

Consequences of ultrafiltration and ultraviolet on the quality of white birch (*Betula platyphylla* var. *japonica*) sap during storage

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Abstract

This study was conducted to evaluate the effect combined with ultrafiltration (UF) and ultraviolet (UV) pasteurization on the quality of white birch (*Betula platyphylla* var. *japonica*) sap during 40th day of storage at 4 and 25 °C. Some samples were treated with UF (a pore size not more than 0.03 µm) or UV independently and the other sample was treated with a combination of UF and UV devices. Total microbial number of control samples was 10⁴ CFU/ml. After treatment, they were 10² CFU/ml in UF, 10³ CFU/ml in UV, and not detected in the mutually treatment of UF and UV devices. After treatment with a combination of UF and UV devices, the pH, total acidity, browning index, turbidity, and total microbial number were very stable until 40th day of storage at both 4 and 25 °C. Also in the UF, UV treatment independently, total acidity, browning index, turbidity, and total microbial number were increased lower than control, while the pH decreased during storage periods. Especially, in the UF, UV treatment independently, microbial number increased continuously, but in the combined consequences of UF and UV, those were not detected at all during 40th day of storage. Hence, combined consequences of UF and UV were considered as the effective method to improve the shelf-life of white birch sap.

Key words: *Betula platyphylla*, shelf-life, ultrafiltration, ultraviolet, white birch sap.

Introduction

Sap is a generic term for liquid in trees, which is divided into xylem sap and phloem sap. In general, only xylem sap is referred as sap, which is a relatively thin solution containing inorganic salt, nitrogenous compound, carbohydrate, enzyme, and plant hormone. It has been used for a long period of time as a folk remedy because of its benefits for treating gastroenteric disorder, neuralgia, hypertension and others (Kim et al., 2010; Kim et al., 2009; Chung et al., 1995a). Currently, *Acer* spp. and *Betula* spp. sap is consumed in Korea, which is previously comprises *Acer mono* and *Acer pseudo-sieboldianum*, and finally comprises *Betula platyphylla* var. *japonica*, *Betula schmidtii*, *Betula davurica*, *Betula costata* and *Betula ermani* as well as bamboo and *Actinidia arguta* (Chung et al., 1995b). *Betula* spp. sap has been widely used as a folk remedy, which has also documented as medicine for strengthening the stomach, promoting urination, quickening the appetite, sedating the nerves, and effectively treating gastroenteric disorders and postnatal symptoms in women (Kim et al., 2009). Previously, many researcher reviewed about sap that the composition of sap including common component such as organic sugar, amino acid and mineral (Kim et al., 2010; Moon et al., 2004a; Hyun et al., 1999; Chung et al., 1995a; Chung et al., 1995b), effect of the anti-cancer and immunity regulating abilities of *Acer mono* sap (Qadir et al., 2007), the active oxygen scavenging activity of bamboo sap (Cho et al., 2008), and the possible functional food which is improves immunity (Lee et al., 2009). Maple sap was used as maple syrup in Canada and the USA, whereas white birch sap is commonly used as energy drink in Hokkaid, Japan (Kim et al., 2009; Moon et al., 2004b). Generally, non-processed natural sap is not consumed in Korea (Yoon 2001). Sap is collected in an agricultural off-season

(Feb~April). All saps are not possible to industrialize due to short period of preservation (Kim et al., 2010). Still, sap processing technology is not marginally improved due to quickly consumed after manufacture, which is really needed to improve for extending shelf life of sap. Recently, most of the food industries were used different type of non-heat pasteurization technologies instead of heat pasteurization (Choi et al., 2010). Among the technology, ultrafiltration is one of the applied methods such as the refine of fruit juice, wine, and enzymes with focus on their clarification, concentration and fractionation (Youn et al., 2000). Ultrafiltration method were used for improving the quality and preservation time of *Acer mono* sap using non-heat treatment technologies (Lee et al., 2010a), this techniques is not so much effective in white birch sap (Lee and Jeong 2011). In this study, the main aim was to apply ultra-filtration and ultraviolet ray pasteurization, which are non-heat processing technologies, in white birch sap, and to improve the storing capacity.

Results and discussions

Physicochemical characteristics before and after UF and UV treatment

Changes in the physicochemical characteristics in white birch sap before and after the application of ultrafiltration (UF) and ultraviolet ray pasteurization (UV) are shown in Table 1. Crude ash was 23.67 mg % in control, 22.50 mg % after UF treatment, 23.50 mg % after UV treatment, and 21.67 mg% after UF and UV treatment. There was slightly decline but not significant difference (p>0.05). Whereas, content of crude ash in white

birch sap was range from 0.04 to 0.09 % (Yoon et al., 1992). Nine types of inorganic components were detected such as copper, iron, manganese, zinc, magnesium, calcium, aluminum, potassium and sodium. The content of potassium and calcium was higher than magnesium and sodium while zinc, aluminum, manganese, copper and iron were very low. The content of potassium was the highest (30.10 mg/L) in control, which is significant decrease after the application of UF (18.91 mg/l), UV (26.03 mg/l), UF and UV (18.84 mg/l) treatments, respectively. The decline rate was greater after UF, UF and UV treatments than after UV treatment ($p < 0.05$). The content of calcium showed a significant difference and decrease after UF and UF+UV treatments since it was initially 25.82 mg/l in control after UF (24.82 mg/l), UV (25.78 mg/l) and UF+UV (24.16 mg/l) treatments, respectively. The content of magnesium, sodium, zinc and copper also showed a significant difference and decrease after UF+UV and UF treatments. These results are consistent with Yoon et al. (2000), whereas demonstrated that the content of major inorganic components of the sap like calcium and potassium decreased slightly after the application of UF, and UV treatment, and also have not a high effect in the sap. The content of organic sugars like fructose and glucose was detected; fructose was 0.33% in controls, and slightly decreases after the application of UF (0.31%), UV (0.31%), and UF and UV (0.30%) treatments. These results are similar with Kim and Kang (2001) that fructose was higher than glucose after UF treatment of tangerine juice, and the loss of organic sugars was significant after UF and UV treatment together. Organic acids like citric acid were at 0.04 mg/ml and malic acid was at 0.17 mg/ml in control, comparatively decreased after the treatments of UF, UV, and UF and UV treatment. Lee and Jeong (2011) reported that the major organic acids like as citric acid and malic acid in white birch sap were slightly decreased after the application of UF. The pH was 5.11 in control; after treatment, the pH was 5.10 in UF and 5.12 in UV with significantly difference and increase up to 5.16 after UF and UV treatment. The total acidity was 0.42 mg/ml in control, and significantly decreased at 0.26 mg/ml in UF, 0.36 mg/ml in UV, and 0.23 mg/ml in UF and UV. Interestingly, organic acid and the pH were increased, while total acidity was decreased after UF and UV treatment in sap. Organic acids and total acidity were decreased, while pH was increased after UF+UV treatment. The level of browning and the turbidity were 0.25 and 0.14 in control, respectively. Those are significantly decreased after the treatments of UF, UV, and UF and UV. To the point of view, turbidity was not detected after treatment of UF and UV. Possible facts of these results, turbid materials or insoluble suspended materials and some nutritive were removed from the sap due to the application of UF and UV. From this circumstance, those treatments have significant effect in the sap, but together UF and UV treatment have highly effect than particular effect of UF and UV.

Total microbial count before and after UF and UV treatment

Changes in the total microbial count in white birch sap during UF and UV treatments are shown in Table 1. The total microbial count was 1.6×10^4 CFU/ml in controls and 2.1×10^3 CFU/ml in UV, 2.0×10^2 CFU/ml in UF treatment. Total microbial number was decreased in UV treatment at 1 log cycle, and 2 log cycle in UF treatment, whereas not detected in UF and UV treatment. Choi et al. (2010) reported that the initial total microbial count in clarified raw rice wine was 1.3×10^5 , but 5.6×10 CFU/ml after the application of UV, which was decreased at 5 log cycles. The total microbial count in UV treatment was decreased in the sap at 1 log cycle due to the

sample variations, UV pasteurization methods and/or treatment periods. Meanwhile, apple wine (Chung et al., 2003a), grape wine (Chung et al., 2003b) and peach wine (Chung et al., 2003c) were not detected microorganism after the application of UF, whereas microorganisms were not completely removed from sap after UF treatment. Microorganisms in white birch sap were not passed through UF membranes with pore size not more than 0.03 μm , but it is deemed that microorganisms are smaller than the pore size, which is exist in the sap. Microorganisms were not detected after UV and UF treatment together. Possibly reason for this results that microorganisms were not removed through UF membranes, pasteurized by treatment of UV. It implies that together use of the UF and UV would be effective and efficient method for removing the microorganisms from *Betula* spp. sap.

Physicochemical characteristics with storage temperature and period

Changes of pH with storage temperature and period on the white birch sap during treatment of UF and UV were shown in Fig. 1. At 4 °C of storage temperature, the pH was 4.77 in control and 4.89 in UV on the 3rd day, whereas after 40th days, the pH was decreased 3.95 and 3.92, respectively. In contrast, the UF treated samples were not difference in the early stage, the pH were decreased (4.05) after 10th days to 40th days, while UF and UV treated samples were not detected any changes in pH until the 40th day. At 4 °C of storage temperature, the pH was showed significantly difference excluding UF and UV samples, gradually decreased until 40th days. Significantly change showed in the total acidity during storage period with temperature (Fig. 2.). The lactic acid was 0.42 eq. mg/ml in control, whereas it was 0.77 eq. mg/ml at 4 °C, and 2.58 eq. mg/ml at 25 °C at 40th days. With this control, lactic acid was production was lower in the early stage, but it was increased as similar to control with extended of the storage period. Lactic acid production was highest at 25 °C than 4 °C. Hence, mutually UF and UV treated samples were slightly changed from 0.18~0.23 eq. mg/ml at 4 °C and 0.23~0.32 lactic acid eq. mg/ml at 25 °C. pH was increased and total acidity decreased during storage period due to microorganisms produced organic acids in the sap (Lee et al., 2010a), not completely removed by UF and UV treated samples. Nevertheless, microorganisms were completely removed by UF and UV treated samples, which showed no change in their pH and total acidity during the storage period. The level of browning and the turbidity were significantly changed during storage time with temperature (Fig. 3 and 4.). The level of browning and the turbidity were the highest value in control; it is slowly increased until 40th days at both of temperature. Unfortunately, the level of browning and the turbidity were not changed during mutually treatment of UF and UV. The turbidity was slightly showed from the 30th days at 25 °C. A slight change in the level of browning and the turbidity of UF and UV mutually treated samples might be contained the high molecular substances, microorganisms and enzymes in the sap, which is had not been removed during filtering. During extended of storage time, microorganisms increased along with the level of browning and turbidity in UV and UF treated samples due to presence of microorganisms.

Changes in total microbial count with storage temperature and period

The changes of the total microbial count in the sap that was treated with UF or UV and stored for 40 days at 4 °C and 25 °C

Table 1. Changes of physicochemical properties and total microbial numbers of white birch sap treated with ultrafiltration (UF) and ultraviolet (UV)

		Control	UF	UV	UF+UV
Crud ash (mg %)		23.67±0.29 ^{NS1)}	22.50±1.32	23.50±0.50	21.67±0.76
Mineral (mg/L)	Cu	0.82±0.10 ^{a2)}	0.72±0.09 ^{ab}	0.82±0.11 ^a	0.55±0.07 ^b
	Fe	0.61±0.09 ^{NS}	0.54±0.03	0.56±0.16	0.49±0.11
	Mn	2.36±0.02 ^a	2.22±0.05 ^b	2.15±0.14 ^{ab}	2.18±0.10 ^b
	Zn	3.82±0.47 ^a	2.89±0.23 ^b	3.52±0.30 ^a	2.80±0.05 ^b
	Mg	11.90±0.15 ^a	11.57±0.12 ^{ab}	11.74±0.10 ^a	11.35±0.27 ^b
	Ca	25.82±0.12 ^a	24.82±0.21 ^b	25.78±0.23 ^a	24.16±0.67 ^c
	Al	2.73±0.34 ^a	2.46±0.22 ^{ab}	2.37±0.01 ^{ab}	2.21±0.26 ^b
	K	30.10±4.81 ^a	18.91±1.05 ^b	26.03±6.22 ^{ab}	18.84±1.18 ^b
Free sugar (%)	Fructose	0.33±0.004 ^a	0.31±0.006 ^b	0.31±0.001 ^b	0.30±0.001 ^c
	Glucose	0.25±0.004 ^{NS}	0.23±0.007	0.23±0.010	0.23±0.011
Organic acid (mg/ml)	Citric acid	0.04±0.001 ^a	0.03±0.001 ^c	0.03±0.001 ^b	0.03±0.001 ^d
	Malic acid	0.17±0.009 ^a	0.12±0.000 ^b	0.13±0.001 ^b	0.12±0.001 ^b
pH		5.11±0.02 ^a	5.10±0.01 ^a	5.12±0.01 ^a	5.16±0.01 ^b
Total acidity (lactic acid eq. mg/ml)		0.42±0.01 ^a	0.26±0.01 ^c	0.36±0.01 ^b	0.23±0.01 ^d
Browning index (OD _{420nm})		0.25±0.008 ^a	0.10±0.001 ^c	0.13±0.004 ^b	0.01±0.001 ^d
Turbidity (OD _{590nm})		0.14±0.005 ^a	0.05±0.001 ^c	0.07±0.002 ^b	ND ³⁾
Total microbial number (CFU/ml).		1.6×10 ⁴	2.0×10 ²	2.1×10 ³	ND

¹⁾ NS; Not significant. ²⁾ Values with different letters (a-d) on the raw are significantly different at the 5% level by one-way ANOVA.

³⁾ ND; Not detected.

is shown in Fig 5. The total microbial count was 1.6×10^4 CFU/ml in controls in the early stage, but increased to 3.2×10^5 CFU/ml at 4 °C and 3.3×10^6 CFU/ml at 25 °C until 40th day along with the temperature and the storage time (Fig. 5). During treatment of UF and UV, the total microbial count (bacteria) was increased until 40th days at both of temperature along with storage period due to slightly presence of microorganisms. But mutually treatment of UF and UV were showed highly significant due to completely removed microorganisms at 4 °C and 25 °C. Therefore, mutually treatment of UF and UV was considered as suitable method for long-term storage of sap.

Materials and methods

Materials

White birch saps were collected for 10 days from April 15 at the central region of Korea (Mt. Sobaek in Chungbuk province). It was collected to put a rubber hose in 8mm hole drilled to a depth of 1.5cm at sapwood portion and stored at -80°C. The samples were quickly thawed and treated with ultra-filtration and ultraviolet ray pasteurization.

Ultrafiltration and ultraviolet ray pasteurization

White birch sap were filtered by pre-filters (polypropylene and pore size not more than 0.5 μm), and hollow fiber membrane filter (polysulfone with a pore size of 0.03 μm). Ultraviolet ray pasteurization was applied using a closed chamber ultraviolet ray disinfection device (NCL-PM-A1, NEOTEC UV, Seoul, Korea). The sap were filtered by ultrafiltration using a pump for supplying pressurized water (EQ-DHM SERIES, DOOCH, Hwaseong, Korea) to make the samples pass at the speed of 2 L/min through three pre-filters and two hollow fiber membrane filters, and then samples were placed in ultraviolet ray pasteurization. After this process, the samples were poured in sterilized container in a clean bench. The samples were used as controls excluding imposed ultrafiltration and ultraviolet ray

pasteurization.

Preservation of the white birch sap

To evaluate the preservation of white birch sap before and after ultrafiltration and ultraviolet ray pasteurization, 50 ml of filtered and pasteurized samples were placed in a sterilized container, and stored at 40 days in 4 °C ± 0.5 and 25 °C ± 0.5. After storing, changes in microorganisms and physical and chemical characteristics were evaluated.

Measurement of crude ash

For determining the content of crude ash in white birch sap before and after ultra-filtration and ultraviolet ray pasteurization, 2 ml of concentrated samples were placed in a crucible with constant weight, and then the samples were preliminarily carbonized on a hot plate for expanding and turned to ash. Also, the samples were burned at 555 °C muffle furnace until reduced to white and light gray ash, and moved into a desiccator for cooling. The crude ash was measured in burn or without burn samples (AOAC 1990).

Measurement of inorganic components

After the samples were turned to ash completely, the ash was dissolved in 10 ml of 0.5 N HNO₃, and homogenized, then filtered through GF/C (90 mm, Cat. No. 1822 090, Whatman International Ltd., Maidstone, England) filter paper. Finally, the samples were dissolved in 50 ml of 0.5 N HNO₃. The contents of Cu, Fe, Mn, Zn, Mg, Ca, Al, K and Na were analyzed using inductively coupled plasma spectrometer (ICP, Thermo Jarrell Ash, Franklin, MA, USA) according to (Lee and Jeong 2011).

Measurement of organic sugar

The organic sugar of the sap was analyzed with minor

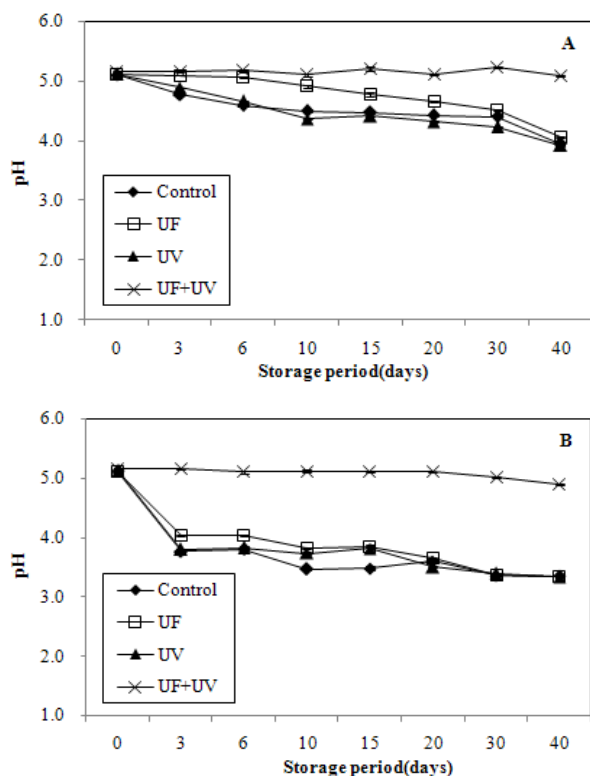


Fig 1. Changes of pH with storage temperature and period on the white birch sap treated with ultrafiltration (UF) and ultraviolet (UV). (A) at 4 °C, (B) at 25 °C.

modification according to (Bae et al., 2001). Samples were filtered with a 0.45 μm pore size membrane filter (Millipore, Billerica, MA, USA) before injection. An Water 2695 HPLC system (Waters 2695, Waters, New Castle, DE, USA) operated by empower software was used. The HPLC used with a ELSD detector (evaporative light scattering detection; Waters 2420, Waters). The column used was a carbohydrate column (4.6 \times 150 mm, Waters). Solvent of the leaking mobile phases were acetonitrile: water (75: 25 % (v/v)) at the velocity of 1.0 ml/min. Twenty (20 μl) micro liter samples were injected into the detector. Triplicate analyses were performed for each sample. Fructose and glucose (Sigma-Aldrich, St. Louis, MO, USA) were used as standards.

Measurement of organic acid

The content of organic acid in the sap was analyzed by filtering the samples through a 0.45 μm syringe filter (Millipore, Billerica, MA, USA), which is employed with HPLC (Thermo Separation Products Inc. Michigan, USA) (Cho et al., 2007). The column used was aminex HPX-87H ion exclusion columns (7.8 \times 300mm; Bio-Rad, Hercules, CA, USA); micro-Guard Cation Hcartridge (4.6 \times 30mm, Bio-Rad). The ultra-violet-visible spectra (Spectra System UV1000, Thermo Separation Products, Waltham, MA, USA) were recorded for all peaks at 215 nm. Solvent of the leaking mobile phases was 0.008 N H_2SO_4 solutions at the flow rate of 0.6 ml/min. Twenty (20 μl) micro liter samples were injected into the detector. Triplicate analyses were performed for each sample. Citric acid and malic acid (Sigma-Aldrich, St. Louis, MO, USA) were used as standards.

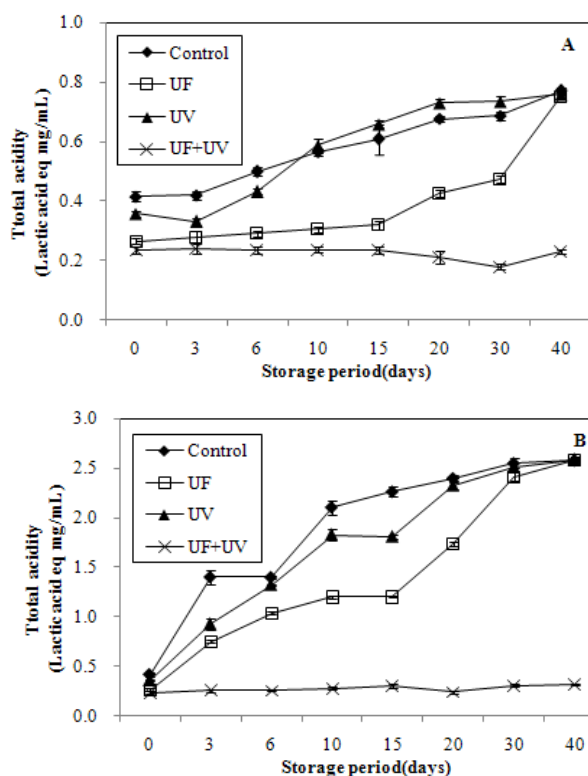


Fig 2. Changes of total acidity with storage temperature and period on the white birch sap treated with ultrafiltration (UF) and ultraviolet (UV). (A) at 4 °C, (B) at 25 °C.

Measurement of pH and total acidity

pH of white birch sap before and after the application of ultrafiltration and ultraviolet ray pasteurization were measured using a pH meter (Orion 4 STAR, Thermo Scientific, BeverBe, MA, USA) at 25 °C. For determining the total acidity, 0.01 N NaOH solutions were titrated into 10 ml of the sap while using 1% phenolphthalein solution as an indicator. The total acidity is shown as the number of ml used NaOH solution, which was converted to the content of lactic acid (Choi et al., 2010).

Measurement of browning index and turbidity

A UV-VIS spectrophotometer (UV-1650PC, Shimadzu, Kyoto, Japan) was used for browning index and turbidity. Browning index was determined at 420 nm, and turbidity at 590 nm in the sap according to (Lee et al., 2010a).

Measurement of total number of microorganisms

The samples were used for counting the total number of microorganisms in white birch sap before and after ultrafiltration and ultraviolet ray pasteurization according to Lee et al.(2010b). The sap was stepwise diluted and cultivated on nutrient agar medium at 37 °C for 24 hours, and then the number of colonies was calculated.

Statistical analysis

The measurements of all experiment items were repeated three times and were shown with the mean value and standard deviation (SD).

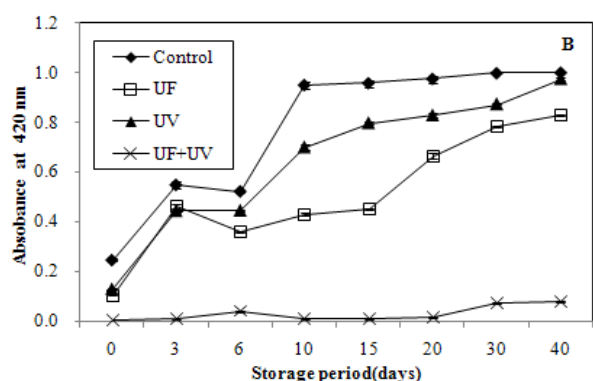
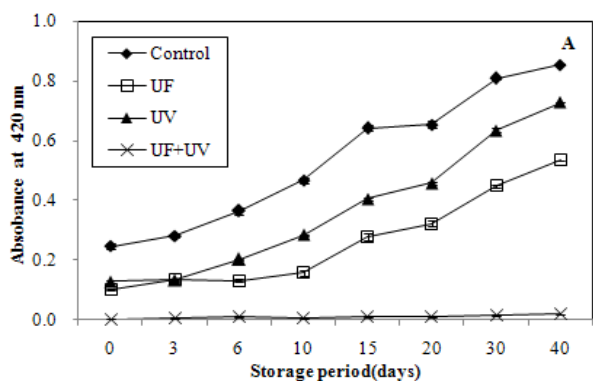


Fig 3. Changes of browning index with storage temperature and period on the white birch sap treated with ultrafiltration (UF) and ultraviolet (UV). (A) at 4 °C, (B) at 25 °C.

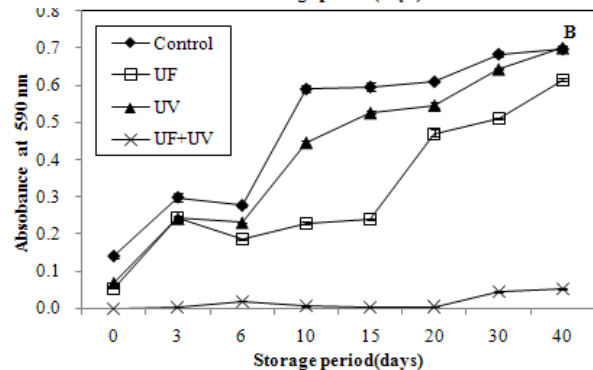
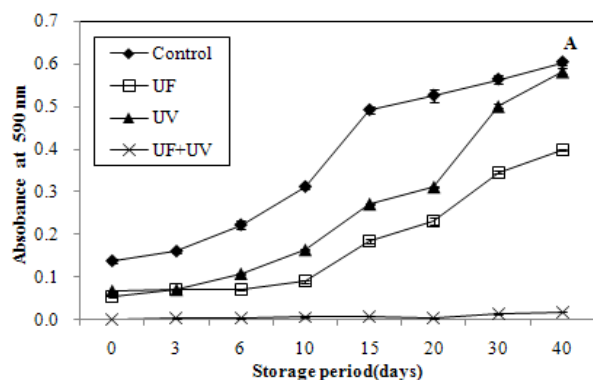


Fig 4. Changes of turbidity with storage temperature and period on the white birch sap treated with ultrafiltration (UF) and ultraviolet (UV). (A) at 4 °C, (B) at 25 °C.

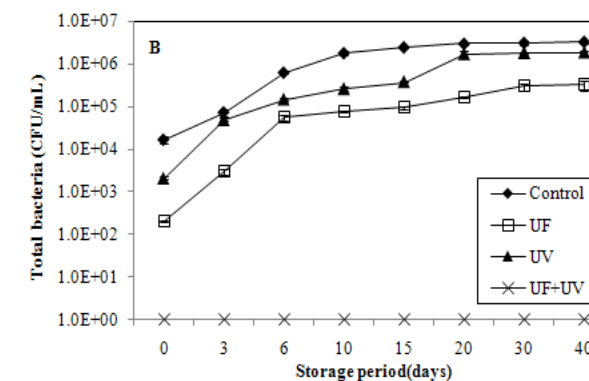
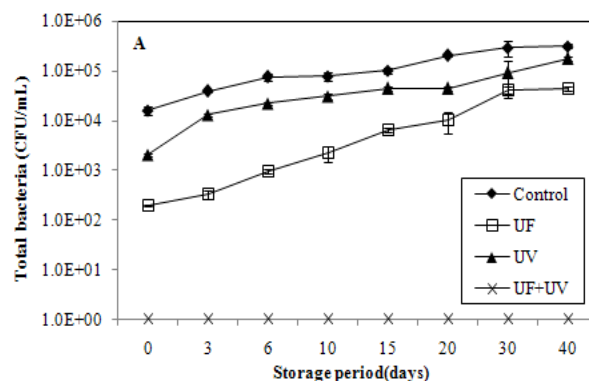


Fig 5. Changes of total bacteria with storage temperature and period on the white birch sap treated with ultrafiltration (UF) and ultraviolet (UV). (A) at 4 °C, (B) at 25 °C.

All data were statistically analyzed by analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) by using statistical package PSAW, Version 17 (SPSS Inc., Chicago, IL, USA).

Conclusion

To improve the quality and preservation of white birch (*Betula platyphylla* var. *japonica*) sap, changes in the quality and preservation time were evaluated after ultra-filtration (UF) and ultraviolet ray pasteurization (UV). The content of crude ash, inorganic components, organic sugars and organic acids were decreased in the sap in UV treatment, but significantly decreased in UF treatment. Additionally, mineral components were decreased more in mutually treatment of UF and UV than treated each UF or UV. Particularly K, Cu, Zn, Na, Fe mineral content were decreased significantly but the changes of Mn, Mg, Ca, Al were insignificant. The level of browning, the turbidity, and the total acidity were decreased while pH increased. The total microbial count were decreased by 2 log cycles in UF and 1 log cycle in UV than controls in the early stage, but not detected in mutually UF and UV treatment. The pH, total acidity, level of browning, and turbidity were slightly altered while the total microbial count in mutually treated samples was not detected until 40th day of storage at 4 °C and 25 °C. However, UV and UF treatments were not completely removed microorganisms, resulted the level of browning and the turbidity increased while the pH declined, and the total acidity increased.

As a final point, mutually treatment of ultrafiltration and ultraviolet ray pasteurization is suitable method in white birch sap, which is improve the shelf life important for sap industrialization even if minerals are slightly decrease.

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