8

Patent Survey

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8.1 Introduction

Over the last decades, various epidemiological studies have reported the direct relationship between antioxidant-rich diet and the decreasing risk of developing certain chronic diseases, such as cardiovascular pathologies, cancer, neurodegenerative disorders, and atherosclerosis (Gómez-Pinilla and Nguyen, 2012; Weng and Yen, 2012). Human intake of bioactive compounds with special health benefits takes place mainly through the daily fruit and vegetable consumption. Foods can also be fortified with bioactive compounds to produce functional foods (Tumbas Šaponjac et al., 2016).

To this direction, there is an increasing interest to find new sources of natural bioactive compounds in order to recover and reutilize them as additives in foods. The fruits and vegetables processing industry produces large amounts of waste materials, such as skins, pulp residue, and seeds (Ćetković et al., 2012) that could compromise a potential source of antioxidant compounds instead of being used as animal feed, fertilizer or sent to sanitary landfill and cause environmental problems (Su and Silva, 2006; Aaby et al., 2005). Phytochemicals from agro-industrial waste may be used as functional food ingredients, natural antioxidants, or colorants in order to replace their synthetic equivalents that have experienced growing criticism due to potential toxicological effects (Iglesias et al., 2010; Vulić et al., 2012).

Among the different fruits, grape annual production is the world's largest one, accounting for >67 million tons, 80% of which is used in winemaking (Fontana et al., 2013; Kammerer et al., 2004). Subsequently with winemaking, wine industry produces millions of tons of leftovers that represent an ecological and economical waste management issue (Fontana et al., 2013). The type of residues produced is closely dependent on the specific vinification procedures. The major residues from winemaking activity are represented by: organic wastes (grape pomace, containing seeds, pulp and skins, grape stems, and grape leaves), wastewater, emission of greenhouse gases (CO_2, volatile organic compounds, etc.), and inorganic wastes (diatomaceous earth, bentonite clay, and perlite). It is estimated that in Europe alone, 14.5 million tons of grape by-products are produced annually (Teixeira et al., 2014). Fig. 8.1 represents the winemaking process and specific points where organic by-products emerge.

Handbook of Grape Processing By-Products. http://dx.doi.org/10.1016/B978-0-12-809870-7.00008-9

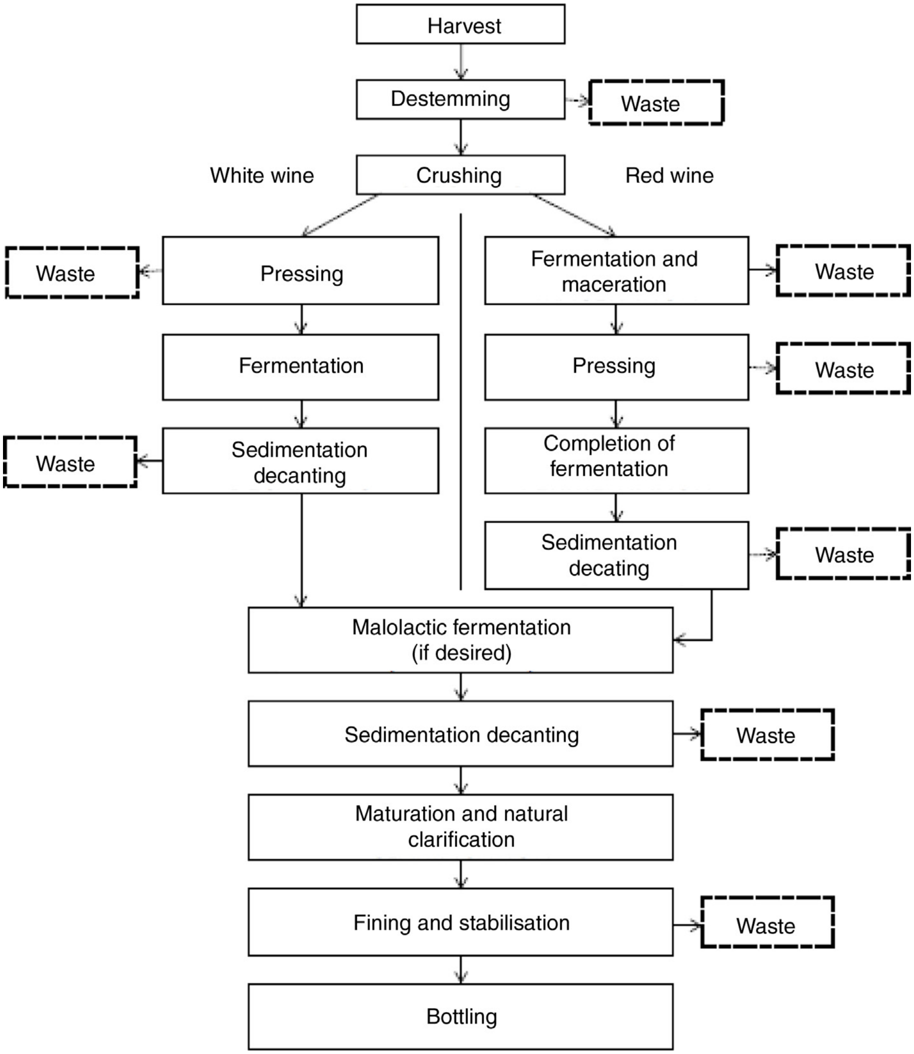

FIGURE 8.1 Diagram of organic waste generated in the process of making red and white wine. *Adapted from Arvanitoyannis, I.S., Ladas, D., Mavromatis, A., 2006. Review: wine waste treatment methodology. Int. J. Food Sci. Technol. vol. 41, 1117–1151; Devesa-Rey, R., Vecino, X., Varela-Alenda, J.L., Barral, M.T., Cruz, J.M., Moldes, A.B., 2011. Valorization of winery waste vs cost of not recycling. Waste Manag. vol. 31, pp. 2327–2335.*

Grape processing by-products (e.g., grape pomace, stems, etc.) are suitable material to be employed in different processes, like the extraction of grape seed oil and polyphenols, production of citric acid, methanol, ethanol, and xanthan via fermentation, and the production of energy by methanization (Teixeira et al., 2014), as a dry fuel for production of electrical energy (Unal and Alibas, 2007) or even biodiesel (Fernández et al., 2010). Also, vinification grape waste can be used to obtain alcoholic drinks by short fermentations and distillation (Brahim et al., 2014; Deng et al., 2011). Grape processing by-products are also known to contain a significant amount of phenolics with health promoting effects, at different concentrations and chemical structures depending on the grape variety. Phenolics comprise an objective of research and extraction for scientists and technologists, as noted in Chapters 1–7. This is happening because industries are looking to find high value and sustainable alternatives for their residues.

To this direction, winemaking by-products have been proposed as an alternative source of natural antioxidants that could be used to fortify foods. For example, Tseng and Zhao (2013) fortified yogurt and salad dressings (Italian and Thousand Italian) with wine grape pomace. Results showed that grape pomace as antioxidant dietary fiber may be used as a functional food ingredient for promoting human health and extending shelf-life of food products. In another study, Mildner-Szkudlarz et al. (2015) used grape processing by-products as additives in muffins. Muffins enriched with appropriate levels of grape pomace (20%) showed lowering of the chronic myeloid leukemia (CML) level and no significant changes in the sensory profile. Zhu et al. (2015) reported the application of dietary fiber from grape pomace as phenolic-rich matrix and dietary supplement with benefits on human health, while Pedroza et al. (2013) added different dehydrated waste grape skins from the juice industry into aged and young red wines as an innovative way of compensating for color loss before bottling. All these examples indicate that there is a potential market attracting the attention of scientists and food industry. To this line, the current chapter focuses on the available patents dealing with the recovery of bioactive compounds from grape processing by-products in order to indicate commercialization prospects in the field.

8.2 Protected Intellectual Property and Innovation

The idea of the industrial property right as a reward for creativity dates back to ancient times. The oldest evidence of inventions being protected originates from the Italian city states. A patent is a right of exclusion, that is, it authorizes its holder to exclude others from commercially making, selling, offering, using, importing for these purposes or owning for these purposes the subject matter of the invention. The inventor does not, however, have the right to prohibit private use of the protected invention or use for the purposes of research (the research privilege). The patent enables its owner to exclude others from exploiting the invention in the territory for which it has been granted (European Patent Office, www.epo.org).

The progress and well-being of humanity depends largely on its capacity to create and invent new works in the areas of technology and culture. The legal protection of new creations encourages the commitment of additional resources for further innovation. The promotion and protection of intellectual property spurs economic growth, creates new jobs and industries, and enhances the quality and enjoyment of life (World Intellectual Property Office, www.wipo.int). Intellectual property has a commercial value since it allows early protection of competitive advantages, applications as a marketing edge, increase of business value and implementation as a potential revenue stream through licensing, or investment attraction. Companies need to take precaution steps to protect intellectual property by filing patent applications where appropriate, registering trademarks and copyrights, as well as taking appropriate steps to protect trade secrets (Galanakis et al., 2015).

Innovation can lead to inventions which can be protected by patent, the only effective way that provides protection of intellectual property. Only registration of inventions in patent and trademark offices provides exclusive rights for use of the product. A strategic planning is required to successfully place a new product or method to the market, as well as to avoid infringement.

Before applying for a patent, some of the basic thoughts that should be addressed include:

- are there any third party rights?
- is the patent already protected?
- is it patentable?
- are there any issues in licensing?
- can it be brought to the market?
- can it be patented in other countries too?
- a question of financing etc.?

The advantages of protected intellectual property rights include securing exclusive right to the invention and competitiveness in developing new products. It also allows finding investors financial and strategic partners, increases the value of the innovation and serves as an indicator of research activity. Careful novelty research is strongly advised in terms of interdiction of any kind of public presentation and publication prior to closure of the patent process. Opposition to patents occurs in small percentage, however, pauses the patent use and is primary to intrigue process.

The World Intellectual Property Office (WIPO) provides international novelty and patentability search, a single place of filing and final decision for countries based on common rules and postponement for 30/31 months from priority date. At the European level, the European Patent Organization (EPO) provides a single place of filing, completion, and patent granting for the 27 EPC member countries at lower costs compared to filing to each country separately (Galanakis et al., 2015). The database search of WIPO (Patentscope) and EPO (Esapcenet) allows an overview on existing technologies, potential suppliers, competitors or customers, potential infringements, and technology trends.

Relevant amounts of phytochemicals and other valuable compounds are still available in the residues of the wine industries, because these are not fully extracted from grape skin and seeds, or they remain in removed or unused grape parts (stems, lees, etc.). This has induced many emerged inventions to be patented. To demonstrate the value of grape by-products reutilization, Fig. 8.2 represents an overview of the number of patents dealing with various grape by-products found through Patentscope and Espacenet search.

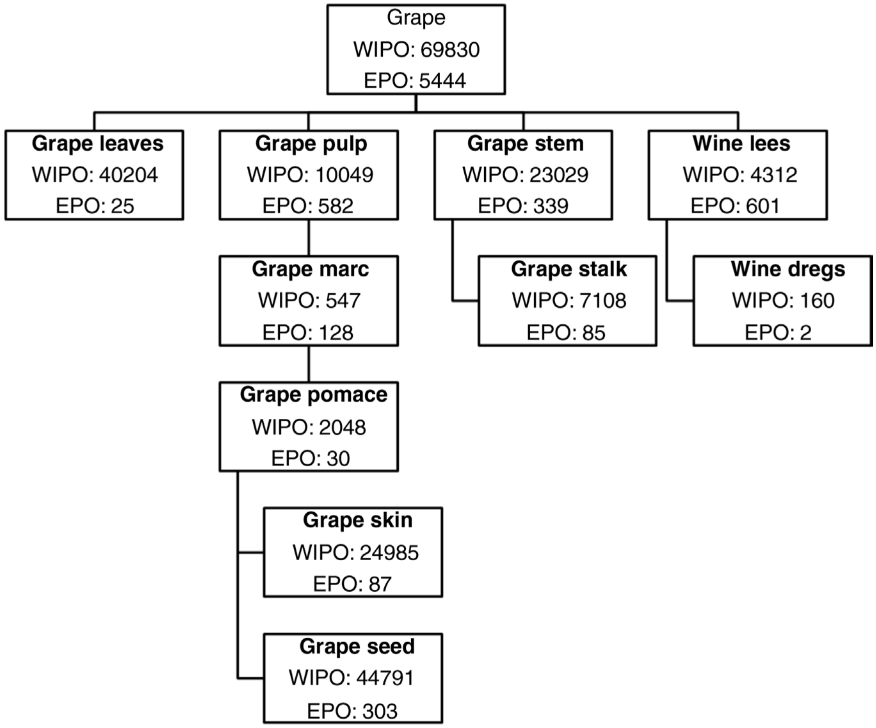

FIGURE 8.2 Number of existing patents in WIPO and EPO applying grape and grape by-products.

Selected patents are summarized in Table 8.1. Further, patents are divided in three subgroups, depending on the aim and outcome, and discussed in the following subsections:

- Patents reporting methods for the recovery of grape processing by-products;
- Patents reporting application of grape processing by-products;
- Patents reporting health benefits of grape processing by-products.

8.2.1 Patents Reporting Methods for the Recovery of Grape Processing By-Products

Grape contains natural pigments up to 300 mg/100 g, or 3.7% of weight. Coloring compounds are comprised of anthocyanins, which beside bright color, have important physiological functions (Puškaš et al., 2010; Tumbas Šaponjac et al., 2014). Therefore, extraction and utilization of pigments from grape and grape by-products is extremely important. Extraction of grape pigments directly from grape skins are disclosed in two Chinese patents, CN104448902 (Tan, 2015) and CN102675904 (Yang, 2012).

The first patent suggests extraction of grape skin powder with 95% ethanol (ratio 1:1–10), acidified with citric acid to pH value to 2–4, at room temperature, for 10–60 min. The process is then repeated, supernatants mixed, centrifuged and freeze-dried into a powder. Mixing for 5–10 min at high-speed is suggested as a pretreatment.

Table 8.1 Patent Survey Disclosing Various Grape By-Products

Grape by-product	Patent Application Number	Applicant	Title	Outcome	Potential Application	References
Grape pomace (marc)	US6,190,716	Galbreath, JSO	Method for preparing a grape derived product	Method for preparation of readily digestible food supplement rich in resveratrol, ellagic acid, and other phenolic compounds and antioxidants	Ingredient of functional food, food supplement	Galbreath (2001)
	CN104287048	Xihua University	Preparation method of grape pomace essence and fruit and vegetable powder combined blending agent	Red powder containing fruit, vegetable and grape pomace extract	Ready to use food, food ingredient or dietary supplement	Xing et al. (2015)
	US6,544,581	Canandaigua Wine Company, Inc.	Process for extraction, purification and enrichment of polyphenolic substances from whole grapes, grape seeds and grape pomace	Method for producing free flowing powder containing monomeric and oligomeric procyanidins, and anthocyanins, flavanols and cinnamic acids in case of red grapes	Ingredient and/or colorant in foods, beverages, dietary supplements, nutraceutical products	Shrikhande et al. (2003)
	EP1288288	Balice Distillati SRL	Process and plant for calcium tartrate extraction from winemaking by-products	Plant for isolation of calcium tartarate from winemaking by-products with improved performances	Food preservative and acidity regulator	Gennaro (2003)
	CN104798854	Northwest A&F University	Method for making grape pomace dietary fiber nutritious biscuits	Process for making confectionery products with improved processing performance, product crisp degree and nutritional characteristics	Functional biscuits, cookies and crackers	Wang et al. (2015)
	CN102268358	Shaanxi University of Science & Technology	Production process for brewing grape fruit vinegar by using grape pomace	Process for making vinegar utilizing grape pomace	Condiment, preservative, flavoring agent, food and beverage ingredient	He et al. (2011)
	US20,060,121,137	Hartle, DK, Greenspan, P, Hargrove, JL, Hofacre, CL & Bralley, EE	Muscadine grape pomace in the treatment of intestinal inflammation	Demonstrated reduction or prevention of colon inflammation in animals by administration of muscadine grape pomace through feed	Antiinflammatory supplements for food and feed	Hartle et al. (2006)

Grape skin	US20,050,002,961	Capri Sun AG	Concentrate comprising green tea, grape skin extract and grape extract, the production thereof and use of the same	Method for producing liquid or solid concentrate with high antioxidant effect and reduced bitterness	For cold or hot beverages, supplementary food to other foodstuffs, such as mueslis, dairy products etc.	Wild and Sass (2006)
	US20,160,067,298	Clark Atlanta University	Muscadine grape skin extract as treatment for bone metastatic cancer	Decrease in Snail expression, CatL and STAT-3 activity in prostate and breast cancer cells and formation of mature osteoclasts.	Treating bone metastatic disease resulting from prostate or breast cancer	Odero-Marah (2016)
	US20,110,311,680	Mercian Corporation	Soft drink, grape skin extract, and methods of preparation of same	The method for preparation of grape skin extract rich in precursors of 3-mercaptohexan-1-ol, and producing soft drinks with fruity aroma and after aroma	Food additives (flavors or flavor enhancing compounds), beverages	Takase et al. (2011)
	CN102675904	Yang Qingsong	Grape skin pigment extracting method	Method for preparation of red grape skin pigment powder	Dyes, food additives (coloring)	Yang (2012)
	CN104448902	Qingdao Zhongren Zhiye Biotechnology Co., Ltd.	Grape skin pigment extraction technology	Method for preparation of red grape skin pigment powder	Dyes, food additives (coloring)	Tan (2015)
	CN1380093	Xinjiang Medical University	Alcohol extraction method for extracting grape skin extract and its grape liver-nourishing capsule	Method for producing capsules with grape skin extract which have demonstrated protective action against chemical hepatic injury	Dietary supplements	Ma et al. (2002)
	CN1039841	Zhang Guangjin	Method of making vinegar using grape skin	Profitable and faster method for producing vinegar from grape skin. Residue can be used as feed.	Condiment, acidity regulator, feed	Zhang and Zhao (1990)
	CN102286061	Not available in English	Method for extracting and purifying oleanolic acid from grape skin	Method for producing oleanolic acid from grape skins with improved efficiency, cost effectiveness and purity of the final product	Dietary supplements, pharmacological preparations	Not available in English
	KR1020130064595	Taegu Technopark Foodwell Co., Ltd.	Antiobesity composition based on grape skin extracts as an effective component and a manufacturing method of the same	Method for preparation of grape skin extract with antiobesity effect	Dietary supplement, functional food ingredient	Sung et al. (2013)

(*Continued*)

Table 8.1 Patent Survey Disclosing Various Grape By-Products *(cont.)*

Grape by-product	Patent Application Number	Applicant	Title	Outcome	Potential Application	References
Grape seeds	US7,226,627	Eckert Peter, Heinen Winfrid, Knaudt Carola	Grapeseed, cold-pressed grape oil, crushed grape and grape flour	Method for producing cold-pressed grape seed oil with increased polyphenol content	Ingredient of food, food supplement, beverages, animal food, medicament, and cosmetics	Eckert et al. (2007)
	US6,291,517	Dry Creek Nutrition, Inc	Method for preventing or reducing stress-induced gastric injury using grape seed proanthocyanidin extract	ability of grapeseed extract to protect against chronic and acute stress-induced gastrointestinal mucosal injury	Recommendation for preferred daily dose to prevent injury: 100–200 mg of GSPE, in two equally divided doses taken within 15–30 min after meals	Bagchi et al. (2001)
	US5,484,594	Tecnofarmaci S.p.A.; Indena S.p.A.	Process for preparing grapeseed extracts enriched in procyanidol oligomers	Process for the preparation of grape seed enriched in procyanidol oligomers, flavanol monomers, (+)-catechin	Procyanidin oligomers–based dietary supplements	Frangi et al. (2007)
	WO2015184291	Shaklee Corporation	Chardonnay grape seed extract	Process for extracting grape seeds, to obtain isolated procyanidin extract with low degree of polymerization and other biologically useful components	Dietary supplement or functional food, natural flavorant, prebiotic nutraceutical, dietary supplement	Ianiro and Fisher (2016)
	WO2015050883	ELC Management LLC	Topical preparations comprising grape seed, folic acid, biotin, *Bifidobacterium longum* and *Echinacea purpurea*	Formulation for topical skin care preparation, with optimal antioxidant activity utilizing grape seed	Ingredient for skin care preparations, hair care preparations, or color cosmetics	Lee et al. (2016)
	AU2016100373 A4	K D Health Food Australia Pty Ltd	"K.D. BIO-BEAUTY"	Formulation of skin serum comprising hyaluronic acid, nano placenta, grape seed extract and collagen serum	Skin care preparation	Lin (2016)
	WO2014141265	Nofar, Gil, Cogan, Uri	Inhibition of neurodegenerative disease by grape seed extract, green tea extract and probiotic bacteria	Demonstrated synergism between green tea and grape seed extract and *Lactobacillus plantarum* in improving mental abilities	Dietary supplements for neurodegenerative diseases	Nofar and Cogan (2016)

	CN105343353	Wuhu Nokan Biotechnology Co LTD	Application of soft capsule containing sheep placenta, *Radix Salviae miltiorrhizae* and grape seeds in preparing cough medicine	Pharmaceutical preparation utilizing grape seeds that reduces the cough variant asthma	Dietary supplement with antitussive effect	Xu (2016)
	CN105342912	Shi Gengwei	Chinese toothpaste and a method for preparing a matrix using grape seed friction modifiers	Formulation of herbal toothpaste with improved friction effect, antibacterial, antiinflammatory, antipyretic activity and reduced cost	Toothpaste	Shi (2016)
Grape leaves	US6,268,002	Michael, Patrick T.	Method for making grape leaves with meat	Device for making grape leaves with meat with improved performances	Food	Michael (2001)
	KR1020110039756	Youngdong University Industry-Academia Cooperation Group	Powder containing high content of polyphenolic substances using grape leaves, and a producing method thereof	Drying method for producing powder rich in polyphenols from grape leaves	Dietary supplements, functional food, and cosmetics	Chang et al. (2011)
	RU02341279	Ehsperester Anke; Shehfer Ehkkkhard; Zakher Fritts	Filmed tablet containing red grape leaves extract	Method for producing filmed tablet of improved stability including dried extract of red grape leaves	Dietary supplements	Ehsperester et al. (2008)
	CN101913995	Zhang Bo	Method for extracting resveratrol from grape leaves	Simple, low cost method for production of resveratrol crystals isolation from grape leaves	Dietray supplements, functional food ingredients	Zhang et al. (2010)
	KR1020090125988	Industry-Academic Cooperation Foundation, Dankook University Ko, Jung Moon	Massive extraction of quercetin glycoside derived from kyoho grape leaves using column chromatography	A method for extraction quercetin glycoside from grape leaves at industrial level	Dietary supplements, functional food, pharmaceutical preparations, cosmetics	Ko et al. (2009)
	RU02367464	Masuda Kendzhi; Matsumoto Kazuki; Okada Minoru; Takakhashi Koichi	Composition for treating chronic venous insufficiencies, containing aqueous extract of red grape leaves and antiinflammatory agent	Novel topical formulation for preventing or alleviating mild/ moderate chronic venous insufficiency of legs	Pharmaceutical products or medicated cosmetic	Masuda et al. (2009)

(*Continued*)

Table 8.1 Patent Survey Disclosing Various Grape By-Products *(cont.)*

Grape by-product	Patent Application Number	Applicant	Title	Outcome	Potential Application	References
Grape stem (stalk)	KR1020090092405	Okcheon Nonghyup	Method for extracting resveratrol using grape stems, capable of using the by-product of grape as a material	Method for recovery of resveratrol from grape stems	Dietary supplements	Shin et al. (2009)
	WO2005048719	Wisconsin Alumni Research Foundation; Folts, John D.; Shanmuganayagam, Dhanansayan; Hutson, Paul R.	Polyphenol-containing stem and vine extracts and methods of use	Method for preparing grape stem and vine extracts and dietary supplements, nutraceutical and pharmaceutical compositions and methods of using the extracts to prevent or treat coronary artery disease	Dietary supplements, nutraceuticals, pharmaceutical compositions	Folts et al. (2005)
	US20,110,185,629	Bettini Charles Randall; Bettini Deborah	Methods and systems for fuel generation	A method of generating a hybrid liquid hydrocarbon fuel distilling the grape stems into ethanol and mixing the ethanol with gasoline or diesel	Energy production	Bettini and Bettini (2011)
	KR1020120116115	Korea Research Institute Of Chemical Technology; Kookmin University Industry Academy Cooperation Foundation	Anticancer drug composition including resveratrol derivative compound separated from grape stem shell	A method for preparation of grape stem extracts rich in resveratrol derivative and dietary supplement with it, in liquid form, with demonstrated anticancer effect	Dietary supplements, beverages	Choi et al. (2012)
Wine lees (dregs)	CN105349297	Sino French Joint Venture Dynasty Winery Ltd	Method for preparing wine from grape juice precipitation solution	Process for storage and processing of grape juice precipitate	Wine and organic fertilizer production	Zhang et al. (2016)
	JP2000135071	Sunstar Inc; University of Kyoto	Food or pharmaceutical compositions containing the wine lees extract	Method for obtaining wine lee extract for safe human use with no side effect, having demonstrated blood vessel-strengthening activity	Functional food, pharmaceutical preparations, dietary supplements	Mizutani et al. (2000)

GB756854	Permutit Co Ltd.	Improvements in the recovery of cream of tartar from wine lees	Method for production of cream of tartar from wine lee solution	Food additive (stabilizing agent, thickening agent), leavening agent, purgative, cleaning agent	Arden (1956)
KR1020150096062	Bobsoonibobsooni	Manufacturing method of pickled yacon using wine lees	Manufacturing method for preparation of pickled yacon using wine lees	Food	Hwang and Jonghwang (2015)
KR1020000049540	Technical Service Institute National Tax Service	Distilled liquor using wine lees	Method for resaccharifying and fermenting the remaining sugar and unresolved starch in wine lees, distilling, deodorizing, and refining to produce a distilled liquor	Beverage	Kwon et al. (2000)
US20,020,102,287	Shanbrom Technologies, LLC	Antimicrobial lees	Method for recovery of pigments from wine lees; demonstrated antioxidant and antimicrobial effects of wine lees	Demulcent formulations, dietary supplements, antimicrobial agents	Shanbrom (2002)
WO1998024878	Oenodev S.A.R.L.; Ducournau, Patrick	Method for making wine on lees in barrel or vat and implementing device	A method for making wine on lees in barrel or vat and an implementing device.	Beverage	Ducournau (1997)
CN101845036	Xinjiang Hairuisheng Bio-Engineering Co., Ltd.	Method for extracting procyanidin from wine lees	Method for isolation of procyanidines from wine lees that overcomes the defects of large solvent consumption, long extracting time and high production costs	Dietray supplements, functional food	Li and Li (2010)

The other patent discloses the method for extracting red pigments from grape skins which uses 50% ethanol. Briefly, the skins of red grapes are washed, dried, crushed and the skin powder is extracted at 50–70°C for 1–2 h with constant stirring. The extraction is repeated twice; filtrates are combined and evaporated to remove ethanol. Remaining water solution is purified through macroporous adsorption resin which is washed with water after adsorption. The pigments are eluted with 95% ethanol. Ethanol is removed and the concentrate is dried in vacuum to obtain grape skin pigment powder.

Seeds comprise 15%–38% of grape pomace, consisting of up to 40% fiber, 16% essential oil, 11% protein, 7% complex phenolic compounds like tannins and other substances like sugars and minerals. Phenolic compounds comprise 60%–70% of extractable compounds from grape seeds, since only minor portion of these have been extracted during pressing of grapes (Teixeira et al., 2014). Grape seed oil consists of 62%–71% is linoleic acid, 14%–28% is oleic acid and 10%–14% saturated fatty acids. The oil also contains 0.03%–0.07% tocopherols, which markedly protect the oil quality during storage (Shi et al., 2002). Grape seed oil may be used as edible oil, ingredient in salad dressings, and mayonnaise, and in baked food. It is used as a cosmetic ingredient for controlling moisture of the skin, hair and as carrier oil for essential oils. Grape seed oil is perhaps the most healthy vegetable oil substitute and is produced worldwide (e.g., Spectrum Naturals from United States, Basso and Costa D'Oro from Italy, Bores from Spain, etc.).

It has been found that the proportion of biologically active compounds (especially polyphenols) in grape seed oils is extremely dependent on the nature of the pressing process, particularly the pressing temperature and the degree of contamination of the starting seed material. Thus, the content of polyphenols in grape seed oil obtained by hot extraction methods is significantly lower as compared to a traditional, "classical" cold-pressing process. In the US7226627 patent, the content of polyphenols of the cold-pressed grape seed oil is improved by a careful purification of the material to be pressed from grape skins, stalks, stems etc., as well as by separating metals and by controlling the pressing power and temperature (Eckert et al., 2007). According to the invention, drying is preferably carried out in a current of air at temperature of 50–70°C, where tissue material of the marc still adhering to the seeds is dried and separated from the seeds by the current of air. The sieving is comprised of several sieving steps or the use of commercially available sieving machines, in addition equipped with an exhausting system for removing the lightweight parts in the seed material and several permanent magnets. Pressing power is in the range 50–100 kN, the temperature of the seeds is in the range 50–80°C and the temperature of the outflowing oil less than 50°C. It was found that normal filtering reduces the content of polyphenol compounds in the pressed oil. It has been proposed that the content of polyphenol compounds in grape seed oil can be further increased by storing the grape seed oil after the pressing process, on its crushed seeds meal (ratio of crushed seeds to grape seed oil 5%–40%) without any auxiliaries, mechanical, or chemical actions, by sedimentation in closed tanks for 10–120 days and without filtration. Food, food supplements, animal food, medicaments and cosmetics in all dosage forms could be composed of the cold-pressed grape seed oil, the crushed grape seeds and/or grape seed meal, or their mixture, or extracting components from the crushed seeds adding a suitable extractant (oils, wine, and other alcoholic and nonalcoholic beverages, as well as other fluids), to the crushed seeds. The weight ratio of grape seed oil to crushed seeds in the food, food supplement, animal food,, and medicament is preferably within a range of from 30:70 to 70:30.

Grape seeds are rich in oligomeric procyanidins which are water-soluble and have low molecular weight (<7000) making them highly bioavailable. Most of the processes presented in patent searches have no selectivity for oligomers with low degrees of polymerization (dp). Chardonnay varietal is one of the most popular wines worldwide and thus abundant quantities of grape seed are generated. Chardonnay seeds are reported to contain a relatively high content of epicatechin (Yilmaz and Toledo, 2004), a flavanol monomer that is reported to mediate beneficial actions on human endothelial function and may underlie, at least in part, the effects of cocoa and grape seed extract on circulatory function (Jimenez et al., 2012). Therefore, in the patent WO2015184291 a method for extracting grape seed (e.g., using Chardonnay variety) to obtain an isolated procyanidin extract with low mean degree of polymerization (mdp), as well as other biologically useful components, is presented (Ianiro and Fisher, 2016). Grape seeds are extracted with water at a temperature 100–200°F, then the extract is enzymatically treated and fine filtered to substantially remove insoluble plant material in the extract. The water extract is clarified prior to ultrafiltration by filtering with a filter that excludes material larger than 1–10 μ. The extract is then concentrated (solids content<10). The enzymatically treated and fine filtered extract is then further processed by ultrafiltration to obtain a first permeate and a first retentate. In the first permeate—a sugar/aromatic phenolic fraction (A), larger molecular weight polymers are removed and it comprises at least 80% sugars. The first retentate is reconstituted, then processed by ultrafiltration to obtain a second permeate (B) that contains polyphenols having a mean degree of polymerization (mdp) of less than 3 or 2.5, and a second retentate (C) enriched in fiber and polyphenols, with mdp of greater than 5. Any or the entire first and second permeates and second retentate may be concentrated and dried to provide a retained end product that is commonly saved in a powdered form.

Grape leaves are also a large volume by-product from viniculture which is either discarded or used as compost. There are some specific uses in food (e.g., in pickles or salads) but in small quantities since the taste is too bitter. However, leaves present a rich source of polyphenols so it has a potential for developing polyphenol-based components of foods and pharmaceuticals. The Patent KR1020110039756 discloses a method for making polyphenol-rich powder (Chang et al., 2011). This invention proposes vacuum freeze-drying from −40 to −80°C, and final drying to the temperature of 30°C. Freeze-dried material is pulverized in a crusher. Using this method, the taste, aroma, color, and valuable bioactive compounds (resveratrol, quercetin, catechin, and epicatechin) are preserved at higher level compared to drying by hot air (for 3 days drying at 50°C). Due to high polyphenols content, the obtained powder is excellent as functional material in various industries including health foods (cosmetics, bath products, food products, etc.).

Resveratrol (3,5,4-trihydroxystilbene) is one of the dominant polyphenols in grapes. It is stilbene (Fig. 8.3), belonging to a subclass of phytoalexins and is synthesized in response

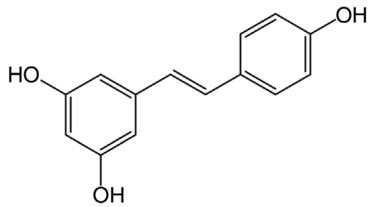

FIGURE 8.3 Chemical structure of resveratrol.

to pathogens and abiotic stress in plants. Its antioxidant, antiinflammatory, anticancer, and phytoestrogenic effect, as well as inhibition of platelet aggregation have been widely reported (Shen et al., 2017).

Grape leaves can be regarded as a good source of resveratrol as well. Patent CN101913995 discloses a method for the isolation of resveratrol from grape leaves (Zhang et al., 2010). The method is comprised of the following steps:

- Grape leaves are collected, stored in dark, and then dried in liquid nitrogen for 2–20 min to be pulverized.
- Extraction is carried out with 30%–95% ethanol by soaking 2–10 parts by weight of leaf powder for 5–24 h and afterward vacuum filtered. The extraction is repeated 2–4 times, each for 3–5 h.
- Collected extracts are then combined, concentrated and purified through macropourous resin column. Washing is carried out with 5%–20% ethanol and elution with 70%–95% ethanol. Eluates are concentrated to obtain resveratrol crystals which have been recrystallized from ethanol two- to threefold and dried.

Benefits of the present invention include simplicity and low cost of the method for the resveratrol isolation from grape leaf, with high extraction rate and without using toxic solvents. In addition, resveratrol is a valuable bioactive compound which is high in demand for its beneficial health effects and this method offers an alternative source taking advantage of discarded grape leaves, broadening sources of income of viticulture.

Another patent disclosing resveratrol isolation from viniculture by-products is KR1020090092405 (Shin et al., 2009) that suggests the following method for the recovery of resveratrol from grape stems:

- grape stalks are crushed;
- 80% ethanol is added (ratio 1:10) and mixed;
- mixture is sonicated for 10 min;
- extract is centrifuged at 3000 rpm for 20 min and evaporated under reduced pressure at less than 40°C;
- extract is filtered.

Since the final form of resveratrol is dissolved in distilled water, it can be dried using a freeze dryer or hot-air drier.

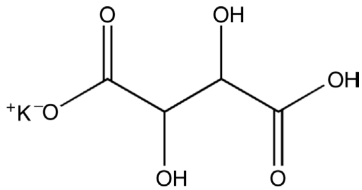

FIGURE 8.4 Chemical structure of potassium hydrogen tartarate.

Tartaric acid is commonly present in grapes and remains in winemaking by-products, such as dregs (lees) or pomace. During winemaking process, naturally occurring tartaric acid develops to its potassium salt, also known as cream of tartar (Fig. 8.4). It crystallizes and precipitates out of winemaking undesirable lees or dregs. Precipitate, that is, lee, is removed by filtration or decanting since it makes the wine cloudy and is unpleasant to swallow. The purified potassium hydrogen tartarate powder may be used in food (stabilizing agent, baking powder, thickening agent), medicine (purgative), chemistry (primary reference standard for a pH buffer), and as cleaning agent.

An older British patent GB756854 from 1956 presented a method for recovering potassium hydrogen tartarate from wine lees (Arden, 1956). According to this patent, passing wine lees through anion-exchange material in the hydroxyl form and elution with potassium or sodium hydroxide or sulfuric acid removes tartarate ions from a solution of wine lees. Similarly, cation-exchange material can be used, too. These adsorbents are regenerated by treatment with an acid and base, respectively. The effluents from adsorbents are electrolyzed in separate cells. Each cell is made up of two or more compartments separated by ion-exchange diaphragms. Tartaric acid from the anion exchange material effluent is separated in the anode compartment and potassium hydroxide from the cation-exchange material effluent in the cathode compartment of the other cell. Tartar cream is formed by the reaction between tartaric acid and potassium hydroxide obtained in anode and cathode compartments. The benefits of the invention include recovery of pure cream of tartar from a by-product, without consumption of chemicals since regenerating solutions may be recovered.

Extraction of calcium tartrate from by-products of a winemaking process, such as dregs and marc in practice is usually carried out in the following way. By-products are preliminarily diluted with water and dealcoholized in fractioning columns, thereby obtaining the so-called wine marc. Calcium tartrate forms as a consequence of neutralization of wine marc with calcium salts (calcium carbonate, calcium sulfate, or calcium chloride), and remains suspended therein in form of crystals. The wine marc is centrifugally treated through banks of serially arranged cyclone separators along which the concentration of the calcium tartrate increases up to the last final cyclone. To achieve a reasonable extraction yield, more than one cyclones are provided, and each bank of separators is fed from a pump. However, the speed (approximately 2800 rpm) of these pumps causes the crystals to crumble, making them more difficult to be separated in the cyclones. In this way, a substantial proportion of the tartrate crumbled by the impellers of the feed pumps driven at a high rotating speed are exhausted irretrievably from the cyclones. Also, frequent stops for maintenance and servicing, due to wear of the pumps and the cyclones from the abrasive action of the tartrate crystals, are necessary that involve considerable downtime costs. The solution to overcome this problem is provided by the patent EP1288288 by an improved process that comprises the steps of vibrating the wine marc or dregs in a vat having a bottom with an outlet conduit, and of discharging the tartrate precipitate from the vat bottom (Gennaro, 2003). It has been found that the tartrate crystals are easily precipitated from the liquid mass due to the vibration that nullifies or reduces drastically, the friction between the tartrate crystals and the liquid in which the tartrate crystals are imprisoned. According to this process, the calcium tartrate collects in the form of a slug at the outlet conduit. The tartrate crystals are of high purity, in the form of substantially entire undamaged crystals, where the tartrate percent recovered has been found to be practically 100%. The plant is regarded as a simple construction, cost-efficient, quiet and with indefinite lifespan.

8.2.2 Patents Reporting Applications of Grape Processing By-Products

Grape pomace (grape marc, press residue) remains after pressing of grapes and making grape must (juice). It contains seeds and skins, and sometimes stems, in variable proportions, depending on many factors (technological treatment, grape variety etc) that represent around 20% of the weight of fresh grapes (Shi et al., 2002). Seeds have much more diverse and complex composition (i.e., much higher monomeric and oligomeric flavan-3-ols composition) than skin (Mandić et al., 2008). Grape pomace is not only rich in dietary fiber, but also nutrients, such as polyphenols, resveratrol, amino acids, and trace elements. The insoluble part of the pomace is mainly constituted from peptic substances, cellulose and lignin (Teixeira et al., 2014). Pomace can be used for the production of ethanol, tartarates and oil from the separated grape seeds. Anthocyanins are considered to be the most valuable components of red grape pomace. The extract from red grape pomace, also called enocyanin, has been commercially produced in Italy since 1879.

A method for the extraction of pigments from grape pomace is provided in patent CN104287048 (Xing et al., 2015). This invention uses microwave vacuum extraction and drying techniques, spray-drying and low-temperature vacuum roasting to obtain stable, delicious, green and healthy, high nutritional value, food ingredient. It comprises of the following components: 60–65 parts of grape pomace, 250–350 parts of fruit, 350–450 parts of vegetables, from 0.1 to 0.2 parts of salt, 20–25 parts corn syrup, 10–15 parts of oligosaccharide syrup. The method consists of the following steps:

1. Extraction of grape pomace essence:
   Grape residue is mixed with distilled water with solid–liquid ratio of 1:2–4, stirred, and boiled at 40–50°C for 5–20 min. Boiled sample is then placed into a vacuum microwave heating apparatus (60–80 KPa; 300–500W) for a rapid pretreatment of 10–30 min, prior centrifuging and filtering the extract.

2. Fruit and vegetable vacuum drying and powder preparation:
   Fruits are vacuum dried under the following conditions: 0.07–0.09 MPa, 500–700 W, 5–15 min. Thereafter, the leaves and stems of vegetables are chopped, blanched, drained, and vacuum dried (0.06–0.08 MPa, 300–500 W, 5–7 min for leaves and 0.07–0.09 MPa, 500–700 W, 7–9 min for stems). Dried plant material is ultimately ground to a fine powder.
3. Fruit and vegetables powder are dissolved in a solution of grape pomace extract, mixed with sugar syrup and salt, homogenized, and sterilized at 130–140°C.
4. Drying: The mixture after sterilization is concentrated by vacuum evaporation at 60–70°C to 40%–60% of the original volume and then spray-dried.
5. Low-temperature vacuum rotary roasting: the mixed powder is placed in rotary roaster, together with milk powder (4%–6.5%), aqueous solution of ascorbic acid (3%–5%) and evacuated [−0.085−(−0.090) MPa]. The temperature program is: 35–40°C for 10–15 min, 60–65°C for 5–10 min, 40–45°C for 5–8 min.

The advantages of the present invention include reducing time and degradation in extraction process. This kind of winemaking waste utilization in a marketable product is claimed to increase 15% of the company's annual revenue. The resulted product has aromas and flavors of fruits and vegetables, essence of grape extract, as well as proteins and dietary fibers, whereas it is low-fat and nutritious. It can be ready-to-use food when reconstituted.

The invention enclosed within patent US6190716 presents a method to prepare food or pharmaceutical products with muscadine grape pomace, rich in red pigments (Galbreath, 2001). The method comprises of the following steps:

1. crushing the muscadine grapes;
2. processing through one of three distinct methods:
   a. *Alpha*: Crushing and pressing muscadine grapes with a bladder press, following standard operational procedures.
   b. *Beta*: First step is the same as in the Alpha method, followed by second step which includes breaking down the pulp of the crushed and dejuiced muscadines through heating the pomace following enzymatic action for 24–36 h, and third step—removing the broken-down pulp (puree) from the pomace by extruders.
   c. *Gamma*: First step is the same as in the Alpha method, followed by second step which is the fermentation of the juice in the crushed grapes, and third step—removing the fermenting juice from the must by pressing.
3. drying the pomace in a modified, rotary drum dryer at about 180°F to less than 1% moisture;
4. bagging the dried material into plastic freezer bags and storing in food-grade containers at 60–70°F;
5. blending materials produced through alpha, beta, or gamma process;
6. fine grinding the dried materials, encapsulating in gelatin capsules or incorporation in food products.

Due to the high content of fibers, as well as remained nutrients and polyphenols, grape pomace can be regarded as a suitable ingredient of functional food, especially bakery and confectionery products (e.g., biscuits, cookies, and crackers), which are convenient for fortification process. The process for producing high-quality dietary fiber biscuits, cookies and crackers with grape pomace is disclosed in patent CN104798854 (Wang et al., 2015).

The dough formulation comprises of: grape pomace superfine powder (6–9 parts), flour (50–60 parts), eggs (8–9 parts), peanut oil (9–11 parts), excipients (baking soda for biscuits, cookies and crackers (0.6–0.7 parts), white sugar for cookies (15–17 parts), and for crackers (4–5 parts), salt for crackers (1.3–1.5 parts), maltitol for crackers (4–5 parts), and water (10–11 parts)). Invention method of the invention is compiled of the following steps:

Grape processing: Grape pomace is separated after collecting wine, skins, and seeds are separated, dried in short-wavelength infrared drying equipment at 60°C for 60 min, and then ground to ultrafine powder;

Dough Modulation: Excipients are mixed with eggs, peanut oil, grape pomace superfine powder, and flour, and stirred.

Rolling: After the initial formation of gluten, dough is rolled and allowed to stand for 10–20 min. Dough is repeatedly rolled and folded.

Forming: The dough was rolled to a thickness of less than 3 mm, and formed with a knife or a homemade shape for biscuit molding;

Baking: On greaseproof baking paper, temperature 180°C, for 4–5 min;

Cooling, packaging: After baking biscuits were gradually cooled to 30–40°C and than packed.

The grape pomace in dough can improve the processing performance, product crisp degree, giving it new nutritional characteristics. Consumption of dietary fiber rich products prevents constipation, helps in loosing weight and achieving nutritional balance.

Beside various bioactive compounds, grape pomace contains a variety of other substances with potential application in the food industry. One of the prospective products made from grape pomace is vinegar and a relevant method is disclosed in patent CN102268358 (He et al., 2011). The latest includes a process of vinegar production employing two-step fermentation of grape pomace with yeast and acetic acid bacteria. This process generates a natural product with soft, sour, and fruity aroma, whereas more bioactive compounds and nutrients are extracted; hence making it more appealing and healthy. According to the method disclosed, the grape pomace is added to an aqueous solution of sulfur dioxide (30 μg/mL) to achieve 14 Brix, and then fermented with yeast (3 mL/100g active dry yeast wine activation solution, 25–28°C, 56–60 h). After alcohol fermentation, the solution (0.4% residual sugar, 7% alcohol) is inoculated with acetic acid bacteria (strain AFA-01), fermented (32–34°C, 128–140 h, until total acid content is at least 3.5%) and at the end of process centrifuged. Supernatant is collected and heated to 50°C, holding 30 min, then stored at 4–6°C for 5–7 days to obtain clarification. The product can be used directly as edible vinegar, but also fruit vinegar beverages can be prepared.

Phenolic compounds have high antioxidant potential and thus a wide range of beneficial health effects (Četojević-Simin et al., 2015). However, their main drawback during their application in food is their bitter taste. For instance, their addition increases not only antioxidant and health potential of the product, but also the degree of bitterness. Subsequently, this effect has an undesirable influence on the consumers' acceptability of the product. The patent US20050002961 presents the production of concentrate with high antioxidant potential and reduced bitterness which can be used in food, particularly in beverages (Wild and Sass, 2006). This concentrate comprises of green tea extract (5%–20%), grape skin (10%–60%) and grape seed extract (0.5%–5%), extracts of edible fungi (0.1%–5%) and fruits, vitamins (0.1%–10%), mineral matter and/or nutritional trace elements (0.1%–10%), and various additives. The green tea extract is obtained by aqueous extraction of the dried tea leaves, at an elevated temperature (from 10 to 90°C). The grape skin extracts are obtained from the marc also by aqueous extraction. Extracts should contain 0.05%–10% of malvidin-3-glycoside. Grape seeds are ground and extracted with water or water/ethanol mixtures. Grape seed extracts should contain 20%–95% of oligomeric procyanidins. Fresh fungi are air-dried, ground, and extracted with water. The fruits are mechanically comminuted, squeezed, and filtered. The concentrate obtained by mixing the aforementioned ingredients can be pasteurized. It can be in the liquid or a dried form produced by spray drying, further processed into tablets, granules, or powders.

Due to its pronounced antioxidant activity, grape seed extract is often employed in topical formulations, such as emollients, emulsifiers, and a skin conditioning agents. The patent WO2015050883 disclosed one or more optimal blends of the chosen five ingredients, that is, folic acid, biotin, bifidus, and *E. purpurea*, in a safe, stable topical skin care preparation, with defined antioxidant activity resulting from their synergistic action (Lee et al., 2016). Various combinations of the active ingredients were tested to determine which formulations gave significant antioxidant activity. It is provided that the combination of folic acid, biotin, and *E. purpurea* (without bifidus) is the most preferred. When all five ingredients are present, then the most preferred ratio of concentrations is 1:1:50:100:10,000 (folic acid:biotin:grape seed:*E. purpurea*:bifidus extract). These formulations may be implemented as skin care preparations, hair care preparations, or as color cosmetics, in the form of lotions, creams toners, gels, solid, or semisolid preparations. They may contain virtually any cosmetically acceptable ingredients, using water and/or alcohol as solvents.

Another patent that provides utilization of grape seed extract in skin care preparations is provided by Australia Pty Ltd.—AU2016100373 (Lin, 2016). "K.D. BIO-BEAUTY" is the serum comprising hyaluronic acid, collagen, nano placenta, argireline, and grape seed extract. Inventors are claiming water-replenishing, whitening, smoothing, hydrating, antiaging, and repairing effects due to a powerful antioxidant and free radical scavenging activities of this serum.

In another patent (CN105342912) the use of grape seeds as an abrasive in toothpaste is proposed (Shi, 2016). The traditional Chinese medicine toothpaste comprises of crushed grape seeds (30–40 kg), *Artemisia annua* hydrogenated oil (2.1–2.5 kg), bamboo vinegar (2–3 kg), water lilies amino acid viscose silk protein (0.3–0.5 kg), lactoferrin (0.2–0.3 kg), polyethylene glycol (2–3 kg), sodium citrate (1–1.2 kg), soy protein (0.3–0.5 kg), cyclohexylamine (0.34–0.4 kg), sodium lauryl sulfate (0.4–0.5 kg), forsythiaside (1.8–2 kg), tea polyphenols (1–1.2 kg), persimmon polysaccharides (0.9–1.1 kg), glycyrrhetinic acid (0.5–0.7 kg), sodium alginate (0.19–0.25 kg), nano-attapulgite (9–11 kg), with the right amount of porous micromolecule high-energy water and deionized water. The process of preparing herbal toothpaste includes the following steps:

1. Forsythoside and polyphenols are mixed with porous micromolecule high-energy water in the high-pressure homogenizer at high speed stirring for 0.5–1 h, than in the nanogenerator for 2–3 h;
2. The crushed grape seeds and nano-attapulgite are mixed with porous micromolecule high-energy water, then heated and stirred at 180°C for 3 h, cooled, milled and sieved over 200 mesh to give matrix composite powder. Vinegar is added and mixed, heated and stirred at 120°C for 2–3 h. After the addition of *Artemisia annua* hydrogenated oil heating is further continued at 120°C and stirred for 1–2 h;
3. The mixtures obtained in (1) and (2) were mixed with water lilies amino acid viscose silk protein, lactoferrin, persimmon polysaccharides, glycyrrhetinic acid, and polyethylene glycol in a magnetic stirring device. Deionized water is added at an amount of three- to fourfold of the total weight of the mixture, stirred in a high-speed magnetic stirrer for 1–1.2 h. Then, soy protein, cyclohexylamine, sodium lauryl sulfate, sodium citrate and sodium alginate are added, with continuous stirring for 1–2 h. Degassing is then carried out for 4–5 h and finally homogeneous and stable paste is vacuum canned and sealed.

The beneficial effects of the invention include antibacterial, antiinflammatory, antipyretic, treatment of common cold, and other effects of forsythiaside. The porous micromolecule high-energy water improves forsythia ester glycoside efficacy and stability. Persimmon polysaccharides and glycyrrhetinic acid as sweeteners improve the taste of toothpaste and increase the effectiveness of herbal ingredients in toothpaste. Grape seeds in its crude crushed form improve friction effect and reduce the cost of the product.

Natural flavoring compounds have been greatly valued as an alternative to synthetic ones, especially if they are recovered from by-products. Grape skin is regarded as a good source of a characteristic 3-mercaptohexan-1-ol (Fig. 8.5) variety aroma, as evidenced in the patent US20110311680 (Takase et al., 2011). This patent discloses a method of extracting this compound from grape skins in order to produce a wine-flavored nonalcoholic beverage. 3-Mercaptohexanol is a thiol aroma compound with very low threshold value (60 ng/L). It has a pleasant odor like grapefruit and passion fruit. The origin of this flavor compound is in grapes, especially skins of grapes where it is abundant in glutathione and

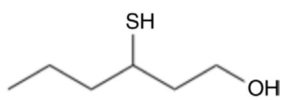

FIGURE 8.5 Chemical structure of 3-mercaptohexan-1-ol.

cysteine forms. During fermentation β-lyase-like yeast enzyme releases the 3-mercaptohexan-1-ol into the wine. It has been determined that wine made from grapes infected with *Botrytis cinerea* has greater 3-mercaptohexan-1-ol contents. The effects of winemaking procedures, the yeast employed, sulfurous acid and oxygen, and storage condition and duration have been examined as factors affecting the content of 3-mercaptohexan-1-ol in wine. Skin contact or pressure control during pressing largely influences transition of cystein precursor of 3-mercaptohexan-1-ol from grapes into juice.

The extraction method comprises of macerating grape skins in water (weight ratio 1:0.5–3) at 0–20°C for 0.5–96 h, followed by grape skin separation (by membrane press, gasket press, centrifuge, or filter press). Due to their astringency and bitterness polyphenols deteriorate flavor of 3-mercaptohexan-1-ol. Thus, the extraction conditions are set to hinder polyphenol extraction. To enhance extraction efficiency and the solid-liquid separation operation, an enzymatic agent, such as pectinase (at 10–500 ppm) can be employed during maceration. Grape skin extract can be concentrated, but at low temperatures because of high decomposition rate of the target molecule. Further, the grape skin extract (concentrated or unconcentrated) can be clarified and sterilized. This invention also discloses the method for production of the soft drink (alcohol content $<1\%$) from grape skin extract, by fermentation with lactic acid bacterium. During fermentation S-(3-hexan-1-ol) glutathione, contained in the grape skin extract, is converted to S-(3-hexan-1-ol)-L-cysteine (concentration 50–600 nM) S-(3-hexan-1-ol)-L-cysteine is a 3-mercaptohexan-1-ol precursor, and is converted to 3-mercaptohexan-1-ol by the action of enzymes in saliva with β-lyase enzymatic activity. It means that this drink, when held in the mouth for few seconds, gives retro-aromatic effect with fruity aroma and after aroma. However, when the concentration of S-(3-hexan-1-ol)-L-cysteine is less than 50 nM, no good fruity aroma or fruity aroma after aroma is produced, while the concentration above 600 nM gives overpowering and ponderous aroma. Having this in mind, the invention presents the method for producing the right amount of 3-mercaptohexan-1-ol, which is in the range 1–100 nM.

The process of winemaking includes pressing the grapes, juice separation, juice clarification and then juice inoculation with yeast to ferment and produce wine. During the clarification phase solid precipitate is generated (15%–20% of grape juice) containing a lot of organic matter. It is rich in glucose, trace elements, organic acids, pectins, vitamins, and other ingredients. This by-product is generally discarded. The invention disclosed in patent CN105349297 presents utilization of this kind of waste by preparing wine from it (Zhang et al., 2016).

To illustrate the invention the following method is proposed:

1. The precipitate (90–110 mg/L) is adjusted to a temperature 13–15°C, then potassium metabisulfite is added and after 15–17 h standing, it was filtered using a vacuum drum filter. Low temperature storage is advised before this step to suppress and kill wild yeast and bacteria accumulated in precipitate, and prevention of oxidation. Time should be enough for settling the particles but not too long because of oxidation and growth of wild yeasts and bacteria;

2. The filtrate is inoculated with yeast.
3. One day after yeast inoculation, fermentation broth goes full circle again, keeping the fermentation temperature to 19°C.
4. The end of fermentation should be regarded when alcohol reaches 11–12°C and reducing sugars less than 4 g/L in the obtained wine. After completion of the fermentation the wine is clarified with bentonite, and respective filtration.

The precipitate remained after filtration in vacuum drum filter in step (1) can be further used to make wine cake for manufacturing organic fertilizer. The present invention disclosed the best method for storage and handling of grape juice precipitate and production of high quality wines with excellent aroma and taste from grape juice precipitate, hence increase production and reduce environmental pollution.

8.2.3 Patents Reporting Health Benefits of Grape Processing By-Products

Grape by-products have been reported to have many health beneficial effects, due to the contained polyphenols. Grape seeds have an extremely high content of polyphenols, especially flavanols, such as procyanidins (epigallocatechin-3-gallates), for example, catechin, its diastereomer epicatechin, the procyanidins B1, B2, and B3, and procyanidin C1, and various proanthocyanidins P1–P2. Grape seeds have one of the highest concentrations of monomeric proanthocyanidins reported in a fruit (Crespo and Brazinha, 2010). Physiologically, procyanidins have hypotensive and antiarteriosclerotic properties, and they are regarded as being very efficient free-radical scavengers, which forms the basis of their antioxidant action (Römpp, 1997). Company Dry Creek Nutritions' [Inter Health Nutraceuticals Incorporated (Concord, CA)] produces ActiVin, a novel IH636 grape seed proanthocyanidin extract, a highly bioavailable form of GSPE (grape seed extract), as a nutritional ingredient used in a wide variety of dietary supplements and nutraceutical food, beverage and cosmetic products. It is the most actively researched grape seed extract on the market today. Many research publications have shown that ActiVin possesses a wide range of antioxidant, chemoprotective and biological properties, including free radical scavenging abilities in vitro and in vivo (Bagchi et al., 1997), anticancer, cardioprotective (Bagchi et al., 2003), reduces pain index and incidence of vomiting in patients suffering from chronic pancreatitis (Banerjee and Bagchi, 2001) and many more. The use of this supplement is included within the patent US6291517 (Bagchi et al., 2001). The invention disclosed the protective ability of GSPE against the gastrointestinal mucosal injury induced by acute and chronic stress in an amount effective to prevent or reduce the injury. The study was investigating the production of superoxide anion, as well as lipid peroxidation, DNA fragmentation and membrane microviscosity (indices of oxidative tissue damage) in the gastric and intestinal mucosa of female Sprague-Dawley rats. Acute and chronic restraint stress was induced by water immersion. In particular, acute stress was induced in the rats for 90 min, where half of the rats received GSPE (100 mg/

kg/day) for 15 days prior to exposure to acute stress. Chronic stress was induced in the rats for 15 min per day for 15 consecutive days, after an overnight fast, where half of the rats were given GSPE (100 mg/kg/day) for 15 days, prior to the exposure to stress. GSPE treatment alone increased gastric and intestinal mucosal lipid peroxidation, DNA fragmentation, membrane microviscosity, and superoxide anion production. On the other hand, pretreatment of the rats with GSPE decreased in gastric and intestinal mucosa acute and chronic stress-induced lipid peroxidation, DNA fragmentation, membrane microviscosities and cytochrome *c* reduction. No lesions occurred in the GSPE-treated animals. The results of these experiments clearly demonstrate GSPE's significant ability to protect against chronic stress-induced gastrointestinal mucosal injury, and some protection against acute stress. In conclusion, the 100 mg/day/kg of body weight dose was found to be effective in reducing stress-induced gastric injury in rats, which is equivalent to a dose of 164 mg of GSPE per day for a 70 kg adult human. Based on these results and other in vivo studies, a preferred daily dose to prevent injury is roughly 100–200 mg of GSPE, preferably in two equally divided doses taken within 15–30 min after meals. If GSPE is taken after (not prior to) onset of stress, a somewhat higher dose may be required to be effective. Such poststress use will likely not be as effective as regular supplementation with GSPE. There are numerous supplements on the market based on grape seed extract. Most of the suppliers produce 100–500 mg grape seed extract supplements. For example, GNC Herbal Plus is available at 100, 300 and 500 mg dosage per capsule, where 95% are polyphenols. ResVitale is a product comprised of 325 mg of grape seed extract (standardized to 95% of polyphenols) and 100 mg of Resveratrol Polyphenol Complex [Wildcrafted Japanese Knotweed Extract, Organic French Whole Red Grape (skin, seeds, fruit, stem, vine), Organic Muscadine (seed and skin)] standardized to contain 25 mg of *trans*-resveratrol.

Synergistic behavior of phytochemicals from various plant extracts has been proven in many in vitro and in vivo studies (Tumbas et al., 2012; Tumbas Šaponjac et al., 2015; Vulić et al., 2012). The patent WO2014141265 discloses joint action of polyphenols from green tea extract and grape seed extract (GSE) on mental abilities and related diseases (Nofar and Cogan, 2016). It has been reported that in addition to radical scavenging, chelation of redox active metals, and regulation of antioxidant protective enzymes, these extracts can be a part of the protective effect against neuronal diseases, such as Alzheimer disease (AD) and Parkinson disease (PD). The merits for these activities have been assigned to epigallocatechin-3-gallate (EGCG) from green tea and polyphenol monomers, oligomers and polymers of catechin and of epicatechin from grape seed extract. Beside medicaments containing EGCG and GSE, some of the tested embodiments contained specific strain of lactic acid bacteria *Lactobacillus plantarum*. The patent suggests GSE manufacturing by the following process:

1. extraction of whole grapes, grape seeds, grape pomace, and mixtures thereof with water at an elevated temperature to obtain a crude grape-water extract;
2. treating the crude grape-water extract with a tannase enzyme at an elevated temperature to obtain a polyphenol grape extract;

3. acidifying the polyphenol grape extract to a pH of about 1.5–2.5 to obtain an acidified polyphenol extract;
4. cooling the acidified polyphenol extract; and
5. filtering the cooled acidified polyphenol extract to obtain a filtered polyphenol extract.

The EGCG is produced by water/EtOH extraction from leaves of *Camellia sinensis*. Preparations and applications of medicaments comprising at least two of EGCG, GSE and *L. plantarum* seem to synergistically improve mental abilities, such as memory and are thus thought to inhibit, or at least reduce, the progression of diseases, such as AD or PD. To implement the claims Capsugel's GSE Meganatural-BP (300 mg/capsule), EGCG (218 mg/capsule) and *Lactobacillus plantarum* R1012—150 (377 mg/capsule) DR Caps (capsules in hydroxypropylmethyl cellulose, acid resistant) were used. The catechins content in the EGCG was at least 95%, and the content of polyphenols in GSE was at least 90%. For 60 days patients were administered two Meganatural-BP capsules (18 days after dose was reduced to one), one EGCG capsule and one *L. plantarum capsule*. Patients' cognitive state and mental capacities appeared to have dramatically improved compared to his condition just prior to the administration of the GSE, EGCG and probiotic bacteria. This invention demonstrated synergy of the (presumably polyphenolic) ingredients in GSE and green tea extracts in improving mental condition, or at least reducing mental deterioration, especially when properly used in conjunction with probiotic bacteria that probably provide metabolic products with improved efficacy. It has been suggested that synergies between the GSE and/or green-tea EGCG and polyphenolic extracts from other plant sources (e.g., pomegranates, dates, and various berries) or vitamin E, can be employed for similar applications.

The application of grape seed extract and synergistic action with sage extract and the sheep placenta in the treatment of cough variant asthma is disclosed in patent CN105343353 (Xu, 2016). To produce the soft capsules the ingredients, sheep placenta freeze-dried powder, grape seed extract, sage (*Salvia miltiorrhiza*) extract, soybean oil, beeswax, gelatin, and glycerin, were crushed, mixed, homogenized, pelleted, processed, and dried. Soft capsules were reddish-brown colored, with characteristic taste, odor, no smell, protein content being ≥6.6 g/100g and anthocyanins ≥3.6 g/100g. However, neither the description of grape seed nor sage extraction procedure is provided in the patent. The invention was illustrated in an experiment with mice, low, medium and high dose group (1.20 g/kg, 2.40 g/kg, 4.80 g/kg, respectively). Sage grape seed soft capsules in each dose group significantly prolonged the incubation period of cough in mice caused by ammonia, reducing the frequency of cough. Also, the test was conducted with humans as well, having three capsules a day for 3 months. The total effective rate was 68.8%.

Muscadine grapes (*Vitis rotundifolia*) differ from other grape varieties primarily by their bronze and purple skin color. Hence, muscadine grape skin extracts (MSKE) have anthocyanins as major bioactive components. The patent US20160067298 disclosed that MSKE possesses chemopreventive activities (Odero-Marah, 2016). Their therapeutic values are proved in treatment of prostate and breast cancer, types of cancers linked with the skeleton leading to bone metastasis. The primary cause of prostate and breast cancer death is metastasis. It has been disclosed that the mechanism of MSKE action includes a decrease in Snail and CatL activity. Cathepsin L (CatL) is a cysteine cathepsin protease that is overexpressed in cancer and increases with tumor grade in prostate and breast patient tissue. Snail overexpression increases CatL activity. Snail transcription factor expression is increased in prostate cancer and associated with increased invasion, migration, and bone turnover. For the illustration of the patent, MSKE was obtained from the Ison cultivar, a purple-skinned variety. Polyphenolic compounds from the dried and pulverized muscadine grape skin were extracted with 50% ethanol/water at a nominal ratio of 9:1 (v/w) by stirring with a magnetic stir bar for 1 h at room temperature. The slurry was allowed to settle for 24 h, and the supernatant was passed through a 0.2 μmol/L membrane filter funnel and collected under a vacuum. The predominant ingredients were anthocyanin 3,5-diglucosides, ellagic acid, and ellagic acid precursors. MSKE contained no significant amount of resveratrol. The novelty of this invention is based on the fact that it is using a natural plant product, MSKE that has never before been tested in preventing the formation of mature osteoclasts which are important in bone resorption and bone metastasis. In conclusion, this extract or its structural analogs may be employed in destroying prostate cancer cells which may prevent bone metastasis.

Among other bioactive effects, muscadine grapes' by-products have been shown to have antimicrobial effects as well. In the patent US20060121137 the effect of muscadine grape pomace on preventing intestine inflammation, most often associated with the bacterium *Clostridium perfringens* and/or protozoa of the genus *Coccidia* (Hartle et al., 2006). In particular, whole powdered muscadine grape pomace was used as a feed additive, in less than 10% of the weight, for treating intestinal inflammation. For example, 2%–0.5% muscadine pomace in chicken feed has been shown to diminish the degree of necrotic enteritis in chickens infected with *Clostridium-perfringens* and *Coccidia*. There was significant improvement in feed conversion and body weight gains and a decreased mortality rate in chickens fed the pomace. Addition of 5% of muscadine grape skin powder to the feed and 5% to the water of animals has been shown to reduce and/or prevent colon inflammation in animals consuming the treated feed and water. This treatment resulted in a decrease in myeloperoxidase activities (a marker of neutrophil invasion and inflammation) by 75% over the control group, a decrease in edema of 40%, and a decrease in macroscopic scores when compared to a control group, when TNBS (2,4,6-trinitrobenzene sulfonic acid) was used to induce colitis in experimental animals. Additionally, the muscadine pomace feed additive performed as well or better than the industrial standard antibiotic (BMD). Therefore, muscadine phytochemicals exert a protective effect over inflammation, and may be used to prevent and treat inflammation. It is demonstrated that a dosage of as little as 0.5% of the diet of the animal may be a therapeutically effective amount of muscadine grape product in treatment of inflammation when administered orally.

The patent RU02367464 includes a claim that the combination of antiinflammatory agent with an aqueous extract of red vine leaves is characterized by increased antiinflammatory and decongestant action (Masuda et al., 2009). It has been disclosed that the innovative formulation for topical use comprising an antiinflammatory agent and an aqueous extract of red vine increase efficiency preventing or alleviating mild/moderate chronic venous insufficiency of legs. The preparation method includes the following steps:

- Leaves are dried and crushed.
- Extraction is performed with purified water at temperature from 60 to 80°C for at least 6–10 h. Extract is filtered and sterilized by heating under pressure, for 1–30 s at 120–150°C.
- It may be concentrated or dried. The total content of flavonoids (quercetin-3-O-β-D-glucuronide) in the extract was from 0.625 to 25%.

The invention discloses also a preparation of weak extract (the content of red vine leaves is reduced from 5–7 parts to 1 part) or liquid extract (dissolving a weak extract in 60% ethanol in 1: 1 ratio) of red vine leaves. In the case of topical application the aqueous extract of red vine leaves in equivalent weak extract of red vine leaves is between 0.1 and 20 g per 100 g (or 100 mL) of the composition, and in equivalent liquid extract of red vine leaves is between 0.2 and 40 g/100 g (or 100 mL) of composition. It is stated that Aloe Vera, extracts of horse chestnut and *Calendulae* spp. and/or lavender oil should be excluded in the current formulations. Examples of antiinflammatory agents are nonsteroidal antiinflammatory drugs, heparinoid, capsaicin, zinc oxide, glycyrrhizic acid, glycyrrhetic acid, allantoin, hyaluronic acid, azulene, and others, alone or as mixtures of two or more components. The total amount of antiinflammatory agent component is from 0.0001 to 50 g/100 g (or 100 mL) of composition. The compositions for external application can include different forms, such as creams, ointments, gel ointments, plasters, solutions for topical use, sprays, lotions, tinctures, etc.

Grape stems and vines are cheaply and readily available resources from wine and juice-making processes with high potential, too. The invention WO2005048719 reports a preparation of food, dietary supplements and pharmaceuticals from these by-products. Specifically, stems and/or vines are frozen and ground prior extraction (Folts et al., 2005). Extractions may be performed using water, alcohols, esters, sulfoxides, ketones, or mixtures of any two or more thereof. Typically, 50%–99% acetone is used. Extraction includes agitating in multiple steps with multiple pH ranges. Fibrous solids and other particulate matter may be removed from the extract by centrifugation and/or filtration. Extract comprises at least 40 mg gallic acid equivalents (GAE)/g dry weight of extract. The resulting extracts may be used in nutraceutical compositions, suitable for the addition to foods or beverages, in dietary supplements (in powder, pill or liquid form), pharmaceutical compositions (with carrier or diluent). A dietary supplement or a nutraceutical composition may be prepared with food grade carrier and other polyphenolic-containing extracts. The weight ratio of the extract to carrier or other extract varies from 0.01 to 100. The composition is introduced into the food in an amount between about 0.1 and 10 mg/g of the active ingredients of the food. When used as a dietary supplement the tablets contain between 0.1 and 1 g or more of active ingredients. The composition can be dispensed as a condiment on the prepared food. The compositions of the inventions may be used to create formulations and prevent or treat coronary artery disease. Such compositions can be in various forms (granules, powders, tablets, capsules, syrup, suppositories, injections, emulsions, elixirs, suspensions, or solutions) for various routes of administration (oral, nasal, rectal, and injections). The formulations of the invention may be designed to be short-acting, fast-releasing, long-acting, and sustained-releasing as described later. The extracts of the invention may be used in the prevention and treatment of heart disease, preventing or treating coronary artery disease, cerebro-vascular disease, peripheral-vascular disease, atherosclerosis, atherosclerosis-related disease, or heart failure including administering to a subject. An effective amount of a compound of the present invention may vary depending upon the route of administration, dosage form, conditions of disease, the age, body weight, general health conditions, sex, and diet of the subject, dose intervals, administration routes, excretion rate, and combinations of drugs.

Lees are obtained from the winery as a suspension of solid material in wine. Solid material is largely potassium tartarate coated with polymerized tannins and pigments. In the patent US20020102287 the antioxidant and antimicrobial effects of the recovered pigments are presented (Shanbrom, 2002). As the first step to achieve the proof, lees are separated by gravity decanting and/or by centrifugation. The invention presents two simple ways to extract pigments/tannins from lees:

- A suspension of lees are carefully brought to an alkaline pH by drop wise addition of sodium hydroxide and the solid material (tartarate) will be transferred into solution. The addition of crosslinked polyvinylpyrrolidone (xPVP) can precipitate the pigments. This way pigments remain insoluble in water. xPVP is removed by filtration or centrifugation and supernatant can be distilled to recover the ethanol.
- Addition of soluble PVP to a suspension of the lees in water. With continued mixing the PVP will bring the pigments/tannins into solution after which the colorless tartarate can be removed by centrifugation. The pigments/tannins can be extracted from the soluble PVP if a PVP-free product is desired (e.g., with butanol). Cholestyramine can be used instead PVP.

Antioxidant measurements were carried out by adding PVP-iodine to the sample and reduction of the iodine to iodide was followed with an iodide electrode. It is disclosed that xPVP effectively captures some of the pigments/tannins and prevents them from reacting with the iodine, while PVP liberates the antioxidant material and facilitates its reaction with the iodine. In general, results point to very high antioxidant levels of wine lees as compared to materials extracted from grape juice. Similarly, the antibacterial tests on *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Bacillus subtilis* show that PVP increases the availability of the substances with antibacterial activity. It appears that the PVP stabilizes and increases the solubility of the antioxidant and antibacterial property of the lees. In this invention, it is reported that wine lees can be used directly as well, as a tonic or demulcent. Potassium tartarates' demulcent effect is enhanced by antioxidant and antimicrobial effect of the polymerized tannins and pigments. The absorption of the antioxidants after ingestion is proved by the increase in the antioxidant properties of excreted urine. Also, the antimicrobial activities have been applied in latex gloves "powdering" them with fine ground dried lees. The lees completely prevented bacterial growth on the gloves.

8.3 Conclusions

Along with the increasing worldwide trend for wine consumption, an increasing volume of accompanied by-products, such as grape pomace, seeds, skins, stems, leaves, and wine lees, arises. Winery by-products, traditionally regarded as waste and environmental problem, are a rich source of polyphenols, such as tannins, procyanidins, anthocyanins, stilbenes, etc. Also, these by-products are valuable sources of dietary fibers, tartarates, essential oils, proteins, tocopherols, etc. Therefore, there are many attempts to utilize these in food (confectionery products, food additives, such as colors, flavors, preservatives, condiments) beverages (wine, juice), dietary supplements, pharmaceutical preparations and cosmetics (skin preparations, toothpaste). Growing need for functional food and biopharmaceuticals and their production has induced the need for protection of the valuable knowledge on grape by-products reutilization by patenting the methodologies to recover and apply them or medicate with them. A vast number of patents already exist in WIPO and EPO databases. Most of them deal with recovery of polyphenols (procyanidin oligomers, anthocyanins, resveratrol, etc.) usually in a powdered form which allows easier application mainly in functional food or dietary supplements. Besides polyphenols, many patents deal with isolation of grape seed oil, which is largely commercialized. However, grape seed extract in the form of a dietary supplement is probably the most commercially utilized grape by-product. Patents dealing with health benefits of grape-byproducts disclosed prevention of stress induced injuries, neuronal and cardiovascular diseases, bacterial infections, inflammation, cancer etc. These activities are, in most cases, attributed to presumable polyphenols' antioxidant mechanisms. Also, many patents include other plants and extracts in combination with grape by-products, proposing synergistic actions of phytochemicals, which is already widely documented in literature. Still, many of these patents are not exploited and commercialized, especially the ones carrying health claims, due to many concerns arising from innovation, such as safety and profitability.

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