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Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 13, 2019, no. 1, p. 167-173
doi: https://doi.org/10.5219/1051
Received: 9 February 2019. Accepted: 01 March 2019.
Available online: 25 March 2019 at www.potravinarstvo.com

ISSN 1337-0960 (online)

NATURAL FRUIT BEVERAGES FORTIFIED BY BIOLOGICALLY ACTIVE SUBSTANCES OF GRAPE VINES

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ABSTRACT

Based on the study of general knowledge of biochemical and all subsequent developmental studies of organic matter, especially products of grapevine and selected fruit products, a comprehensive study of processing technologies is prepared. Use of a combination of vine products and fruit products in the form of natural grapes. Beverages are researched and developed to be purely natural on the basis of grape musts, blue and white, either individually and again separately in targeted combinations, both biochemically, organoleptically and colorfully, with fruit sources. The core of grape value of biologically active substances is an integral and essential new part and condition of designing these beverages. Their increased biological values, which create the preconditions for containment and if properly managed on the basis of scientific knowledge, may in some cases almost result in the elimination of synthetic additives. It should be noted that 20 – 25% of the adult population suffers from many unexpected allergies, for example, to the sulphite content, although its content in the final product does not exceed the health-approved normatives. And there are many other, interrelated relationships. Beverages are technologically dealt with both without alcohol fermentation and with this fermentation, but only based on their compositional natural resources. They are therefore suitable for the entire population profile. The whole set contains 7 variants and a combination of natural beverages from different fruits. Including natural beverages with or without alcoholic fermentation from the must of white wine grapes, the juice of apple puree with those of biologically active substances from the products grapevine. Three months of monitoring and determination of basic (oenological) values and biologically active substances were performed on these products. The high-performance liquid chromatography method with a refractometric detector determined amount of sugar and alcohol, whilst titrating determined total and volatile acids and free sulfur dioxide. Yeast assimilable nitrogen, total anthocyanins and polyphenols were determined by spectrophotometry, antioxidant activity by DPPH and ABTS methods.

Keywords: biologically active substances; natural stability; isolation of selected substances; natural beverages with or without alcohol

INTRODUCTION

The values of biologically active substances are monitored by biochemical analysis in connection with the determination of the oenological, microbiological and other characteristics. Critical points of instability of the biologically active substances value are determined, possibilities of restraint until the causes of this instability are eliminated. Values of endogenous sulfur dioxide content and possibilities of reduction until its elimination **(Wells and Osborne, 2011; Ivanova, Petruseva and Mitrev, 2015)**. Correlation of concurrent laboratory and organoleptic values, which create the first sense of customer interest and interest in manufacturing and commercial applications. The equilibrium of the total polyphenol values demonstrates the stability of the biologically active substances values, thus demonstrating the correctness of the design of the technological processes **(Mlček et al., 2016; Tarko et al., 2015)**. The protein content test proves their stability, which is a measure of protection against sunburn.

In this case, however, completely different technological processes, due to the prepared conditions for yeast activity, are also of completely original origin **(Hernández et al., 2018)**. The yeast for the preparation of the fermentation support comes from the same variety and locality, including agrotechnical practices and technology for the cultivation of grapevine **(Lachman et al., 2009)**. The fermentation process is prepared from grapes harvested 5 to 7 days before the harvest for the entire production process. The exact start date of the preparation of the

fermentation support is determined by the temperature relations of the habitat and the duration of the preparatory processes for the technological process of fermentation and its course **(Yu et al., 2018)**. The temperature sessions need to be regulated in the range of 18 – 28°C and YAN conditions (assimilable N). In the case of unfermented must in the range of 120 – 200 mg.L^{-1} (on the experimental parcels of the submitted project, it ranged in three sampling times at 177 – 166 – 134 mg.L^{-1}, the yeasts were not referred to as "life", they did not consume nitrogen). In fermentation, nitrogen value has fallen further since the beginning of the fermentation process and reached 50 – 44 – 40 mg.L^{-1}. However, it is necessary to emphasize that the resources for obtaining the raw material needed for its processing are consistent but the technological procedures for the respective goals are quite varied.

The technological bond of the basic raw material, ie the apple pulp, has been created to obtain apple juice and the sources of tripenten biological substances contained in apple peel. Apples contain a range of substances that the technologist must register and monitor their development **(Baron, Dénes and Durier, 2006; Valdramidis et al., 2009)**. Mutual relationships of organic acids and sugar content, which is represented by fructose, glucose. During fruit maturation, sucrose passes gradually into fructose. Ripening also creates conditions for the transformation of starch (in immature fruit) into sugar. Furthermore, it is necessary to monitor proteins, pectin substances, fiber and others, especially vitamins and aromatics **(Qin, Petersen and Bredie, 2018)**. Biological activity is heavily enriched with biologically active substances from grape vines, which which belongs to high-incidence plants. The combination of both components – i.e. apples and natural additives, i.e. biologically active substances from grapevine products, creates a value that is purposefully controlled on the basis of the relevant analyzes **(Vrancheva et al., 2018)**.

Scientific hypothesis

The aim of the research is to increase the share of biologically active substances and to increase their stabilization throughout the technological processes from the stage of their sources, the analytical procedures in the course of research and development and the complete observance of the development rules throughout the production process. Monitor biological agents and their development during storage and validate values using appropriate matrix-adapted methods.

MATERIAL AND METHODOLOGY

Materials

The whole set contains 4 variants and a combine natural beverages from different fruits. Includes natural beverages with or without alcoholic fermentation from the must of white wine grapes, the juice of apple puree with those of biologically active substances from the products grapevine.

Methods

Determination of free SO_2 by OIV-MA-AS323-04B : R 2009

The modified method of **Snopek et al. (2018)** which is based on the methodology of **OIV (1990)**. 50 mL of wine sample is pipetted into a 500 mL volumetric flask, we add 3 mL of 16% H_2SO_4 (Penta, Prague, Czech Republic), 1 mL EDTA 3 solution having a concentration of 1%, 5 mL of starch solution is titrated against a white background I_2 (Ing. Petr Lukeš, Uherský Brod, Czech Republic) solution having a concentration of 0.02 mol.L^{-1} to blue color.

Spekctrophotometric methods

Spectrophotometric measurements were performed on a Lambda 25 UV-VIS spectrophotometer (PerkinElmer, USA) in 10 mm optical quartz cuvettes.

Determination of total polyphenol content (TPC)

The spectrophotometric method using the Folin-Ciocalteau reagent is used to determine the total phenolic compound (TPC) content. The essence is the reduction of the phosphomolybdate-tungsten complex by phenolic substances in an alkaline environment. The modified method of **Singleton and Rossi (1965)** according to **Snopek et al. (2018)** was used. Determination is carried out after a 30 min incubation at a wavelength of 765 nm. TPC of substances was expressed as gallic acid equivalent (GAE) in mg.L^{-1}. For the preparation of calibration solutions, use distilled water (20 mL), four volumetric flasks (volume 50 mL) and a micropipette. Split the standard solution, then the Folin-Ciocalteau (Penta, Prague, Czech Republic) reagent (1 mL) and mix. After 2 minutes, 5 mL of 20% sodium carbonate solution are added. The volumetric flask thus prepared is filled with distilled water to the mark. Incubation is followed (60 minutes) and we measure dye intensity in a 10 mm diameter cell at 765 nm against a blank. In the same way, determine the absorbance of the samples. According to the regression curve equation we calculate the content of polyphenols, expressed as mg of gallic acid equivalent (GAE).L^{-1}.

Determination of total antioxidant activity (TAA) by DPPH metod

Total antioxidant activity was assessed by modification method of **Rop et al. (2010)** according to **Snopek et al. (2018)**. The stock solution is prepared by dissolving 24 mg of 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) (Penta. Ing. Petr Švec, Prague, Czech Republic) in 100 mL of methanol (Penta, Prague, Czech Republic). To prepare the working solution, use 10 mL of stock solution and 45 mL of methanol. The working solution is measured spectrophotometrically at a wavelength of 515 nm against methanol as blank. A sample of 450 µL of beverage is pipetted into a test tube. 8.55 mL of DPPH working solution are added. Incubation is followed (60 min) in the dark and then the sample is measured at the indicated wavelength. Absorption loss was recalculated using the linear regression equation to equivalent Trolox (TE).L^{-1}.

Determination of total antioxidant ativity by ABTS metod

The analysis was performed using the modified method of **Re et al. (1999)** and **Hosu, Cristea and Cimpoiu, (2014)**. The $ABTS^{+}$ cationic radical was obtained by reacting a 7 mmol.L^{-1} 2,2'-azino-bis(3-

ethylbenzothiazoline-6-sulphonic acid) ABTS (Merck KGaA, Darmstadt, Germany) solution of a diammonium salt with a solution of 2,45 mmo.L^{-1} $K_2S_2O_8$ mixed 1:1 (v/v). The solution was incubated for 16 hours at room temperature in the dark. Subsequently, 0.5 mL of the sample was added to 3 mL of $ABTS^+$ solution diluted to give an absorbance of less than 0.800. Absorbance was measured at 734 nm for 30 minutes. Absorption loss was recalculated using a linear regression equation to the Trolox equivalent (TE).L^{-1}.

Determination of total anthocyanins content (TAC)

A sample extract was prepared for the assay, to which 5 mL of the extraction mixture (70 methanol : 29 distilled water : 1 acetic acid) was added to 1 mL sample. Subsequently, they were placed in a water bath and shaken for 1 hour at 50 °C and placed for ultrasound for 20 minutes. The extract thus prepared is pipetted in a volume of 0.5 mL into two tubes. To one was added 2.5 mL of 0.025 M KCl buffer of pH 1 (0.186 g of KCl was dissolved in 98 mL of distilled water, followed by pH adjustment by concentrated HCl), and the same quantity of 0.4 M acetate buffer pH 4.5 (5.443 g of sodium acetate trihydrate was dissolved in 96 mL of distilled water, the pH value checked by pH meter and optionally adjusted with HCl). Absorbance was measured in all tubes on a spectrophotometer at a wavelength of 510 nm (absorption maximum of major cyanidine-3-glucoside) and at 700 nm against the extraction mixture. This determination was carried out by a modified method according to **Giusti and Wrolstad (2001)** and **Orsavová et al. (2019)**

Determination of sugar and alcohol

Before sampling, samples were diluted 1:10 with distilled water and then filtered through nylon micro filters (Syringe Filter, Nylon 13 mm x 0.45 μm). The determination of ethanol was carried out using a high performance liquid chromatography (RP-HPLC) method on UltiMate 3000 (Dionex, Sunnyvale, CA, USA) using a Phenomenex Rezex RCM-Monosaccharide Ca + 2 (100 x 7.8 mm, 8 μm) Torrance, CA, USA) with a RI detector. Water, isocratic elution, with a flow rate of 0.4 mL.min^{-1}, was used as the mobile phase. Column temperature 80 °C. Detector temperature RI 35 °C.

The qualitative evaluation was performed on the basis of analysis of the individual sugars and ethanol standards. A quantitative evaluation where the resulting value was determined as the average of the six measurements was performed by the calibration curve method and subsequent calculation of the concentration of the substance in the sample. The fructose, glucose and sucrose content was expressed as the equivalent amount of g standard in a 1 liter sample. The alcohol content was expressed as volume %.

Determination of volatile and total acids

To measure total acids, measure 30 mL of water, 1 mL of indicator and 20 mL of sample (CO_2-free) into a conical flask. We will titrate in green blue with a standard 0.1 M NaOH solution (pH = 7.0). All titratable acids are calculated as the tartaric acid content in g.L^{-1}.

The Berh S2 apparatus is used for volatile acids, a 25 mL sample (CO_2-free) is dripped into the distillation vessel. The heated distillate is heated to 60 – 70 °C, add 2 drops of phenolphthalein and titrate with 0.1 M NaOH to pink color. Calculate the volatile acid content as acetic acid in g.L^{-1}.

Determination of yeast assimilable nitrogen (YAN)

According to **Gump et al. (2002)** and **Petrovica et al. (2018)** free amine nitrogen and ammonia as well as YAN components are measured separately by an enzymatic test using K-PANOPA Megazy (Ireland) and ammonia using Enzytec Fluid Ammonia (R-Biopharm). This was done spectrophotometrically on a 20XT instrument (Thermo Fisher Scientific, Walthan). These individual values for free amine nitrogen and ammonia provide the total amount of available YAN and expressed as the nitrogen content in mg .L^{-1}.

Statisic analysis

The data obtained was expressed as mean value ± standard deviation (SD) and the Microsoft Office Excel program (Redmond, WA, USA) was used to calculate them. All analyzes were performed five times in two replicates. Differences between observed results were detected by t-test (Statistica, 2018, StatSoft, USA). A $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Samples of natural beverages from grape vines and apple juice were supplemented with biologically active substances from grapevine and free sulfur dioxide, total antioxidant activity (TAA) by methods using DPPH and ABTS, total polyphenol content (TPC), total anthocyanins content (TAC), sugar (fructose, glucose and sucrose), alcohol (ethanol), free and volatile acids and yeast assimilable nitrogen (YAN) were determined at regular monthly intervals during beverage storage under the prescribed conditions at 5 ° C.

Free sulfur dioxide in test samples of natural beverages (Table 1 – 4) ranges from 1.84 to 15.05 mg.L^{-1}. The smallest content was found in the natural beverage from apple juice with values of biologically active substances from vine products without alcoholic fermentation after 3 months of storage, on the contrary, the highest value was in natural beverage from white grape must with alcoholic fermentation after the first determination. If we compare the achieved values for free SO_2 (Table 1 – 4) with a study of sulfur dioxide **(Snopek et al. 2018)** the measured values are compared to those in organic quality.

To determine the total polyphenols content, a spectrophotometric method using Folin-Ciocalteau reagent was used in samples of a natural beverages of grape vines and fortified beverages of apple juice, using gallic acid as a standard. For beverages tested, the higher value of total polyphenols in fortified apple beverages was made by biologically active substances from grapevine and its change during storage was only fractionally. For alcoholic beverages, they exceeded 677.26 mg GAE.L^{-1} after first measurement to 619.54 mg GAE.L^{-1} after 3 months of storage.

Table 1 Results of individual provisions for natural beverage from white grape must without alcoholic fermentation.

Length of storage [month]		1st	2nd	3rd
Free SO_2	**(mg.L^{-1}) ±SD**	2.25 ±0.12^{a}	2.21 ±0.18^{a}	2.02 ±0.24^{a}
TPC	**(mg GAE.L^{-1}) ±SD**	303.55 ±10.14^{a}	339.30 ±8.17^{b}	329.30 ±12.87$^{a, b}$
TAA (DPPH)	**(mg TE.L^{-1}) ±SD**	436.60 ±12.54^{a}	503.42 ±17.24^{b}	431.77 ±11.72^{a}
TAA (ABTS)	**(mg TE.L^{-1}) ±SD**	632.53 ±14.57^{a}	616.58 ±18.74^{a}	660.63 ±20.14^{a,b}
TAC	**(mg C3G.L^{-1}) ±SD**	5.95 ±0.98^{a}	6.26 ±1.01^{a}	6.14 ±0.52^{a}
Sugar (fru,glu,suc)	**(g.L^{-1}) ±SD**	226.64 ±2.41^{a}	228.18 ±3.24^{a}	226.11 ±2.25^{a}
Alcohol	**(% vol.)**	0.21 ±0.01^{a}	0.22 ±0.01^{a}	0.19 ±0.02^{a}
Volatile acids	**(g.L^{-1}) ±SD**	0.04 ±0.01^{a}	0.06 ±0.01^{b}	0.06 ±0.01^{b}
Total acids	**(g.L^{-1}) ±SD**	8.81 ±0.25^{a}	8.85 ±0.31^{a}	8.91 ±0.27^{a}
YAN	**(mg.L^{-1}) ±SD**	177.04 ±1.24^{a}	166.58 ±2.01^{b}	134.45 ±2.18^{c}

Table 2 Results of individual provisions for natural beverage from white grape must with alcoholic fermentation.

Length of storage [month]		1st	2nd	3rd
Free SO_2	**(mg.L^{-1}) ±SD**	15.05 ±0.74^{a}	7.45 ±0.51^{b}	6.74 ±0.59^{b}
TPC	**(mg GAE.L^{-1}) ±SD**	305.03 ±8.25^{a}	334.00 ±8.87^{b}	306.58 ±9.24^{a}
TAA (DPPH)	**(mg TE.L^{-1}) ±SD**	552.38 ±11.29^{a}	621.34 ±14.52^{b}	472.02 ±9.93^{c}
TAA (ABTS)	**(mg TE.L^{-1}) ±SD**	825.32 ±18.47^{a}	810.04 ±17.74^{a,b}	785.70 ±15.98^{b}
TAC	**(mg C3G.L^{-1}) ±SD**	4.35 ±0.42^{a}	3.91 ±0.33^{a}	3.23 ±0.28^{b}
Sugar (fru,glu,suc)	**(g.L^{-1}) ±SD**	10.44 ±0.74^{a}	9.58 ±0.98^{a}	9.37 ±0.74^{a}
Alcohol	**(% vol.)**	11.74 ±1.01^{a}	11.76 ±0.94^{a}	11.72 ±0.89^{a}
Volatile acids	**(g.L^{-1}) ±SD**	0.12 ±0.01^{a}	0.15 ±0.01^{b}	0.15 ±0.01^{b}
Total acids	**(g.L^{-1}) ±SD**	9.24 ±0.71^{a}	9.08 ±0.67^{a}	8.84 ±0.55^{a}
YAN	**(mg.L^{-1}) ±SD**	50.15 ±2.17^{a}	44.84 ±1.87^{b}	40.18 ±0.98^{c}

Table 3 Results of individual provisions for natural beverage from apple juice with values of biologically active substances from vine products without alcoholic fermentation.

Length of storage [month]		1st	2nd	3rd
Free SO_2	**(mg.L^{-1}) ±SD**	2.84 ±0.25^{a}	2.37 ±0.71^{a,b}	1.84 ±0.46^{b}
TPC	**(mg GAE.L^{-1}) ±SD**	677.26 ±12.02^{a}	672.34 ±8.07^{a}	619.54 ±8.96^{b}
TAA (DPPH)	**(mg TE.L^{-1}) ±SD**	644.64 ±10.99^{a}	609.95 ±9.92^{b}	655.58 ±10.85^{a}
TAA (ABTS)	**(mg TE.L^{-1}) ±SD**	975.73 ±15.94^{a}	916.60 ±13.34^{b}	956.89 ±14.20^{a}
TAC	**(mg C3G.L^{-1}) ±SD**	8.29 ±0.57^{a}	9.51 ±0.74^{b}	9.19 ±0.84^{a,b}
Sugar (fru,glu,suc)	**(g.L^{-1}) ±SD**	156.54 ±5.51^{a}	148.24 ±4.23^{b}	147.65 ±4.63^{b}
Alcohol	**(% vol.)**	0.12 ±0.01^{a}	0.13 ±0.01^{a}	0.09 ±0.01^{b}
Volatile acids	**(g.L^{-1}) ±SD**	0.01 ±0.01^{a}	0.03 ±0.01^{b}	0.03 ±0.01^{b}
Total acids	**(g.L^{-1}) ±SD**	10.45 ±0.47^{a}	10.40 ±0.53^{a}	10.47 ±0.61^{a}
YAN	**(mg.L^{-1}) ±SD**	156.42 ±5.17^{a}	133.81 ±4.33^{b}	124.37 ±3.86^{c}

Table 4 Results of individual provisions for natural beverage from apple juice with values of biologically active substances from vine products with alcoholic fermentation.

Length of storage [month]		1st	2nd	3rd
Free SO_2	**(mg.L^{-1}) ±SD**	10.02 ±0.74^{a}	6.87 ±0.67^{b}	6.21 ±0.71^{b}
TPC	**(mg GAE.L^{-1}) ±SD**	312.94 ±6.24^{a}	328.41 ±8.57^{b}	312.18 ±7.09^{a}
TAA (DPPH)	**(mg TE.L^{-1}) ±SD**	459.22 ±7.41^{a}	553.96 ±9.04^{b}	479.44 ±7.61^{c}
TAA (ABTS)	**(mg TE.L^{-1}) ±SD**	825.36 ±11.27^{a}	794.44 ±9.98^{b}	741.36 ±9.54^{c}
TAC	**(mg C3G.L^{-1}) ±SD**	3.31 ±0.23^{a}	2.57 ±0.19^{b}	2.66 ±0.21^{b}
Sugar (fru,glu,suc)	**(g.L^{-1}) ±SD**	25.78 ±0.89^{a}	24.14 ±0.91^{a}	22.97 ±0.95^{b}
Alcohol	**(% vol.)**	9.02 ±0.78^{a}	9.25 ±0.69^{a}	9.37 ±0.91^{a}
Volatile acids	**(g.L^{-1}) ±SD**	0.52 ±0.02^{a}	0.54 ±0.05^{a}	0.52 ±0.04^{a}
Total acids	**(g.L^{-1}) ±SD**	9.92 ±0.68^{a}	9.84 ±0.88^{a}	9.93 ±0.76^{a}
YAN	**(mg.L^{-1}) ±SD**	99.45±3.54^{a}	97.17 ±2.94^{a}	76.34 ±3.58^{b}

Note: Table 1 to 4: SO_2 – sulfur dioxide; TPC – total polyphenol content; TAA – total antioxidant ativity using DPPH and ABTS – radical scavenging activity; TE – trolox equivalent; GAE – gallic acid equivalent; C3G – cyanidine-3-glucoside equivalent; fru – fructose; glu – gluctose, suc – sucralose; YAN – yeast assimilable nitrogen; ±standard deviation. The different superscripts in rows indicate statistically significant differences between data groups (statistically tested on level of significance $\alpha = 0.05$).

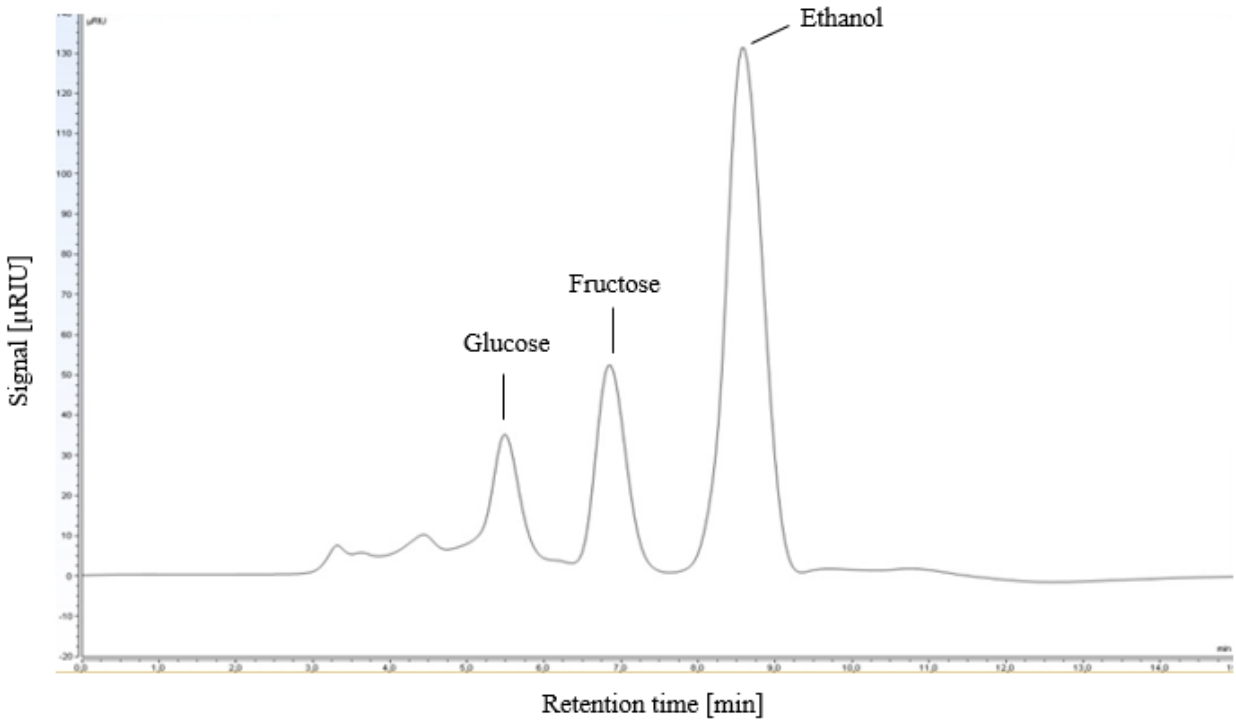

Figure 1 Chromatogram of determination of sugars and alcohol in a sample of white grapes (HPLC – RID).

The least total polyphenols were determined for a natural drink of white grape vines with alcoholic fermentation. It reached from 305.03 mg GAE.L^{-1} after the first measurement to 306.58 mg GAE.L^{-1} after three months of storage.

Paixao et al. (2007) evaluated the content of polyphenols in a fermented beverage of white grape vines. The average measured content was 369 mg GAE.L^{-1}, another study **(Hurtado et al., 1997)** reported the TPC of the beverage of 292 mg of GAE.L^{-1}. These published values are comparable to our values. The results of the work by **Ricci, Parpinello and Versari (2017)** for a TPC were reported at 222 mg GAE.L^{-1} which is in correlation with our results. Fortified beverage achieved above-average results and can be evaluated very positively. **Tarko et al. (2015)** published comparable values for apple juice, indicated as raw materials with high TPC.

To determine the antioxidant activity of natural beverage samples, the DPPH method was used, based on the reaction of the test substance with a stable 1,1-diphenyl-2-picrylhydrazyl radical. In addition, the ABTS method was used. It is characterized by reacting the test substance with 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid). Both methods used trolox as standard. The highest antioxidant activity in natural beverages (Table 1 – 4) was determined by both methods in non-alcoholic natural beverage from apple juice with fortified bioactive substances from white grape vines. The value ranged from 655.58 – 644.48 mg TE.L^{-1} determined by the DPPH method and 975.73 – 956.89 mg TE.L^{-1} using the ABTS method during storage. The smallest antioxidant activity was determined in a beverage sample of white grapes without alcohol.

The determination of total anthocyanins content was another of analyzes that support the high content of biologically active substances in a natural beverage based on fortified apple juice. Its content ranged from 9.51 mg C3G.L^{-1} to 8.29 mg C3G.L^{-1}. There followed a non-alcoholic natural drink of white grape vines with values ranging from 5.95 mg C3G.L^{-1} to 6.14 mg C3G.L^{-1}. A decrease in total anthocyanin values was observed for alcohol drinks during a three-month storage period with a white grape drink from 4.35 mg C3G.L^{-1} to 3.23 mg C3G.L^{-1} and for fortified apple juice with alcohol then 3.31 mg C3G.L^{-1} to 2.66 mg C3G.L^{-1}. Our measured values of the content of anthocyanin in apples reached the published values by **Wolfe, Wu and Liu (2003)**. Losses were attributed primarily to the technological modifications that took place during the production of the beverage.

Chromatographic techniques were most suitable and accurate for the identification and quantification of mono- and oligosaccharides in food **(Duarte-Delgado et al., 2015)** and according to the guidelines of the Association of Official Analytical Chemists **(AOAC, 1993; Sims, 1995)** for quantification of sugars. Table (1 – 4) lists the sugars (fructose, glucose and sucrose) content in natural beverages samples at the beginning of storage determined by the HPLC method. Figure 1 shows the chromatogram of determination of sugars and alcohol in a sample of white grapes (HPLC – RID). When the highest peak is ethanol, the other peaks represent the individual sugars. Table 1 – 4 shows that alcohol-free beverages contain up to ten times more sugars than alcohol drinks. Most of the sugars were determined for a grape wine without alcohol 226.64 g.L^{-1}, which was retained after 3 months of storage and the alcohol content fluctuated between 0.19% (vol.) and 0.22% (vol.). In fortified apple juice the sugar value dropped from the initial 156.54 g.L^{-1} to 147.65 g.L^{-1}, and its alcohol content also reduced from 0.12% (vol.) to 0.09% (vol.). Conversely, for a white grape alcohol drink,

which reached an average of 11.74% (vol.) alcohol content, it contained only 9.37 g.L^{-1} to 10.44 g.L^{-1} of sugars.

Fortified apple juice contained less alcohol from 9.02 – 9.37% (vol.), the sugar content ranged from 25.78 – 22.97 g.L^{-1}. **Pickering et al. (1998)** determined the effect of ethanol concentration (range 0-14% vol.) on perception of the "fullness" of beverages. It has been found that ethanol concentrations were highly correlated with perceived intensity, physical viscosity and density measurements **(Nurgle and Pickering, 2005).**

Volatile and total acids were also determined. Volatile acids were determined in very small amounts in all analyzed samples. Most of them were determined for fortified apple juice with an alcohol of 0.52 – 0.54 g.L^{-1}, on the other hand, the least amount in the same sample without alcohol, and in the range of 0.01 – 0.03 g.L^{-1} during the three month storage. Total acids were at highest as measured for fortified apple juice without alcohol in the range of 10.45 – 10.47 g.L^{-1}. For all other beverages, the total acid value was approximately the same as 9 g.L^{-1}.

Asymilatable nitrogen were considered to be the initiator of fermentation, affecting its kinetics, aromatic content, acetic acid and others. Table 1 – 4 shows the results of determining the amount of assimilable nitrogen in natural beverages. Highest values of assimilated nitrogen were determined for non-alcoholic beverage variants. The highest was determined for a drink from white grapes wine 177.04 mg.L^{-1} and dropped to 134.45 mg.L^{-1}. The fortified apple juice was 156.42 mg.L^{-1} and dropped to 124.37 mg.L^{-1} during 3 month storage. Conversely, for alcoholic beverages, the values were lower. Table 2 shows a decreasing value from 50.15 mg.L^{-1} to 40.18 mg.L^{-1}. Table 4 shows a high drop from 99.45 mg.L^{-1} to 76.34 mg.L^{-1}.

This confirmed **(Steidl, 2002)** that the more ammonium ions are in the environment, the more yeast consume them.

CONCLUSION

It is essential to select and evaluate sources to prepare raw material base for the production of natural beverages without and with alcoholic fermentation using exclusively natural starting values. During production it is necessary to assess natural values and quality of these basic rescources.

The high content of biologically active substances from grape vines and their incorporation into all musts is an integral part of the entire technological, production process and in combination with the corresponding fruit sources it appears to be a positive step towards enriching food with natural additives.

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Acknowledgments:

This work was supported by grant IGA/FT/2019/004

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