Antioxidant and antimicrobial properties of wine by-products and their potential uses in the food industry

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ABSTRACT

Wine pomace (WP) is one of the agricultural by-products that has received most attention from food scientists due to the wide range of interesting compounds that remain after the winemaking process. Different powdered products rich in phenolic compounds, with interesting antioxidant and antimicrobial activities were obtained from WP by applying processes that are both environmentally friendly and economically affordable for the food industry. The products obtained showed high global antioxidant activities (ABTS assay), successfully delayed the onset of lipid oxidation in the Rancimat test, and showed different antimicrobial properties. Products derived from seed-free WP showed bactericidal effects against total aerobic mesophilic bacteria (TAMB) and lactic acid bacteria (LAB) and inhibited *Enterobacteriaceae* growth completely. The product derived from whole WP presented bacteriostatic activity against the three microorganism groups tested, whereas the product obtained from grape seed promoted TAMB and LAB growth but delayed *Enterobacteriaceae* proliferation.

KEYWORDS: wine by-products; antioxidant capacity; Rancimat; antimicrobial activity; spoilage population**.**

INTRODUCTION

The food industry needs to extend the shelf-life of its products in order to reduce the amount of food that is wasted. The use of antioxidants and antimicrobials is therefore required in order to produce microbiologically safe foods while maintaining adequate sensory properties. Synthetic additives have traditionally been used due to their low price and high effectiveness [1]. However, consumer awareness concerning the potential risks of long-term intake of synthetic additives is increasing [2]. This fact has led the food industry to search for natural products that possess antioxidant and antimicrobial activity and can be used to replace synthetic additives in food formulations. Plant extracts and by-products from different industries have emerged as potential replacements for synthetic additives since they may exert similar effects and are preferred by consumers due to their natural nature. The preservative effects of such extracts have been attributed to their elevated content in bioactive compounds, including polyphenols, which are well-known antioxidants and antimicrobials (Cowan [3] and Brewer [4], amongst others).

The use or reuse of by-products, especially those from plant-based materials, presents several advantages such as the low cost of these by-products, reduced storage and elimination costs, their environmentally friendly nature, and revalorization of both the process and its by-products [5]. Wine pomace (WP) has been one of the most widely studied plant-based by-products for many years and many applications have been proposed for its revalorization, as recently reviewed by Yu et al. [6].

The solids remaining after the fermentation of red grapes, racking-off the wine, and subsequent pressure is usually known as WP, which mainly comprises solid grape parts (skin, rest of pulp and seeds) along with small pieces of stalk. WP also contents residual yeasts and bacteria which were the main agents to carry out alcoholic and malo-lactic fermentations. WP revalorization is usually approached by producing extracts rich in antioxidants, which can be incorporated into different food

matrixes and also used in the cosmetics and pharmaceutical industries due to their antioxidant properties [7] and antimicrobial effects [8]. Although the use of these extracts is usually claimed to be a "green" alternative for the food industry, extracts are often obtained using organic solvents, thus meaning that this "green" status is questionable and could lead to their use in food formulations being refused [9]. Furthermore, extraction steps could considerably increase production costs and complicate broader applications in the food industry.

In addition to such extracts, other authors have proposed the use of wine by-products without any prior extraction of the phenolic compounds, an approach that presents clear economic and environmental advantages [5]. The most widespread use of these products in the food industry is to increase the fiber and the global antioxidant capacity of the product they are added to [6]. However, few studies have investigated the ability of such products to improve the stability of foodstuffs, and there is a lack of information regarding the effect of these products on the spoilage population of food systems.

In light of the above, the main aim of this work was to evaluate the ability of products obtained directly from wine by-products (specifically WP) to extend the shelf-life of foodstuffs by preventing oxidative degradation and controlling the growth of spoiler microorganisms.

MATERIALS AND METHODS

Material

Wine pomaces from red winemaking were kindly supplied by seven different wineries located in the Burgos region. Wine pomaces from all wineries were well mixed and dehydrated to achieve a final water content of less than 10%.

Three products were obtained from the dry material: one from the global or whole wine pomace, termed "whole wine pomace product" (WWPP), another from the seed-free WP, termed "Skins wine

pomace product" (SkWPP), and the third from the isolated seeds, termed "Seeds wine pomace product" (SdWPP). The dried materials were milled and sieved, and powdered products with particle sizes of less than 0.250 (SkWPP and WWPP) and 0.355 mm (SdWPP) were used to carry out this study. Ultraviolet (UV-C, 254 nm) and thermal (90 ºC) treatments were applied to inactivate the microbial flora present in the products obtained. Different heat and UV treatment times (15, 30, 60, 90 and 120 minutes) were tested in order to reach the optimum microbial inactivation.

Analytical Methodologies

Main composition analysis

The moisture content was evaluated by the difference in the sample weight before and after drying at 105ºC to constant weight. The fat content was determined after Soxhlet extraction using petroleum ether (Lab-Scan, Gliwice, Poland) as extraction solvent in a Buchi B-811 extraction system (Buchi, Switzerland). The protein content was determined using the Kjeldahl method, which measures the total nitrogen content after digestion with boiling sulfuric acid. A conversion factor of 6.25 was used to convert nitrogen into protein values. The total dietary fiber content was evaluated using the kit provided by Sigma (St Lois, USA) according to the manufacturer's instructions based on AOAC method 985.29. Total SO_2 was determined using the enzymatic kit developed by R-Biopharm AG (Darmstadt, Germany) according to the manufacturer's instructions.

Mineral content

The ash content was determined by incineration of the samples at 525ºC in a furnace (P-selecta, Barcelona, Spain). Sodium and potassium contents were determined according to the dry ashing method proposed by AOAC [10] using flame photometry (Flame Photometer 410, Corning, UK), whereas the calcium content was measured in the same acid solution using a polarized Zeeman atomic

absorption spectrophotometer Z-8200 (Hitachi; Japan). Phosphorus determinations were conducted by reaction of the acid solution with vanadate-molybdate reagent (Panreac, Barcelona, Spain) [11].

Main phenolic families

Previously rehydrated powders were extracted in methanol/HCl (97:3) for 24 hours. The resulting extract was centrifuged and filtered. The content of four phenolic groups was determined: total polyphenol content (TPC) was determined by reaction with Folin–Ciocalteu reagent (Merck, Darmstadt, Germany) and expressed as mg/g of gallic acid [12]; total catechin content (TCC) was determined by treatment with vanillin reagent (Sigma) and expressed as mg/g of D-catechin [13]; total anthocyanin content (TAC) was quantified by measuring the difference in the absorbance (525 nm) of the sample diluted in 1 N HCl and in phosphate citrate buffer (pH 3.5) [14] and the results expressed as mg of malvidin-3-glucoside/g; and total proanthocyanidin content (TPAC) was measured after acid hydrolysis at 95ºC for 40 minutes and expressed as mg of procyanidin B1/g [15].

Antioxidant capacity

$ABTS^{\cdot+}$ method: The radical scavenger activities of the powdered products were studied by measuring the decrease in the absorbance at 734 nm after incubation of methanolic extracts with a solution of ABTS (2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid). The ABTS radical solution was prepared by mixing ABTS and $K_2O_8S_2$ in water (1:1) [16].

Fat oxidation inhibition capacity: The ability of the extracts to protect fats against oxidation was evaluated using the rancimat test, which was performed using a Rancimat 743 Instrument (Metrohm, Switzerland). The air flow-rate and temperature were set at 20 L/h and 110ºC respectively. The tests were conducted in triplicate on commercial olive oil and melted pork lard. Controls and fats with 2% (w/w) of the different wine pomace products (WPPs) under study were conducted. The Rancimat test evaluates the conductivity caused by the short-chain fatty acids produced during lipid oxidation.

123 Although this test has been criticized due to the use of high temperatures, which may affect the
124 mechanism of lipid oxidation [17], it remains a valuable tool for predicting the protection exerted by an
125 antioxidant in real food systems. The results were expressed as protection factor, which was calculated
126 by dividing the induction period of the WPPs with that for controls (fat without WPPs).

127 ***Microbial analysis***

128 Samples were weighed in a Stomacher bag, decimally diluted with Ringer solution (Oxoid,
129 Basingstoke, Hampshire, UK) under sterile conditions and homogenized in a laboratory blender
130 (Stomacher 400, Colworth, London, UK). Total aerobic mesophilic bacteria (TAMB) and total yeasts
131 and moulds were evaluated on plate count agar (PCA) (Merck) and Sabouraud agar with
132 chloramphenicol (Pronadisa, Madrid, Spain) respectively. PCA plates were incubated at 30ºC for 72
133 hours and Sabouraud plates at 25ºC for 7 days.

134 ***Antimicrobial potential of products***

135 The antimicrobial capacity was measured using meat homogenates, which were prepared by
136 homogenizing commercially available beef in sterile water (1:3). A comparative study was carried out
137 using homogenates of control meat (additive-free), meat with 2% of each WPP, and meat with 300
138 ppm of SO_2. Homogenates were incubated while stirring for 32 hours in an incubator (New Brunswick
139 Scientific, Edison, U.S.A.) at 37ºC. For each sample, decimal dilutions were prepared in Ringer
140 solution and plated onto the appropriate culture medium. TAMB were determined using pour plates on
141 PCA after incubation at 30 ºC for 72 h. Lactic acid bacteria (LAB) were enumerated using de Man
142 Rogosa Sharpe Agar (MRS) (Oxoid) after incubation at 25ºC in an atmosphere of 5% CO_2 for 5 days.
143 Those colonies that reacted positively to the catalase test were not counted. *Enterobacteriaceae* counts
144 were determined using a double layer of violet red bile glucose agar (Pronadisa) incubated at 37 ºC for

24 hours. Antimicrobial experiments were repeated three different times with duplicate homogenates each time.

Statistical analysis

Statistical analysis was performed using StatGraphics ® Centurion XVI. Fisher's least significant difference (LSD) test was performed in order to identify significant differences.

RESULTS AND DISCUSSION

WP presents a sufficiently high water content to promote both microbial growth and enzyme-related degradation, therefore a dehydration process was required in order to obtain a raw material that remains stable until final transformation into the desired powder products. Thermal drying was chosen over freeze-drying for the following reasons. Previous studies have suggested that, in contrast to freeze-drying, oven drying does not affect total extractable polyphenols and condensed tannins as long as the temperature does not exceed 60ºC [18]. Tseng & Zhao [19] reported that freeze-dried products had the lowest stability during storage, probably due to their higher porosity and higher exposure to oxygen. Furthermore, the costs of freeze-drying, which is estimated to be 4-8 times more expensive than thermal drying [20], was also considered. WP was dehydrated to a water content of less than 10%.

Particle size determines the use and application of different ingredients in food manufacture [21]. The handling safety of the ingredient, palatability of the resulting foodstuff, and the release of active compounds are some of the factors involved in this aspect. Furthermore, stability of products and manufacturing costs need to be considered. The likelihood of oxidative reactions and microbiological contamination increases with the reduction of particle size. Besides, the manufacturing costs also increase, due to longer manufacturing times and higher energy levels required to obtain smaller particle size. To balance the advantages and drawbacks of a very small particle size, SkWPP and WWPP were milled and sieved through a mesh with a size of 0.250 mm, whereas SdWPP was milled

and sieved through a mesh with a size of 0.355 mm. The reason for choosing a higher particle size in SdWPP was the low yield obtained using a size of 0.250 mm. This could be explained by the caking and sticking of the seeds powder as a consequence of the high fat content of grape seeds. These phenomena are commonly found for dairy-based powders with high lipid contents. Surface fat may melt during the grinding process due to the temperature increase, which leads to the formation of fatty bridges between particles. When the temperature drops the fat solidifies, forming bridges between particles and resulting in a lumpy product [22, 23]. Furthermore, the tough structure of grape seed endosperm [24] could also contribute to the low yield obtained.

Characterization of the products obtained

The three products obtained showed significant differences in terms of composition (Table 1). Dietary fiber was the main component in all three products, with the highest content being found for SdWPP (±59%) (Table 1), probably due to the presence of lignin in the seeds. These results agree with those of Saura-Calixto et al. [25] and Tseng & Zhao [19], who indicated that WP mainly contains insoluble fiber and, more specifically, Klason lignin. WP fiber is associated with a high amount of antioxidants, thus making it a valuable source of dietary antioxidant fiber [26]. Similar protein contents were found for SkWPP and WWPP (±14% and 13% respectively), with the value for SdWPP (12%) being slightly lower due to the higher fiber and lipid content. Protein values were comparable to those obtained by other authors [26, 27]. Similar differences in the content of the various components of WP (mainly between skins and seeds) have been described previously [19, 27, 28].

SkWPP showed the highest ash content (±14%), with the values obtained being similar to those reported for Spanish grape pomaces by Saura-Calixto [26], thus indicating a high mineral content in grape skins. Potassium was the predominant macroelement, especially in the products derived from raw material containing grape skins (± 43 mg/g), which correlates with the well-known high potassium

content usually found in grape skins. It is widely accepted that during grape ripening, potassium accumulates in the skin and forms both soluble and insoluble salts with organic acids [29]. Seeds are considered to be the strongest sinks for calcium and phosphorous in grape berry which explains the higher content of these minerals observed in SdWPP in comparison to SkWPP [30]. The low sodium levels detected also agree with the lack of accumulation of these mineral during grape ripening [30].

The incorporation of sulfating agents is widespread in wineries due to the microbial and oxidative stability provided by them. However, sulfites were not detected in any of the WP products obtained.

The phenolic composition of the products obtained (Table 2) agrees with the well-known fact that grape seeds are a richer source of polyphenols, especially catechins and proanthocyanidins, than grape skins and that only grape skins contain a relevant amount of anthocyanins [31, 32]. It should be noted that the values obtained are lower than those reported in grapes [33] but are in agreement with the quantity of phenolic compounds transferred from grapes to wine during red winemaking [34].

Control of the microbial load of the products obtained was considered both convenient and necessary in order to obtain the safe products required by the food industry. Despite their wide range of applications, the microbiological contribution of WP-derived ingredients to foodstuffs has been underestimated as it has been assumed that the drying process is sufficient to ensure the safety of the powdered products [35]. However, other agricultural products with similar water contents and water activities, such as herbs and spices, have been involved in different outbreaks of food poisoning caused by the presence of pathogens [36]. Moreover, it is also important to consider that fungi are able to produce mycotoxins even at low water activities [37].

Our results showed that SkWPP had the highest microbial load, most likely due to the higher degree of manipulation and exposure to microbial contamination (Table 3). Although the microbial load

observed is usually considered to be acceptable in spices [38], microbial inactivation is recommended to ensure the quality of the powdered products obtained.

Two inactivation methods were conducted: thermal treatment and UV treatment. Although thermal treatment is the most common means of inactivating microorganisms, the low thermal stability of phenolic compounds may limit its application in WP products. Ultraviolet radiation has been mainly applied in liquid foods, although its use has also been proposed in powdered products [39]. In addition, it has successfully been used in leafy vegetables and is a clean and relatively inexpensive alternative [40, 41]. As such, it may be a valuable option for WP products.

Preliminary studies were conducted in order to establish the most efficient conditions for thermal and UV treatments. Firstly, the efficiencies of different UV-treatment times were tested. The differences between the microbial load of untreated and UV-treated WWPP and SdWPP were not significant (Table 3). The highest effectiveness of UV treatment was observed for SkWPP. These differences in effectiveness could be due to the difference in the particle size of each type of product [42]. A progressive reduction in microbial load of SkWPP was observed from 15 to 60 minutes of treatment, while no significant reduction was observed during the second hour (from 60 to 120 minutes) of UV treatment. Thus, after 60 minutes of UV treatment, SkWPP showed reductions of 1.16 and 1.26 log CFU/g in the counts of TAMB and yeasts and moulds, respectively (Table 3). The thermal treatment at 90 ºC produced a progressive reduction in the microbial load of the three products under study. The complete inactivation of the studied microbial flora was achieved after 90 minutes of treatment.

UV and thermally treated products showed similar main compositions to untreated ones, although some differences in phenolic composition were detected (Table 2). WWPP showed the highest decrease in TPC (16% in UV-treated and 6% in thermally treated), whereas the TPC of SkWPP was affected similarly by both treatments. In contrast, only UV treatment affected the TPC of SdWPP. The

cause of this higher decrease in UV-treated samples might be the photodimerization and isomerization of phenolic compounds induced by UV radiation [43]. Furthermore, UV radiation may also promote the formation of new linkages between hydroxycinnamic acids and lignin units [39], thereby decreasing their extractability. As far as thermal processing is concerned, previous studies found no significant decrease after heating to 100ºC for 250 min and even a significant increase at 150ºC [44]. This fact can be explained by the degradation of cell-wall polysaccharide structures, thus facilitating polyphenol release and extractability from the matrix structure [45]. Similarly, Chamorro et al. [46] found no significant decrease in TP after thermal treatment at 100ºC for 1 hour.

Catechin was one of the groups most affected by treatments (26% after UV treatment and 17% after thermal treatment). Epimerization and autoxidation seem to be the most likely mechanism for catechin degradation. The observed degradation agrees with the rates reported by Volf et al. [47]. Thermal treatment produced the highest degradation in TAC (27% in WWPP and SkWPP), which may be explained by the well-known thermal lability of anthocyanins [48]. Sólyom et al. [44] reported degradation rates of 75% in similar thermal treatments of non-dehydrated WP. Water availability plays a key role in anthocyanin degradation and the low water activity of the studied products may explain the relatively limited degradation of anthocyanins observed in this study [49, 50]. UV treatment did not decrease the TAC of either WWPP or SkWPP. Thermal and UV treatments produced similar decreases in the TPAC for all products studied (approximately 15%). Previous literature data in this regard are contradictory. Significant reductions in TPAC levels were reported by Khanal et al. [51] for grape pomace after thermal treatment at temperatures of 60ºC or higher. These authors observed degradation of the oligomeric procyanidins in WP upon increasing the temperature from 60ºC to 125ºC. In contrast, Chamorro et al. [46] found no significant reductions after heating grape pomace at 100ºC for 60 minutes. Overall, thermal treatment produced less intense reductions in TPC, TCC and TPAC than UV treatment, whereas it affected TAC more extensively. These reductions can be considered acceptable

since the WPPs obtained retain the vast majority of their phenols and their safety is markedly improved. Consequently, thermal treatment was selected as the best option and thermally stabilized products were used in the subsequent study of antioxidant and antimicrobial activities.

Properties of interest for food industry applications

In addition to use of the products obtained as a source of fiber, antioxidant, and possibly some minerals, such as potassium, WPPs exhibit other properties of interest to the food industry. In this regard, antioxidant and antimicrobial properties were considered to be of particular importance for application as natural food preservatives.

The ABTS method was used to evaluate the global antioxidant capacity of the products studied from amongst the large number of possibilities available due to its simplicity and the complete information provided by it [16]. All WP products showed interesting antioxidant capacities but with statistically significant differences. SdWPP exhibited the highest antioxidant activity (141.99 ± 2.09 μmol/g), followed by WWPP (103.29 ± 0.23 μmol/g) and SkWPP (75.65 ± 1.98 μmol/g). ABTS results were positively and strongly correlated with TPC, TCC and TPAC, as it is indicated by Pearson correlation coefficient, which showed values of 0.9951, 0.9968 and 0.987, respectively. This fact agrees with the attribution of antioxidant activity to the phenolic composition of WP. For example, Bonilla et al. [52] reported that gallic acid exerted the highest protection amongst all the phenols extracted from crushed grape pomace when added at the same concentration. Lafka et al. [53] also concluded that catechins and gallic acid contents are the main factors that determine the antioxidant activity of WPPs.

Considering the importance of preventing fat oxidation in the food industry, the ability of the products obtained to protect against fat oxidation was evaluated using the Rancimat method. Two fat systems with different oxidation sensitivities (olive oil and pork lard) were used. Olive oil mainly contains unsaturated fatty acids, which are more susceptible to oxidation than the saturated fatty acids found in

283 pork lard. However, olive oil also contains polyphenols and tocopherols with antioxidant activity [54],
284 whereas metals with pro-oxidant activity can be found in pork lard [55]. Furthermore, both fats can be
285 considered as good representatives of the different types of fat used in the food industry (plant and
286 animal fats).

287 Olive oil and pork lard gave average induction times of 26.07 and 14.65 hours respectively. The three
288 products studied delayed the onset of lipid oxidation in both types of fats (Table 4). These results could
289 be correlated to the global antioxidant capacities of the products, most likely to the phenol content. The
290 protection exerted by SdWPP was significantly lower than that exerted by SkWPP despite their higher
291 TPC, TCC and TPAC values. These findings are in agreement with the findings of Shaker [56], who
292 reported a higher inhibition for grape skin extracts in comparison to grape seed extracts when added to
293 sunflower oils at the same polyphenol concentration. The data obtained appear to indicate a key role
294 for anthocyanins in the protection against lipid oxidation [57]. These same authors found that grape skin
295 anthocyanins present a higher protective capacity against lipid oxidation than catechin and α-
296 tocopherol at the same concentration. Although anthocyanins are not soluble in fat, their protective
297 properties could be associated with their excellent ability to scavenge the free radicals formed during
298 fat oxidation. Wine anthocyanins showed a particularly intense hydroxyl radical scavenging capacity,
299 which was similar to their superoxide radical scavenging activity [58]. Furthermore, the possible lipid
300 oxidation of seed fat during the production and storage of SdWPP and WWPP should be also
301 considered, due to the fact that the presence of oxidized fatty acids may initiate oil and fat oxidation [59],
302 counteracting the antioxidant effects of polyphenols.

303 Antimicrobial activities were studied in meat homogenates since meat is a good system for studying
304 spoiler growth. The inhibitory effect of the WP products on the growth of potential spoilage
305 microorganisms was studied and compared with the antimicrobial effect of sulfites (Figure 1), a
306 standard and well-known antimicrobial food additive, as control. The antimicrobial activity of sulfites

307 appears to be related to their ability to induce changes in protein structures. Other antimicrobial
308 mechanisms of sulfites include blockage of transport, inhibition of glycolysis, nutrient destruction and
309 inhibition of microbial metabolism. [60].

310 Firstly, it is worth noting that the incorporation of SdWPP did not affect the initial load of
311 homogenates, whereas addition of SkWPP, WWWP and sulfites produced an immediate decrease in
312 TAMB and LAB counts. This finding suggests that the WP products obtained were in optimal
313 conditions for use as food additives. Significant protective effects were detected for all three products
314 studied, with these effects being very similar for all three assays performed.

315 Meat homogenates incubated with sulfites exhibited a significant decrease in TAMB count, a complete
316 inactivation of LAB after 32 hours of incubation, and no *Enterobacteriaceae* growth. SkWPP
317 produced similar inhibitions of TAMB and *Enterobacteriaceae* growth to those produced by sulfites.
318 The decrease in TAMB and LAB counts in the meat incubated with SkWPP suggests its bactericidal
319 capacity. The addition of WWPP delayed the onset of TAMB and LAB growth (by 15 and 22 hours
320 respectively), thereby confirming its bacteriostatic activity against spoilage flora. Moreover, addition
321 of WWPP delayed *Enterobacteriaceae* growth and decreased the final counts in comparison with
322 controls. These results could be correlated with the phenolic compounds present in SkWPP and
323 WWPP. The effect of grape polyphenols on the growth of different meat spoiler microorganisms, such
324 as LAB, has been widely studied and appears to depend on the polyphenol concentration, medium, and
325 species (or even strains) under study [61-63].

326 The antimicrobial effects of plant materials containing phenolic compounds have been associated with
327 different mechanisms of action [3], such as the ability to inhibit cell wall synthesis, thereby producing
328 cell membrane alterations and the consequent loss of crucial intracellular material; the ability to chelate
329 essential metals such as iron; and the ability to bind polysaccharides and proteins, thereby producing

compounds that cannot be metabolized by microorganisms [64]. Furthermore, Friedman [65] reported the ability to bind vital components in the cell, such as enzymes and cell transport proteins. Previous studies have shown an ability to increase the microbial stability of meat products but using grape pomace extracts. For instance, Sagdic et al. [66] observed the inhibitory effect of ethanolic grape pomace extract on the spoiler growth of beef patties. The incorporation of high levels of grape pomace extracts (10%) led to the complete inactivation of spoilage flora, whereas the addition of 1% grape pomace extract delayed the onset of microbial growth. The microbial results obtained with WWPP were worse than those obtained with SkWPP but better than those observed for SdWPP. The incorporation of SdWPP promoted the growth of TAMB and LAB and reduced their lag phases. These results were surprising in light of the higher phenolic content of this product. However, they are in agreement with the stimulatory role of grape seed extract on *Lactobacillus acidophilus* growth [67] and with the improved growth caused by gallic acid and catechin in *Lactobacillus hilgardii* [68]. Alberto et al. [69] have suggested that polyphenols may improve sugar metabolism in LAB, thereby stimulating proliferation. The results obtained are satisfactory and novel.

From a microbial stability and spoilage protection point of view the best product was SkWPP, although WWPP also showed good results. Moreover, the results obtained upon addition of SdWPP were also interesting, especially considering that the promoting effect on LAB could result in an increase in the production of certain bacteriocins with antimicrobial activity against pathogens [70]. In addition, it has been found that the bacteriocins produced by gallic acid and catechin adapted LAB are particularly potent inhibitors of the growth of some food-borne pathogens such as *Staphylococcus aureus* and *Salmonella enterica* [71].

In conclusion, wine pomace can be readily transformed into a series of products that meet the requirements of the food industry, namely cheap, environmentally friendly and natural, and with good antioxidant and antimicrobial abilities. Furthermore, these products can be used as a natural source of

fiber, antioxidants and potassium. The bactericidal activity of SkWPP, which is similar to that for sulfites, suggests the possibility of using this product as a sulfite substitute, thereby reducing the allergenic risk. The ability of the products studied to inhibit fat oxidation also suggests potential applications in fatty food with a high tendency to rancidity, thereby extending their shelf life.

ABBREVIATIONS USED

ABTS: 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid; **LAB:** Lactic acid bacteria; **MRS:** de Man Rogosa Sharpe Agar; **PCA:** Plate count agar; **SdWPP:** Seed wine pomace product; **SkWPP:** Skin wine pomace product; **TAC:** Total anthocyanins; **TAMB:** Total aerobic mesophilic bacteria; **TCC:** Total catechin content; **TPAC:** Total proanthocyanidins; **TPC:** Total phenolic content; **WP:** Wine pomace; **WPPs:** Wine pomace products; **WWPP:** Whole wine pomace product

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TABLES AND ARTWORK

Table 1. Proximate composition of skin wine pomace product (SkWWP), whole wine pomace products (WWPP) and seed wine pomace product (SdWPP).

Parameter	**SkWPP**	**WWPP**	**SdWPP**
Moisture (%)	6.78 ± 0.43 a	7.12 ± 0.25 a,b	7.57 ± 0.09 b
Total dietary fibre (% DM)	48.6 ± 0.7 a	49.4 ± 0.9 a	58.9 ± 0.5 b
Total lipid (% DM)	3.69 ± 0.07 a	10.61 ± 0.18 b	16.99 ± 0.18 c
Total protein (% DM)	14.35 ± 0.81 b	13.09 ± 1.51 a,b	12.04 ± 0.21 a
Ash (% DM)	14.37 ± 0.27 c	10.73 ± 0.13 b	2.94 ± 0.21 a
Mineral matter (mg/g DM)			
Potassium	43.34 ± 2.53 c	38.20 ± 1.26 b	4.39 ± 0.13 a
Calcium	1.82 ± 0.11 a	3.13 ± 0.20 b	3.4 ± 0.27 b
Phosphorous	1.93 ± 0.11 a	2.57 ± 0.18 b	2.75 ± 0.24 b
Sodium	1.31 ± 0.11 c	0.98 ± 0.07 b	0.12 ± 0.02 a

Different letters (a,b,c) denotes significant differences (LSD test and $P<0.05$) between products. Values are means ± standard deviation of three replicate determinations.

Table 2. Phenolic composition of untreated, UV-treated, thermally treated wine pomace products[a].

	Untreated	**UV-treated**	**Thermally treated**
Total polyphenol content (mg gallic acid/g)			
SkWPP	25.87±0.34 b	23.95±0.27 a	24.43±0.15 a
WWPP	32.49±0.26 c	27.13±0.11 a	30.62±0.45 b
SdWPP	42.72±0.79 b	38.59±0.67 a	41.66±0.34 b
Total catechin content (mg D-catechin/g)			
SkWPP	10.52±0.17 c	7.78±0.25 a	8.78±0.22 b
WWPP	18.86±0.49 c	14.16±0.36 a	16.93±0.33 b
SdWPP	33.44±1.29 c	26.22±0.92 a	30.25±0.36 b
Total anthocyanin content (mg of malvidin-3-glucoside/g)			
SkWPP	3.38±0.13 b	3.12±0.27 b	2.47±0.10 a
WWPP	1.42±0.06 b	1.62±0.04 c	1.02±0.10 a
SdWPP	0.18±0.02 b	0.09±0.05 a	0.09±0.02 a
Total proanthocyanidin content (mg of procyanidin B1/g)			
SkWPP	43.45±0.79 c	35.92±1.63 a	39.62±1.03 b
WWPP	56.87±6.23 b	46.90±1.63 a	51.37±1.60 a,b
SdWPP	81.43±11.52 a	77.36±3.33 a	76.67±1.61 a

Different letters (a,b,c) denotes significant differences (LSD test and $P<0.05$) among treatments. SkWPP = Skin wine pomace product, WWPP = Whole wine pomace product, SdWPP = Seed wine pomace product. [a] Values are means ± standard deviation of three replicate determinations.

Table 3. Microbial load of untreated, UV-treated and thermal-treated treated wine pomace products[a]. Results are expressed as log CFU/g of each product.

	Product	Untreated	UV-treated	Thermally treated
Total aerobic mesophilic bacteria	SkWPP	4.65 ± 0.06	3.49 ± 0.17	nd
	WWPP	4.00 ± 0.09	3.95 ± 0.06	nd
	SdWPP	3.27 ± 0.15	3.33 ± 0.08	nd
Yeasts and moulds	SkWPP	3.42 ± 0.09	2.16 ± 0.02	nd
	WWPP	1.75 ± 0.21	1.53 ± 0.09	nd
	SdWPP	1.60 ± 0.43	1.15 ± 0.21	nd

nd: not detected

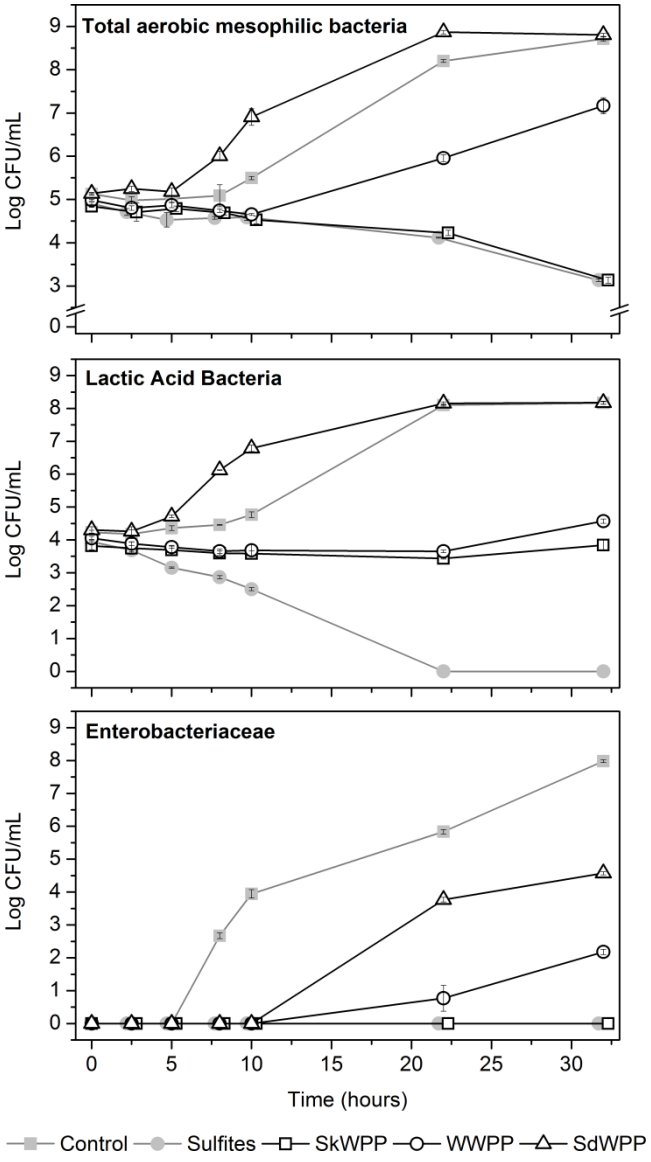
SkWPP = Skin wine pomace product, WWPP = Whole wine pomace product, SdWPP = Seed wine pomace product. [a]Values are means ± standard deviation of three replicates.

Table 4. Protection factor of wine pomace products in olive oil and pork lard measured by Rancimat method [a].

	SkWWP	**WWPP**	**SdWPP**
Olive oil	1.087 ± 0.028 b	1.061 ± 0.026 a,b	1.024 ± 0.016 a
Pork lard	1.145 ± 0.028 b	1.086 ± 0.036 a,b	1.052 ± 0.010 a

Protection factor = induction time of fat with product under study / induction time of control fat. Different letters (a,b) denote significant differences (LSD test and $P<0.05$) among products. SkWPP = Skin wine pomace product, WWPP = Whole wine pomace product, SdWPP = Seed wine pomace product. [a] Values are means ± standard deviation of three replicate determinations.

Figure 1. Effect of incorporation of sulfites, skin wine pomace product (SkWPP), whole wine pomace product (WWPP) and seed wine pomace product (SdWPP) on the total aerobic mesophilic bacteria, lactic acid bacteria and *Enterobacteriaceae* counts of beef homogenates incubated at 37ºC.



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