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THE CHEMICAL COMPOSITION OF TWO KINDS OF GRAPE JUICE WITH MEDICINAL PLANT ADDITION

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ABSTRACT
The safety of plant-based food with an herbal origin is a priority for producers and final consumers these days. The interest in the high biological value of the final food products enriched with herbal ingredients is rising. We focused on the study of physico-chemical composition and antioxidant activity of two kinds of grape juice with medicinal plant addition in our study. We used 2 varieties of grapes - Welschriesling and Cabernet Sauvignon, six species of medicinal plants - *Calendula officinalis* L., *Ginkgo biloba*, *Thymus serpyllum*, *Matricaria recutita*, *Salvia officinalis* L., and *Mentha aquatica* var. *citrata* in our experiment. There were14 samples prepared, two of them were control samples and 12 samples were treated with medicinal plants. We tested each of the selected parameters triplicate with an interval of one week. We evaluated the results statistically in 4 levels of significance p <0,01, p <0,001, p <0,0001 and p <0,00001. The content of fructose, glucose, dry matter, density, malic acid, pH, potential alcohol, total acids, and total sugars in the treated samples was significantly lower compared to the control sample, which was probably due to the degree of dilution of grape juice with extracts gained from medicinal plants. The antioxidant effect was demonstrably higher in the samples enriched with medicinal plants than in the control samples. The highest antioxidant effect was measured in the second test in the samples with the addition of *Thymus serpyllum* (80.93 % - white grape must, 82.33 % - blue grape must), *Calendula officinalis* L. (79.29 % - white grape must, 80.49 % - blue grape must) and *Ginkgo biloba* (79.10 % - white grape must, 83.3 % - blue grape must). Generally, we found out that the selected medicinal plants increase the biological quality of grape juice.

Keywords: grape juice; *Calendula officinalis* L.; *Ginkgo biloba*; *Thymus serpyllum*; *Matricaria recutita*; *Salvia officinalis* L.; *Mentha aquatica* var. *citrate*; chemical parameters

INTRODUCTION
Grapes have a high level of moisture and sugar in their fresh form and these aspects can lead to their deterioration **(Farias et al., 2021)**. The grapes belong to fragile fruits therefore the degree of their damage during harvest and the distribution chain is very high **(Huwei et al., 2021)**. In addition, grape berries are sensitive to pathogenic infections, which result in significant economic losses **(Zhao et al., 2021)**. Grape juice mainly consists of water and several metabolites with an emphasis on sugars, organic acids, minerals, phenolic and aromatic compounds **(Dutra et al., 2021)**. Organic acids and sugars are associated with the chemical balance of grape juice and they also have a significant effect on taste balance and sensory properties **(Coelho et al., 2018)**.

Grape juice can be generally divided into fresh, concentrated, and reconstituted juice. Fresh grape juice is undiluted and unfermented pure juice made from the pulp of fresh grapes. Concentrated grape juice is a product that has undergone a physical process to remove water. Reconstituted grape juice is juice obtained by diluting concentrated or dehydrated juice to the original concentration of fresh juice **(Dutra et al., 2021)**.

Grape juices can be prepared from any grape variety (white or purple) as soon as they are properly ripened. Many observational studies suggest that intake of pure (100%) grape juice may reduce the risk of hypertension, cardiovascular disease, and diabetes mellitus, suggesting the importance of these drinks for maintaining health **(Zuanazzi et al., 2019)**.

Consumption of grape juice gradually increases due to factors such as characteristic taste, aroma, color, or refreshment. In addition, grape juice is gaining popularity due to the functional properties related to phenolic compounds which are associated with consumer health **(Dutra et al., 2021).**

The bioactive composition of the juice depends primarily on the grapes used in its preparation. Each variety has an individual phenolic composition. However, the amount of these compounds can vary and this variability depends on several factors, such as the species, the climatic conditions of the region, or the stage of ripening of the grapes at the time of harvest **(Silva et al., 2019)**.

The most important nutrients in grape juice are vitamins, minerals, proteins, carbohydrates, soluble sugars, organic acids, fatty acids, and amino acids **(Gamboa et al., 2019)**. The health benefits of these nutrients and bioactive substances depend on their level of intake and bioavailability **(Haas et al., 2019)**. Amino acids play a key role in human health because the human body needs them for the synthesis of proteins, peptide hormones, and some neurotransmitters in the body. In addition, amino acids are essential for the catabolism of the intestinal mucosa. The most important amino acids found in grape juice are proline, arginine, glutamine, glutamic acid, and alanine **(Gamboa et al., 2019).**

The chemical composition of the juices is influenced by several factors including processing techniques **(Dutra et al., 2021)**. The processing and preservation of fruit juices need to be gentle and efficient to preserve the original properties of the fruit as much as possible **(Mesquita et al., 2020)**.

Grape juice is rich in flavonols, anthocyanins, procyanidins, and phenolic acids. Therefore, it has beneficial effects on human health **(Azimi et al., 2021)**.

Medicinal plants are used in the food industry to improve appearance and attractiveness. Scientific studies over the last few decades have shown that extracts from plants and herbs can be used as natural preservatives. In addition, it is possible to develop new food products with new and interesting flavor variants by applying various herbs **(Haugaard et al., 2014; Sayed et al., 2019)**.

Our study was focused on the chemical properties and antioxidant activity of some grape juice samples enriched with selected medicinal plants for two weeks.

Scientific hypothesis

We supposed that the medical plant addition could reduce the chemical parameters of grape juice. We set the hypothesis that the medical plant addition increases the antioxidant activity of grape juice.

MATERIAL AND METHODOLOGY

Samples

There were two types of grapes used in the test - white grapes (variety Welschriesling) and blue ones (variety Cabernet Sauvignon), which were obtained from the Slovak University of Agriculture, Institute of Horticulture.

Chemicals

2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma Aldrich, Germany).

Animal and Biological Material:

Welschriesling, Cabernet Sauvignon (the Slovak University of Agriculture, Institute of Horticulture).

Instruments

Glomax spectrophotometer (Promega Inc., Madison, USA), MALDI-TOF MS Biotyper, Alpha FT-IR analyzer (Bruker, Daltonics, Bremen, Germany).

Laboratory Methods

There were analyzed 9 parameters based on a certain calibration by using the Alpha FT-IR analyzer. The calibration is based on more than 1,700 wines from around the world. The determined parameters of grape juice were as follows: fructose ($g.L^{-1}$), glucose ($g.L^{-1}$), dry matter (°Brix), density ($g.L^{-1}$), malic acid ($g.L^{-1}$), pH, potential alcohol (%), total acids ($g.L^{-1}$) and total sugars ($g.L^{-1}$). There were three replicates of measurements on each sample performed.

This used device automatically drew a 10 mL sample of grape juice into a sterile syringe, squeezing about ¾ of the total contents of the syringe into the appropriate hole. The sample of grape juice flowed through the analyzer and it was finally collected through a thin tube into a beaker designated later during this process. The Alpha FT-IR analyzer using Opus software (Bruker Optics, Germany) is checked only with one click. The Alpha FT-IR analyzer was set at a constant temperature of 40 °C, measuring one sample up to 127 times for approximately 2 minutes.

The measurement procedure to determine the antioxidant activity of our samples using the DPPH method was as follows:

- 3.6 mL of DPPH radical (prepared by dissolving 0.025 g in 100 mL of ethanol and then diluting as needed) and 0.4 mL of sample were pipetted into the cuvette.
- the mixture thus formed was mixed quickly and stored in the dark for 10 minutes,
- after 10 minutes, the absorbance at a wavelength of 515 nm on a spectrophotometer was measured

We repeated the measurement 3 times. The results of antioxidant activity were determined as the percentage of radical inhibition.

Description of the Experiment

Sample preparation:

The juice from the grapes was prepared by using the following grape dressing procedures: harvesting, stalking, maceration, pressing, sludge removal, and pasteurization.

The grapes from the Welschriesling variety and the Cabernet Sauvignon variety were collected in October 2020 in the morning in warm and sunny weather. The grapes were hand-harvested in crates with a volume of 25 kg. The grapes were immediately transported to the AgroBioTech research center laboratory of beverages intended for processing to prevent possible steaming of the grapes after harvesting. The sugar content of the Welschriesling grape after harvest was 19.87 ° for Brix and 16.35° for the Cabernet Sauvignon. Then the grapes were put into a mill hopper, where the bunch was separated from the must and berries. The maceration of the grape mash lasted 1 hour. The grape mash was placed in closed containers with a volume of 100 liters during maceration. The pressing was carried out in a gentle way using the maximum pressing pressure - 0.1 MPa. There was discontinuous hydrolysis used for compression. The aim of pressing at a lower pressure is to eliminate damage of the grape seeds and the subsequent transfer of immature phenolic substances to the pressed must. In the case of pressing at a higher pressure than 0.15 MPa, undesirable substances can pass into the pressed must. These substances could negatively affect the character of the must and its sensory properties. The grape must be pumped into a sludge tank using a pump, where static sludge removal was placed without the use of oenological auxiliaries. Subsequently, both experimental musts were pasteurized to eliminate possible contamination by unwanted microcenosis, in particular by fermentation of the must under the influence of wild yeasts.

The extracts were prepared in a ratio of 1:10. We mixed 10 g of the medicinal plant with 100 mL of ethanol. The prepared herbal mixture was stored for 2 weeks. After these 2 weeks, the herbal mixture was treated by evaporation on a vacuum evaporator and subsequent dissolution in distilled water.

We used prepared samples of musts from the varieties of Welschriesling and Cabernet Sauvignon in a volume of 200 mL and extracts from selected medicinal plants in a volume of 20 mL for the sample preparation of grape juice enriched with medicinal plants. We prepared the mixtures of grape juice with herbal extracts in a ratio of 1:10. We used the samples prepared in this way to monitor the microbiological quality and antioxidant activity.

The chemical parameters of the control samples of grape juice, as well as the samples of grape juice enriched with selected medical plant addition, were measured by using Alpha FT-IR is a wine analyzer. The Alpha FT-IR analyzer analyses the sample based on the infrared spectrometry method. It means that the analyzer measures the absorption of infrared radiation that passes through the sample. There were many various changes in the energy states of organic or inorganic molecules during this process which depend on the change in the dipole moment of the molecule. The output of the measurement is a graphical presentation of the infrared spectrum, which is expressed as percent transmittance or absorbance units.

There were used 14 grape juice samples, 2 of them were used as a control (from white and blue grapes) and the 12 samples were enriched with selected medicinal plants (6 juices from white and 6 juices from blue grapes).

Number of samples analyzed: 14

Number of repeated analyses: 3

Number of experiment replication: 2

Statistical analyses

We used the basic statistical method using Microsoft Office Excel 2016 (arithmetic mean calculation of 3 measurements) and the statistical program GraphPad Prism 6 (GraphPad Software, San Diego, USA) for the evaluation of individual parameters and test data as well as two-factor analysis of variance ANOVA (Dunnett test) at 4 levels of significance $p < 0.01$, $p < 0.001$, $p < 0.0001$ and $p < 0.00001$.

RESULTS AND DISCUSSION

There are only a few studies that were focused on the evaluation of the grape juice with the medicinal plant addition. Our work aimed to monitor the chemical parameters of grape juice with the addition of medicinal herbs, as well as to determine the antioxidant activity.

The chemical composition of grape juice depends on the variety of used grapes, the handling and processing of the grapes **(Morris, 1998; Soyer et al., 2003; Camargo, 2004; Camargo and Maia, 2004; Camargo et al., 2005; Yier et al., 2010; Stalmach et al., 2011; Ribeiro et al., 2012; Rizzon and Miele, 2012; Cosme et al., 2018)**.

The following tables process the averages of the measured values of the basic chemical parameters of grape juice (Table 1, Table 2, Table 3 and Table 4). The measurements were repeated after the week because of a better evaluation of the influence of medicinal plants on the chemical parameters of grape juice. Grape juice generally contains a relatively high amount of total sugars **(Fügel et al., 2005; Navarro-Pascual-Ahuir et al., 2015 a, b; Yi et al., 2017; Navarro-Pascual-Ahuir et al., 2017; Nikolaou et al., 2017; Marszałek et al., 2018; Li et al., 2020)**. The amount of total sugar in the control sample was 191.78 $g.1^{-1}$ of white grape juice. In contrast, medicinal plants contain a minimum of sugars, and therefore, after their addition to grape juice, this amount decreased in each sample to the limit of evidence $p < 0.0001$. The lowest measured amount of total sugars was in the sample of grape juice with the addition of *Calendula officinalis* L. (175.21 $g.L^{-1}$). The lowest decrease of glucose content was observed after the addition of *Ginkgo biloba*, where the glucose value of 84.30 $g.L^{-1}$ was measured.

The glucose content of the control sample decreased slightly from 92.15 $g.L^{-1}$ to 91.62 $g.L^{-1}$ after one week. The differences in glucose values between the control sample and the samples with the addition of medicinal plants were at the level of $p < 0.00001$. The highest glucose loss was recorded by the sample with the addition of *Calendula officinalis* and the lowest in the sample with the addition of *Salvia officinalis* in comparison with the second measurement testing. The lowest decrease in dry matter compared to the control sample was achieved by juice with the addition of *Ginkgo biloba* with a value of 84.02 °Brix. Nevertheless, the decrease in dry matter in the samples compared to the indicator reached a high level at the level of significance $p < 0.00001$. The content of potential alcohol decreased slightly one week apart in treated plant samples. On the contrary, its value in the control sample increased slightly from 10.33% to 11.25%. However, these changes were insignificant in terms of statistical significance. Density and pH values did not show any significant differences even after a week.

Blue grape juice with the addition of medicinal plants showed a significant difference compared to the control sample in almost all tested chemical parameters. The fructose content varied approximately equally between the individual treated samples. The differences between the treated samples in the glucose content were minimal, only by 0.6 $g.L^{-1}$. The measured values of density, pH, and total acids did not show statistically significant differences compared to the control sample. Nevertheless, the best-preserved amount of total acids was in the sample of grape juice with the addition of *Salvia officinalis* and at least in the sample of grape juice with the addition of *Mentha aquatica* var. *citrata*.

The measured values of density, pH, and total acids did not show significant differences between the control sample of grape juice and the treated samples of juice even one week after the first measurement. The fructose content of the control sample was 79.32 $g.L^{-1}$. The fructose content with a statistical significance of $P<0.00001$ was observed in other samples treated with medicinal plants. The loss of glucose amount compared to the control sample was in all treated samples at approximately the same level, around 8.54% with a level of significance $p < 0.0000.1$. The differences in the dry matter content of the samples enriched with medicinal plants and the control sample ranged at 2 levels of significance. The potential alcohol did not significantly change compared to the first test, it decreased slightly. After the second measurement, it ranged from 8.62 to 8.88% in the samples enriched with medicinal plants and 9.40% in the control sample.

Table 1 Chemical parameters of white grape after first week.

Sample	Fructose ($g.L^{-1}$)	Glucose ($g.L^{-1}$)	Dry matter (°Bx)	Density ($g.L^{-1}$)	Malic acid ($g.L^{-1}$)	pH	Potential alcohol (%)	Total acids ($g.L^{-1}$)	Total sugars ($g.L^{-1}$)
CW	101.85	92.15	19.87	1.080080	5.18	3.19	10.33	7.58	191.78
W1	92.13**	83.34****	18.23***	1.073160	4.47	3.25	10.22	6.49****	176.67***
W2	93.31**	84.30****	18.47***	1.074427	4.36*	3.26	10.30	6.37****	178.95***
W3	90.86***	82.80****	18.29***	1.073723	4.25*	3.25	10.26	6.44****	177.84***
W4	90.89***	82.65****	18.60***	1.072107	4.32*	3.25	10.17	6.44****	175.21***
W5	92.43**	83.61****	18.29***	1.073530	4.51	3.25	10.26	6.65***	177.17***
W6	92.32**	83.60****	18.29***	1.073800	5.16	3.20	10.32	7.34	176.39***

Note: CW - white grape juice control sample, W1 - white grape juice with the addition of *Calendula officinalis* L., W2 - white grape juice with the addition of *Ginkgo biloba*, W3 - white grape juice with the addition of *Thymus serpyllum*, W4 - white grape juice with the addition of *Matricaria recutita*, W5 - white grape juice with the addition of *Salvia officinalis* L., W6 - white grape juice with the addition of *Mentha aquatica* var. *citrata*, significance to the control sample at the level of evidence * p <0.01, ** p <0.001, *** p <0.0001.

Table 2 Chemical parameters of white grape after second week.

Sample	Fructose ($g.L^{-1}$)	Glucose ($g.L^{-1}$)	Dry matter (°Bx)	Density ($g.L^{-1}$)	Malic acid ($g.L^{-1}$)	pH	Potential alcohol (%)	Total acids ($g.L^{-1}$)	Total sugars ($g.L^{-1}$)
CW	101.23	91.62	19.82	1.079677	5.27	3.21	11.25	7.77	190.71
W1	91.82**	82.58****	18.28***	1.073113	4.63	3.25	10.21	6.81***	176.41***
W2	92.95**	84.02****	18.43***	1.073580	4.51	3.25	10.33	6.53****	177.05***
W3	90.28***	82.52****	18.33***	1.073937	4.25*	3.24	10.29	6.63****	178.38***
W4	92.27**	82.71****	18.29***	1.073147	4.57	3.24	10.27	6.73***	176.95***
W5	91.04***	82.67****	18.36***	1,.73120	4.65	3.25	10.25	6.89***	176.09***
W6	90.78***	82.71****	18.25***	1.073327	4.85	3.24	10.30	7.43*	174.97***

Note: CW - white grape juice control sample, W1 - white grape juice with the addition of *Calendula officinalis* L., W2 - white grape juice with the addition of *Ginkgo biloba*, W3 - white grape juice with the addition of *Thymus serpyllum*, W4 - white grape juice with the addition of *Matricaria recutita*, W5 - white grape juice with the addition of *Salvia officinalis* L., W6 - white grape juice with the addition of *Mentha aquatica* var. *citrata*, significance to the control sample at the level of evidence * p <0.01, ** p <0.001, *** p <0.0001.

The highest decrease in total sugars compared to the control sample was in the sample of grape juice with the addition of *Ginkgo biloba*, namely by 7.68%.

Burini et al. (2009) focused on the study of grape juice in terms of physicochemical aspects of grape juice. They used 12 samples of grape juice for the research, of which 7 were commercial, 2 of them were organic juice samples, and 3 samples were homemade. All tested parameters were performed in 3 series and the results were expressed as the average of individual measurements. The pH of the samples ranged from 3.30 to 3.64, which is a slightly higher pH than in our samples, where the pH values did not exceed 3.28. The measured dry matter content was varied in the individual samples. The highest amount of dry matter was observed in homemade grape juice, up to 21 °Brix, and the lowest amount of dry matter was contained in commercial grape juice with the lowest measured value of 9.5 °Brix. Compared to our samples of dark grape juice, samples of organic grape juice with a value of 15 °Brix achieved a similar amount of dry matter. In contrast, the values of our white grape juice samples ranged similarly to **Burini et al. (2009)** home-made juices, around 19 °Brix. The total titratable acids in the samples ranged from 5.60 to 9.84 $g.L^{-1}$.

Table 3 Chemical parameters of blue grape after first week.

Sample	Fructose (g.L^{-1})	Glucose (g.L^{-1})	Dry matter (°Bx)	Density (g.L^{-1})	Malic acid (g.L^{-1})	pH	Potential alcohol (%)	Total acids (g.L^{-1})	Total sugars (g.L^{-1})
CB	79.77	71.43	16.30	1.067480	10.47	3.28	9.39	13.37	152.94
B1	73.82****	65.14****	15.12***	1.062827	9.42***	3.28	8.69****	12.60	142.34****
B2	74.26****	65.36****	15.11***	1.062470	9.43***	3.29	8.69****	12.42	142.63****
B3	73.44****	65.74****	15.49**	1.064340	9.34***	3.28	8.96****	12.65	147.15***
B4	73.96****	65.32****	15.12***	1.062270	9.41***	3.28	8.70****	12.63	142.75****
B5	73.58****	65.39****	15.80*	1.062293	9.60**	3.28	8.70****	12.75	141.83****
B6	74.13****	65.14****	15.60**	1.062640	9.47**	3.27	8.70****	12.45	142.05****

Note: CB - blue grape juice control sample, B1 - blue grape juice with the addition of *Calendula officinalis* L., B2 - blue grape juice with the addition of *Ginkgo biloba*, B3 - blue grape juice with the addition of *Thymus serpyllum*, B4 - blue grape juice with the addition of *Matricaria recutita*, B5 - blue grape juice with the addition of *Salvia officinalis* L., B6 - blue grape juice with the addition of *Mentha aquatica* var. *citrata*, significance to the control sample at the level of evidence * p <0.01, ** p <0.001, *** p <0.0001.

Table 4 Chemical parameters of blue grape after second week.

Sample	Fructose (g.L^{-1})	Glucose (g.L^{-1})	Dry matter (°Bx)	Denstity (g.L^{-1})	Malic acid (g.L^{-1})	pH	Potential alcohol (%)	Total acids (g.L^{-1})	Total sugars (g.L^{-1})
CB	79.32	70.98	16.25	1.066927	10.26	3.26	9.40	12.74	152.54
B1	73.13****	64.79****	15.05***	1.062210	9.51**	3.26	8.62****	12.56	141.88****
B2	73.72****	64.88****	15.04***	1.061960	9.50**	3.26	8.65****	12.54	140.82****
B3	73.09****	65.43****	15.46**	1.063993	9.35**	3.25	8.88****	12.40	147.17**
B4	73.51****	64.85****	15.05***	1.061830	9.47**	3.26	8.63****	12.65	142.40****
B5	73.05****	64.88****	15.52**	1.061833	9.51**	3.25	8.63****	12.43	140.83****
B6	73.82****	64.67****	15.36**	1.062030	9.57**	3.25	8.64****	11.98	141.46****

Note: CB - blue grape juice control sample, B1 - blue grape juice with the addition of *Calendula officinalis* L., B2 - blue grape juice with the addition of *Ginkgo biloba*, B3 - blue grape juice with the addition of *Thymus serpyllum*, B4 - blue grape juice with the addition of *Matricaria recutita*, B5 - blue grape juice with the addition of *Salvia officinalis* L., B6 - blue grape juice with the addition of *Mentha aquatica* var. *citrata*, significance to the control sample at the level of evidence * p <0.01, ** p <0.001, *** p <0.0001.

In contrast, the values of total acids in samples of blue grape juice ranged from 11.98 - 12.74 g.L^{-1}. On the contrary, samples from white grapes ranged in similar values as **Burini et al. (2009)** samples, specifically from 6.63 - 7.77 g.L^{-1}.

Nassur et al. (2014) investigated the number of chemical components contained in grape juice, specifically the content of dry matter, anthocyanins, and total sugars. The number of total sugars ranged from 24.19 gl-1 to 30.85 g.L^{-1}, which is a significantly smaller amount than we measured in our samples, where we measured the values of pure juice immediately after processing 191.78 g.L^{-1} in the sample from white grapes and 152.94 g.L^{-1} in a sample of blue grapes. The measured dry matter content in the tested samples was approximately the same (**Nassur et al. (2014)** as in our samples of pure blue grape juice sample (16.30 °Brix). We found out that the lowest dry matter content of samples was 14.10 °Brix and the highest dry matter content was 15.83 °Brix. On the contrary, the dry matter content of our sample of pure white grape juice was higher in the first measurement with the measured value of 19.87 °Brix than in the second measurement.

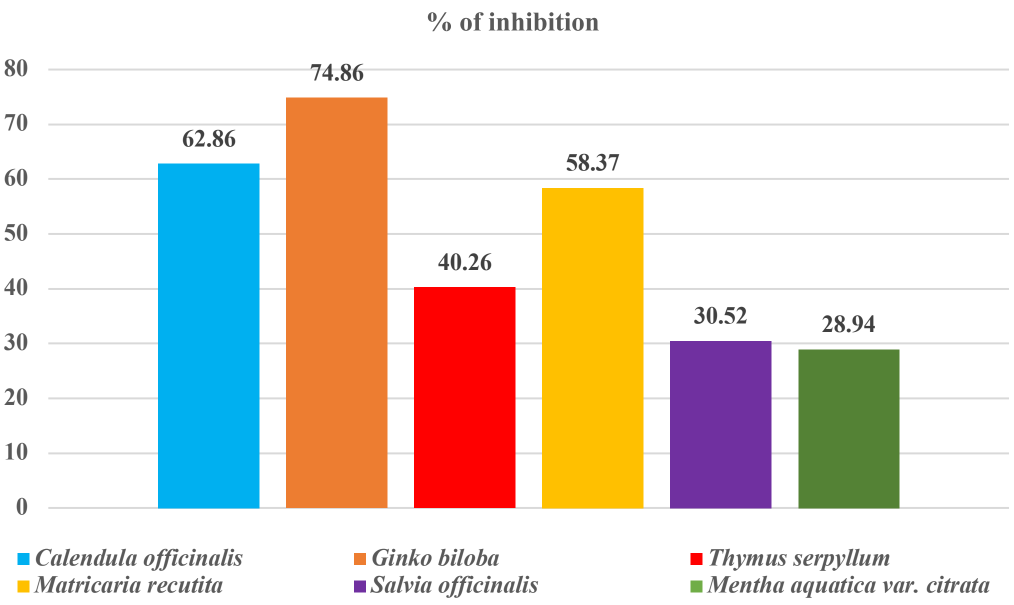

Figure 1 Antioxidant activity of medicinal plants extract.

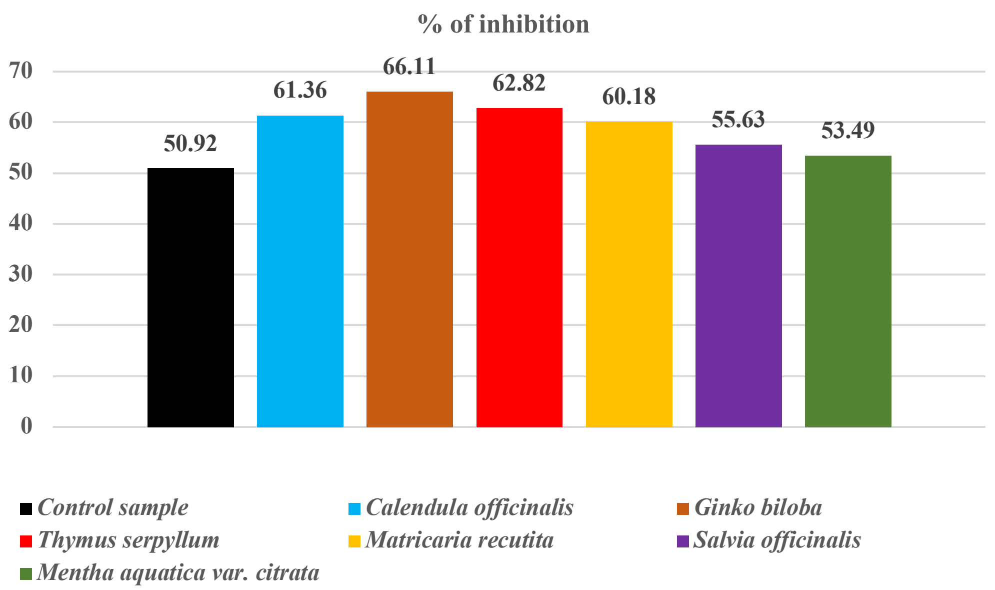

Figure 2 Antioxidant activity of white grape juice with addition of medicinal plants after one week.

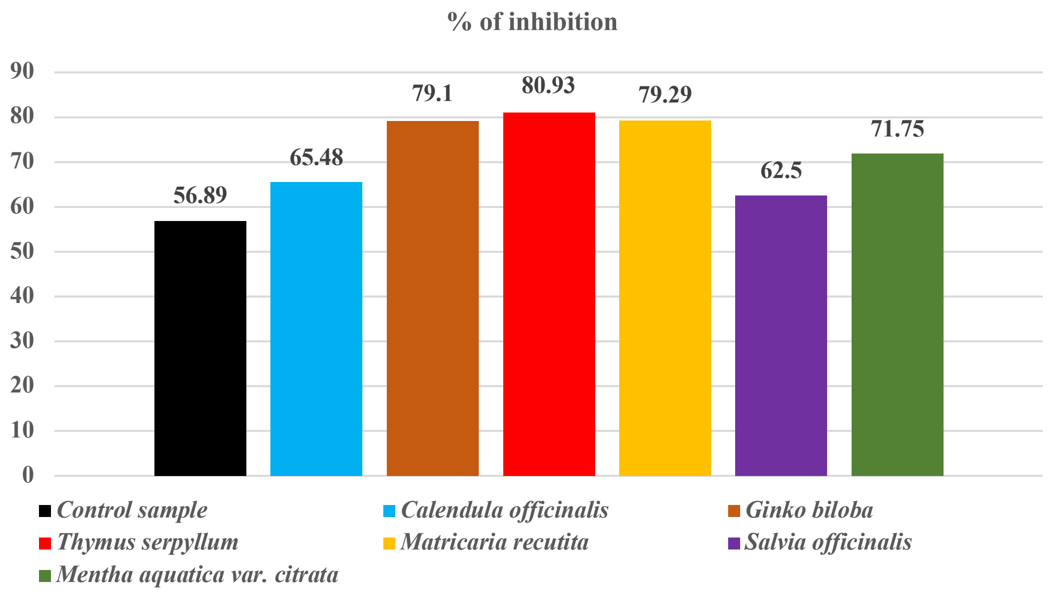

Figure 3 Antioxidant activity of white grape juice with addition of medicinal plants after two weeks.

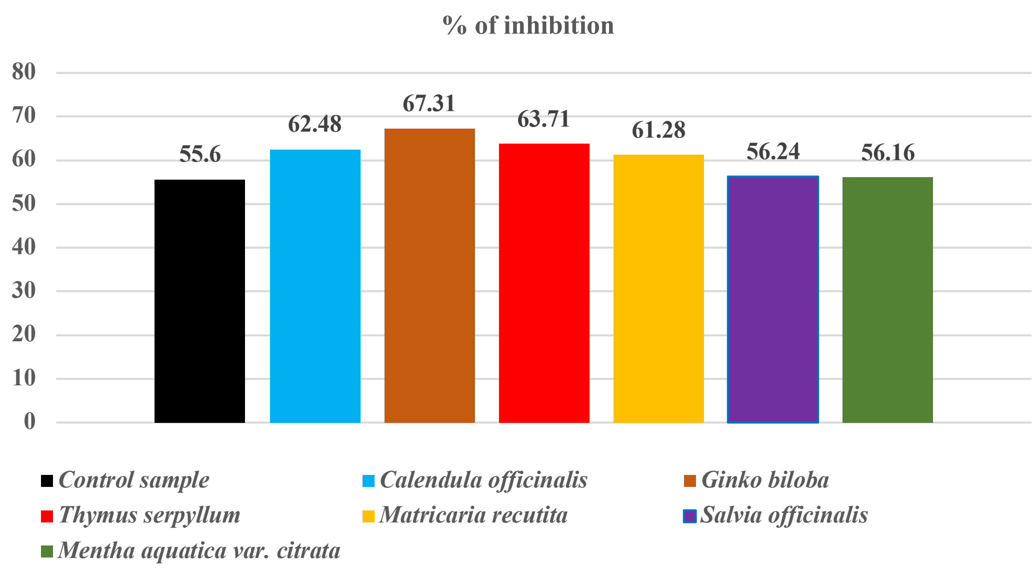

Figure 4 Antioxidant activity of blue grape juice with addition of medicinal plants after one week.

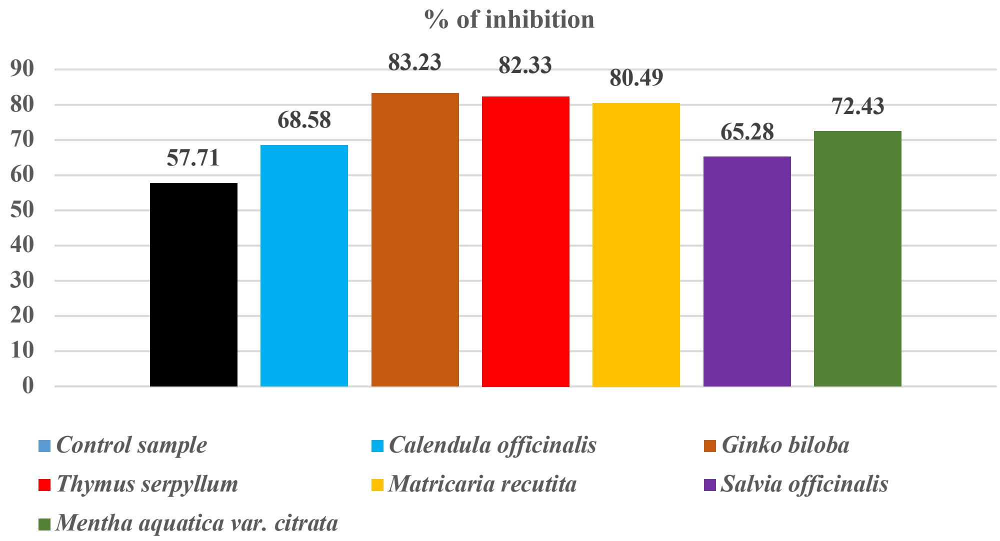

Figure 5 Antioxidant activity of blue grape juice with addition of medicinal plants after two weeks.

Costa et al. (2019) focused their research on the physicochemical aspects of grape juice produced in Brazil in the Cmpanha Gaucha region. They got a type of grape *Vitis labrusca* (blue grapes) from two different places - Dom Pedrito and Santana do Livramento. The study aimed to determine juice density, pH, dry matter, and color intensity. In general, grape juice obtained from both places reached a significant color intensity, Dom Pedrito juice 8.65 and Santana do Livramento juice 10.76. The pH values were the same as in the study by **Burini et al. (2009)** slightly higher than ours, namely 3.59 to 3.67 for Dom Pedrito and 3.45 to 3.63 for Santana do Livramento. The measured density of grape juice samples from *Vitis labrusca* grapes ranged from 1049.25 g.ml^{-1} to 1055.75 g.ml^{-1} (Dom Pedrito) and 1053.25 g.ml^{-1} by 1054.25 g.ml^{-1}. Our samples of blue grapes reached density values slightly higher, in particular, these values ranged from 1.06227 g.L^{-1} to 1.06748 g.L^{-1}. In addition to these parameters, **Costa et al. (2019)** devoted another important parameter - dry matter content. The Dom Pedrito samples measured an average of 12.37 °Brix and the Santana do Livramento samples measured an average of 13.14 °Brix. Compared to our research, the dry matter content in individual samples was lower by up to 20-25%.

Gurak et al. (2010) focused their research on the quality of grape juice, which was concentrated by reverse osmosis. The research was performed at different pressures (40, 50, and 60 bar) and different temperatures (20, 30, and 40 °C). The following parameters were tested to measure the quality of individual samples of grape juice: pH, dry matter, acidity, the concentration of phenolic compounds, anthocyanin content, and color index. However, none of the measured results achieved significant differences in the basic chemical-physical characteristics of grape juice samples. The concentration of phenolic acids averaged 1.98 g.L^{-1}, the concentration of anthocyanins averaged 110.86 mg.L^{-1}. The color based on absorbance averaged 2,088 ABS.

The pH of the samples was significantly more acidic lower in all differently treated samples in comparison with other studies **(Costa et al. 2019**; **Burini et al., 2009)**. The pH values ranged from 2.95 (at 30 °C and 50 bar) to 2.98 (at 20 °C and 40 bar). In contrast, our pH values were around 3.25. The amount of total acids was also not so different due to the process of concentrating grape juice. The average amount of total acids was 7.0 g.L^{-1}. In contrast, our samples showed more varied values of total acids, namely the values in the samples of grape juice from white grapes ranged between 6.37 and 7.77 g.L^{-1} and from 8.62 and 9.40 g.L^{-1} in the samples of grape juice from blue grapes. The dry matter content had the highest value in samples of grape juice at a temperature of 20 °C and a pressure of 60 bar, as well as at a temperature of 30 °C and a pressure of 50 bar, namely 14.3 °Brix. The lowest value of dry matter was found at a temperature of 20 °C and a pressure of 40 bar, namely 13.5 °Brix. Our samples of blue grape juice reached slightly higher dry matter values (on average 15.45 °Brix) and samples of white grape juice contained on average up to 18.56 °Brix dry matter.

The antioxidant activity of individual types of food is based on the amount of antioxidants **(Athukorala et al.,2003; Shahidi and Zhong, 2005; Liyana-Pathirana et al., 2006; Cumby et al., 2008; Pereira and Abreu, 2020).** The function of antioxidants is to slow down or cancel unwanted oxidative reactions in the body and thus protect it from unwanted radicals that can harm it **(Young and Woodside, 2001; Lobo et al., 2010)**.

The antioxidant activity of substances can be determined physically or chemically. In our case, we chose a chemical method using the DPPH method to determine the antioxidant potential of grape juice enriched with medicinal plants. We also used a control sample prepared for a more thorough determination of the antioxidant activity of enriched grape juices with medicinal plants. We repeated the testing of individual samples after one week.

The antioxidant activity of the extracts from medicinal herbs determines the extent of their inhibitory effects on radicals (Figure1). The results show that the most significant antioxidant effect was available in *Ginko biloba* with a value of 74.86%. *Calendula officinalis* and *Matricaria recutita* also reached relatively high values, namely 62.86% (*Calendula officinalis*) and 58.37%

(*Matricaria recutita*). *Mentha aquatica* var. *citrata* and *Salvia officinalis* showed a significantly lower antioxidant effect in comparison with *Ginkgo biloba*. *Thymus serpyllum* showed a medium effect and the least significant antioxidant effect was measured with *Mentha aquatica* var. *citrata* from all 6 extracts.

The antioxidant activity of juice samples after one and two weeks is shown in Figure 2-5. A control sample of white grape juice achieved an antioxidant activity of 50.92%. The antioxidant potential increased after the addition of the extracts. The highest activity was achieved by the juice with the addition of *Ginkgo biloba*, namely 66.11%. We measured a mean percentage of radical inhibition, which ranged from 60.18% to 62.82% in the samples of grape juices with the addition of *Calendula officinalis*, *Thymus serpyllum* and *Matricaria recutita*. The lowest values among the treated samples with medicinal plants were measured in *Salvia officinalis* and *Mentha aquatica* var. *citrata*.

It can be seen after the second testing of the samples, that the antioxidant activity of the individual samples increased. There was an increase of the percentage of radical inhibition noticed in the control sample, in percentage 5.97% compared to the first test. The lowest antioxidant activity among the samples treated with medicinal plants was achieved by *Calendula officinalis* and *Salvia officinalis*, which percentage of radical inhibition did not exceed 65.48%. The antioxidant activity of the juice with the addition of maternal sip significantly improved, namely by 18.11% after a week of storage, making this sample also the most effective among all samples of white grape juice. The samples with the addition of *Ginkgo biloba* (79.10%) and *Matricaria recutita* (79.29%) were approximately at the same level of efficiency. There was also a significant increase in antioxidant activity occurred in the sample with the addition of *Mentha aquatica* var. *citrata*, where the antioxidant effect increased by up to 18.26%. This recorded increase was the highest in comparison to the other samples. On the contrary, the lowest increase since the first testing was measured in the sample treated with *Calendula officinalis*, where the antioxidant effect achieved a 77.44% smaller increase than the sample with the addition of *Mentha aquatica* var. *citrata*.

We can say based on the results of testing samples that blue grapes have better antioxidant activity than white ones. Therefore, other measured values of samples treated with medicinal plants also achieved higher efficiency with samples from blue grapes. The most significant antioxidant effect was shown in a sample of grape juice from blue grapes with the addition of *Ginkgo biloba* (67.31%). The minimum difference compared to the control sample was achieved by samples of juices with the addition of *Mentha aquatica* var. *citrata* and *Salvia officinalis*, where the increase in efficiency was only 0.6%. On the contrary, relatively significant differences compared to the control sample were achieved by samples of juices with the addition of maternal sap (difference 8.11%), *Calendula officinalis* (difference 6, 88%), and *Matricaria recutita* (difference 5.68%).

Nile et al. (2013) focused in their study on the phenol content and antioxidant activity of various grape species. They used 20 types of grapes, which were received from Gyeonggi-do province located in Korea and processed into grape juice. They extracted the juice from the pulp and the skins of individual grapes. Research shows that, in general, juice made from grape pulp has greater antioxidant activity. The results of antioxidant activity were different, ranging from 35% to 87%. However, the most of samples showed high antioxidant activity, from 62% up to 17 samples of grape juice. Our samples of grape juice also showed a relatively high value of antioxidant activity from 56.89% to 80.93% for samples of grape juice from white grapes and from 57.71% to 83.23% for samples of grape juice from blue grapes.

The most significant increase in efficiency was achieved in the samples with the addition of *Ginkgo biloba* (83.23%) and *Thymus serpyllum* (82.33%), which is up to 30.27% more compared to the control sample. The weakest inhibitory effect of radicals among all treated samples with medicinal plants was shown by the sample with the addition of *Salvia officinalis* (65.28%). There was obtained a very significant antioxidant effect in the blue grape juice samples in the sample with the addition of *Matricaria recutita*, which percentage of radical inhibition increased to 19.21% compared to the first test in contrast to the white grape juice samples. The smallest difference between the first and second testing occurred in the control sample, where the value increased by 2.11%, which is 17.11% less compared to the increase in the sample with the addition of *Matricaria recutita*.

CONCLUSION

Grape juice is becoming more and more popular with consumers every day, not only for its unique taste, aroma, or clear color but especially for its rich content of minerals, vitamins, and antioxidants, which have a positive effect on the body. We evaluated 9 parameters - fructose, glucose, total sugar, dry matter, density, malic acid, total acids, potential alcohol, and pH. In general, the pH and density values were not different. We consider the storage temperature, sludge removal during storage, and dilution of grape juice by medicinal plants as the reasons for the reduction of the values of chemical parameters in the treated samples. The larger molecules settle to the bottom during storage entraining some compounds in certain amounts and therefore there is an increase in loss. We consider the dilution of grape juice by medicinal plant extracts to be the greatest factor that influences the decrease of measured chemical parameters. We suppose that if we add a larger amount of medicinal plant extract to grape juice, the loss of individual chemicals will be higher and vice versa. We tested the antioxidant activity of grape juice samples using the DPPH method. The antioxidant activity of individual samples gradually increased, as is clear from the results of the second testing. In general, the antioxidant activity was higher in the samples of grape juice from the Cabernet Sauvignon variety than in the samples of grape juice from the Welschriesling variety. The best antioxidant results in juice samples from both varieties were found in juice samples with the addition of *Thymus serpyllum*, *Matricaria recutita,* and *Ginkgo biloba*. It is clear from obtained test data that the enrichment of grape juice with medicinal plants increases, resp. improves their biological properties. Medical plants have a high potential to be bioactive compounds therefore a deeper study focused on the improvement of the food functionality is needed.

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