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New technologies for extending shelf life of birch tree sap

M. Bilek[1], E. Cebula[1], K. Krupa[1], K. Lorenc[1], T. Adamowicz[2], S. Sosnowski[1]

[1] Department of Food and Agriculture Production Engineering, Faculty of Biology and Agriculture,
University of Rzeszów, Zelwerowicza 4, PL 35 601 Rzeszów, Poland, e-mail: mbilek@ur.edu.pl,
ewelina.cebula07@wp.pl, krpklaudia16@gmail.com, kamilaa.katarzyna@gmail.com, ssos@ur.edu.pl
[2] MA, Białostoczek 26, PL 15 592 Białystok, Poland; e-mail: tomasz@tma.pl

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Abstract. Three experiments were carried out to assess the effectiveness of the so-called new technologies in extending birch tree sap shelf life. In the first one, microfiltration of birch sap was carried out using 0.22 μm, 0.45 μm and 0.8 μm filters. In the second experiment, ultraviolet radiation was applied for 5, 15 and 45 minutes. In the third experiment ultrasound was applied with one-, two-, three- and four-fold use. Regardless of the pore diameter, all the microfiltered samples were non-perishable for at least a month at room temperature. The efficacy of ultrasound was also demonstrated. The four-fold use of ultrasound proved to be the most effective and prolonged the shelf life of birch sap from one to two days at room temperature and from four to twelve days in refrigeration conditions. In turn, the use of ultraviolet radiation did not extend the shelf life of birch sap, although in refrigeration conditions the clouding was significantly inhibited. However, there is no doubt that among the so-called new technologies, microfiltration is the most advantageous in extending shelf life of birch sap and has perspectives for use in food industry.

Keywords: birch tree sap, microfiltration, ultraviolet, ultrasound, shelf life, clean label.

INTRODUCTION

Food products that were not subjected to high temperature treatment and are free from sweeteners and additives, have become increasing popular in the XXI century. Such requirements guarantee maintaining the unchanging composition of the starting raw material and protection of pro-health properties. These consumer demands have become the basis for major trends in food industry, i.e. "clean label food products" and "non-processed foods" [1, 15]. In this context, the processing of birch sap seems to be an exceptional issue. Birch sap is a well-known traditional food product of Central and Eastern Europe with scientifically confirmed nutritional and medical benefits [16, 19]. The main problem associated with the consumption of birch sap is its low shelf life. The loss of shelf life takes place after about one day at room temperature and after three to four days under refrigeration conditions. This phenomenon is associated with the presence of numerous species of bacteria and yeasts, that contaminate sap at the time of collection [13]. Therefore, a wide range of bottled birch sap with high shelf life is available. However, all these beverages are pasteurized. They also contain sweeteners, flavour additives and food additives to extend shelf life. Simultaneously, a number of non-perishable and not pasteurized beverages have also been developed. They can be prepared by consumers who collect birch sap on their own. The shelf life of these beverages is extended by the addition of food acids, such as citric, lactic and malic acid. In turn, their taste is balanced with sweetening additives with a pro-healthy character, e.g. honey [3, 4]. In both cases the original composition and taste of sap is thoroughly changed. This, in turn, is inconsistent with the trend of "non-processed foods" and "clean label food products". Consequently, attempts have been made to extend the shelf life of birch sap using the so-called new technologies of the food industry, specifically microfiltration. As a result, months of shelf life were obtained, analogously to pasteurized sap. This effect was achieved without any interference with the sap composition and without thermal processing [2].

Membrane techniques, including microfiltration [21, 22], are not the only ones that may have potential application in the shelf life extending of food products. In this context the most often mentioned possibility is the use of ultrasound [8, 10] and ultraviolet radiation [6, 23]. For this reason, the aim of this work was to estimate the usefulness of ultraviolet radiation and ultrasound in extending the shelf life of birch sap, as well as optimizing the previously developed microfiltration method of birch sap.

MATERIAL AND METHODS

Birch sap collecting. Tree sap of silver birch (*Betula pendula* Roth.) was collected in Łukawiec village (50.097559, 22.168178), in accordance with the suggestions of Yoon et al. [25]. The collection was carried out using the drilling technique, with the drill sized 16 mm, to the depth of 4–5 cm, on the south side of tree trunk. The tree sap was collected simultaneously from four trees, mixed together and then frozen. The birch sap was placed in glass vessels and thawed in water bath just before the experiments described below.

Microfiltration experiment. In this experiment, we used the procedure described in detail in the earlier work [2], and thus the method of sterilization and filling of cuvettes was the same. The difference was that we used filters with three different pore diameters, i.e. 0.22 μm, 0.45 μm and 0.8 μm made of Mixed Cellulose Esters membrane (Millex, Merck Millipore). 24 samples were prepared, i.e. six samples of unfiltered birch sap, six samples filtered with 0.22 μm filters, six samples filtered with 0.45 μm filters and finally six samples filtered with 0.8 μm filters. The experiment was conducted only at room temperature.

Ultraviolet experiment. The experiment was carried out in the V9 sterilizer (TMA, Białostoczek) dedicated for the decontamination of drinking water, using a 15 W radiator, a radiation power (at wavelength 254 nm) of 4 W and the capacity of about 980 mL. The sap was stored inside ultraviolet (UV) sterilizer, which in each case was disinfected with ethanol 70 %. After filling with sap, the inlet and outlet of UV sterilizer were permanently protected with aluminium foil, which was carefully fired. Sap completely filled the interior of the sterilizer, so there was no air space inside. This security scheme forced only a single opening of the sterilizer, just before the measurement. The experiment was carried out in two temperature conditions, i.e. at room temperature and refrigeration conditions (4 °C). The scheme of UV application is presented in Table 1.

Table 1. The scheme of the UV application

Sample name	*Room temperature / refrigeration conditions*
Birch sap	Without the use of ultraviolet
Birch sap UV x 5 min.	Application of ultraviolet for 5 minutes
Birch sap UV x 15 min.	Application of ultraviolet for 15 minutes
Birch sap UV x 45 min.	Application of ultraviolet for 45 minutes

Ultrasound experiment. The sap was treated with ultrasound (US) directly in the sterilized cuvettes of an optical densitometer. Cuvettes were protected against air access with aluminium foil. The protocol of filling the cuvettes was the same as in the case of microfiltration [2]. The source of ultrasound was Sonic 0.5 ultrasonic bath (PolSonic) with the following work parameters: 40 kHz, 2x80 W, 15 minutes. The scheme of US application has been presented in Table 2. Five batches consisted of six samples each. Three of them were stored at room temperature and three in the refrigeration conditions (4 °C).

Table 2. The scheme of the US application

Sample name	*Room temperature / refrigeration conditions*
Birch sap	Birch tree sap without the use of ultrasound
Birch sap US x 1	Birch tree sap after ultrasound application once
Birch sap US x 2	Birch tree sap after ultrasound application twice at two-hour interval
Birch sap US x 3	Birch tree sap after ultrasound application three times at two-hour intervals
Birch sap US x 4	Birch tree sap after ultrasound application four times at two-hour intervals

Instrumental analysis. All the samples were tested using the so-called optical density meter DEN-1B (BioSan), which measured the absorbance at 565±15 nm, within the measuring range from 0 to 15 MFU (McFarland Unit), at the resolution of 0.01 MFU and with the measurement accuracy of±3 %. The measured value of the optical density is the result of the turbidity of the sample. Before each measurement, the cuvettes in which the experiment was conducted were shaken several times. Each measurement was performed three times and the average values are presented in the Figures.

RESULTS AND DISCUSSION

Microfiltration experiment. The initial optical density of all the tested samples was zero. A significant turbidity was observed just after three days of the storage test for all the unfiltered samples in the range 0,38 to 0.47 MFU. In subsequent measurements, a gradual increase in the optical density was observed for all the samples in the unfiltered batch, with the values on the last day of the test ranging from 0.39 to 0.77 MFU. For all the samples to which microfiltration was applied, independently of pore diameter, the initial zero value of the optical density was maintained throughout the one-month test (Fig. 1).

Ultraviolet experiment. The initial optical density of all the tested samples was zero. For the samples stored at room temperature, both after UV treatment for 5, 15 and 45 minutes, a significant optical density was observed on the third day of the test. It was respectively 0.42 MFU, 0.8 MFU and 0.3 MFU. Thus, it has been shown that the samples after UV treatment do not differ in terms of shelf life from the sample to which UV has not been applied (0.61 MFU) (Fig. 2). In turn, in the case of refrigeration conditions, the inhibitory effect of UV on the clouding of birch sap has been clearly demonstrated. On the tenth day of the storage test, the turbidity of the samples after UV treatment for 15 and 45 minutes was significantly lower than the turbidity of the sap reference sample. However, in both cases, trace turbidity was observed. It was respectively 0.04 MFU and 0,01 MFU, wherein the optical density of the birch sap reference sample was 0.2 MFU on the tenth day of storage test (Fig. 3).

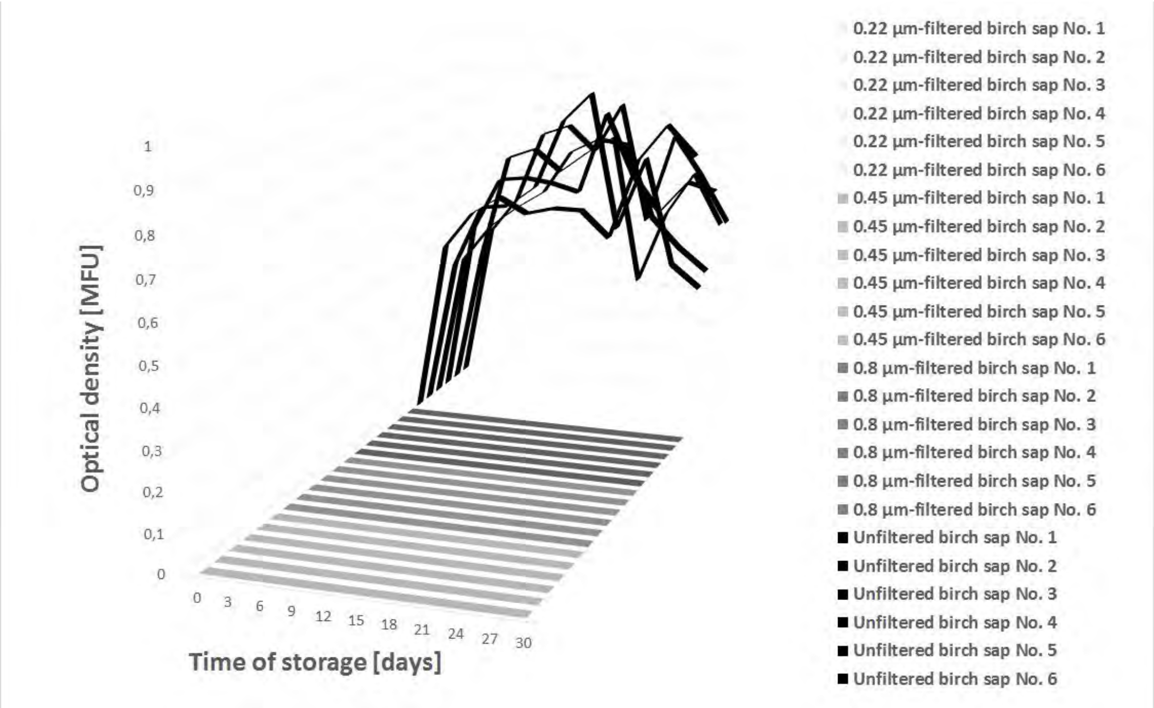

Fig. 1. Changes in the optical density of unfiltered birch sap and microfiltered birch sap during the one-month storage test at room temperature

Ultrasound experiment. On the second day of storage test conducted at room temperature the increase in turbidity was observed in untreated (control) birch sap samples and birch sap samples treated with US one, two and three times (Fig. 4–7). Only four-fold application of ultrasound was successful in extending the shelf life at room temperature. It allowed to retain turbidity one day longer, i.e. the shelf life of three samples was three days (Fig. 8). The effectiveness of ultrasound application was clearly visible in refrigeration conditions. The shelf life of birch sap to which US was not applied was four days (Fig. 4). The single US application did not affect the shelf life of birch sap in refrigeration conditions (Fig. 5), The US application twice extended the shelf life period of birch sap to five days, but only for one of the three samples tested (Fig. 6). The US application three times extended the shelf life of all the samples to five days. However, the shelf life of one of them was seven days (Fig. 7). For the samples to which the US was applied four times, the shelf life was twelve days (Fig. 8).

Thermal effect of new technologies. The thermal effect of new technologies use should also be mentioned. It is important because of possible thermal decomposition of many birch sap components. In the case of microfiltration we did not observe any differences in the birch sap temperature before and after the filtration. In turn, the 15 minutes application of ultrasound caused an increase in the birch sap temperature from 22.4 °C to 27 °C. The use of UV for 5 minutes did not affect the temperature of birch sap, while the use of UV for 15 minutes increased it by 1 °C, and for 45 minutes by 3.9 °C. As it can be seen, the thermal effect observed has no effect on the results obtained.

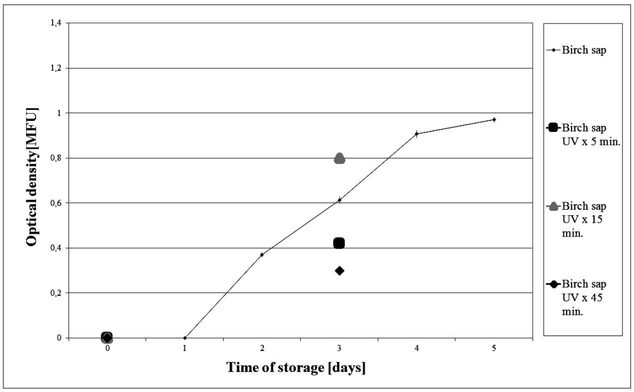

Fig. 2. Changes in the optical density of birch sap at room temperature during the 5-day storage test. Markers indicate the results of optical density measurements of birch sap treated with UV

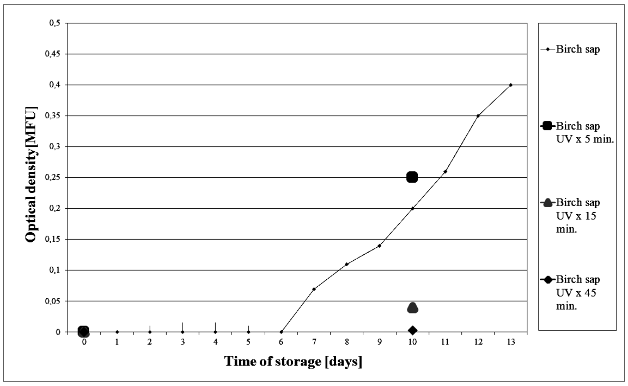

Fig. 3. Changes in the optical density of birch sap at refrigeration conditions during the 13-day storage test. Markers indicate the results of optical density measurements of birch sap treated with UV

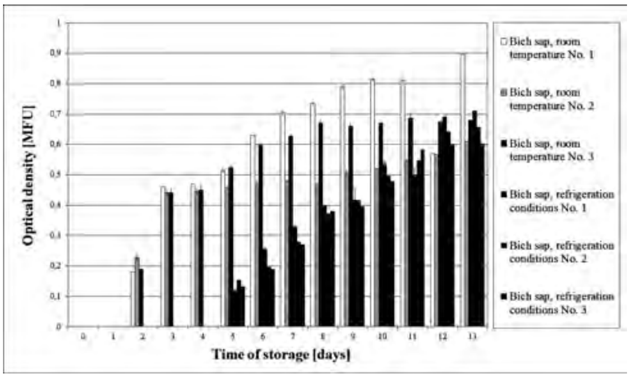

Fig. 4. Changes in the optical density of birch sap at room temperature and refrigeration conditions

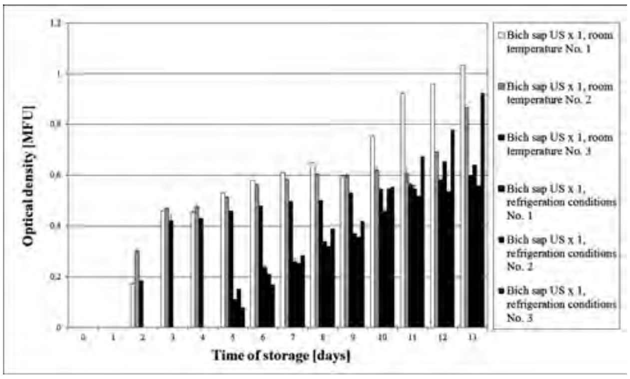

Fig. 5. Changes in the optical density of birch sap at room temperature and in refrigeration conditions after a single ultrasound application

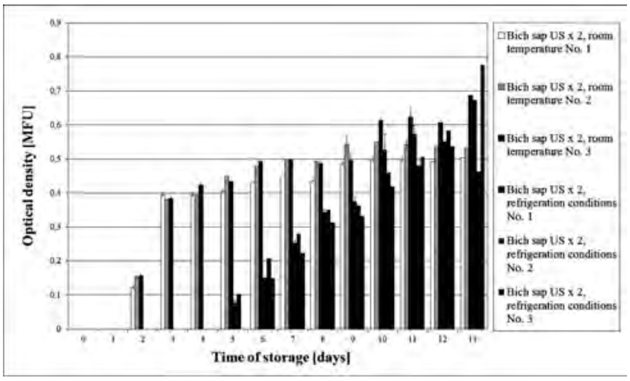

Fig. 6. Changes in the optical density of birch sap at room temperature and refrigeration conditions after applying ultrasound twice

The use of UV is commonly practiced in the food industry, primarily for the decontamination of water, air and production habitats. The use of UV to sterilize meat for example is prohibited by the European Union, but for unclear reasons [23]. A lot of controversies associated with the use of UV for food sterilization are caused by the possibility of lowering vitamin C content, causing oxidative changes in fats and the formation of free radicals [5]. Despite this, the so called "authorized novel food" such as UV-treated milk, UV-treated mushroom, UV-treated bread and UV-treated baker's yeast were placed on the "Union list of novel foods". Thanks to the use of UV, these products have an increased content of vitamin D_2 [24]. There are also further studies on various aspects of the UV effect on food. Among others, there are experiments on te decontamination of egg shells [18, 19]. In the study cited above [7], the influence of UV on the shelf life of birch sap was also investigated. Jeong et al. showed that UV did not extend the shelf life of birch sap at room temperature, while in refrigeration conditions it ensured a three-day shelf life [7]. It can be assumed that such a low efficiency of UV radiation is associated with the contamination of birch sap not only by bacteria, but mainly by yeast [13]. It is known that in the case of yeasts, the doses of UV radiation should be several times higher than in the case of bacteria [26]. The specificity of our research does not allow to determine the total shelf life of birch sap after UV treatment. But we proved that with the use of our V9 sterilizer it was not possible to extend the shelf life of birch sap in the four-day storage test at room temperature, as well as in the ten-day storage test at refrigeration conditions. Thus, our results are consistent with Jeong cited work [7]. However, it should be emphasized that in refrigeration conditions UV treatment for 15 and 45 minutes significantly inhibit clouding.

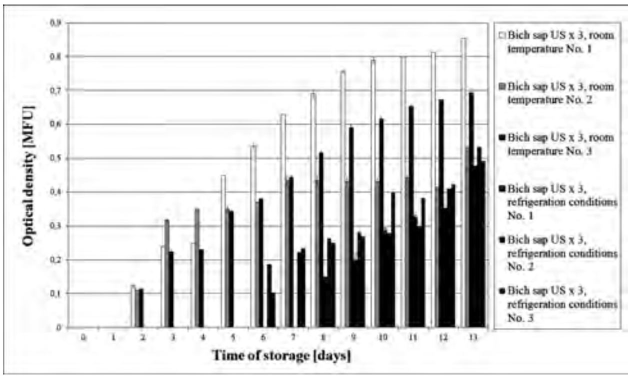

Fig. 7. Changes in the optical density of birch sap at room temperature and refrigeration conditions after applying ultrasound three times

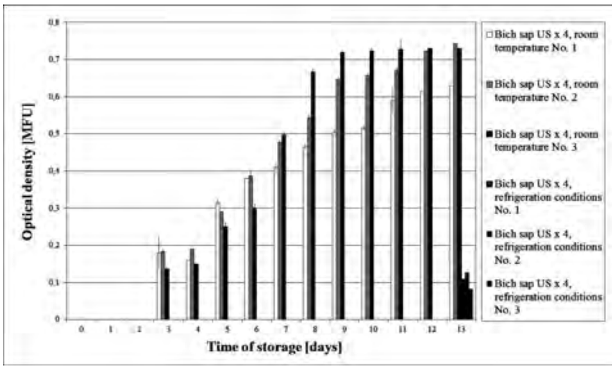

Fig. 8. Changes in the optical density of birch sap at room temperature and refrigeration conditions after applying ultrasound four times

It is also worth paying attention to the nearly two-week shelf life extension of birch sap by the application of ultrasound four times. This shelf life period may be comparable to the shelf life of sap with the addition of citric acid, stored in the refrigeration conditions [4], but when using ultrasound, we are consistent with the "clean label food products" idea, i.e. without the addition of substances that extend shelf life. This result seems to be the most interesting as until now it has been generally claimed that the US is not suitable for extending the shelf life of plant-based food products [14], and their effectiveness has been demonstrated only in the deactivation of yeast [9].

As a conclusion of our work, it should be clearly stated that among the presented new technologies only microfiltration creates real perspectives for practical use. In contrast to US and UV it allows to obtain shelf life at room temperature and, in terms of effectiveness, can be compared to pasteurization. However, it is worth continuing research into the application of other new technologies in extending the shelf life of birch sap. These could include, for example, the use of US with a higher power or repeated more frequently than only four times, as well as the use of modifications in the applied construction in the case of extending shelf life with UV.

CONCLUSIONS

1. The birch tree sap microfiltered under sterile conditions and into a sterile vessel is stable at least one month without any turbidity, at zero optical density and regardless of the pore diameter.
2. The use of ultraviolet radiation does not extend the shelf life of birch sap. However, the use of UV for 15 and 45 minutes and next storage under refrigeration conditions significantly inhibits the microbiological cloudiness of birch sap.
3. A sufficient 12-day shelf life was obtained with the use of ultrasound, but only under refrigeration conditions and at four-fold application.
4. It appears that among the presented new technologies only microfiltration can be a way to develop methods on an industrial scale, enabling the consumer to receive birch tree sap with significantly longer shelf life and at the same time without any chemical additives.

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