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Applications of Wine Pomace in the Food Industry: Approaches and Functions

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Abstract: Winemaking generates large amounts of wine pomace, also called grape pomace. This by-product has attracted the attention of food scientists and the food industry, due to its high content in nutrients and bioactive compounds. This review mainly focuses on the different published approaches to the use of wine pomace and its functions in the food industry. Traditionally, wine pomace has been used to obtain wine alcohol, food colorings, and grape seed oil. More recently, research has focused in the production of other value-added products, such as extracts of bioactive compounds, mainly phenols, recovery of tartaric acid, and the making of flours. The most common functions associated with wine pomace products are their use as antioxidants, followed by their use as fortifying, coloring, and antimicrobial agents. These products have mainly been applied to the preparation of meat and fish products and to, a lesser extent, cereal products.

Keywords: antimicrobial, antioxidant, coloring, fortification, wine pomace

Introduction

Grapes are one of the most extensively cultivated crops in the world with almost 63 million tons produced worldwide, and the vast majority of the total grape production (75%) is used to produce wine (FAOSTAT 2013). Approximately 20% of the grapes (by weight) constitute the main winemaking by-product, the grape or wine pomace (Laufenberg and others 2003). Wine pomace, also called grape pomace, is the residue of pressed grapes, small pieces of stalks, and yeast cells from the wine fermentation process.

Wine pomace has for a long time been an undervalued product due to lack of alternative uses with economic benefits. Traditionally, wine pomace has been distilled to produce different types of "wine alcohol" (Silva and others 2000), which are used to make well-appreciated and valorized distilled spirits, liquors, and liqueurs (González-SanJosé 2014), so as to fortified wines. Other traditional applications of wine pomace have been its use as fertilizer or as animal feed (Arvanitoyannis and others 2006). For instance, Diaz and others (2002) proposed the use of composted wine pomace to increase the organic matter, nitrogen and mineral contents of vineyard soils. However, these solutions present some drawbacks, mainly related to the presence of antinutritive compounds that can negatively affect crop yields and animal weight gain. In addition, they fail to exploit the full potential market of this by-product (Dwyer and others 2014).

The idea of revalorizing wine pomace is not new and different alternatives have been proposed since the 1970s. All of them have focused on the exploitation of the interesting compounds retained in the wine pomace. The production of "enocyanine" was probably one of the 1st interesting proposals with international acknowledgment in the food industry as well as in the pharmaceutical and cosmetics industries. So much so that there are currently available several commercial "enocyanines" (anthocyanins isolated from red wine pomace), and those used in food industry are recognized in Europe as the food colorant E-163. Alongside this product, other alternatives have been developed, although none of them have had the same success as enocyanins. For example, proanthocyanidins extracted from grape seeds have been commercialized in France since 1970 (for example, Endotelon) for medical uses, but nowadays the use of similar products in the food industry is not common. Grape seed oil has also been produced for decades, and it is gaining market as a gourmet product (Dwyer and others 2014).

Up until the end of the 1990s, practically all alternatives included extraction processes followed by concentration and separation processes, in order to obtain products containing specific compounds (for example, tartaric acid or proanthocyanidins). However, over the past few decades, other alternatives, to avoid extraction phases, have been proposed to the generation and use of minimally processed wine pomace derivative products (Martin-Carron and others 1997; Cheng and others 2007; Duque and others 2011; García-Lomillo and González-SanJosé 2013; Jang and others 2015). Considering the above comments, the objective of this work was to review the most interesting proposals to revalorize wine pomace by promoting the development of useful products for the food industry.

Composition of Wine Pomace

The composition of grapes may vary depending on extrinsic factors such as edaphoclimatic conditions (Kliewer 1977) and viticultural practices (Freeman and others 1979), as well as

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intrinsic factors such as variety, maturity, and sanitary conditions (Philip and Kuykendall 1973; González-SanJosé and others 1986, 1990a, 1990b, 1993; Robredo and others 1991) . Similarly, both the type of process and the conditions under which winemaking is carried out notably influence the composition of wine pomaces (González-SanJosé and others 1990b; Pérez-Magariño and González-SanJosé 2000). Variability between grape varieties and the different effects of each winemaking process on the composition of wine explain the variations reported in the literature on the composition of wine pomace and its main components: skins and seeds. Furthermore, it is important to note that red wine pomace is a by-product that has been fermented, while white and rosé pomaces are removed before alcoholic fermentation. Since they are not fermented separately, fermentable sugars remain in both white and rosé wine pomace.

Water content and microbial stability

Depending on the origin and the intensity of the pressure applied in the pressing operation, wine pomace will show important differences in its water content, ranging between 55% and 75%. Despite these possible differences, its water content is in all cases sufficient to promote microbial and enzymatic degradation (González-Centeno and others 2010), which may compromise the subsequent application of fresh pomace.

The presence of microorganisms in wine pomace has been poorly studied. Available data indicate relatively low values of spoilage microorganisms, with counts of total aerobic mesophilic bacteria (TAMB) ranging between 3 and 6 logs colony forming units (CFU) per gram and loads between 3 and 6 logs CFU/g of yeasts and molds (Ayed and others 1999; Augustine and others 2013; Özlem and others 2014).

Dietary fiber

Dietary fiber is the main component of dried wine pomace, with concentrations ranging between 43% and 75%. Dietary fiber is mainly constituted of cell wall polysaccharides and lignin. Generally, seeds are richer in fiber than skin, and red wine pomace is richer in fiber than white wine pomace (Gül and others 2013). Saura-Calixto and others (1991) reported that insoluble dietary fiber, especially acid insoluble lignin (Klason lignin) is the main component of dietary fiber in both red and white wine pomace. Moreover, the fiber contains a considerable proportion of tannins and proteins (Arnous and Meyer 2008).

Protein

The protein content of wine pomace may range between 6% and 15% (dry matter) depending on grape variety and harvesting conditions. The proportion of protein in the skins and seed is similar, but the skins from wine pomace are slightly richer than the seeds separated from the wine pomace. Wine pomace has an amino acid profile similar to that of cereals, being rich in glutamic acid and aspartic acid and deficient in tryptophan and sulfur-containing amino acids. Furthermore, the skin protein content is rich in alanine and lysine, a fact that is not observed in the proteins of seeds (Igartuburu and others 1991a, 1991b). Gazzola and others (2014) have published a complete characterization of the proteins present in grape seeds.

Lipids

The major lipid contribution in wine pomace is from the seeds. Seeds from wine pomace have contents ranging between 14% and 17% (Gül and others 2013; Mironeasa and others 2016). Furthermore, the lipid fraction presents an interesting fatty acid profile rich in polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acids, with low levels of saturated fatty acids (SFAs). Linoleic acid (C18:2; approximately 70%), oleic acid (C18:1; approximately 15%), and palmitic acid (C16:0; approximately 7%) are the predominant fatty acids in grape seed oil (Fernandes and others 2013).

Minerals

The mineral content of wine pomace may present even wider variations than in the case of the other components, due to the strong influence of the edaphoclimatic conditions, viticultural practices, and the winemaking process (Ortega-Heras and others 1999; Taylor and others 2003; Lachman and others 2013). The type and mainly the duration of maceration processes have a strong influence on the extraction and reabsorption of minerals during the winemaking, notably affecting the mineral content remaining in wine pomace (Ribéreau-Gayon and others 2006).

Minerals in grapes are usually classified in groups depending on their mobility in phloem. Potassium, phosphorus, sulfur, and magnesium show high mobility and are accumulated and mainly localized in the skin of the grape berry during ripening. In consequence, grape skins present higher levels than grape seeds, mainly due to their high content of potassium salts localized in grape skins, specifically in the hypodermal cells (Rogiers and others 2006). In contrast, seeds are the strongest reservoir of calcium, phosphorus, sulfur, and magnesium (Coombe 1987; Gül and others 2013; García-Lomillo and others 2014).

The most abundant potassium salts are tartrate, mainly potassium bitartrate ($KC_4H_5O_6$). Tartrates may represent a relevant amount of the wine pomace (between 4% and 14 % in dry matter), with high differences depending on the ripening stages and the culture practices applied on winemaking grapes. Tartrate salts are mainly in the form of potassium bitartrate ($KC_4H_5O_6$), although calcium tartrate ($CaC_4H_6O_6$) can also be in significant concentrations (Rice 1976; Nurgel and Canbas 1998).

Phenolic compounds

The phenolic composition of wine pomace has been extensively described (Kammerer and others 2004; Peralbo-Molina and Luque deCastro 2013; Teixeira and others 2014), with notable qualitative and quantitative differences. The large dispersion of published data is directly correlated with 2 well-known facts: the strong influence of all the factors that affect grape compositions on the phenolic profile of grapes (Andrades Rodríguez and González-SanJosé 1995; Pérez-Magariño and others 1999; Pérez-Magariño and González-SanJosé 2006) and the effect of diverse enological practices on the extraction of phenolic compounds during the winemaking process (Revilla and González-SanJosé 2002; Pérez-Magariño and others 2009).

Phenols are usually classified according to their chemical structure and molecular weight in the following groups: simple phenols (mainly C6-C1 and C6-C3), flavonoids (C6-C3-C6 and oligomers), polymeric compounds (including hydrolyzable and condensed tannins, lignin, and so on) and miscellaneous phenol groups with very different structures (xanthones, stilbenes, betacyanines, and so on) (El Gharras 2009).

Regarding simple phenols, skins from wine pomace are generally richer in phenolic acids than from white grapes. Grape skins are rich in hydroxycinnamic acids (C6-C3) and especially rich in tartaric esters of these acids, mainly caftaric acid and coutaric acid followed by fertaric acid. In contrast, seeds are rich in gallic acid and protocatechuic acid (Kammerer and others 2004; Teixeira and others 2014). The presence of tartaric ester in the skins is probably associated with the remains of pulp sticking to them, as pulp generally has the highest levels of those types of compounds (Lee and Jaworski 1987; Kammerer and others 2004).

Flavonoids are a very extensive group of phenolic compounds that include a wide range of different families or subgroups, mainly differentiated by the degree of oxidation of their oxygenated heterocycle. Anthocyanins (in red pomace) and flavanols are the most abundant in wine pomace, leaving all others in a minority. According to the normal composition of *Vitis vinifera* red varieties, the predominant anthocyanin is malvidin-3-*O*-glucoside that is usually followed by peonidin, petunidin, or delphinidin-3-glucoside depending on the grape variety (González-SanJosé and Diez 1987,1993; Kammerer and others 2004; Pérez-Magariño and González-SanJosé 2004; Amico and others 2008). The absence of anthocyanins in white grapes leaves flavanols as the most abundant phenols in white wine pomace. Flavanols are mainly located in the seeds, whose levels range 56% to 65% of the total flavanols of grapes against 14% to 21% present in grape skins. The seeds are rich in gallocatechins (Czochanska and others 1979; Rodríguez Montealegre and others 2006), whereas the presence of epigallocatechin (tri-hydroxyl catechin) has only been described in skins (Escribano-Bailón and others 1994; Rodríguez Montealegre and others 2006). In addition, oligomers (from 2 to 5 units) and polymers of flavanols are in relevant concentrations, with significant predominance of type-B proanthocyanidins (Ricardo-Da-Silva and others 1991). Proanthocyanidins from seed wine pomace have a lower average degree of polymerization (10 to 20 units) than the skins (25 to 35 units) (Ky and others 2014). Oligomers and polymers with low levels of solubility are not extracted during winemaking processes and remain in the wine pomace.

The clear relevance of quercetin 3-*O*-glucuronide in comparison with other flavonols has been described in the wine pomace of some specific varieties (Ruberto and others 2007; Amico and others 2008); although other authors indicated similar concentrations of quercetin 3-*O*-glucuronide and quercetin 3-*O*-glucoside with slight differences between grape varieties (Kammerer and others 2004).

Apart from the phenolic fraction that is easily extractable by conventional methods (aqueous-organic methods), wine pomace presents important quantities of nonextractable polyphenols (NEPP) including hydrolyzable polyphenols (HPP) and nonextractable proanthocyanidins (NEPA) (Pérez-Jiménez and others 2009). NEPA are those proanthocyanidins that are associated with other components of wine pomace, especially fiber. HPP are monomeric phenols bound to protein, polysaccharides, or cell walls via hydrophilic/hydrophobic interactions, hydrogen bonds, or covalent bonds (Brenes and others 2008). The low solubility of these fractions means that they are not extracted during winemaking and are left in the wine pomace.

Approaches in the Applications of Wine Pomace in the Food Industry

The large amounts of wine pomace obtained from the winemaking process and their potential market has led food researchers to look for new alternatives that exploit this by-product. Nevertheless, various factors should be considered in order to obtain satisfactory applications for the food industry.

Wine pomace stability

Fresh wine pomace presents a high content of water, limiting its chemical and microbiological stability. This fact is very important in view of the large amounts of wine pomace produced over short periods all of which cannot be processed. There are only a few applications of fresh pomace limited to the ripening of some traditional Italian cheeses (Di Cagno and others 2007; McGuigan 2015). Then, wine pomace needs to be stabilized to prevent degradations that could compromise subsequent uses and applications. The shelf-life of wine pomaces has been traditionally extended removing the oxygen by compacting the wine pomace until it is finally processed (Da Porto 2002). Different acids (sulfuric, tartaric, or phosphoric acids) or sulfites can also be sprayed over pomace in order to avoid wine pomace degradation (Silva and Malcata 1998; Ayed and others 1999). Gamma-irradiation in combination with other synthetic preservatives such as sulfites (Ayed and others 1999) and sodium benzoate (Augustine and others 2013) has also been proposed to increase its shelf-life. However, different drying methods are the most common ways of processing wine pomace.

Due to the low thermal stability of bioactive compounds, freeze-drying is considered to retain the highest levels of bioactive compounds in comparison to oven-drying (Tseng and Zhao 2012). However, freeze-dried samples present the highest losses of bioactive compounds during subsequent storage, most probably caused by the porosity of freeze-dried products that increases air contact and their susceptibility to oxidation. In contrast, Larrauri and others (1997) found no significant differences between total extractable polyphenols and condensed tannin contents of freeze-dried and oven-dried (under 60 °C) wine pomace. However, significant decreases were observed in processes at 100 and 140 °C. Interestingly, heat treatment over lengthy periods may induce the release of certain low molecular weight compounds increasing the level of certain phenolic compounds (Pedroza and others 2012; Planinic and others 2015). Considering the above comments, the low rate of processing of freeze-drying and its higher cost (4 to 8 times more expensive than conventional drying (Ratti 2001)), freeze-drying is not actually a suitable technique to process large amounts of wine pomace. Recently, Sui and others (2014) also proposed the suitability of infrared-drying to retain the highest bioactivity in comparison to convective drying, but this technology is also more expensive than conventional drying methods.

Despite the low water activity and pH of dehydrated wine pomace, this may not be enough to assure complete stability during storage. Products with similar characteristics, such as herbs and spices, have already caused outbreaks (Vij and others 2006) and may decrease the microbial quality of the products where they are added (Kneifel and Berger 1994; García and others 2001). Moreover, molds are also capable of producing mycotoxins even at low levels of water activity (Romagnoli and others 2007). García-Lomillo and others (2014) observed the satisfactory application of heat treatment at 90 °C to completely eliminate the microbial flora of dehydrated wine pomace with minimal losses in the bioactive content. Ultraviolet treatment also reduced the microbial counts, but the reduction was not sufficient to obtain a safe product.

Products obtained from wine pomace

A wide range of products has been developed from wine by-products over recent decades. The most common approach is by obtaining extracts, other than seed oil, using organic solvents or water for the production of enriched extracts of high interest in food applications. However, other nonextracted products have also been proposed to be applied by the food industry (Figure 1). Extractive processes may be combined with purification and concentration steps to obtain concentrated extracts of specific compounds. Due to the high concentrations of

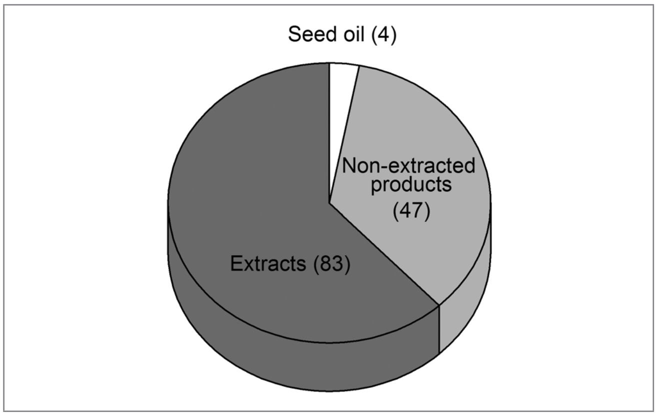

Figure 1–Number of the main wine pomace approaches to be applied by food industry published from 2000.

bioactive compounds in the obtained extracts, low concentrations are needed for successful applications in food systems.

The 1st extracted components were probably anthocyanins, and the 1st commercial product "enocyanin." The excellent coloring properties of this product enabled its use in different matrixes such as dairy desserts, ice creams, drinks, juices, and other food preparations.

Grape seed oil is another successful approach and corresponds to a solvent extracted product obtained from grape seeds. However, although consumers perceive grape seed oil as a healthier alternative than other oils, this product is not widely used probably because of its high price and it is not extensively utilized by the food industry (Dwyer and others 2014).

Tartaric acid is another interesting product recovered from wine pomace (Nurgel and Canbas 1998; Braga and others 2002). The yield of the recovery of tartaric acid ranges between 50 and 75 g of tartaric acid per kilogram of wine pomace (Braga and others 2002). Tartaric acid is widely applied in various food categories including dairy products, edible oils and fats, fish and meat products, fruit and vegetable products, and soft and alcoholic drinks. It is used according to its antioxidant, pH regulatory, and preservative activities. Furthermore, it presents a pleasant sour taste, and it is able to enhance some positive flavors. Potassium tartrates are also used in baked products due to their ability to react with sodium bicarbonate producing carbon dioxide without requiring fermentation (Stephanie 2005; Doores 2011).

Excluding a fraction of grape seed protein with interesting solubility and emulsifying activity for application in soups, sauces, beverages, and meat products (Zhou and others 2011), wine pomace-derivative products have mainly been focused on the extraction of different phenolic compounds. The influence of different factors, including solvent polarity, temperature, solid particle size, ratio of solid:solvent, and other more, on the final yield and on the composition of extracts have been studied for many years (Bonilla and others 1999) and several reviews have described in depth the factors affecting polyphenol extraction (Pinelo and others 2005; Spigno and De Faveri 2007).

Other techniques applied to enhance extraction of polyphenols from wine pomace include enzyme attack (Meyer and others 1998), high hydrostatic pressure and ultrasonic techniques (Corrales and others 2008), microwaves (Krishnaswamy and others 2013), high-voltage electrical discharges (Boussetta and others 2009), pulsed electric field (Corrales and others 2008), and gamma-irradiation (Ayed and others 1999).

In contrast to extracts, other researchers have proposed the use of products without a previous extraction process. This approach enables a more complete reutilization of the by-products, and enables intense fortification with fiber, minerals, protein, oil, and other constituents of wine pomace, such as phenols, including nonextractable phenolic compounds. In this way, the nutritional value and the potential health benefits can be improved. Furthermore, since extraction is not required, the process of obtaining these powdered products is more economic and has a lower impact on the environment, resulting in a sustainable approach.

Probably the 1st approach to nonextracted products was the concept known as "grape antioxidant dietary fiber" (Saura-Calixto and García-Laurrari 1999). This approach focused on the healthy benefits of the fiber combined with the grape antioxidant, but technological applications, mainly due to their antioxidant activity, were also proposed (Martin-Carron and others 1997).

Different authors have also proposed the use of wine pomace flours obtained after milling whole wine pomace or their main components (seeds and skins) (Hoye and Ross 2011; Özvural and Vural 2011; Mironeasa and others 2012; Rosales Soto and others 2012). Increasing consumer demands for alternatives to wheat flour, and especially for flours with high fiber and mineral levels, have prompted the development of these products.

Another approach to the application of wine pomace in the food industry is the development of seasonings (González-SanJosé and others 2015), which have antioxidant and antimicrobial activity in food matrices (García-Lomillo and others 2014). The use of these types of seasonings can reduce the salt levels in various foodstuffs without compromising their microbial stability or their sensory quality.

Functions in the Food Industry

The content of wine pomace in diverse compounds with different properties enables a wide range of potential functions and technological uses of this by-product. Many of these options are a consequence of the content in phenolic compounds with high bioactivity (antioxidant, antimicrobial, vitamin P effect among others). However, other components such as fiber, minerals, and fat may play a relevant role in determining some of the possible functions of wine pomace products in foodstuffs. The number of published works applying wine pomace in foodstuffs has been increasing since 2010 (Figure 2), with applications describing its antioxidant effects being predominant. Interestingly, fortification applications are those with the highest increases in the recent years, followed by those exploiting the antimicrobial effects of wine pomace (Figure 2).

Improvement of nutritional properties and possible health effects

Fortification involves the incorporation of nutrients to foods whether or not the nutrients are originally present in the food. According to its composition, wine pomace may be a source of different and interesting nutrients. Fortification with wine pomace may contribute to reducing certain nutritional problems detected in western societies, such as low average intakes of antioxidants, fiber, and minerals (Flagg and others 1995; He and MacGregor 2008; Slavin 2008). Furthermore, the presence of beneficial compounds, such as phenolic compounds, may also improve its perceived value among consumers on the look-out for functional foods (Morley 2013). In fact, wine pomace products have mainly been applied to enrich foodstuffs with antioxidants.

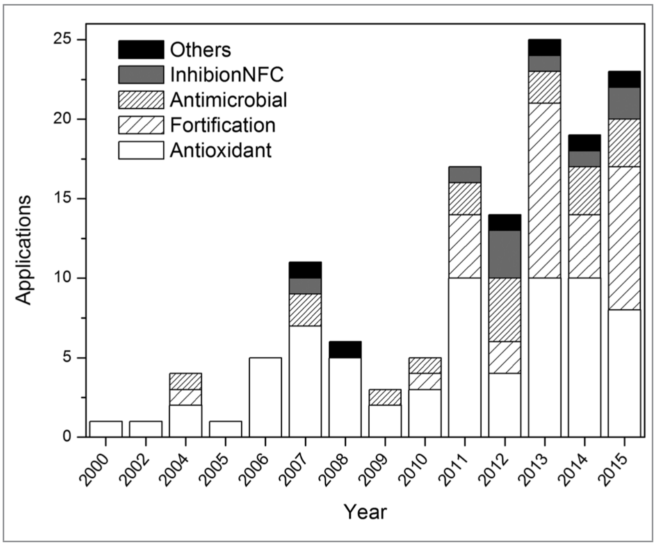

Figure 2–Trend in the number of published studies applying wine pomace in food products from 2000 to 2015.

Enrichment with polyphenols. Phenolic compounds are not considered nutrients, but many reports in the literature have revealed their potential benefits (Xia and others 2010; Teixeira and others 2014). The most commonly cited function is their antioxidant activity; however other capacities, as antimicrobial and anti-inflammatory activities, have been also indicated. Antioxidant capacity has been linked to the prevention of diseases associated with oxidative stress (Shrikhande 2000; Teixeira and others 2014). So, wine pomace polyphenols have been associated with anti-cancer activities, as they are able to induce apoptosis in tumor cell lines and may exert antiproliferative activity (Jara-Palacios and others 2015) and avoid the formation of carcinogen-induced DNA adduct and DNA synthesis in cancer cells (Xia and others 2010).

Generally, the health benefits of wine pomace have been associated with the consumption of the so-called extractable polyphenols. However, the contribution of extractable phenols is usually overvalued, to the detriment of NEPP. NEPP were shown to decrease cholesterol absorption and increase fat excretion (Bravo and Saura-Calixto 1998; Bobek 1999; Saura-Calixto 2012). Moreover, they are more stable and less influenced by digestion and food processing conditions than extractable polyphenols that are degraded during the process.

Limited research has been conducted to determine whether the health benefits ascribed to isolated phenols are also obtained by consuming foods enriched with wine pomace products. This fact is very interesting due to the interaction between polyphenols and matrix ingredients that may reduce the bioactivity of wine pomace phenols. Moreover, food processing may also degrade phenolic compounds, especially during high-temperature processes (Surh and Koh 2014). Degradation during storage may also induce a relevant decrease in phenolic content. Mildner-Szkudlarz and Bajerska (2013) reported that breads enriched with freeze-dried wine pomace made from skin reduced total cholesterol levels, low-density lipoprotein, lipid peroxidation, and increased antioxidant activity in rats with diet-induced hypercholesterolemia. Recently, grape seed extract incorporated into white bread was proposed to reduce the postprandial glycemic response and increase satiety (Coe and Ryan 2016). Grape seed extract was able to reduce sugar release from starch and glycemic response as well as increase satiety, perhaps of even greater relevance in high glycemic index foods such as white bread. There is also an increasing trend toward the utilization of fruit by-products in extruded snacks, due to the release of simple sugars during extrusion that increases the glycemic index (Rohm and others 2015). The use of the complete product (high in fiber) rather than extracts can also contribute to a reduction in the glycemic response.

Several food categories have been successfully enriched in phenols by incorporating wine pomace products (Table 1). Cereal products, mainly bread and cookies, are the category with the highest number of applications that mainly make use of wine pomace flours (Hoye and Ross 2011; Munteanu and others 2013; Acun and Gül 2014; Aghamirzaei and others 2015; Mironeasa and others 2016).

Cookies incorporating seedless and wine pomace flours obtained higher values of acceptability than those made with seed flours (Acun and Gül 2014), whereas cereal bars were reported as an excellent option to include grape seed flour (Rosales Soto and others 2012). However, the incorporation of wine pomace flour requires the adaptation of recipes and processing conditions to preserve the quality of baked products. Various works have noted the modifications induced by these types of flours, such as the increase of α-amylase activity, leading to a lower falling number (an indicator of enzymatic activity); and the possible interaction of seed lipids with gluten, starch, and hydrophobic components resulting in weaker consistency of the dough, increased viscosity, and delayed gelatinization of starch (Mironeasa and others 2012, 2016). In contrast, Meral and Doğan (2013) described contrary results such as a strengthening activity of grape seed flours, ascribed to the covalent or noncovalent bonds between gluten proteins and phenols, obtaining stronger flours with higher extensibility and resistance.

Dairy products are the 2nd food category with significant phenol-enrichment applications using wine pomace products (Table 1). In this case, the effectiveness was lower than in cereal products due to instability and loss of phenols during processing and storage of dairy products, as well as other technological problems. Tseng and Zhao (2013) described that the addition of grape pomace flour to milk produced excessive syneresis (levels higher than 3%) of yogurt, and no coagulation was observed at levels higher than 5%. Moreover, periods of storage as low as 1 wk induced relevant drops in total phenolic and antioxidant activity of yogurt. These results were ascribed to polyphenol degradation at yogurt pH and to phenol–casein interactions. Loss of phenol contents over short periods were also observed by other authors (Karaaslan and others 2011; Aliakbarian and others 2015), and the decrease in the phenolic content was ascribed to consumption of phenols to prevent lipid oxidation (Chouchouli and others 2013). However, Marchiani and others (2016) noted that levels of quercetin increased during storage, probably due to its solubilization in the yogurt.

Grape seed extract was satisfactorily used in cheese manufacturing, where hydrophobic interactions between caseins and phenols contributed to retention of phenols in the curd, reducing the losses of phenolic compounds (levels in whey around 20%) (Han and others 2011), although the kinetics of gel formation were retarded due to the decrease in the pH. The pH decrease caused by the incorporation of organic acids from grape pomace also induces the degradation of casein at the end of ripening of Toma-like cheeses (Marchiani and others 2015). Similarly, the incorporation of pomace extracts and flours into milk delayed milk clotting and decreased the clotting rate and syneresis, which was explained by

Table 1–Products fortified with wine pomace products found in the literature.

Category	Foodstuff	Wine pomace product	Level	Fortification with	Complementary aims	References
Cereal products	Bread	Seed flour	2.5% to 10%	Phenols	Physical/sensory properties	Hoye and Ross (2011)
			5% to 25%	Phenols/fiber	Physical properties	Aghamirzaei and others (2015)
			2.5% to 7.5	Phenols	Physical/sensory properties	Meral and Doğan (2013)
			10%	Fatty acids/minerals	Physical/sensory properties	Munteanu and others (2013)
		WWP powder	5% to 10%	Fiber/phenols	Mineral fortification Physical/sensory properties	Smith and Yu (2015)
		SkWP powder	4% to 10%	Phenols/fiber	Physical/sensory properties	Mildner-Szkudlarz and others (2011)
	Biscuits	SdWP powder	5%	Phenols Mineral	Prevention of lipid oxidation Physical/sensory properties	Aksoylu and others (2015)
		WWP powder	10% to 30%	Phenols/fiber	Physical/sensory properties	Mildner-Szkudlarz and others (2013)
		WWP powder	20% to 30%	Fiber/protein	Physical/sensory properties	Karnopp and others (2015)
		Seedless, whole, and seed WP	5% to 15%	Fiber/protein	Physical/sensory properties	Acun and Gül (2014)
		Skin wine pomace powder	5% to 10%	Mineral/fiber	Sensory properties	Canett Romero and others (2004)
			10% to 50%	Fiber/protein	Sensory properties	Mieres Pitre and others (2011)
	Bread and muffin	WWP powder	5% to 20%	Phenols/fiber	Physical/sensory properties	Walker (2013)
	Muffins	SkWP powder	20%	Phenols	Reduction of CLM	Mildner-Szkudlarz and others (2015)
	Breakfast cereals	WP flours	5% to 20%	Phenols/fiber	Physical/sensory properties	Oliveira and others (2013)
	Bars, Pancakes, and Noodles	SdWP flour	5% to 30%	Phenols	Sensory analysis	Rosales Soto and others (2012)
	Pasta	WP extract	–	Phenols	Physical/sensory properties	Marinelli and others (2015)
Dairy products	Fermented milk	WP extract	100 mg GAE/L	Phenols	Sensory properties	Aliakbarian and others (2015)
		WP flour	10 to 50 g/L	Phenols	Fermentation kinetics	Frumento and others (2013)
	Yogurt	SdWP extract	50 to 100 mg/kg	Phenols	Physical properties	Chouchouli and others (2013)
		WWP powder and extract	1% to 3% 2%	Phenols/fiber	Inhibition of lipid oxidation	Tseng and Zhao (2013)
		WWP extract	1%	Phenols		Karaaslan and others (2011)
		SkWP flour	6%	Phenols	Physical/sensory properties	Marchiani and others (2016)
	Cheese	WWP powder	0.8% to 1.6%	Phenols	Microbial/physical properties	Marchiani and others (2015)
		WWP extract	0.5 mg/mL	Phenols	Physical properties	Han and others (2011)
		SkWP, WWP and SdWP extracts	0.1% to 0.3%	Phenols	Physical properties	Felix da Silva and others (2015)
	Ice cream	Grape seed extract	0.4%	Phenol	Sensory	Sagdic and others (2012a)
Meat products	Sausages	SdWP flour	0.5% to 5%	Fiber	Antioxidant and sensory	Özvural and Vural (2011)
	Beef loin	Grape seed oil	10%	Fatty acids	Enhance quality and safety	Jung and others (2012)
Others	Seafood	SkWPP powder	3%	Fiber/phenols	Antioxidant antimicrobial	Ribeiro and others (2013)
	Puree	SkWP powder	3.2%	Phenols	Sensory	Lavelli and others (2014)
	Infusions	SkWP extract	50% to 100%	Phenols	Color, antiviral activity	Bekhit and others (2011)

WP, wine pomace; WWP, whole wine pomace; SkWP, skin wine pomace; SdWP, seed wine pomace.

hydrophobic interactions between proteins and polyphenols that reduce the amount of hydrophobic groups in casein (Felix da Silva and others 2015). An increase in syneresis was also observed (Marchiani and others 2016). However, authors explained this observation in terms of a matrix gel rearrangement, caused by the insoluble dietary fiber of the skin flour.

Another potential limitation of wine pomace products could be their antimicrobial effect on lactic acid bacteria (LAB) growth during the fermentation. Viable counts of LAB should be kept above 10^7 CFU/g during the commercial shelf-life of the product (WHO 2011). However, studies found in the literature show satisfactory results with no or little effect on microbial growth or on LAB survival during storage (Chouchouli and others 2013; Casarotti and Penna 2015; Marchiani and others 2016).

Other products have also been fortified with wine pomace products, such as marmalade or candies (Guzmán Nieves 2011; Cappa and others 2015), salad dressing (Tseng and Zhao 2013), and tomate puree (Lavelli and others 2014). A new seafood functional sausage was also developed based on meagre (*Argyrosomus regius*), (Ribeiro and others 2013), and red skin extracts were also incorporated into a tea infusion (Bekhit and others 2011) at different concentrations, ranging between 50% and 100% in order to increase the phenolic profile and antioxidant activity of the infusions.

Enrichment with fiber. An adequate fiber intake has been associated with the prevention of some diseases such as hypertension, diabetes, and obesity (Anderson and others 2009). Soluble dietary fiber is able to decrease glycemic responses and cholesterol levels in plasma. Since it is fermented in the large intestine, fiber improves the colonic environment. Moreover, it delays and interferes in the absorption of cholesterol and bile acids. Dietary fiber also limits carbohydrate absorption, reducing insulin response and triacylglycerol levels, which are risk factors for coronary diseases. Insoluble dietary fiber increases fecal bulk and presents benefits in terms of intestinal motility, lowering gastric emptying, and promoting satiety (Rodríguez and others 2006).

Nonextracted products such as wine pomace flours have mainly been used to increase the fiber content of different foods (Table 1). The incorporation of these products is limited due to similar factors to those described in the previous section. Hence, the most common matrix in which these products have been incorporated is cereal products.

Fortification with minerals. Currently, the intake of minerals in western populations presents a clear deficiency in some minerals such as potassium, while sodium among others is consumed in excess (EFSA 2005). Potassium is an essential mineral, involved in the electrolyte balance and normal cell functioning, and it is required to maintain muscular and neurological functions due to its role in neuromuscular excitability (Rüdel and others 1984). Moreover, adequate potassium intake could contribute to reduced blood pressure, decreasing stroke risk and cardiovascular diseases, especially in hypertensive populations (Karppanen and Mervaala 2006; He and MacGregor 2010). Due to its relatively high mineral content, products derived from wine pomace provide interesting alternatives for the fortification of foodstuffs and to increase the intake of minerals; especially of potassium, but also of calcium, magnesium, zinc, copper, manganese, and phosphorus, all of which have essential human health functions. However, the mineral composition of winemaking by-products has often been undervalued and there are no applications that focus on mineral content. Some isolated studies (2 of those are shown in Table 1) have pointed to changes in the mineral composition of cereal products following the incorporation of wine pomace flours. Furthermore, the application of a new seasoning from wine pomace enabled an improvement in the mineral content of meat products, as it enabled salt reduction. Consequently, the meat product contained lower levels of sodium and higher levels of potassium and calcium in comparison with the control samples (González-SanJosé and others 2015).

Mineral fortification is also relevant to the food industry from a technological point of view. For instance, the calcium content of seed flours may stabilize enzymes such as proteases and α-amylases that are essential in the quality of cereal products (Mironeasa and others 2012, 2016).

Improvement of fatty acid profile. The high levels of essential fatty acids in grape seed oil may contribute to lowering the risk of such diseases as cardiovascular disease, diabetes, arthritis, immune disorders, and cancer (Simopoulos 2003). The current intake of SFAs is higher than recommended (10% of total energy), so grape seed oil can balance the PUFA/SFA ratios of the human diet (Williams 2000). However, grape seed oil is deficient in omega-3 fatty acids showing a ratio of omega-6/omega-3 higher than recommended (Fernandes and others 2013). Diets with ratios higher than 6:1 may be linked to cancer, cardiovascular, inflammatory, and autoimmune diseases (Simopoulos 2002). Then, the incorporation of grape seed oil in food formulation should be balanced with others rich in essential omega-3 fatty acids to achieve an optimum ratio of essential fatty acids. Interestingly, grape seed oil is free of cholesterol (Choi and others 2010), and it is associated with antioxidants such as phenolic compounds, tocopherols (especially α-tocopherol), and tocotrienols.

Grape seed oil was proposed as an innovative food ingredient in various food formulations (Jung and others 2012). Apart from its previously described nutritional characteristics, grape seed oil has interesting properties for the food industry due to its high smoking point. It has been used to reduce animal fat contents in meat products, improving the nutritional properties as well as reducing cooking loss, and increasing protein solubility (Choi and others 2010). The incorporation of grape seed oil (up to 10%) was also proposed to replace beef fat and improve the fatty acid profile of frankfurters and beef loin steaks (Jung and others 2012; Özvural and Vural 2014).

Protection against oxidative processes

Different food components may undergo oxidation during food storage or food processing, reducing the quality and the nutritional properties of foods. Food matrix composition (metal content, water activity, fatty acid profile, and so on), and formulation strongly affect the susceptibility of foods to oxidation (Ladikos and Lougovois 1990). Storage conditions such as atmosphere, light exposure, and temperature play a key role in the development of oxidative processes. Furthermore, manufacturing processes such as grinding or cooking may also increase oxidative instability due to the loss of physical structure and the release of prooxidant compounds from the intracellular medium (Alfawaz and others 1994; Kanner 1994).

The food industry has traditionally used synthetic antioxidants such as butylhydroxyanisole (BHA), butylated hydroxytoluene (BHT), and ascorbyl palmitate to mitigate food oxidation (Decker and Mei 1996). However, some of these synthetic antioxidants have been linked to different toxicological effects, including tumor-promoting activity (Kahl and Kappus 1993), and current food policies of various countries are increasingly restricting the use of these types of additives. Furthermore, there is an increasing consumer concern over the potential risks of chemical additives, increasing the demand for products obtained from nature without synthetic additives (Carocho and others 2015). For these reasons, natural additives, and mainly alternative natural antioxidants, with similar efficacy than synthetic additives, have received high interest from the food industry. Among the natural additives, wine pomace products have been reported as an excellent alternative to synthetic antioxidants, mainly due to their high content of phenolic compounds. Different mechanisms have been used to explain the antioxidant properties exerted by wine pomace products: donating hydrogen atoms (Bors and others 1990), scavenging free radicals (Kanner 1994), quenching intermediate compounds of oxidative reactions (Kanner and others 1994), scavenging the superoxide ion $*O_2^-$ (Chen and others 1990), chelating metal initiators (Gülçin 2010), inhibiting the enzymatic activity of oxidative enzymes such as lipoxygenase (Duque and others 2011), and the preservation of endogenous antioxidants (Pazos and others 2005).

Lipid oxidation. Lipid oxidation is, with microbial spoilage, one of the main factors limiting the shelf-life of food products, causing large loses during storage. Lipid oxidation involves 2 phases: primary oxidation that induces the formation of lipid hydroperoxides, diene and triene conjugates, and secondary oxidation that leads to the formation of volatile compounds (Frankel 1983). Consequently, the sensory quality deteriorates, the nutritional value is reduced (due to the destruction of nutrients such as PUFA and vitamins), and the technological properties may also be affected (Kanner 1994). Moreover, some compounds derived from lipid oxidation, especially those from the primary oxidation, can present toxic effects.

In general terms, the literature reports that wine pomace products present stronger inhibition against the secondary lipid oxidation phase than against the primary phase (Sánchez-Alonso and others 2006). Although pure phenolic compounds may present interesting antioxidant activity, wine pomace products usually show higher activity than isolated compounds (Shaker 2006; Maestre and others 2010). This fact suggests a synergistic effect between phenolic compounds. Regarding concentration, relatively low levels of wine pomace extracts are required to achieve an antioxidant effect, although the published data vary depending on the type of product in use. So, different wine pomace products have been effective from levels as low as 10 ppm up to levels as high as 10% (Rojas and Brewer 2007; Shirahigue and others 2010; Hasani and Alizadeh 2015). Generally, a low health risk of prooxidant activities has been described, which facilitates the application of these products as antioxidants.

The antioxidant efficacy against lipid oxidation of wine pomace products has also been tested in combination with other natural extracts such as essential oils (Adams and others 2002; Moradi and others 2011; Tajik and others 2015) and green tea extracts (Rababah and others 2010, 2011a) , and with possibly objectionable additives such as sulfites (Bañón and others 2007). Generally, additive effectiveness has been observed suggesting a synergistic action between the different antioxidants.

Various researchers have tested the potential of incorporating wine pomace products into films. These types of products present a gradual release of active compounds into the matrix, and the effect is observed for prolonged periods (Borderías and others 2005). Chitosan is the most commonly used material in such films due to its high versatility and its excellent film-forming properties. Furthermore, chitosan possesses relevant antioxidant and antimicrobial properties (Ulbin-Figlewicz and others 2014). The effectiveness of chitosan films has been improved by the incorporation of grape seed extracts (Moradi and others 2011). Moreover, grape seed extract was incorporated into a carboxymethylcellulose coating with satisfactory results (Raeisi and others 2014).

Meat and meat products are the food categories in which wine pomace products have been most widely used to prevent lipid oxidation. Meat products usually contain high levels of fat and prooxidants such as salt and metals. In the case of raw meat, atmospheres rich in oxygen, used to keep the red color, may also enhance lipid oxidation. Furthermore, processes such as grinding, mixing, and cooking may also increase the oxidative instability of products during storage afterwards (Alfawaz and others 1994; Kanner 1994).

Wine pomace products have been applied in meat products from different species such as beef (Ahn and others 2002, 2007), pork (Carpenter and others 2007; Sasse and others 2009), chicken (Shirahigue and others 2011), turkey (Mielnik and others 2006), goat (Rababah and others 2012a), and buffalo (Tajik and others 2015), usually in patties or sausages that permit an acceptable homogeneity of the product in the matrix (Ryu and others 2014; Liu and others 2015; Wagh and others 2015). In the case of intact muscles (such as chicken breasts or steaks), the products can be applied by pressurized tumbling (Rababah and others 2006, 2010), by rubbing with the dehydrated product (Wong and Kitts 2002), by dipping the meat product into a mix or solution containing the antioxidant (Vaithiyanathan and others 2011) such as frying batters (Cagdas and Kumcuoglu 2014) and marinades (Gibis and Weiss 2012), and by spraying the antioxidant onto the surface of the meat (Camo and others 2011). Other meat products that have been tested include liver (Pateiro and others 2014), restructured mutton (Reddy and others 2013), chorizo (Lorenzo and others 2013), pâté (Pateiro and others 2014), dry-cured bacon (Wang and others 2015), mortadella-type sausages (Moradi and others 2011), and Milano-type salami (Mendes and others 2014), as well as dehydrated meat (Nissen and others 2000) and lard (Schevey and Brewer 2015).

Despite the differences in the mechanisms and the kinetics of lipid oxidation in meat products, wine pomace products have shown antioxidant activity in a wide range of meat products, storage conditions, and processes, revealing their suitability for these sorts of products. For instance, grape antioxidant dietary fiber has presented interesting antioxidant activity in muscle samples stored at room temperature (Yu and others 2013), under refrigeration (Ahn and others 2004; Sáyago-Ayerdi and others 2009), or under frozen conditions (Sánchez-Alonso and others 2006; Brannan and Mah 2007; Colindres and Susan Brewer 2011; Kulkarni and others 2011). Other grape antioxidant products also have shown successful results in raw and cooked products (Nissen and others 2004; Colindres and Susan Brewer 2011; Selani and others 2011). Furthermore, the antioxidant activity of different products derived from wine pomace has been described in samples packaged in air (Price and others 2013; Gómez and others 2014), under vacuum (Rojas and Brewer 2008; Sánchez-Alonso and others 2008), and under modified atmosphere conditions (Garrido and others 2011; Jongberg and others 2011).

Apart from the protection exerted during meat storage, wine pomace products may also limit the lipid oxidation produced in different treatments such as high-pressure processing (Montero and others 2005), electron-beam radiation (Rababah and others 2006), gamma-irradiation (Schevey and others 2013), extrusion (Camire and Dougherty 1998), restructuring (Reddy and others 2013), microwave exposure (Rababah and others 2012a), salting (Lau and King 2003; Brannan 2008), dehydration (Nissen and others 2000), freeze-thaw cycles (Nirmal and Benjakul 2010), and curing (Wójciak and others 2011; Lorenzo and others 2013; Wang and others 2015).

Apart from meat products, wine pomace products were effective at inhibiting lipid oxidation in different fish species, including horse mackerel (Sánchez-Alonso and others 2006), rainbow trout (Gai and others 2015), silver carp fillets (Shi and others 2014; Hasani and Alizadeh 2015), chub mackerel (Özalp Özen and others 2011), bonito fillets (Yerlikaya and Gokoglu 2010), and cod (Sánchez-Alonso and others 2007a). Fish products usually have a lipid profile with a high content of PUFA; and high levels of prooxidants such as free iron, which may promote lipid oxidation.

Regarding the prevention of lipid oxidation in fats and oils, wine pomace products have been successfully incorporated in oils with different fatty acid profiles: rich in linoleic acid, such as grape seed oil (Jang and others 2015), sunflower oil (Poiana 2012), and soybean oil (Bakota and others 2015), and in oils rich in oleic acid such as olive oil (Bonilla and others 1999) and canola oil (Schevey and Brewer 2015). Grape seed extract was also incorporated into solid systems like pork lard and into oil–water emulsions (Altunkaya and others 2013), which would be highly interesting in food systems such as mayonnaise and salad dressing (Tseng and Zhao 2013). Generally, wine pomace products were less effective in oil than in muscle systems. For instance, grape seed extract promoted the formation of conjugated dienes in a canola oil system, and it effectively inhibited lipid oxidation in beef patties (Schevey and Brewer 2015). Bakota and others (2015) found no antioxidant activity in soybean oils, which was due to the poor solubility of phenolic compounds in oil systems. In contrast, Poiana (2012) observed relevant proportions of phenols that remained in the oil (between 30% and 60%), which exerted significant antioxidant effect and Bakota and others (2015) also observed lipid protection in oil-in-water emulsions. Different factors can contribute to explain such contradictory data; those factors with the highest relevance probably being the polarity of the treated samples and of the products that are used.

Wine pomace also successfully inhibited lipid oxidation in dairy products such as yogurt (Ersöz and others 2011) and cheese (Shan and others 2011), and in vegetable-derived products such as corn (Rababah and others 2011b) and potato chips (Rababah and others 2012b).

Protein oxidation protection. Protein oxidation involves the formation of amino acid derivatives, increases of carbonyl derivatives, loss of thiol groups, changes in protein structure, protein denaturation, and polymerization (Lund and others 2011). Therefore, the technological quality of proteins is reduced and solubility decreases, as well as the gelation and water-holding capacities, enzymes are deactivated, and drip loss increases. Furthermore, protein oxidation presents several implications for human health such as reduced digestibility, loss of essential amino acids, and increased cytotoxicity. Protein oxidation, at an advanced stage, can also affect sensory quality including loss of tenderness and formation of dark pigments (Lund and others 2011; Soladoye and others 2015).

Generally, the efficacy of wine pomace products at inhibiting protein oxidation is lower than against lipid oxidation. Several studies have observed no protection in solubility loss (Brannan 2008; Yu and others 2013) and Sánchez-Alonso and others (2007b) noted no effect against myosin loss. White wine pomace extract promoted the loss of thiol groups, but inhibited carbonyl formation and myosin cross-link formation, suggesting that grape phenols may interact with thiols thereby avoiding protein aggregation (Jongberg and others 2011). Red skin wine pomace products also reduced protein radical accumulation; mitigating thiol loss and the formation of cross-linked myosin (García-Lomillo and others 2016b). Grape seed polyphenols were also effective inhibiting carbonyl formation and protecting thiol groups (Yu and others 2013).

Interaction with microorganims in food

Wine pomace contains different constituents, such as fiber, acids, salts, and phenolic compounds that can interact with food microorganisms, mainly due to their positive or negative capacities to influence the growth of microorganims. Generally, antimicrobial activity is the most extensively studied and, among wine pomace components, phenolic compounds are the most widely studied agents, due to their well-known antibacterial and antimold activities.

Antimicrobial action against food spoilage microorganisms. The spoilage flora includes those microorganisms that deteriorate food quality by reducing consumer acceptance of the final product and limiting shelf-life (Gill and others 1996). Deterioration caused by the spoilage flora is usually associated with the formation of volatiles that cause off-flavors, color deterioration, acidification, slime formation, and gas production.

The most relevant conditions that may affect the development of the spoilage flora in foods are the initial microbial population, food processing contaminations, and storage conditions (temperature, packaging) (Gill and others 1996). In air-packaged atmospheres, *Pseudomonas* is usually the predominant population causing putrid and sulfur odors, due to the formation of ethyl esters and sulfur compounds. *Pseudomonas* growth may be inhibited by using CO_2 in the packaging atmosphere or by using vacuum packaging. In these cases, LAB (which are facultative anaerobic and have a high tolerance to CO_2) become the predominant microbial group (Schillinger and others 2006). The genera most frequently involved in food spoilage are *Lactobacillus*, *Pediococcus*, *Streptococcus*, and *Leuconostoc*. Enterobacteriaceae are also very resistant to CO_2 and anaerobic conditions and are responsible for putrefactive deteriorations, thus shortening the shelf-life of food products. Other microorganisms involved in food spoilage include *Brochothrix thermosphacta*, *Aeromonas* spp., and *Alteromonas putrefaciens* (Borch and others 1996).

Due to the problems caused by the spoilage organisms, the food industry is constantly looking for new strategies to inhibit their growth. Over recent years, the strategies have been focused on new natural compounds with antimicrobial activity to replace the use of chemical preservatives. Different products obtained from wine pomace, especially grape seed extracts, have been proposed to control spoilage. The growth of TAMB, LAB, *Pseudomonas*, and psychrotrophic populations in pork patties was delayed by the incorporation of seed extracts (Lorenzo and others 2014). In comparison to other natural extracts such as tea, seaweed, and chestnut extracts, higher antimicrobial action was exerted by grape seed extract. In addition, Bañón and others (2007) also described delaying activity against TAMB and total coliform count, and an effect against *Pseudomonas* was also described by Király-Véghely and others (2009). Sagdic and others (2011) studied the effect of 5 wine pomace extracts at different concentrations between 1% and 10% and noted bactericidal effects against the populations of TAMB, psychrotrophic, lipolytic, and proteolytic bacteria, as well as yeasts and molds, micrococcaceae, lactobacilli, and lactococci after 2 h of applications. Wine pomace showed antimicrobial activity against spoilage populations such as TAMB and psychrotrophic hydrogen sulfide-producing microorganisms (Ribeiro and others 2013). A seasoning derived from seedless red wine pomace was able to mitigate the instability caused by salt reduction in beef patties (González-SanJosé and others 2014). The seasoning was effective at inhibiting the growth of TAMB, LAB, and Enterobacteriaceae, considerably increasing the shelf-life. Similar results have recently been pointed out by Hasani and Alizadeh (2015), however, in this case, the effect was less relevant than in other studies. Other researchers showed that low concentrations of grape extracts (lower than 0.2%) had no effect on the final population or only produced slightly lower final counts of TAMB (Garrido and others 2011; Kumar and others 2015). The lack of any antimicrobial effect was ascribed to the low level applied and to matrix–phenol interactions that may limit the antimicrobial capacity of phenolic compounds. Wine pomace products may also exert an inhibitory effect against yeasts and molds, (Corrales and others 2010; Yadav and others 2015), and grape pomace showed antimicrobial activity against spoilage populations (Ribeiro and others 2013).

Apart from the capacity of wine products to limit microbial growth, they may be able to induce metabolic changes and to mitigate some of the deteriorative reactions and effect caused by spoilage organism's metabolism, such as gas formation (Yamakoshi and others 2001), slime formation (Furiga and others 2014), acid production (Thimothe and others 2007), and the formation of biogenic amines (Alberto and others 2007; Wang and others 2015).

Antimicrobial action against foodborne pathogens. The consumption of food contaminated with pathogens causes more than 320000 outbreaks each year in the European Union, with eggs and meat and fish products provoking the highest number of cases (EFSA, ECDC 2015).

A large number of studies have been published describing the *in vitro* effect of wine pomace products against foodborne pathogens, but evaluations in food matrices are more limited. The published results show that the efficacy against pathogens depends on the product concentration, the microorganism species under study (even at the strain level), and the pH and polarity of the matrix (Rhodes and others 2006; Vaquero and others 2007; Al-Habib and others 2010).

Grape seed extracts (at 1%) showed bactericidal effects against *Escherichia coli* and *Salmonella typhimurium*, and they delayed the growth of *Listeria monocytogenes* and *Aeromonas hydrophila* (Ahn and others 2007); but when incorporated into films they only presented slight activity against *B. thermosphacta* (Corrales and others 2009). They were also effective in cheese inoculated with *L. monocytogenes*, *Staphylococcus aureus*, and *Salmonella enterica* (Shan and others 2011). The concentrations required to observe the antimicrobial effect were higher than with *in vitro* assays, which suggested a decrease in the antimicrobial effect when the products were added to foods. Probably the low solubility in certain foods and the interaction of polyphenols with other food components can explain the lower effect (Shelef 1984; Corrales and others 2009). Red grape pomace extract and powder also showed activity against *E. coli* and *S. aureus* at concentrations ranging between 2% and 10% (Sagdic and others 2012b), and wine pomace extracts were shown to inhibit the formation of microbial films (Xu and others 2014). Among the powdered products, a seasoning produced from grape skin wine pomace also exerted a bactericidal effect against *S. aureus*, *Listeria innocua* at 4%, and an intense inhibition against *E. coli*. The lag phase was extended, and the maximum growth rate was reduced in the 3 microorganisms by incorporating 2% of the seasoning (data not published).

It has been noted in the literature that grape seed extracts exhibit higher inhibition than the corresponding skin extracts (Rhodes and others 2006; Xu and others 2014). In the case of nonextracted products, grape pomace also presented activity against *S. aureus* and *E. coli* (Sagdic and others 2012b). In contrast, Kim and others (2012) reported that wine pomace only had antimicrobial effects after being fermented by *Lactobacillus casei*.

Generally, Gram-positive bacteria exhibit higher sensitivities toward wine pomace products than Gram-negative bacteria (Corrales and others 2009; Delgado Adámez and others 2012; Xu and others 2014), although contradictory results have also been reported (Katalinić and others 2010; Cueva and others 2012). These different sensitivities could be explained by the presence of the lipopolysaccharide cell wall in Gram-negative bacteria, which can limit the penetration of phenolics into the cell. Furthermore, the presence of efflux pumps in some Gram-negative bacteria like *E. coli* could contribute to their higher resistance (Xu and others 2014). Moreover, some Gram-negative bacteria are able to metabolize certain phenolic compounds, such as hydroxycinnamic acids, by deactivating their antimicrobial effect (Vaquero and others 2007).

The antimicrobial effect of wine pomace products is usually ascribed to different phenolic compounds. Several studies have shown the predominant role of phenolic acids (mainly gallic acid, followed by *p*-hydroxybenzoic and vanillic acids) in comparison to flavonoids. In this sense, gallic acid was found to be the strongest antimicrobial agent of grape seed extracts (Tesaki and others 1999). Corrales and others (2009) suggested the higher potential of hydroxycinnamic acids, in comparison to their corresponding hydroxybenzoic acids, due to their lower polarity, which means they can cross through the cell membrane. Mingo and others (2016) pointed to epicatechin gallate and resveratrol as the most active compounds against *Campylobacter*. In contrast, polymeric compounds seem to be more active than the corresponding monomeric compounds in grape seed and skin extracts (Rhodes and others 2006).

It is interesting to point out that generally pure compounds have a much lower activity than the wine pomace products, which reveals a synergistic effect of all the phenolic compounds (Silván and others 2013; Xu and others 2014). For example, a fractionated extract rich in caftaric acid had a high antimicrobial effect against *Campylobacter jejuni*, while pure caftaric acid was not active itself (Silván and others 2013). Pure phenolic compounds, including gallic acid, caffeic acid, catechin, ellagic acid, and quercetin, showed little or no effect against *S. aureus*, *S. enterica*, and *E. coli* in comparison to grape seed and skin extracts (Xu and others 2014). Rodríguez Vaquero and others (2010) observed that combinations of flavonoids and phenolic acids produced a synergistic effect against *E. coli*, which was corroborated in a meat system. The combinations showed bactericidal effect in contrast to individual phenolic compounds with only bacteriostatic activity. Consequently, it appears that a combination of different compounds is more effective than the use of pure compounds.

Different mechanisms have been suggested to explain the antimicrobial effect observed for wine pomace products. Partially, hydrophobic phenols are able to penetrate into the phospholipid bilayer and induce several changes in cell functions including membrane disruption and structural changes (Cowan 1999). The presence of an outer membrane of a hydrophilic nature in the Gram-negative bacteria seems to prevent polyphenols from entering through the cytoplasmic membrane. Modified structures of *S. aureus* producing larger, rougher, and more irregular cells were observed after being incubated with grape seed extracts (Al-Habib and others 2010). Wine pomace products may also be able to enter the cell and deactivate intracellular components such as enzymes

(Thimothe and others 2007) or intercalate into the microbial DNA (Sivarooban and others 2008). Other potential mechanisms of phenolic compounds, especially high molecular weight compounds, are metal chelation (Chung and others 1998) and protein precipitation (Shibambo 2008), limiting the transference of these nutrients into the cell. Furthermore, phenolic compounds may inactivate extracellular microbial enzymes, thus limiting microbial growth (Scalbert 1991).

Protection of probiotics. In contrast to the more widely studied effect of growth inhibition of spoilage and foodborne pathogenic organisms, some published studies also showed the capacity of wine pomace products to promote the activity of or to protect probiotic microorganisms against different altering external factors.

The effect of phenolic compounds on the growth of LAB may vary widely according to the chemical structure and the concentration of each phenolic compound. It also depends on the microorganism species or, strain, its growth in the medium, and the growth phase (Rodríguez and others 2009). Wine pomace and grape seed extracts were able to promote *Lactobacillus acidophilus* according to Hervert-Hernández and others (2009). The authors highlighted that as LAB do not require heme enzymes in their metabolism, the chelating activity of phenolic compounds would not affect their growth. Growth of *Lactobacillus hilgardii* was also enhanced by the presence of catechin and gallic acid (Alberto and others 2001). These effects were ascribed to their ability to metabolize these phenolic compounds and to enhance sugar metabolism.

Regarding food applications, grape pomace enhanced *L. acidophilus* fermentation, by stimulating lactic acid production, and reduced the fermentation time (Frumento and others 2013). A similar effect was observed by Aliakbarian and others (2015) who reported higher counts of *Streptococcus thermophilus* and *L. acidophilus* after fermentation. Pomace flour had no effect on the fermentation time, but it enhanced their resistance to simulated gastrointestinal conditions (Casarotti and Penna 2015), and grape seed extract may protect probiotic LAB against cell injury caused by freezing and prevent the decay of bacterial counts (Sagdic and others 2012a).

Effect on neo-formed contaminants

Industrial and household heat treatments are required in order to develop an acceptable taste, increase digestibility, and assure the safety of some food products. However, processes at high temperature also involve the formation of the so-called neo-formed contaminants (NFCs) that may have toxicological effects (Birlouez-Aragon and others 2010). Many of these compounds are related to Maillard reactions, which involve a condensation reaction between free amino groups and carbonyl compounds (from reducing sugars, aldehydes, or ketones). Generally, anti-Maillard activity has been ascribed to polyphenols, which seems to be due to polyphenol–amine and polyphenol–sugar interactions (Totlani and Peterson 2005; Ortega-Heras and González-Sanjosé 2009) and their radical scavenging activities (Mildner-Szkudlarz and others 2015). Products derived from wine have shown inhibition against the formation of different NFCs: heterocyclic amines (HAs), polycyclic aromatic hydrocarbons (PAHs), acrylamide, advanced glycation end products (AGEs), and furans.

Generally, HAs are compounds with 3 fused aromatic rings and at least 1 nitrogen atom in the ring as well as 1 exocyclic amino group. Different types of HAs may be formed depending on the cooking temperature (thermic and pyrolytic HAs). Thermic HAs are formed through the reaction of creatine and reducing sugars as part of the Maillard reaction, whereas pyrolytic HAs are formed through pyrolytic reactions (preferably formed at temperatures above 250 °C). One thermic HA (2-amino-3-methylimidazo[4,5-f]quinolone, IQ) has been listed as "probable human carcinogen" (Group 2A), and 3 thermic and 6 pyrolytic ones are listed in Group 2B as a "possible human carcinogens" according to the Intl. Agency for Research on Cancer (IARC 1986, 1993).

Different studies have shown the high effectiveness of grape seed extract at limiting the formation of HAs, and proanthocyanidins are of high relevance in the inhibitory effect (Cheng and others 2007; Rounds and others 2012). Marinating with red and white wine also mitigated the formation of HAs in pan-fried beef (Melo and others 2008; Viegas and others 2012). The activity was ascribed to the ability of phenols to scavenge radicals from the Strecker degradation reactions, and it was highlighted that other components, such as hexoses and pentoses, can also contribute to the observed inhibition (Gibis and Weiss 2012). However, other studies show only low protection against nonpolar HAs and even some promoting effect (Busquets and others 2006; Gibis and Weiss 2012), due to the presence of metals.

PAHs may also be formed in relevant amounts during the heat treatment of meat and meat products. Substances rich in antioxidants have been suggested to inhibit their formation (Janoszka 2011; Viegas and others 2014), and the effect was positively correlated to the radical scavenging activity of the product that was used (Viegas and others 2014). However, the literature on this is still scarce, and no studies regarding the activity of wine pomace products have been conducted on food. Seasoning derived from skin red wine pomace was also able to inhibit the formation of PAHs in those samples that had been stored for 9 d (data submitted).

Acrylamide is also classified as a "probably carcinogenic" agent (2A) found in starchy food cooked at high temperatures such as potatoes and cereal products (IARC 1994). Grape seed proanthocyanidins were shown to inhibit the formation of acrylamide in starch-based models (Zhu and others 2011), and wine pomace skin and seed extracts mitigated the formation of acrylamide in model systems and during the frying of potato chips (Xu and others 2015). Skin extracts presented higher activity than seed extracts, and the authors related this effect to a possible combination between polyphenols and Maillard reaction products blocking the formation of acrylamide.

During heat treatments, AGEs were also formed, with $N(\varepsilon)$-carboxymethyl-lysine (CML) being the most abundant in food due to the reaction between lysine and carbonyl groups of reducing sugars and of lipid oxidation products (Goldberg and others 2004). It was reported that AGEs derived from the diet are absorbed and accumulated in human body tissue, increasing the risk of diabetic and cardiovascular complications and renal diseases (Nguyen and others 2014). Grape polyphenols have demonstrated inhibitory activity against the formation of CML in muffins. The effect was dependent on the formulation, with the lowest effect in formulations rich in protein (Mildner-Szkudlarz and others 2015). The results were ascribed to their scavenging of free radicals as well as their ability to trap intermediate compounds with carbonyl groups. The formation of fructosamine, another AGE, was also inhibited by a red skin wine pomace extract (Jariyapamornkoon and others 2013).

Plant polyphenols were also able to limit the formation of furans (including furfural and hydroxymethylfurfural) in a glycine–glucose model system (Oral and others 2014), probably due to the capacity of phenolic compounds to interact and to block the Maillard reaction.

Natural food coloring

Various products derived from wine pomace may be used to modify the sensory properties of food products, mainly the chromatic characteristics. In this regard, the most common application is the use of the anthocyanins recovered from wine pomace (enocyanin) as natural food coloring.

The satisfactory use of enocyanin as food coloring strongly depends on the food matrix in which it is incorporated. As it is well known, anthocyanins are compounds with very different color depending on the pH of the medium (flavilium cation shows intensive red color to pH lower than 3.5, whereas carbinol pseudobase and chalcones are colorless structures, which are formed to pH higher than 5). Furthermore, the degree of acetylation, polymerization, and copigmentation also affect the color properties, as intensity, hue, and color stability. The presence of sulfites that are able to react with anthocyanins may produce drastic color reduction. Other parameters that may limit the stability of these pigments are contact with O_2, light, and heat that can occur during food processing and storage (Mateus and de Freitas 2009).

Anthocyanins extracted from wine pomace generally have adequate intensity and stability for being used in the food industry, although other resources such as red cabbage have shown better properties (Mateus and de Freitas 2009). These parameters can be improved by copigmentation or by encapsulation of the extract (Stoll and others 2016). Wine pomace extracts have been successfully used as food colorings at concentrations between 20 and 60 ppm in a wide range of food categories including beverages (soft drinks, wine, and liqueurs), dairy products (yogurts, desserts, ice creams, and so on), and jam and fruit preparations (Calvi and Francis 1978; Clydesdale and others 1978; Prudencio and others 2008; Mateus and de Freitas 2009).

Other functions

Antipolyphenol oxidase activity. Polyphenol oxidase (PPO) is a copper-containing enzyme distributed in different food products that catalyzes the oxidation of o-diphenols to o-quinones. *o*-Quinones can undergo polymerization and form brown pigments, limiting the shelf-life of some products. Melanosis is one of the main problems related to PPO within the food industry, due its high impact on the visual appearance of the product. Melanosis of shrimp was inhibited by immersion in solutions of 2.5 to 15 g/L of grape seed extract (Gokoglu and Yerlikaya 2008; Sun and others 2014). Other products rich in antioxidants were used in dipping solutions to inhibit PPO in other matrices such as fruits (Soysal 2009). The protective activity against PPO may be ascribed to the high capacity to reduce the o-quinones formed by the action of PPO, forming colorless o-diphenol. Furthermore, wine pomace products were also suggested to inhibit the activity by chelating copper, scavenging free radicals, or directly inactivating the enzyme (Nirmal and Benjakul 2011).

Reduction of residual nitrites and nitrosamines. Nitrates and nitrites are added to some foods, especially to meat products due to their reaction with myoglobin producing the typical color of cured products. Furthermore, nitrites and nitrates present antioxidant and antimicrobial activities that inhibit the growth of *Clostridium botulinum*. Nitrite consumption, as such, can be toxic when in excess producing methemoglobinemia ("blue baby disease") and lowering blood pressure (Lundberg and others 2008). Furthermore, the nitrite that does not react with myoglobin can react with other free amines to form nitrosamines that are considered potential carcinogens (IARC 1978).

Grape seed extract was able to reduce the residual levels of nitrite after ripening of dry-cured sausages and to decrease the formation of nitrosamines (Li and others 2013), and to inhibit *N*-nitrosodimethylamine formation (Wang and others 2015). It seems that polyphenols may remove residual nitrite by reduction or by direct reaction (Fernández-López and others 2007; Viuda-Martos and others 2009). Polyphenols may also mitigate the formation of nitrosamines by inhibiting microbial activity and by scavenging the radicals involved in amine formation (Dong and others 2013).

Effect on the formation of pyrazines. Polyphenols present in wine pomace may also affect the formation of pyrazines that are compounds formed during cooking, such as pyrazines involved in the development of acceptable food flavors. Generally, polyphenols have been considered to be inhibitors of the reactions involved in pyrazine formation and lower levels of formation have been reported in different products (Porter and others 2006). However, the incorporation of a seasoning derived from wine pomace was found in the formation of pyrazines in barbecued beef patties (García-Lomillo and others 2016a). The observed results were explained by the promoting effect in the formation of α-dicarbonyls derived from carbohydrates, due to their high capacity to reduce metals (Wilker and others 2015). Polyphenols, in their quinone state, can also participate in the Strecker degradation of amino acids, contributing to the formation of pyrazines (Rizzi 2006).

Sensory repercussion of adding wine pomace products to foods

Beyond the satisfactory and desired coloring effect, the use of products derived from wine pomace can also induce modifications on food colors, leading to unusual effects. This fact may limit their application in some food categories. Generally, in the case of beef and pork meat, applications below 0.2% do not have negative effects on color, odor, or taste attributes; however higher concentrations (1%) produced significant increases in redness, although this was not always perceived as negative (Rojas and Brewer 2007; Ahmad and others 2015). For white meats such as chicken, relevant modifications in the color were observed even at 0.1% (Brannan 2008; Sáyago-Ayerdi and others 2009). Higher intensity of redness and darker crusts (brightness decreases) are usually reported in baked products, due to a higher degree of Maillard reaction (Hoye and Ross 2011).

Apart from the modifications caused in food appearance, wine pomace may also induce other types of modifications on the sensorial properties, which are usually linked to bitter and astringent taste perceptions. In some products, the increase in the astringency may be positive, such as in chocolate, soft drinks, or wine (Lesschaeve and Noble 2005). However, the increase of astringency and bitterness of food usually is not a well-accepted effect, and this fact may limit the application of wine pomace products on a certain food matrix. Several alternatives can mitigate these problems such as the use of sweeteners, a protein to complex polyphenols to limit their interaction with taste receptors and salivary proteins, and increasing fat content to provide some lubricity (Ares and others 2009).

Products derived from wine pomace may also enhance or suppress other aromas of the food. For instance, grape seed extract enhanced wine woody aroma and suppressed those related to fruity notes (Cliff and others 2012). However, Pasqualone and others (2014) reported that biscuits enriched with wine pomace had higher sensory scores of fruity odor and sour taste.

Furthermore, more volatile compounds derived from Maillard reaction were formed.

Although consumers' studies are not abundant, acceptance tests usually reported positives or neutral results, although this depends on the concentration used and on the type of products. Rosales Soto and others (2012) conducted an extensive hedonic study on the effect of grape seed flour on several parameters of 3 cereal products: cereal bar, pancakes, and noodles. Among the 3 products, cereal bar with 5% grape seed flour was pointed out as the best option to incorporate the flour. In this case, grape seed flour increased the acceptance rates of appearance, flavor, taste, mouthfeel, and texture attributes. Acun and Gül (2014) also reported that incorporation of 5% of grape pomace flours (seedless wine pomace, whole wine pomace, and seed flours) in cookies improved their acceptability. In contrast, higher levels led to consumers' rejection due to darker crust and bitterness. Grape extract at 1% also improved the acceptability of yogurt (Karaaslan and others 2011), whereas grape seed extract at 1000 ppm also improved overall acceptability of dry sausages (Lorenzo and others 2013). In other cases, no significant differences were reported such as in ice cream (Sagdic and others 2012a) or in potato chips (Rababah and others 2012b). Replacement of up to 10 g grape seed flour/100 g increased the firmness of bread, but this did not cause any effect on consumer acceptance of hardness, and concentrations of 6 g/100 g did not modify consumer acceptance of astringency or bitterness (Mildner-Szkudlarz and others 2011).

On the other hand, decreased liking scores were also observed in the aroma, aftertaste, flavor, and appearance of pasta enriched with wine pomace (Sant'Anna and others 2014). In the case of dairy products, fortification with skin wine pomace at 6% induced decrease in the liking score, especially for the taste and flavor (Marchiani and others 2016).

Furthermore, the use of the products derived from wine pomace may contribute to keep the sensory properties associated to fresh food during storage due to their ability to inhibit oxidative and microbial reactions (Ahn and others 2002; Shirahigue and others 2011; Rababah and others 2012a).

It is worth remarking that the overall acceptability of food products also depends on extrinsic factors such as health claims. Then, the potential nutritional benefits and the natural origin of wine pomace products may contribute to improve the acceptability of the products and consumer' willingness to pay more for the product (Lesschaeve and Noble 2005).

Conclusions

The large number of wine pomace applications described in this review shows the high potential of the revalorization of this by-product in the food industry. These alternatives may contribute to reduce winery residuals, improve environmental aspects, so as to reduce production costs, and offer new ways to diversify the production. Furthermore, the food industry is provided with natural products that are able to inhibit different microbiological and chemical reactions, enabling the reduction in the use of synthetic food preservatives and antioxidants without compromising the stability of the final product. This fact can contribute to the higher consumer's perceived value that would balance the cost of the development of new formulations and optimization of the food-making processes.

The potential advantages of the applications described are compelling reasons for further research on this topic. Recent trends shows the potential interest of new research on nonextracted products such as flours or seasonings to take advantage of the wide range of nutrients in wine pomace including fiber, minerals, phenolic compounds, and so on. Special attention deserves the use in bakery products as alternatives to integral flours. Further research requires the optimization of food formulation (other ingredients, food processing, and packaging) in order to achieve the highest quality possible, especially those related to sensory parameters. Furthermore, it will be required to study the response of consumers and their willingness to pay more for these types of products. According to the last proposed, studies focused on health aspects that will be able to demonstrate the real effect of wine pomace derivate products on different diseases and health alterations will be required.

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