Plant Growth, Fruit Quality Analysis and Consumer Evaluation of Blueberry Grown in an Advanced Plant Factory

先進植物工場で栽培したブルーベリーの成育と果実品質の解析および消費者評価

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Chapter 1

General Introduction

1.1. Blueberry in general

Blueberries belong to the family Ericaceae and the genus *Vaccinium*, section Cyanococcus. The genus includes about 400 species (Camp, 1945), about 40% of which are native to Southeast Asia, 25% to North America, and 10% to Central and South America, with the rest scattered worldwide (Luby et al., 1991). Highbush (*V. corymbosum* L.), and southern highbush (*V. corymbosum* interspecific hybrid), lowbush (*V. angustifolium* Ait.), and rabbiteye (*V. ashei* Reade) blueberries are grown commercially. Figure 1.1 shows world acreage distribution of blueberry production regions throughout the world and North America is the leading country of global highbush blueberry production.

Blueberries are amazing fruits and no other food tastes quite like blueberry. Not only the fruits can be eaten fresh but also it has many uses such as jams, jellies, syrups, wine, salad dressings and countless other food items. Moreover, unlike most other fruits, blueberries do not have a 'seed' problem or must be peeled or cored. Nowadays, blueberries have been capturing worldwide attention including health scientists and consumers because of their high antioxidant capacity, high concentration of anthocyanins and other phenolic compounds, which regards the fruit as one of the most desirable and nutritious among fresh fruits and vegetables (Prior et al., 1998). In recent years, researchers have discovered several links between blueberries and health; and various types of scientific evidence suggested that components found in blueberries may be effective in protecting the body against oxidative stress and other conditions that are involved in the development of various diseases (e.g., heart disease and various cancers) and the aging process (Hara et al., 2014; Joseph et al., 1999; Kalt, 2006; Mainland and Tucker, 2002; Tsuda et al., 2013). Compared to other fruits and vegetables, blueberries contain an abundance and great variety of polyphenolics (i.e. phytochemicals) of both the flavonoid and non-flavonoid types (Cao et al., 1996; Kalt et al., 2001; Prior et al., 1998; Wang et al., 1996). The possible health benefits of blueberries are seemingly endless and the fruits fit perfectly with today's lifestyle where consumers want convenient, healthy and flavourful foods. Therefore, consumption of blueberries is going to be increased throughout the world.

1.2. Blueberry cultivation in Japan

In Japan, at least 18 native species of the genus *Vaccinium* were distributed from the north to the south (Ito, 2000; GRIN, 2005) (Table 1.1). According to Tamada (2009), highbush blueberry was introduced in 1951, and rabbiteye blueberry in 1962, both from USA. However, commercial blueberry culture did not begin until 1968 and the progress was very slow. Then, blueberry culture was developed due to the concentrated efforts of Dr. Iwagaki Hayao who is called "Father of Japanese blueberry". Dr. Iwagaki was a Professor at Tokyo University of Agriculture and Technology (TUAT) who conducted researches on the production of highbush and rabbiteye blueberries, and basic researches on breeding characteristics, pollination and fruiting etc., from 1966 to 1984. The results of his findings, especially on plant propagation, fruit setting and marketing have been built up today's blueberry industry in Japan. In the early 1970s, some researchers started trials on blueberry growing in local areas and production expanded more rapidly during the mid 1980s (Iwagaki and Ishikawa, 1984; Japan Blueberry Association, 2001). According to the MAFF statistics 2011 data, blueberry growing areas and production have increased rapidly from about 1995 (Fig. 1.2). Recently, different cultivars of northern highbush (NHB), half-high highbush (HHB), southern highbush (SHB) and rabbiteye (RB) were planted throughout the country. Among them, Tokyo is included in the main area where all three blueberry types can be successfully grown according to Che et al. (2009). Figure 1.2 shows annual growing area and total production (ton) from year 1976 to 2011 in Japan (MAFF Statistics, 2011).

1.3. Current status of blueberry production and import necessary

In Japan, blueberry can usually be produced in summer time and the harvest duration of blueberry fruits is limited for only four months (from June to September) for open field production from all suitable elevations by use of a range of species throughout the country. However, the domestic blueberry is primarily used for processing rather than for fresh sale of which only about ⅓ to ¼ of the total production is shipped to the fresh market (MAFF, Statistics, 2011). Figure 1.3 shows total marketable fruits divided by the amount of fresh fruits selling in the market and the amount of fruits for jam making from 1976 to 2011 in Japan. Therefore, blueberry was begun to import from 1988 in order to meet increased demand of Japanese consumers who are eating blueberries for their health benefits (Fig. 1.2).The amount of imported blueberry fruit (cultivated and wild) occupies much more than the domestic production according to MAFF statistics data (2011).

1.4. Some blueberry research and development in Japan

In 1998 and 1999, new Japanese cultivars for northern highbush blueberries, ‘Ootsubu-boshi’ (Ootsubu means larger size and boshi means the star in English) and ‘Amatsubu-boshi’ (Amatsubu means sweeter) were released from Gunmaken Horticultural Research Station (Horigome and Sato et al., 1999, 2000). Then, new intersectional hybrids were released from crossing between colchicine-induced tetraploid Shashanbo (*V. bracteatum* section *Bracteata*) and tetraploid highbush blueberry ‘Spartan’ with the efforts of Tsuda et al. (2013) and they reported these new cultivars contain high sugar content, abundant phytochemicals and extensive environmental adaptability.

In blueberry propagation, Kunitake et al. (2006) tried to investigate possibility for cultivation of northern highbush blueberry in warm region by grafting on to wild blueberry 'Shashanbo' (*V. braceatum* Thunb.). Moreover, Kunitake et al. (2006) also found that 41 blueberry cultivars including highbush blueberries and rabbiteye blueberries were successfully grafted on the 'Shashanbo' rootstock which was a suitable rootstock for cultivation of northern highbush blueberries in southern Kyushu. Moreover, cultivar test have been studying in 35 northern highbush cultivars, 18 southern highbush cultivars and 11 rabbiteye cultivars to evaluate growth and fruit quality in Tokyo by Che et al. (2009). Besides, Vano et al. (2011) reported that the effectiveness of incorporation of peat moss and sawdust compost with ferrous sulfate in raised bed cultivation can decrease soil pH and increased yield in upland blueberry growing. In order to support cultivar development for different weather conditions and to fulfill consumers' needs, breeding through interspecific hybridization between V. *corymbosum* x *V. virgatum* and *V. corymbosum* x *V. ashei* have been carried out (Miyashita et al., 2009; 2012). Furthermore, tissue culture researches such as *in vitro* chromosome doubling of multiple shoots in *Vaccinium* species (Tsuda et al., 2012) and *in vitro* shoot proliferation and *ex vitro* rooting of Japanese wild *Vaccinium* species and cultivated varieties were investigated by Sato-Yamauchi et al. (2012). Currently, wild *Vaccinium* species has been attracted for their diverse antioxidants contents among the researchers' and Tsuda et al. (2013) investigated a comparative study of antioxidant activities between wild species in Japan and commercial blueberry cultivars. In addition, Hara et al. (2014) reported the tumor suppression effects of billberry extracts for prophylactic use in human cancer. Therefore, the areas of doing various researches on blueberry by scientists are seemingly endless.

1.5. Constraints in blueberry production

All types of blueberries are grown on three types of soils in Japan; brown forest soil, red and yellow soil, and volcanic ash soil. In successful blueberry growing, one of the vital things necessary to be careful is low soil pH. According to Williamson et al. (2006), optimum soil pH may depend on different species and soil type but the best growth can be achieved where pH ranged between 4.2 and 5.2. Williamson et al. (2006) also suggested if the pH could be maintained a narrower range of 4.5 to 4.8 and soil pH adjustments should be made at least six months to a year before planning blueberry if it is necessary. Generally, Japanese blueberry farmers try to overcome soil condition problems through soil amendments in the planting hole but they do not adjust the soil pH which is a big problem in blueberry growing.

Moreover, the major ripening period of highbush blueberry is coinciding with the rainy season so that it is difficult to produce high quality fruits. Also the harvest duration of blueberry in open field is rather short but the demand for blueberry by Japanese consumers has been gradually increasing. Since there is a potential of highly profitable market during off-season time (October to May), farmers desired to produce more blueberries. Meanwhile, growers and researchers seek cultural methods that modify microclimate conditions and enable season extension to some extent. Rain protected culture in plastic houses has been used successfully and some blueberries are able to harvest before the rainy season begins (around May) by heating culture starting from February after the completion of endo-dormancy (Ozeki and Tamada, 2006). However, it is still lacking of sufficient supply for all year round including off-season time. The possibility of future blueberry production in Japan will be for the high quality table fruits and the consumption will likely be increased continuously. Therefore, it is worth to take efforts for off-season production of blueberry fruits in any possible ways.

1.6. Establishment of blueberry factory in Tokyo University of Agriculture and Technology

In 2011, supported by Ministry of Economy, Trade and Industry, 'Tokyo University of Agriculture Campus Blueberry Factory' was established as a pioneer for realizing the model of 'fruit tree factory' focusing on research facilities with expectations for profitable blueberry cultivation among the orchards (Ogiwara and Arie, 2010).

According to Dr. Ogiwara (2010), the factory is a two-storied building composed of a ground floor and underground floor comprising six rooms that reproduced seasons in Japan artificially; i.e. Early-spring, Spring, Summer, Autumn, Late- autumn and Winter; (Fig. 1.4; 1.5). The rooms with artificial seasons of Spring, Summer and Autumn are located as glasshouses under natural sunlight in the ground floor. Underground floor consists of the rest three rooms that reproduced the seasons of Late-autumn, Winter and Early-spring under artificial fluorescent lights. Artificial seasons are created by the effective usage of environmental controlling systems (Fig. 1.6). The equipments such as a cooling machine, curtain and heater for controlling temperature; dehumidifier and dry fog generator for controlling humidity; high-pressure sodium lamp and LED for controlling light intensity; black curtain and high-pressure sodium lamp for controlling daylength and a carbon dioxide generator for controlling CO_2 concentration are using in the factory (Fig. 1.7). Growing under different environments, the whole life cycle of blueberry plants under four seasons in Japan can be accreted in the factory of TUAT (Fig. 1.8).

1.7. Possibility of year-round blueberry production

1.7.1. Experiment on shortening life cycle by cultivation method

Ogiwara et al. (2014) reported that ‘flowering and shoot growth responses of blueberry grown under different day-lengths and temperatures after harvest’ as the first experiment in the blueberry factory in TUAT campus. In their study, southern highbush ‘Sunshineblue’ plant which was completed harvesting in June for natural open growing conditions was used. The plants were moved to the controlled rooms with four seasons in the plant factory on 25th August, and observation was done for 108 days until 8th December. Ogiwara et al. (2014) tried to carry out four experiments by rotating the blueberry plants to different artificial seasons in the factory.

1) The plants were constantly kept in Summer room and it was found that the shoots were

continuously growing.

2) The plants were rotated in the controlled rooms in the order of Summer to Autumn, Autumn to Winter, and Winter to Spring finally. It was found that some leaves were shedding in the Winter room and the sprouting of new shoots in the Spring room.
3) The plants were rotated in the controlled rooms in the order of Summer to Autumn, Autumn to Spring and Spring to Summer finally. The results showed that the flower buds formation at the shoot tip and fruiting occurred in November.
4) The plants were rotated in the controlled rooms in the order of Summer to Autumn, Autumn to Winter, Winter to Spring and Spring to Summer finally. However, the period of keeping the plants in Winter room was half period of the second experiment. The results showed that no shedding of the leaves and flowering occurred in December.

From the findings of Ogiwara et al. (2014), it is possible to shorten the life cycle for producing blueberry fruits in a year as flowering and fruiting was observed in December. The results showed that blueberry fruits were able to harvest twice a year by keeping the blueberry plants under low temperature and short-daylength from July to December. From the findings, it was known that the life cycle of blueberry can be shortened by dormancy breaking according to Ogiwara et al. (2014).

1.7.2. Experiment on continuous flowering and fruiting method

Generally, blueberry flowers bloom in mid-April from flower clusters of the axillary buds on the branches which passed the dormancy period in previous year in Japan. One variety generally took about 3 weeks of harvesting period and it is possible to harvest from June to August in Kanto region by using different varieties. Usually, flowering and fruiting are not occurring during autumn and winter season in natural environment of open field condition. However, it was very hot autumn (September) of 2010 and 2013 and it was observed blueberry flowering at the tip of the water sprouts (shoots with vigorous growth) in October. Therefore, Horiuchi et al. (2013) and Ogiwara et al. (2014) tried to start an experiment for continuous flowering and fruiting of blueberry plants. In 2010, the flowering plants were moved to a plastic greenhouse and the night time temperature was set to the lowest 10°C in order to harvest fruits. To extend daylength for 13 to 14 hours, high pressure sodium lamp was used for the supplemental irradiation two hours before sunrise and after sunset. Horiuchi et al. (2013) and Ogiwara et al. (2014) found that subsequent flowering occurred from the tip of water sprouts and fruits could be harvested in December. Furthermore, new shoots were generated from the axillary buds in February. When the daylength was set again to the natural condition; 8 to 10 hours from March to April, the occurrence of new shoots stopped and apical flower buds occurred again (Mayumi et al., 2012). When the daylength was extended with the supplemental light again, flowers bloomed at the tip of the shoots and the fruiting occurred. Therefore, blueberry cultivars such as 'Emerald', 'Sharpblue' and 'Sunshineblue' were treated with the high temperature and the long-daylength in September and then the results showed that it is possible to harvest fruits continuously from December to July by subsequent flowering from the mother branch. According to the results of Mayumi et al. (2012), it is possible for flowering and fruiting of the water sprouts by post-harvest treatment with the high temperature and the long-daylength to the plants. In other words, it can be called continuous flowering and fruiting method (making four seasons) by Mayumi et al. (2012).

Finally, it was found that the year-round production of blueberry was possible according to the results of Ogiwara et al. (2014) and Mayumi et al. (2012). Based on the findings of their results, the comparison of growth characteristics and the fruit quality analysis of blueberry plants under different growing conditions in the plant factory were possible to carry out in this study.

1.8. Thesis outlines

This study was carried out in a controlled environment under two different growing conditions; in a glasshouse under natural sunlight and a controlled room under artificial light in the TUAT blueberry factory. Then, determinations on characteristics of plant growth and fruit quality analysis in Chapter 2, fruit quality changes throughout the year to evaluate fruit quality in Chapter 3, questionnaire survey on eating quality test of the factory produced fruits in Chapter 4 and consumers' opinion of the fruit tree factory in Chapter 5 were carried out.

In Chapter 2, to study the characteristics of the plant growth and the fruit quality, a comparative study of two environmental conditions, growth characteristics, the photosynthetic capacity and the fruit quality analysis of one northern highbush cultivar and two southern highbush cultivars were carried out under different growing conditions; in a glasshouse under natural sunlight and in a controlled room under artificial light in the factory.

Since the fruit quality analysis of Chapter 2 was conducted in summer season, the fruit quality under different conditions during winter season should be checked out. Then, in Chapter 3-1, investigation of flowering time, harvesting time and fruit quality including anthocyanin and antioxidant activity during off-season of two southern highbush cultivars was conducted again in the plant factory.

In the next step, fruit quality changes throughout the year to evaluate fruit quality in Chapter 3-2 was possible to carried out in the factory for 11 months from May, 2013 to March, 2014. Parameters for evaluation of fruit quality such as fruit weight, diameter, different firmness, seed number per berry, soluble solids content, titratable acid and total anthocyanin contents were collected for two southern highbush cultivars under two different growing conditions.

From the above findings, it was found that the possibility of producing blueberry fruits in a glasshouse under natural sunlight and in a controlled room under natural sunlight. Then, in Chapter 4-1, a cooperative study of the instrumental and sensory analysis of blueberry fruit produced under two different growing conditions in the TUAT factory was made together with a famous sensory company (Intelligent Sensor Technology, Inc. (INSENT)). An analysis of various tastes and panelists' score for visual, textural and tastes etc. were carried out to the blueberry fruits produced in winter season in Japan. Next, in Chapter 4-2 a questionnaire survey of eating quality test was conducted for fruits produced under two different growing conditions in TUAT factory in winter season and in summer season in Japan. The objective was to predict consumers' acceptability of factory produced fruits in order to understand the market potential. Altogether 100 participants (50 in winter and 50 in summer) from different classes were participated.

Finally, in Chapter 5, an extensive internet survey for consumers' opinions and expectations for the fruit tree factory was carried out working together with a Japan research center throughout Japan. Total number of 1,318 women participants of different ages (20 to 69 years old) living in different regions (Hokkaido, Tohoku and Kanto etc.) was participated in the survey.

Chapter 6 recounts the possibilities, some constraints in blueberry growing and fruits production in a controlled room in the TUAT factory and future prospects of were discussed.

Based on the results of all findings, the summary of all Chapters and the final conclusion was mentioned in Chapter 7.

World acreage distribution of highbush blueberry

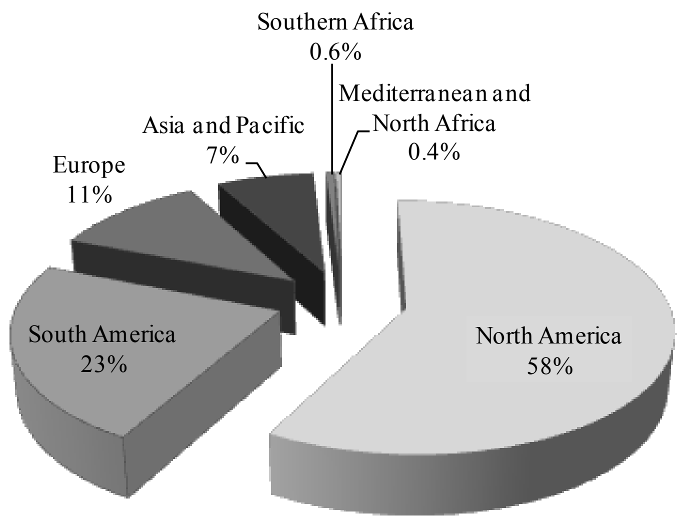


Fig. 1.1. World acreage distribution of highbush blueberry (Source: 2008 World Blueberry Acreage and Production Report).

Table 1.1. Native species of *Vaccinium* in Japan (Ito, 2000; GRIN, 2005).

Vaccinium species	Common name
V. boninense Nakai	Munin shashanbo
V. bracteatum Thunb.	Shashanbo
V. ciliatum Thunb.	Arage natsuhaze
V. emarginatum Hayata	Yadori-kokemomo
V. erythrocarpum subsp. Japonicum (Miq) Vander Kloet	Akushiba
V. hirtum Thunb.	Usunoki
V. myrtillus L. (syn. *V. yatabei* Makino)	Hime-usunoki
V. oldhamii Miq.	Natsuhaze
V. ovalifolium Sm.	Kurousugo
V. oxycoccus L. (syn *V. microcarpum* [Turcz. ex Rupr.] Schmalh.)	Tsuru kokemomo
V. praestans Lamb.	Himetsuru kokemomo (Iwatsutsuji)
V. sieboldii Miq.	Honaga natsuhaze
V. shikokianum Nakai	Maruba usugo
V. smallii A. Gray	Ooba sunoki
V. uliginosum L.	Kuromamenoki
V. vitis-idaea L.	Kokemomo
V. wrightii A. Gray	Giima
V. yakushimense Makino	Akushiba modoki

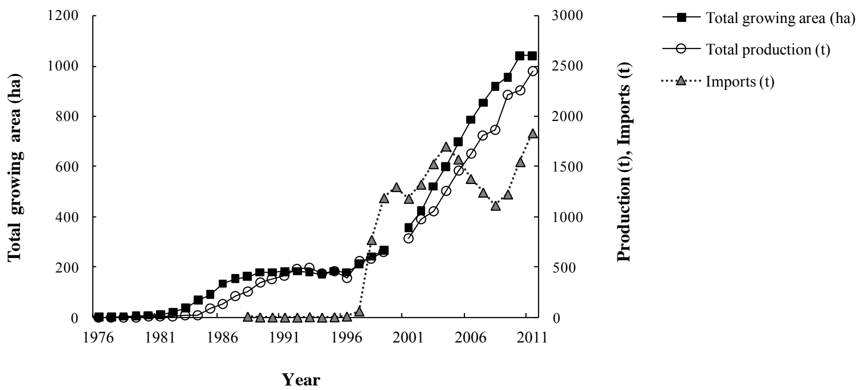


Fig. 1.2. Annual growing area (hectare) and total production (ton) (data was not collected in 2000) and imports (ton) of blueberry from 1976 to 2011 in Japan (Source: MAFF Statistics, 2011).

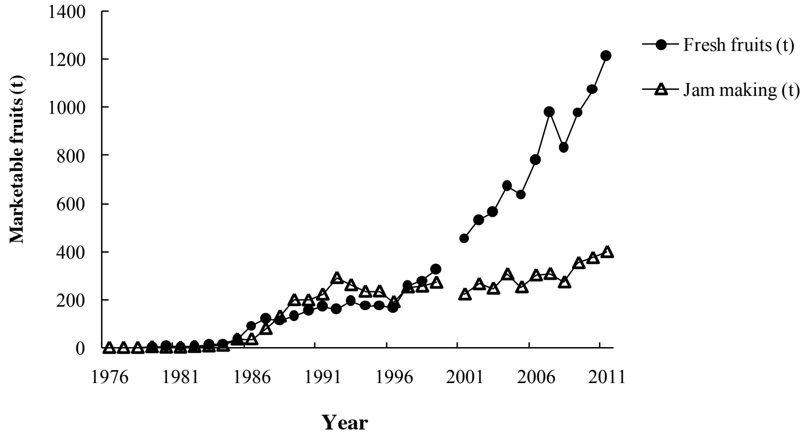


Fig. 1.3. Comparison of fresh fruits and fruits for jam making from total marketable fruits selling in the market from 1976 to 2011 (data was not collected in 2000) in Japan (Source: MAFF Statistics, 2011).

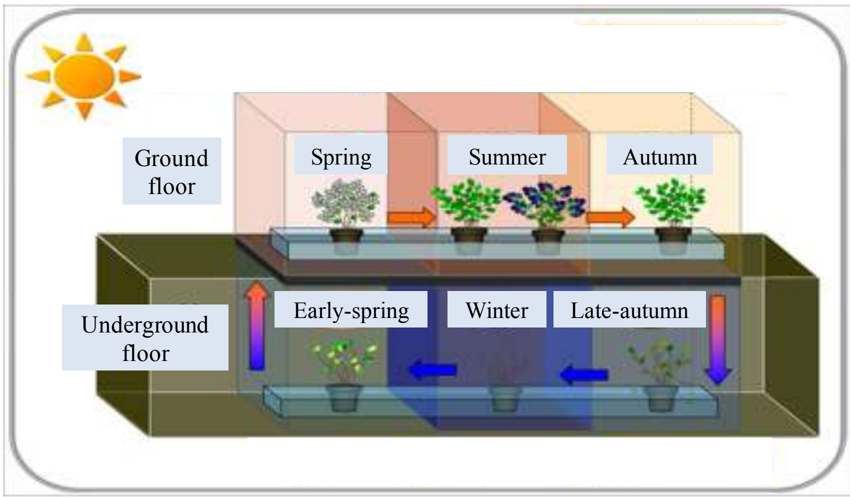


Fig. 1.4. Design of the advanced plant factory with artificial seasons focusing for year-round production of blueberries in Tokyo University of Agriculture and Technology campus, Tokyo, Japan (Source: Ogiwara and Arie, 2010).

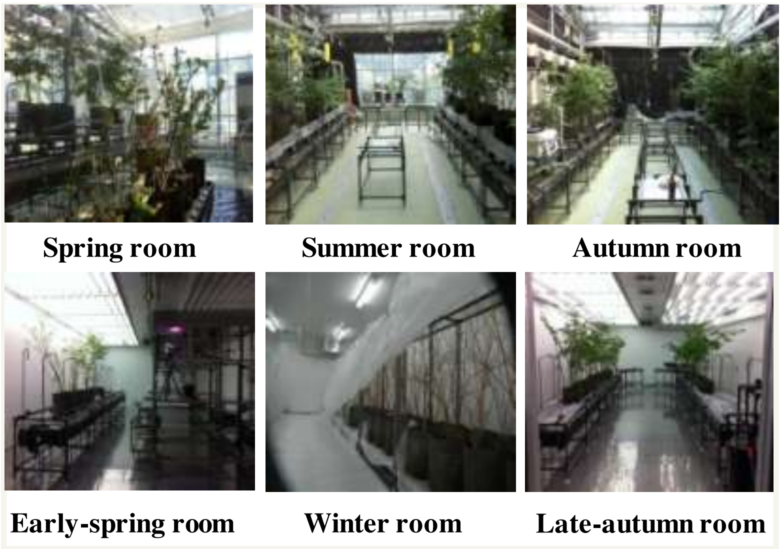


Fig. 1.5. Growing conditions of blueberry plants in rooms stimulated with artificial seasons in the blueberry factory, Tokyo University of Agriculture and Technology (Source: Ogiwara and Arie, 2010).

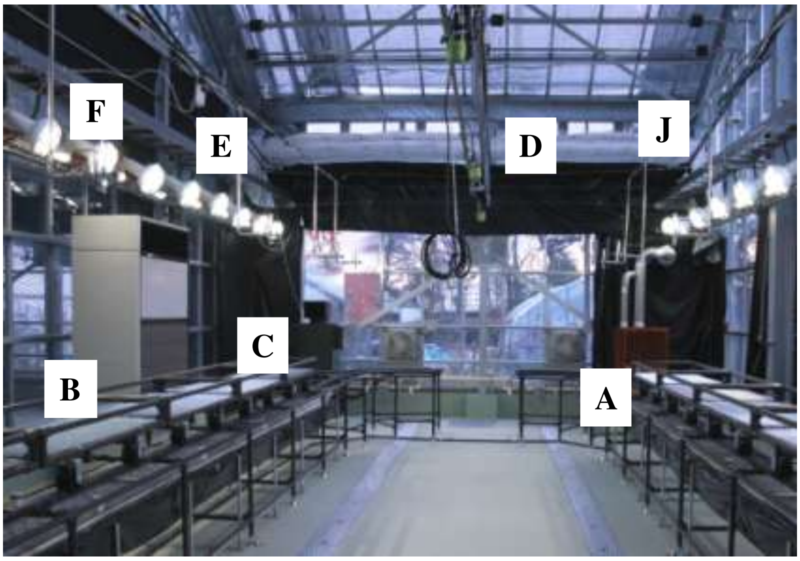


Fig. 1.6. Environmental controlling system in cultivation rooms (e.g. autumn room) of the blueberry factory, Tokyo University of Agriculture and Technology (Source: Ogiwara and Arie, 2010).

A: Heater,

B: Dehumidifier,

C: Carbon dioxide generator,

D: Dry fog generator,

E: High-pressure sodium lamp,

F: Light Emission Diode (LED) and

G: Curtain.

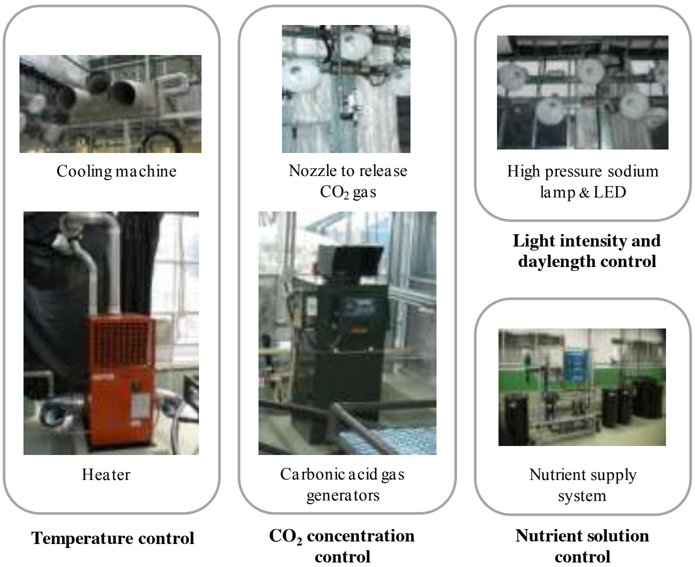


Fig. 1.7. Controlling equipments that are used in the blueberry factory, Tokyo University of Agriculture and Technology (Source: Ogiwara and Arie, 2010).

Autumn **Summer** **Spring** **Winter**

Fig. 1.8. Year-round conditions of blueberry plants under four seasons in the blueberry factory in Tokyo University of Agriculture and Technology (Source: Ogiwara and Arie, 2010).

Chapter 2

Plant Growth and Fruit Quality of Blueberry in a Controlled Room under Artificial Light

2.1. Introduction

In Japan, blueberries (family Ericaceae; genus *Vaccinium* spp.) were first introduced from the United States of America in 1951. From about 1995, blueberry culture and consumption increased rapidly because blueberries were attracting worldwide interest due to their high antioxidant content and their validated link to human health (Joseph et al., 1999; Kalt et al., 2001; Mainland and Tucker, 2002). Consequently, the demand for blueberries as a nutritional food and beverage has increased markedly. Many cultivars comprising four species, northern highbush blueberry, half-high highbush blueberry, southern highbush blueberry and rabbiteye blueberry, have been introduced mainly from the USA, Australia, and New Zealand, and planted throughout the country (from Hokkaido to Kyushu). Investigations were conducted on the character traits of those introduced cultivars and their adaptability to Japanese climate and soil conditions (Fukushima et al., 1989; Ishikawa and Koike, 2006). Blueberry growing may be divided into three zones in Japan based on air temperature: a) northern highbush and half-high highbush blueberries in the northern region (Hokkaido) and the highland areas of the main island (Tohoku), which are relatively cool during the growing season; b) three blueberry types (northern highbush, southern highbush and rabbiteye) in the central regions (Kanto, Kinki, Chugoku, and northern Kyushu) of the main island, which are the leading growing areas in Japan; and c) mainly rabbiteye and southern highbush blueberries in the southern regions (southern Kyushu and Shikoku), which are warmer during the winter.

Nevertheless, the harvest duration of blueberry fruits in Japan is limited to June to

September (only four months) for open-field production from all suitable elevations by using a range of species. Due to the increased demand of Japanese customers, during the off-season (October to May), about 12,000 tons of frozen blueberry fruits need to be imported from countries including the USA, Canada, China, Australia, New Zealand and Chile at high cost (Ishikawa and Koike, 2006). As a result, some blueberry farmers are trying to extend the production period by artificial heating in plastic houses starting from February after the completion of endo-dormancy. This enables the blueberry harvest to commence from May. However, year-round production of blueberry fruit, including the off-season, is required in Japan. In 2011, an advanced plant factory focusing on year-round production of blueberry fruits was established at Tokyo University of Agriculture and Technology, Tokyo, Japan (Ogiwara and Arie, 2010). The factory consists of six rooms (three glasshouses under natural sunlight and three controlled rooms under artificial light) that can duplicate the four seasons in Japan. In order to produce blueberry fruits year round, it is necessary to use both glasshouses under natural sunlight and controlled rooms under artificial light. Therefore, it is essential to establish a cultivation system in a controlled room under artificial light, and the purpose of this study was to clarify the plant growth and fruit quality of blueberry in a controlled room under artificial light.

2.2. Materials and Methods

2.2.1. Experimental site

The research was carried out in an advanced plant factory, which is a two-storied building composed of a ground floor and an underground floor located in Tokyo University of Agriculture and Technology, Tokyo, Japan (Ogiwara and Arie, 2010). The ground floor includes the glasshouses under natural sunlight and the underground floor consists of controlled rooms under artificial fluorescent lights (FHF32EX-N-H; Panasonic Co. Ltd., Osaka, Japan).

2.2.2. Plant materials and growing management

Four- to 5-year-old plants of northern highbush 'Blueray', southern highbush 'Misty' and 'Sharpblue' grown in 30 L pots containing Kanuma soil:peatmoss:volcanic ash (1:1:1, v/v) were used in this experiment. Three replicate pots of each cultivar grown in an open field until May 2, 2012 after finishing anthesis were moved to two growing conditions, a glasshouse under natural sunlight or a controlled room under artificial light, in order to compare vegetative growth characteristics, photosynthetic potential and fruit quality analysis between the different growing environments. Automated drip irrigation with a conventional liquid fertilization system for blueberry was used (Otsuka AgriTechno Co. Ltd., Tokyo, Japan). By using pH reducing solution containing potassium phosphate ($P_2O_4^-$) (Otsuka AgriTechno Co. Ltd.), pH was constantly controlled between 4 to 5 and electric conductance (EC) ranged from 0.7 to 1.2 mS•cm^{-1}. Irrigation was provided at 1 to 1.5 L per pot per day and a water wash system was applied to plants if insect-like aphids were found, and no pesticides or insecticides were used. Fruit loading of each cultivar was set at the same level.

2.2.3. Setting of environmental conditions

In the glasshouse under natural sunlight, day and night temperatures were controlled to 30°C and 18°C using a ventilator and heater, respectively. There was no setting for humidity control but daylength was controlled to 14 hours using a high pressure sodium lamp. In the controlled room under artificial light, temperatures were controlled to 25°C (lighting period) and 15°C (dark period) using an air conditioner, humidity was set at 50–70% during the light period using a dehumidifier. Light intensity was set around 300 μmol•m^{-2}•s^{-1} at the top of the plants and the photoperiod was set at 10 hours. The minimum and maximum environmental conditions of temperature, humidity, light intensity and daylength of the two different growing conditions throughout the experiment are shown in Table 2.1. Temperature and humidity were measured by using a single block relative humidity and temperature microprocessor transmitter (HD 9009TRR; Delta Ohm S.r.L., Padova, Italy). Light intensity was measured with a sensor for photosynthetic photon flux density (MIJ-14PAR Type 2; Environmental Measurement Japan Co. Ltd., Fukuoka, Japan).

2.2.4. Experiment 1. Determination on vegetative growth characteristics

For each cultivar, 20 newly produced shoots were randomly selected and shoot lengths were measured on June 28 for 'Misty' and 'Sharpblue', and on July 4 for 'Blueray'. Likewise, 20 leaves (upper, middle and lower position of the plant) were selected on each cultivar and estimated leaf areas were determined by leaf length and width based on the method of Fallovo et al. (2008). Leaf length (cm) was measured from the lamina tip to the point of intersection of the lamina and the petiole, along the midrib of the lamina, whereas leaf width (cm) was measured from end-to-end between the widest lobes of the lamina perpendicular to the lamina midrib. Estimated leaf area (LA) was calculated by following the formula LA = 0.54 + 0.68 LW (length and width). Chlorophyll content was determined by using a chlorophyll meter SPAD 502 (Konica Minolta Co. Ltd., Tokyo, Japan). Leaf thicknesses (mm) were measured by a 0.01–10 mm dial thickness gauge (Model G; OZAKI MFG. Co. Ltd., Tokyo, Japan).

2.2.5. Experiment 2. Comparison of photosynthetic ability

An A/C_i regression curve compared growth between the glasshouse under natural sunlight and the controlled room under artificial light for 'Sharpblue'. Three replicate measurements were taken and data were collected on May 8, 12, 13, June 10, 13, 14 and July 3, 8, 10 using fully expanded leaves. A portable photosynthesis system (LI-6400; LI-COR, NE, USA) with an LED light source (LI-6400-02B; LI-COR) was used. The photosynthetic rate was measured under base conditions of humidity 50–70%, leaf temperature 20°C, light intensity 2000 $\mu mol•m^{-2}•s^{-1}$ and air flow 350 $\mu mol•s^{-1}$. Ambient CO_2 concentration (C_a) in the cuvette was controlled with a CO_2 mixer across the series of 0, 50, 100, 300, 500, 800, 1000, 1500, and 2000 μmol•mol^{-1}. The intercellular CO_2 concentration (C_i) was calculated as follows.

$$C_i = \frac{\left(g_{tc} - \frac{E}{2}\right)C_s - A}{g_{tc} + \frac{E}{2}}$$

Whereby

C_i = intercellular CO_2 concentration, μmol CO_2•mol/air

A = net assimilation rate, μmol CO_2•m^{-2}•s^{-1}

C_s = mole fraction of CO_2 in the sample IRGA, μmol CO_2•mol/air

E = transpiration, mol H_2O•m^{-2}•s^{-1}

g_{tc} = total conductance to CO_2, mol CO_2•m^{-2}•s^{-1}

In addition, the diurnal changes of the photosynthesis rate (one-hour interval) of 'Blueray' and 'Misty' under two growing conditions were analyzed under base conditions of humidity 60–70%, temperature 25°C, air flow 350 μmol•s^{-1}, and CO_2 concentration 350 μmol•mol^{-1}; from 6:00–19:00 for the glasshouse under natural sunlight and 6:00–22:00 for the controlled room under artificial light. Finally, in order to understand the photosynthetic rates under different light intensity levels in the controlled room, the diurnal changes (7:00–23:00) under the same conditions were analyzed for 'Sharpblue' by measuring the leaves in different positions; bottom, middle, and top; of the plant depending on the availability of light intensity levels (171–323 μmol•m^{-2}•s^{-1}) for each position under artificial light.

2.2.6. Experiment 3. Fruit quality analysis

For both growing conditions, fruits started to harvest on June 28 for 'Misty' and 'Sharpblue', and on July 9 for 'Blueray'. Mature blueberries assessed by full color development of the fruit and blue at the pedicel base were harvested under each growing condition. For relative fruit size, the weight (g) and diameter (mm) of 20 randomly selected fruits were measured individually for each cultivar, 'Blueray', 'Misty' and 'Sharpblue'. Fruit firmness was determined by measuring the force to penetrate (with 2 mm Φ) the fruit positioned on its side with a rheometer (RT-3005 D; RHEOTECH Co. Ltd., Tokyo, Japan). The maximum peak of the recorded force-time curve, measured in kilogram force (kg_f), was taken as the firmness of the blueberry. Twenty replicates were taken for each measurement, and SDs were obtained. Soluble solids content (SSC) and titratable acid (TA) were determined using freshly prepared juice. One gram of pulp per five fruits was mixed with 3 mL distilled water and centrifuged at 13000 rpm for 10 minutes. The SSC (Brix %) was measured using a digital refractometer (model PR101; Atago Co. Ltd., Tokyo, Japan) standardized with distilled water. TA was determined by diluting each 1mL aliquot of blueberry juice to 5 mL with distilled water, then titrating to pH 8.1 using 0.01 N NaOH. Acidity was expressed as g citric acid/100 mL juice. Three replications were taken for each result. In order to assess total anthocyanin contents, three 0.05 g sub-samples of fruit skin were extracted with 3 mL 2% TFA methanol (methanol containing 2% trifluoacetic acid). A 100 μL aliquot was diluted again with 1400 μl of 2% TFA solution to dilute 15-fold and total anthocyanin content was determined using a Shimadzu UV-visible spectrophotometer (UV-1200; Shimadzu Co. Ltd., Kyoto, Japan) at 530 nm and the results were expressed as the absorbance (Abs) value. In addition, the surfaces of fruit skins of 'Blueray' and 'Misty' were observed under an Olympus microscope (magnification: ×6, SZX-ILLK100; Olympus Optical Co. Ltd., Tokyo, Japan) to compare the differences in bloom formation of the fruits between different growing conditions.

2.2.7. Statistical analysis

Growth characteristics and fruit quality data under two different growing conditions were statistically analyzed by Student's *t*-test. Differences with *P* values of < 0.001, < 0.01 and < 0.05 were considered significant.

2.3. Results

2.3.1. Environmental conditions

Diurnal changes (10-minute intervals) of environmental conditions between different growing systems (glasshouse under natural sunlight and controlled room under artificial light) were monitored every day. However, only the data for diurnal changes on 10 May, June, and July are shown in Figure 2.1. The day and night temperatures under natural sunlight changed markedly with the highest day temperatures in July, June, and May, respectively. However, the temperatures under artificial light were 25°C with slight variations over 10 hours during the light period and 15°C during the dark period. Daytime humidity under natural sunlight ranged between 30% and 60%, and under artificial light it ranged from 50% to 70%. Under artificial light, the maximum humidity in May and June was similar. However, it was 100% in July from midnight to early morning because the artificial room was located underground and high humidity occurred in the rainy season, especially at night time. Although PPFD values under natural sunlight showed great variations, the values under artificial light ranged from minimum 335 $\mu mol•m^{-2}•s^{-1}$ to maximum 521 $\mu mol•m^{-2}•s^{-1}$ for 10 hours during the light period of 10:00 to 20:00. In sum, the growing environment under artificial light was in conditions of low temperature (15–25°C) and low light intensity (335–521 $\mu mol•m^{-2}•s^{-1}$) but high humidity (50–70%). The conditions under natural sunlight had more variation.

2.3.2. Experiment 1. Vegetative growth characteristics

All the tested cultivars (northern highbush and southern highbush) grew healthily in the controlled room under artificial light (Fig. 2.2). The comparison of growth characteristics between two different growing conditions (glasshouse under natural sunlight and controlled room under artificial light) are presented in Table 2.2. Regardless of the growth habit of each cultivar, all the tested cultivars except 'Misty' showed no significant difference in shoot length between the two growing conditions. Shoots growth of 'Misty' grown under natural sunlight (19.43 cm) was greater than under artificial light (7.53 cm), although the leaf area and leaf thickness of all cultivars were the same under different growing conditions. When grown under artificial light, Chl content changed in 'Blueray' with a slight decrease in plants grown under artificial light (38.38).

2.3.3. Experiment 2. Photosynthetic ability

In both production methods (glasshouse under natural sunlight and controlled room under artificial light), the net photosynthetic (Pn) rate of 'Sharpblue' increased linearly between 0 to 400 $\mu mol \cdot mol^{-1}$ of intercellular CO_2 concentration (C_i), slightly increased between 400 to 700 $\mu mol \cdot mol^{-1}$, and finally remained constant at 1500 $\mu mol \cdot mol^{-1}$ of C_i (Fig. 2.3). There were no differences in the Pn rate up to 400 $\mu mol \cdot mol^{-1}$ of C_i in both growing conditions for three months (from May to July). Even in the controlled room under artificial light with low PPFD value, the photosynthetic ability of 'Sharpblue' showed the same tendency as in the glasshouse under natural sunlight.

In the determination of diurnal changes of photosynthetic ability between the two growing conditions, both 'Blueray' and 'Misty' showed nearly the same pattern (Fig. 2.4). In the glasshouse under natural sunlight, the Pn rate of both cultivars increased in accordance with increasing sunlight and decreased with a low PPFD value according to sunlight (Fig. 2.4A, C). Therefore, Pn rates under natural sunlight were not constant and markedly changed depending on the sunlight intensity, although it had the highest peak value of 5.18 $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$ for 'Blueray' and 6.85 $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$ for 'Misty' during the day. In contrast, in the diurnal changes of photosynthetic ability in the controlled room under artificial light, the Pn rate was quite constant for 'Blueray' with a value of about 2 $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$ with a PPFD value 182 to 193 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Fig. 2.4B). Likewise, the Pn rate was also constant for 'Misty' with a value of around 4 $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$ with a PPFD value

193 to 279 $\mu mol \bullet m^{-2} \bullet s^{-1}$ (Fig. 2.4D).

In the findings of the Pn rate depending on different positions of the plant for 'Sharpblue' in the controlled room under artificial light, the Pn rate gradually increased from bottom to top positions depending on the nearness to the light sources (PPFD value 171 to 323 $\mu mol \bullet m^{-2} \bullet s^{-1}$) and all the results remained constant during the light period (Fig. 2.5). Moreover, the Pn rate of the leaves in the middle and top position of the plants were relatively high, about 5 $\mu mol\ CO_2 \bullet m^{-2} \bullet s^{-1}$ for the former case and about 6 $\mu mol\ CO_2 \bullet m^{-2} \bullet s^{-1}$ for the latter case.

2.3.4. Experiment 3. Fruit quality analysis

Fruit size from different cultivars varied under different growing conditions (Table 2.3). The larger fruit weights and diameters were produced by 'Blueray' under artificial light and 'Misty' under natural sunlight. No significant differences occurred in fruit firmness of all cultivars under different growing conditions; however, 'Sharpblue' (0.19 kg_f) produced the firmest fruit under natural sunlight. Higher SSC (%) and anthocyanin contents (Abs value) combined with low acid were observed in 'Blueray' and 'Misty' under artificial light (Table 2.3). In contrast, 'Sharpblue' produced higher SSC (%), higher anthocyanin (Abs value) and lower TA (%) under natural sunlight. Among all the tested cultivars, cultivar 'Blueray' produced the largest fruit (2.58 and 3.12 g) and the highest anthocyanin content (1.18 and 1.60 Abs) in both growing conditions (Fig. 2.6). 'Sharpblue' under natural sunlight has the lowest TA (0.16%). Moreover, much more bloom appeared on the surfaces of the fruits grown under artificial light than those grown under natural sunlight (Fig. 2.7). By checking 'Blueray' and 'Misty' under the microscope, the surfaces of both fruits produced under artificial light (Fig. 2.7B, D) had a larger amount of bloom than those under natural sunlight (Fig. 2.7A, C).

2.4. Discussion

As the plant factory for blueberries was established in 2011, it was necessary to perform studies on how to effectively control and adjust the suitable environmental conditions for the growing rooms. The study group performed some preliminary tests on various settings of temperature, daylength and photoperiod in order to understand the plant response under different conditions in the controlled room. Kameari et al. (2010) suggested that the most suitable temperature for maximum Pn on blueberry plants grown in a phytotron was 25°C. Furthermore, through research on the responses of plants, the optimal temperature was set at 15°C (dark period) and 25°C (light period) in the controlled room. Although the saturated point of Pn was reached under a PPFD value of 800–1000 $\mu mol \bullet m^{-2} \bullet s^{-1}$ (Kameari et al., 2010), the temperature increased if PPFD was set at 1000 $\mu mol \bullet m^{-2} \bullet s^{-1}$ in a controlled room and a lot of energy consumption was lost. Based on this result, PPFD was then set at around 300 $\mu mol \bullet m^{-2} \bullet s^{-1}$ at the top of the plants and the photoperiod was set at 10 hours. Low humidity might cause stomatal closure directly by causing water stress in the epidermal tissue and guard cells due to excessive loss of water by a high rate of transpiration (Dai et al., 1992; Loreto and Sharkey, 1990; Rawson and Begg, 1977). Finally, relative humidity was set between 50% and 70% during the light period in order to open more stomata. In the findings of monthly determination (from May to July) of leaf photosynthesis via A/C_i curve regression analysis, photosynthetic abilities of blueberry leaves in the glasshouse under natural sunlight and in the controlled room under artificial light showed no differences for three months (Fig. 2.3). Then, in the comparison of diurnal changes of photosynthetic rates between the two growing conditions, Pn values in the glasshouse varied depending on changes of sunlight intensity, although there were some peaks during the day (Fig. 2.4A, C). In contrast, diurnal changes of photosynthesis rates in the controlled room under artificial light were constant during the light period (Figs. 2.4B, D and 5). Although Pn values of bottom leaves in the controlled room were low, the values were quite constant for 10 hours during the light period and Pn values of middle leaves and top leaves were relatively high (Fig. 2.5). Therefore, it can be assumed that the same photosynthetic capacities of blueberry plants were obtained in the controlled room under artificial light throughout the growing period (only three months) compared to those plants in the glasshouse under natural sunlight. In addition, according to the results of environmental monitoring, the values of temperature, humidity, and light intensity under natural sunlight changed markedly depending on climate (Fig. 2.1), but the values under artificial light were under conditions of constantly low temperature and low light intensity, but high humidity, which may have helped to maintain the constant Pn rate (Fig. 2.1).

‘Misty’ and ‘Sharpblue’ are included among the recommended cultivars for forcing and/or heating culture in Japan (Ozeki and Tamada, 2006). In the report on the fruit ripening and quality profile of 64 cultivars in three species of blueberries grown in Tokyo (Che et al., 2009), the mean fruit sizes were 2.49 g in ‘Misty’ and 2.13 g in ‘Sharpblue’, respectively. Fruit weights in a recent study were a little lower than those reported in the study under field conditions by Che et al. (2009), which was thought to be because of the differences of the crop load. However, higher soluble solids content of fruit in ‘Misty’ (11.33% under natural sunlight and 15.73% under artificial light) and in ‘Sharpblue’ (14.80% under natural sunlight and 13.73% under artificial light, respectively) than those reported on both cultivars (9.67% in ‘Misty’ and 10.4% in ‘Sharpblue’) in the test of field conditions by Che et al. (2009) were observed in this study. In contrast, TA of the fruits in this study resulted in lower values (0.39% under natural sunlight and 0.26% under artificial light) for ‘Misty’ and (0.16% and 0.42% respectively) for ‘Sharpblue’ than those reported by Che et al. (2009); 0.76% for ‘Misty’ and 1.23% for ‘Sharpblue’. Beaudry (1992) demonstrated that a 0.1% decrease in acid concentration is known to be equivalent to a 1% increase in perceived sweetness in blueberry fruit and also suggested that blueberries should contain > 10% SSC and 0.3–1.3% TA. Based on these quality standards, fruit quality of all cultivars in this study except for TA % of ‘Misty’ (0.26%) under artificial light and that of ‘Sharpblue’ (0.16%) under natural sunlight showed acceptable SSC and TA values. As a whole, all cultivars grown under both growing conditions had high SSC %. However, the effect of environmental condition (natural sunlight and artificial light) on fruit quality was different among the tested cultivars. Since the environmental condition of the controlled room under artificial light was suitable for producing high-quality fruits of ‘Blueray’ and ‘Misty’, the condition in the glasshouse under natural sunlight was suitable for obtaining high-quality ‘Sharpblue’ fruits according to the results (Table 2.3). Krüger and Josuttis (2014) reported that differences in the accumulation of chemical components in berry fruits are related to genotype × environmental interactions. Moreover, they demonstrated that pre-harvest factors such as day and night temperature, light intensity and light quality, protected cultivation with various types of plastic film, irrigation and fertilization etc. can influence the contents and composition of the berry fruits. Therefore, in this study, the different results in fruit quality among the tested cultivars might be not only because of their genotype effects but also the external environmental influences, especially temperature and light during fruit development under different growing conditions.

Fruits with high SSC % and low TA % taste sweet and their quality is high. Interestingly, the point to consider here is why ‘Blueray’ and ‘Misty’ contained higher SSC % and lower TA % in the controlled room under artificial light. A possible reason is that, in the controlled room under artificial light, the growing environment was kept mainly under a constant temperature and light intensity, which might help to increase the accumulation of carbon in leaves that have a low respiration rate. SSC % of fruit could also be increased due to the constant Pn rate and due to the low respiration rate in the dark period in the controlled room under artificial light. However, TA % may have decreased due to the low respiration rate under low temperature and low light intensity in the controlled environment. Moreover, many studies have indicated that artificial irradiation by ultra violet (UV) fluorescent light increased fruit coloration and produced anthocyanin pigments in skin of apples (Ubi et al., 2005), sweet cherries (Arakawa, 1993; Kataoka et al., 2005), and grapes and peaches (Kataoka and Beppu, 2004; Kataoka et al., 2004). In this study, the UV length under artificial light was 380–430 nm, which may have increased the amount of anthocyanin in blueberry fruits. Finally, these overall conditions could produce “high-quality” fruits. However, the effect of the interaction between light intensity and temperature was not clearly differentiated and further study is still necessary to elucidate these effects. In addition, there was less fruit drop than in plants in the glasshouse under natural sunlight (data not shown). The air flow in the controlled room was created by a ventilator to cycle 80–100 $cm•s^{-1}$ to increase the photosynthetic rate of blueberry leaves. In the open field, strong winds sometimes affect fruits, causing them to fall. In a recent study, there was no strong wind so fruit drop was low in the tested cultivars. In the future, this could be a benefit to increase the total harvest weight of blueberry plants by growing in a controlled environment compared to open-field production. In addition, the formation and amount of bloom on the surface of blueberry fruit may depend on genetic variability or environmental variables (Albrigo et al., 1980; Shepherd and Griffiths, 2006). In this study, it was assumed that the occurrence of more bloom on the surfaces of ‘Blueray’ and ‘Misty’ fruits (Fig. 2.7B, D) might be the effect of environmental conditions in the controlled room under artificial light. However, the different amounts of bloom between the two growing conditions could not be estimated in this study unfortunately. Therefore, bloom between the two growing conditions should be clarified by scanning electron microscopy in a future study.

In summary, it is possible to establish a new cultivation system for blueberry in a controlled room under artificial light by controlling conditions to a low temperature (15–25°C), high humidity (50–70%) and short daylength (10 hours) under constantly low light intensity (150 to 350 $\mu mol•m^{-2}•s^{-1}$) to produce a vigorous fruit tree by maintaining leaves with constant Pn activity in order to develop normal fruits with high quality. Finally, the study confirmed that successful continuous blueberry production is possible throughout the year, including the off-season by a combination of an open field, plastic houses, glasshouses, and controlled rooms.

Table 2.1. Environmental conditions of the glasshouse and controlled room throughout the experiment.

Growing condition	Temperature (°C)	Humidity (%)	PPFD ($\mu mol \bullet m^{-2} \bullet s^{-1}$)	Daylength (hour)
Natural sunlight	17 – 38	23 – 94	sunlight (0 – 1000)[z]	14
Artificial light	9 – 28	36 – 100	0 – 504	10

[z] Supplementary light was given from 6:00 to 6:30 and 17:30 to 18:00.

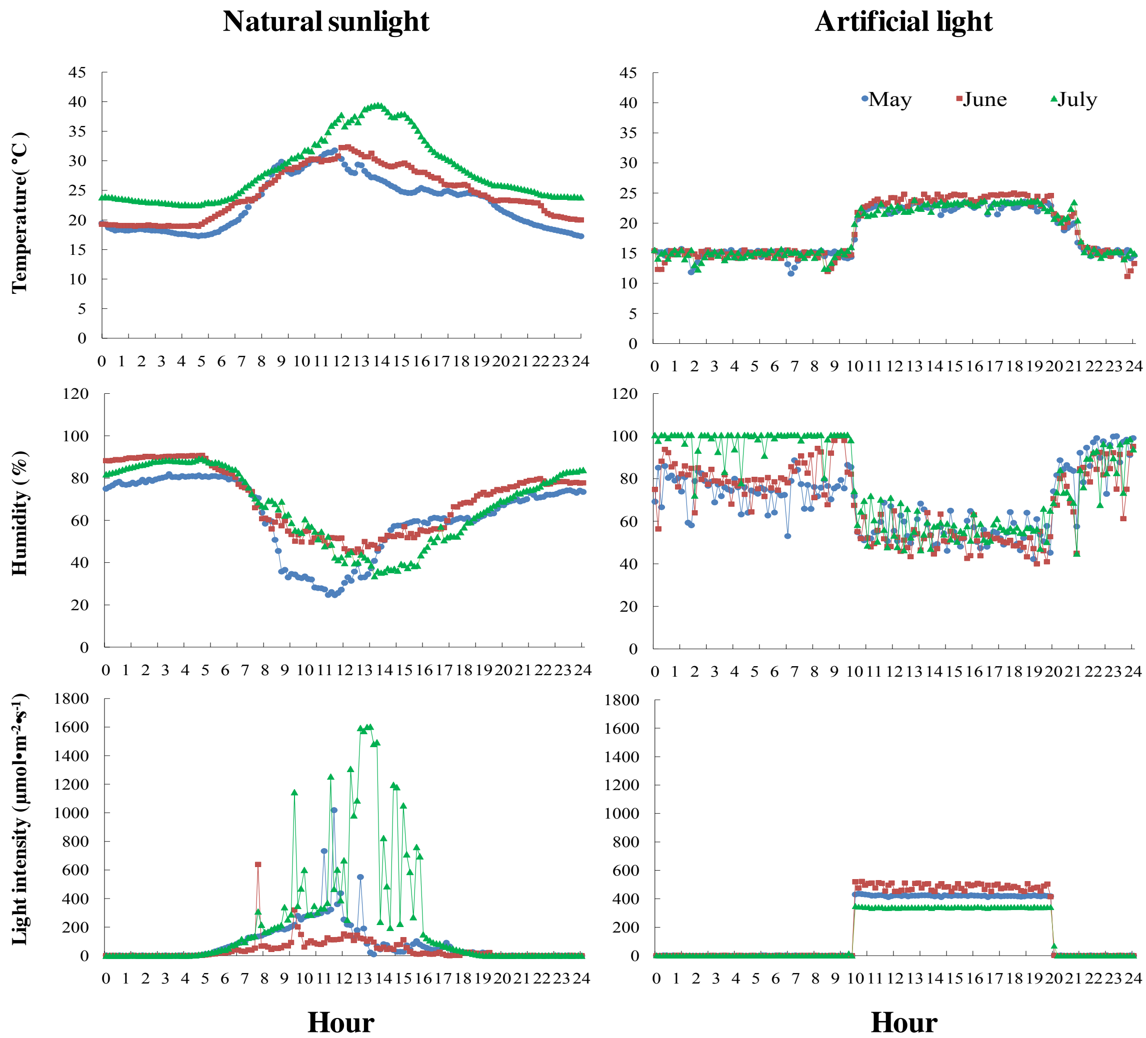


Fig. 2.1. Diurnal changes of environmental conditions in the glasshouse under natural sunlight and in the controlled room under artificial light in the plant factory on 10th May, June, and July, 2012 at ten-minute intervals using a real-time environmental monitoring system.

Fig. 2.2. Comparison of blueberry cultivars under two different growing conditions: plants in a glasshouse under natural sunlight (above) and plants in a controlled room under artificial light (below).

Table 2.2. Growth characteristics of blueberry cultivars under different growing conditions (glasshouse and controlled room).

Cultivar	Growing condition	Shoot length[z] (cm)	Leaf[z] Length (cm)	Width (cm)	Area (cm)	Thickness (mm)	Chl (SPAD value)
Blueray	Natural sunlight	10.48	7.29	3.65	18.87	0.34	47.42
	Artificial light	11.45	7.22	3.74	19.03	0.37	38.38
	t -test[y]	NS	NS	NS	NS	NS	**
Misty	Natural sunlight	19.43	5.63	2.96	12.03	0.45	58.91
	Artificial light	7.53	5.52	2.89	11.60	0.41	56.16
	t -test[y]	*	NS	NS	NS	NS	NS
Sharpblue	Natural sunlight	24.29	6.77	4.35	21.12	0.33	49.15
	Artificial light	25.66	6.76	4.49	21.86	0.37	49.80
	t -test[y]	NS	NS	NS	NS	NS	NS

[z] Values show the mean.

[y] NS, **, * indicate not significant, significant at $P < 0.01$, and $P < 0.05$, respectively.

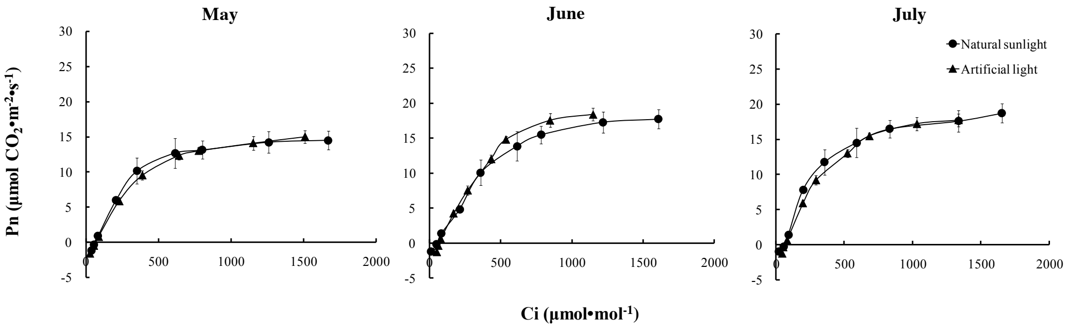


Fig. 2.3. Monthly determination of the net photosynthetic rate (May to July) in response to intercellular CO_2 concentration (C_i) of 'Sharpblue' under different growing conditions: (●) glasshouse under natural sunlight and (▲) controlled room under artificial light. Vertical bars are standard errors of the mean (n = 3). In some cases, the error bars are obscured by the datum symbol.

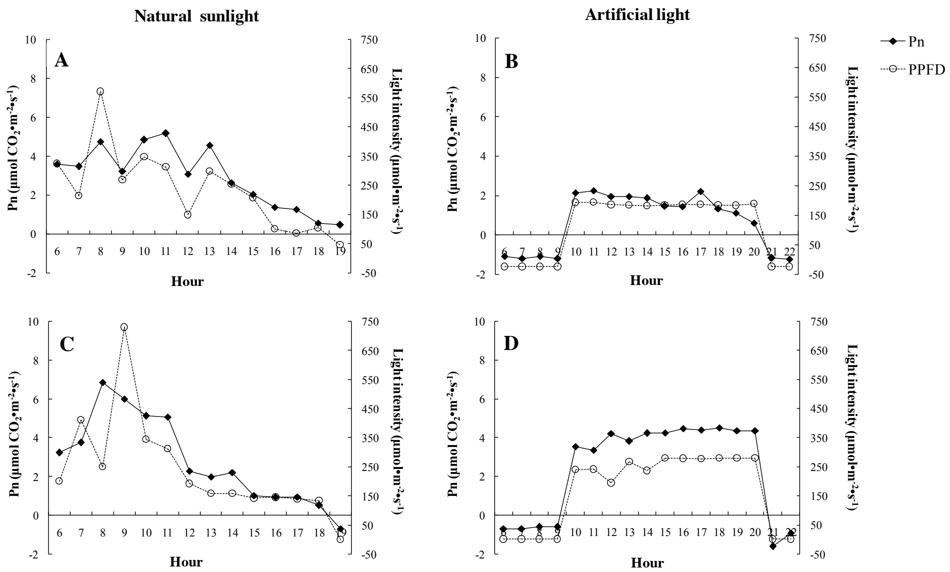


Fig. 2.4. Diurnal changes in the net photosynthetic rate (◆) and light intensity (○) of ‘Blueray’ (A and B) and ‘Misty’ (C and D) grown under different conditions.

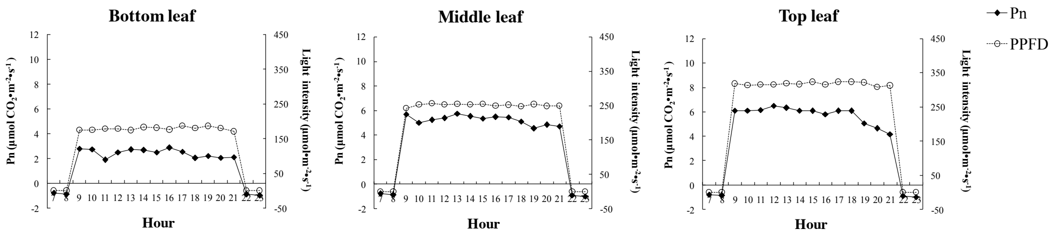


Fig. 2.5. Diurnal changes in net photosynthetic rate (◆) of different-positioned leaves in 'Sharpblue' grown in the controlled room under artificial light depending on available light intensities (○).

Table 2.3. Fruit characteristics of blueberry cultivars under different growing conditions (glasshouse and controlled room).

Cultivar	Growing condition	Fruit[z]					
		Weight (g)	Diameter (mm)	Firmness (kgf)	SSC (%)	Titratable acid (%)	Anthocyanin (Abs value)
Blueray	Natural sunlight	2.58	17.98	0.10	12.47	0.58	1.18
	Artificial light	3.12	19.74	0.10	16.47	0.51	1.60
	t -test[y]	**	*	NS	**	*	*
Misty	Natural sunlight	2.43	17.36	0.16	11.33	0.39	0.75
	Artificial light	1.35	14.13	0.15	15.73	0.26	0.89
	t -test[y]	**	***	NS	***	***	*
Sharpblue	Natural sunlight	1.50	14.53	0.19	14.80	0.16	0.66
	Artificial light	1.74	15.35	0.16	13.73	0.42	0.42
	t -test[y]	NS	NS	NS	*	**	***

[z] Values show the mean.

[y] NS, ***, **, * indicate not significant, significant at $P < 0.001$, $P < 0.01$, and $P < 0.05$, respectively.

Fig. 2.6. Comparison of ‘Blueray’ fruits produced under two growing conditions.

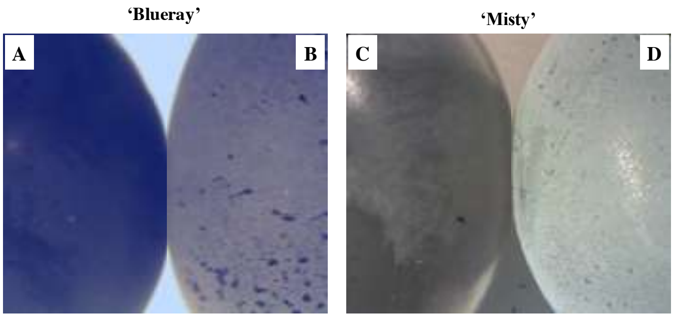


Fig. 2.7. Microscopic photos of blueberry fruits ('Blueray' and 'Misty') produced under two growing conditions: fruits under natural sunlight (A and C), fruits under artificial light showing bloom on the fruit skins (B and D).

Chapter 3

Flowering and Fruit Setting during Off-season and Fruit Quality Analysis of Blueberry throughout the Year in a Plant Factory

Chapter 3-1: Flowering, Fruit Setting and Fruit Quality Analysis of Blueberry Grown in a Plant Factory during Off-season

3.1.1. Introduction

Naturally, blueberry plants alternate their annual growth cycle between a period of active growth in the spring and summer, and one of the dormancy in the fall and winter. Also, they require a period of low temperature (chilling) following the onset of dormancy to occur normal growth and development. Species and cultivars vary with the amount of chilling requirement (number of hours below 7.2°C) ranging from 800～1500 hours for northern highbush (Norvell and Moore, 1982); 200～600 hours for southern highbush (Williamson et al., 2002) and 300～600 hours for rabbiteye (Darnell and Davies, 1990). To make earlier harvest in fruit trees such as grape, cherry, etc. in Japan, farmers carried out forcing culture with heating treatment in houses after completion of the dormancy around late-December (Kubota, 2006; Shiraishi, 2006). In strawberry, heating treatment was carried out after the completion of flower bud formation at the tip of main shoot around mid-September before starting of the dormancy period (Fujishige, 2006). And then, controlling the dormancy and fulfilling chilling requirements are the potential cultural management to extend more harvest period of blueberry. Ogiwara et al. (2014) reported that the life cycle of some southern highbush blueberry cultivars with low chilling requirement can be shortened without dormancy breaking. According to their findings, it is possible to harvest blueberry fruits twice a year in some southern highbush cultivars by keeping the plants under low temperature and short-daylength after the end of harvesting in the open field. Moreover, Ogiwara et al. (2014) reported that continuous flowering and fruiting from the water sprouts (shoots with vigorous growth) by post-harvest treatment with high temperature and long-daylength after the completion of flower bud formation to the plants. Based on the findings of Ogiwara et al. (2014), a preliminary experiment was trying to test and the results showed that the possibility of flowering and fruit setting of blueberry plants under artificial light during off-season time in the TUAT factory. In the Chapter 2, a comparative study was carried out on growth, photosynthetic capacity and fruit quality analysis of blueberry and then the results showed that it is possible to harvest fruits with high quality in a controlled room under artificial light. Moreover, it is necessary to investigate the quality of fruits produced during off-season time. Therefore, in order to get detail information, this study was carried out again:

1) To observe flowering, fruit setting and harvest duration, and
2) To investigate fruit quality analysis of two southern highbush cultivars grown in a glasshouse under natural sunlight and in a controlled room under artificial light in the TUAT factory during off-season time.

3.1.2. Materials and Methods

The study was carried out from September, 2012 to April, 2013 in an advanced plant factory for blueberries in TUAT campus. Four and five year-old southern highbush cultivars 'Misty' and 'Sharpblue' were obtained from a commercial grower in August, 2012. On 19th September, plants were placed in Winter room (< 5.5°C) for one week to ensure chilling for breaking endo-dormancy. After the chilling period, three replicate pots of each cultivar were moved to two different growing conditions; glasshouse under natural sunlight (Summer room) and controlled room under artificial fluorescent lights (Early-spring room). Care and management of the plants were the same as Chapter 2.

3.1.2.1. Investigation on flowering and fruit setting record

In order to compare flowering and fruit setting conditions under different growing environments, altogether 20 flower clusters for each cultivar, ‘Misty’ and ‘Sharpblue’, were started marking for flowering date when more than 50% of flower buds per cluster were opened. To get successful fruit setting, assisted pollination was accomplished by honey bees in the glasshouse and by bumblebees in the controlled room. When individual flowers were swelling and ensure for fruiting, fruit setting was recorded immediately. All fruit clusters were regularly checked for 3 days interval until complete harvesting to understand harvest duration. Furthermore, determination on individual flower longevity of cultivar ‘Misty’ between two different growing conditions, 20 flowers per each growing, was carried out simultaneously.

3.1.2.2. Determination of fruit quality analysis

Fruit weight (g), diameter (mm) and firmness (kg_f) of randomly selected 20 fruits were measured individually for both cultivars. Fruit juice pH was determined by using freshly prepared juice. 20 to 30 fruits were ground with a homogenizer ‘Psyscotron’ (Micotec Co. Ltd., Chiba, Japan), then centrifuged at 13,000 rpm for 15 minutes and the supernatant was measured for pH (Mettler Toledo pH electrode InLab Expert Pro-ISM, Tokyo, Japan). Soluble solids content (SSC %) and titratable acid (TA %) were determined the same procedure in Chapter 2. Three replications were taken for each result.

In addition, high performance liquid chromatography (HPLC) analysis was carried out for finding 15 kinds of anthocyanin. Cyanidin-3-glucoside used as standard for anthocyanin, trifluoroacetic acid (TFA) and HPLC-grade methanol and acetonitrile were purchased from Wako. Sample extraction of fruit powder has been subjected by freeze drying process of the whole fruits (including peel, pulp and seeds). Twenty fruits kept in −80°C freezer (KM-DU73Y1J; Panasonic Co. Ltd., Osaka, Japan) were immediately placed in freeze dryer linked with vacuum pump (FRD-82M, Iwaki Co., Ltd.) and allowed to run for 24 hours. After making powder with grinder (model T351, Rong Song Iron, Co.), anthocyanin were extracted by adding 150 μL of 2% TFA/methanol to 150 mg powder for more than 4 hours in dark condition at 4°C; centrifuged at 13,000 rpm for 10 minutes at 4°C (Hitachi, Ltd.) and the supernatant was filtered through a 13 mm syringe filter with a 0.45 μm PVDV (polyvinylidene difluoride) membrane (Whatman, Japan). Then, placed in a 1.5 ml vial and stored in −30°C before HPLC analysis.

Anthocyanin identification was performed by Shimadzu HPLC system (Shimadzu Co., Kyoto, Japan) equipped with a Prominance HPLC system (LC-20AD pumping system, a SIL-20AHT autosampler, SPD-M20A diode array detector (200～600 nm detection range). Shim-pack VP-ODS column (4.6 mm Φ x 150 nm, Shimadzu, Co., Kyoto, Japan) at 40°C was used to chromatographically separate anthocyanin species using 0.5% TFA in ultrapure water as solvent A and 0.1% TFA in acetonitrile as solvent B. The solvent gradient was %B, initial 8%; 50 min, 15%; 60 min, 30%; 65 min, 60% and 75 min, 65% (run time 75 min); flow rate 1 $mL•min^{-1}$ and injection volume 10 μL. Analysis was done in three replicates and results were expressed in $μg•g^{-1}$ of dry weight.

Finally, antioxidant activity was measured by the oxygen radical absorbance capacity (ORAC) assay as described previously (Prior et. al, 2003). A 20 μL trolox standard (6.25, 12.5, 25, 50 μM/75 mM potassium phosphate buffer pH 7.4) were added to a 96-well microplate. To each well, 200 μL of fluorescein solution (94.4 nM/75 mM potassium phosphate buffer pH 7.4) was added and fluorescence intensity was measured by using fluorescence plate reader at 37°C. Thereafter, 75 μL 2, 2-azobis 2-amidinoropane dihydrochloride (AAPH) solution (31.7 mM/75 mM potassium phosphate buffer pH 7.4) was added, stirred and then the plate was read at 485 ± 20 nm (excitation wavelength) to 530 ± 25 nm (emission wavelength) at 2 minutes interval for 90 minutes at 37°C using a Tecan infinite® M200 microplate reader. Each ORAC value of the samples was calculated by using a regression equation between Trolox concentration and the net area under the fluorescence decay curve (AUC). The net AUC corresponding to a sample was calculated by subtracting the AUC corresponding to the blank. Results were expressed in Trolox equivalents, μmol TE•g^{-1} of dry weight. All analysis was done in three replicates and the results were expressed as mean values.

3.1.3. Results

3.1.3.1. Flowering and fruit setting conditions

The duration of flowering period and harvesting records is shown in Table 3.1.1. Both cultivars started flowering during the end of September to early-October in 'Sharpblue' and early-October in 'Misty'. Fruit setting period of 'Misty' took 16 days (22^{nd} Oct. to 6^{th} Nov.) under natural sunlight and 19 days (18^{th} Oct. to 5^{th} Nov.) under artificial light and; that of 'Sharpblue' was 20 days (10^{th} to 29^{th} Oct.) under natural sunlight and 21 days (12^{th} Oct. to 1^{st} Nov.) under artificial light respectively. Fruit setting percentage of both cultivars during off-season was quite high; 98% to 100% and fruit number per each cluster was about 8 fruits. However, fruit number per cluster was increased in 'Misty' under artificial light (10 fruits). From 25^{th} January, 'Sharpblue' could be able to harvest fruits under both growing conditions and harvest duration lasted 68 days (25^{th} Jan. to 2^{nd} Apr.) under natural sunlight and 69 days (25^{th} Jan. to 3^{rd} Apr.) under artificial light respectively. Harvesting of 'Misty' under natural sunlight was started on 13^{th} February and harvest duration was 58 days (13^{th} Feb. to 11^{th} Apr.). On the other hand, under artificial light, fruit harvesting could be started on 20^{th} February and harvest duration lasted 42 days (20^{th} Feb. to 2^{nd} Apr.). Ripening period; i.e. total number of days from flowering period to end of harvest duration; of 'Misty' under both growing conditions was 163 days and that of 'Sharpblue' was 138 days (under natural sunlight) and 136 days (under artificial light). Although the ripening period of 'Sharpblue' under both growing conditions was shorter than 'Misty', there were no significant differences for each cultivar under natural sunlight and under artificial light. However, in the observation of individual flower longevity of 'Misty' between two growing conditions, it was significantly different. Since the average single flower longevity of 'Misty' under natural sunlight was 101 days, the duration under artificial light was 118 days (data not shown in Table 3.1.1; n=20).

3.1.3.2. Fruit quality attributes

Table 3.1.2 shows fruit quality analysis results of blueberries between different growing conditions during off-season time. Although fruit weight in artificial light was larger than fruits under natural sunlight, there was no difference in fruit weight of tested cultivar 'Misty' but the diameter of fruits under artificial light was significantly larger than fruits under natural sunlight. In cultivar 'Sharpblue' the fruit weight and diameter under artificial light were significantly larger than the values under natural sunlight. The parameters of firmness, pH, SSC %, TA% and ORAC values were not significantly different in both cultivars between different growing conditions. However, total anthocyanin content of 'Sharpblue' was significantly different under different growing conditions although the content of 'Misty' was not different. Figure 3.1.1 presents various kinds of anthocyanin by HPLC analysis. For both growing conditions, the anthocyanin contents of 'Sharpblue' were higher than 'Misty' by increasing malvidin-3-glucoside, peonidin-3-arabinoside, malvidin-3-galactoside and delphinidine-3-galactoside and the content of 'Sharpblue' under natural sunlight was the highest among the results.

3.1.4. Discussion

In the report on fruit ripening and quality profile of 64 cultivars in three species of blueberries grown in Tokyo (Che et al., 2009), the ripening period of 'Misty' and 'Sharpblue' was 71 days and 74 days respectively for open field condition during the on-season. In recent study, the ripening period during off-season (101 days for 'Misty' under natural sunlight and 118 days under artificial light) was about 30 days longer than the report under field condition by Che et al. (2009). It was assumed that the environmental conditions of the usual harvest season of blueberries under field condition had high temperatures; 30～35°C in the day time, and the night time temperature was >20°C during the coloration period to harvesting. Therefore, it can possibly take about 2½ months (from the middle of April to the end of June) for both cultivars from the flowering to the harvesting time. In this study, although the day time temperature (setting temperature under 30°C by ventilation system) was not so much different, especially the night time temperature (setting temperature 15°C by heater) was lower than field conditions. Therefore, it can take longer time from the flowering to the harvesting time. In detail, for a flower cluster, it took about 2 months from the flowering to the fruit development and the coloration period; and the harvest duration took about 3½ months. The same result of the long harvest duration was reported in the northern Spain by Ciordia et al. (2006). In open field condition, the harvest duration per cluster generally took 3 weeks for blueberry in Japan. Although the growing duration was longer than usual growing period due to the low temperature especially at night time, this finding might be beneficially usable for extending the harvest duration of blueberry fruits in future production.

In the report of Che et al. (2006), the mean fruit sizes were 2.49g in 'Misty' and 2.13 g in 'Sharpblue' in open field. In this study, fruit thinning was not carried out so that the values of the fruit weight were a little smaller than that reported by Che et al. (2006). The average fruit weight of 'Misty' was 1.67 g under natural sunlight and 1.86 g under artificial light; for 'Sharpblue', it was 1.87 g and 2.19 g respectively. It seems that fruit sizes of both cultivars under artificial light were larger under artificial light compared to under natural sunlight based on the fruit weight and diameter.

Beaudry (1992) suggested that blueberries should contain > 10% SSC and 0.3～1.3% TA and a pH between 2.25 and 4.25. Based on these quality standards, not only 'Misty' but also 'Sharpblue' under both growing conditions contained acceptable SSC % except 'Misty' (8.73%)

under artificial light and pH value. TA % (0.23 to 0.37%) in this study was shown in low tendency. Compared to the results of Chapter 2, the opposite result was found in ‘Misty’ under different growing conditions and SSC % of ‘Sharpblue’ was also low in the recent study. And TA % was also opposite result in ‘Misty’. The definite reason of why fruit quality was low especially in SSC % in this study was not understood clearly. As above-mentioned, it seems that the values of SSC % and TA % in this study were affected by fruit loading and due to the plant vigour. Then, to clarify the reason, fruit quality of SSC % and TA % was determined monthly in the next experiment (Chapter 3-2).

Besides fiber and micro-nutrients, blueberries contain a large number of phytochemicals and are a rich source of antioxidant. Krüger and Josuttis (2014) demonstrated differences in the accumulation of these bioactive compounds in berry fruits are related to genotype x environmental interactions. Nevertheless, Krüger and Josuttis (2014) also suggested genotype is the main factor affecting the formation of bioactive compounds and the antioxidant capacity in berries and hence their nutritional value. Therefore, the differences in anthocyanin content and ORAC values between ‘Misty’ and ‘Sharpblue’ in recent study were assumed due to their genotype difference. However, in cultivar ‘Sharpblue’, the amount of anthocyanin under natural sunlight was relatively higher than that of under artificial light although antioxidant value was not significantly different. This finding agreed with the result found in Chapter 2; i.e. total anthocyanin content of ‘Sharpblue’ under natural sunlight was higher than artificial light. Therefore, it was assumed that the growing environment especially temperature may affect the anthocyanin contents of ‘Sharpblue’ since there are some reports, for berry fruits, of either positive (Wang and Zheng, 2001; Remberg et al., 2010) or negative relationships (Åkerström et al., 2010) between anthocyanin contents in fruit and temperature.

Table 3.1.1. Flowering period, fruit setting and harvest duration per cluster of blueberries between different growing conditions during off-season time.

Cultivar	Growing condition[z]	Flowering period[y]		Fruit setting period[x]		Fruit/cluster	Fruit setting	Harvest duration		Ripening period[w]
		(days)	(Date - month)	(days)	(Date - month)	mean ± SD	(%)	(days)	(Date - month)	mean ± SD
Misty	Natural sunlight	8	(01-Oct ~ 09-Oct)	16	(22-Oct ~ 06-Nov)	8 ± 2	100	58	(13-Feb ~ 11-Apr)	163 ± 17
	Artificial light	6	(04-Oct ~ 10-Oct)	19	(18-Oct ~ 05-Nov)	10 ± 2	98.78	42	(20-Feb ~ 02-Apr)	163 ± 17
Sharpblue	Natural sunlight	9	(26-Sep ~ 05-Oct)	20	(10-Oct ~ 29-Oct)	8 ± 2	98.1	68	(25-Jan ~ 02-Apr)	138 ± 22
	Artificial light	8	(26-Sep ~ 04-Oct)	21	(12-Oct ~ 01-Nov)	8 ± 1	100	69	(25-Jan ~ 03-Apr)	136 ± 24

[z] Number of checked cluster for each growing condition = 20.

[y] Start marking when >50 % of flower buds were opening.

[x] all flower buds were swelling and sure for fruit setting.

[w] Days from flowering period to end of harvest duration (cluster longevity).

Table 3.1.2. Fruit characteristics of blueberries between different growing conditions during off-season time.

Cultivar	Growing condition	Fruit[z]							
		Weight (g)	Diameter (mm)	Firmness (kgf)	pH	SSC (%)	TA[y] (%)	TACN[x] (μg•g-1 DW)	ORAC[w] (μmol TE•g-1 DW)
Misty	Natural sunlight	1.67	15.08	0.18	4.40	10.00	0.23	4972.04	331.69
	Artificial light	1.86	15.87	0.18	4.39	8.73	0.37	5490.08	330.76
	t -test[v]	NS	*	NS	NS	NS	NS	NS	NS
Sharpblue	Natural sunlight	1.87	15.12	0.20	4.26	12.07	0.28	9063.39	267.11
	Artificial light	2.19	16.17	0.19	4.37	11.67	0.31	6341.31	242.42
	t -test	*	*	NS	NS	NS	NS	*	NS

[z] Values show the mean.

[y] Titratable acid.

[x] Total anthocyanin content expressed in μg cyanindin 3-glucoside equivaltents g-1 dry weight.

[w] Oxygen radical absorbance capacity expressed in Trolox equivalents, μmolTE•g-1 of dry weight.

[v] NS,**, * indicate not significant, significant at $P < 0.05$ and 0.01, respecitvely.

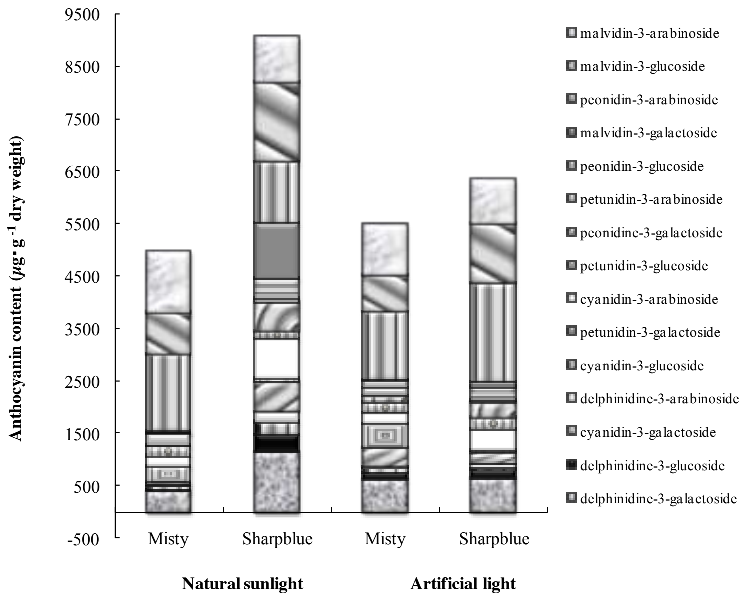


Fig. 3.1.1. Comparison of 15 kinds of anthocyanin between 'Misty' and 'Sharpblue' by high performance liquid chromatography (HPLC) analysis.

Chapter 3-2: Quality Evaluation of Blueberry Fruit Harvested throughout the Year in a Plant Factory

3.2.1. Introduction

Nowadays, agricultural production systems have been incredibly developed around the world. Various attempts have been made by growers to produce many crops continuously so that it can be fulfilled not only for consumers' needs but also for reducing the price fluctuation of the crop. Compared to the cereal crops, horticultural crops stood top position in growers' attention to produce continuously. Due to the perishable nature the horticultural crop and poor in quality for long storage, more efforts have been put on these crops to produce all year round. Moreover, by supplying high quality products, growers can make more profits from horticultural crops. Therefore, there are enormous efforts of year-round production of different vegetables and various flowers in recent horticultural production around the world including Japan. There are different types of plant factories for producing vegetables such as 'sunlight type', 'artificial light type', 'combined light use type' and 'fully sunlight type' in the world. In Japan, the very first plant factory was established in 1974 and about 127 vegetable plant factories are currently running (Kurihara et al., 2014). Until now, growers can produce many horticultural crops such as leafy vegetables, tomato, pepper and cucumber etc. in the plant factories successfully. However, there is no venture for year-round supply of fruit tree among horticulturists until 2011. Possibly due to difficulties to control their sizes, different natures in their life cycle and need to take years for commercial production, it seems so far for year-round production of fruit trees among orchardists.

Despite that, in 2011, the very first fruit tree factory was established in Tokyo (Ogiwara and Arie, 2010). Since the established year, various researches were on-going in the factory with expectations for profitable blueberry cultivations among the orchards (Ogiwara et al., 2014). Possibility of producing high quality blueberry fruits continuously all year round was included one of the major targets in the factory. Currently, by adjusting the suitable environmental conditions and / or controlling the dormancy period, the life cycle of the southern highbush blueberry plants could be possibly rotated two times a year (Ogiwara et al., 2014). Certainly, in the factory, blueberry fruits could be harvested twice a year from one plant and there were some cultivars that showed ever-bearing nature by producing fruits continuously especially under artificial light.

Then, blueberry fruits could be successfully produced throughout the year by using a glasshouse under natural sunlight and a controlled room under artificial light. The fruit quality during on-season (summer season) and off-season (winter season) were investigated in Chapter 2 and Chapter 3-1. However, the opposite results were shown in fruit quality between the Chapters. Therefore, in this study (Chapter 3-2) monthly fruit quality evaluation was tired to carry out throughout the year:

1) To investigate the size and quality of the fruits from two southern highbush cultivars grown under different growing conditions in the TUAT factory.

3.2.2. Materials and Methods

Monthly determination of the fruit quality analysis of blueberry fruits was conducted for 11 months; from May, 2013 to March, 2014. Cultivar used were southern highbush cultivars ‘Misty’ and ‘Sharpblue’ grown under two different growing condition in the advanced plant factory in TUAT campus. Well matured blueberry fruits from both cultivars were continuously harvested monthly. As soon as after harvest, fruit weight (g), diameter (mm), firmness (kg_f) and seed number per berry were collected immediately. Altogether 20 fruits per sample were used for measuring fruit weight and diameter. Ten fruits per sample were used for finding different firmness and counting seed per berry. Monthly determination of soluble solids content (SSC %), titratable acid (TA %) and total anthocyanin contents (Abs value) was carried out the same procedure in Chapter 2.

3.2.3. Results and Discussion

Table 3.2.1 shows the comparison of monthly average values ('mean' in table), monthly variance values, minimum average values and maximum average values of fruit weight, fruit diameter, firmness, SSC % etc. of 'Misty' and 'Sharpblue' fruits harvested between two growing conditions. In 'Misty' except fruits were not available in the glasshouse under natural sunlight in March, 2014, fruits could be harvest for 11 months continuously under both growing conditions (Table 3.2.2). However, fruits of 'Sharpblue' were not able to harvest in some growing conditions; i.e. natural sunlight growing condition in August, 2013 and artificial light growing condition in December, 2013; and under both growing conditions in November, 2013 (Table 3.2.3). In the weight and the diameter of the fruits, the maximum average value and the monthly average values were high in both cultivars under artificial light (Table 3.2.1). However, in 'Misty', the minimum average values were small and the maximum average values were high in the fruit diameter under artificial light so that the variance values were large. In 'Sharblue', the weight and diameter of fruits under artificial light were high almost in all months (Table 3.2.3). In 'Misty', the fruit weight and diameter under artificial light were higher than fruits under natural sunlight almost in all months except June, 2013 and November, 2013 (Table 3.2.2). Then, it was found that the seed number per berry in June and November, 2013 were very small under artificial light in 'Misty' (Table 3.2.2). When the seed number per berry of 'Misty' under artificial light was checking in Table 3.2.1, the monthly average values under artificial light were small in both cultivars. Therefore it can be assumed that the pollination for seed setting by bumblebees under artificial light was lower than the pollination by honey bees under natural sunlight. Possibly due to this reason, the size of fruits under artificial light might be small by the poorness of the pollination by using bumblebees. Therefore, it is necessary to check the activity of bumblebees for pollination of the blueberry plants grown in a controlled room under artificial light. In future study, it is also essential to consider for the suitable environmental conditions for the bumblebees. In addition, it is assumed that the small fruit weight of ‘Misty’ only under artificial light in Chapter 2 might be not only by the effect of weakness in plant vigour and heavy fruit loading but also by poorness in pollination for seed setting.

The results of firmness (fruit firmness, flesh firmness and skin firmness) are also shown in Table 3.2.1 and it was not found large differences between different growing conditions for each cultivar.

In addition, according to the results, SSC % of both cultivars was high under artificial light (Table 3.2.1). In ‘Misty’, SSC % were high under artificial light in all months except May, 2013 but the SSC % of ‘Sharpblue’ under artificial light were high only in four months; May, June and July, 2013 and March, 2014 (Fig. 3.2.1). In the case of TA %, the monthly average of both cultivars under artificial light was smaller than the values under natural sunlight. In ‘Misty’, TA % in May, July, August and November, 2013 under artificial light were low but the values were high in September, 2013 and January, 2014 (Fig. 3.2.2). Whereas, in ‘Sharpblue’, TA % in May, June, July and October, 2013 were low under artificial light but the value was high in September, 2013 (Fig.3.2.2). In generally, the amount of titratable acid % was decreased during the fruit maturation in most fruits so that the differences of the harvesting time might be affected to the final concentration of TA % in fruits. In this study, the fruit harvesting time was decided by colouration and so there were variations in harvesting time for fruit quality analysis during data collection. Therefore, it is necessary to consider for suitable harvesting date of the fruits by checking from the flowering time to the suitable harvest stage.

In the checking of the ratio of SSC % and TA %, the monthly average values in both cultivars under artificial light were higher because SSC % was larger and TA % was lower under artificial light (Table 3.2.1).

From the findings of anthocyanin contents, the monthly average values in ‘Misty’ under artificial light had less variations and the values were high in May, September, October and December, 2013. In ‘Sharpblue’ the monthly average values were low under artificial light except

October, 2013 (Fig. 3.2.3).

To sum up, the fruit sizes, SSC % and ratio of SSC % and TA % were larger, and TA % was lower in both cultivars in a controlled room under artificial light growing condition. However, not only various firmness of fruits in both cultivars but also the anthocyanin content (except 'Sharpblue') were not have any difference under two growing conditions. In the comparison of cultivar responses for different growing conditions, cultivar 'Misty' showed constant and sustainable response to the environment than cultivar 'Sharpblue' which showed many changes to the growing environment.

From the above findings, it was found that the nature of fruit setting period of the southern highbush blueberry 'Misty' and 'Sharpblue' was changing according to the conditions of the growing environment. It was observed that blueberry plants were continuously productive in the controlled environment under both natural sunlight and artificial light. Throughout the whole study period, it was noticed that both cultivars showed that the continuous production of new shoots and new flower formation while the plant itself was with load of different stages of fruits. Therefore, it was supposed that the deciduous nature of the blueberry plant became changed to show ever bearing nature. From the measurements of the fruits, it was found that large fruits were able to produce continuously for many months in both cultivars under artificial light. However, favourable growing environment might be different between two cultivars for fruit quality. Since the growing environment under artificial light might be suitable for 'Misty', the environment under natural sunlight might be preferable for 'Sharpblue' which showed the same results in previous Chapter 2. On top of that, the recent study had to stop after 11 months unfortunately. However, it was found that the tested southern highbush cultivars were still productive continuously in both growing conditions. Since the knowledge gained from this study was limited for 11 months, it would be better go continuous study of the fruits and shoots for both cultivars for long term understanding.

To be concluded finally, the information gained from this study would be hopefully supported for possibility of year-round production of blueberry fruits in Japanese orchards in future.

Table 3.2.1. Comparison of 'Misty' and 'Sharpblue' under different growing conditions from May, 2013 to March, 2014.

Cultivar	Growing condition	Month	Fruit weight (g)				Fruit diameter (mm)			
			Mean	CV	Min	Max	Mean	CV	Min	Max
Misty	Natural sunlight	10	2.25	0.19	1.48	3.00	17.29	1.99	14.59	19.35
	Artificial light	11	2.71	0.33	1.42	3.25	18.38	3.07	14.40	20.29
Sharpblue	Natural sunlight	9	1.74	0.06	1.32	2.07	15.39	0.70	13.70	16.34
	Artificial light	9	1.98	0.08	1.51	2.39	16.16	0.72	14.68	17.84
Cultivar	Growing condition	Month	Fruit firmness (kg_f)				Flesh frimness (kg_f)			
			Mean	CV.	Min.	Max.	Mean	CV.	Min.	Max.
Misty	Natural sunlight	10	0.16	0.00	0.12	0.19	0.04	0.00	0.03	0.05
	Artificial light	11	0.16	0.00	0.13	0.19	0.04	0.00	0.02	0.05
Sharpblue	Natural sunlight	9	0.19	0.00	0.16	0.22	0.04	0.00	0.03	0.04
	Artificial light	9	0.18	0.00	0.16	0.20	0.04	0.00	0.03	0.04
Cultivar	Growing condition	Month	Skin firmness (kg_f)				Seed number/ berry			
			Mean	CV.	Min.	Max.	Mean	CV.	Min.	Max.
Misty	Natural sunlight	10	0.11	0.00	0.09	0.14	54.67	490.98	29	68
	Artificial light	11	0.11	0.00	0.04	0.14	43.28	437.56	6	66
Sharpblue	Natural sunlight	9	0.15	0.00	0.13	0.18	83.38	432.31	60	109
	Artificial light	9	0.14	0.00	0.12	0.17	76.04	581.94	60	112
Cultivar	Growing condition	Month	Soluble solids content (%)				Titratable acid (%)			
			Mean	CV.	Min.	Max.	Mean	CV.	Min.	Max.
Misty	Natural sunlight	10	13.27	1.83	12.13	15.07	0.42	0.02	0.28	0.6
	Artificial light	11	14.82	2.63	10.13	15.86	0.33	0.01	0.16	0.53
Sharpblue	Natural sunlight	9	13.72	1.01	12.13	15.3	0.46	0.01	0.35	0.68
	Artificial light	9	14.41	4.41	12.4	17.33	0.37	0.01	0.3	0.58
Cultivar	Growing condition	Month	SSC/TA				Anthocyanin (Abs value)			
			Mean	CV.	Min.	Max.	Mean	CV.	Min.	Max.
Misty	Natural sunlight	10	34.5	134.8	21.8	50.82	1.89	0.01	1.71	2.05
	Artificial light	11	50.5	379.9	28.93	93.6	1.85	0.11	1.14	2.01
Sharpblue	Natural sunlight	9	31.9	78.7	19.9	43.81	1.49	0.16	0.91	1.98
	Artificial light	9	41.6	163.0	21.94	60.89	1.30	0.19	0.7	1.69

Table 3.2.2. Measurements of 'Misty' fruit under different growing conditions (May, 2013 - March, 2014).

Month	Growing condition	Misty[z]					
		Fruit wgt. (g)	Fruit dia. (mm)	Fruit frim. (kg_f)	Flesh firm. (kg_f)	Skin frim. (kg_f)	Seed /berry
May	Natural sunlight	1.82	15.59	0.19	0.05	0.14	x[y]
	Artificial light	1.95	16.28	0.19	0.05	0.14	x
	t -test[x]	NS	*	NS	NS	NS	–
Jun.	Natural sunlight	2.48	17.48	0.16	0.05	0.10	59
	Artificial light	1.42	14.40	0.16	0.04	0.12	35
	t -test	***	***	NS	**	NS	*
Jul.	Natural sunlight	2.28	18.16	0.13	0.03	0.05	x
	Artificial light	3.20	19.66	0.16	0.02	0.04	64
	t -test	***	**	NS	NS	NS	–
Aug.	Natural sunlight	2.20	17.63	0.12	0.03	0.09	68
	Artificial light	3.04	19.12	0.15	0.04	0.11	61
	t -test	***	***	**	NS	NS	NS
Sept.	Natural sunlight	1.87	16.35	0.15	0.03	0.12	64
	Artificial light	2.79	17.97	0.16	0.03	0.13	66
	t -test	***	**	NS	NS	NS	NS
Oct.	Natural sunlight	2.43	18.00	0.15	0.04	0.11	78
	Artificial light	2.33	17.52	0.17	0.04	0.13	60
	t -test	NS	NS	NS	NS	NS	*
Nov.	Natural sunlight	3.00	19.35	0.16	0.05	0.11	66
	Artificial light	2.19	16.98	0.16	0.04	0.12	6
	t -test	***	***	NS	**	NS	***
Dec.	Natural sunlight	2.67	18.37	0.16	0.04	0.11	x
	Artificial light	3.09	19.74	0.13	0.03	0.10	x
	t -test	*	**	*	*	NS	–
Jan.	Natural sunlight	2.31	17.34	0.17	0.04	0.13	29
	Artificial light	3.07	19.48	0.17	0.05	0.12	20
	t -test	***	***	NS	NS	NS	NS
Feb.	Natural sunlight	1.48	14.59	0.18	0.05	0.13	18
	Artificial light	3.25	20.29	0.17	0.04	0.13	42
	t -test	***	***	NS	NS	NS	*
Mar.	Natural sunlight	x	x	x	x	x	x
	Artificial light	2.73	18.59	0.16	0.04	0.11	37
	t -test	–	–	–	–	–	–

[z] Values show the mean.

[y] Data was not collected; No fruit was available.

[x] NS, ***, **, * indicate not significant, significant at $P < 0.001$, $P < 0.01$, and $P < 0.05$, respectively.

Table 3.2.3. Measurements of 'Sharpblue' fruit under different growing conditions (May, 2013 - March, 2014).

Month	Growing condition	Sharpblue[z]					
		Fruit wgt. (g)	Fruit dia. (mm)	Fruit frim. (kg_f)	Flesh firm. (kg_f)	Skin frim. (kg_f)	Seed /berry
May	Natural sunlight	2.07	15.84	0.21	0.03	0.18	x[y]
	Artificial light	2.39	16.74	0.19	0.04	0.15	x
	t -test[y]	*	*	**	*	***	–
Jun.	Natural sunlight	1.73	15.41	0.21	0.03	0.18	109
	Artificial light	1.96	15.80	0.16	0.04	0.12	64
	t -test	NS	NS	***	NS	***	***
Jul.	Natural sunlight	1.32	13.70	0.17	0.04	0.14	x
	Artificial light	1.51	14.68	0.17	0.03	0.13	x
	t -test	NS	**	NS	NS	NS	–
Aug.	Natural sunlight	x	x	x	x	x	x
	Artificial light	2.28	17.84	0.17	0.04	0.13	60
	t -test	–	–	–	–	–	–
Sept.	Natural sunlight	1.84	16.34	0.16	0.04	0.13	73
	Artificial light	1.64	15.90	0.19	0.04	0.15	48
	t -test	**	*	**	NS	**	*
Oct.	Natural sunlight	1.50	15.10	0.17	0.04	0.13	63
	Artificial light	1.98	16.30	0.19	0.04	0.15	62
	t -test	**	**	**	NS	**	NS
Nov.	Natural sunlight	x	x	x	x	x	x
	Artificial light	x	x	x	x	x	x
	t -test	–	–	–	–	–	–
Dec.	Natural sunlight	1.93	15.85	0.17	0.03	0.14	x
	Artificial light	x	x	x	x	x	x
	t -test	–	–	–	–	–	–
Jan.	Natural sunlight	1.54	14.64	0.21	0.04	0.17	101
	Artificial light	2.08	16.12	0.18	0.04	0.14	104
	t -test	***	***	*	NS	*	NS
Feb.	Natural sunlight	2.02	16.29	0.20	0.04	0.16	95
	Artificial light	2.13	16.34	0.20	0.05	0.15	112
	t -test	NS	NS	NS	NS	NS	NS
Mar.	Natural sunlight	1.71	15.32	0.22	0.04	0.18	60
	Artificial light	1.89	15.74	0.20	0.04	0.17	82
	t -test	*	NS	NS	NS	NS	NS

[z] Values show the mean.

[y] Data was not collected; No fruit was available.

[x] NS, ***, **, * indicate not significant, significant at $P < 0.001$, $P < 0.01$, and $P < 0.05$, respectively.

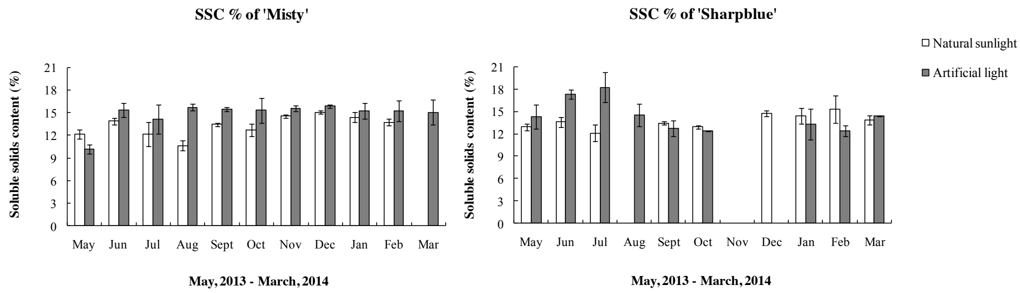


Fig. 3.2.1. Soluble solids content of 'Misty' and 'Sharpblue' fruits under different growing conditions.

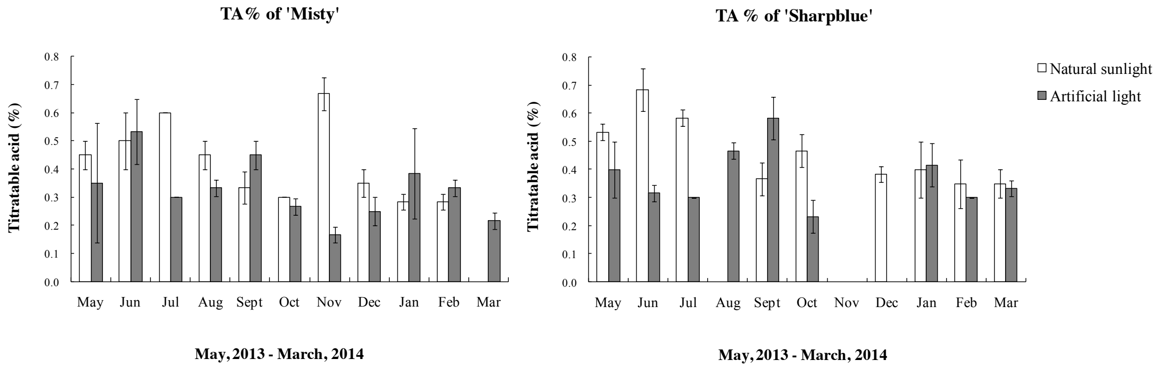


Fig. 3.2.2. Titratable acid percentage of 'Misty' and 'Sharpblue' fruits under different growing conditions.

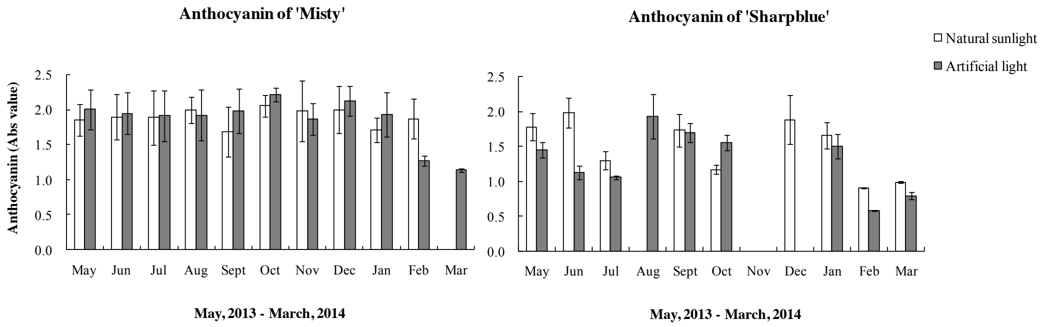


Fig. 3.2.3. Total anthocyanin content of 'Misty' and 'Sharpblue' fruits under different growing conditions.

Chapter 4

Sensory Taste Analysis and Questionnaire Survey on Eating Quality Test of Factory Produced Blueberry Fruits for Consumer Acceptability

Chapter 4-1: Different Tastes and Sensory Analysis of Blueberry Fruits Produced in a Plant Factory during Off-season

4.1.1. Introduction

An underlying goal for the long-term research of blueberry factory in TUAT campus is to produce high quality blueberry fruits all year round. The results from previous Chapters mentioned that blueberry fruits could be able to produce continuously in the Blueberry factory in TUAT campus and the fruits produced from some cultivars generally contained high soluble solids content, low acid amounts and high in quality especially for the fruits produced under artificial light. However, it is desirable to have an analysis by instrumental quality measurements for different tastes and evaluation of trained panelists for the taste of fruits produced from the factory for sound confirmation of previous results. Moreover, a comparative study between fruits from TUAT factory and other blueberry fruits available in Japan should be carried out. To that end, a cooperative study was carried out with Intelligent Sensor Technology, Inc. (INSENT) to analyze different tastes of blueberry fruits produced from the factory during winter season under two main objectives:

(1) To compare blueberries from different production areas, and

(2) To compare fresh (raw) blueberries and frozen blueberries.

4.1.2. Materials and Methods

The analysis was carried out during February, 2013 (winter season) and the evaluation performance was observed the following samples which were available during the experiment (Fig. 4.1.1).

(1) Fresh fruits imported from Mexico,

(2) Fresh fruits (mixture of 'Misty' and 'Sharpblue') from TUAT blueberry factory produced under natural sunlight during winter season,

(3) Fresh fruits (mixture of 'Misty' and 'Sharpblue') from TUAT blueberry factory produced under artificial light during winter season,

(4) Frozen fruits from Hokkaido, and

(5) Frozen fruits from Poland.

Taste information was analyzed in two ways;

(1) Instrumental measurement by using different taste sensors with artificial lipid-based membrane, and

(2) Sensory evaluation test by 14 panelists from the Intelligent Sensor Technology, Inc. (INSENT).

Thereafter, physiological properties of blueberry fruits were also analyzed finally.

4.1.2.1. Sample preparation and instrumental analysis

Chemical components and taste information of taste sensors are presented in Table 4.1.1. For fresh fruits, 120 g of fruits were put in a stomacher bag and add same amount of water and carried out three stomaching process with stroke 0.5 s/s for 30 seconds. Then filtrate with tea strainer and the samples were analyzed by the sensors. For frozen fruits, the samples were thawed and transferred to the refrigerator the day before testing and 120 g of individual sample was taken after removal of the extract. Then the same procedure was repeated like fresh fruit samples. Figure

4.1.2 shows measurement procedure by different sensors followed by the method of Kobayashi et al. (2010). First, the sensor was immersed in the reference solution of 30 mM potassium chloride (KCL) and 0.3 mM tartaric acid to obtain the membrane potential, V_r. Second, the sensor was immersed in the sample solution for getting the potential, V_s. Third, the sensor was rinsed lightly with the reference solution and it was immersed again in the reference solution to get the potential, V_r'. The difference in potential ($V_s - V_r$) was called the relative value and the difference in potential ($V_r' - V_r$) was called CPA (Change of membrane Potential caused by Adsorption) value. Finally, the sensor was rinsed well in alcohol solution to remove adsorbed substances before measuring the next sample and the same procedure was repeated for all samples.

4.1.2.2. Sensory test by panelists

Attribute of the participated panel for sensory evaluation test is shown in Table 4.1.2. Sensory tests of panelists from Intelligent Sensor Technology, Inc. (INSENT) were carried out to rate various tastes, texture and overall attributes of all samples using a 7 point-hedonic score (1, dislike extremely to 7, like extremely).

4.1.2.3. Physical property analysis

Additionally, some physical property data such as fruit weight (g), diameter (mm) and firmness (N), electric conductance, pH and °Brix of fruit juice were analyzed at Japan food research laboratory. To measure firmness, creep meter (Yamaden Co., Ltd.) with plunger diameter 20 mm and rising bar rate 1 mm•s^{-1} was used, and the unit was expressed in newton (N).

4.1.3. Results

4.1.3.1. Taste information of instrumental analysis

Taste information of different blueberry samples by taste sensors (Intelligent Sensor Technology, Inc. (INSENT)) is shown as radar charts (Fig. 4.1.3, 4.1.4). While reference solution (30 mM KCL + 0.3 mM tartaric acid) was set as a tasteless sample (control), the fruits produced from TUAT factory were sweeter, more savory (Umami) and less sour than other samples (Fig. 4.1.3). In the comparison between fresh and frozen blueberries, frozen fruits were sourer and less sweet although some other tastes were more or less the same except fruits from TUAT factory. When the taste of Mexico fruit was used as a control with taste information set to zero (Fig. 4.1.4), the same result was observed; fruits from TUAT factory were sweeter, more savory (Umami) and less sour among the samples. In the comparison of TUAT fruits between two growing conditions, the fruits produced under natural sunlight were sweeter, less sour and more savory (Umami) than fruits produced under artificial light.

4.1.3.2. Result of human sensory score

The result of sensory evaluation test by panelists is shown in Figure 4.1.5. From the results of eating quality evaluation among samples, fruits from TUAT factory both under natural sunlight and under artificial light got the highest score in sweetness (5.15, under natural sunlight and 5.05, under artificial light) and lowest score in sourness (2.85, under natural sunlight and 2.95, under artificial light) in all samples. Mexico fruits stood second rank in high sweetness (3.20) and low sourness (4.80) next to TUAT fruits. Fruits from Poland had the lowest sweetness (1.90) and the highest sourness (6.03) among the samples. On the other hand, firmness (5.23) and richness (4.95) of Mexico fruits was the highest in all samples while richness of other samples was more or less the same. In the comparison between fresh and frozen fruits, fresh fruits were sweeter, firmer and less sour than frozen fruits. Comparison between fruits from TUAT under two growing conditions, fruits under artificial light (3.88) were firmer than fruits under natural sunlight (3.42) although other parameters were not different.

In the scores of preference choice results, Mexico fruits got the highest score values in all parameters among the samples. However, fruits from TUAT factory were also getting high scores and not significantly different with Mexico fruits by LSD test. In the comparison of fresh and frozen samples, the panelists gave higher scores for fresh fruits than frozen ones in all parameters. Moreover, fruits from TUAT factory were getting the same scores for both growing conditions and not significantly different according to the preference choice by the panelists.

4.1.3.3. Result on physical property analysis

Table 4.1.3 shows the results of physical property analysis by Japan food research laboratory. Among all sample, Mexico fruits were the largest fruit size (weight and diameter) and the highest firmness. Then, TUAT fruits under artificial light stood in second rank. Fruits from Poland had the lowest in fruit size and firmness but the highest EC value. For pH and °Brix, the values of fresh fruits were higher than frozen fruits.

4.1.4. Discussion

From the above findings, it was found that blueberry fruits from TUAT factory were sweeter, more savory (Umami) and less sour compared to other fruit samples. Although the results were satisfactory, the fruits produced under natural sunlight were sweeter and less sour by taste sensor analysis in this study. However, there were no differences in sensory score by panelist between two growing conditions. In previous Chapters, it was learned that the southern highbush blueberries ‘Misty’ and ‘Sharpblue’ had different response to their favourable environment for getting high quality fruits. During the sample preparation of the fruits for this study, fruits from both cultivars for each growing conditions were unfortunately mixed due to the insufficient amount of the necessary samples. Therefore, the results observed in this study were the combination of tastes between ‘Misty’ and ‘Sharpblue’. If the samples could be prepared separately for each cultivar, the detail understanding of taste information could be obtained for individual one. However, this study was a pioneer of making comparison of blueberry fruits from TUAT blueberry factory with other blueberries from different places for instrumental analysis of various tastes, and panelists’ score for visual, textural and taste quality characteristics, respectively. Therefore, the study could determine how well the instruments could predict the sensory scores. The results showed that there were sensory-instrumental relationships on sweetness, sourness and appearance etc. Saftner et al. (2008) has shown that soluble solids content values were not correlated with sensory scores for intensity of sweetness or to any other sensory quality characteristics. In contrast, SSC % was highly correlated with sensory scores for sweetness in this study. Various textural and visual quality characteristics also influenced assessment of panelists for overall eating quality of blueberries. Finally, instrumental and sensory quality characteristics determined the fruits produced from TUAT factory were high in quality.

Fig. 4.1.1. Blueberry samples for instrumental and sensory analysis.

(1) Fruits from Mexico (Fresh)

(2) Fruits from TUAT blueberry factory produced under natural sunlight (Fresh)

(3) Fruits from TUAT blueberry factory produced under artificial light (Fresh)

(4) Fruits from Hokkaido (Fresh)

(5) Fruits from Poland (Fresh)

Table 4.1.1. Chemical components and taste information of taste sensors used by the sensor technology company.

Taste sensor	Chemical components		Taste information	
	Artificial lipid	**Plasticizer**	**First taste** **(Relative value)**	**Aftertaste** **(CPA value)**
Umami sensor AAE	Phosphoric acid di(2-ethylhexyl)ester Trioctylmetylammonium chloride	Dioctyl phenylphosphonate	**Umami(savoriness)** Amino acid, nucleic acid derivatives	Umami koku (richness)
Saltiness sensor CT0	Tetradodecylammonium bromide 1-Hexadecanol	Dioctyl phenylphosphonate	**Saltiness** Inorganic salt such as sodium chloride	None
Sourness sensor CA0	Phosphoric acid di(2-ethylhexyl) ester Oleic acid Trioctylmetylammonium chloride	Dioctyl phenylphosphonate	**Sourness** Acetic acid, citric acid and lartaric acid	None
Bitterness sensor C00	Tetradodecylammonium bromide	2-Nitrophenyl octyl ether	**Acidic bitterness** Unpleasant bitter taste substances derived from rough taste or secret ingredients	Aftertaste from acidic bitterness (Beer, coffee etc.)
Astringency sensor AE1	Tetradodecylammonium bromide	Dioctyl phenylphosphonate	**Astringency** Stimulations of astringent substances derived from rough taste or secret ingredients	Aftertaste from astringency (Tea, wine etc.)
Sweetness sensor GL1	Tetradodecylammonium bromide Trimellitic acid	Dioctyl phenylphosphonate	**Sweetness** Sugar alcohol	None

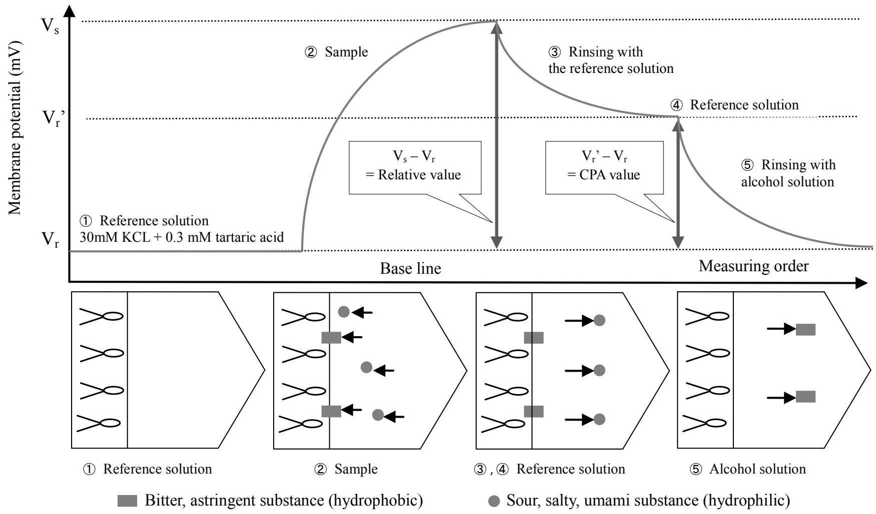


Fig. 4.1.2. Measurement procedure of taste sensors (Source: Kobayashi et al., 2010).

Table 4.1.2. Attribute of panel from the sensor technology company.

Panel[z]	**Like/dislike of sour fruits**	**Like/dislike of blueberry taste**	**Experience of eating fresh blueberry**
A	x	△	○
B	x	△	x
C	○	△	x
D	△	△	○
E	△	△	x
F	○	○	○
G	△	○	○
H	○	△	○
I	△	△	○
J	○	△	○
K	△	○	○
L	○	○	○
M	○	○	○
N	△	△	x

○ = Like; Yes, △ = Nither like nor dislike, x = Dislike; No.

[z] Male: A～E, Female: F～N.

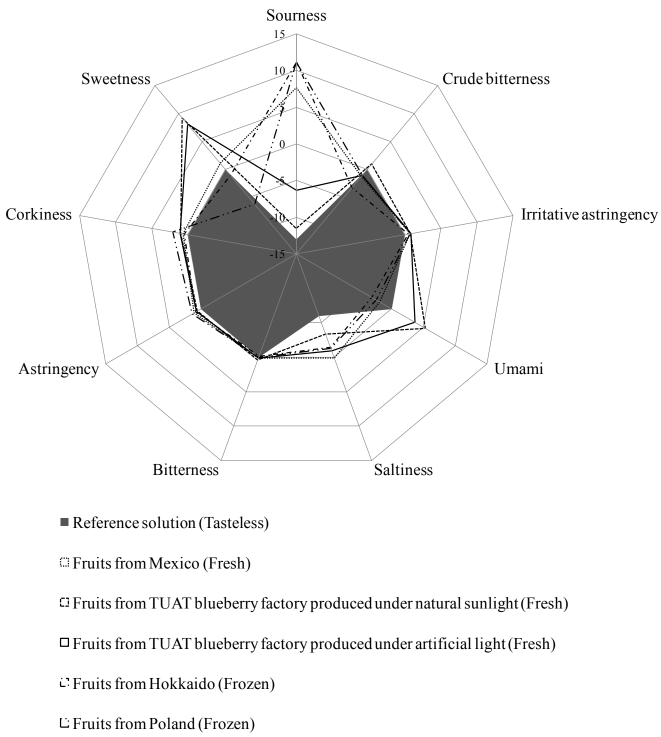


Fig. 4.1.3. Radar chart on the various taste items of the blueberry samples relative to reference solution (30 mM KCl + 0.3 mM tartaric acid) used as a tasteless sample with taste information set to zero.

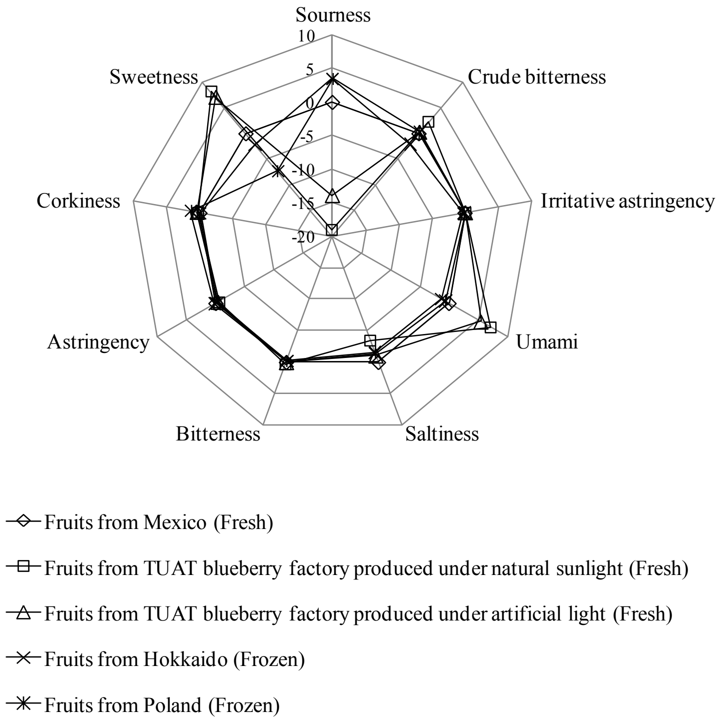


Fig. 4.1.4. Radar chart on the various taste items of the blueberry samples relative to fresh fruits from Mexico used as a control with taste information set to zero.

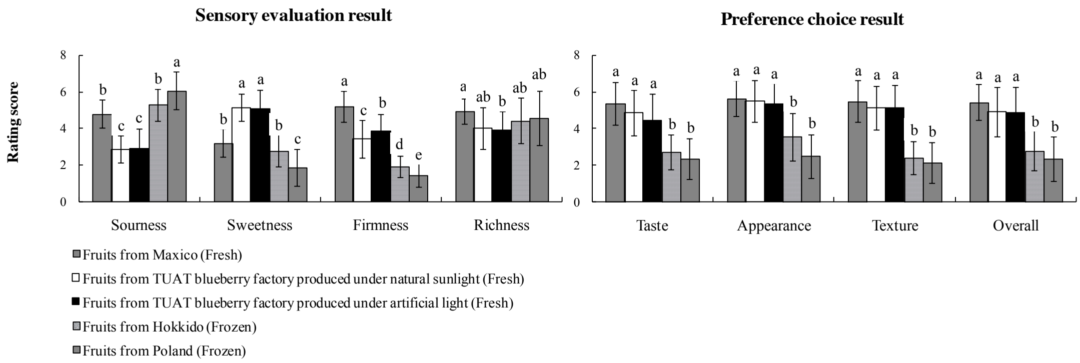


Fig. 4.1.5. Sensory evaluation test by panelists with grading scores from level 1 to 7.

Table 4.1.3. Physiological property data test by Japan food research laboratory.

Blueberry samples[z]	Weight (g)	Diameter (mm)	Firmness (N)	Electric conductivity (mS•cm $^{-1}$)	pH	°Brix
1	1.89	17.35	25.20	1.61	2.98	13.60
2	1.27	13.85	6.97	1.94	4.00	11.90
3	1.53	15.45	8.80	1.68	3.93	13.10
4	1.15	14.50	5.00	1.78	2.84	10.30
5	0.37	9.40	1.90	2.52	2.81	9.80

[z] 1 = Fruits from Maxico (Fresh)

2 = Fruits from TUAT blueberry factory produced under natural sunlight (Fresh)

3 = Fruits from TUAT blueberry factory produced under artificial light (Fresh)

4 = Fruits from Hokkido (Frozen)

5 = Fruits from Poland (Frozen)

Chapter 4-2: Questionnaire Survey on Eating Quality Test of Factory Produced Blueberry Fruits for Consumer Evaluation

4.2.1. Introduction

Nowadays, taste evaluation is gathering attention worldwide in many fields such as foods, beverages and pharmaceuticals to evaluate consumers' acceptability of a product. Sensory evaluation and chemical analysis are commonly used to evaluate taste qualities of the products (Kobayashi et al., 2010). Although sensory evaluation using a panel of tasters is susceptible to human physical and psychological conditions as well as individual preference, human sensory evaluation is still widely used in every large industrial food and beverage processing company (Citterio and Suzuki, 2008). In addition, although routine quality control of known food samples without human panelists might be possible with commercialized taste sensors, more challenging tasks, such as designing a new product or adapting the taste of a globally manufactured product to specific regional consumer preferences, are probably still beyond the limits of most artificial taste sensors. Therefore, adaptation to human taste is extremely important since the ultimate goal is to satisfy the consumer. The results from Chapter 4-1 mentioned that instrumental and sensory quality characteristics by trained panelists determined the fruits produced from TUAT factory were high in quality. However, it is necessary to know local consumers' response of factory produced blueberry and perception of the plant factory which is vitally important for long term future of the factory. Therefore, the questionnaire survey on eating quality test was carried out in this study and the main objectives were:

1) To predict consumers' evaluation of blueberry fruits produced from the TUAT factory in order to understand the market potential.
2) To check consumers' response of blueberry fruits between two different seasons (summer season vs. winter season).

4.2.2. Materials and Methods

The questionnaire survey on eating quality test was carried out two times during two different seasons; summer season, 2012 and winter season, 2013. Fruits of 'Blueray' and 'Misty' produced under two different growing conditions; glasshouse under artificial light and controlled room under artificial light were used in the survey. Altogether 100 participants (50 participants for summer season and 50 participants for winter season, respectively) from different classes such as business people, office staff, students (domestic and international) and housewives; ranged in age from 10 years old to more than 50 years old were participated. Although some participants had experience of eating fresh blueberry fruits, some had never experienced. Figure 4.2.1 shows sex ratio of the participants between different seasons and the age distribution of the participants was shown in Figure 4.2.2. In the background parameter, 'like' or 'dislike' of sweet taste preference was taken for further information. Sample number of blueberry fruits was two fruits per each growing condition per participant and asked to rate sweetness, sourness, flavour, texture and preference choice. To understand market potential, information of desirable purchase price (ranged from 100 to 500 yen) for 100 g of fresh blueberry fruits during Summer time (on-season) and Winter time (off-season) was also obtained from the participants in both seasons.

4.2.3. Results and Discussion

In both summer season and winter season, all participants accepted factory produced blueberry fruits and were delightful to join in the survey. The results on 'like' or 'dislike' of sweet taste preference are shown in Figure 4.2.3. In summer season, 72% of total participants liked sweet tastes and, among them, male participants (77%) preferred more sweet tastes compared to female participants (65%). During winter season, 82% of total participants preferred sweet taste and 100% of female participants would like to have sweet taste compare to male participants (72%). There are no participants of 'dislike' of sweet taste during winter season in female participants. In the comparison of the results between two seasons, the results showed that more participants wanted to have sweet taste during winter season compared to summer season. Form this finding, it can be assumed that the desire of human perception on sweet taste was changed depending on the seasons and they wanted to have more sweet taste when the weather was cold.

Figure 4.2.4 shows the results on sensory tastes; sweetness, sourness, flavour and preference choice of fruits produced under different growing conditions. According to the results on taste, all participants reported that fruits produced under artificial light were sweeter (96% in summer season and 68% in winter season) with less sour taste (12% in summer season and 20% in winter season, respectively). In the case of flavour, most participants could not identify whether they got flavour or not and how to differentiate blueberry-like flavour of the fruits. However, some participants answered they got blueberry-like flavour from fruits produced in both growing conditions according to the results. In both summer and winter season, all participants agreed that fruits under artificial light were more flavoursome than the fruits produced under natural sunlight. Therefore, according to the survey from the sensory taste results for two different seasons, it can be concluded that blueberry fruits produced in the controlled room under artificial light were sweeter, less sour and more falvoursome than those fruits produced under natural sunlight. Moreover, in the results on preference choice of fruits between two growing conditions, 68% of total participants (in summer season) and 70% of total participants (in winter season) selected the fruits produced under artificial light for their preference choice (Fig. 4.2.4). However, there were some participants who wanted to have sour taste in blueberries so that they selected the fruits produced under natural sunlight for their preference choice; 32% in summer season and 27% in winter season.

During the survey collection, it was noticed that some participants wanted to get more sour taste on the fruits produced under artificial light and they thought that it was not real blueberry taste if the fruits did not have some sour taste even the fruits were really delicious for them. Anyway, from overall results of preference choice, all participants who selected fruits produced under artificial light answered the reasons of choosing the fruits produced under artificial light in the same way. They evaluated the fruits produced under artificial light were more tender, delicate, juicy and easier to eat. As a whole, blueberry fruits produced under artificial light had high eating quality and consequently the fruits tasted better than those under natural sunlight according to the results of questionnaire survey. Subsequently, all members accepted the fruits produced under artificial light.

The inquiry of market survey between two seasons is shown in Figure 4.2.5. In both seasons, all participants said that it would be the best if they could get the quality blueberry fruits with the low price as cheap as possible. Due to the high price and limited supply of fresh blueberry fruits in the market, it was out of their reach to buy blueberry fruits frequently. From the survey results, many participants preferred to pay nearly the same price (300 yen) for 100 g of fruits in both on-season (40% for summer season survey and 36% for winter season survey) and off-season (38% for summer season survey and 34% for winter season survey). However, some participants were willing to pay a higher price (500 yen) if they could get blueberry fruits during off-season time (12% for summer season survey and 2% for winter season survey). In the comparison of the results between summer season and winter season, it was observed that people would like to buy more fruits and were willing to pay higher price during summer season than winter season.

To sum up the questionnaire survey of two seasons, it was speculated that gender, age and like or dislike of sweet taste might influence consumers' preferences so that these were included on the ballots. No age bias was shown for any of the sensory quality characteristics, but there were some gender bias and sweet taste preference, with females scoring higher on visual quality characteristics for acceptability of appearance, colour, sweetness and fruit size compared to males who wanted to have sour taste. Moreover, it is also observed that the human desire of sweet taste and desire of taking fruits was changing depending on the seasons. Finally, almost all participants suggested their desire of 'safe and security', 'quality', 'pesticide-free' and 'low price' for factory produced blueberry fruits.

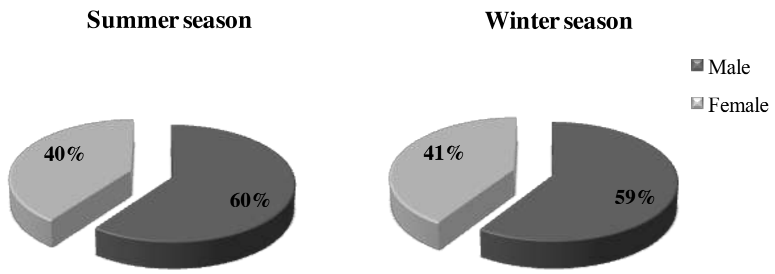


Fig. 4.2.1. Background parameter on sex ratio of survey participants between two seasons.

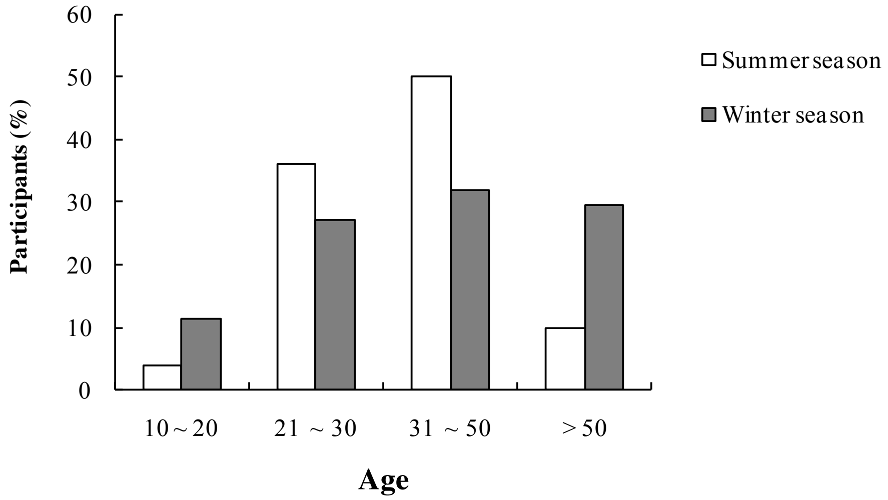


Fig. 4.2.2. Background parameter on age (10 to > 50 years old) of survey participants between two seasons.

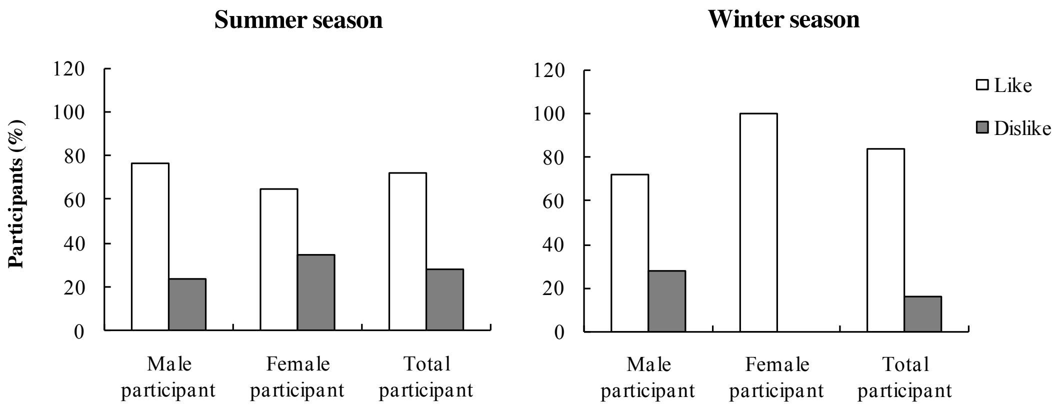


Fig. 4.2.3. Results on 'like' or 'dislike' of sweet taste preference by survey participants between two seasons.

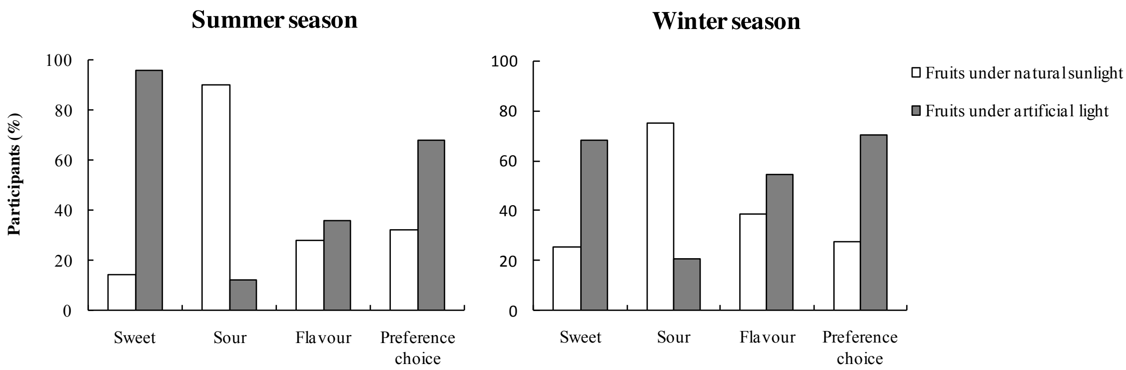


Fig. 4.2.4. Results on sensory taste and preferable choice of blueberry fruits by survey participants between two seasons.

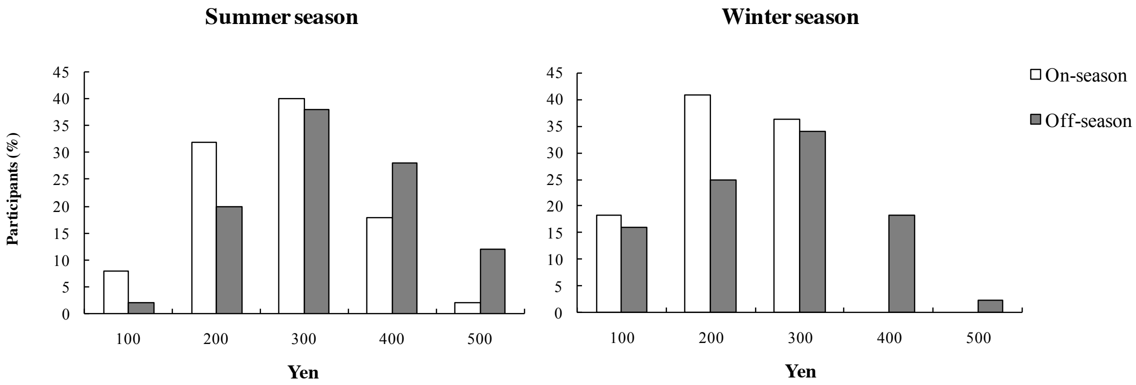


Fig. 4.2.5. Results on preferable price to buy 100g of blueberry fruits during on-season and off-season by participants between two survey seasons.

Chapter 5

Investigation Report on Consumer Opinion and Expectation for Realization of a Fruit Tree Factory

5.1. Introduction

In Japan, the development of vegetable plant factories was started in the second half of the 1970s and currently, Japan is one of the most active countries in the world in technology development in this field (Watanabe, 2011). About 127 vegetable plant factories are recently running and their operations have been successful in their development of consistent and efficient production technology but insufficient market information remains one of the obstacles for factory produced vegetables (Kurihara et al., 2014). In the report of consumer evaluation of plant factory produced vegetables by Kurihara et al. (2014), they mentioned that 60% of current commercial plant factories operate with a deficit balance and less than 10% are actually profitable according to some survey results. In 2011, the very first fruit tree plant factory focused on producing blueberry fruit was established in Tokyo (Ogiwara and Arie, 2010). Till now, blueberry fruits could be successfully produced twice a year from some cultivars (Ogiwara et al., 2014) and the fruits produced were high in quality according to instrumental and sensory analysis results (Chapter 2; 4). However, in order to conduct further development on technologies of blueberry factory in TUAT campus and for the successful commercialization of fruit tree factory throughout the country, an extensive survey of consumers from different areas and their opinions on fruit tree factories (hereafter: blueberry factory) and their expectations for the fruits produced should be found out. Therefore, a survey on internet findings of consumers’ response to fruit tree factory was conducted working together with Nippon Research Center to understand the market potential for the fruit tree factory.

5.2. Materials and Methods

An internet survey was conducted to the women participants living in Japan and the survey period was from 8th to 11th March, 2013. Data collection was mainly divided into two classifications; age and residence area. As for age, a total of 500 participants were participated between 20 and 69 years old; 100 participants for individual age level. For the second class, it was divided into 7 different regions according to the residence areas throughout the country; 1) Hokkaido and Tohoku, 2) Kanto, 3) Chubu, 4) Kinki, 5) Chugoku, Shikoku and Kyushu, 6) Tokyo metropolitan area and 7) Kansai area. Total participants number depending on the residence areas was 818. Data reviewing was performed on analysis mainly focus on the following points:

(1) Recognition of the plant factory,

(2) Acceptance of fruits from the fruit tree factory,

(3) Reasons of resistance or no resistance to fruits from the fruit tree factory and

(4) Expected cultivation methods for fruit tree plant factory.

5.3. Results and Discussion

Figure 5.1 shows that the recognition of the plant factory and factory products by the participants. The results showed that the plant factory was well-recognized depending on old age; more than 30% know well by 50s and 60s. In terms of residence areas, the participants from Kanto, Chubu and Tokyo metropolitan regions know well about the plant factory. However, 33.3～60.6% of the participants answered that they know somehow about the factory and 7～19% of them had heard about the factory. The unknown % about the factory was ranged from 3～22%. In the report of Kurihara et al. (2014), the unknown % of the factory produced vegetables in Tokasu region was 20%. Therefore, it was noticed that there were some people who have not had knowledge about the plant factory in Japan.

Figure 5.2 displays the acceptance of the plant factory products by the participants. The ages of 20s, 50s and 60s took in top positions of acceptability of the fruit tree factory (> 32%). Participants from Kanto and Tokyo metropolitan area answered the highest values of acceptability among the tested regions (> 35%). Delightfully, there were no participants for unacceptability of the plant factory by age 40s and Hokkaido and Tohoku region. The participants who did not want to eat factory products somehow was 6～21.2%. However, the percentage of unacceptability of the plant factory among overall participants was less than 5%. Therefore, from this finding, it can be assumed that the consumers can generally accept and are desirable the fruits from the fruit tree factory.

The reasons of the survey participants why they could accept factory produced fruits and no resistance to the fruit tree factory can be seen in Figure 5.3. Firstly, ‘constant price’ was the most common positive image for no resistance to the fruit tree factory production; the highest in age 50s (65%). Then, ‘safety’, ‘constant quality’ and ‘edible regardless of the season’ stood as a second preferable choice for no resistance of the factory products. As a third rank, ‘constant taste’, ‘sanitary with no dirt’, ‘stable nutritional value’, ‘beautiful appearance’ and ‘cheap price’ were recommended by the survey participants. There were some percentages for ‘equal size and shape’, ‘more delicious’, ‘same feeling with factory produced vegetables’ and ‘no particular feelings’ of the factory produced fruits have been found out finally.

In contrast, there were some participants who could not accept the factory produced fruits and had resistance to the fruit tree factory (Fig. 5.4). The main resistant reason of the participants to the fruit tree factory was they wanted to eat seasonal fruits by nature (63.2%). Those participants seemed to love nature according to their thoughts of ‘high nutritional value of open field products’, ‘open field products are more delicious’, and ‘feel safety growing in soil’. It was found that some participants had doubts on the factory produced fruits such as ‘using lots of chemicals for disinfection’, ‘imagination of using lots of chemical fertilizers’, ‘using genetically modified plants’ and ‘the products seem evil to their body’. Moreover, they did not think shape and size of the fruits were not different. Few participants had no particular reason but they disliked factory produced fruits. However, it was delightful to get the results of which the number of participants who resisted to the fruit tree factory was less than the participants who did not resist (Fig. 5.3, 5.4).

Finally, Figure 5.5 presents the consumers' expectations for their desirable fruits from the fruit tree factory. 'Food safety', 'availability' and 'stable cheap price' seemed to be the highest desire they want to get from the factory products because the highest percentage of 'no pesticide', 'stable price', 'edible all the time', 'low price', 'no fungicide' and 'no post-harvest chemicals' were found in the results. 'Non laborious' and 'nutritional expectation' could be classified as a second important desire because from the answers of 'edible fresh fruit', 'easy to eat without washing', 'more delicious', 'able to get rare fruits' and 'high nutritional values' and 'high functional ingredients'. Moreover, some participants had the extra expectations such as 'more components of beauty', 'reduce allergic components', 'lower calories' and 'new ingredients' to the factory produced fruits.

From the overall findings, it was clear that the participants' desire for 'food safety'. This result agreed with the finding of Kurihara et al. (2014) from the survey of 168 participants resided in metropolitan Chiba. In general, a common perception of consumers' opinion of the factory produced products is 'sanitary but high price'. Therefore, it is necessary to find the system for reducing production costs and promoting the safety of the factory produced fruits for commercializing of the fruit tree factories. Watanabe (2011) demonstrated that using high pressure sodium lamps and fluorescent lights accounts approximately 40% of the total running cost for vegetable production. Moreover, Watanabe (2011) reported the effectiveness of using light-emitting diodes (LEDs) not only for reducing the production costs but also for other benefits by using LEDs in his study. Therefore, thorough considerations and further researches for reduction of the production costs and constant supply of safety fruits are necessary for successful commercial running and the future development of fruit tree plant factory in Japan.

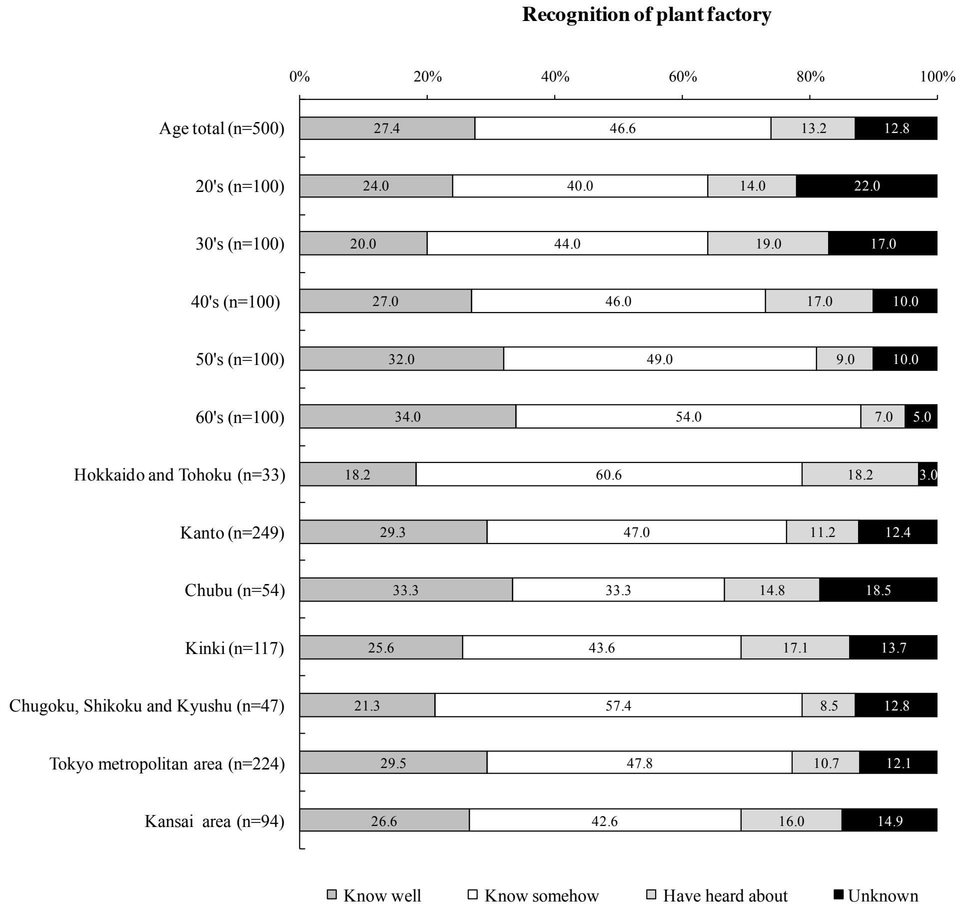


Fig. 5.1. The percent recognition of the plant factory by the participants depending on ages and residence areas according to the results of internet survey.

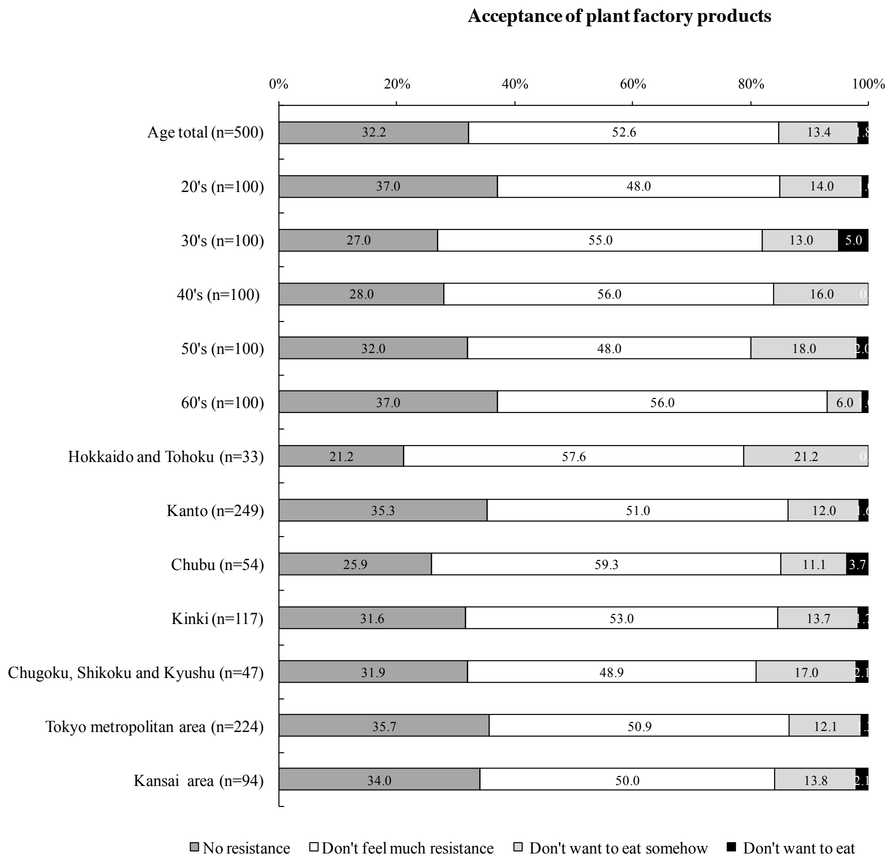
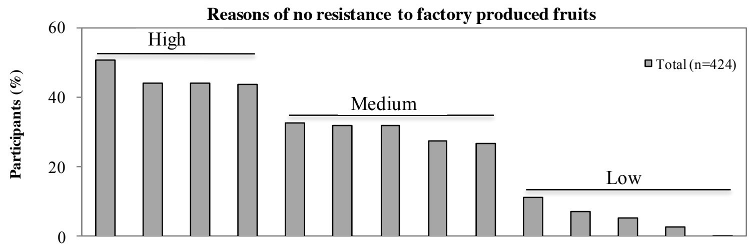
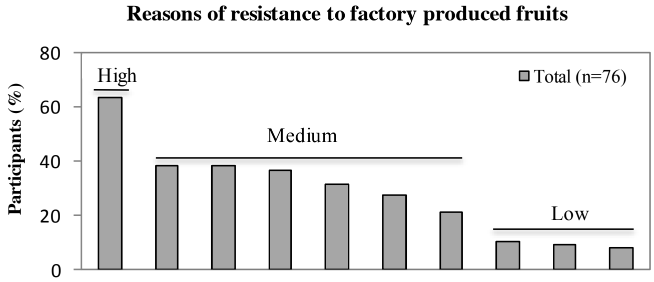


Fig. 5.2. The percent acceptance of the plant factory products by the participants depending on ages and residence areas according to the results of internet survey.



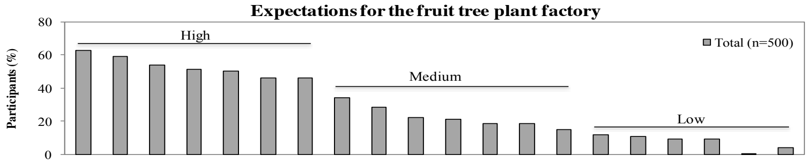
Classification		Number	The price is constant, not limited by seasonal price	Safe, pesticide-free, low-pesticide	Quality is constant throughout the year	Edible regardless of the season	Taste is constant throughout the year	There is no dirt, such as soil	Nutritional value is stable throughout the year	Appearance is beautiful	The price is cheap	Size and shape are aligned	There is no particular feelings	Vegetables are usually produced in plant factory	More delicious than open frield products	Other
Classification	Total (age)	424	50.9	44.1	44.1	43.9	32.5	32.1	32.1	27.4	26.7	11.3	7.3	5.4	2.8	0.2
Age	20's	85	40.0	25.9	27.1	35.3	23.5	21.2	21.2	16.5	43.5	10.6	9.4	4.7	3.5	-
	30's	82	45.1	34.1	28.0	35.4	22.0	26.8	18.3	23.2	24.4	9.8	12.2	7.3	3.7	-
	40's	84	45.2	46.4	41.7	44.0	27.4	32.1	35.7	31.0	23.8	8.3	7.1	3.6	1.2	1.2
	50's	80	65.0	51.3	57.5	51.3	37.5	33.8	30.0	26.3	27.5	10.0	2.5	7.5	3.8	-
	60's	93	59.1	61.3	64.5	52.7	50.5	45.2	52.7	38.7	15.1	17.2	5.4	4.3	2.2	-
Residence area	Hokkaido and Tohoku	26	46.2	38.5	38.5	34.6	23.1	23.1	15.4	11.5	26.9	11.5	19.2	-	-	-
	Kanto	215	50.7	43.7	47.0	45.1	35.3	31.2	33.5	28.4	25.1	13.0	5.6	6.0	2.3	0.5
	Chubu	46	47.8	43.5	41.3	50.0	39.1	37.0	45.7	34.8	26.1	13.0	10.9	8.7	4.3	-
	Kinki	99	57.6	46.5	37.4	42.4	29.3	36.4	30.3	25.3	32.3	10.1	6.1	5.1	3.0	-
	Chugoku, Shikoku and Kyushu	38	42.1	44.7	52.6	39.5	23.7	26.3	23.7	28.9	21.1	2.6	7.9	2.6	5.3	-
	Tokyo metropolitan area	194	50.5	44.8	47.4	43.8	36.1	32.5	33.5	28.9	22.7	13.4	4.6	6.2	2.6	0.5
	Kansai area	79	54.4	49.4	30.4	43.0	29.1	31.6	31.6	24.1	31.6	11.4	6.3	5.1	3.8	-

Fig. 5.3. Reasons for no resistance to the fruit tree factory production by the participants depending on ages and residence areas according to the results of internet survey.



Classification		Number	Want to eat seasonal fruits	Think nutritional value of open field product is high	Open field product is delicious	Feel safety of vegetables grown in the soil	Imagine lots of chemicals are using such as disinfection	Imagine lots of chemical fertilizers are using	Seem having genetically engineered crops	Seem evil to body	Shapes and sizes are the same, as artificial	No particular reason, but dislike somehow	Other
Classification	Total (age)	76	63.2	38.2	38.2	36.8	31.6	27.6	21.1	10.5	9.2	7.9	-
Age	20's	15	40.0	40.0	33.3	53.3	46.7	26.7	20.0	20.0	6.7	-	-
	30's	18	66.7	33.3	16.7	33.3	16.7	22.2	22.2	11.1	11.1	16.7	-
	40's	16	75.0	50.0	56.3	31.3	43.8	31.3	31.3	6.3	12.5	6.3	-
	50's	20	55.0	30.0	40.0	35.0	20.0	25.0	10.0	5.0	5.0	10.0	-
	60's	7	100	42.9	57.1	28.6	42.9	42.9	28.6	14.3	14.3	-	-
Residence area	Hokkaido and Tohoku	7	71.4	42.9	57.1	42.9	57.1	28.6	28.6	14.3	14.3	-	-
	Kanto	34	61.8	38.2	38.2	38.2	29.4	35.3	32.4	14.7	14.7	5.9	-
	Chubu	8	75.0	25.0	25.0	37.5	25.0	12.5	-	-	-	12.5	-
	Kinki	18	55.6	22.2	33.3	22.2	22.2	16.7	-	-	5.6	16.7	-
	Chugoku, Shikoku and Kyushu	9	66.7	77.8	44.4	55.6	44.4	33.3	33.3	22.2	-	-	-
	Tokyo metropolitan area	30	63.3	36.7	40.0	36.7	23.3	30.0	26.7	10.0	13.3	6.7	-
	Kansai area	15	53.3	26.7	26.7	20.0	20.0	20.0	-	-	6.7	20.0	-

Fig. 5.4. Reasons for resistance of the fruit tree factory by the participants depending on ages and residence areas according to the results of internet survey.



Classification		Number	No pesticides usage	Stable price throughout the year	Edible regardless of the season	No chemicals usage	Low price	Products without using fungicide	No chemicals, such as wax	Edible fresh fruits	Ready-to-eat without washing	More delicious than open frield products	Able to get rare fruits	Sweeter than open field products	Higher nutritional values than open field products	Higher functional ingredients than open field products	Higher components of beauty than open field products	Reduce in allergic components	Lower calories than open field products	Contain ingredients not included in open field products	Other	No expectation in particular
Classification	Total (age)	500	63.0	59.4	54.0	51.4	50.2	46.0	46.0	34.2	28.6	22.4	21.4	18.6	18.6	15.0	11.8	10.8	9.4	9.4	0.4	4.2
Age	20's	100	45.0	56.0	40.0	36.0	58.0	28.0	26.0	19.0	16.0	19.0	12.0	15.0	18.0	12.0	10.0	7.0	6.0	9.0	-	4.0
	30's	100	54.0	54.0	52.0	41.0	49.0	44.0	41.0	30.0	24.0	22.0	24.0	17.0	20.0	13.0	13.0	10.0	12.0	12.0	-	5.0
	40's	100	66.0	59.0	50.0	58.0	51.0	48.0	52.0	36.0	29.0	21.0	20.0	16.0	12.0	12.0	12.0	10.0	8.0	10.0	1.0	5.0
	50's	100	70.0	64.0	65.0	63.0	43.0	50.0	53.0	42.0	30.0	21.0	24.0	21.0	19.0	14.0	14.0	12.0	6.0	8.0	1.0	5.0
	60's	100	80.0	64.0	63.0	59.0	50.0	60.0	58.0	44.0	44.0	29.0	27.0	24.0	24.0	24.0	10.0	15.0	15.0	8.0	-	2.0
Residence area	Hokkaido and Tohoku	33	63.6	54.5	57.6	36.4	57.6	36.4	30.3	30.3	21.2	27.3	27.3	18.2	15.2	9.1	6.1	3.0	6.1	9.1	-	9.1
	Kanto	249	65.5	59.4	57.4	52.6	46.2	46.6	50.2	37.3	32.1	26.1	21.3	20.5	20.5	16.5	14.1	10.8	9.2	12.0	0.4	4.4
	Chubu	54	59.3	57.4	44.4	53.7	57.4	57.4	48.1	40.7	25.9	18.5	33.3	18.5	18.5	18.5	14.8	16.7	9.3	11.1	-	1.9
	Kinki	117	62.4	60.7	56.4	55.6	51.3	47.0	42.7	29.9	29.1	17.9	18.8	17.1	17.1	12.8	7.7	12.0	10.3	5.1	0.9	4.3
	Chugoku, Shikoku and Kyushu	47	55.3	61.7	38.3	42.6	55.3	34.0	40.4	23.4	17.0	14.9	10.6	12.8	14.9	12.8	10.6	6.4	10.6	4.3	-	2.1
	Tokyo metropolitan area	224	67.4	61.6	59.4	54.5	46.0	47.8	50.9	38.4	32.1	26.3	21.4	20.5	21.4	17.9	15.2	11.2	9.8	12.9	0.4	3.6
	Kansai area	94	63.8	63.8	56.4	55.3	53.2	44.7	40.4	28.7	26.6	17.0	19.1	19.1	18.1	12.8	7.4	12.8	11.7	6.4	1.1	3.2

Fig. 5.5. Expectations on the fruit tree factory by the participants depending on ages and residence areas according to the results of internet survey.

Chapter 6

General Discussion

6.1. Future prospect of blueberry production in Japan

In Japan, the growing areas and blueberry production have been dramatically increased in recent years although blueberries stand a minor fruit crop among the orchardists (MAFF Statistics, 2011). Day by day, increased blueberry consumption among the Japanese consumers has been caused by superior blueberry fruit functionality and availability, thus creating demand for increased blueberry production. However, there is still lacking of self-sufficiency within the country and blueberries are needed to import yearly (MAFF Statistics, 2011). Therefore, it is necessary to find ways to produce more blueberry fruits including year-round supply of the crop. From the findings of the recent study, it is possible to formulate a successful blueberry fruits production system in a controlled room under artificial light in an advanced blueberry factory. Subsequently, the fruits produced were high in quality especially from the plants grown under artificial light. Moreover, Japanese consumers also expect the constant supply of the high quality and safe fruits produced from the fruit tree factory.

By looking the commercial strawberry growing in Japan, seasonal production in the open field condition is June to late-July. However, recently, farmers are able to harvest more strawberries from late-October to early-November by the forcing culture and from April to July by the semi forcing culture (Fujishige, 2006). In the comparison of strawberry fruit quality between the open field and the forcing culture in a controlled environment, results showed that the fruits produced by the forcing culture are high in quality. The reason is that the difference between day and night temperatures is very large so that the duration from the flowering to the fruit maturation is longer than open field conditions. Moreover, plant respiration rate is very high under high temperature during May and June in the open field. In the forcing culture in the controlled environment, more sucrose can accumulate from the leaves to the fruits to contain more SSC % due to the low respiration rate of the plants under the low temperature and the longer growing season.

Ogiwara et al. (2014) reported that the life cycle of some southern highbush blueberry cultivars with low chilling requirement can be shortened without dormancy breaking by keeping the plants under low temperature and short-daylength after the end of harvesting in the open field. Moreover, they demonstrated that continuous flowering and fruiting from the water sprouts (shoots with vigorous growth) by post-harvest treatment with high temperature and long-daylength after the completion of flower bud formation to the plants. In recent study, it was also found that the possibility of off-season production of blueberry fruits by using low chilling requirement cultivars such as ‘Misty’ and ‘Sharpblue’ (Chapter 3-1). However, according to the result findings, it is necessary to choose the suitable cultivar with sustainable response to the growing environment to produce the constant yield and the constant high quality. Moreover, it is also essential to create the favourable environmental conditions for the pollinators such as bumblebees in a controlled room.

In future, it is hoped that blueberry farmers will be able to produce off-season blueberry fruits from December to May or June in the plastic houses. Moreover, the profitable commercial blueberry production will be improved to have more sugar contents and low acid percentage by growing in a controlled room under artificial light especially for production in the summer season compared to the usual open field growing in which the fruits quality is poor under the high temperature.

6.2. Points to consider for future development of the blueberry factory

Until now, although the blueberry factory in TUAT campus is running well without any serious problems yet, the following areas should be considered for sustainable long term development.

6.2.1. Energy consumption

Of course various kinds of plant factories cannot be established without a large amount of financial investment and it still needs inputs to continue proper running for long term. Recently, there are about 127 plant factories producing vegetables throughout Japan (Kurihara et al., 2014). Compared to fruit tree, the life cycles of vegetables are generally short so that care and management procedure will be much easier than fruit tree factory. Moreover, growers are quite familiar with the operation systems of vegetable plant factories for successful and consistent production since it has been started for about 40 years in Japan. In contrast, the life cycle of fruit trees are generally long (hereafter: blueberry) and it is necessary to alternate their annual growth cycle between a period of active growth and dormant period. Therefore, careful and detail considerations have to be made in environmental controlling systems. For example, ethylene emission is necessary to carry out for shedding leaves and to accelerate the fruit maturity. Moreover, low humidity condition, CO_2 emission and air flow is necessary to create for increasing photosynthetic capacity of blueberry leaves. In vegetable factories such as lettuce production, the PPFD values is generally ranged between 100–200 $\mu mol \bullet m^{-2} \bullet s^{-1}$and the distance between light source and the plants is about 30 cm or less. Due to the small size of the plants, it is possible to grow in layers and total yield per production area can be increased. However, for the fruit tree factory like blueberry factory, the plant size grown in the pot is generally about 1 m so that it is necessary to use the enormous amount of light intensity to get the leaves from different positions and more spaces for growing. In recent vegetable factories, one constraint with using the artificial lighting is the running cost, especially the costs for the lighting and the air conditioning. Estimates based on high-pressure sodium lamps and the fluorescent light indicate that the lighting costs approximately 40% of the total running cost in growing vegetables (Watanabe, 2011). Therefore, the use of light-emitting diodes (LEDs) has been popular in vegetables factories these days. Although LED lamps are relatively expensive, the long term benefits of using LED lamps is likely to become better than that of using high-pressure sodium lamps and fluorescent lights. For example, the amount of electricity costs was reduced to 30% or less by using LEDs in growing chrysanthemums (Watanabe, 2011). Since the blueberry factory in TUAT campus is the very special one equipped with controlling systems to create artificial seasons, the energy consumption is quite large and especially the electricity consumption is about 70% of the total costs. Estimates show that 30% of energy consumption will be decreased if the lighting system will be changed to LEDs. Therefore, in order to reduce the production costs as low as possible, various researches should be oriented not only to reduce the cost but also to produce high quality blueberry fruits continuously under the least energy consumption by using effectively and efficiently.

6.2.2. Pests and disease

In the blueberry factory in TUAT campus, no pesticide or insecticides have been used for any pest or disease problems. Manual controls such as removing insects by hand or washing the plants were practiced regularly. Until now, nearly three year of growing blueberries in the factory, it is still lacking of serious disease problems so far. However, cranberry fruitworm and aphids were the most serious pests occurring in the factory. Since the occurrence of cranberry fruitworm was serious in the glasshouses especially in summer time, aphid was the most serious pest found in the rooms of the underground floor throughout the year due to high humidity percentage of the growing rooms especially rainy days. Therefore, it should be considered for practicing the suitable integrated pest management (IPM) systems for sustainable long run production of blueberry fruits in future.

6.3. Future expected research of the blueberry factory

The recent study confirmed that blueberry fruits with high sugar content, high anthocyanin content but low acid amount could be produced in a controlled room under artificial light. Therefore, the production system for high quality food library is expected to develop in blueberry cultivars in future. Recently, a lot of knowledge was gained from one northern highbush blueberry ‘Blueray’ and two southern highbush blueberries ‘Misty’ and ‘Sharpblue’. However, it was found that the cultivar response to their preferable environment for getting high quality fruits is different and their capacity of sustainability. Therefore, further researches are still necessary to work with the other cultivars to find out the suitable cultivars for continuous production of fruits with sustainable high quality since over 100 blueberry cultivars are collected in TUAT campus. Although blueberries were continuously produced from some plants, some plants looked exhausted by rotating their cycle without resting periods. Therefore, the suitable nourishment system to replenish the weak plants should be considered for using the same trees continuously. Moreover, detailed studies on shoot growth patterns, flowering natures and low input energy consumption systems etc. should be made in the factory. Finally, not only in vivo but also in vitro studies such as DNA analysis of blueberries in the factory are worth to carry out.

Chapter 7

Summary

Blueberries have recently become recognized as one of the foremost health foods around the world including Japan. The consumption of blueberry by Japanese consumers is gradually increasing due to their desire for health benefits. In nature, due to the deciduous nature of the crop; i.e. shedding leaves and rotating their cycle, necessity of chilling requirements and passing of dormant periods etc.; blueberry can be harvested only one time a year in Japan. Moreover, major harvesting period is coinciding with the rainy season so that it is difficult to get high quality fruits. In 2011, in order to support blueberry production in Japan, 'Tokyo University of Agriculture Campus Blueberry Factory' was established for realizing the model of 'fruit tree factory' and focusing on year-round blueberry production. Although there are a lot of plant factory successfully running for year-round production of leafy vegetables (e.g. lettuce), vegetable fruits (e.g. tomato) and various flowers throughout Japan, the advanced blueberry factory in TUAT campus was the very first factory to produce a fruit tree (i.e. blueberry) all year round. For that reason, various kinds of research have been needed to carry out in the blueberry factory in TUAT campus urgently. In this study, determinations on:

(1) Plant growth and fruit quality analysis of blueberry in the controlled room under artificial light,
(2) Possibility of off-season production and continuous harvesting,
(3) Instrumental and sensory fruit quality analysis of factory produced fruits, and
(4) Survey on consumers' opinions of factory produced fruit

were carried out.

First of all, a comparative study was carried out among different blueberry cultivars; one

northern highbush blueberry and two southern highbush blueberries; for growth characteristics, photosynthetic potential and fruit quality analysis under different growing environments, in particular focusing on plants growing in a glasshouse under natural sunlight and those in a controlled room under artificial light. Environmental conditions of the controlled room under artificial light were determined 15 to 25°C, 50 to 70% humidity, 150 to 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light intensity and 10 hour photoperiod from primary experiment. Under these growing environments, normal fruits were developed in all the tested cultivars by successful growth without decreasing any plant vigour and leaf photosynthetic ability until fruit harvesting time compared to the cultivars grown in the glasshouse under natural sunlight condition. Moreover, it was confirmed that high quality fruits could be harvested in controlled environment to increase fruit production profits with high soluble solid content and high anthocyanin content but low acid concentration in some cultivars (Chapter 2).

From the beginning of the establishment of the blueberry factory in TUAT campus, various kinds of research have been conducted continuously. From the findings of Ogiwara et al. (2014), the life cycle of the blueberry plants could be controlled by adjusting moving time of the plants to the suitable growing environments and blueberry fruits could be successfully produced two times a year from the same plant in some southern highbush cultivars with low chilling requirement. Therefore, to get information on off-season blueberry production and to check whether blueberry fruits can be produced continuously or not, further study was carried out again. Attempts had been focused on two southern highbush blueberries in the factory. The results confirmed that it is possible to produce off-season blueberry fruits successfully in controlled environment by fulfilling necessary chilling requirements to low chilling requirement blueberry cultivars. Various fruit quality analysis was also investigated. Subsequently, monthly determination on possibility of blueberry fruit harvesting between two growing conditions, quality measurements of fruits were investigated on two southern highbush cultivars for 11 months. The results showed that it was possible to harvest blueberry fruits continuously by keeping the plants under suitable environmental conditions. Moreover, it was learned that the natural deciduous nature of the southern highbush blueberry plant could be controlled and able to be changed into ever bearing habits by fulfilling the suitable conditions of the growing environment as necessary in the controlled room (Chapter 3).

Nowadays, consumers' awareness and their knowledge have been extended in choosing various kinds of foods. Especially in getting raw foods such as fruits and vegetables, their desires have been wisely focused on not only for getting 'freshness', 'large size' and 'good taste' but also for 'safety', 'natural' and 'healthy' etc. Therefore, as a next step, instrumental analysis for different tastes, and sensory and eating quality test for the fruits produced in TUAT factory was conducted. In Chapter 4-1, blueberry fruits produced from TUAT factory were analyzed for different tastes by a cooperative study with a sensory company and panelists' score for visual, textural and taste etc. was also carried out to determine how well the instruments could predict the sensory scores. The results showed that there were sensory-instrumental relationships on sweetness, sourness and appearance. In addition to this, instrumental and sensory quality characteristics determined fruits from TUAT factory were high in quality.

Beyond this, in Chapter 4-2, a questionnaire survey on eating quality test between two different seasons (summer season and winter season in Japan) was investigated in order to estimate market potential and consumers' acceptability of blueberry fruits produced in a controlled room under artificial light in the TUAT factory. Delightfully, all participants accepted factory produced blueberry fruits. According to the sensory taste results, it can be concluded that blueberry fruits produced in the controlled room under artificial light were sweeter, less sour and more falvoursome. From the market survey, participants wanted to buy high quality blueberry fruits with the low price as cheap as possible but some participants were willing to pay higher price if they could get blueberries during off-season.

For the last step in Chapter 5, an extensive internet survey for consumers' opinions and expectations for the fruit tree factory was carried out working together with a research center throughout the country. Total number of 1318 women participants of different ages and from different regions was participated in the survey. The survey was performed mainly focus on: 1) recognition of the plant factory, 2) acceptance of fruits from the fruit tree factory, 3) reasons of resistance or no resistance to fruits from the fruit tree factory and 4) expected cultivation methods for fruit tree plant factory. From the results, it can be assumed that future extension of fruit tree factory is possible to carry out and it was also learned that consumer's desire of 'safety', 'quality' and 'low price' etc. for the fruits produced from the fruit tree factory.

Conclusion

Nowadays, blueberries have been recognized as one of the foremost health fruits around the world. In Japan, blueberry growing area and production have been increased rapidly from about 1995 due to the increased demand of Japanese consumers who are eating blueberries for their health benefits. In the open field production, the harvest duration of blueberry fruits in Japan is limited from June to September (only four months) form all suitable elevations by use of a range of species. Due to the efforts of some blueberry farmers by heating culture in plastic houses, some blueberries could be harvested in May. However, self-sufficiency is still lacking within the country and blueberries are needed to import every year (Ministry of Agriculture Statistics, 2011). In 2011, to upgrade recent blueberry production, an advanced blueberry factory was established in Tokyo University of Agriculture and Technology (TUAT) as the very first factory to grow a fruit tree in a controlled environment under four seasons (Ogiwara and Arie, 2010). From the attempts of off-season production and continuous harvesting of some cultivars of southern highbush blueberries, the result showed that it was possible to produce off-season fruits in a controlled environment (Ogiwara et al., 2014).

This study was carried out in a controlled environment under two different growing conditions in the TUAT blueberry factory and determinations on characteristics of plant growth and fruit quality analysis in Chapter 2, fruit quality changes throughout the year to evaluate fruit quality in Chapter 3, questionnaire survey on eating quality test of the factory produced fruits in Chapter 4 and consumers' opinion of the fruit tree factory in Chapter 5 were carried out, and the possible constraints of the fruit production in the plant factory were discussed.

First of all, a comparative study of one northern highbush and two southern highbush blueberries for growth characteristics, photosynthetic potential and fruit quality analysis was carried out. The results showed that normal fruits were developed in all the tested cultivars by successful growth without decreasing any plant vigour and leaf photosynthetic ability until fruit harvesting time. Moreover, it was confirmed that fruit quality was high in some cultivars with high soluble solids content and high anthocyanin content but low acid % under artificial light growing condition (Chapter 2).

Moreover, fruits which could be continuously harvested throughout the year were investigated for quality analysis. The results showed that fruit quality was different depending on growing condition and the cultivar; fruit quality of ‘Misty’ was constantly high but the quality of ‘Sharpblue’ was changing in controlled room. From this finding, it is essential to choose a suitable cultivar like ‘Misty’ for sustainable year-round production of high quality fruit (Chapter 3).

As a next step, blueberry fruits produced from TUAT factory in winter season were offered to make a comparative study between instrumental analysis of various tastes and panelists’ score for visual, textural and taste etc. The results showed that there were sensory and instrumental relationships on sweetness, sourness and appearance. In addition, instrumental and sensory quality characteristics determined blueberry fruits from TUAT factory were high in quality. Based on this finding, a questionnaire survey was carried out to 100 participants (50 in winter season and 50 in summer season) in order to estimate market potential and consumers’ acceptability of blueberry fruits produced from TUAT factory. Delightfully, all participants accepted factory produced blueberry fruits and then the eating quality results showed that fruits produced under artificial light were sweeter, less sour and tastier (Chapter 4).

Finally, an extensive internet survey for consumers’ opinions and expectations for fruit tree factory was carried out working together with a Japan research center throughout the country. Total number of 1,318 women participants of different ages (20 to 69 years old) living in different regions (Hokkaido, Tohoku and Kanto etc.) was participated in the survey. The survey results showed that over 80% of participants accepted factory produced fruits. Moreover, the reason of acceptance was to produce fruits with ‘safe and security’, ‘high quality’, ‘pesticide-free’ and ‘low price’ etc. for factory produced fruits (Chapter 5).

In conclusion, a fruit tree factory will be realized in future if it is possible to produce year-round with ‘high yield’, ‘high quality’, ‘safe’ and ‘security’ fruits constantly by choosing suitable cultivars.

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