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Flour Fortification With Grape Must for Nutritional And Health Benefits

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Abstract: Currently, dietary health natural supplements have increasingly used in the prevention and treatment of chronic disorders. Moreover, deficiencies of macro and micronutrients, (vitamins and minerals), and non-nutrients (polyphenolics) have mainly cause several illnesses, especially in children and women worldwide. World organizations have focused great efforts to address these shortcomings and improve the health of the populations. Grape must, or commonly known as grape must juice, becomes a valued ingredient with nutritional and chemical qualities already recognized. Four portuguese grapevine must (Touriga Nacional; Touriga Franca; Tinta Roriz and Vinha Velha) were analysed and results of phytochemical screening revealed that Touriga Nacional grape must contains a number of medicinally active secondary metabolites, with highest total phenolic and flavonoids contents (325.2 mg GAE/L and 218.3 mg CE/L, respectively). In contrast, Tinta Roriz exhibited the lowest values (144.4 mg GAE/L and 18.3 mg CE/L, respectively). Natural pigments were also investigated, with significantly different levels of anthocyanins in all four portuguese grapevine must: Touriga Nacional (23%), Touriga Franca (63%), Tinta Roriz (70%), and Vinha Velha (19%). Apart from the sensory attributes that grape must can impart in commercial flours, the presence of non-nutrient compounds (polyphenols) is an asset in the development of new functional foods, namely in flours for infant formulations, preteens, teens, adult and athletes.

Keywords: Portuguese grapevine must, Phenolics, Natural colorants, Sugar content, Functional ingredient, Flour fortification.

1. INTRODUCTION

Macro and micro nutrients are essential elements to provide the energy and growth, metabolism and maintenance of a healthy body. The deficiencies of these nutrients, mainly vitamins and minerals have resulted in irreparable physical and cognitive *effects*, since these compounds play an important role in the normal functioning of almost all organs (Vinha AF *et al.*, 2021; Sousa C *et al.*, 2019). The WHO (2018) have estimated that billions of people are deficient in several types of nutrients and non-nutrients. Revealing that in several countries, women and children suffer from severe deficiencies caused by inadequate amounts of nutrients, such as vitamins, essential amino acids and minerals, more precisely vitamin A, vitamin D, iron, zinc and probably non-caloric substances identified as non-nutrients (polyphenols, phytosterols, saponins, and phytates) (Ribeiro PVM *et al.*, 2019). For these reasons, many efforts have been made to employ food by-products and/or natural raw materials, chemically enriched. In fact, industrial processing of plant-based food materials generate a lot of by-products including wheat bran (Onipe OO *et al.*, 2015), grape pomace (Sporin M *et al.*, 2018), pineapple pomace (Banerjee S *et al.*, 2018), broccoli by-products (stalk and leaves) (Lafarga T *et al.*, 2019) and oil seed cakes from flaxseed (Wirkijowska A *et al.*, 2020), among others. Most of these plant-based food by-products possess low economic value as they are either mostly fed to livestock (Yang K *et al.*, 2021). So, the large quantity of plant-based food by-products generated by the food industry warrants the need for exploitation of their potential use in new food product development including bread and bakery products with flour. Bread and bakery products are a commonly consumed foods across the globe (Graça C *et al.*, 2019). Thus, bread and bakery products could be targeted as an essential delivery medium for bioactive compounds and essential nutrients present in plant-based food by-products or nautral raw plant materials (Nour V *et al.*, 2015). There is an increased acceptability and demand from consumers for bread and new bakery products produced with flour fortification with beneficial health effect beyond normal nutritional nourishment. Thus, products made with enriched flour are referred to as "functional foods". The incorporation of plant-based food by-products or natural raw materials in bread and bakery products are a viable way of enhancing the nutritional value of those foods improving the nutritional status of consumers.

Vitis vinifera is one of the most important fruit crops worldwide (Grassi F & De Lorenzis G, 2021). According to International Organisation of Vine and Wine (OIV) in the global wine industry, covering approximately 7.5 million hectares in 2019 and producing more than 67 million tons of grapes. Two subspecies are recognised: the wild form, *V. vinifera* subsp. *sylvestris*, and the domesticated one, *V. vinifera* subsp. *vinifera* (or *sativa*). Also, in the European Union (EU), weather conditions favoured a potentially large 2020 harvest; albeit, that has been limited, taking into account the direct and indirect negative impacts of the Covid-19 pandemic on the global wine market. However, it is necessary to state that grapes can be eaten fresh as table grapes or they can be used for several industries, in the production of wine, jam, grape juice, jelly, grape seed extract, raisins, vinegar, and grape seed oil, among others (Spinei M & Oroian M, 2021). Food and Agriculture Organization (FAO) statistics indicate that around 76 000 square kilometers of agricultural land is used for grape production and about 21% of total grape production is for table grapes. Moreover, table grapes consumed in Europe are mainly produced in Mediterranean countries and also countries in the Southern Hemisphere (mainly South Africa, Australia, Argentinia, Brazil, Chile and New Zealand). In the last years, Portuguese wines have grown in quality and exportation, gaining increasing recognition in the international market (Martins I, 2018). In fact, the wide variety of portuguese grape varieties (about 250) allows the production of a diversity of wines, marked by unique characteristics, which enhances its competitiveness in international markets (Martins I, 2018). For these reasons, the viticulture and vinification are very important to wine and food industries, once there are many factors that limit the selection of grape varieties. Also, grape varieties selection is based in their chemical profile, and in other conditions, including geographic, geological, historical, legal, financial and commercial terms. Moreover, the resources and availability and cost of local labour will have a major impact upon the decisions made, including up the grape varieties selection and wine production operations.

Grape Must

Consumer interest in the consumption of health promoting foods is growing worldwide due to the realization of the link between diet and human health. Grape must (from the Latin *vinum mustum*) is freshly crushed grape juice that contains the skins, seeds, and stems of the grape (Kocher GS & Nikhanj P, 2019). The liquid fraction is called grape must or grape must juice and, the solid portion is known as pomace, wich generally may represents 7-23% of the total must weight (Spinei M & Oroian M, 2021). In wine production, grape must is the first product to be obtained, and for this reason it is not considered a food by-product, unlike grape pomace. In fact, from grape pomace it is possible to obtain other by-products, including grape skin, grape seeds and stalks (Figure 1). Despite not being highly valued, the liquid fraction of grape must contains high contents of glucose and frutose, and therefore is commonly used as a sweetener or a natural dye in a variety of cuisines (Khan N *et al.*, 2021).

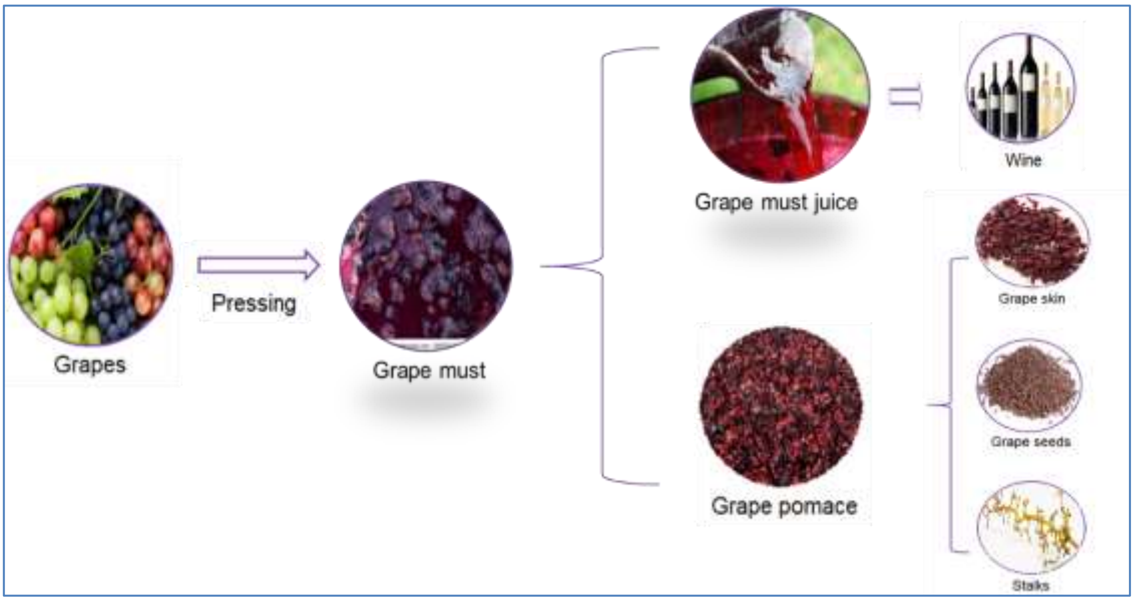

Figure 1. Representative scheme of grape must production and its division into wine and industrial by-products.

Unlike commercially grape juice, which is filtered and pasteurized, grape must juice is thick with particulate matter, opaque, and comes in several shades of brown and purple. Exactly due to grape must is not an industrial processed food, its nutritional and chemical qualities become enriching from the perspective of a valued ingredient and natural preservative for food industry. The phenolic composition in grapes and, consequently, grape must juice is very important, as it allows the determination of the wine color, the evolution of wine and the final wine perception by the consumers. Moreover, in grapes and grape must, polyphenols and quality are closely interconneted in order to provide good qualilty to wines (final product). Indded, these compounds possees a critical role due to their contribution to wine organoletic quality as color, astringency, and bitterness. Also, phenolics content in grapes depend on several factors like variety and maturity level of grapes as well as viticulture practices. Regading chemical composition of grape must, it may be argued that it is identical to grape fruits. Phenolics in grape and grape must are generally classified into two main groups: flavonoids and nonflavonoids. The major flavonoids include flavan-3-ols, flavonols, and anthocyanins. Flavonoids, such as anthocyanins, are principally localized in the skins, while the flavan-3-ols are present in pulp, skins and seeds. The nonflavonoids enclose phenolic acids and stilbenes.

The red grape varieties present higher content of flavonoids than white grape varieties, mainly anthocyanins which accumulate in the skin and pulp of red berries, usually present as glucoside derivatives of delphinidin, cyanidin, petunidin, peonidin and malvidin (Fournand D *et al.*, 2006). Flavanols like catechin, epicatechin, epicatechin-gallate and epigallocatechin are also present in fruit pulp, skins and seeds as free monomers and polymeric forms, which are called proanthocyanidins or tannins (Watrelot AA & Norton EL, 2020). However, the composition and concentration of phenolics in grapes may vary with variety, botanical species, viticultural and environmental factors, like soil conditions, climate, and crop load (Berrueta LA *et al.*, 2020; Soares S *et al.*, 2017). Regarding nonflavonoids composition, stilbenes are present in grapes and grape must, mainly the resveratrol and piceid (resveratrol glucosides) in *cis* and *trans* isomeric forms. In addition to biological properties, stilbenes have proved to be good discriminants of the grape variety (Ragusa A *et al.*, 2019; Ragusa A *et al.*, 2017) and grape species (Magdas DA *et al.*, 2019).

As a matter of fact, the interest on the natural antioxidants from natural resources has recently increased, especially phenolics such as quercetin, carnosol, thymol, catechin, anthocyanins and resveratrol, due these compounds present great interest as dietary supplements or food preservatives.

In view of the above, the aim of this study was to value the grape must as a possible natural ingredient in the development of new enriched food products, not only in a chemical but also in a sensory perspective. Free sugars content, total phenolic, total flavonoids, and total anthocyanins contents in four red grapevine cultivars must juice (Touriga Nacional; Touriga Franca; Tinta Roriz; Vinha Velha) were analysed. In fact, as far as we know, this is the first work to be described in these Portuguese varieties.

2. MATERIALS AND METHODS

2.1. Materials

The used water was treated in a Milli-Q purification system (Millipore, Bedford, MA, USA) to obtain ultra-pure water. Chemicals and reagents were of analytical grade. Sodium carbonate, absolute ethanol, hydrochloric acid and sodium hydroxide were obtained from Merck (Darmstadt, Germany). *Folin-Ciocalteu′*s phenol reagent, gallic acid, catechin, sodium nitrite, and aluminum chloride were acquired from SigmaAldrich (St. Louis, USA).

Four red grape varieties (Touriga Nacional, Touriga Franca, Tinta Roriz, Vinha Velha) grown in Carrazeda de Ansiães (North region and sub-region of Douro, Portugal) were harvested between the end- August to the mid-September 2020, transported to Sociedade Agrícola Trigo de Negreiros Lda, a certified wine industry. The grape must juices (samples) were collected before the beginning of wine production, ie free alcoholic fermentation. Sugar levels ranged from 25.3 to 30.4° Brix at harvest.

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2.2. Phytochemicals Analysis

2.2.1. Free sugars Content

Liquid chromatography (LC) with refractive index (RI) detection is the most common technique used in sugar analysis and quantification. In this study, sugars were determined using an HPLC (Hewlett Packard Series 1050, Hewlett Packard GmbH, Waldbronn, Germany). The high-performance liquid chromatograph using a refractive index was used to analyse glucose and fructose of four grapevine varieties must. Sample preparation and chromatographic procedure were followed as described in AOAC. Samples were injected directly after filtration. Chromatographic condition: Detector: Hewlett-Packard refractive index detector-HP 1047 A RI detector (Hewlett Packard HP 1047, Tokyo, Japan); Column: HPLC carbohydrate analysis column-(BIO-RAD) aminex HPX-87 ºC carbohydrate column (300 mm × 7.8 mm) (Catalog 125-0095); Mobile phase: 100 % ultra-distilled water (obtained from Millipore Synergy-185, quality of water is 18.2 MΩ cm); Flow rate: 0.6 mL/min; Column compartment: 85 ºC; Injection volume: 20 µL.

2.2.2. Total Phenolics Content

Total phenolics content was determined according to the Folin-Ciocalteau assay described by Costa ASG et al. (2018) with minor modifications. 1 mL of each grape must were mixed with 5 mL of *Folin-Ciocalteu′*s phenol reagent (1:10) and 20 mL of sodium carbonate (Na_2CO_3) 7.5% (m/V). Solutions were incubated at room temperature for 30 min, protected from light. Absorbances were measured at 750 nm in a UV-vis spectrophotomete (Serie 4200 Generic Laboratory, UK), and gallic acid was used as a standard. A calibration curve (y= 0.0104 x + 0.0008; R^2=0.9976) was used to determine the total phenolic contents of grape must samples. Results are expressed in gallic acid equivalents (GAE mg/L fresh weight [fw]).

2.2.3. Total flavonoids content

Total flavonoids content was evaluated by a spectrometry assay according to Costa ASG et al. (2014). Therefore, 1 mL of grape must jiuce was mixed with 4 mL of deionized water and 300 µL of sodium nitrite ($NaNO_2$) 5% (m/V). After 5 min at room temperature, 300 µL of aluminium chloride ($AlCl_3$) 10% (m/V) were added to the previous mixture. After incubation at room temperature (1 min), 2 mL of sodium hydroxide (NaOH) 1 M, and 2.5 mL of deionized water were added. The absorbances were measured at 510 nm in a UV-vis spectrophotomete (Serie 4200 Generic Laboratory, UK), and a catechin calibration curve was prepared (y= 0.0012 x + 0.0128; R^2=0.9951). Total flavonoids contents are expressed in mg catechin equivalents/L of fw (mg CE/L).

2.2.4. Total anthocyanins content

As a powerful antioxidant and natural colorant, anthocyanins are being used increasingly as a component of food supplements and nutraceutical products. Their quantification was based in a spectrometry assay, based by preparing two solutions with different pH values. Breafly, two solutions of the same grape must were prepared: i) 1 mL of sample + 1 mL of ethanol with 0.1% concentrated HCl + 10 mL of a buffer solution at pH 3.5; ii) 1 mL of sample + 1 mL of ethanol with 0.1% concentrated HCl + 10 mL of 2% HCl (pH ≅ 0.6). Absorbances (Abs) were measured a 520 nm, and total anthocyanins content was calculated by the following formula: Total anthocyanin compounds (mg/L) = 400 (Abs_{ii}-Abs_i).

2.3. Statistical analysis

Statistical analysis was performed using the program SPSS V.21.0® (SPSS Inc., Chicago, IL). ANOVA was used to assess significant changes in the dependent variables. The level of significance for all hypothesis tests (*p*) was 0.05.

3. RESULTS AND DISCUSSION

The search for food products that promote health benefits has grown in the last years. Phenolic compounds present in grapes and in their derivatives, such as grape must, grape juices, and grape pomace represent a wide range of research, given the benefits that these compounds present on the human health (Merkyté V *et al.*, 2020). Grape must can be produced from any grape variety once it has attained appropriate maturity. However, only in traditional wine producing regions, grape must juices are produced from *Vitis vinifera* grape varieties [21]. Further, grapes are one of the richest sources of phenolic compounds among fruits. Therefore, grape juices have been broadly studied due to their composition in phenolic compounds and their potential beneficial effects on human health, specifically the ability to prevent various diseases associated with oxidative stress, including cancers, cardiovascular and neurodegenerative diseases [22-25]. Therefore, our results will address grape must phenolic composition, with a special focus on the potential beneficial effects on human health and on the sensory impact as na ingredient for other foods, as well as free sugars content, sine grape must juice can also be used as natural sweetener in bakery products.

Quantitative analysis (g/100 mL) of major carbohydrates, along with the ratio glucose/ fructose in the four Portuguese grapevines must varieties are presented in Table 1. In this study, interparietal comparison of results showed that the glucose variables were significantly different. Among analysed samples, glucose content of all grapevines must varieties varied from 5.91 % (Vinha Velha) to 12.21 % (Touriga Nacional). Glucose content of Touriga Franca was 10.69% and Tinta Roriz presented 6.50%. Sugar accumulation, especially the concentration of high level of fructose, is a very important physiological process that determines the dessert fruit quality. The mean glucose value of grape varieties was determined as 8.83%. Regarding the fructose content in the analysed grapevine must varieties was ranged between 5.93 % (Tinta Roriz) and 12.66 % (Touriga Nacional). Vinha Velha and Touriga Franca grapevines must were found as the lowest fructose content. The mean fructose content was determined as 9.07% in all four different grapevines must varieties. No additional literature is found regarding the glucose and fructose contents of these Portuguese grape varieties, therefore, the total sugar content of some varieties were compared.

Table 1. Amounts of free sugars: glucose, fructose, and sucrose contents (%) presented in four Portuguese grapevines must varieties.

Grape must varieties	Glucose (%)	Frutose (%)	Sucrose (%)	Glucose/Frutose
Touriga Nacional	12.21^{aA}	12.24^{aA}	nd	0.99
Touriga Franca	10.69^{bA}	10.71^{bA}	nd	0.99
Tinta Roriz	6.50^{cA}	7.26^{cB}	nd	0.90
Vinha Velha	5.91^{dA}	6.06^{dB}	nd	0.97

nd-not detected. Means followed by different lower superscripts in same column for different grape cultivar were significantly different at p = 0.05. Means followed by different capital letter within the columns for the same grape cultivar were significantly different at p = 0.05.

The total phenolic and flavonoids contents of four Portuguese red grapevine cultivars must are shown in Table 2.

Table 2. Total phenolic and flavonoids contents of four Portuguese red grapevine cultivars must.

Grapevine cultivars	**Total phenolics (mg GAE/L)**	**Total flavonoids (mg CE/L)**
Touriga Nacional	325.2 ± 7.3^{aA}	218.3 ± 6.6^{aB}
Touriga Franca	195.4 ± 5.1^{bA}	35.0 ± 0.7^{bB}
Tinta Roriz	144.4 ± 3.5^{cA}	18.3 ± 8.3^{cB}
Vinha Velha	243.1 ± 5.6^{dA}	123.9 ± 1.7^{dB}

Means followed by different lower superscripts in same column for different grape cultivar were significantly different at $p = 0.05$. Means followed by different capital letter within the columns for the same grape cultivar were significantly different at $p = 0.05$.

The comparison of phenolic profiles of different grape cultivars and, consequently grape must, is the most studied application regarding phenolics as chemical markers. In fact, the most important classes of flavonoids that have been applied as chemical markers are anthocyanins, flavonols, and flavan-3-ols (Merkyté V *et al.,* 2020; Cosme F *et al.,* 2018). Regarding the results, total phenolic contents of all red grape varieties must were higher than total flavonoids content, revealing significant differences between them ($p<0.05$). Among the grape varieties, the highest content of total phenolics (325.2 mg GAE/L) was found in Touriga Nacional, being Tinta Roriz the cultivar with the lowest amount (144.4 mg GAE/L). The same trend, although with lower levels, was observed in flavonoids content. In fact, Touriga Nacional presented the highest amount and Tinta Roriz exhibited the lowest (218.3 mg CE/L and 18.3 mg CE/L, respectively). Our results cannot be compared with other identical studies, since there are no studies with Portuguese varieties. However, it is known that in the grape berries, the phenolic compounds are distributed in the different parts of the fruit and, consequently, the phenolic compounds present in grape must juice are mainly those ones extracted from the grape skins and pulp, and to a lesser extent, those extracted from the grape seeds (Terral JF *et al.,* 2010). Pastrana-Bonilla E et al. (2003) found a total amount of phenolic compounds of 2178.8, 374.6 and 23.8 mg/g GAE (gallic acid equivalent) in skin, pulp, and seed, respectively. According to Pandey KB & Rizvi SI (2009) thereabout 100 mg of polyphenols are identified in a cup of coffee or tea or a glass of red wine. Also, resveratrol (3,5,4′-trihydroxystilbene) being a natural polyphenol with a stilbene structure, may be quantify as a total phenolic compound. Although resveratrol can be found in other fruits, which are part of the human diet, such as blueberries (*Vaccinium spp*.), blackberries (*Morus spp*.), and peanuts (*Arachis hypogaea*), the grapes, grape products and red wine are the main source of resveratrol in the Mediterranean diet (Kim Y *et al.,* 2016). As mentioned above, total phenolics and flavonoids depend on the grape variety, grape maturity, geographical origin and soil type, sunlight exposure, and many other factors (Garrido J & Borges F, 2013) besides the grape must juice processing technology, such as grape must juice extraction, contact time between juice and the grape solid parts (skins and seeds). Also, the thermal treatment of intact or crushed grapes enhances the release of phenolic compounds, as a consequence of both the increase mass transfer and higher solubility of cell components. Ordinarily, the temperatures commonly used in the extraction process are superior than 60°C for different times, according to the processing technology. This factor may influence the phytochemical composition present in grape must, once flavonoids are heat-sensitive polyphenolic compounds, being reported that heat treatment can induce their thermal breakdown and oxidation. This reason may support our results, since the levels of flavonoids found in our samples are much lower than the content of total phenolics. Accoding to Celotti E & Rebecca S (1998) the combination of time/temperature affects the extraction yield of the phenolic compounds, according to the molecular type; therefore, at 55 °C, tannin extraction is favoured over red pigment extraction, but with higher temperatures (63°C), the maximum extraction of anthocyanins (red pigments) occurs after 20 min. Given the importance of anthocyanin extraction during grape must production, the adequate combination of skin contact time and temperature treatment during maceration is crucial to achieving grape must organoleptic characteristics and chemical composition. Given the importance of organoleptic and beneficial characteristics that natural pigments can provide, total anthocyanins were analysed (Table 3).

Table 3. Total anthocyanins content obtained from four portuguese red grapevine cultivars must.

Grapevine cultivars	Total anthocyanins content (mg/L)
Touriga Nacional	50.3 ± 0.2^{a}
Touriga Franca	22.1 ± 0.9^{b}
Tinta Roriz	12.8 ± 0.6^{c}
Vinha Velha	23.7 ± 0.8^{b}

Means followed by different lower superscripts in same column for different grape cultivar were significantly different at $p = 0.05$.

Results confirm the same trend as in the previous results. Touriga Nacional was the grape must with the highest anthocyanins content (50.3 mg/L) and Tinta Roriz presented the lowest content (12.8 mg L). No significant differences were found in Touriga Franca and Vinha Velha (p>0.05). Despite results show a low concentration of total flavonoids, the anthocyanins percentage prove to be interesting: Touriga Nacional (23%), Touriga Franca (63%), Tinta Roriz (70%) and Vinha Velha (19%). In this sense, grape must can be considered as a functional ingredient with attractive organoleptic properties. According to Vinha AF et al. (2018), various types of colorants (natural and synthetic) are available in the market as coloring agents to food commodities. However, consumer behavior is increasingly demanding and natural colorants are now gaining popularity and considerable significance due to consumer awareness, as synthetic ones may cause severe health problems.The fingerprint composed by phenolic acids, flavonoids, tannins, and stilbenes can be used for the classification on the basis of mainly local grape variety in terms of producing country. Moreover, these fingerprint may be useful to select grape varieties and, consequently, optimize the ideal grape must juice to be used as valued ingredient for the development of new food products, including a new flour formulation.

Flour formulation with grape must

Nowadays, food industrial processing of plant-based food materials is related with by-products and/or undervalued products that may have a negative impact on the environment but could add value to bread-based products (Rauf A *et al.*, 2018). The chemical composition and related biological properties of plant-based food by-products improve the properties of bread, as well as bioavailability/bioaccessibility leading to potential health effects in human nutrition. Taking this into account and, through the chemical analysis of the grape must, in this experimental work, an attempt was made to start a pilot study by adding grape must to wheat flour. As already mentioned, bioactive compounds (phenolic acids, flavonoids, tannins, stilbenes, lignins, and coumarin) promote health benefits such as preventing the start of oxidative stress (Rauf A *et al.*, 2018; Imran M et al., 2017), reducing inflammatory markers (Champ CE & Kundu-Champ A, 2019; Imran M et al., 2017; Pinheiro LC *et al.*, 2017), evidencing antidiabetic properties through glycaemic response attenuation (Rauf A *et al.*, 2018), and exhibiting cardiovascular protective effects (Rauf A *et al.*, 2018; Pinheiro LC *et al.*, 2017) demonstrated through *in vitro* and human clinical trials. Besides their established biological properties, several phenolic compounds may exhibit significant antibacterial activity. Several polyphenols present activity against foodborne pathogenic or food-spoiling bacterial strains. For instance, resveratrol revealed activity against *P. aeruginosa*, *B. subtilis*, *S. aureus*, *E. coli*, *S. enteritidis* (Bouarab-Chibane L *et al.*, 2019). Also, epigallocatechin gallate and caffeic acid exhibited antibacterial activity against *E. coli*, *L. monocytogenes*, *S. aureus* and *B. subtilis*. Among the Gram-negative bacteria, *S. enteritidis* was the bacterial strain most affected by the phenolic compounds (Bouarab-Chibane L *et al.*, 2019). Consequently, phenolics can potentially promote health and improve the well-being of consumers following their consumption and, at the same time, be natural food preservatives. Moreover, in this work, as a pilot study, two cakes were developed. A traditional homemade cake recipe was selected and two cakes were elaborated (traditional cake and a cake containing flour added with grape must). Sugar was not added but replaced by grape must (Touriga Nacional), and results are shown in Figure 2.

Figure 2. Traditional wheat flour cake and wheat flour cake enriched with Touriga Nacional grape must.

From Figure 2, it becomes evident that the incorporation of grape must significantly affects the sensory characteristics of a tradicional wheat flour cake. Besides the addition of the grape must to the wheat flour, and consequent change in the cake appearance, another relevant aspect focuses on the absence of addition of common sugar (sucrose). As previously mentioned, grape must contains high contents of glucose and frutose, both natural sugars. An important issue in this respect is the effect of sugars on postprandial glucose and insulin responses (Gomez M & Martinez MM, 2018). The glycaemic index values of hundreds of foods, including glucose, sucrose and fructose, have already been determined, showing that many starchy foods produce higher glycaemic responses than sucrose, and that fructose bring out a lower glycaemic response than most other foods (Foster-Powel I & Miller JB, 1995).

The development of food products using composite flour has increased and is attracting much attention from researchers, especially in the production of bakery products and pastries. Apart from being a good source of calories and other nutrients, wheat flour is nutritionally poor, for example, in essential amino acids such as lysine and threonine (Martins ZE *et al.,* 2017). Grape must proves to be interesting as an ingredient enriching wheat flour for its nutritional richness, phytochemical and sensory properties. In the future, more studies need to be carried out, in order to optimize the amount of grape must to be added, as well as in the preparation of different food products, which should be subjected to a sensory analysis.

4. CONCLUSION

Flour fortification has the potential to improve the nutritional status of all population, regardless of the social class, as they are a staple food widely distributed and consumed worldwide, and, this fortification does not require changes in existing food patterns of populations, being a very cost-effective method. It is also more efficient in reducing the risk of multiple deficiencies that can result from deficits in food supply or a poor diet. It is a major advantage primarily for women of childbearing age, during periods of pregnancy and lactation (increasing the rate of vitamins and minerals in breast milk and reducing the use of supplements) that need adequate amounts of micronutrients, as well as growing children, which need nutrients daily for growth and developmen. It is also of great interesnt to all the athletes, particularly high-competiton athletes. The importance of each class of phenolics, flavonoids and anthocyanins to wine quality has been known for a long time. Anthocyanins are pigments responsible for red wine colour, and their contribution could be direct, due to their spectral properties, or indirect after copigmentation reaction with other phenolic compounds, such as flavonols which are present in minor quantities with respect to other flavonoids. Despite the grape must not be a food by-product, this can be used not only by the wine industry but it can also be an excellent food ingredient. Its chemical and organoleptic properties show that grape must can be incorporated into food product formulations, like flour and pasta. The results of the phytochemical assays indicate that Portuguese grape cultivars must juice have a great potential to be exploited by the grape processing industry and other foods industries. Moreover, it is also usually possible to add grapevine must without adding significantly to the total cost of the flour at the point of manufacture. In general, flours are a potential vehicle for fortification, because of their high consumption worldwide. Nevertheless, the success of flour fortification is based on the correct evaluation of the prevalence of micronutrient deficiency, political opinions and their implementation, selection of fortifiers, levels of fortification, usual level of flour consumption and products derived from that staple food, fortification of other food vehicles, feasibility, cost and acceptability studies. So, further studies focused on the chemical, biochemical and pharmacological properties of the components in the different grape varieties are needed to clarify the beneficial effects of these functional ingredient on human health.

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