ABSTRACT

The consumption of natural bioactive compounds, such as dietary fibre and polyphenols, offers health benefits including protection against cardiovascular disease, cancer and other degenerative diseases. Indeed, a relevant challenge for food innovation is the development of foods with optimal dietary fiber and antioxidant contents. One of the recent directions in the cereal sector is the use of flours deriving from other cereals or non grain plants (vegetables and oily seeds) in the baking industry, in order to produce composite flours.

In this context, the potential use of wine-industry by-products (mainly grape-skins and seeds) as a source of dietary fibre and polyphenols in breadmaking, has been investigated in this research. In particular, two grapes (*Vitis vinifera* L.) varieties were considered: Barbera (red wine) and Chardonnay (white wine). The recovery processes of grape skins and seeds were designed to maximize the fibre content while preserving the antioxidant substances. Correlations between the composition of wine industry by-products and their NIR-MIR spectral data, together with the creation of predictive quality model, have been established, and the ability of these models in terms of 'sample classification' was tested. These wine-industry by-products were then adopted at high integration levels (10-30% w/w), for the production of baked goods, such as bread (both by straight-dough and sourdough process) and biscuits. Baked goods, in fact, represent an interesting model system for studying the rheological, macro- and micro-structural effects of fiber fortification on a food matrix, and they are also characterized by a worldwide consumption. They could be therefore a potential vehicle for important amounts of fiber and antioxidants. Positive and encouraging results were obtained from the use of grape-skin powders in breadmaking and biscuit production, highlighting their potential use for the production of innovative and nutritionally convincing baked goods.

RIASSUNTO

L´assunzione di composti bioattivi naturali, quali la fibra alimentare e i polifenoli, offre interessanti benefici alla salute, come la protezione contro malattie cardiovascolari, cancro ed altri disturbi cronico-degenerativi. Un'importante sfida per l'innovazione alimentare è dunque rappresentata dallo sviluppo di alimenti con un contenuto ottimale di fibra e sostanze antiossidanti. In questo contesto, una delle recenti principali direzioni nel settore della panificazione è la miscelazione delle farine di grano con altre farine derivanti da altri cereali o piante di diverso tipo (vegetali o semi oleaginosi), per l'ottenimento di farine composite.

In questo contesto, in questa ricerca è stato valutato il potenziale utilizzo dei sottoprodotti della filiera enologica, rappresentati per lo più da vinacce ottenute da due differenti varietà di uva (*Vitis vinifera* L.; Barbera e Chardonnay), per l´ottenimento di 'ingredienti' ricchi in fibre alimentari e polifenoli da impiegarsi in panificazione. In particolare, i processi di recupero di tali frazioni sono stati progettati per massimizzare il contenuto di fibra e la conservazione delle sostanze antiossidanti. La composizione dei sottoprodotti di lavorazione è stata valutata, oltre che con le tecniche analitiche classiche, anche mediante spettroscopia NIR-MIR, la arrivando a creare dei modelli predittivi della qualità dei sottoprodotti, testandone anche la capacità classificatoria. Tali sottoprodotti sono stati poi impiegati in alte percentuali (10-30% p/p) per lo sviluppo di prodotti da forno 'innovativi', quali pane (ottenuto con metodo diretto e mediante sourdough) e biscotti. I risultati ottenuti sono apparsi molto positivi ed incoraggianti, consentendo di ipotizzare il trasferimento di tali integrazioni anche ad altri prodotti alimentari.

PREFACE

A relevant challenge for food innovation is the development of foods with optimal dietary fiber and antioxidant contents. Scientific studies have demonstrated that a high fiber intake is associated with body weight control and a reduced risk of diseases such as colon cancer, diabetes and atherosclerosis (Bingham et al., 2003; Dana et al., 2005; Slavin, 2005). Not all fibers are equally good: the benefits of a fiber-rich diet greatly depend on the solubility and fermentability of the fiber (Blackwood et al., 2000) as well as on its association with other functional constituents, particularly polyphenols (Tourino et al., 2009). A number of health benefits have also been attributed to natural antioxidants such as phenolics, ascorbic acid, carotenoids, tocols and tocotrienols (Diplock et al., 1998; Hertog et al., 1995).

To this regard, by-products of plants food processing are potential advantageous sources since they combine the presence of large amounts of dietary fibers and natural antioxidants. Large accumulation of these materials during the processing season represents a problem since they are susceptible to microbial spoilage. In addition, the costs of drying, storage and transportation are economically limiting factors. Therefore, these wastes are often used as feeds or fertilizers or burned. Hence, the efficient recovery and re-utilization of these materials in the food chain is becoming more and more important, both to reduce their environmental impact and to exploit their potential health benefits.

Due to the importance of grapes, apples and tomatoes in the Italian production, managing of their processing by-products is of primary importance. Some applications have considered the use of these by-products, exclusively submitted to drying and milling, as food ingredients (Sudha et al., 2007; Calvo et al., 2008). On the other hand, to successfully develop new foods enriched with these materials, it is necessary to carefully evaluate the effects of their incorporation, particularly undesired color variations and micro- and macro-structural changes, which affect consumers' liking. Carotenoids that are present in tomato skins can be used as natural food colorants; however, in some matrices they undergo oxidation and cause color discoloration (Lavelli et al., 2010). Phenolics, that are abundant in apples and grape by-products can oxidize both enzymatically and non-enzymatically, and cause product browning (Lavelli et al., 2011). Enhancing fiber content could be a mean to improve food texture, however it could also lead to an increase in viscosity and a decrease in stability of food microstructure (emulsions, foams or dispersions). By using simplified model systems, it has been demonstrated that thermal (blanching, drying) and mechanical treatments (high-shear treatments and extrusion) can cause significant variation in fiber properties (Redgwell et al., 2005). Grinding can also affect the hydration properties of the same material, depending on the particle size obtained (Raghavendra et al., 2006).

Hence, it can be hypothesized that specifically designed treatments of food processing by-products can modulate and improve their "technological functionality", such as soluble/insoluble dietary fiber ratio, water binding capacity, and emulsifying properties.

Wine-making process generates a substantial volume of solid by-products which are produced in a limited period of the year and have some pollutant characteristics that complicate their management. The possibility of valorizing oenological by-products for the production of high-added value compounds with broad food applications would provide an extra income for wine makers coming from the selling of wastes at a profitable price (while at present the price often does not either cover transportation costs) and, at the same time, reduce the environmental impact of such wastes. During grape processing, in fact, a substantial volume of solid by-products is produced. On average, the pressing of 100kg of grapes originates about 25kg of solid materials consisting in skins (50%), stalks (25%) and seeds (25%).

Scientific works reported in literature and research projects devoted to the utilization of agro-food by-products testify the importance of such issues and reveal the feasibility of extracting high-value components such as phenolic substances, fibers and oils. In order to be realistically implemented at industrial scale, a recovery strategy should be set-up following an integrated approach that finally leads to the development of a system in which the by-products are completely converted to new products/ingredients/additives with economic-efficient and environmental-friendly technologies. Furthermore, recovered products need to have standard characteristics in order to be successful market products. The properties of wine-making by-products, for instance, could be greatly influenced by grape cultivars, vinification processes and climate conditions. It comes that appropriate technologies able to produce standard high-added products starting from multiple and variable raw materials are required.

Currently, grape stalks are generally distributed on fields as fertilizers (without any income for the winery which has instead to pay for transport costs), grape marcs are primarily sold to distilleries for alcohol production, and distilled skins and seeds are used for tartaric acid and oil extraction, respectively, or burned. Distilled products are wastes on their own, and must comply with the Directive 2006/12/EC. The trade value of grape marcs depends on humidity, grape stalks presence and amount of sugars and alcohol, but often the price is neither enough to cover transportation costs.

Globally, different works have shown the feasibility of valorizing the grape processing wastes for the production of common and novel products for other sectors: color and tanning extracts, oils, inks and pigments, antibacterial agents, skin, hair and healthcare products, functional ingredients are just some examples. In fact, grape pomace contains valuable substances which have beneficial health effects such as fibers (17-21%),

tannins (16-27%), polyphenolic compounds (2-6.5%), lipids (7-12%), sugars (3%) and tartaric acid. Particularly, polyphenols (resveratrol, anthocyanins, procyanidins, catechins, and others) but also grape seeds oil and defatted flour have outstanding importance due to their antioxidant and free radical scavenging properties (Rice-Evans et al., 1997; Shrikhande, 2000; Vermerris & Nicholson, 2006).

Stalks, due to their lignocellulosic nature, represent a precious renewable organic source from which it is possible to fractionate the main components - mainly lignin, cellulose and hemicelluloses - by means of chemical/physical and/or enzymatic treatments. Cellulose can be used for fiber production; lignin can be transformed into many chemical agents or hydrolyzed to obtain phenolic compounds to be used as natural antioxidants and microbial growth inhibitors. Hemicelluloses can be purified, by removing phenols, and further hydrolyzed (chemically or enzymatically), to increase the concentration of fermentable sugars, and used for the production of food additives like lactic acid, xylitol, xylose and other compounds (Jimenez et al., 2008; Max et al., 2009).

Grape seeds oil contains a large percentage of unsaturated fatty acids, such as linoleic and oleic acids, and large amounts of tocopherols and tannins. Grape seeds oil finds large applications into the cosmetic, food and pharmaceutical industries for its ability to contrast free-radicals, cardiovascular diseases, cholesterol. It is indicated for human consumption and in particular for infants and elderly people (Vermerris & Nicholson, 2006).

High-added value compounds recovered from food by products have a great market potential for the production of natural additives and functional foods, due to the increasing attention of consumers towards the quality of what they eat, their increasing concern for chemical risks due to food additives and also their increasing demand of functional foods. The term *"functional food"* means a food made using basic nutrients added with ingredients that have an effect on one or more functions of the human organism so that they can improve the general and physical condition or/and decrease the risk of the evolution of diseases. The global functional foods market continues to be a dynamic and growing segment of the food industry, and in 2015 it is expected to represent five percent of the total global food market. At the moment, generally, functional foods are characterized by a higher price compared to their corresponding basic foods, being in this way accessible only to minor population groups. Addition of polyphenolic compounds, fibers, conjugated linoleic acid (CLA) and omega-3 fatty acids has been one of the main diffused strategies for *"functionalisation"*, and it has been traditionally applied to yogurt, due to its nutritional profile and ease of consumption. Only recently, great interest and research have been developed on multi-functional ingredient incorporation into fruit-based products, due to the significant

growth in the fruit juice market (Bazinet et al., 2009; Shemer et al., 2008) and bakery foods, due to their high frequency of consumption (Peng 2010; Wang et al., 2007).

For an industrial scale application of wine by-products recovery strategies, the heterogeneity of processed batches of raw materials by different cultivars might poses serious problems when aiming to obtain high-quality standardized phenolic extracts able to compete with the extracts present on the market but obtained from fresh grapes or wine. The possible advantages related to the obtainment of specific high-added compounds from specific grape cultivars has never been fully investigated and demonstrated, since this approach might comport a huge increase in management and processing costs. Grape cultivars might have a strong influence on chemical and sensorial quality of seeds oil, which has never been investigated, as well.

Regarding food applications, the Europe functional food market (with Germany, France, United Kingdom and Netherlands as the most important countries) takes up the second place after the USA and before the Japanese market. Market and consumers are still demanding for new functional products and Italy should enhance this market in order to gain competitiveness in the European and world market. Economical analysis and process optimization are required for the production of functional ingredients at a low price, in order to enhance the production and diffusion of low-cost health added value available also to low-income populations. Recovery of high added health compounds from by-products has been demonstrated in many literature works and research projects, but their actual employment for the production of commercial ingredients is still missing.

Therefore, besides the optimization of processing technologies to convert by-products into multi-functional ingredients, a complementary research area is that involving the strategy for adding value to these ingredients through the development of novel foods. To this regard, for instance, bread is an interesting model food to study the rheological, macro- and micro-structural effect of fiber fortification (Mariotti, 2006). In addition, due to bread popularity, fortification strategies would be convenient to deliver beneficial compounds for human health to a large number of people.

The general aim of this PhD project was the recovery of fractions rich in antioxidants and fibers from the wine industry by-products. In particular, the recovery processes were designed to maximize the fiber content while preserving the antioxidant substances. Correlations between the chemical parameters of by-products and MIR-NIR spectral data were studied, and predictive models - for their quality assessment - were set up. The use of these fractions in the production of bakery products of high technological and nutritional quality was then assessed.

Specific aims of this PhD research could be, therefore, resumed as follows:

Recovery of grape skin powders (GSPs) from wine industry by-products and their chemical-physical characterization
Development of rapid, non-destructive methods for predictive purposes
Development of innovative methods for increasing the soluble fiber fraction of GSPs
Use of GSPs in the formulation of innovative baked goods.

This research was supported by the *"VALORVITIS"* (*Valorizzazione dei sottoprodotti della filiera vitivinicola per la produzione di composti ad alto valore aggiunto"* Project AGER PROJECT, Grant n. 2010-2222.

REFERENCES

Bazinet, L., Cossec, C., Gaudreau, H., & Desjardins, Y., (2009) Production of a phenolic antioxidant enriched cranberry juice by electrodialysis with filtration membranes. *Journal of Agricultural and Food Chemistry*, 57: 10245–10251

Bingham, S.A., Day, N.E., Luben, R., Ferrari, P., Slimani, N., Norat, T., Clavel-Chapelon, F., Kesse, E., Nieters, A., Boeing, H., Tjnneland, A., Overvad, K., Martinez, C., Dorronsoro, M., Gonzalez, C. A., Key, T.J., Trichopoulou, A., Naska, A., Vineis, P., Tumino, R., Krogh, V., Bueno-de-Mesquita, H.B., Peeters, P.H.M., Berglund, G., Hallmans, G., Lund, E., Skeie, G., Kaaks, R., & Riboli, E. (2003). Dietary fibre in food and protection against colorectal cancer in the European prospective investigation into cancer and nutrition (EPIC): an observational study. *Lancet*, 361:1496–1501.

Blackwood, A.D., Salter, J., Dettmar, P.W., & Chaplin, M.F. (2000). Dietary fibre, physicochemical properties and their relationship to health. *Journal of the Royal Society of Health*, 120: 242-247.

Calvo, M.M., Garcia, M.L., & Selgas, M.D. (2008). Dry fermented sausages enriched with lycopene from tomato peel. *Meat Science*, 80:167–172.

Dana, E.K. (2005). Dietary fiber, inflammation, and cardiovascular disease. *Molecular Nutrition and Food Research*, 49:594-600.

Diplock, A.T., Charleux, J.L., Crozier-Willi, G., Kok, F.J., Rice-Evans, C., Roberfroid, M., Sthal, W., & Vina-Ribes, J. (1998). Functional food science and defence against reactive oxygen species. *British Journal of Nutrition*, 80:S77-S112.

Hertog, M.G.L., Kromhout, D., Aravanis, C., & Blackburn, H. (1995). Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. *Archives of International Medicine*, 155:381–386.

Jimene, L, Rodriguez, A, Pérez, A, Moral, A, & Serrano, L. (2008) Alternative raw materials and pulping process using clean technologies. *Industrial Crops and Products*, 28(1):11-16.

Lavelli, V., & Corti, S. (2011). Phloridzin and other phytochemicals in apple pomace: stability evaluation upon dehydration and storage of dried product. *Food Chemistry*, 129:1578–1583.

Lavelli, V., & Torresani, M. (2011). Modelling the stability of lycopene-rich by-products of tomato processing. *Food Chemistry*, 125:529–535.

Mariotti, M., Lucisano, M., & Pagani, M.A. (2006) Development of a baking procedure for the production of oat-supplemented wheat bread. *International Journal of Food Science and Technology,* 41:151-157.

Max, B, Torradoa, A.M., Moldes, B., Converti, A., & Dominguez, J.M. (2009). Ferulic acid and p-coumaric acid solubilisation by alkaline hydrolysis of the solid residue obtained after

acid prehydrolysis of vine shoot prunings: effect of the hydroxide and pH. *Biochemical Engineering Journal*, 43(2):129-134.

Peng, X., Ma, J., Cheng, K.W., Jiang, Y., Chen, F., & Wang, M. (2010) The effects of grape seed extract fortification on the antioxidant activity and quality attributes of bread. *Food Chemistry.* 119:49-53.

Raghavendra, S.N., Swamy, S.R., Rastogi, N.K., Raghavarao, K.S.M.S., Kumar, S., & Tharanathan, R.N. (2006). Grinding characteristics and hydration properties of coconut residue: a source of dietary fibre. *Journal of Food Engineering*, 72:281–286.

Redgwell, R.J., & Fisher, M. (2005). Dietary fiber as a versatile food component: an industrial perspective. *Molecular Nutrition and Food Research*, 49:421- 535.

Rice-Evans, C., Miller, N.J., & Paganga, G., (1997). Antioxidant properties of phenolic compounds. Trends in Plant Science 2, 152-159.

Shrikhande, A.J. (2000). Wine by-products with health benefits. *Food Research International,* 33:469-474.

Slavin, J.L. (2005). Dietary fiber and body weight. *Nutrition*, 21:411-418.

Sudha, M.L., Baskaran, V., & Leelavathi, K. (2007). Apple pomace as a source of dietary fiber and polyphenols and its effect on the rheological characteristics and cake making. *Food Chemistry*, 104:686–692.

Tourino, S., Fuguet, E., Vinardell, M.P., Cascante, M., & Torres, J.L. (2009). Phenolic metabolites of grape antioxidant dietary fiber in rat urine. *Journal of Agricultural and Food Chemistry*, 57:11418-11426.

Vermerris, W. & Nicholson, R. (2006) Phenolic Compounds Biochemistry. Springer Edition.

Wang, R., Zhou, W., & Isabelle, M. (2007) Comparison study of the effect of green tea extract on the quality of bread by instrumental analysis and sensory evaluation. *Food Research International* 40:470-479.

Yuval Katzir, Eli Budman, Noa Lavid, Yuval Shoham, Michael Shemer, Ronit Shemer (2008). Fruit juice and puree with a lowered amount of available sugars. [Patent] PCT International Patent Application. WO 2008/102336 A2.

1. Recovery and characterization of fibrous fractions from wine industry by-products

1.1 INTRODUCTION

Issues as food availability, food security, natural resources preservation and so on have been further and further argued for the last decades all around the world. Furthermore, the world population is growing and is simultaneously facing an increasing competition for land, water, and energy. For these reasons, one of the main goals for the world is finding the way to produce more efficiently more food and, at the same time, equitably ensuring the preservation of the world resources (Hall et al., 2009; Arvanitoyannisa et al., 2014).

1.1.1 New food ingredients from vegetable by-products

The sectors of citrus processing and wine cover an important part of the food industry, both at an Italian and European level; furthermore, these product lines generate a significant amount of by-products that need to be disposed of (Fig. 1). Although these residues are not per se dangerous, their organic nature and the fact that their mass is in some cases concentrated in certain periods of the year, give rise to problems of great environmental impact. Nevertheless, vegetable by-products are particularly interesting as potential food ingredients thanks to the simultaneous presence of antioxidants and dietary fibers (DF) (Deng et al., 2011; Mussato et al., 2006; Sendra et al., 2010; Sri Harsha et al., 2013).

As reported by Camire et al. (2001), establishing a definition of dietary fiber has been a long process as it depends both on the nutrition knowledge and on the analytical methods adopted, and no international consensus has been reached (Redgwell, & Fischer, 2005). However, the definition proposed by the American Association of Cereal Chemistry (AACC) is often accepted. According to this one, DF can be defined as "*the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated plants substances*". Therefore, in accordance with the AACC definition, all vegetable by-products could be a source of DF, but the structural features of fiber have to be considered, too. In fact, DF promote many beneficial physiological effects, including body weight control, laxation, blood cholesterol and glucose attenuation (Anderson et al., 2009; Camire et al., 2001; Slavin, 2005), that depend on the relative amount of individual fiber contents, mainly in terms of soluble (SDF) and insoluble fractions (IDF), as reported by Elleuch et al. (2011). Furthermore, the same authors underlined the differences in DF technological functionality: the soluble fraction, particularly when compared with the IDF, demonstrates greater capacity to provide viscosity, ability to form gel and to act as emulsifier, together with an easier incorporation in foods and beverages. In addition of the several benefits associated

with high fiber content, the orange peel and pulp and marc are also rich in polyphenols, which have antioxidant properties. Clearly, the origin of the various raw materials (e.g. grape cultivar and citrus variety), as well as the process conditions used (e.g. wine-making and juice preparation), strongly influence the composition of the by-products. For grape pomace, for example, wide ranges of variation in dietary fiber and polyphenols amounts have been evidenced depending on the cultivar (Saura-Calixto, 1998), while for citrus fruits, the varieties, the fruit ripeness and the extraction procedure adopted are the most critical parameters (Marin et al., 2002).

Stability of antioxidants obtained from vegetables is another critical aspect. Wang and Zhou (2004) investigated the stability of green tea catechins in bread-making process founding that green tea catechins were relatively stable in dough during freezing and frozen storage at −20°C for up to 9 weeks and no further detectable losses of tea catechins in bread during a storage of 4 days at room temperature were evidenced. Otherwise, Amendola et al. (2010) compared antioxidants compounds of different vegetable extracts under different storage and usage conditions underlining different stability; in particular, they found that red grape marc extracts were stable and could be potentially used as functional ingredients in low-pH and thermally treated beverages.

Figure 1. Reducing wastes reusing by-products: powders obtained from two vegetable industrial by-products.

1.1.2 Relevance of the recovery technology

Antioxidants are frequently commercialised as solid-liquid extracts (Amendola et al., 2010) to improve their activity. However, when optimizing the extraction of phenolic compounds to obtain solid-liquid extracts many processing variables have to be considered. For example, Spigno et al. (2007) studied the effects of various extraction kinetics and solvents on yield and quality of phenols extracts from grape skins. They underlined that to maximise recovery yield it could be better to work for longer times at 45°C rather than at 60°C, while from an economical point of view it would be advisable to work at 60°C for shorter times (<8 h). It appears evident that the process to obtain polyphenols extracts from plant materials is time-consuming and expensive,

and therefore it has been carried out principally for pharmaceutical and cosmetic applications up to now, and only moderately for foods.

The direct addition of powders or purees in food preparations is more sustainable in terms of process' resources used. For the recovery and stabilization of vegetable by-products, the technology of drying (eventually after a homogenization step at high-shear), followed by grinding and sieving, is often used. The grinding method as well as the extent of grinding has to be defined, as they affect the physicochemical properties of the resulting powder (Chau et al., 2007). On the other hand, depending on the final application of the new ingredients, also the technique of wet grinding to obtain purees (Del Bo' et al., 2012; Lavelli et al., 2008) could be an interesting alternative solution. Sangnark and Noomhorm (2004) evidenced that by means of mechanical treatments, such as stirring, fiber structure changes and its water holding capacity decreases. In addition, Raghavendra et al. (2006) underlined how the particle size is an important aspect to be considered; in fact, for coconut fibers, good hydration properties were found for the medium particle size, whereas for particle sizes below 550μm the hydration decreased. Chen et al. (1988) studied the addition of apple fiber, obtained after juice extraction, both as powder and as hydrated material on traditional bread. The researchers found a general reduction of the loaf volume (lower technological properties) but with a less harmful effect of the hydrated apple fibre, underlining the great importance of the recovery technology. Furthermore, they compared apple fiber with wheat and oat bran, and they evidenced how the use of apple fiber as DF source and humectant in certain foods production was better than the use of cereal fibers, because apple fiber resulted more rich in total DF and with a good water binding capacity.

Therefore, the processing conditions adopted to recover vegetable by-products are critical, as they can modify the technological (e.g. in terms of dispersible properties of powders into food matrices and water binding capacity) and nutritional properties of the new ingredients (e.g. soluble and insoluble fibre contents, total fibre amount, antioxidant properties, vitamins and minerals). Unfortunately, it is not possible to define a general way of by-products recovering, but the optimum technology has to be carefully established from time to time on the basis of the waste type and the food application.

1.2 MATERIALS AND METHODS

1.2.1 Grape skins powders

In order to valorise the typical grapes production of the Italian regions (Piemonte, Lombardia and Trentino) involved in the "VALORVITIS Project", wine industry by-

products were collected from the most important cultivars of the areas. In particular, one red wine grape pomace (**RWGP**) and one white wine grape pomace (**WWGP**) were used in this study, *Vitis vinifera* L. cv. Barbera (**Ba**) and cv. Chardonnay (**Ch**), respectively.

Fresh grape skins samples were sieved (with a 5 mm sieve) to separate the skins from the seeds, and then frozen, to inhibit microbial growth responsible of product degradation (Lavelli et al., 2006). Then, the samples were transported frozen (Fig. 2) to the various laboratories involved in the Project.

At DeFENS laboratories, samples were dried in a oven at 54°C for 48h. Dried grape skins were ground, using a hammer mill, and sieved through an automatic sifter (10min, amplitude 8; Octagon Digital, Endocotts LtD, GB) to obtain 3 different particle sizes: 0.250 ≤ large (**L**) ≤ 0.500mm; 0.125 ≤ medium (**M**) ≤ 0.250mm; small (**S**) ≤ 0.125mm (Fig. 2). Six different grape skins powders (GSPs) were thus obtained: Ba-L, Ba-M, Ba-S; Ch-L, Ch-M, Ch-S.

The powders were fractionated to allow a rapid and homogeneous dispersion in the food matrix and to evaluate the potential influence of particle size on the physical-chemical properties of the sample. The powders were then vacuum packaged (Reepack S.r.l., Seriate, BG, Italy), and stored in the dark at 4°C until characterization.

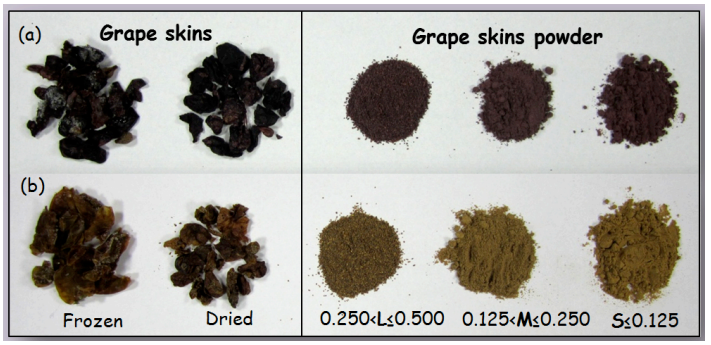

Figure 2. Barbera (a) and Chardonnay (b) grape skins, and related milled fractions (L, large; M, medium; S small; mm)

1.2.2 Chemical-physical evaluations

Samples were characterized partly at DeFENS and partly (when specified) during a research period spent at VTT (Technical Research Center of Finland).

a) Moisture, ash, protein and fat

The moisture content of the various fractions was determined according to the official standard method AACC 44–15A (2000). The total nitrogen content was determined according to the official standard method AOAC 920.87 (1995), and the protein content was calculated adopting 6.25 as a conversion factor. Lipids were determined according to the official standard method ICC n.136 (1992), after extraction using a Soxhlet equipment. Ash content was determined after calcination in a muffle furnace at 550°C for 16 hours. All these analyses were performed at least in duplicate ($n \geq 2$).

b) Dietary fibre

Total dietary fibre (TDF) was determined according to the official standard method AOAC 991.43 (1995) on duplicate samples of dried material. This method determines soluble, insoluble and total dietary fibre content in processed foods.

Samples are heated at ~100°C with heat stable α-amylase to give gelatinisation, hydrolysis and depolymerisation of starch, incubated at 60°C with protease (to solubilise and depolymerise proteins) and amyloglucosidase (to hydrolyse starch fragments to glucose), and treated with ethanol to precipitate soluble fibre (SDF) and remove depolymerised proteins and glucose (from starch). The residue is filtered; washed with 78% ethanol, 95% ethanol, and acetone; dried; and weighed obtaining the insoluble dietary fibre (IDF). One duplicate is analysed for protein and the other is incubated at 525°C to determine ash. The TDF is the weight of the filtered and dried residue less the weight of the protein and ash. DF was measured at least in duplicate ($n \geq 2$).

c) Soluble sugars (VTT)

A representative 0.5g sample of grape skin powder in a 35mL centrifuge tube was extracted 3 times in succession with 25mL of 80% ethanol for 15min at room temperature in an ultrasonic unit (AOAC 994.13, 2007). Each extraction was terminated by centrifugation (10,000×g for 10 min) and collection of the resulting supernatant. The combined supernatants, from the three extractions, were evaporated at 50°C under vacuum by a rotary evaporator, to remove ethanol, and then diluted to 50mL with water. The product obtained all the way here represents '*free sugars*'.

The soluble sugar content was then determined by thoroughly mixing 1mL of extract with 2mL 75% H_2SO_4, and incubating the reaction mixture at 100°C for 15min, then cooling to room temperature.

The free and soluble sugars extracts were diluted in distilled water for obtaining the correct dilution range for the spectrophotometric reading. Subsequently, 1mL of the appropriate diluted sample was added to 1.5mL of DNS reagent (3,5-dinitrosalicylic acid) in a test tube, and the solution was mixed. After incubation for 5min in

vigorously boiling water, the sample was cooled in a cold-water bath. The absorbance was measured at 540 nm. Absorbance values were converted to "*glucose equivalents*" using a calibration curve prepared with a D-(+)-glucose. Results are the average of at least 2 replicates.

d) Total lignin (VTT)

Total lignin (TL) was determined by extracting a dry sample with heptane for 5h in a Soxhlet apparatus. After extraction, the klason lignin (KL) content was determined gravimetrically evaporating the solvent at room temperature (Plus et al., 1985). Afterwards, the acid hydrolysis was applied and the soluble lignin (SL) was measured from the hydrolysate by UV absorbance (Goldschmid, et al., 1971).Results are the average of at least 2 replicates.

e) Solubility (VTT)

A representative amount of GSP (0.2g) was mixed with distilled water (1mL) for 1h at room temperature. After centrifugation (10000×g for 10min), the supernatant residue was collected and dried (overnight in an oven at 105°C) for solubility measure, obtained by weight.

f) Water binding capacity

GSP water binding capacity (WBC) was determined according to Anderson et al. (1969). The sample (1g) was suspended in 25mL distilled water at room temperature in a previously weighed 50mL centrifuge tube, stirred intermittently over a 30min period and then centrifuged at 5200rpm for 10min. After pouring the supernatant into a tared tube, the WBC ($n \geq 2$) was calculated as follows: WBC(%d.b.) = (g H_20/ g sample d.b.).

g) Pasting properties

This analysis was performed by means of a Micro Visco-Amylograph (MVA; Brabender OHG, Duisburg, Germany). An aqueous suspension of 20%GSP (at 14% moisture in 100mL distilled water) was evaluated under constant conditions (stirring: 250 min^{-1}, sensitivity: 300cmg_f) according to the following time-temperature profile: heating from 50°C to 95°C at a uniform rate of temperature increase of 6°C/min; on reaching 95°C, the sample was maintained at this temperature for 5min (first holding period); the paste was then cooled to 50°C at 6°C/min and held at this temperature for 5min (second holding period). The pasting properties of the samples were characterised using the following parameters taken from the curves: beginning of gelatinisation (pasting time, min; pasting temperature, °C), maximum viscosity (Brabender Unit, BU), breakdown (defined as the difference between the maximum viscosity and the viscosity at the end of the first holding period; BU), setback (defined as the difference

between the viscosity at the end of the cooling period and the viscosity at the end of the first holding period; BU).

1.2.3 Soluble phenolic compounds: extraction and quantification

a) Sample extraction

In order to investigate the phenolic compounds, dried and milled grape skins (50g) were extracted with the solvent (absolute ethanol:water:HCl, 60:40:0,5 v/v/v) at a 4:1 (v/wet weight of oven-dried marc) solvent-to-sample ratio under continuous stirring (2 h) on a magnetic stirrer (825rpm) at 60°C, as proposed by Amendola et al. (2010). The liquid extract was separated from the solids by centrifugation (10,000g for 15min; Centrikon T-42K, Kontron Instruments) and the supernatant was used for the phenolic characterization. Extractions were performed in quadruple (n=4).

b) Total phenolic

The Folin–Ciocalteu assay (Ribéreau-Gayon et al., 2000) was performed on the GSPs. The reaction mixture contained 6.0mL of distilled water, 0.5mL of the extracts diluted with ethanol:water:HCl (80:20:0.1, v/v/v) or 0.5mL of the reference products dissolved in methanol, 0.5mL of Folin–Ciocalteu reagent and 3mL of 10% Na_2CO_3. The mixtures were incubated for 90min at room temperature and then the absorbance was recorded at 750nm against a blank with no extract addition. For each extract, 2–4 dilutions were assessed in duplicate. A calibration curve was built using gallic acid. Total phenolics were expressed as g of gallic acid equivalents (GAE) per 100g of dry product. The determination was performed in quadruple (n=4).

c) Total anthocyanins

Total anthocyanins for Ba grape skins were estimated by dilution with acid ethanol (ethanol:water:HCl, 80:20:0.1, v/v/v), reading the absorbance at 540nm. The value was multiplied by the dilution factor and by a conversion coefficient for a mixture of grape anthocyanins (Di Stefano and Cravero, 2001). Total anthocyanins were expressed as g of malvidin (MALE) per 100g of dry product. Results are the average of four replicates (n=4)

d)Tannins

Condensed tannins were estimated by the acid-butanol assay based on the ability of monomer and condensed 3–4 flavandiols to oxidise in acid and alcoholic medium at high temperature to give coloured procyanidins. Briefly, in a glass tube 2mL of the extract were mixed with 6mL of n-butanol:HCl (95:5, v/v) and 0.2 ml of 0.04 mol/l $NH_4Fe(SO_4)_2.12H_2O$ in 2mol/L HCl the iron reagent (150 mg/L of iron (II) sulfate in a

solution of n-BuOH-conc. HCl (50:50, v/v). The tube was hermetically sealed, shaken and the reaction developed within 30min in a boiling water bath. The solution was cooled and the absorbance measured at 550nm. The value was corrected by the blank (a sample prepared in the same way but left for 30min in the dark without heating) and multiplied by 0.1736 (mg/mL) (a conversion factor calculated from a non-commercial grape procyanidins solution) to give the g/100g of cyanidin (CYAE) (Glories, 1978). Results are the average of four replicates (n=4)

e) Ferric ion reducing antioxidant power (FRAP) assay

The FRAP assay was performed on the GSP extracts, according to a procedure described previously (Benzie and Strain, 1996). Briefly, FRAP reagent was prepared by adding 25mL of 300mM acetate buffer, pH 3.6; 2.5 mL of 10mM 2,4,6-tripyridyl-*s*-triazine in 40mM HCl and 2.5mL of 20mM $FeCl_3$. The reaction mixture contained 0.4mL of extracts opportunely diluted with methanol:water:HCl (80:20:0.1, v/v/v) and 3mL of FRAP reagent. The increase in absorbance at 593 nm was evaluated against a blank with no extract addition. For each extract, 2–4 dilutions were assessed in duplicate. Results were expressed as mmol Fe(II) sulphate equivalents per kg of dry product. The antioxidant activities were expressed in Trolox mmol/100g (TEmmol/100g).

1.2.4 GSPs enzymatic processing (VTT)

GSPs were enzimatically processed, aiming to increase their SDF fractions. Four commercial enzyme preparations were used at this purpose: Econase CE (AB Enzymes; Rajamäki, Finland); Pectinex Smash (Novozymes; Bagsvaerd, Denmark); Pectinex (Novozymes; Bagsvaerd, Denmark); Ultra SP-L from. Depol 740L (Biocatalysts; Wales, UK). The activity of each enzyme has been reported in Tab. 1.
For this evaluation, 1.3 g of GSP were weight in a plastic tube (50mL), and added with the enzyme and the buffer (0.05M ammonium acetate, pH=4.0). The tubes were then subjected to a heat treatment (50°Cx24h) under continuous stirring with a magnetic stirrer. Subsequently, the samples were centrifuged at 9500rpm for 10min. The supernatant was recovered and the amount of reducing groups was determined by the DNS method, according to Bernfeld (1955).

Table 1. Specific activity of each enzymes

Enzyme	Activities
Econase CE	Cellulase
Pectinex Smash	Pectinases
Pectinex Ultra SP-L	Pectinases
Depol 740L	Xylanases

1.2.5 Statistical analysis

Experimental data were analyzed by one-way ANOVA using the least significant difference (LSD) as a multiple range test, and by linear regression analyses using Statgraphics 5.1 (STCC Inc.; Rockville, MD). Results are reported as average ± SE.

1.3 RESULTS AND DISCUSSION

1.3.1 Chemical-physical properties of the grape skin powders

Data related to the chemical-physical properties of the various grape skin powders (GSPs) are here reported.

a) Chemical features

The results obtained from the chemical characterization of the samples have been produced in Tab. 2. In general, interesting differences were evidenced both between the two varieties and, within the same variety, among the different particle sizes.
Different moisture levels were evidenced between the two varieties, ranging between 3.44±0.21 and 4.27±0.24 g/100g for Barbera (Ba) and between 6.20±0.09 and 6.44±0.27 g/100g for Chardonnay (Ch).
The protein content was high (around 10 g/100g for both the varieties), and significant differences ($P<0.05$) were evidenced in relation to the different particles sizes. As reported in literature, glutamic acid is the main aminoacid of grape skins proteins, while the limiting ones are lysine, tryptophan and the sulphur containing aminoacids (Ingartuburu et al., 1991; Valiente et al., 1995; Bravo et al., 1998). It has been suggested that the composition and properties of these proteins make them suitable to be used in foods (Ingartuburu et al., 1991). However, some scientific data reported in literature show that these proteins are poorly digestible, which should be kept in mind when considering their potential applications.
The fat content of grape pomaces was quite high, too, even though the highest values correspond to grape seeds. Linoleic acid is usually the main fatty acid, followed by

oleic, palmitic, stearic and myristic acids (Izzo et al., 1993). A significant influence ($P<0.05$) of the particle size was evidenced, for both the varieties. It has to be underlined that fat, in this case, also include waxes from the cuticle and fatty acids derived from the seeds.

Ch was significantly ($P<0.05$) richer in soluble sugars in comparison to Ba: values between 9.19±0.20 and 10.40±0.24 g/100g db were found, in fact, for Ch, while those of Ba were in the range 1.64±0.14 – 1.97±0.18 g/100g db. The high soluble sugar content for Ch was also reported in a previous publication (Deng et al., 2011) and it is given to the different winemaking procedures applied and to the different grape varieties used. In fact, Ch grape skins were obtained right after pressing the juice, while Ba grape skins were obtained after fermenting with juice for several days in order to extract colour pigments and polyphenols. Hence, the unfermented juice remaining on Ch grape skins rendered the considerably higher amount of soluble sugar of this sample. However, it must also be underlined that the soluble sugar content of the Ba grape skins investigated in this study was relatively lower than those of commercial wine in Europe (Bravo & Saura-Calixto, 1998; Baumgärtel, et al., 2007; Llobera & Cañellas, 2007). Data in literature report that the main constituent of the soluble sugar fraction is glucose (Deng et al., 2011).

According to previous studies (Bravo et al., 1998), dietary fiber (DF) was the major component, with a prevalence of the insoluble fraction (insoluble dietary fiber, IDF), if compared to the soluble fraction (SDF). Generally, IDF consists of lignin, cellulose, hemicelluloses and some pectins, while SDF consists of pectins and arabinogalactans. Generally, the smaller the particle size the lower the IDF level and the higher the SDF content. Therefore, the particle size seems to play an important role on the IDF and SDF content of the samples. The ratio SDF:IDF increased when the particle size decreased, in particular for Ba. The same trend was observed for the ash content, having Ba the highest level.

Table 2. Chemical composition of Chardonnay (Ch)) and Barbera (Ba) grape skins powders

	ChL	ChM	ChS	BaL	BaM	BaS
Moisture (%)	$6.20^{a} \pm 0.09$	$6.37^{a} \pm 0.07$	$6.44^{a} \pm 0.27$	$4.27^{b} \pm 0.24$	$3.64^{a} \pm 0.13$	$3.44^{a} \pm 0.21$
Protein (%db)	$9.77^{a} \pm 0.39$	$10.41^{ab} \pm 0.14$	$11.41^{b} \pm 0.27$	$8.23^{a} \pm 0.50$	$9.61^{b} \pm 0.31$	$9.13^{b} \pm 0.10$
Sugar (%db)	$10.40^{b} \pm 0.24$	$9.19^{a} \pm 0.20$	$9.92^{a} \pm 0.14$	$1.76^{a} \pm 0.10$	$1.64^{a} \pm 0.14$	$1.97^{a} \pm 0.18$
Fat (%db)	$4.22^{a} \pm 0.21$	$5.89^{b} \pm 0.28$	$7.32^{c} \pm 0.21$	$4.07^{a} \pm 0.16$	$6.78^{b} \pm 0.21$	$7.71^{c} \pm 0.29$
Soluble dietary fibre (%db)*	$7.70^{a} \pm 0.50$	$9.40^{b} \pm 0.60$	$9.20^{b} \pm 1.10$	$6.50^{a} \pm 1.00$	$12.90^{b} \pm 2.80$	$20.02^{c} \pm 2.30$
Insoluble dietary fibre (%db)*	$44.00^{a} \pm 2.20$	$44.90^{a} \pm 0.30$	$42.80^{a} \pm 3.10$	$53.80^{c} \pm 4.00$	$41.80^{b} \pm 1.20$	$36.10^{a} \pm 3.00$
Ash (%db)	$3.52^{a} \pm 0.24$	$4.33^{a} \pm 0.23$	$5.10^{b} \pm 0.22$	$3.74^{a} \pm 0.35$	$6.92^{b} \pm 0.28$	$8.97^{c} \pm 0.03$
WBC (gH_2O/g db)**	$2.95^{a} \pm 0.09$	$3.28^{a} \pm 0.31$	$2.89^{a} \pm 0.05$	$2.69^{a} \pm 0.07$	$2.60^{a} \pm 0.15$	$2.23^{a} \pm 0.05$

Note: particle sizes: 0.250 < large (L) ≤ 0.500mm; 0.125 < medium (M) ≤ 0.250mm; small (S) < 0.125mm.
In the same raw, within the same GSP variety, values followed by different letters are significantly ($P<0.05$) different.

In order to study more in depth the dietary fibre fractions of the GSPs, further evaluations were performed, during a research period at VTT (Technical Research Centre of Finland). In particular, the lignin content of the GSPs was determined. Lignin is usually quantified as the sum of two fractions: the acid-insoluble lignin (or Klason lignin) and the acid-soluble lignin fraction. The main constituent of DF in GSPs was Klason lignin (KL), for both Ba and Ch, with values ranging from 20.46g/100g db (BaS) to 37.5g/100g db (ChL) (Fig. 2). These results agree with those reported previously by Saura-Calixto et al. (1991) and others (Valiente et al., 1995). The gravimetric residue determined as KL, however, includes other substances such as tannins and proteins. The KL observed in Ch IDF was higher than that in Ba, for all the particle sizes considered. In addition, the large (L) fraction of both the varieties had the higher content of KL, in comparison to the medium and small fractions.

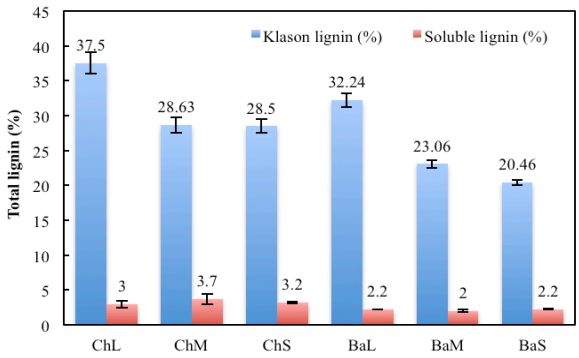

Figure 2. Klason lignin and soluble lignin content of Chardonnay and Barbera grape skin powders, in relation to the various particle sizes.

After KL determination, the soluble lignin (SL) was measured spectrophotometrically from the hydrolyzed samples (Fig. 2). SL was significantly lower ($P<0.05$) than the insoluble lignin. SL values ranged from 3.0 to 3.7g/100g db? for Ch, and from 2.0 to 2.2 g/100g db for Ba. However, according to literature, some other compounds have been reported to solubilize in the analytical conditions adopted for lignin evaluation, causing interferences (Reeves, 1993). Since grape pomaces are rich in these compounds, an accurate lignin value could not be achieved. Nevertheless, even taking into account these interferences, the values for SL were much lower than KL values. Consequently, it can be assumed that the errors due to interferences probably did not affect the analysis.

b) Water solubility, water absorption and pasting properties

Dietary fibres are classified as soluble or insoluble, based on whether they from a solution when mixed with water (soluble) or not (insoluble). Soluble dietary fibres include pectin, gum, mucilage, and some hemicelluloses, whereas cellulose, other types of hemicelluloses and lignin, as previously reported, are included in the insoluble fraction (Davidson & McDonald, 1998; Roehrig, 1988; Schneeman, 1987).
Compared with insoluble dietary fibre, in food processing the soluble fraction, demonstrates greater capacity to provide viscosity, ability to form gels and emulsions, it has neither bad texture nor bad taste, and it is easier to be incorporated into processed foods and drinks.
The hydration properties of DF are related to the chemical structure of the component polysaccharides, as well as to other factors such as porosity, particle size, ionic form, pH, temperature, ionic strength, type of ions in solution and stresses upon fibres. In addition, chemical, mechanical, thermal and enzymatic processings can modify such properties. Partial delignification of lignocelluloses by alkaline hydrogen peroxide treatment, enzymatic treatment and extrusion, for instance, are among the processes that improve the functionality of fibre (Larrauri, 1999).
In particular, mechanical treatments, such as stirring, opens the fibres structure by mechanical shear, making free hydroxyl groups from cellulose available to bind water (Sangnark & Noomhorm, 2004). Grinding can also affect fibre properties: it can damage the regions of potential water binding and, therefore, decrease the capacity to hold water. On the other hand, however, it can improve these properties as a consequence of the increase in the exposed superficial area. Grinding can also increase or decrease the hydration properties of the same material, depending in its particle size.
For all these reasons, GSPs properties such as water solubility, water absorption and pasting properties have been evaluated, too.

The soluble solids of the various samples has been reported in Fig. 3. Ch exhibited higher solubility values, in comparison to Ba, for each particles range. Furthermore, the solubility increased when the particles size of GSPs decreased. In fact, for both the grape varieties, the highest solubility value was evidenced for the 'S' fraction (15.86±0.051 g/100g db for Ch; 10.63±0.261 g/100g db for Ba). This could be probably related to the increased amount of soluble matters (such as SDF) due to the mechanical reduction of particle sizes in the smallest fraction of GSPs.

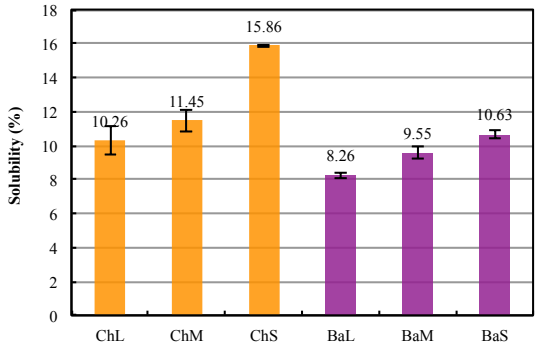

Figure 3. Soluble solids (%) of Chardonnay and Barbera grape skin powders, in relation to the various particle sizes.

The water binding capacity of the various GSPs has been reported in Fig. 4.The results obtained from Chardonnay and Barbera were very similar, independently from the particle size. While for Ba, the ability in binding water decreased when the particle size decreased, it was not possible to observe a clear trend for Ch. In general, these values were close to those normally recorded for wheat bran, but much lower than those generally observed for fibers deriving from other fruits and vegetables (Elleuch et al., 2011). This could be probably due to the fact that the GSPs here investigated were not only made of fiber (Tab. 2).

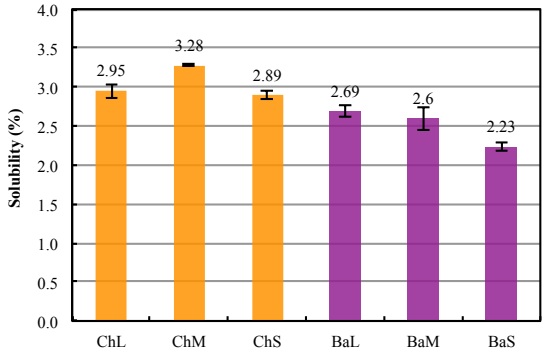

Figure 4. Water binding capacity of Chardonnay and Barbera grape skin powders, in relation to the various particle sizes.

The 'pasting' properties of the various GSPs have been reported in Fig. 5. Viscosity, the resistance to flow, is defined as the ratio of shear stress to shear rate. Most

polysaccharide solutions exhibit non-Newtonian flow and an increased shear rate can increase or decrease viscosity (Sanderson, 1981). Water soluble fibres are the major components that would increase the viscosity of a solution.

Generally, starchy slurries increase their viscosity as the temperature raises, due to starch swelling and rupturing and to the release of amylose outside the granules. Swelling is characterized by an initial phase of slight swelling, a second phase of rapid swelling and a final stage in which maximum swelling of starch granule is reached (Tester & Morrison, 1990). When starch rupture becomes prominent, a decrease of viscosity is observed. Then, on cooling, a further viscosity increase is experienced as the hot paste turns into gel.

The various GSP have been submitted to a conventional time-temperature profile. The resulting profiles have been reported in Fig. 5. Test were performed on 20% GSPs dispersed in water. As GSPs do not contain starch, their viscosity profile is very different from those of a flour. The hydration of the samples went on during the heating and holding phase, and when the temperature started to decrease, an increase in the viscosity profile of some samples was observed. In particular, this was evidenced for BaL, BaM and ChL, where the formation of a gel took place under the adopted conditions. Minimal variations, on the contrary, were highlighted for all the other samples. The fractions of lower sizes exhibited the same phenomenon at higher concentrations. These results are in accordance with the previous findings, since the 'L' fractions were able to absorb the higher amounts of water. In addition, usually there is a positive relationship between the molecular weight of dietary fibers in solution and viscosity: the 'L' fraction could reasonably contain fibers of higher molecular weight.

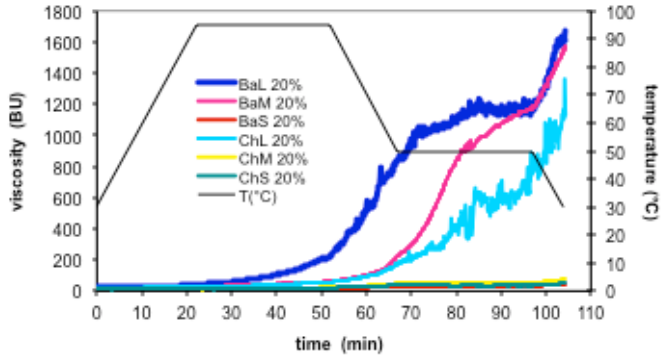

Figure 5. Viscosity profiles of the various grape skin powders

1.3.2 Phenolic compounds in the grape skin powders

Wine phenolics are important quality components that contribute to the colour, taste, and feel of wines. Although phenolic compounds found in wine can also originate from microbial and oak sources, the majority of the phenolic constituents found in wine are grape-derived (Kennedy, 2008). Usually, in white wine, the most important phenolic compounds are the hydroxycinnamic acids and of minor quantities, the flavan-3-ol monomers; in red wine, tannins and anthocyanins are the most important phenolic classes (Kennedy, 2008). Certainly, the phenolic composition of grapes depends on multiple factors, including climate, degree of ripeness, berry size and grape wine variety. Once grapes are harvested, phenolic composition becomes dependent upon processing in the winery.
Results (total phenolics, total anthocyanins, procyanidin and antioxidant activity) related to the various GSPs here investigated have been reported in Fig. 5a.

The total phenolics of the two grapes in skins ranged between 3.58 and 5.54 GAEg/100g, with significant differences ($P<0.05$) among the genotypes. The main differences, as expected, were evidenced between the two GSPs varieties and, to a lower extent, among the various particles fractions. Total soluble polyphenols, in fact, were higher in white grape skins than in red grape skins. This is due to the fact that red grape pomaces were with their juice, during the winemaking process, allowing phenols extraction from the pomace. On the contrary, white grapes were only crushed and pressed to extract juice and no further contact with the juice occurred, therefore the phenolic extraction could not be prolonged.
Because anthocyanins are localized in the skin tissue of most grape cultivars, fermentation and maceration have a profound effect on the amount of anthocyanin present in the final wine. An extreme example of this is the separation of the solid parts of the grape berry from the juice with little or no maceration resulting in a wine with little or no red colour (Kennedy, 2008). This is the reason why no anthocyanins were detected in Ch, while high levels of these components were evidenced in Ba (Fig. 5b).

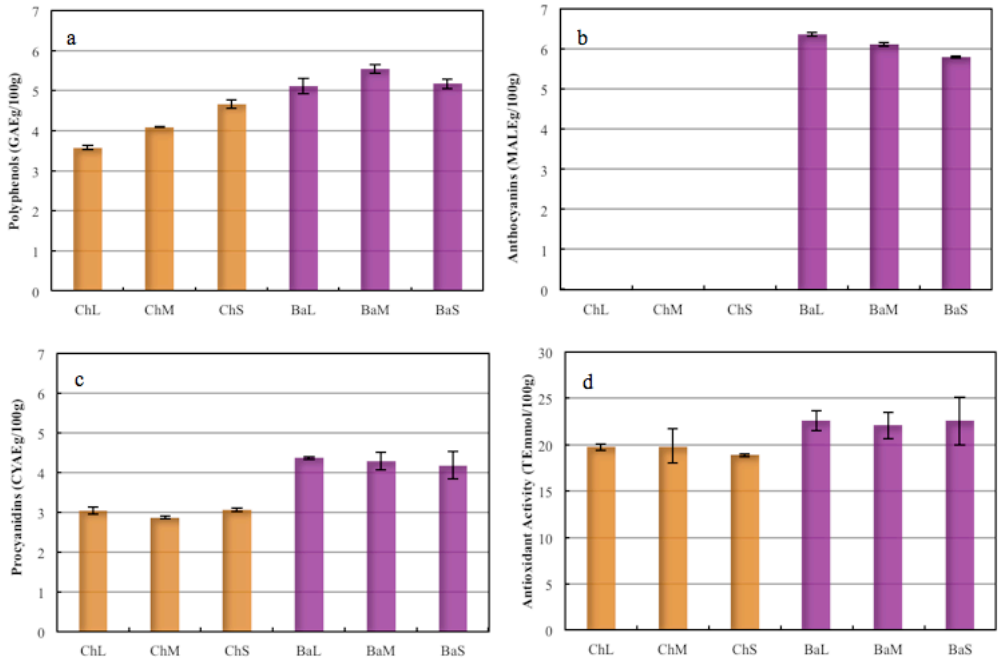

Figure 5. Total polyphenols (a), anthocyanins (b), procyanidins (c) and antioxidant activity (d) of Chardonnay and Barbera grape skin powders, in relation to the various particle sizes.

The content of procyanidin (as condensed tannins; Fig. 5c) was very high in all the samples, the higher values corresponding to the red grape skins, independently from the particles size. It should be pointed out that condensed tannins are also included in the total dietary fibre value.

Generally, a positive relationship between total phenolics and antioxidant activities has been reported previously (Alonso et al., 2002; Borbalàn et al., 2003). Hence, the higher antioxidant activity of Ba would be attributed to its higher values of total phenolics, total anthocyanins, and procyanidins.

Since total phenolic content is an index of potent antioxidant capability (Kiselova et al., 2006), Barbera and Chardonnay GSPs - possessing higher total phenolics - can be good resources as beneficial health materials, whereas wide ranges of grape skins contained lower amounts of procyanidin monomer with no significant differences among the genotypes.

1.3.3 Grape skin powders enzymatic processing

The general purpose in submitting GSPs to enzymatic treatments was the potential increase of the soluble fiber fraction.

Carbohydrates are more resistant to enzymatic hydrolysis; the degree of solubilisation with one-step hydrolysis is only 28–30% (Forssell et al., 2008; Treimo et al., 2009). The resistance of carbohydrates to hydrolytic enzymes is probably due to several factors, including inaccessibility caused by the cross-linking and substitution of the polymers in a complex, lignin-rich matrix. The xylan backbone in cereals is substituted with arabinose, xylose, galactose, glucuronyl and acetyl residues (Collins et al., 2010), which restrict the action of xylanase. Some of the arabinose residues are further substituted with ferulic acid either in mono or dimeric, and higher oligomeric forms. Ferulic acids form diferulate cross links between arabinoxylan molecules and also between arabinoxylan and lignin (Ralph et al., 1995), and such cross linking hinders the access of enzymes to the cell wall polysaccharides (Grabber et al., 1998). In addition, cellulase activity is inhibited by the presence of lignin through the non-specific binding of the enzyme to the polymer (Palonen et al., 2004), and this has been shown to negatively affect the hydrolysis of GSP (Mussatto et al., 2008). Also pre-treatments, that decrease the particle size, and open up the cell wall structures and reduce cellulose crystallinity, make the biomass more accessible to enzymes and thus improve the digestibility (Hendriks & Zeeman, 2009).

Results of the enzymatic treatments performed on the GSPs have been reported in Tab. 3. If the 'control sample' is taken into account, Ch GSPs, as expected, released higher levels of soluble sugars (6.45% - 7.45%), in comparison to Ba (1.52% - 2.07%). The total yield of soluble carbohydrates was increased after the enzymatic treatments for Ba, while it decreased for the variety Ch. For both the varieties, when the GSPs particle size decreased the solubility of carbohydrates decreased as well, and then the use of enzymes has the milling effect. Enzymes Pectinex Smash and Pectinex Ultra (pectinases) had a higher solubilizing effect than Depol (xylanase) and Econase (cellulose), for both the varieties.

Table 3. Solubilisation of GSPs carbohydrates by various enzymatic treatments: Pectinex Smash (pectinase), Pectinex Ultra (pectinase), Depol (xylanase) and Econase (cellulase)

Enzyme	Substrate	ChL	ChM	ChS	BaL	BaM	BaS
		(% of carbohydrates solubilized)					
Pectinex Smash	PG (pH 3,5) 35 000 nkat/ml	6.67 ± 0.15	5.89 ± 0.31	5.71 ± 0.05	3.10 ± 0.06	2.92 ± 0.05	2.59 ± 0.11
Pectinex Ultra	PG 81870 nkat/ml	5.62 ± 1.60	5.81 ± 0.36	5.35 ± 0.17	3.01 ± 0.03	2.39 ± 0.52	2.42 ± 0.05
Depol	KSY pH 5 11837 nkat/ml	4.91 ± 0.40	4.35 ± 0.02	3.96 ± 0.24	2.04 ± 0.16	1.82 ± 0.03	1.79 ± 0.22
Econase	Ksyl pH5 27997 nkat/ml	5.49 ± 0.12	4.86 ± 0.01	5.08 ± 0.47	2.30 ± 0.09	2.04 ± 0.06	1.88 ± 0.12
CONTROL		7.07 ± 0.13	7.45 ± 0.01	6.45 ± 0.15	2.07 ± 0.12	1.52 ± 0.01	1.63 ± 0.10

To be highlighted is the increase in varietal compounds during wine fermentation, possibly attributable to enzymatic hydrolysis by the endogenous enzymes present in the grapes or by the yeast enzymes under the conditions of winemaking. The effect of both these enzyme sources is low, but nevertheless certain other researchers have observed this same increase under the conditions of fermentation (Castro Vazquez, et al., 2002).
Furthermore, the effect of the commercial glycosidic enzymes used for the treatments was to increase the concentrations of most of the components.

1.4 CONCLUSIONS

The first part of this research project was targeted to the recovery of fractions rich in antioxidants and fibers from the wine industry by-products, taking into account typical production of the Italian regions. In particular, the recovery processes were designed to maximize the fiber content while preserving the antioxidant substances. The results obtained provided the baseline data for developing innovative utilizations of GSP.

Overall, the recovered WGSP and RGSP exhibited high contents of antioxidant compounds and dietary fibre, making them excellent candidates for nutraceutical, medical and food applications. They were also good sources of lignin, cellulose, and hemicellulose, thus having great potentials of being environmental-friendly supporting materials. The distinctively high amount of soluble sugar in WGSP could also enable them to form innovative products, for example innovative biodegradable packaging materials with excellent flexibility. Nevertheless, more studies on WGP should be carried out, for instance, to further investigate the differences in chemical compositions due to the different wine making processing, as well as to the cultivars. On the other hand, more efforts should be made to improve WGP preparation procedures, in order to obtain a bright and stable colour, a pleasant aroma, with higher soluble fractions and nutrient compounds.

REFERENCES

AACC (2000). Official Standard Method 10-10B “Optimized Straight-Dough Bread-Baking Method” Official Standard Method “Moisture: Air-Oven Methods” 44-15A. In: Approved Methods of the American Association of Cereal Chemists, 10th Edn. St Paul, MN: American Association of Cereal Chemists.

AACC (2001). American Association of Cereal Chemistry. Dietary Fiber Technical Committee (2001). The definition of dietary fiber. *Cereal Foods World*, 46, 112.

AOAC (1995). Official Standard Method 920.87 “Protein (total) in fluor”. Official Standard Method 991.43 “TotalDietary Fiber in Foods” In: Official Methods of Analysis, 16th Edn. Gaithersburg, MD: Association of Official Analytical Chemists.

AOAC (2007). Official Standard Method 994.13 “Total dietary fiber”. Official Methods of Analysis of AOAC International (17th Ed.). Washington, DC, USA.

Alonso, A.M., Guillèn, D.A., Barroso, C.G., Puertas, B., & Garcìa, A. (2002). Determination of antioxidant activity of wine by-products and its correlation with polyphenolic content. *Journal of Agriculture and Food Chemistry*, 50:5832-5836.

Amendola, D., De Faveri, D. M., & Spigno, G. (2010). Grape marc phenolics: extraction kinetics, quality and stability of extracts. *Journal of Food Engineering*, 97(3):384-392.

Anderson, J.W., Baird, P., Davis Jr., R.H., Ferreri, S., Knudtson, M., Koraym, A., Waters, V., & Williams, C.L. (2009). Health benefits of dietary fiber. *Nutrition Reviews*, 67(4):188-205.

Anderson, R.A., Conway, H.F., Pfeifer, V.F., & Griffin Jr., E.L. (1969). Roll and extrusion cooking of grain sorghum grits. *Cereal Science Today*, 14:372-376.

Arvanitoyannisa, I. S., Kotsanopoulosa, K. V., & Veikou, A. (2014). Life Cycle Assessment (ISO 14040) Implementation in Foods of Animal and Plant Origin: Review. Critical Reviews in Food Science and Nutrition, 54 (10)

Baumgärtel, T., Kluth, H., Epperlein, K. & Rodehutscord, M. (2007). A note on digestibility and energy value for sheep of different grape pomace. *Small Ruminant Research*, 67:302- 306.

Bernfeld, P.(1955). Amylases, α and β. Methods in Enzymology:149-158.

Borbalán A, Ángeles M., et al. "Study of the polyphenol content of red and white grape varieties by liquid chromatography–mass spectrometry and its relationship to antioxidant power." Journal of Chromatography A 1012.1 (2003): 31-38.

Camire, M.E., Cho, S., Craig, S., Devrie, J., Gordon, D., Jones, J., & Tungland, B. (2001). The definition of dietary fiber. *Cereal Foods World*, 46(3):112-124.

Castro Vazquez, L., Perez-Coello, M.S.,& Cabezudo, M.D. (2002). Effect of enzyme treatment and skins extraction on varietal volatiles in Spanish wines made from Chardonney, Muscat, Airen, and macabeo grapes. *Analytica Chimica Acta*, 458:39-44.

Chau, C-F., Wang, Y-T., & Wen Y-L. (2007). Different micronization methods significantly improve the functionality of carrot insoluble fibre. *Food Chemistry*, 100(4):1402-1408.

Chen, H., Rubenthaler, G.L., Leung, H.K, & Baranoski, J.D. (1988). Chemical, physical, and baking properties of apple fiber compared with wheat and oat bran. *Cereal Chemistry*, 65(3):244-247.

Collins, H.M., Burton, R.A., Topping, D.L., Liao, M., Bacic, A., & Fincher, G.B. (2010). Variability in fine structures of noncellulosic cell wall polysaccharides from cereal grains: potential importance in human health and nutrition. *Cereal Chemistry*, 87:272-282.

Davidson, M. H., & McDonald, A. (1998). Fiber: forms and function. *Nutrition Research*, 18:617-624

Del Bo', C., Riso, P., Brambilla, A., Gardana, C., Rizzolo, A., Simonetti, P., Bertolo, G., Klimis- Zacas, D., & Porrini, M. (2012). Blanching improves anthocyanin absorption from ighbush blueberry (accinium corymbosum .) Pur e in ealthy uman olunteers: A Pilot Study. *Journal of Agricultural and Food Chemistry*, 60(36):9298-9304.

Deng, Q., Penner, M. H., & Zhao, Y. (2011). Chemical composition of dietary fiber and polyphenols of five different varieties of wine grape pomace skins. *Food Research International*, 44:2712- 2720.

Di Stefano, R., & Cravero, M.C.(2001). Metodi per lo studio dei polifenoli dell'uva. *Rivista di Viticoltura ed Enologia*, 2:37-45.

Elleuch, M., Bedigian, D., Roiseux, O., Besbes, S., Blecker, C., & Attia, H. (2011). Dietary fibre and fibre-rich by-products of food processing: characterisation, technological functionality and commercial applications: A review. *Food Chemistry*, 124(2):411-421.

Forssell, P., Kontkanen, H., Schols, H.A., Hinz, S., Eijsink, V.G.H., Treimo, J., Robertson, J.A.,

Waldron, K.W., Faulds, C.B., &Buchert, J. (2008). Hydrolysis of brewers' spent grain by carbohydrate degrading enzymes. *Journal of the Institute of Brewing*, 114:306-314.

Glories, Y., 1978. Recherche sur la matière colorante des vins rouges. *Thèse d'ètat de l'Universitè de Bordeaux II*.

Goldschmid, O. (1971). Ultraviolet spectra. In: Lignins: Occurrence, Formation, Structure and Reactions; Sarkanen, K. V., Ludwig, C. H., Eds.; John Wiley & Sons: New York; pp 241−298.

Grabber, J.H., Ralph, J., & Hatfield, R.D. (1998). Ferulate cross-links limit the enzymatic degradation of synthetically lignified primary walls of maize. *Journal of Agricultural and Food Chemistry*,46:2609.2614.

Kennedy, J.A. (2008). Grape and wine phenolics: observations and recent findings. *Ciencia e Investigaciòn Agraria*, 35(2):107-120.

Kiselova, Y., Ivanova, D., Chervenkov, T., Gerova, D., Galunska, B., & Yankova, T.(2006). Correlation between the in vitro antioxidant activity and polyphenol content of aqueous extracts from Bulgarian Herbs. *Phytotherapy Research*, 20:961-965.

Hall, K. D., Guo, J., Dore, M., & Chow, C. C. (2009). The progressive increase of food waste in America and its environmental impact. PLoS One, 4(11), 7940.

Hendriks, A.T.W.M., & Zeeman, G.(2009). Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresource Technology*, 100:10-18.

ICC (1992). Official Standard Method No. 115/1 "Method for using the Brabender Farinograph" Official Standar Method 136 "Cereals and cereal products – Determination of total fat content". In: ICC Standard Methods. Vienna: International Association for Cereal Science and Technology.

Izzo, R., & Muratore, G (1993). Seed lipids from some varieties of grapes grown in Sicily. I. Fatty acid composition. Rivista Italiana delle Sostanze Grasse, 70(12):601-604.

Igartuburu, J.M.,del Rio, R.M., Massanet, G.M., Montiel, J.A., Pando, E., & Rodriguez, L.F. (1991). Study of agricultural by-products. Extractability and amino acid composition of grapeseed (*Vitis vinifera*) proteins. *Journal of the Science of Food and Agriculture*, 54(3):489-493.

Llobera, A. & Cañellas, J. (2007). Dietary fiber content and antioxidant activity of Manto Negro red grape (*Vitis vinifera*): pomace and stem. *Food Chemistry*, 101:659-666.

Larrauri, J.A. (1999). New approaches in the preparation of high dietary fibre powders from fruit by-products. *Trends in Food Science and Technology*, 10:3-8

Lavelli, V., Pagliarini, E., Ambrosoli, R., Minati, J.L., Zanoni, B. (2006). Physicochemical, microbial, and sensory parameters as indices to evaluate the quality of minimally-processed carrots. *Postharvest Biology and Technology*,. 40:34-40.

Lavelli, V., Pompei, C., & Casadei, M.A. (2008). Optimization of color and antioxidant activity of peach and nectarine puree: Scale-up study from pilot to industrial plant. *Journal of Agricultural and Food Chemistry*, 56(16):7091-7099.

Marın, F.R., Martinez, M., Uribesalgo, T., Castillo, S., & Frutos, M.J. (2002). Changes in nutraceutical composition of lemon juices according to different industrial extraction systems. *Food Chemistry*, 78:319-324.

Montealegre, R.R., Peces, R.R., Vozmediano, J.L.C., Gascuena, J.M., & Romero, E.G.(2006). Phenolic compounds in skins and seeds of ten grape *Vitis vinifera* varieties grown in a warm climate. *Journal of Food Composition and Analysis*, 19:687-693.

Mussatto, S.I., Fernandes, M., Milagres, A.M.F., & Roberto, I.C.(2008). Effect of hemicellulose and lignin on enzymatic hydrolysis of cellulose from brewer's spent grain. *Enzyme and Microbial Technology*,43:124-129.

Mussatto, S.I., Dragone, G., & Roberto, I.C. (2006). Brewers' spent grain: generation, characteristics and potential applications. *Journal of Cereal Science*, 43(1):1-14.

Palonen, H., Tjerneld, F., Zacchi, G., Tenkanen, M. (2004). Adsorption of Trichoderma reesei CBH I and EG II and their catalytic domains on steam pretreated softwood and isolated lignin. *Journal of Biotechnology*,. 107:65–72.

Puls, J.. Poutanen, K., Körner, H., & Viikari, L. (1985). Biotechnical utilization of wood carbohydrates after steaming pretreatment. *Applied Microbiology and Biotechnology*,. 22:416.423.

Ralph, J., Grabber, J.H., Hatfield, R.D.(1995). Lignin-ferulate cross-links in grasses: active incorporation of ferulate polysaccharide esters into ryegrass lignins. *Carbohydrate Research*, 275:167-178.

Raghavendra, S.N., Ramachandra Swamy, S.R., Rastogi, N.K, Raghavarao, K.S.M.S., Sourav Kumar, & Tharanathan, R.N.(2006). Grinding characteristics and hydration properties of coconut residue: a source of dietary fiber. *Journal of Food Engineering*, 72(3):281-286.

Redgwell, R.J., & Fischer, M. (2005). Dietary fiber as a versatile food component: an industrial perspective. *Molecular Nutrition & Food Research*, 49(6):521-535.

Reeves, J.B. (1993). Chemical studies on the composition of fiber fractions and lignin determination residues. Journal of Dairy Science, 76:120-128.

Ribéreau-Gayon, P., Glories, Y., Maujean, A., & Dubourdieu, D. (2000). Handbook of Oenology. The chemistry of wine Stabilization and Treatments, vol. 2. John Wiley and Sons Ltd., West Sussex, England.

Roehrig, K.L. (1988). The physiological effects of dietary fiber. *Food Hydrocolloids*, 2:1-18.

Sanderson, G.R. (1981). Polysaccharides in foods. *Food Technology*, 35(1):50-57.

Sangnark, A., & Noomhorm, A. (2004). Chemical, physical and baking properties of dietary fibre prepared from rice straw. *Food Research International*, 37(1):66-74.

Saura-Calixto, F. (1998). Antioxidant dietary fiber product: a new concept and a potential food ingredient. *Journal of Agricultural and Food Chemistry*, 46(10):4303-4306.

Saura-Calixto, F.,GoSi, I., MaSas, E., & Abia, R (1991). Klason lignin, condensed tannins and resistant protein as dietary fibre constituents: determination in grape pomaces. Food Chemistry, 39:299-309.

Schneeman, B.O. (1987). Soluble and insoluble fibre-different physiological responses. *Food Technology*, 41:81-82.

Sendra, E., Kuri, V., Fernández-López, J., Sayas-Barberá, E., Navarro, C., & Pérez-Alvarez, J.A. (2010). Viscoelastic properties of orange fiber enriched yogurt as a function of fiber dose, size and thermal treatment. *LWT-Food Science and Technology*, 43(4):708-714.

Sri Harsha, P.S.C., Gardana, C., Simonetti, P., Spigno, G., & Lavelli, V. (2013). Characterization of phenolics, in vitro reducing capacity and anti-glycation activity of red grape skins recovered from winemaking by-products. *Bioresource Technology,* 140:263-268.

Slavin, J.L. (2005). Dietary fiber and body weight. *Nutrition*, 21(3):411-418.

Spigno, G., Tramelli, L., & De Faveri, D.M. (2007). Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. *Journal of Food Engineering*, 81(1):200-208.

Tester, R.F., & Morrison, W.R. (1990). Swelling and gelatinization of cereal starches. I. Effects of amylopectin, amylose, and lipids. *Cereal Chemistry*, 67(4)_551-557.

Treimo, J., Westereng, B., Horn, S.J., Forssell, P., Robertson, J.A., Faulds, C.B., Waldron, K.W., Buchert, J., & Eijsink, V.G.H. (2009). Enzymatic solubilization of brewers' spent grain by combined action of carbohydrases and peptidases. *Journal of Agricultural and Food Chemistry*, . 57:3316-3324.

Wang, R., & Zhou, W. (2004). Stability of tea catechins in the breadmaking process. *Journal of*

Agricultural and Food Chemistry, 52(26):8224-8229.
Valiente, C., Arrigoni, E., Esteban, R.M., & Amado, R. (1995). Grape pomace as a potentital food fiber. Journal of Food Science, 60(4):818-820.