Attempt for postharvest ripening of immature fruits of Haskap (*Lonicera caerulea* L. var. *emphyllocalyx* Nakai), an emerging fruit in Northern Japan

Y. Yamamoto[1], Y. Hoshino[2], H. Masago[3], T. Kawano[1,3,4,*]
[1] *Graduate School of Environmental Engineering, The University of Kitakyushu, Kitakyushu, Japan.*
[2] *Field Science Center for Northern Biosphere, Hokkaido University, Sapporo, Japan.*
[3] *LINV Kitakyushu Research Center (LINV@Kitakyushu), Kitakyushu, Japan.*
[4] *Université Paris Diderot, Sorbonne Paris Cité, Paris 7 Interdisciplinary Energy Research Institute (PIERI), Paris, France.*

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Abstract: Haskap or Japanese blue honeysuckle (*Lonicera caerulea* L. var. *emphyllocalyx* Nakai) is a deciduous shrub berry crop, which is recently listed as one of promisingly emerging berry crops. In the present short survey, an attempt for postharvest ripening of immature fruits of haskap was testified by examining the changes in fruit hardness, peel color, pigment synthesis and sugar-acid balance during storage at 5, 10, 15 or 20°C. Softening and coloring were shown to be induced during postharvest storage, especially at 20°C. The extent of maturation was largely enhanced by longer storage period. It is conclusive that haskap berries can be harvested at premature stage if postharvest maturation was allowed during storage and/or transportation to the markets.

1. Introduction

Haskap (Japanese blue honeysuckle; *Lonicera caerulea* L. var. *emphyllocalyx* Nakai), a deciduous berry shrub, growing to 1.5-2.0 m tall, bearing a type of blue-berried honeysuckle about 1 cm in diameter, has recently been listed as one of four promising, emerging berry crops with commercial potential despite its current status of low economic importance (Hummer *et al*., 2012). Therefore, these crops have attracted the attention of world agriculturalists and breeders even if the history of their agricultural handling is still brief (Hummer *et al*., 2012). Blue-berried honeysuckles, including haskap, are native throughout the cool temperate Northern Hemisphere; haskap is cultivated in Hokkaido, the coldest local region in Japan. Haskap berry is now known as the earliest fresh fruit harvested in Hokkaido prefecture, Japan, starting in late June (Fu *et al*., 2011).

In the last two decades, an American research team has conducted a survey on the adaptability of various blue honeysuckle plants to the northwestern United States. They have considered qualities from several geographic sources including botanical varieties *edulis*, *kamtchatica*, *altaica*, and *boczkarnikovae* from Russia, *edulis* and *boczkamikovae* from northeast China, and *emphyllocalyx* from Hokkaido, Japan, and concluded that the Japanese variety *emphyllocalyx* (Haskap) has superior adaptability in Oregon (Thompson, 2006; Thompson and Barney, 2007). Therefore, a breeding program utilizing this variety was initiated in 2003 in the states of Oregon and also Idaho, aiming for outstanding selections in hopes of identifying superior individuals to release as cultivars as the basis for a new berry industry in the United States.

To date, a research team at Hokkaido University has surveyed the ploidy level and geographical distribution of wild haskap, based on the flow cytometric analysis revealing the presence of DNA diploid and DNA tetraploid plants sampled in Japan (Miyashita *et al*., 2011). Accordingly, chromosomal analysis confirmed that diploid and tetraploid plants showed 2n=2x = 18 and 2n = 4x = 36, respectively. The DNA diploid populations were found only in lowland mires, Betsukai, Bekanbeushi, Kushiro and Kiritappu located in eastern Hokkaido prefecture. On the other hand, DNA tetraploid populations were widely distributed in most areas in Hokkaido prefecture, and also in mainland Japan.

In fact, commercial cultivars of haskap have been selected only from wild plants and thus, fruit traits and other agricultural characteristics have been largely limited until recently (Miyashita and Hoshino, 2010). Breeding and plant biotechnological attempts to obtain novel haskap cultivars have followed, resulting in enhanced yield and quality. Around a decade ago, selection of haskap wild lines showing notable edible qualities and some horticultural char-

Corresponding author: kawanotom@kitakyu-u.ac.jp
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acteristics were performed for a further breeding program (Takada *et al*., 2003). Accordingly, interspecies crosses between *L. caerulea* var. *emphyllocalyx* and *Lonicera gracilipes* var. *glabra* Miquel were examined to increase genetic variability of *L. caerulea* var. *emphyllocalyx* (Miyashita and Hoshino, 2010). Furthermore, Miyashita *et al*. (2009) reported the regeneration of haskap plantlets from endosperm culture of selected lines to enhance the breeding strategy.

Although haskap is one of most promising berry crops in Hokkaido, the amount of cultivation and supply to the market is largely limited due to the attention required to handle soft ripe berries. In Japan's small-scale orchards, farmers carefully harvest the haskap berries by hand so as to avoid losing any juice through damage of the delicate peel (Fu *et al*., 2011). To date, conventional agricultural machinery has failed to be introduced for the harvest. There could be two distinct approaches applicable to improve harvesting efficiency: (1) development of novel machinery or devices for automated or semi-automated harvesting with maximal care from aids such as sensors and actuators; and (2) development of novel working algorithms to simplify the overall processes so that conventional agricultural machines or equipment can be readily introduced, as recently proposed for other horticultural crops (Kawano *et al*., 2012).

Some examples of recent approaches can be found in a series of studies conducted by Fu *et al*. (2011). After testing various combinations of separating, collecting and cleaning methods, they concluded that the harvesting rate for haskap went from 1.45 kg/h (conventional hand picking) to a maximum of 10.36 kg/h.

The present study aims to contribute to the documentation, designing a novel harvesting strategy based on the ripening physiology of haskap berries, which could be readily automated with minimal efforts. Mature haskap berries are easily damaged by mechanical operations during harvest, selection and transportation. Therefore, mature haskap berries are hard to handle by conventional harvesting machines and those harvested in the Hokkaido region bear transportation with difficulty over great distances to markets in large cities in mainland Japan. To avoid these problems or difficulties which could be attributed to fruit maturity, it is tempting to propose harvesting immature green berries which are less sensitive to mechanical stresses, to then be followed by postharvest forced maturation during storage or transportation. In this brief survey, we report an attempt at postharvest ripening of immature fruits of haskap by examining the changes in fruit hardness, peel color, pigment synthesis and sugar-acid balance during storage at different temperatures.

2. Materials and Methods

Plant materials

Mature and immature haskap berries were picked from two-year-old shrubs cultivated at the Experiment Farm at Hokkaido University. The berries, harvested by hand, were immediately packed for transportation under temperature-controlled conditions (exposure to heat or cold was avoided), and used for the experiments within two days after harvest.

Color measurements

As described elsewhere (Kawano and Shimokawa, 2003), changes in color of the fruit peel were monitored using a handy CIELAB color reader (CR-13, Konica Minolta Sensing Inc., Osaka, Japan) by measuring the a^* and b^* values of CIE (Commission International de l'Eclairge) 1976 $L^*a^*b^*$ color space units (CIELAB system), at 0, 1, 3, and 7 days of storage. As the a^* value corresponds to a red-green scale (red, positive; green, negative) and the b^* value corresponds to a yellow-blue scale (yellow, positive; blue, negative) (Kawano, 2013), we assumed that the a^* and b^* values represent degreening due to chlorophyll loss and blue color development due to the synthesis of anthocyanin, respectively.

Hardness

Changes in fruit hardness during storage were determined using a universal fruit hardness meter (max., 1 kg; model KM, Fujiwara Scientific Co. Ltd, Tokyo).

Storage

As the studied species are native to a cold region, we tested the effect of a low to moderate range of temperature, namely, 5, 10, 15 and 20°C, by keeping the samples in temperature-controllable cool incubators (model CN-25C, Mitsubishi Electronic Engineering Co. Ltd., Tokyo, Japan).

Sugar and acid contents

Freshly squeezed berry sap was used for determination of sugar content using a pocket sugar meter (model PAL-1, ATAGO Co. Ltd., Tokyo, Japan) and organic acid content using a portable amperometric acid sensor (model FS-101N, ATAGO Co. Ltd., Tokyo, Japan). For determination of sugar and organic acid, 0.5 mL and 0.1 mL of berry sap were used, respectively. Quantification of organic acids is based on the voltammetric reduction of 3,5-di-tert butyl 1,2-benzoquinone (in quinone reagent mixture provided by ATAGO Co. Ltd.) in the presence of acids (Kotani *et al*., 2008).

Pigment analysis

Anthocyanins were extracted from 0.1 g fresh weight of homogenates in 20 ml of 50% (w/v) acetic acid kept at 23°C for 24 h. Then, 1 ml of crude extract was subjected to centrifugation at 10,000 rpm (8217 x *g*) at room temperature for 10 min and the resultant supernatant was used for optical reading at 550 nm using a spectrophotometer (U-3310, Hitachi, Tokyo). Total anthocyanin content (expressed as of cyanidine-3-glucoside) was estimated according to Matsuzoe *et al*. (2006).

3. Results and Discussion

Appearance of berries before and after the storage

Prior to and after storage, the color of berry peels was compared with that of mature samples with the naked eye

(Fig. 1). While the peel of mature berries were apparently highly pigmented with a deep blue color, the immature berry samples lacked the blue pigmentation but were green due to the remaining chlorophylls. After a week of storage at 20°C, the peel of immature berry samples was slightly colored blue (Fig. 1 bottom). These observations suggest that postharvest ripening of haskap berries could likely be achieved under controlled conditions.

Softening and coloring

The progress in postharvest maturation in haskap berries kept under various temperature was scored by the changes in mechanical hardness (Fig. 2) and peel color (Fig. 3). The results suggest that the softening of berries is affected by the storage temperature. At time 0, the difference in hardness between mature and immature samples was significantly large. The hardness of the immature samples kept at 20°C was gradually lowered and finally attained a level comparable to naturally-matured berry samples (Fig. 2).

Similarly, coloring was enhanced in the samples stored at higher temperature. Peel color was non-destructively determined by a colorimeter and the changes in peel color were expressed as values in CIELAB system (Fig. 3). At 5°C, neither lightness (*L** values), greenness (negative *a** values), nor blueness/yellowness (*b** values) showed significant change during seven days of storage. In contrast, under higher temperatures, all coloring values were altered with time, suggesting that chlorophyll degradation and anthocyanin biosynthesis were induced. Loss of chlorophylls (de-greening) is one key feature of fruit ripening found in various fruits with ethylene-producing climacteric (Kawano

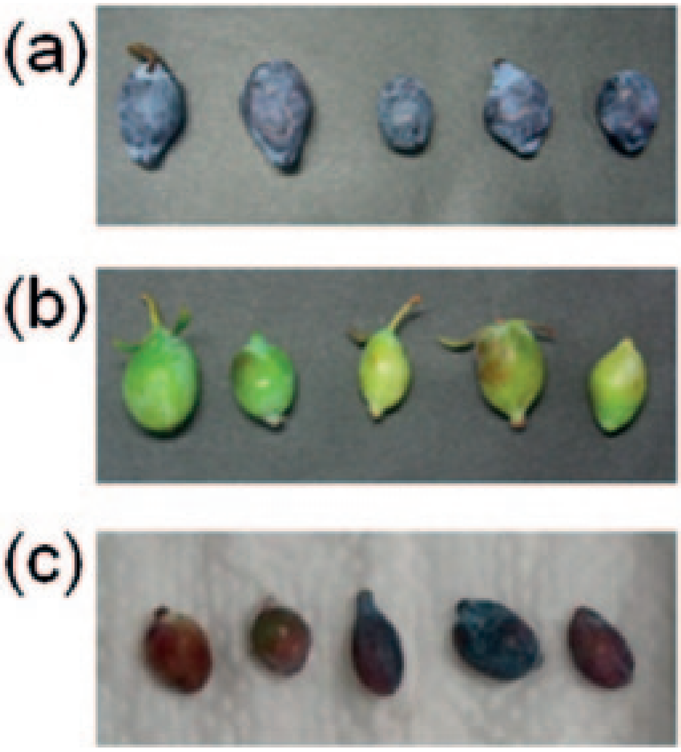

Fig. 1 - Images of typical haskap berries at three different stages of maturation. (a) Mature berries. (b) Immature berries prior to storage. (c) Semi-colored berries obtained after storing the immature samples for 1 week at 20°C.

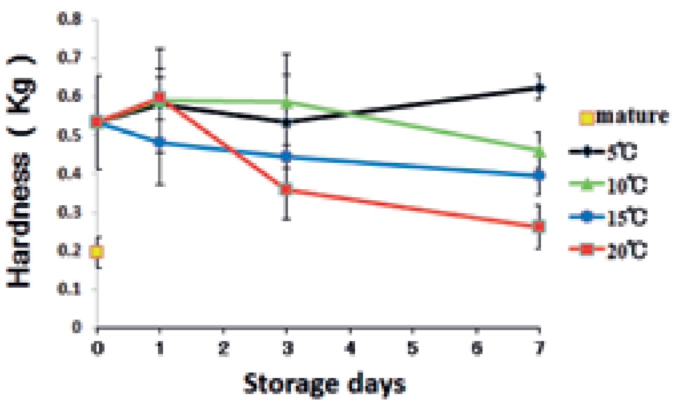

Fig. 2 - Changes in mechanical hardness of haskap berries during storage under various temperatures. Vertical bars on the graph indicate SE (n=4).

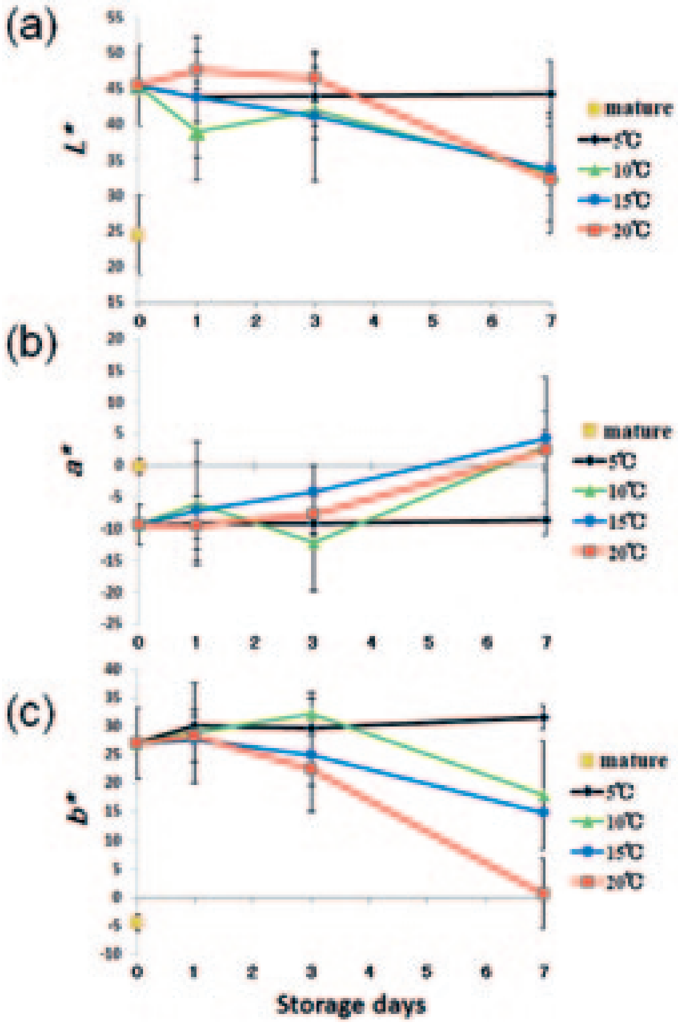

Fig. 3 - Colorimetric changes in peel color in haskap berries during storage under various temperatures. Peel color was determined by colorimeter and expressed as values in CIELAB system, namely *L** (a), *a** (b), and *b** (c). Vertical bars on the graph indicate SE (n=4).

and Shimokawa, 1994; Kawano *et al.*, 1999; Kawano and Shimokawa, 2004, 2005 a) and non-climacteric natures (Kawano and Shimokawa, 2003, 2005 b). Thus, further investigation of the de-greening mechanism is encouraged.

Anthocyanin content

As the decrease in *b** value in the peel color (Fig. 3 c) reflects the increase in blue pigments, the changes in anthocyanin content during postharvest storage were monitored (Fig. 4). The mature samples were rich in anthocyanin (Fig. 4 a-i) and immature samples contained no detectable anthocyanin (Fig. 4 a-ii). Among the samples which were subjected to postharvest maturation, only the samples kept at 20°C showed signs of induced pigmentation (Fig. 4 a-vi).

Similarly to the colorimetrically determined blueness (Fig. 3), the blue pigmentation in the samples stored at 20°C was shown to be linearly increased with time (Fig. 4). Based on the observed tendency, we can further expect that enhanced pigmentation can be manifested by longer storage.

Sugar/acid contents

It is well known that both the absolute values and balance in Brix index and acidity in the berry sap largely determine the consumption quality of fresh haskap berries (Takada *et al.*, 2003). During postharvest maturation under various temperatures, no significant increase in sugar content in the immature berries was observed (Fig. 5 a). Instead, compared to green immature samples examined prior to storage, there was significant decrease in acidity over the seven days of storage in most samples kept at different temperatures (Fig. 5 b), thus contributing, although slightly, to the increase in sugar/acid ratio (Fig. 5 c). In the end, significance between the sugar acid ratio in mature samples and that in stored samples was lost, suggesting that sugar/acid ratio was amended by the postharvest maturation process employed.

Texture, taste, flavor, and attractive components

Softening of the berries is one of the important factors determining the texture of haskap berries. As described above, the mechanical hardness of berries was significantly lowered during postharvest incubation at 20°C and attained a level comparable to naturally-ripened samples (Fig. 2). As shown in figure 5 a, the postharvest maturation approach was barely successful for enhancing the sweetness increase, although sweetness and sourness represented by the contents of sugar and organic acids are major factors determining the tastes of berries (Takada *et al.*, 2003). However, due to the induced decrease in total acid

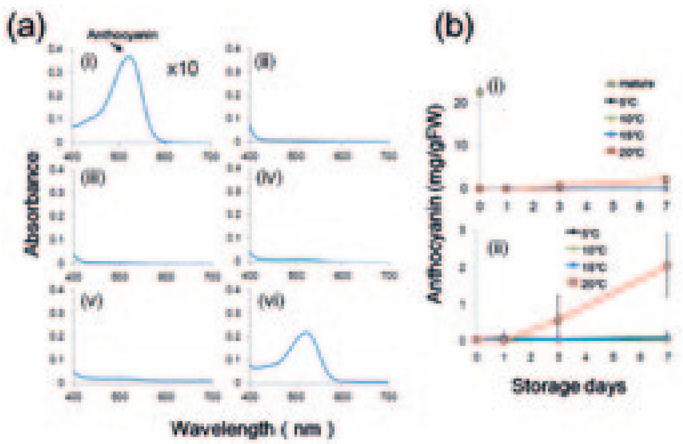

Fig. 4 - Changes in anthocyanin content in haskap berries during storage under various temperatures. (a) Typical spectral profiles of extracts from different samples. (i) Mature berries. (ii) Immature berries prior to storage. (iii, iv, v, vi) Immature berries subjected to one- week-long storage under 5, 10, 15, 20°C, respectively. (b) Increase in anthocyanin content in haskap berries during storage under various temperatures. (i) Comparison of anthocyanin content between mature samples and immature samples subjected to storage. (ii) Data in (i) were enlarged for ease of comparison among the samples stored under different temperatures. Vertical bars in (b) indicate SE (n=4).

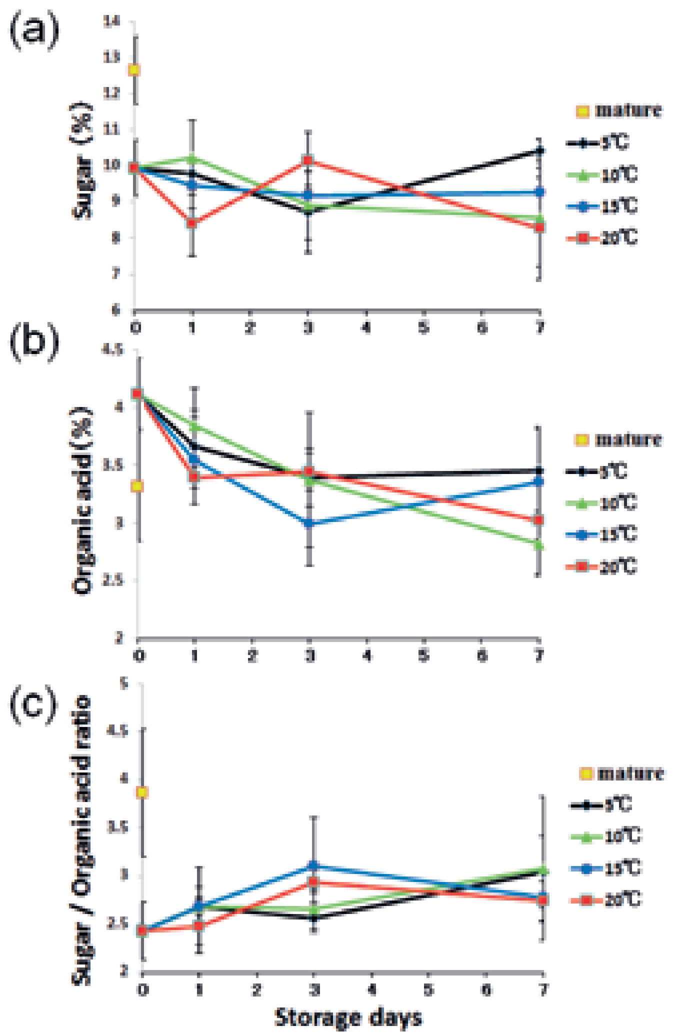

Fig. 5 - Changes in sugar and acid contents in haskap berries during storage under various temperatures. (a) Sugar content (percentage by weight). (b) Organic acid content (percentage by weight). (c) Sugar/acid ratio are compared. Vertical bars on the graph indicate SE (n=4).

level (with limited extent, Fig. 5 b), the sweetness/acidity balance might be enhanced reaching a level relatively close to the level of naturally-matured samples (sugar/acid ratio in Fig. 5 c). A preliminary organo-lip test performed by laboratory members is in support of the data on sugar/acid balance, but it is still early to provide any conclusion from such a limited survey.

One of major uses of haskap in Hokkaido is in bakery goods as sour taste accents and as color-attractive toppings. Therefore, rather than sweetness, production of pigments, chiefly anthocyanin, is of more importance from this point of view.

It is notable that haskap is considered a new berry crop with high antioxidant capacity due to its wealth in anthocyanins and related substances: anti-oxidative scores for haskap varieties were the highest among commercial fruits examined through multiple methods such as ferric reducing antioxidant power (FRAP) assay, oxygen radical absorbance capacity (ORAC) assay, the 1,1-diphenyl-2- picrylhydrazyl (DPPH) free radical scavenging assay, the aluminum chloride colorimetric method and the Folin-Ciocalteu method (Rupasinghe *et al*., 2012). More recently, Takahashi *et al*. (2014) examined the effects of dietary intake of anthocyanin-rich phenolic phytochemical (containing 13.2% anthocyanin) purified from haskap fruit on postprandial serum triglyceride and blood glucose levels in rats, concluding that a decrease in postprandial blood lipids and blood glucose by short or long-term haskap phytochemical ingestion is due to anthocyanin and other polyphenols contained in the haskap phytochemical. Reports on the antioxidant capacity of haskap and other health-related studies regarding haskap-derived pigments have been reviewed elsewhere (Celli *et al*., 2014).

In addition to pigments, the flavors or aromas of haskap berries are of commercial importance and have recently gained attention from food industries. For instance, haskap residues after juice extraction have been used preliminary as natural flavoring for teas (Sakamoto *et al.*, 2012). Thus, the impact of postharvest maturation of haskap berries on the production of flavors and aromas should be documented in future studies.

4. Conclusions

Two key parameters of fruit maturation, namely softening and coloring, were significantly amended by postharvest storage at moderate temperature and the extent of maturation is likely to be enhanced by a longer storage period. As enhanced coloring represents an increase in anthocyanin content, valued for its antioxidant action, the postharvest maturation approaches presented here may contribute to the market quality of this crop. However, the sugar content in the berry sap could not be altered during storage and the change in sap acidity induced during storage was limited. In conclusion, haskap can be harvested at premature stage if postharvest maturation is allowed during storage and/or transportation to the markets.

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