape skins and seeds contain flavonoids (catechin, epicatechin, procyanidins and anthocyanins), phenolic acids (gallic acid and ellagic acid) and stilbenes (resveratrol and piceid). Grape seed procyanidin extract has in vivo antioxidant activity and could be as important as vitamin E in preventing oxidative damage in tissues by reducing the lipid oxidation and/or inhibit the production of free radicals.

Grapeseed oil is a vegetable oil pressed from the grape seeds (figure 5, table 3). It has a relatively high smoke point, approximately 216 °C, so it can be safely used for heating. In addition, it has a clean, light taste that has been described as 'nutty' and is safe for cooking food. Less grape seed oil is needed for cooking porposes, compared with other oils.

Grape seed oil is reputed to contain plentiful antioxidants, as well as substanses which lower cholesterol levels. It also cotains vitamin E (0.8 to 1.2 g/kg), vitamin C and Beta-Carotene. There is an unconfirmed information that grape seed oil also contains vitamin D. Grape seed oil also contains 0.8 to 1.5% unsaponifiables rich in phenols (tocopherols) and steroids (campesterol, beta-sitosterol, stigmasterol).

According to current knowledge, grapeseed oil, a high linoleic (76 %) product, is the only food known to raise HDL (good cholesterol) and lower LDL (bad cholesterol). Low level HDL is also a risk facror for impotence. Linoleic acid is one of two essential fatty acids people cannot manufacture themselves. Linoleic acid is an omega-6 fatty acid.

Grape seed oil is a preferred cosmetic ingredient for damaged and stressed tissues, for possessing regenerative and restructuring qualities which allow a better control of skin moisturization and protection.

Table 3. Grape seeds composistion

Quantity: ~30% of wet pomace)
Composition:

- Water 25-45%
- Sugars, polisaccharides 34-36%
- Organic acids 2-7%
- Oils, fatty acids 13-20% [76% linoleic acid (omega-6 fatty acid) - unsaturated oils]
- Phenolics 4-6% (proanthocyanidin, flavonoids, phenolic acids, stilbenes)
- Nitrogen substances 4-6,5%
- Minerals, inongranic 2-4%
- Vitamines [E (tocopherol), A, C, PP, P, B1, B2, B5, B6, B9], b-Carotene

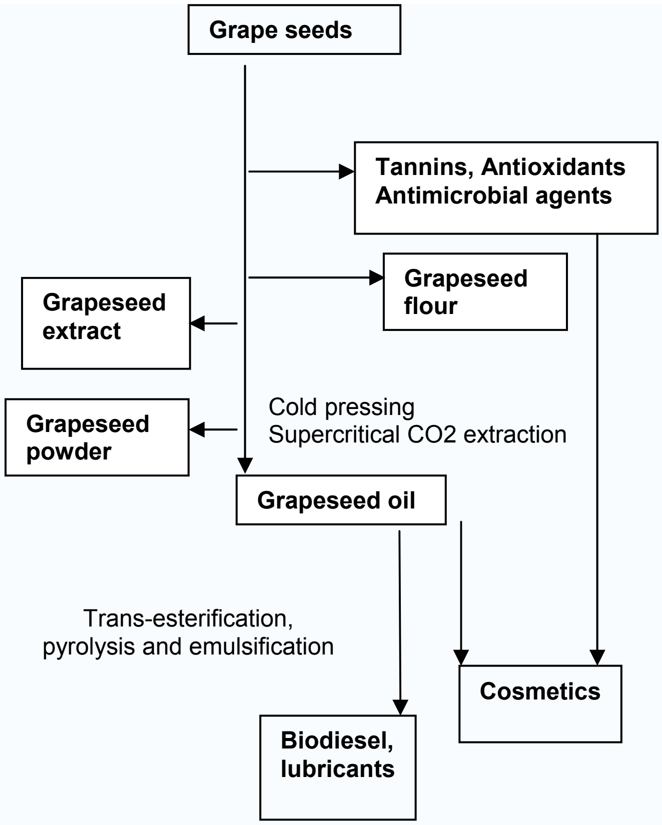

Figure 5. Grape seed uses and products.

2.5 Yeast lees and subproducts

Yeasts have been used as a probiotic supplement (at 0,1 %) in fish food (Lara-Flores et al., 2003) as well in animal feed. They contain protein, carbohydrates and vitamins.

Approximately 20 % of the total dry weight of yeast is cell wall material, yeast cell walls contain 30-60 % 1,3-b-D-glucan in dry weight. Beta-1,3-glucans are non-toxic and potent non specific stimulators of the immune response (against infectious diseases cancer, viruses, radiation, e.t.c.) acting as a microphage activator (Kogan, 2000, Peppler, 1983). New non degrading isolation methods which could be used for production of b-1,3-glucans from wine yeast lees are available (Freimund et al., 2003). The uses and products which can be recovered from yeast lees can be seen in figure 6.

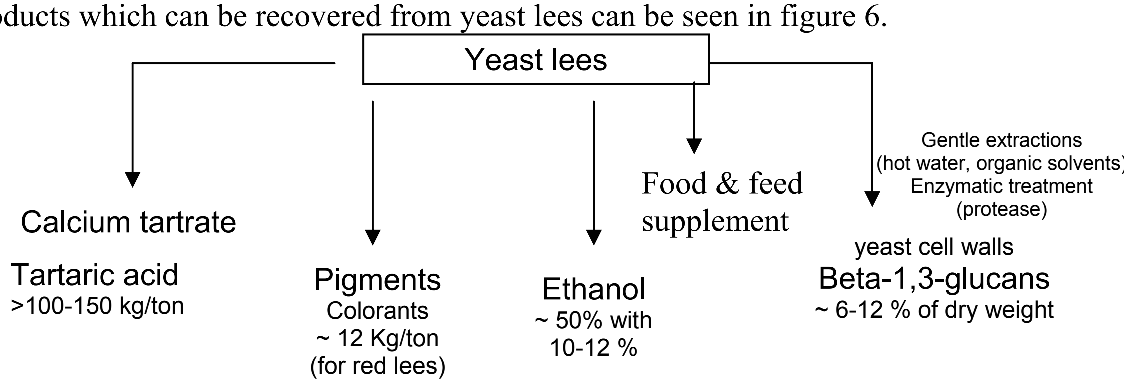

Figure 6. Yeast lees products

2.6 Tartrate recovery

Winery residues are also used for tartrate recuperation (Braga et al., 2002). Results indicate values ranging from 100 to 150 kg/t (wine lees) and 50 to 75 kg/t (grape pomace) with respect to the potential production of calcium tartrate.

2.7 Fuel and biofuel

Fuel ethanol production from grapes is already a reality in Europe. This situation arises as a result of surplus wine production and is a continuing problem. Bioethanol is an octane enhancer. Although bioethanol production from wine has negative energy yield, it is possible to improve the performance of grapes as an energy crop, principally by raising yields. It was estimated that a net energy output of 69 GJ/ha is possible (with an energy output:input ratio of 1.8), and production costs (after deduction of revenue for co-products) could be reduced to $0.80 per liter. (Scrase et al., 1993).

Biodiesel production is also possible. After mechanical separation, the seeds are pressed to yield (at approx. 12% by weight) poly-unsaturated oils. While this oil can be burned directly in internal combustion engines (possibly resulting in some fouling or glazing over time), splitting the tri-glyceride molecule can result into cleaner-burning single-stranded methyl or ethyl esters (Leber, 2004). Beacause of the high production cost seed derived oils are more attractive as friction-reducing fuel additives that improve delivered horsepower and extend engine life by reducing internal heating and wear.

The "press cake," from the seed press, also has energy potential. It can be pelletized and burned, yielding – per initial calorimetric studies – approximately 50% of the heating value of wood pellets. Many of the organic components of the press-cake pellets can also be volatilized to form a biogas for subsequent heat and power production. Grape seed with the oil removed also has uses as fuel pellets which have 133 percent the heating value of wood pellets (Leber 2004).

The suitability of incineration for dried grape peels and exhausted grape-stalks has been tested with good results (Mariani et al., 1992)

Dried grape can be converted to carbon products by low-temperature pyrolysis. The calorific value of these products was determined and compared with that of commercial barbeque briquettes. The gross heat of combustion of grape charcoal briquettes was approximately 90% of that for the commercial briquette, while the dried press-cakes contained approximately 65% (Walter and Sherman, 1976).

Biogas can also be produced through thermophilic anaerobic digestion of alcohol distillery wastewaters. The methane concentration of the biogas is high (76%), thus making it a valuable fuel (Vlissidis and Zouboulis, 1992).

3. CONCLUSIONS

The value-added conversion of the bio-products from winemaking can help in reducing the negative costs and demonstrating sustainability in winemaking. An important number of the above mentioned processes are already used commercially while others might prove useful with time as the price of rival processes increase (ex. oil price).

In order to use any technique on an industrial scale it is necessary to undertake laboratory and pilot plant trials, life cycle analysis and feasibility studies. Studies should also be undertaken regarding the combined or sequential use of different processes in order to maximize product recovery and minimize the secondary wastes. Most importantly a consensus of the industry stakeholders is necessary based on the understanding of the potential benefits from the reuse of winery wastes and by-products.

REFERENCES

1. Bertran, E., X. Sort, M. Soliva, I. (2004). Trillas Composting winery waste: sludges and grape stalks, Bioresource Technology, 95, pp. 203–208.
2. Bisson, L.F., A.L. Waterhouse, S.E. Ebeler, M.A. Walker, J.T. Lapsley. (2002). The present and future of the international wine industry, Nature, 418, pp. 696-699.
3. Botella, C., I. de Ory, C. Webb, D. Cantero, A. Blandino. (2005). Hydrolytic enzyme production by Aspergillus awamori on grape pomace. Biochemical Engineering, 26, pp. 100–106.
4. Braga F.G, Fernando A. Lencarte Silva, Arminda Alves. (2002). Recovery of Winery By-products in the Douro Depomaceated Region: Production of Calcium Tartrate and Grape Pigments. Am. J. Enol. Vitic., 53, 1, pp. 41-45.
5. Diaz, M.J., E. Madejo, F. Lopez, R. Lopez, F. (2002). Cabrera Optimization of the rate vinasse/grape pomace for co-composting process, Process Biochemistry, 37, pp. 1143–1150.
6. Dominguez, J., C. A. Edwards and S. Subler. A. (1997) Comparison of vermicomposting and composting, BioCycle, April, pp. 57-59
7. Einion, T. (1999). Mixed Residual Waste, Pre-Treatment and Recycled Organics. Recycled Organics – Expanding the Horizons, 23-25th November, Hyatt Coolum, Australia.
8. Famuyiwa, O., Ough, C.S. (1981). Grape pomace: Possibilities As Animal Feed. Am. J. Enol. Vitic., 133, 1, pp. 44-46.
9. Freimund S., M. Sauter, O. Kappeli, H. Dutler. (2003). A new non-degrading isolation process for 1,3-b-D-glucan of high purity from baker's yeast Saccharomyces cerevisiae, Carbohydrate Polymers, 54, pp. 159–171.
10. Giulio, M., E. Benfenati, R. Fanelli, A. Nicoli, I. E. Bonfitto, S. Jacopone. (1992). Incineration of agro-industrial wastes and macro- and micropollutants emission, Chemosphere, 24, ll, pp. 1545-1551.
11. Hang, Y.D. (1988). Recovery of food ingredients from grape pomace. Process Biochem., 22, pp. 2-4.
12. Hang, Y.D., E.E. Woodams. (1985). Grape pomace a novel substrate for microbial production of citric acid, Biotechnol. Lett., 7, pp. 253–254.
13. Hazell, P. (2000). Winery Environmental Management Regulation: A State Comparison. 1st National Wine Industry Environment Conference. 31 October – 1 November. Royal Adelaide Showgrounds. Australia.
14. Inbar, Y., Y. Hadar and Y. Chen (1992). Characterization of humic substances formed during the composting of solid wastes from wineries, The Science of the Total Environment, 113, pp. 35-48.
15. Kogan, G. (2000). 1-3,1-6-b-D-Glucans of yeasts and fungi and their biological activity. Studies in Natural Products Chemistry, 23, pp.107–152.
16. Lara-Flores, M., M.A. Olvera-Novoa, B.E. Guzman-Mendez, W. Lopez-Madrid. (2003). Use of the bacteria Streptococcus faecium and Lactobacillus acidophilus, and

the yeast Saccharomyces cerevisiae as growth promoters in Nile tilapia (Oreochromis niloticus) Aquaculture, 216, pp. 193–201.
17. Leber, E. (2004). Recovering Value from Winemaking By-Products. ASEV 55th Annual Meeting San Diego, California June-July.
18. Louli, V., N. Ragoussis, K. Magoulas. (2004). Recovery of phenolic antioxidants from wine industry by-products, Bioresource Technology, 92, pp. 201–208.
19. Nicolini, L., C. Volpe, A. Pezzotti, A. Carilli. (1993). Changes in in-vitro digestibility of orange peels and distillery grape stalks after solid-state fermentation by higher fungi, Bioresource Technology, 45, pp. 17 -20.
20. Nogales R., C. Cifuentes, E. Benitez. (2005). Vermicomposting of Winery Wastes: A Laboratory Study. Journal of Environmental Science and Health. 40, 4, pp. 659 - 673.
21. Ntalos, G.A., A. H. Grigoriou. (2002). Characterization and utilisation of vine prunings as a wood substitute for particleboard production, Industrial Crops and Products, 16, pp. 59–68.
22. Ozkan, G., O. Sagdic, N. Goturk Baydar, Z. Kurumahmutoglu. (2004). Antibacterial activities and total phenolic contents of grape pomace extracts," Journal of the Science of Food and Agriculture, 84, 14, pp. 1807-1988.
23. Palenzuela, B., L. Arce1, A. Macho, E. Muñoz, A. Ríos, M. Valcárcel. (2004). Bioguided extraction of polyphenols from grape pomace by using an alternative supercritical-fluid extraction method based on a liquid solvent trap, Analytical and Bioanalytical Chemistry, 378, 8, pp. 2021–2027.
24. Peppler, H. J. (1983). Fermented feeds and feed supplements. In Biotechnology vol 5, G. Reed (ed.), VCH Publishing Co., Weinheim, West Germany.
25. Rodriguez Couto, S., Elena Lopez, M. Angeles Sanromán. (2006). Utilisation of grape seeds for laccase production in solid-state fermentors, Journal of Food Engineering, 74, pp. 263–267.
26. Scrase, J. I., D. Hall, D. C. Stuckey. (1993) Bioethanol from grapes in the European Community, Biomass and Bioenergy, 5, 5, pp. 347-358.
27. Shrikhande, A. J. (2000). Wine by-products with health benefits. Food Research International, 33, pp. 469–474.
28. Tataridis, P., K. Apostolopoulos, E. Moraitaki, D. Spanou, E.T. Nerantzis, S. Papakonstantinou. (2006). Utilization of grape pomace for the production of bacterial cellulose. Pan-Hellenic Union of Bioscientists Congress “Biosciences in the 21st century”, 13-15 April, Athens, Greece, pp. 114, (in greek).
29. Tataridis, P., Mylonas P., Mylona D., E..T. Nerantzis. (2005). Bioethanol Production from Solid State Fermentation (SSF) of Grape Pomace. International Conference Renewable resources and biorefineries, Ghent – Belgium, September, 19-21, p. 92.
30. Vaccarino, C., Lo Curto, R.B., Tripodo, M.M., Bellocco, E., Lagan, G., Patan, R., 1987. Effect of SO2, NaOH and Na2CO3 pretreatments on the degradability and cellulase digestibility of grape pomace, Biol. Wastes 20, pp. 79-88.
31. Vaccarino, C., Lo Curto, R.B., Tripodo, M.M., Patan, R., Ragno, A. (1992). Grape pomace as a source of feestuff after chemical treatments and fermentation with fungi, Bioresource Technol., 40, pp. 35-41.

32. Villaescusa, I., N. Fiol, M. Martinez, N. Miralles, J. Poch, J. Serarols. (2004). Removal of copper and nickel ions from aqueous solutions by grape stalks wastes, Water Res., 38, pp. 992–1002.
33. Vlissidis, A. and Zouboulis A.I. (1993). Thermophilic anaerobic digestion of alcohol distillery wastewaters, Bioresource Technology, 43, pp. 131-140.
34. Xenopoulou, K. (2001). Use of the mediterranean sea grass posidonia oceanica for the production of organic compost and compost, for agriculture with co-composting of organic waste from agricultural, animal or industrial units. Patent No. GR99100348.
35. Yilmaz, Y., R.T. Toledo. (2006). Oxygen radical absorbance capacities of grape/wine industry byproducts and effect of solvent type on extraction of grape seed polyphenols, Journal of Food Composition and Analysis, 19, pp. 41–48