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Qualitative Changes in Birch Sap after Freezing and Thawing

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Abstract: In this study, the qualitative changes in raw birch sap after freezing and thawing were determined. Ten-liter bottles and one-ton plastic containers with six replications were used for the freezing of birch sap and thawing of frozen sap. During and after the thawing, the physical and physical–chemical properties of the sap were measured. According to the results, as the ice melts, the concentration of acids and other soluble substances in the sap decreases, but changes in qualitative indicators indicate the beginning of fermentation processes through color changes and pH as the temperature of the melting sap becomes positive. As a result, to freeze raw sap in large-volume containers, it is necessary to develop fast thawing technology using auxiliary means—circulation, external energy sources, and mechanical ice crushing.

Keywords: biochemical composition; microbiology; physicochemical indicators

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1. Introduction

Birch sap, also known as birch water, is a clear liquid that is obtained by tapping the birch tree during the spring season. It has been consumed for centuries in various regions around the world, particularly in Northern Europe and Asia, for its refreshing taste and potential health benefits [1,2]. Birch sap is rich in vitamins, minerals, and antioxidants, making it a popular natural beverage and ingredient in traditional medicine. As with other natural products, the popularity of birch sap is increasing in the market [3,4].

Birch sap is a sensitive, perishable product. During its extraction, there are technological challenges in preserving the properties of fresh sap; as a result, industrially extracted sap must be processed before the start of fermentation, because fermentation causes changes in the sap's color and odor and its chemical composition [2,5].

Birch sap has gained attention for its high nutritional value and various health benefits. It is a rich source of vitamins, minerals, and antioxidants that contribute to overall well-being [6]. Birch sap is particularly known for its high content of vitamin C, which helps boost the immune system and promotes collagen production. Additionally, it contains potassium, calcium, magnesium, and manganese, which are essential minerals for maintaining healthy bodily functions [7]. Furthermore, birch sap has been found to have diuretic and detoxifying properties, supporting kidney health and aiding in the elimination of toxins from the body [3]. These nutritional components and health benefits make birch sap a valuable addition to a balanced diet and a potential natural remedy for promoting overall health and well-being.

Harvesting and processing birch sap is a delicate and precise procedure that requires a careful understanding of the birch tree's biology and the optimal time to tap into its sap. Researchers have found that the chemical composition of birch sap depends on the type of birch habitat [8,9], the age of the birch tree, the daily sap volume, and the date of sap collection [10]. Typically, sap is harvested in early spring when the sap begins to rise and before the buds open. This is the period when the sap flow is at its peak, yielding the highest quantity and quality of sap [11]. The most common method of sap extraction is through tapping, which involves drilling small holes into the tree trunk and inserting a spout or tap to collect the sap. Once collected, the sap is then processed to extend its shelf life and enhance its flavor. Common processing methods include pasteurization, filtration, and evaporation to remove impurities and concentrate the sap.

Fresh birch sap can be kept for a long time simply by freezing it [7]. Bilek et al. [12], after studying sap microfiltration and treatment with ultrasound and UV radiation, stated that microfiltration is the most advantageous in extending the shelf life of birch sap and has potential for use in the food industry, but microfiltration extends the shelf life by only one month. Additionally, the sap can be fermented to create traditional birch wine or syrup. Fermentation involves the conversion of sugars in the sap into alcohol by the action of yeast, resulting in a unique and flavorful beverage [4]. Fresh birch sap ferments quickly and can be self-fermented (auto fermentation). Spontaneous fermentation can be suppressed by ozonating the juice. The main difference between kombucha and fermented birch sap is the ratio of predominant bacteria to yeast. Colonies of mesophilic lactic acid bacteria predominate in birch sap fermented under aerobic conditions [4]. Lactic acid fermentation has also long been known as a preservative, shelf-life-extending process that also produces antimicrobial agents against pathogenic organisms [13,14].

The research on sap exudation is important because as the popularity of forest cutting within society decreases and environmental ideas are fostered, alternative ways of farming are becoming more popular in forests [15]. Alternative forest uses help to increase income from traditional, timber-based forestry [16,17]. With the increasing demand for sap and the intensification of sap exudation in Lithuania, it is important to research the main problems related to the sap exudation process. One of the main problems faced by the gatherers and processors of industrial birch sap is the storage of the production yield, because the sap is extracted in a short period of about one month, usually in March–April, and processing often takes place throughout the year. Due to the sugars, amino acids, and microelements contained in birch sap, it is an excellent medium for various microorganisms, such as lactic acid bacteria; therefore, the extracted sap is kept frozen until processing [18]. The suitability of refrigeration for preserving the original properties of the sap was also affirmed by the Latvian scientists Nikolajeva and Zommere [19]. Sancho et al. [20] studied the influence of freezing on the microbiological safety and protein composition of birch sap, but there have been no studies, or only episodic studies, on the influence of freezing and thawing on the chemical composition and physicochemical indicators of birch sap.

This work aims to evaluate how the quality indicators of the sap change after it is frozen for a long storage period and then thawed for use.

2. Materials and Methods

Birch sap was extracted in the mature birch forest of the Radviliškis forest district of Lithuanian State Forest Enterprise 487,565, 6,132,629 (LKS) 55.328831, 23.804034 (WGS) 55°19′43.79″, 23°48′14.52″ (WGS) in the spring of 2021. Freezing of birch sap and defrosting of frozen sap were performed at the Open Access Center for Modeling of Fruit and Vegetable Processing Technologies at the Lithuanian Research Centre for Agriculture and Forestry (LAMMC). The tests were performed in 10 replicates of 10 L in MX 1000 one-ton-capacity plastic containers (Schütz GmbH & Co. KGaA, Selters, Germany).

In mature birch stands, mature silver and downy birch trees with a diameter (at a height of 130 cm measured from the root collar) of at least 20 cm were chosen. The device used for sap extraction (ref. No. LT 5813 B) ensures minimal contact with the environment

so that the sap does not contain forest litter, precipitation water, or any other material that could affect the results. The sap was collected daily and immediately frozen. The chemical composition and quality indicators were analyzed in the laboratory of the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry. The following test methods were used for birch sap quality studies:

Determination of total soluble solids. The soluble solids content was measured directly with a PR-32 digital refractometer (Atago Co., Ltd., Saitama, Japan).

Determination of titratable acidity. Titratable acidity was estimated by titrating with 0.1N NaOH solution to pH 8.2 and was expressed as a mg-equiv/100 mL of citric acid [21].

Birch sap color measurement. Color indexes in the space of even contrast colors were measured with a MiniScan XE Plus spectrophotometer (Hunter Associates Laboratory, Inc., Reston, VA, USA), and the chroma ($C = (a^{*2} + b^{*2})^{1/2}$) and hue angle ($h^\circ = \arctan(b^*/a^*)$) were calculated [22]. The hue angle h° was defined as the angle between the reference and 0° on the a* axis. The values L*, a*, b*, and C* were measured in NBS units; hue angle h° was expressed in degrees from 0 up to 360°. The NBS unit is a unit of the U.S. National Bureau of Standards and meets one color resolution threshold, i.e., the smallest difference in a color that can be captured by a trained human eye. Before each series of measurements, the spectrophotometer was calibrated with a light trap and a white standard with the following color coordinates in the XYZ color space: X = 81.3, Y = 86.2, and Z = 92.7. The value of L* indicated the ratio of white to black, the value of a* indicated the ratio of red to green, and the value of b* indicated the ratio of yellow to blue. The ΔE* value was calculated using the CIE76 formula:

$$\Delta E^*_{ab} = \sqrt{\left(L^*_2 - L^*_1\right)^2 + \left(a^*_2 - a^*_1\right)^2 + \left(b^*_2 - b^*_1\right)^2}$$

where $\Delta E^*_{ab} \approx 2.3$ corresponds to a JND (just noticeable difference) [23]. The color coordinates were processed by Universal Software V.4–10.

Determination of the amount of mineral elements. The mineral element contents in birch sap were determined with inductively coupled plasma optical emission spectrometry (ICP-OES) [24]. Sap samples were diluted to 50 mL with deionized water and analyzed by an ICP–OES spectrometer (Spectro Genesis, SPECTRO Analytical Instruments GmbH, Kleve, Germany) at 1300 W RF power, 12 L/ min plasma flow, 1 L/min auxiliary flow, 0.8 L/min nebulizer flow, and 1 mL/min sample uptake rate. The mineral content (mg/L) was evaluated according to the respective analytical wavebands for each element.

Active acidity (pH) was measured with an "inoLab pH Level 1" pH meter with a SenTix 81 (WTW) electrode.

The electrical conductivity of the birch sap was measured with an ECTestr 11+ conductometer (Oakton, Vernon Hills, IL, USA).

Temperature measurement. The temperature of the birch sap was measured and recorded using the Omega OM-DAQPRO-5300 Portable Handheld Data Logger (Omega Engineering Inc., Norwalk, CT, USA) with K-type thermocouple temperature sensors.

FLIR photos of the containers (thermal imaging and visible-light imaging) were taken with a FLIR T650sc thermal imager (FLIR Systems, Inc., Wilsonville, OR, USA).

Microbiological tests. The total microorganism count was determined according to ISO 4833-1:2013 [25]. The total number of yeasts and molds was determined according to ISO 21527-1: 2008 [26]. Six replicate samples were taken from each juice sample for analysis. Colonies were counted, and numbers were given in colony-forming units per milliliter (CFU/mL) [27].

The statistical methods of data processing. Means and standard deviations were calculated with STATISTICA 10 (StatSoft, Inc., Tulsa, OK, USA) and Excel (Version 2403, Microsoft, Redmond, WA, USA) software. One-way analysis of variance (ANOVA) along with the post hoc Tukey's HSD test was employed for statistical analysis. Differences were considered to be significant at $p < 0.05$. Trends and relationships between some birch sap quality properties were visually investigated by making a scatter plot of the data.

3. Results

3.1. Quality Indicators of Birch Sap

The chemical composition of the fresh birch sap (n = 3) was determined immediately after the collection of the samples. The chemical composition and physicochemical indicators of the birch sap are presented in Table 1.

Table 1. Quality indicators of fresh birch sap.

Indicator	Mean Value	Standard Deviation
Soluble solids, %	0.82	0.24
Titratable acidity, mg-equiv./100 mL	0.11	0.04
Electrical conductivity, µS/cm	486	38
pH	6.62	0.48
Mineral elements, mg/L	188	8.25
CIELab color coordinates:		
L*, %	51.61	0.31
a*, NBS units	−0.5	0.07
b*, NBS units	0.3	0.15
C, NBS units	0.6	0.13
h, °	145.4	9.31
Total number of microorganisms, log CFU/mL	1.60	0.98
Yeasts, log CFU/mL	0	-
Molds, log CFU/ml	0	-

3.2. Freezing and Thawing of the Sap

Birch sap was collected in 10 L plastic containers, in which it was transported from the forest to the freezer. In the freezer, the sap could be frozen in the same capacity, but freezing large amounts is not technologically convenient; therefore, the sap was frozen in food-grade plastic containers of 1000 L capacity. If birch sap is frozen in 10 L containers, it freezes at the top, bottom, and sides of the container at the beginning and later freezes in the middle (Figure 1).

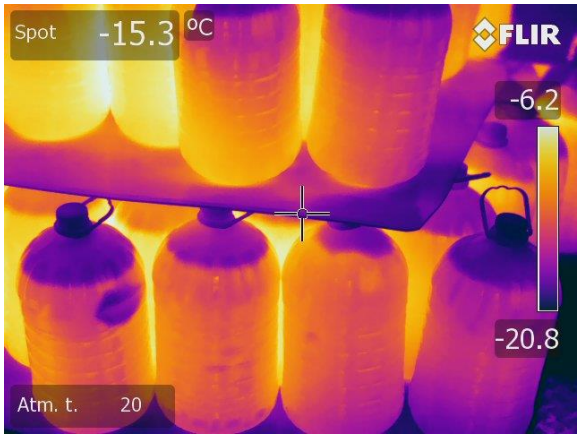

Figure 1. Thermographic (FLIR) image of the birch sap freezing process when the sap was frozen in 10 L containers.

If the sap is frozen in 1000 L containers when the temperature is maintained at −18 ± 0.5 °C, it freezes completely within 14 days (Figure 2). The sap freezes first at the top, bottom, and sides of the container, while the center of the container freezes last.

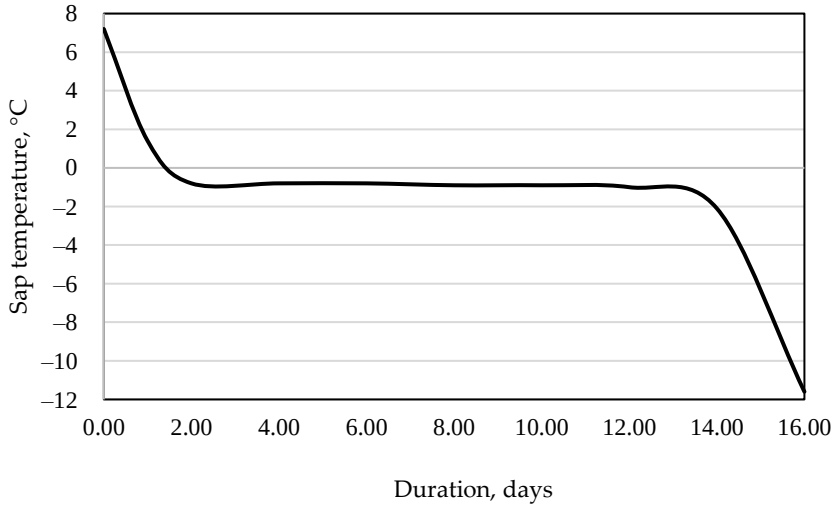

Figure 2. Dynamics of temperature changes in the center of the 1000 L container during freezing of birch sap.

During the concentration of the birch sap, samples of sap with different amounts of soluble solids were taken, for which the freezing temperature was determined (Figure 3). It was established that as the amount of soluble solids in birch sap increased in a linear relationship, its freezing temperature decreased. When the soluble solids concentration was extrapolated to zero, the freezing point was not zero, which should be the case for pure water, but this can be explained by the chemical composition of the sap. The amount of soluble solids basically reflects the amount of sucrose, and birch sap contains only 0.12 percent sucrose, although the soluble solids concentration is 0.76 percent. The amount of soluble solids in the sap was calculated based on the sucrose equivalent. In addition to sucrose and monosugars, the sap also contains organic acids and micro- and macroelements, which leads to a lower freezing point of the juice than can be expected from the amount of soluble solids (sucrose). These studies assume that even in a 1000 L container, when ice crystals form, almost pure water freezes first, and only later do sap crystals with an increasing amount of soluble solids begin to form.

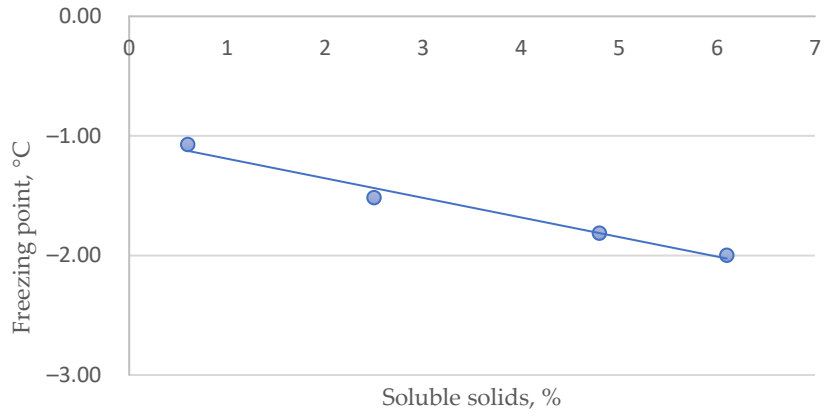

Figure 3. Influence of soluble solids on the sap freezing point.

The thawing of birch sap was carried out in both 10 L and 1000 L containers. When thawing was carried out at a room temperature of 18–20 °C (Figure 4), it was found that the thawing temperature at depths of 20 cm, 40 cm, and 60 cm was the same, only the duration

of thawing varied: at a depth of 20 cm, it fully thawed in 6 days; deeper, at 40 cm, it thawed in 8 days; and in the middle of the container, at a depth of 60 cm, it thawed in 10 days.

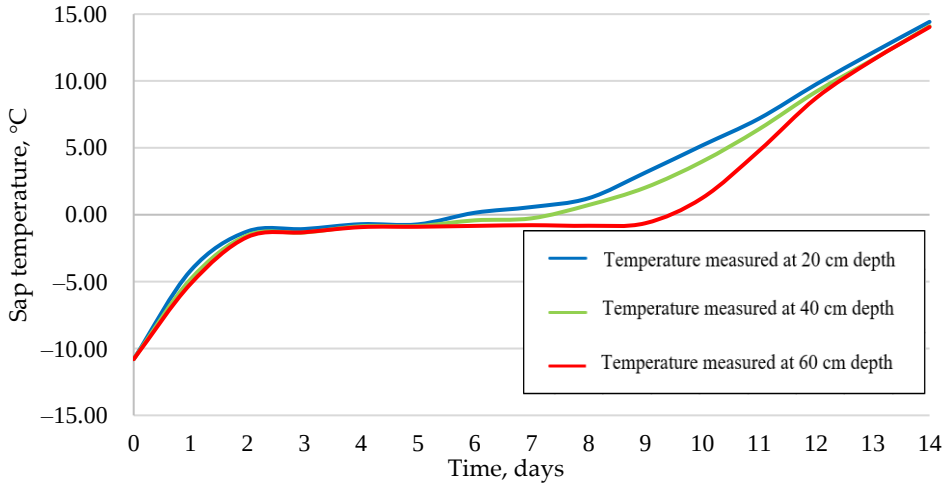

Figure 4. Dynamics of sap thawing at various depths of the container.

To determine the dynamics of the amount of naturally thawed sap and changes in quality indicators, the amount of thawed sap was measured every day by draining the dissolved sap and studying its chemical composition and physical–chemical indicators (Figure 5). The sap container thawed completely within 14 days at room temperature without any additional effort. A positive sap temperature was reached within 10 days.

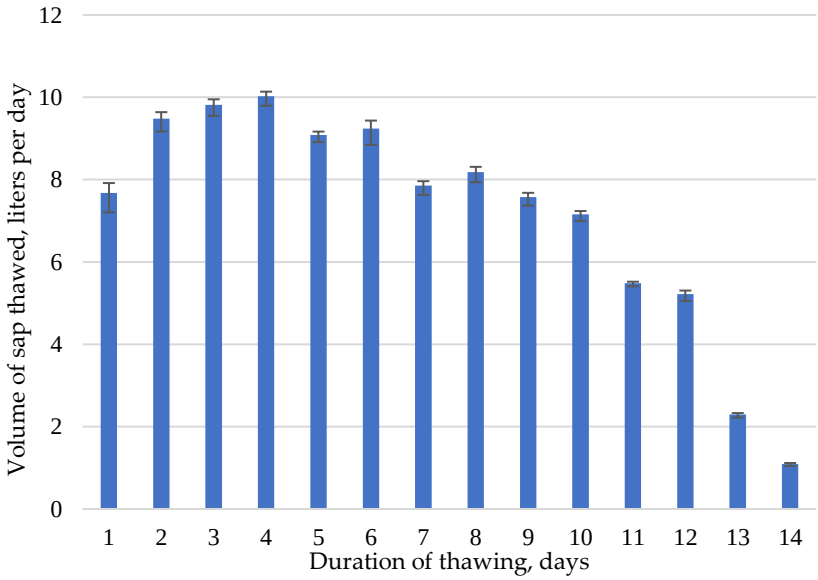

Figure 5. Dynamics of the amount of sap defrosted at room temperature, depending on the day of thawing.

3.3. Effect of Thawing on Sap Quality Indicators

The changes in the chemical composition of the sap during thawing were evaluated every day (Figure 6). The highest amount of soluble solids was found in the sap thawed during the first day, as much as 6.4 percent. On the second day, the soluble solids concentration measured almost three times lower and was equal to 2.1 percent.

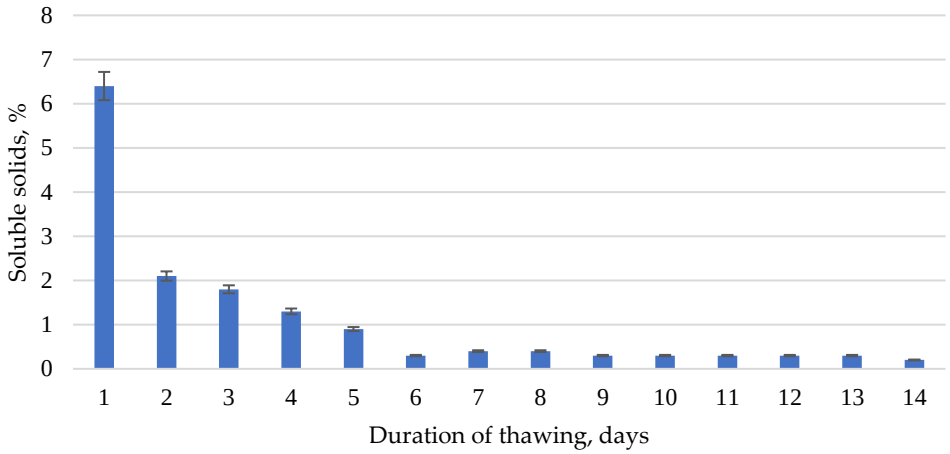

Figure 6. Changes in the soluble solids concentration in birch sap, depending on the day of thawing.

The results of this study support the assumption that when birch sap freezes, water freezes first, followed by sap with an increasing concentration of soluble solids. During thawing, sap with a high concentration of soluble solids melts first; then, sap with a decreasing soluble solids concentration thaws later. During thawing at room temperature, the concentration of soluble solids in the sap stabilized from the 6th day, because the concentration of dissolved substances in the remaining ice was very low.

The active acidity pH of the thawed birch sap did not change for the first 6 days and was about 5.5; then, the pH rose (Figure 7), which indicates that, at the very end of melting, the remaining ice consisted of almost pure water with a pH of or close to 7.0.

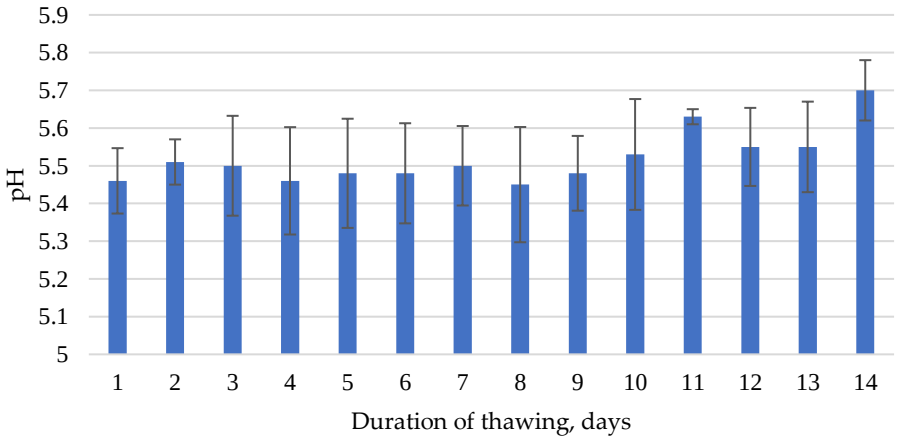

Figure 7. Changes in the active acidity (pH) of birch sap during thawing.

As sap thaws, it should ferment as the thawing time increases, and, therefore, the pH should drop, but the research results show the opposite. This is explained by the fact that the part of the frozen juice that contains a lot of sugars, organic acids, and micro- and macroelements thaws at the beginning, and then the liquid that melts later consists of almost pure water, which does not ferment without sugar, or ferments slowly; therefore, the pH increases as the time of thawing increases.

During thawing, the amount of organic acids, i.e., the titratable acidity, decreased exponentially alongside the concentration of total soluble solids (Figure 8). On the first day, the titratable acidity of the thawed sap was as much as 13.1 mg-equiv/100 mL; on the second day, it reached only 0.48 mg-equiv/100 mL. From the 6th day of dissolution, the titratable acidity practically did not change. This shows that during thawing, birch sap with a high sugar content ferments quite actively, while sap with a low soluble solids concentration ferments very slowly.

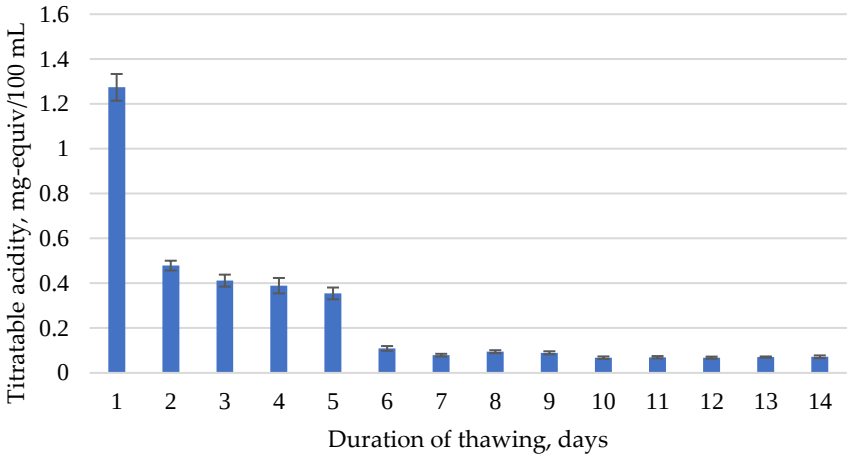

Figure 8. Changes in the titratable acidity of birch sap during thawing.

Like the pH, the electrical conductivity of the thawed sap also changed. As the thawing time increased, the conductivity decreased (Figure 9); this is because every day, as the sap thawed, it contained less sugars, organic acids, and micro- and macroelements.

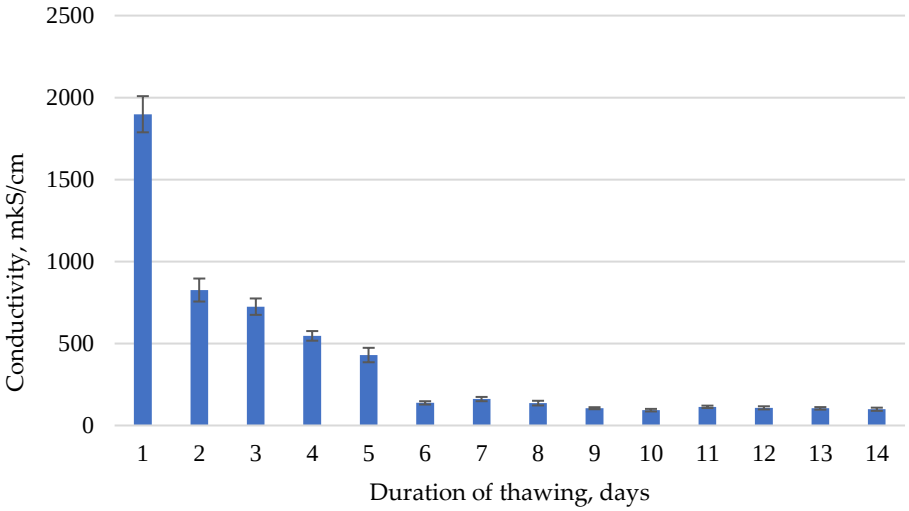

Figure 9. Changes in the electrical conductivity of birch sap during thawing.

3.4. Composition and Physicochemical Indicators of Thawed Sap at Different Depths of the Container

During the thawing of frozen birch sap at room temperature, the chemical composition of the sap and its physicochemical indicators at different depths of the container were investigated. It was determined that on the surface of the container (0–10 percent of the depth), the sap contained only 0.2 percent soluble solids. The concentration of soluble solids increased very little with depth, but on the bottom of the container (90%–100% of the depth), the concentration of soluble solids reached 5.7–6.1 percent (Figure 10).

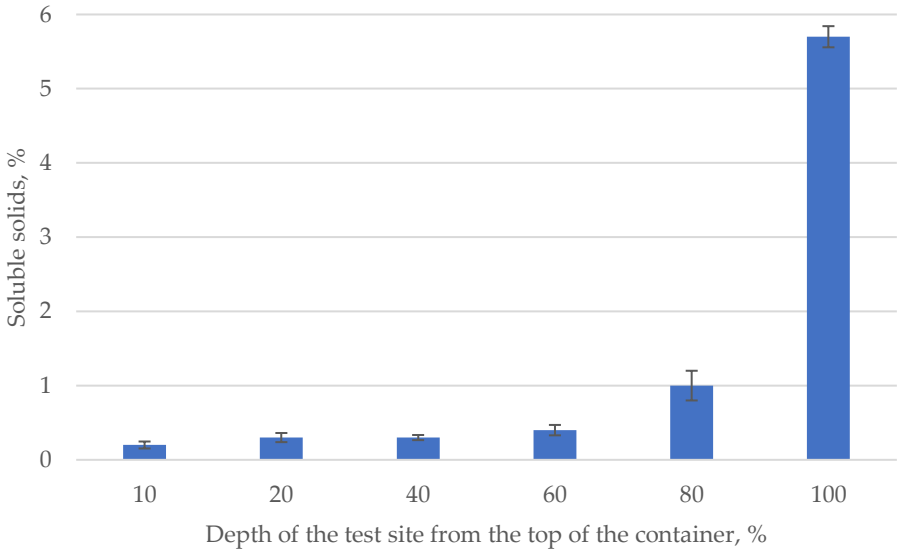

Figure 10. Distribution of soluble solids at different depths of the thawed sap container.

The active acidity (pH) of the sap at the top of the container was measured to be neutral and equal to 6.6 (Figure 11), but in the deeper layers, a lower pH was determined; i.e., the deeper the layer of thawed sap, the more acidic it was. This can be explained by the fact that a higher soluble solids concentration, and, accordingly, a higher concentration of sugars, leads to faster/more intense fermentation of natural, unpasteurized, frozen and thawed sap. At the bottom of the container, where the concentration of soluble solids reached 5.7–6.1 percent (Figure 11), the pH reached only 4.66.

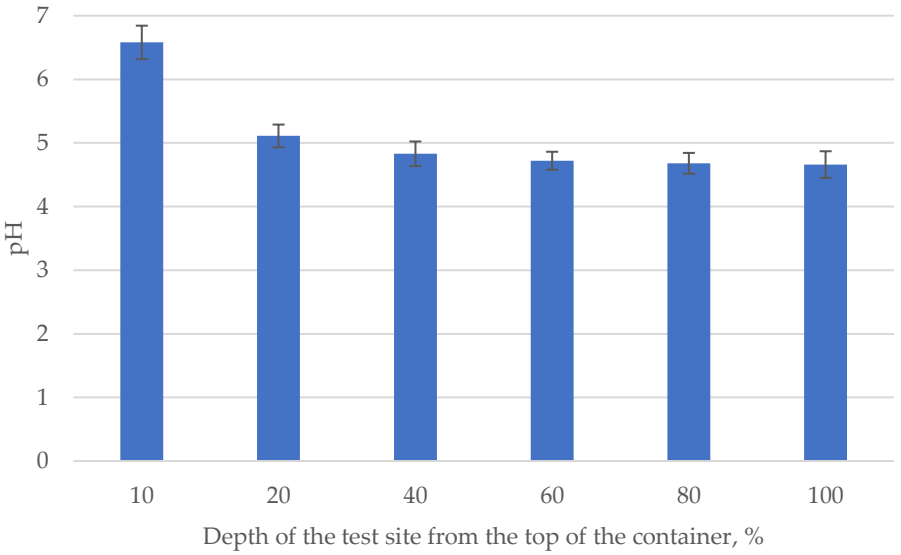

Figure 11. Active acidity (pH) of unfrozen sap at different depths of the container.

A corresponding but inverse regularity was also found when studying the titratable acidity in different layers of the depth of the container (Figure 12): at the top of the container, almost no organic acids were detected, while in the lower layer of the container, the titratable acidity of the sap reached 63.4 mg-equiv/100 mL.

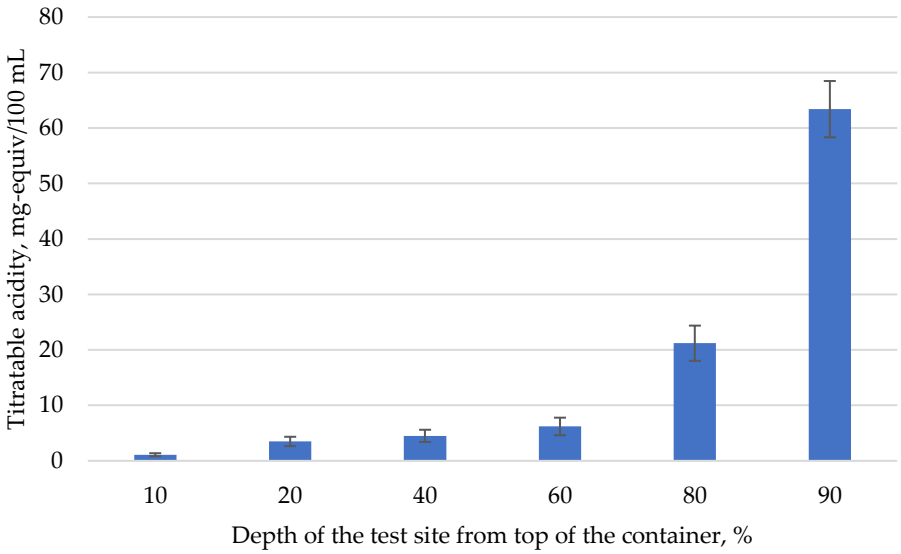

Figure 12. Titratable acidity within different layers of unfrozen sap.

Changes similar to those for the titratable activity were also determined when examining the electrical conductivity of the thawed sap in different depth layers (Figure 13): The conductivity increased slowly from 235 µS/cm in the upper layer of the thawed sap, but near the bottom, the conductivity increased significantly and reached a value of 2750 µS/cm. The average conductivity value of the sap in the mixed container was 748 ± 37 µS/cm.

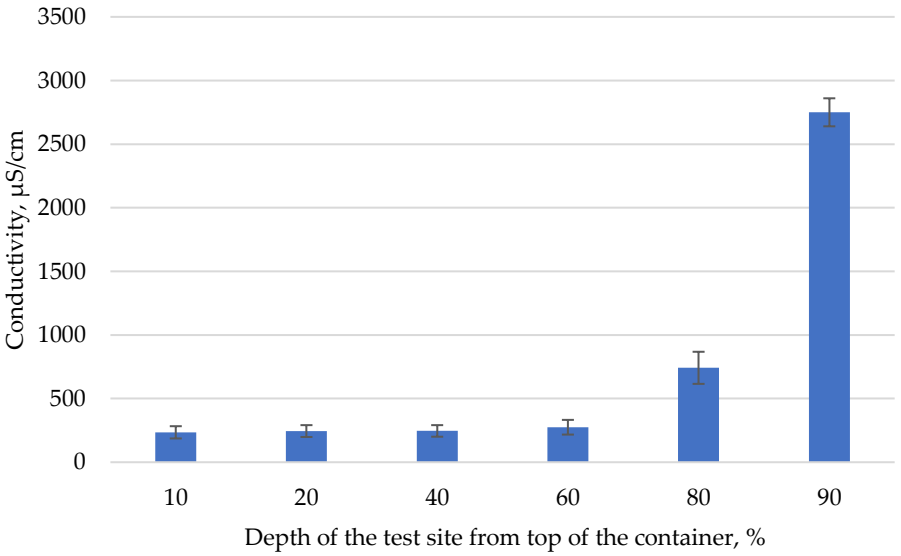

Figure 13. Changes in electrical conductivity within different depths of the unfrozen sap container.

Color is an important indicator used by consumers when choosing products. In industry, color indicators are studied with colorimetric devices. The light factor L* represents the brightness of the medium under study from black (L* = 0) to white (L* = 100) [22]. During the investigation, it was found that L* = 48.8 for the thawed sap on the surface of the container. Together with the amount of matter dissolved in the sap, the brightness coefficient also increased and reached a maximum value of 51.5 in the middle of the container, remaining stable until the bottom (Table 2). The darker color of the sap indicates the intensity of the fermentation process; with more dissolved material, fermentation takes place more intensively in the deeper layers of the thawed sap.

Table 2. CIELab color coordinates of the thawed sap depending on the depth in the container and comparison to fresh sap.

Depth	L*	a*	b*	C*	h°	ΔE*ab
Fresh	51.6 ± 0.31 a	−0.5 ± 0.07 a	0.3 ± 0.15 c	0.6 ± 0.13 c	145.4 ± 9.31 a,b	-
10	48.8 ± 0.01 c	−0.6 ± 0.18 a	2.5 ± 0.09 a	2.6 ± 0.12 a	102.9 ± 3.28 c	3.48
20	50.5 ± 0.00 b	−0.6 ± 0.15 a	0.7 ± 0.09 b	0.9 ± 0.13 b	127.3 ± 7.19 b,c	1.13
30	51.2 ± 0.04 a	−0.7 ± 0.07 a	0.4 ± 0.09 c	0.8 ± 0.09 b,c	153.1 ± 5.36 b	0.44
40	51.3 ± 0.03 a	−0.7 ± 0.11 a	0.4 ± 0.21 c	0.8 ± 0.19 b,c	153.1 ± 9.62 b	0.30
50	51.6 ± 0.06 a	−0.7 ± 0.03 a	0.3 ± 0.05 c	0.8 ± 0.01 b,c	155.3 ± 4.02 b	0.26
60	51.6 ± 0.02 a	−0.7 ± 0.06 a	0.2 ± 0.11 c	0.8 ± 0.04 b,c	162.2 ± 8.86 b	0.29
70	51.5 ± 0.04 a	−0.6 ± 0.04 a	0.2 ± 0.13 c	0.6 ± 0.03 c	164.8 ± 12.2 b	0.23
80	51.7 ± 0.02 a	−0.7 ± 0.07 a	0.3 ± 0.07 c	0.7 ± 0.09 b,c	157.0 ± 2.91 b	0.23
90	51.6 ± 0.01 a	−0.6 ± 0.13 a	0.2 ± 0.02 c	0.6 ± 0.13 b,c	159.5 ± 2.89 b	0.19
100	51.1 ± 0.61 a	−0.5 ± 0.09 a	0.4 ± 0.17 c	0.7 ± 0.05 b,c	147.7 ± 16.68 a,b	0.42

Note: Different letters in the same column indicate statistically significant differences between the individual color coordinates depending on the depth in the container ($p < 0.05$).

Color coordinates a* and b* at a value of 0 indicate that the medium is colorless or grey. On the horizontal axis, positive a* values indicate that the test medium is pink, and negative values indicate that the medium is bluish/greenish [22]. During the investigation, it was found that the thawed sap was slightly greenish/bluish (Table 2), but this is not related to the fermentation process due to the uniform distribution of this shade throughout the container.

On the vertical axis of the CIELAB color coordinates (L*, a*, b*), a positive indicator b* indicates yellow, while a negative value indicates blue colors [22]. The small (0.18–0.82) positive values of the indicator b* obtained during the thawing study (Table 2) showed that the thawed sap was characterized by a slightly yellowish shade, which often occurs in fresh sap as well. At the top of the container, this shade was more intense (0.82–0.73); in deeper layers, it ranged within 0.2–0.3. It can be concluded that a higher lightness (or turbidity, in the case of sap) L* obscures the yellowish shade.

The hue angle h° and chroma C* are considered more appropriate indicators in color measurements. In the case of this study (Table 2), the highest chroma value was found at the surface layer; the chroma values of deeper samples decreased, as did the yellowness b*, with increasing brightness L* (turbidity).

The tendency of the value of the hue angle in this study was inversely proportional to the tendency of the b* and C indicators, depending on the depth of the container.

In birch sap with higher levels of soluble solids in its deeper layers, the electrical conductivity was also higher, as depicted in Figure 14. Furthermore, the electrical conductivity increased in proportion to the total amount of micro- and macro-elements, as shown in Figure 15. These results indicate that upon thawing, not only sugar but also micro- and macro-elements accumulate at the bottom of the container.

Birch sap is an ideal medium for microorganisms because it contains enough available organic compounds and mineral substances, so the sap ferments rather quickly [10,20]. It was determined that in the upper part of the container (where the depth of the thawed sap was 10%–30% the depth of the sap in the container) the total amount of microorganisms was even lower than that in fresh birch sap, at 1.27 log colony-forming units (CFU) per milliliter and 1.60 log CFU/mL, respectively. Meanwhile, at the bottom of the container (at which the depth of thawed juice in the container was 80%–100%), where the soluble matter content measured up to 7.5%, the total amount of microorganisms reached as much as 3.25 log CFU/mL. Pathogenic microorganisms *E. coli* and *Salmonella* were not detected in the thawed sap at various depths, while molds were found in amounts of <1 log CFU/mL.

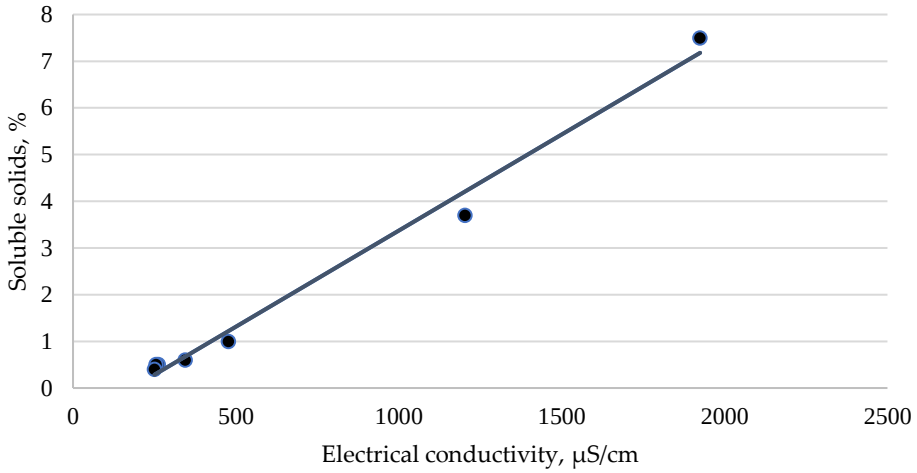

Figure 14. Effect of soluble solids in unfrozen sap on electrical conductivity.

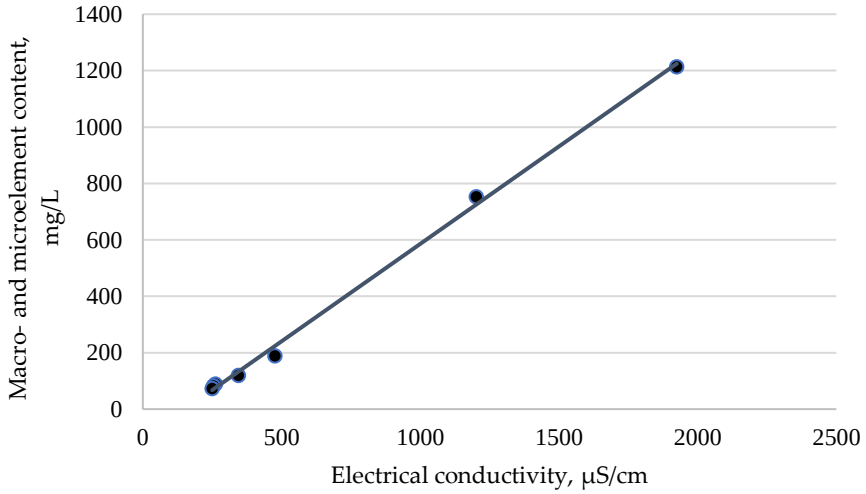

Figure 15. Effect of the total amount of micro- and macroelements in thawed sap on electrical conductivity.

4. Discussion

Birch sap is known for its nutritional value and health benefits; therefore, to better use birch sap as a beverage or food ingredient, it is important to consider its quality preservation during storage [7,28–30]. One of the best ways to preserve the quality of birch sap is to freeze it and, if necessary, thaw it before use or any further processing. Thus, the study of changes in the quality of birch sap during freezing and thawing is very important [31]. According to our data and other researchers [19,20], the quality indicators of frozen and thawed birch sap in containers with a capacity of 10 L or less do not differ from those of fresh birch sap or differ within the error limits. A container of 1000 L of sap with an initial temperature of 8 °C freezes within about 14 days in a freezer where the temperature is maintained at −18 °C. When the process of ice formation begins, the proportion of soluble solids in the solution begins to increase because pure water freezes first; that is, the sap concentrates.

During the process of thawing, the quality indicators of the sap changed. The highest amount of soluble dry matter was found in the sap thawed during the first day, as much as 6.4 percent. On the second day, the concentration of soluble matter was almost three times lower and was equal to 2.1 percent.

After examining the color indicators, it was found that the lightness coefficient was L* = 48.8 for the thawed sap on the surface of the container. Together with the amount of substances dissolved in the sap, the brightness coefficient also increased, reaching a

maximum value of 51.5 in the middle of the container and remaining stable until the bottom (Table 2). The brightness of the color was associated with an increase in turbidity. The darker color of the juice indicated the intensity of the fermentation process; with more dissolved substances, fermentation took place more intensively in the deeper layers of the thawed sap.

At the top of the container, this shade was more intense (0.82–0.73); in the deeper layers, it varied within 0.2–0.3. It can be concluded that a higher lightness (or turbidity, in the case of sap) L* obscures the yellowish shade.

As the ice melted, the concentration of acids and other soluble substances in the sap decreased, but changes in qualitative indicators indicated the beginning of fermentation processes; a change in color and pH occurred as the temperature of the thawing sap became positive. As a result, to freeze raw sap in large-volume containers, it is necessary to develop fast defrosting technology using auxiliary means—circulation, external energy sources, and mechanical ice crushing.

5. Conclusions

Birch sap is a beverage known for its nutritional value and health benefits. It is important to preserve the quality of birch sap during storage to ensure its optimal use as a food ingredient. One of the best methods to achieve this is to freeze the sap and then thaw it before further processing or consumption. Therefore, the study of how freezing and thawing affects the quality of birch sap is crucial. Based on the obtained results of birch sap freezing research, it can be assumed that even in a container with a capacity of 1000 L, when ice crystals form, pure water freezes first, and only later do sap crystals with an increasing amount of soluble solids begin to form. After freezing birch sap in 1000 L containers, thawing at room temperature can take up to 14 days. During thawing, the quality of the birch sap changes: The sap becomes layered, and different amounts of soluble solids, sugars, organic acids, and micro- and macroelements are found in different layers. Similarly, various physicochemical indicators, such as the pH, electrical conductivity, and CIELab color coordinates, change. However, when birch sap is frozen in containers of 10 L capacity or less, its quality is not very different from that of fresh sap. Therefore, it is necessary to use auxiliary means to thaw large quantities of sap faster, such as circulation, external energy sources, or mechanical crushing of ice.

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References

1. Jeong, S.; Lee, C.; Kim, H.; Lee, S.; Hwang, I.; Shin, C.; Lee, J.; Jeong, H. Quality Characteristics of the White Birch Sap with Varying Collection Periods. *Korean Soc. Food Sci. Nutr.* **2012**, *41*, 143–148. [CrossRef]
2. Bilek, M.; Stawarczyk, K.; Olszewski, M.; Kędziora, K.; Cieślik, E.; Kuźniar, P. Evaluation of the Content of Inorganic Anions in Tree Saps. *J. Elem.* **2016**, *12*, 1277–1288. [CrossRef]
3. Svanberg, I.; Sõukand, R.; Łuczaj, Ł.; Kalle, R.; Zyryanova, O.; Dénes, A.; Papp, N.; Nedelcheva, A.; Šeškauskaitė, D.; Kołodziejska-Degórska, I.; et al. Uses of Tree Saps in Northern and Eastern Parts of Europe. *Acta Soc. Bot. Pol.* **2012**, *81*, 343–357. [CrossRef]
4. Semjonovs, P.; Denina, I.; Fomina, A.; Patetko, A.; Auzina, L.; Upite, D.; Upitis, A.; Danilevics, A. Development of Birch (*Betula pendula* Roth.) Sap Based Probiotic Fermented Beverage. *Int. Food Res. J.* **2014**, *21*, 1763–1767.

5. Jeong, S.J.; Jeong, H.S.; Woo, S.H.; Shin, C.S. Consequences of Ultrafiltration and Ultraviolet on the Quality of White Birch (‘*Betula platyphylla*’ var. Japonica) Sap during Storage. *Aust. J. Crop Sci.* **2013**, *7*, 1072–1077.
6. Shen, Y.B.; Kazusaka, A.; Fujita, S.; Terazawa, M. Preventative properties of birch sap against oxidative stress in rats. In *Tree Sap II*; Terazawa, M., Ed.; Hokkaido University Press: Sapporo, Japan, 2021; pp. 147–151.
7. Viškelis, P.; Rubinskienė, M. Changes of Birch Sap Quality Indices during Storage. *Sodininkystė Daržininkystė* **2012**, *31*, 63–73. (In Lithuanian)
8. Grabek-Lejko, D.; Kasprzyk, I.; Zaguła, G.; Puchalsky, C. The bioactive and mineral compounds in birch sap collected in different types of habitats. *Balt. For.* **2017**, *23*, 394–401.
9. Mingaila, J.; Čiuldienė, D.; Viškelis, P.; Bartkevičius, E.; Vilimas, V.; Armolaitis, K. The Quantity and Biochemical Composition of Sap Collected from Silver Birch (*Betula pendula* Roth) Trees Growing in Different Soils. *Forests* **2020**, *11*, 365. [CrossRef]
10. Staniszewski, P.; Bilek, M.; Szwerc, W.; Tomusiak, R.; Osiak, P.; Kocjan, R.; Moskalik, T. Effect of tree age, daily sap volume and date of sap collection on mineral and heavy metal content in silver birch (*Betula pendula* Roth) tree sap. *PLoS ONE* **2020**, *15*, e0244435. [CrossRef]
11. Ozolinčius, R.; Bareika, V.; Rubinskienė, M.; Viškelis, P.; Mažeika, R.; Staugaitis, G. Chemical Composition of Silver Birch (*Betula pendula* Roth.) and Downy Birch (*Betula pubescens* Ehrh.) Sap. *Balt. For.* **2016**, *22*, 222–229.
12. Bilek, M.; Cebula, E.; Krupa, K.; Lorenc, K.; Adamowicz, T.; Sosnowski, S. New technologies for extending shelf life of birch tree sap. *Econtechmod* **2018**, *7*, 3–8.
13. Ross, R.P.; Morgan, S.; Hill, C. Preservation and Fermentation: Past, Present and Future. *Int. J. Food Microbiol.* **2002**, *79*, 3–16. [CrossRef]
14. Sionek, B.; Szydłowska, A.; Küçükgöz, K.; Kołożyn-Krajewska, D. Traditional and New Microorganisms in Lactic Acid Fermentation of Food. *Fermentation* **2023**, *9*, 1019. [CrossRef]
15. Weiss, G.; Emery, M.R.; Corradini, G.; Živojinović, I. New values of non-wood forest products. *Forests* **2020**, *11*, 165. [CrossRef]
16. Purwestri, R.C.; Hájek, M.; Šodková, M.; Jarskỳ, V. How are wood and non-wood forest products utilized in the Czech Republic? A preliminary assessment of a nationwide survey on the bioeconomy. *Sustainability* **2020**, *12*, 566. [CrossRef]
17. Enescu, C.M. Collection and use of birch sap, a less known non-wood forest product in Romania. *Sci. Pap. Ser. Manag. Econ. Eng. Agric. Rural Dev.* **2017**, *17*, 191–194.
18. Christensen, K.V.; Valgren, M.; Toft Hansen, L.; Hansen, K. Concentration of Birch Sap using MF, RO and DCMD. In Proceedings of the Nordic Filtration Symposium, København, Denmark, 25–26 August 2008.
19. Nikolajeva, V.; Sommerer, Z. Changes of Physicochemical Properties and Predominant Microbiota during Storage of Birch Sap. *Int. Food Res. J.* **2018**, *25*, 527–533.
20. Sancho, A.I.; Birk, T.; Gregersen, J.M.; Rønne, T.; Hornslet, S.E.; Madsen, A.M.; Bøgh, K.L. Microbial safety and protein composition of birch sap. *J. Food Comp. Anal.* **2022**, *107*, 104347. [CrossRef]
21. AOAC. Acidity (titratable) of fruit products. In *Official Methods of Analysis*, 17th ed.; Helrich, K., Ed.; Official Method 942.15; Association of Official Analytical Chemists Inc.: Arlington, TX, USA, 2000.
22. McGuire, R.G. Reporting of Objective Color Measurements. *Hort Sci.* **1992**, *27*, 1254–1255. [CrossRef]
23. Yoo, W.S.; Kang, K.; Kim, J.G.; Yoo, Y. Extraction of Color Information and Visualization of Color Differences between Digital Images through Pixel-by-Pixel Color-Difference Mapping. *Heritage* **2022**, *5*, 3923–3945. [CrossRef]
24. Viršilė, A.; Laužikė, K.; Sutulienė, R.; Brazaitytė, A.; Kudirka, G.; Samuolienė, G. Distinct Impacts of UV-A Light Wavelengths on Nutraceutical and Mineral Contents in Green and Purple Basil Cultivated in a Controlled Environment. *Horticulturae* **2023**, *9*, 1168. [CrossRef]
25. *ISO 4833-1:2013*; Microbiology of the Food Chain. Horizontal Method for the Enumeration of Microorganisms Part 1: Colony Count at 30 °C by the Pour Plate Technique. ISO: Geneva, Switzerland, 2013.
26. *ISO 21527-1:2008*; Microbiology of Food and Animal Feeding Stuffs—Horizontal Method for the Enumeration of Yeasts and Moulds—Part 1: Colony Count Technique in Products with Water Activity Greater than 0.95. ISO: Geneva, Switzerland, 2008.
27. Pitt, J.I.; Hocking, A.D. *Fungi and Food Spoilage*, 3rd ed.; Springer: New York, NY, USA, 2009.
28. Kūka, M.; Čakste, I.; Geršebeka, E. Determination of bioactive compounds and mineral substances in Latvian birch and maple saps. *Proc. Latv. Acad. Sci. Sect. B Nat. Exact Appl. Sci.* **2013**, *67*, 437–441. [CrossRef]
29. Bilek, M.J.; Wawer, J.; Szwerc, W.; Słowik, K.; Sosnowski, S. Birch sap concentrate as a potential modern food product. *Econtechmod Int. Q. J. Econ. Technol. Model. Process.* **2018**, *7*, 5–9.
30. Peev, C.; Dehelean, C.; Mogosanu, C.; Feflea, S.; Corina, T. Spring drugs of *Betula pendula* Roth: Biologic and pharmacognostic evaluation. *Stud. Univ. Vasile Goldis Arad Ser. Stiintele Vietii* **2010**, *20*, 41–43.
31. Bilek, M.; Vietoris, V.; Ilko, V. Shelf life extension and sensory evaluation of birch tree sap using chemical preservatives. *Potravin. Slovak J. Food Sci.* **2016**, *10*, 499–505.