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ORIGINAL RESEARCH PAPER

EVALUATION OF BIRCH SAP (*BETULA PENDULA*) QUALITY DURING STORAGE

Liliana Norocel[1], Sergiu Pădureț[2*]

[1] "Ştefan cel Mare" University of Suceava, Department of Health and Human Development, 13 University Str., 720229, Suceava, Romania
[2] "Ştefan cel Mare" University of Suceava, Faculty of Food Engineering, Suceava, 13 University Str., 720229, Suceava, Romania

*Corresponding author: sergiu.paduret@fia.usv.ro

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Abstract: This study presents the quality assessment of fresh and heat treated birch sap (*Betula pendula*) during 4 weeks of storage at 4 ºC. The entire quantity of birch sap was divided into three equal parts: a part was subjected to a pasteurization process, a part was subjected to a direct heat treatment at 60 °C and a part was kept fresh. The total microbial number of fresh collected birch sap was 94 CFU·mL^{-1} and after applying heat treatment the total microbial number decreased significantly (maximum 1 CFU·mL^{-1}).
For quality evaluation of birch sap samples during storage the element minerals content, *p*H, total acidity, conductivity, total microbial number, antioxidant capacity and polyphenols content were determined. The pasteurized birch sap presented a greater stability during storage than the other two samples, the *p*H varied slightly (3.47 - 3.16) and the sap conductivity showed a slight increase from 672.4 µS·cm^{-1} to 759 µS·cm^{-1}.

Keywords: *antioxidant activity, birch sap, minerals, PCA, thermal treatment*

INTRODUCTION

Birch sap is considered a spring beverage in central and northern countries of the Europe, frequently consumed fresh, only occasionally concentrated into syrup [1, 2].
In Romania, Transylvania region the sap of *Betula pendula* is called birch water and is extracted by different methods, according to some studies since the 18th to the 20th century [3]. Tree sap, especially birch sap contains a range of various mineral elements, enzymes, amino acids, carbohydrates, organic acids, and some bioactive substances such as phenolic compounds, vitamin C and vitamins from B complex. All these compounds along with betulinic acid which has a range of biological effects, such us antitumor activity represents a great importance for health of human body [4]. Minerals are among the most important substances promoting the beneficial effect of tree sap on human health; the minerals that can be found in birch sap are potassium, calcium, magnesium, manganese, copper, iron, zinc, aluminum and nickel [5, 6]. The most common amino acids analyzed in birch sap are valine, glutamine, isoleucine, citrulline and asparagine. Oligosaccharides such as: fructosyl/glucosyl-sucrose, gentiobiose, melibiose and manninotriose were found in birch sap. In birch sap were also identified organic and inorganic acids namely malic, phosphoric, succinic and citric; the concentration of these acids varies during the flow season [7, 8].
In recent studies was demonstrated that this beverage can be used against liver stones and kidney anemia, arthritis, tuberculosis, hypertension, gout, rheumatism and colds, scab, constipation, headache and pneumonia. A consumption of 200–300 mL birch sap/day could have a diuretic, anti-infectious, anti-rheumatic and anti-inflammatory effect. Birch sap has been widely used as a remedy or as a medicine for, promoting urination, quickening the appetite, strengthening the stomach sedating the nerves, treating gastroenteric disorders and postnatal symptoms in women. Besides these, birch sap was used as a bioactive compound in cosmetic products for skin and hair care [3, 7].
Sap is collected from February to April, the highest flow has been observed before the burst of buds burst and development of leaves; nowadays all types of saps cannot be industrialized because of the short preservation period [9]. Sap processing technology is not so improved due to quickly consumed after manufacture, which is really needed for extending shelf life of sap [10]. Tree and especially birch sap have been used for its nutrients and as a revitalizing and energizing beverage in many countries, but nowadays only north countries are collecting birch sap and maple sap. Tree sap can be added in other beverages or food products such as beer, wine and syrup [7]. The sap can be consumed fresh, stored in the refrigerator at 4 - 8 °C for a limited term (5 - 7 days), and may be boiled until syrup is obtained or by fermentation with sugar, yeast and other aromatics compounds are produced alcoholic beverages [4].
Studies demonstrated that one birch produces 4 $L \cdot day^{-1}$ of sap. The scientific investigation and the current research regarding the tree sap are limited into the european production technology of tree sap and lesser of these research have been written in English, mainly are in Finnish, so the physicochemical properties of the European trees sap is much less known. The sugar content in the European species of birch saps can reach to 0.8 % of their weight [1, 3].
The aim of this paper was to analyze some important elements which are present in Romanian birch sap (*Betula pendula*), the changes of these during storage and choosing

an optimal conservation method, so that consumers can enjoy this drink for a longer period of time throughout the year.

MATERIALS AND METHODS

In this study it was used a sample of birch sap (*Betula pendula*), without thermal treatment (fresh), a sample of birch sap with a thermal treatment applied in the laboratory (60 °C for 20 minutes) and a sample pasteurized under industrial conditions. All samples were stored at 2 - 4 °C. It was collected in early spring, when the sap moves intensively, from the forests of Bucovina (Bukowina - the land of beeches), following the collection process described by Bilek *et al.*, 2016 [11].
For determination of total polyphenol content and for determination of antioxidant activity, a Shimadzu spectrophotometer 3600 UV-Vis-NIR was used and for mineral element analysis, the Agilent Technologies 7500 Series spectrometer (Agilent, USA) was used. The concentration of birch sap in soluble substances (°Brix) and the refractive index were determined with a Leica Mark II Plus refractometer (accuracy of 0.1 %). The conductivity measurement was performed with Accumet conductivity meter XL30 and the *p*H was measured with Hach *p*H-meter (HQ11d). Microbiological analysis of total microbial number (TC - total count) was performed according to SR EN ISO 6222/2004 [12].

Total acidity

Total acidity was determined according to Semjonovs, 2014 by alkaline titration and the results was expressed as Thörner degrees (°T) [7].

Total polyphenol content (TPC)

Total polyphenol content (TPC) was measured according to Sripakdee *et al.*, 2015 [13] by Folin–Ciocalteu method. The absorbance was measured at 765 nm using 1 mL of sample, 5 mL of Folin Ciocalteu reagent, and 4 mL of Na_2CO_3 (7.5 %), then mixed for 5 min and stored in dark room at ambient temperature for 60 min prior to analyzing. The results were expressed as mg of Gallic Acid Equivalents (GAE)/100 mL sample [13, 14].

Antioxidant content evaluation

For antioxidant content evaluation, the DPPH assay was used, and the scavenging effect was calculated. A volume of 0.5 mL birch sap was added to 2.5 mL DPPH ethanol solution ($6 \cdot 10^{-5}$ M) and after 5 min the absorbance were measured at 517 nm.

$$DPPH_{\ scavenging\ effect}\ (\%) = (A_c - A_{5min})/A_c \times 100$$

where A_c was the absorbance of the control sample and A_{5min} was the absorbance in the presence of the sample [15, 16].

Minerals content determination

The birch sap minerals were analyzed by coupled plasma-mass spectrometer (7500 Series Agilent Technologies, SUA) and following the protocol described by Fernandez-Turiel *et al.*, 2000 [17] which implies the acidification of the sample with HNO_3 (1 % v/v HNO_3). Double deionized water (18.2 $M\Omega \cdot cm^{-1}$, Thermo Fisher-Germany) was used for preparation and/or dilution of solutions. All reagents were purchased from Sigma Aldrich (Germany).

Statistical analysis

Unscrambler 9.7 software was used for Principal Component Analysis (PCA), which describe the relationship between measured parameters and the analyzed birch sap samples. The Pearson correlation was made by SPSS 13 software (SPSS Inc. Chicago, IL) and the variance analysis (ANOVA) was performed using STATGRAPHICS CENTURION XVI software (Trial Version). The results are expressed as means of three measurements.

RESULTS AND DISCUSSIONS

In this study the usefulness of birch sap consumption was analyzed and for this purpose the minerals concentration, *p*H, total acidity, conductivity, total microbial number, antioxidant activity and total polyphenol content were determined. The analyzed birch sap samples were fresh, thermally treated in the laboratory and pasteurized.

In Table 1 are presented the concentrations of mineral elements and as we can see, calcium content was the highest, even higher than sodium (18.513 $mg \cdot L^{-1}$), magnesium (29.481 $mg \cdot L^{-1}$) or chromium while zinc, selenium, iron, aluminum, copper and manganese showed a lower concentration, similar to the results presented by Jeong *et al*., 2013 [10].

The calcium content varies between 94.811 - 84.545 $mg \cdot L^{-1}$, which makes the birch sap to be an important source of calcium for those who consume it, one liter of birch sap providing 10 % of an adult's daily requirement. The amount of selenium ranges between 0.112 - 0.103 $mg \cdot L^{-1}$, selenium being a powerful antioxidant and together with zinc (0.697 - 0.614 $mg \cdot L^{-1}$), which also has antioxidant properties protects the body from free radicals and stimulates the immune system [18]. The presence of heavy metals in birch sap samples such as lead, mercury and nickel are insignificant and uranium is not detected, the sap respecting European Union rules on heavy metal limits [19]. The ANOVA analysis showed that the differences between analyzed samples are not significant ($p > 0.05$).

The evolution of physicochemical parameters, the total polyphenol content (TPC) expressed as Gallic acid and the antioxidant capacity of birch sap samples during storage are shown in Figure 1.

Tabel 1. *Minerals in birch sap samples*

Elements [mg·L^{-1}]	Fresh birch sap	Thermally treated in the laboratory birch sap	Pasteurized birch sap
Li	0.007^{b}	0.008^{a}	0.007^{b}
Na	17.073^{a}	18.513^{a}	16.033^{b}
Mg	27.077^{b}	29.481^{a}	28.705^{a}
Al	3.368^{a}	3.456^{a}	3.671^{a}
Ca	86.058^{b}	94.811^{a}	84.545^{c}
Cr	5.894^{a}	5.915^{a}	5.307^{b}
Mn	0.112^{a}	0.159^{a}	0.169^{a}
Fe	0.097^{b}	0.107^{a}	0.081^{b}
Ni	0.051^{a}	0.077^{a}	0.053^{a}
Cu	2.266^{b}	2.467^{a}	2.447^{a}
Zn	0.644^{a}	0.697^{a}	0.614^{b}
Se	0.105^{a}	0.112^{a}	0.103^{a}
Hg	0.001^{a}	0.005^{a}	0.002^{a}
Pb	0.077^{a}	0.090^{a}	0.081^{a}
U	ND	ND	ND

Different lowercase letters (a–c) in a row show significant differences between the groups ($p < 0.05$). The results are expressed as means of three measurements. ND - not detected

The samples of birch sap without thermal treatment (fresh) shows a strong increase of total acidity, total numbers of germ, conductivity, a decrease in *p*H and a decrease in antioxidant activity during the 4 weeks of testing. The samples of birch sap without thermal treatment are recommended to be consumed within the first 2 weeks of harvesting, in the 4th week the total microbial numbers (Figure 1c) reached the value of 480 CFU·mL^{-1}, the total acidity has increased considerably up to 7.6 °T and the antioxidant activity decreased from 81.86 % to 57.86 %. In contrast, the sap samples with thermal treatment are not differenced in the early stage (the first two weeks), the total microbial number was insignificant, and the conductivity showed close values (767.9 - 680.55 µS·cm^{-1}).

The pasteurized samples of birch sap show a greater stability during storage than the other two categories of samples, the *p*H varies between 3.47 - 3.16, the total acidity varies from 2.19 °T in first week to 2.95 °T in last week and the conductivity shows a slight increase from 672.4 µS·cm^{-1} to 759 µS·cm^{-1}. The birch sap samples concentration of soluble substances expressed as °Brix showed similar results, between 0.95 - 1.35 °Bx, birch sap should be considered as a low-calorie diet supplement and a good substitute for water [20], the main monosaccharides identified in birch sap being represented by fructose, glucose and sucrose [11].

The greatest antioxidant capacity measured by DPPH method is presented by the pasteurized samples of birch sap, in the first week the sap showed the highest antioxidant capacity with an average of 90.20 % inhibition of DPPH solution and until the last week of analysis the inhibition capacity decrease with 7.70 % (82.49 %).

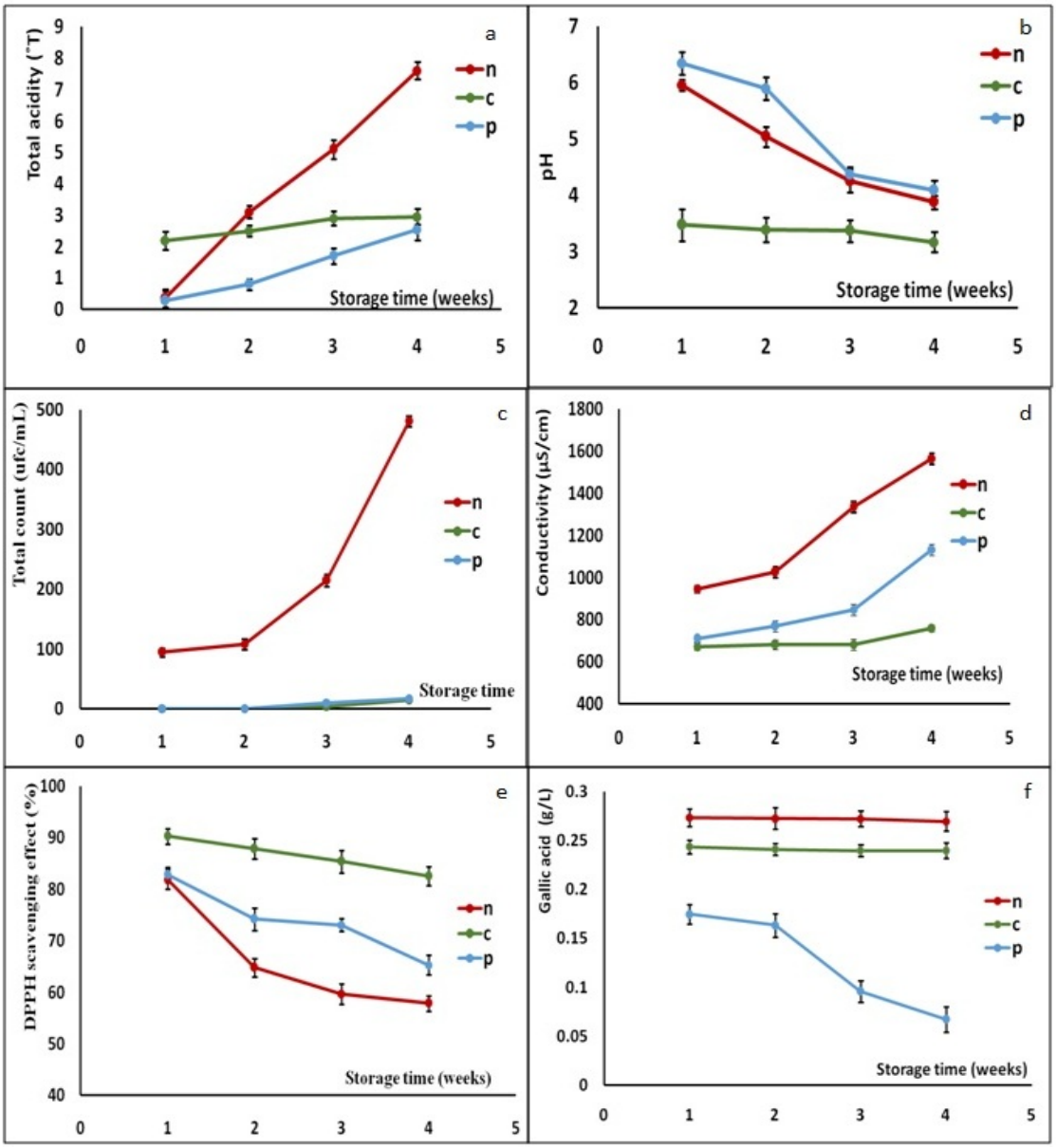

Figure 1. *The evolution of physicochemical parameters, total polyphenol content and the antioxidant capacity of birch sap samples: n - birch sap sample without thermal treatment (fresh), p - birch sap sample with thermal treatment, c - pasteurized birch sap sample*

Another advantage of birch sap consumption it is represented by TPC, the average value of three measurements resulted by Folin–Ciocalteu assay was 0.273 $g \cdot L^{-1}$ (expressed as gallic acid equivalent) for fresh birch sap with a very low decrease during storage, 0.243 $g \cdot L^{-1}$ for pasteurized birch sap sample also with a very low decrease during storage. Unlike the other two samples, the birch sap sample with thermal treatment applied in the laboratory has a phenolic content of 0.174 $g \cdot L^{-1}$ in the first week with a decrease of 38.5 % during storage, the pasteurization being the best method of birch sap preservation. The total phenolic content of analyzed samples is similar to that reported for fresh melons juice (watermelon and sweet melon) by Al-Musharfi *et al.*, 2015 [21].

The above results strengthen the fact that birch sap is an exceptional beverage with a high content of bioactive compounds, which does not involve the addition of additives in the technological process and respect the current trend of clean label.
The Pearson correlation matrix (Table 2), showed a strong positive correlation of total acidity with conductivity ($p < 0.01$, $r = 0.776^{**}$), total microbial number ($p < 0.01$, $r = 0.842^{**}$) and also a positive correlation with storage time ($p < 0.05$). Repeatedly, the antioxidant content of birch sap samples measured by DPPH assay was negatively influenced by acidity ($p < 0.05$, $r = -0.633^{*}$), conductivity ($p < 0.01$, $r = -0.901^{**}$) and total microbial number ($p < 0.05$, $r = -0.703^{*}$). A high positive correlation exists also between conductivity and total microbial number at a level of $p < 0.01$, $r = 0.895^{**}$.

Table 2. *Pearson correlation matrix of measured parameters*

	A	**C**	***p*H**	**r**	**B**	**P**	**D**	**TC**	**W**
A	1	0.776**	-0.54	-0.418	-0.026	0.384	-0.633*	0.842**	0.648*
C		1	-0.02	-0.144	0.235	0.165	-0.901**	0.895**	0.508
***p*H**			1	0.652*	0.328	-0.141	-0.107	-0.072	-0.567
r				1	0.686*	-0.013	-0.115	-0.177	-0.567
B					1	-0.303	-0.581*	0.000	-0.516
P						1	0.070	0.438	0.061
D							1	-0.703*	-0.540
TC								1	0.374
W									1

A - total acidity, W - storage time in weeks, C - conductivity, TC - total microbial number, r - refractive index, B - concentration of soluble substances expressed as °Brix, P - total polyphenol content, D - antioxidant capacity
*Correlation is significant at the 0.05 level (2-tailed)
**Correlation is significant at the 0.01 level (2-tailed)

The physicochemical parameters and bioactive compounds influence on the birch sap samples was evaluated by Principal Component Analysis (PCA). The two principal components (PCs) explain all the data variation; PC1 explains 93 % while PC2 explains 7 % of the variance in the obtain data. The results of principal component analysis (correlations loadings and scores) are presented in Figure 2 and Figure 3.
Based on this analysis we can observe that birch sap samples groups are well defined, distributed in different quadrants depending on applied thermal treatment (Figure 2). The central parameters from correlations loadings matrix (Figure 3): *p*H, total acidity (A) and storage time in weeks (W) have insignificant influence on differentiation of the analyzed sap samples, whereas those from the outside such as: conductivity (C), total microbial number (TC), refractive index (r), concentration of soluble substances expressed as °Brix (B), total polyphenol content (P) and antioxidant capacity (D) have a strong influence.

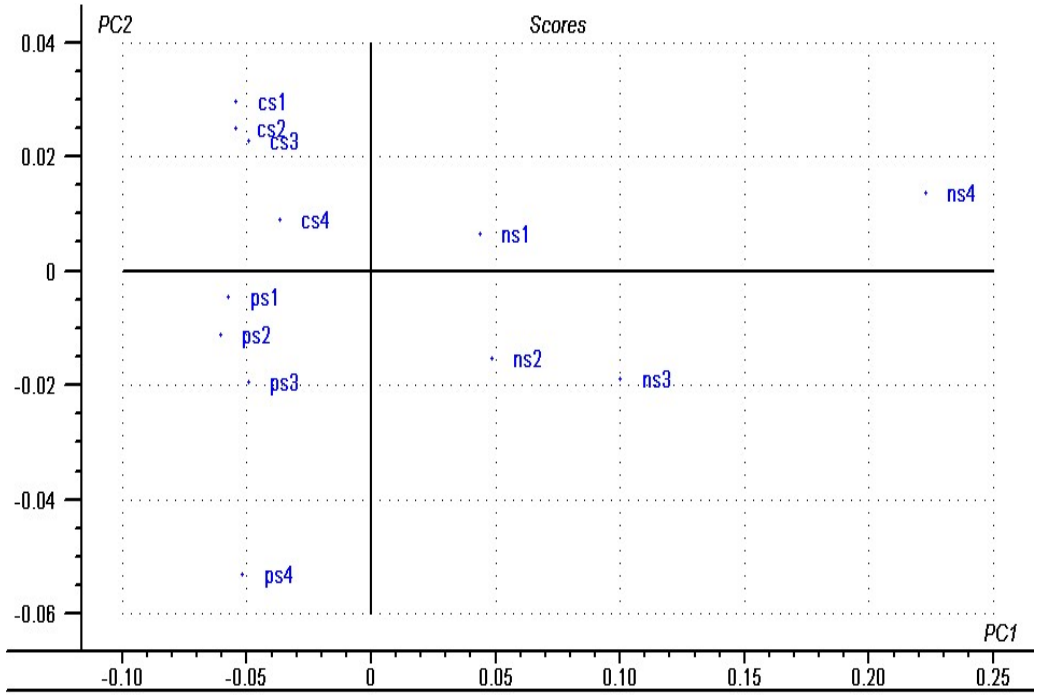

Figure 2. *PCA scores of birch sap samples: n - birch sap sample without thermal treatment (fresh), p - birch sap sample with thermal treatment, c - pasteurized birch sap sample, s1, s2, s3, s4, s5-time in weeks*

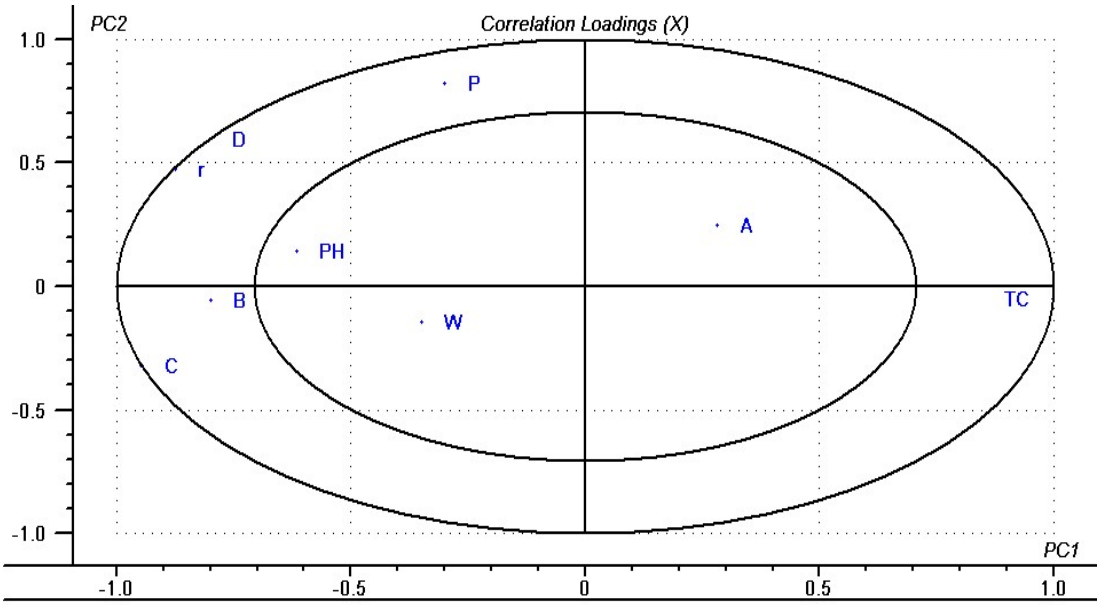

Figure 3. *PCA loadings of birch sap samples (A - total acidity, W - storage time in weeks, C - conductivity, TC - total microbial number, r - refractive index, B - concentration of soluble substances expressed as °Brix, P - total polyphenol content, D - antioxidant capacity)*

From Figure 2, it can be noticed that PC1 separates the fresh birch sap samples from the thermally treated ones, PCA being a powerful method for samples differentiation. From

the PCA correlation loadings analysis (Figure 3), it can be observed that the fresh birch sap samples projection is significantly influenced by total microbial number (TC) and less influenced by total acidity (A). The pasteurized birch sap sample projection was influenced by antioxidant activity and total polyphenol content, while the projection of birch sap samples with thermal treatment were influenced by conductivity.

CONCLUSION

Birch sap is a beverage with exceptional properties that should be consumed fresh to a maximum of two weeks, kept under refrigeration conditions and if we want to consume it for a longer time, it must be subjected to a pasteurization process. Birch sap is a valuable natural beverage representing a minerals source for human consumption and besides these shows antioxidant properties which together with polyphenols content and minerals like selenium and zinc helps the organism in fight against free radicals and oxidative stress. Future research is suggested to explore the addition of birch sap in food manufacturing processes such as bread and pastry making.

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