

BIOLOGICALLY ACTIVE COMPOUNDS CONTAINED IN GRAPE POMACE

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ABSTRACT

A healthy lifestyle and gastronomic trends based on traditional and local foods accompanied by waste-free technologies are currently in the primary focus. One of the raw materials with properties in alignment with such requirements is grape pomace. This paper evaluates the antioxidant activity of grape pomace (which is homogenized into a brown powder) and selected commonly available commercial flours – wheat bread, rye plain, and rye whole grain flour – using DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) and total polyphenol content method, where was used Folin-Ciocalteure agent and then it was determined by spectrophotometric method (the measure of absorbance). The total amount of polyphenols in grape pomace was measured of 47.94 mg GAE.g⁻¹, but the value 0.27 mg GAE.g⁻¹ was measured in wheat bread flour. Grape pomace performed the antioxidant activity of 57.45 mg AAE.g⁻¹, whereas wheat bread flour of only 0.21 mg AAE.g⁻¹. Compared to selected commercial flours, the total amount of polyphenols in grape pomace was up to 150 times higher and the ratio of antioxidant activity between grape pomace and wheat bread flour was even more than 280 times higher. This makes it possible to fortify commercial, commonly available flours with different amount of grape pomace so that products with a higher amount of biologically active substances can be prepared. Another benefit could be a reduction in health risks and a contribution to improving consumer health.

Keywords: grape pomace; polyphenolic compounds; antioxidant activity; flour

INTRODUCTION

The interest in healthy and balanced nutrition has been increasing. The society is striving to find suitable food beneficial to the human body – a diet rich in vitamins and minerals. The WHO recommends 600 g of fruit and vegetable, including cooked vegetables, as a daily intake (WHO, 2013). The ratio between fruit and vegetable should be 1:2 (The Czech Society for Nutrition, 2012). Unfortunately, in many European countries, their income is lower. However, fruit and vegetable are rich in biologically active substances (Juriková et al., 2016). These include antioxidants, such as vitamins C, E, provitamin A, minerals with antioxidant properties, polyphenols, tannins, and other groups of substances that can reduce health risks and contribute to health enhancement (Salter, Wiseman and Tucker, 2012; Tomás-Barberán and Gil, 2008). One source of these substances may be grapevine and products from it (Katalinić et al., 2010; Kim et al., 2011; Liang et al., 2014; Stockham et al., 2013).

Grapevine is one of the best known and most widespread fruits in the world (Yang et al., 2009; Yang, Martinson and Liu, 2009; Soto, Brown and Ross, 2012) and includes four species: the European type (*Vitis vinifera* L.),

American grape type (*Vitis labrusca* L.), Muscadine type (*Vitis rotundifolia* Michx.) and Amurensis (*Vitis amurensis*). Out of these, the European type has spread worldwide and accounts for 71% of total grape production in the wine industry (Yang, Martinson and Liu, 2009). An example of producing of grapes can be mentioned in Brazil, where the production was approximately 534 million kg in the year 2009. This number increased by 24%, which means that the number was 709 million kg two years later (in the year 2011). Logically, when the production of grapes increased, automatically the amount of grape pomace increased as well (Casagrande et al., 2019). Grapes serve as a suitable source of phenolic compounds with antioxidant effects, such as anthocyanins, flavonoids, and resveratrol (Yang, Martinson and Liu, 2009; Hernández-Salinas et al., 2015).

It is a fruit consumed all over the world. It can be eaten in a form of fresh table fruit and also in a form of diverse processed products including wine, juice (Kim et al., 2017), grape flour, or grape pomace. Grape pomace, dried and homogenized grape seeds and skin, is a by-product of wine production applied for further processing. It can be used as a substrate to produce alcoholic beverages due to sufficient sugar content. The most famous liquor prepared

from grape pomace is Italian Grappa. In France, brandy originated from grape pomace is known as Marc, in Spain it is named De orujo and in Portugal Bagaceira (Piras, 2008; Gasnier, 2005). Furthermore, the inedible components such as seeds and skins can be used as a food additive, feed, fertilizer, seed source for oil production, or fuel. Therefore, grape pomace is considered to be a favorable alternative product allowing waste-free management. Grape pomace is rich in polyphenols, especially flavonoids such as gallic acid, catechin, and epicatechin (Özvural and Vural, 2014).

Polyphenolic compounds are plant secondary metabolites present in plant foods and they can affect human health (Ziauddeen et al., 2019; Pinto and Santos, 2017; Zehiroglu and Sarikaya, 2019). It is a complex group of phytochemical compounds ranging from simple phenols and phenolic acids to high molecular weight polymer structures (e.g. hydrolyzed and condensed tannins) (Ziauddeen et al., 2019; Zamora-Ros et al., 2016). From a chemical point of view, it is a diverse and heterogeneous group of organic compounds (Llobera and Cañellas, 2007). Their distribution in different parts of plants is diverse. Commonly, they are present in outer layers of fibrous plant material; insoluble substances in the cell walls, and soluble in vacuoles (Pandey and Rizvi, 2009). The number of phenolics which has been found in plants is over 8000. The particular profile of polyphenols depends also on specific cultivar within a species. An example can be given grape varieties where the composition can depend on factors, such as geographical region, soil composition, or terroir. This is the reason why are different polyphenolic compositions but also the success of growing specific species of wine (Bakota et al., 2015).

As a by-product, grape pomace is an inexpensive source of polyphenolic antioxidants (Bakota et al., 2015) attracting growing interest as it offers a wide range of usable products in the food, cosmetic and pharmaceutical industries (Milella et al., 2019). Several phenolic compounds have a role in preventing lifestyle diseases, such as cardiovascular diseases, diabetes mellitus type 2, neurodegeneration, and certain types of cancer (Bončíková et al., 2012; Ziauddeen et al., 2019).

The process of obtaining polyphenols from fruit or vegetable is usually extraction. It can be for example Soxhlet extraction, maceration, or hydrostillation. Nowadays there are also new non-conventional methods, due to the effort to use a method that is environmentally friendly to compare to the classical method. An example can be ultrasound, microwave, and pressure-assisted extractions (it can be applied alone or together with various solvents for the sake of reducing solvent requirements and the energy) (Milella et al., 2019).

Furthermore, apart from phenolic compounds with antioxidant effects, grape pomace contains other antioxidants neutralizing free radicals. In the human body, free radicals may harm compounds altering cell membranes, cause cell damage and cell inflammation, promote abnormal cell growth including certain types of cancer and even cause cell death. Free radicals are formed in the body in natural metabolic processes. In their formation, exogenous factors, including ultraviolet light,

radiation, fumes, and air pollution, play an important role (Krishnaswamy et al., 2013). Antioxidants, comprising phenolic compounds, flavonoids, and carotenoids, can reduce the risk of oxidative damage by converting free radicals into inactive molecules (Štípek et al., 2000).

These beneficial properties of grape pomace are in accordance with current nutritional trends emphasizing the application of traditional, local and organic food in compliance with waste-free management (Burg, Masan and Ludin, 2017; Makris et al., 2008).

Due to the fear of the unfavorable effects and the safety of synthetic antioxidants, the studies started to be more interested in studies that were focused on natural products. Namely, it was for example herbs, vegetables, fruits, or agro-industrial waste. The reason for attention on these products is because they are rich in polyphenols and they can be used instead of synthetic antioxidants that are used in the pharmaceutical, food, or cosmetic industry (Casagrande et al., 2019).

It is assumed that polyphenol content is about 60 – 70% in grape seeds, 30% in the skin, and merely 6% in the flesh (Kim et al., 2017).

Homogenized grape pomace has reached the market and allows fortification of common commercial flours or their complete replacement. The purpose of fortification is to increase the content of biologically active substances. First, it is necessary to identify the original content of biologically active substances in the individual basic materials.

This paper aims to compare polyphenolic substances in grape pomace and their antioxidant effects with commercial, commonly available flours on the market.

Scientific hypothesis

Hypothesis No. 1: The total content of polyphenolic substances and antioxidants in grape pomace is higher than in the commercial flours.

MATERIAL AND METHODOLOGY

Material for analysis

The analysis employed material samples from grape pomace (Figure 1), wheat bread flour, rye flour, and rye wholemeal flour. A presented grape pomace sample came from viticulture Ludwig, vineyard track "Zbavce", village Němčičky, region Velkopavlovická, area Moravia. It was homogenized from grapes of the Riesling variety which was manually harvested in October 2018. Next, the grapes were processed using a time-driven horizontal press. Drying and grinding were performed at 70 °C. The grinding was performed until the grape pomace reached similar size as the flours which were bought for the analysis.

The flours were bought in a supermarket as a commonly available commercial material:

– wheat bread flour – wheat flour "Babiččina volba", GoodMills Česko inc., Prague, the Czech Republic, with the following nutritional values per 100 g: energy 1461 kJ/345 kcal, fats 1.7 g, of which saturated fatty acids 0.2 g, carbohydrates 69 g, of which sugar 2.0 g, fiber 3.1 g, proteins 12 g, salt <0.01 g;



Figure 1 The grape pomace sample.

– rye plain flour – rye plain dark flour “Babiččina volba” GoodMills Česko inc., Prague, the Czech Republic, with the following nutritional values per 100 g: energy 1435 kJ/339 kcal, fats 1.0 g of which saturated fatty acids 0.2 g, carbohydrates 70 g, of which sugars 6.0 g, fiber 9.0 g, proteins 8.0 g, salt <0.01 g;

– rye wholemeal flour – “Pernerka” rye wholemeal fine flour, Mlýn Perner, Svijany, the Czech Republic, with the following nutritional values per 100 g: energy value 1449 kJ/342 kcal, fats 1.3 g of which saturated fatty acids 0.13 g, carbohydrates 72.1 g, of which sugars 1.8 g, proteins 6.1 g, salt 0 g.

An extract (5 g of sample extracted in 50 mL of methanol (MERCI, s.r.o., Brno, the Czech Republic)) was prepared from all flour samples and used for further determination.

Total phenolic content analysis

To determine the total phenolic content (TPC) Folin-Ciocalteu agent (Sigma Aldrich, the Czech Republic) was used. The extract of the amount of 0.1 mL was mixed with water in a 10 mL volumetric flask. Then, 0.5 mL of Folin-Ciocalteu agent and 1.5 mL of 20% solution of Na_2CO_3 (Sigma Aldrich, the Czech Republic) were added to the solution. Specord 50 Plus (Analytik Jena, Jena, Germany) was used to measure absorbance at a wavelength of 765 nm. Pure water was used as a reference (Thaipong et al., 2006). To express the results, unit milligrams of Gallic acid (GAE) per grams of fresh mass (FM) were used. TPC was measured four times.

Total antioxidant capacity analysis

Total antioxidant capacity (TAC) was established applying DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) (Sigma Aldrich, the Czech Republic) assay in accordance with the study by Brand-Williams, Cuvelier, and Berset (1995).

The stock solution was prepared by the solution of 24 mg of DPPH in 100 mL of methanol (MERCI, s.r.o., Brno, the Czech Republic) which was stored at $-20\text{ }^\circ\text{C}$ until it was needed. The absorbance of DPPH radical without any added extract was recorded and corrected every day. The sample solution used for measuring was prepared by

mixing 10 mL of the stock solution with 45 mL of methanol to obtain the absorbance of 1.1 ± 0.02 units at 515 nm using spectrophotometer Specord 50 Plus (Analytik Jena, Jena, Germany). The extract (210 μL) was allowed to react with a 4 mL DPPH solution for 1 hour in the dark. Then, the absorbance was recorded at a wavelength of 515 nm. TAC was measured three times.

For the calculation of antioxidant capacity, a decrease of the absorbance value was used following the formula:

$$\text{Antioxidant capacity (\%)} = (A_0 - A_i/A_0) \times 100,$$

where A_0 is the absorbance of a blank without the sample and A_i is the absorbance of the mixture containing the sample. The calculated antioxidant capacity was converted using a calibration curve of the standard and expressed in ascorbic acid equivalents (AAE) (Vasanth Rupasinghe, Jayasankar and Lay, 2006).

Statistical analysis

Excel 2013 (Microsoft Corporation, USA) and STATISTICA CZ version 12 (StatSoft, Inc., USA) were used for data analysis. The results were expressed by mean \pm standard deviation. The comparison of TPC and total antioxidant activity (TAA) of grape pomace with those of flour samples were calculated by non-parametric tests – Kruskal-Wallis test Wald-Wolfowitz test, Kolmogorov-Smirnov test and Mann-Whitney U Test ($\alpha = 0.05$).

RESULTS AND DISCUSSION

The comparison of total polyphenol content of grape pomace and selected commercial, commonly available flours are shown in Table 1. The lowest value of polyphenols was established in wheat bread flour with a value of $0.27\text{ mg}\cdot\text{g}^{-1}$. Rye flour showed a slightly higher value in both plain and whole grain flour. The total amount of polyphenols in grape pomace was measured to be 150 times higher, namely $47.94\text{ mg}\cdot\text{g}^{-1}$. This indicates a statistically significant difference between grape pomace and commercial flours.

Due to the non-compliance with the prerequisites for the ANOVA type statistical evaluation, the Kruskal-Wallis test based only on the order of the individual samples had to be applied to compare obtained data as can be seen in Table 2. For this reason, a statistically significant difference was observed only between grape pomace and wheat bread flour, although the measured values indicated a statistically significant difference between grape pomace and all the commercial flours. In case that only two individual samples are compared, there was a statistically significant difference ($p < 0.05$) between all flour samples both using non-parametric tests (Wald-Wolfowitz test, Kolmogorov-Smirnov test, Mann-Whitney U Test) and using the parametric Welch t-test. However, the homogeneity condition is not met in this test.

Published studies devoted to the issue of this study have revealed a wide range of values. For instance, Costa et al. (2019) declared the value of $4.7 \pm 0.0\text{ mg GAE}\cdot\text{g}^{-1}$ for grape pomace of red wine from Brazil. In contrast, Theagarajan et al. (2019) established the total content of polyphenols in dry compacts from ripe red grapes of the Muscat variety grown in India at the amount of $280.6 \pm 3\text{ mg GAE}\cdot\text{g}^{-1}$. Chamorro et al. (2012) determined the total content of extractable polyphenols in grape seeds

from France of 296 ± 9 mg GAE.g⁻¹ in dry material and 23.6 ± 0.8 mg GAE.g⁻¹ in grape pomace originated from Spain. Similar values of total polyphenol content as found in this study are documented in the article by **Casagrande et al. (2019)** in the range of 17.91 to 35.10 mg GAE.g⁻¹. Considering grape seeds from different cultivars, **Sung and Lee (2010)** reported polyphenol levels varying from 7.92 mg GAE.g⁻¹ to 43.69 mg GAE.g⁻¹. **Rockenbach et al. (2011)** measured the total phenolic content in various wines and the measured range is 32.62 – 74.75 mg GAE.g⁻¹.

In a comparison of different types of flour, **Li et al. (2015)** measured different coloured wheat, where the values were in the range of 0.51 to 0.66 mg GAE.g⁻¹. **Ragae, Abdel-Aal and Noaman (2006)** state that in wheat flours and whole grain cereals is the range of 0.50 to 4.22 mg GAE.g⁻¹, which is similar to the value of wheat flour which was measured in this paper, and again lower than the values for grape pomace.

For gluten-free flours, in the paper **Rocchetti et al. (2019)** the values were similar too – the total polyphenolic content was in the range 0.52 to 5.00 mg GAE.g⁻¹. **Alvarez-Jubete et al. (2010)** measured the polyphenols of seeds of amaranth, quinoa, buckwheat, and wheat in the range 0.21 – 3.23 mg GAE.g⁻¹ dry-weight basis.

Interesting results for comparing were also analyzed in **Ky and Teissedre (2015)** where was measured total phenol content in grape pomace seed and skin extracts. The measured amount was 128.22 – 215.93 mg GAE g⁻¹ of dry weight in grape seed pomace extract and 71.88 – 196.71 mg GAE g⁻¹ of dry weight in grape pomace skin extracts when for the measuring was used the extraction method which is appropriate for the preparation of edible extracts.

For comparison, there are some studies where were measured the amounts of polyphenols in wines, where the unit was in mg GAE.L⁻¹. In the study **Boussenna et al. (2016)** three different grape pomace extracts were used. The amount of polyphenols was in the range of 140 to 400 mg GAE g⁻¹ grape pomace extract. **Snopek et al. (2018)** were analyzing wine where the values for white wine were 203.06 – 678.78 mg GAEL.L⁻¹ and for red wine 905.21 – 1349.12 mg GAEL.L⁻¹. The average amount of measured wine in **Bajčan et al. (2016)** was 2,424 mg GAEL.L⁻¹. **Bajčan, Čéryová and Tomáš (2012)** measured range 1579 – 2734 mg GAEL.L⁻¹ in Blaufränkisch. The range of wine from various Slovak vineyard areas which was measured by **Bajčan et al. (2015)** was 2064 – 4274 mg GAEL.L⁻¹. In the study **Lapčíková, Lapčík and Hupková (2017)** was measured the range 549.44 to 2832.78 mg GAEL.L⁻¹ for a variety of wines. The higher values were measured by **Garaguso and Nardini (2015)**, where the mean was 4417 mg GAEL.L⁻¹ for organic wines and 4225 mg GAEL.L⁻¹ for conventional wines. **Faitová et al. (2004)** measured the amount of total polyphenolic content in twelve bottles of Traminer, and the values were in the range 257.7 – 282.5 mg GAEL.L⁻¹. The study **Ivanova-Petropulos et al. (2015)** is showing that the differences can be seen in the same country (Macedonia) but different regions, where the values were measured in the range of 1394 to 3097 mg.L⁻¹. The wide range is shown in **Fanzone et al. (2012)** where were used thirty red

Table 1 Total Polyphenol Content in individual samples.

Flour	Total Polyphenol Content	
	<i>M</i> (mg GAE.g ⁻¹)	<i>SD</i> (mg GAE.g ⁻¹)
wheat bread	0.27	0.01
rye plain	0.40	0.01
rye wholemeal	0.51	0.01
grape pomace	47.94	0.11

Note: *M* – mean, *SD* – standard deviation.

Table 2 Total phenolic content (TPC) in individual analysed samples – Multiple comparison of *p* values.

Flour	wheat bread	rye plain	rye wholemeal	grape pomace
wheat bread		<i>p</i> >0.05	<i>p</i> >0.05	<i>p</i> <0.05
rye plain	<i>p</i> >0.05		<i>p</i> >0.05	<i>p</i> >0.05
rye wholemeal	<i>p</i> >0.05	<i>p</i> >0.05		<i>p</i> >0.05
grape pomace	<i>p</i> <0.05	<i>p</i> >0.05	<i>p</i> >0.05	

Table 3 Total antioxidant activity (TAA) in individual analysed samples.

Flour	Total antioxidant activity	
	<i>M</i> (mg AAE.g ⁻¹)	<i>SD</i> (mg AAE.g ⁻¹)
wheat bread	0.21	0.06
rye plain	0.41	0.06
rye wholemeal	0.63	0.03
grape pomace	57.45	0.42

Note: *M* – mean, *SD* – standard deviation.

Table 4 Total antioxidant activity (TAC) in individual samples – Multiple comparison of *p* values.

Flour	wheat bread	rye plain	rye wholemeal	grape pomace
wheat bread		<i>p</i> >0.05	<i>p</i> >0.05	<i>p</i> <0.05
rye plain	<i>p</i> >0.05		<i>p</i> >0.05	<i>p</i> >0.05
rye wholemeal	<i>p</i> >0.05	<i>p</i> >0.05		<i>p</i> >0.05
grape pomace	<i>p</i> <0.05	<i>p</i> >0.05	<i>p</i> >0.05	

wines from the 2010 vintage, the values were 1585.6 – 4203.2 mg.L⁻¹.

Kondrashov et al. (2009) measured different varieties of Merlot and Cabernet wine, and the total phenolic content was in the range from 1447 to 2414 mg GAEL.L⁻¹. **Burin et al. (2010)** measured Cabernet as well and the results of their measurements are in the range of 1750.9 – 2424.1 mg GAEL.L⁻¹. **Yoo et al. (2011)** reported wide range, namely 1417.14 – 3588.57 mg GAEL.L⁻¹ for Cabernet Sauvignon and 1181.43 – 3288.57 mg GAEL.L⁻¹

for Shiraz wines. **Paixão et al. (2007)** compared five red wines, one rosé wine and five white wines and the result was in the range 282 – 1936 mg GAE.L⁻¹. As can be seen, there are differences between wine (the liquid product of grapes) as well, which shows the variety of polyphenols which can be seen in the different types of grapes, that were grown in the different conditions.

The amount of TPC is affected by a number of factors, so the results presented in this article may be different compared to the others. The content of polyphenols can be affected by environmental factors such as soil type, sunlight, climate, etc., TPC is further affected by storage, during which polyphenols oxidize easily. Concentrations of phenolic acids generally decrease during maturation, while concentrations of anthocyanins increase. Another parameter that affects the content of polyphenols is culinary treatment – grinding of plant tissues degrades polyphenols and maceration can increase the content of polyphenols due to diffusion in the juice (**Manach et al., 2004; Pandey and Rizvi, 2009**).

Table 3 summarizes antioxidant activities which were up to 280 times higher in grape pomace than in other commercial flours. Grape pomace performed the antioxidant activity of 57.45 AAE mg.g⁻¹, whereas wheat bread flour of only 0.21 AAE mg.g⁻¹. Samples of rye flour differed from wheat bread flour by only 0.20 AAE mg.g⁻¹, which is a negligible difference if compared to grape pomace.

Similarly to the determination of the total amount of polyphenols, it was not possible to apply the ANOVA test. Therefore, a comparison of the obtained data was performed using the Kruskal-Wallis test as displayed in Table 4. The results follow the same trend as for total polyphenols content. As with the TPC determination, non-parametric tests (Wald-Wolfowitz test, Kolmogorov-Smirnov test, Mann-Whitney U test) and parametric Welch t-test between individual pairs of samples were performed again. There was a statistically significant difference ($p < 0.05$) between all pairs in all tests.

Concerning grape seeds from different cultivars, **Sung and Lee (2010)** reported antioxidant activity ranging from 28.2 ("Agawam") to 121.2 ("Cabernet Sauvignon") $\mu\text{mol Trolox.g}^{-1}$. **Costa et al. (2019)** documented $160.9 \pm 22.3 \mu\text{mol Trolox.g}^{-1}$ in unprocessed grape seeds and $105.5 \pm 2.0 \mu\text{mol Trolox.g}^{-1}$ in defatted grape seeds. Grapes pomace samples of Château Roslane (Meknes, Morocco) were employed in the study by **Aziz et al. (2019)** publishing the values ranging from 0.12 ± 0.0 to $0.23 \pm 0.0 \text{ mmol Trolox.g}^{-1}$ dry weight.

Even though more studies are examining this issue, data comparison is very problematic as different methodologies and their modifications, different grape varieties, and sample processing techniques of TPC and TAA determination were applied. It is essential to consider specifics of biological material that may be influenced by the ambient conditions (climate, temperature, sunshine), cultivation technologies (soil fertilization, harvest time, harvesting method), and by post-harvest processing and storage.

For future practical applications, it would be appropriate to monitor TAA and TPC in the mixtures of common commercial meals and grape pomace in various ratios to enable fortification of traditional flour with a small

percentage of grape flour. Furthermore, it is necessary to identify specific characteristics of such mixtures, including sensory properties and leavening, and subsequently, to examine properties in baked goods, such as shelf life, to distinguish their mutual influences.

CONCLUSION

This research has examined the content of antioxidants and polyphenolic substances in grape pomace which were gained from the local farmer and it was thoroughly compared with three different commercials, commonly available flours, where two of them were from one company and the last one was from the different one for more valuable comparison. It evaluates differences in existing products and products containing non-traditional alternatives to commercial ones. The results indicate that grape pomace performed magnitude higher values of both polyphenolic contents and antioxidant properties than commercial flours.

As this study has revealed, grape pomace provides a diverse array of applications in the food industry; it plays a role as a food additive improving sensory and technological properties and works as a dietary supplement reducing the risk of civilization diseases including cardiovascular diseases, diabetes mellitus, and obesity. Newly, further advantageous attributes still required to investigate more is the possibility to employ grape pomace in gluten-free and lactose-free products.

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