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Blueberries Quality Evaluation during Maturation Using Image Processing

Aryanis Mutia Zahra [1)*], Tadashi Chosa [2)], Seishu Tojo [2)]

[1)]Department of Agricultural and Biosystems Engineering, Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta, Indonesia
[2)]Division of Environmental and Agricultural Engineering, Institute of Agriculture, Tokyo University of Agriculture and Technology, Tokyo, Japan
[*)] Correspondence Email: aryanismutiazahra@ugm.ac.id

ABSTRACT: Blueberries' quality parameters do not change uniformly during ripening. Blueberries should be harvested fully ripened at the post-climacteric stage with consistent color, taste, and ease of removal from the plant as excellent indicators. Therefore, the blueberries are not harvested until it has the desired blue color. The reliance on human perception on the fruit's taste and appearance might cause inconsistency and inaccurate judgment of the fruit maturation. This study aimed to develop an image processing algorithm capable of classifying blueberry maturity stages. The Bluecrop Northern highbush blueberries were harvested at five different stages of maturity based on visual grading of the fruit color (green, green-red, red, red-blue, and blue) from various fruit positions on the tree. Image processing with discriminant analysis accurately classified maturity stages at 98.3% accuracy. The image quality attributes of blueberries changed significantly at different maturity stages. Overall, most image quality attributes correlated strongly with blueberry physicochemical properties. This study showed that image processing during the blueberry maturation process could be a reliable and comprehensive method for estimating changes in color, shape, weight, and ultimately changes in specific physicochemical properties. This study also provided a practical evaluation of the maturity stages and physicochemical properties, which were predicted using image processing.

Keywords: blueberries, discriminant, image processing, maturity stages, visual evaluation.

INTRODUCTION

Blueberries' consumption has increased rapidly due to their high amounts of bioactive compounds and antioxidant activity, such as anthocyanins (Wang et al., 2017). Five stages of physiological and biochemical changes in blueberries occur during maturation. The stages mainly vary in color, such as green, green-red, red, red-blue, and blue (Lin et al., 2020). The biochemical and physiological changes during fruit maturation affect fruit size, color changes, and changes in sweetness and acidity (Eichholz et al., 2015). Color changes in blueberries are related to the pigment diversity in the peels, exclusively in anthocyanin forms (Lin et al., 2020; Guofang et al., 2019). Furthermore, the sweetness of blueberries is associated with late harvesting. The sweetness correlates to soluble solids resulting from converting starch and acids into sugar due to the natural maturing process (Mallik and Hamilton, 2017).

The blueberries are usually let in the bushes to ripe and evaluated manually by a human. However, human perception in taste and appearance causes inconsistency and inaccurate maturation (Moggia et al., 2018). One method for determining the maturity stage is image processing, which provides rapid, consistent, and non-destructive inspection. Combining fruit appearance with external quality can be classified automatically to reduce subjective from human experts (Mallik and Hamilton, 2017). Many studies have successfully applied image processing for fruit classification at different maturity stages. Pre-mature, early-mature, mature, and over-mature fruits based on color in image processing were successfully studied with 60 – 63% accuracy in cherry and strawberry fruit by thresholding technique (Raut and Bora, 2016). Fresh market tomato color values (RGB and HSI) with different maturity stages and varieties were successfully classified using image processing with 99.31% accuracy (Wan et al., 2018).

Digital image processing successfully determined the maturity of papaya fruit based on color values (RGB) using normalization, image segmentation, and pixel calculation (Simbolon et al., 2019). Therefore, the evaluation of external quality parameters, such as chromatic attributes (skin color) and geometric attributes (shape and size) at different maturity stages using image processing, is needed for blueberry cultivars. This research aimed to implement an image processing algorithm to classify the maturity stages of blueberries as the state of coloration and quality changes during maturation using image processing.

MATERIALS AND METHODS

Materials

Blueberry fruits (Bluecrop Northern highbush, Vaccinium corymbosum) were manually hand-harvested from the university orchard on Fuchu Campus of the Tokyo University of Agriculture and Technology, Japan. All the fruits were harvested from a tree planted eight years after being cultured in the pot for three years. Blueberries were harvested and sorted into five different maturity stages, based on visual grading and observation of the fruit color categories, green, green-red, red, red-blue, and blue, during fruit development and ripening (Table 1). The fruits were screened to eliminate defects as well as mechanical damage. The fruits from each stage were harvested on the same day, followed by the image capturing and the analysis process. For each stage, 12 fruits were collected randomly from different heights and areas of the clusters. For each stage, 12 fruits were collected randomly from different heights and areas of the clusters. Following that, 60 fruits were used as calibration and validation samples.

Fruit Position on Sample Grouping

The bunches from each cluster were measured on the height of the tree as the Z-axis for grouping into three positions as upper third (X > mean + SD), middle third (X = mean – SD ≤ X ≤ mean + SD), and lower third (X < mean – SD). All the clusters, including the sample, were then measured on the coordinates of the X-axis (east +X; west –X) and the Y-axis (north +Y; south –Y). The fruit position was determined by measuring the distance from the bunches to the tree's base.

Analysis of Physical Attributes

The weight and size of the berries from each stage and cultivars were measured. The fruit was weighed using an analytical balance (Shimadzu Analytical Balanced ATY-224, Japan). The size of the berries was calculated based on the average diameter from the maximum height and width to get spherical volume (Equation 1)(Mallik and Hamilton, 2017).

$$(1.333 \times \pi \times (\tfrac{d}{2})^3) \quad (1)$$

Total Soluble Solid Content, The Acidity of Juice, and Titratable Acidity

Each sample was ground in a mortar, and the juice was used for total soluble solids content (TSS) analysis. The TSS of blueberry juice was determined using Atago 3810 Hand Pocket Refractometer with a 0–32Brix measurement range and 0.2Brix increment at room temperature.

Total titratable acidity (TA) was measured by titration of 0.1N NaOH solution and blueberry juice with 10mL of dilution to a pH of 8.2 using 0.3mL of 1% phenolphthalein indicator. The amount of titrant required to achieve this pH was used to determine the concentration of citric acid in g/L. The pH of the juice before titration was also recorded. Solution containing 0.1N NaOH as thestandard base (12.65 pH approximately) prepared by diluting 2.722g NaOH (97%; ρ 1.515g/mL; MW 40 g/mol) with 1 L distilled water (modification from Mallik and Hamilton, 2017; Catrin and Sadler, 2019). TA was then calculated using Equation 2.

$$(\%TA\ (w/v) = (\frac{\text{mL NaOH x N NaOH x DF x sample amount x Eq weight of citric acid}}{1000})\ \text{x}\ 100\% \quad (2)$$

Table 1. Image classification for blueberry on different maturity stages

Maturity Stages	Characteristics
	Fully green color, smallest size, berries are expanding, highest acidity, and lowest Brix.
	Red in color with 25-75% greenish, berries are expanding, higher acidity and lower Brix, contain extra minuscule of anthocyanin.
	Fully red color, berries are expanding, have higher acidity and lower Brix, and contain very little anthocyanin.
	Red in color with 25-75% bluish, berries are expanding, moderate acidity and Brix, and contain small amount of anthocyanin.
	Completely blue in color, maximum weight, fruit size, Brix, higher anthocyanin content, and significantly lower acidity.

Total Anthocyanin Content

Total anthocyanin content was quantified using the pH differential method on pH 1.0 and pH 4.5, with the result expressed as mg (100g)-1 of cyanidin-3-glucoside equivalents on a fresh weight basis. The anthocyanin from blueberry juice was extracted with 10 mL of 70% acidified ethanol (EtOH:H2O:HCl concentration; 70:24:6; v/v/v; pH 2) for an hour. Centrifugation of filtrate was conducted at 6000 rpm for 15 min, and collected supernatants were used for further analysis (modification method from Lin et al., 2020; Mallik and Hamilton, 2017; Spinardi et al., 2019).

0.025 M KCl as the solution of pH 1.0 was obtained by dissolving 0.962 g of KCl (99.5%; ρ 1.98 g/mL; MW 74.55 g/mol) with 1 L distilled water. 0.4 M CH3COOK as the solution of pH 4.5 was obtained by dissolving 22.223 g of CH3COOK (98.5%; ρ 1.53 g/mL; MW 82.03 g/mol) with 1 L distilled water. 0.2 N HCl, which was added to reach pH requirements on both solution and 70% ethanol was obtained by diluting 16.7 mL HCl (37%; ρ 1.19 g/mL; MW 36.5 g/mol) to 100 mL with distilled water.

Total anthocyanin content was measured by diluting 1 mL of -

fresh blueberry extract supernatants with 5 mL of pH 1.0 and pH 4.5 solutions. The absorbance of the diluted solutions was measured at 520 and 700 nm using Shimadzu UV–1601Double Beam UV Visible spectrophotometers.

Total anthocyanin content was expressed in mg/100 g of fresh fruit and calculated using Equation 3 dan 4 (Lee et al., 2005 with modification).

$$\text{Anthocyanin (mg/100 g)} = (\text{absorbance x MW x initial volume})/(\varepsilon \text{ x sample weight}) \quad (3)$$

$$\text{Absorbance} = (A_{510}\text{ nm} - A_{700}\text{ nm})_{\text{pH 1.0}} - (A_{510}\text{ nm} - A_{700}\text{ nm})_{\text{pH 4.5}} \quad (4)$$

Where MW = 449.2 g/mol for citric acid; and ε = 26900L/(mol cm) for cyd-3-glu.

Image Capturing

The image is taken from three positions of the blueberries (calyx, stem, and side) using DSLR Canon 500D kit (Lens EFS 18 – 55 mm, Mode Single-Frame Shooting, ISO 100, Shutter Speed 1/15, Aperture 5.0, White Balance Auto, Exposure Comp. 0.0) for colors and object size analysis. Blueberries were placed horizontally and vertically above the whiteboard as a background. The image capturing was done inside the box, which prevents the light from outside entering the system. A camera with a 15 – 20 cm distance above the enclosure floor was set and focused on blueberries. Two LED lights were located at the upper corners to illuminate inside the enclosure (Figure 1).

Image Processing

The image, taken by the DSLR camera, was entered into the image processing program, which was made in SharpDevelop 5.1 RC, with a 400 x 300-pixel resolution (Figure 1). The image quality attributes were analyzed by changing the fruit image into a binary image through the thresholding process. The object is set to white, while the background was set to black. In order to separate between objects and images, to improve pixel accuracy, and to reduce the background noise, an opening operation on the binary image was performed with a structured element of 7 by 7 pixels, followed by a closing operation using the same structured element to maintain the original object size. The resulting clean binary image was used to obtain a blueberry-color image by masking the binary picture in the original image, as illustrated in Figure 2. Finally, the image quality attributes of the fruit were extracted from the masked image in the normalized RGB and HSI color models.

Data Analysis

Analyses of variance (ANOVA) were used to evaluate the effect of different maturity stages on each parameter with a Factorial Randomized Design. Duncan's multiple range test (DMRT) was used for further testing. The probability value of $P \leq 0.05$ was used to denote significant differences. Research data were processed using SPSS. Correlations and multiple linear regression analyses were also used to determine the relationship between physicochemical properties and image quality attributes.

The discriminant analysis method was used to perform the classification functions and validation processes. In this research, discriminant analysis was used to identify image attributes that can classify blueberry fruit at each maturity stage as categorical. SPSS also used the Leave-one-out-cross (LOO) validation technique, as all data sets have a small sample size. LOO is the logical extreme of K-fold cross-validation, with the fold count equaling the number of data points in the original data sets. The LOO method was used for all training and validation data sets. If cross-validation accuracy is similar to original accuracy, the discriminating model is more accurate in performance evaluation (Everitt and Landau, 2004).

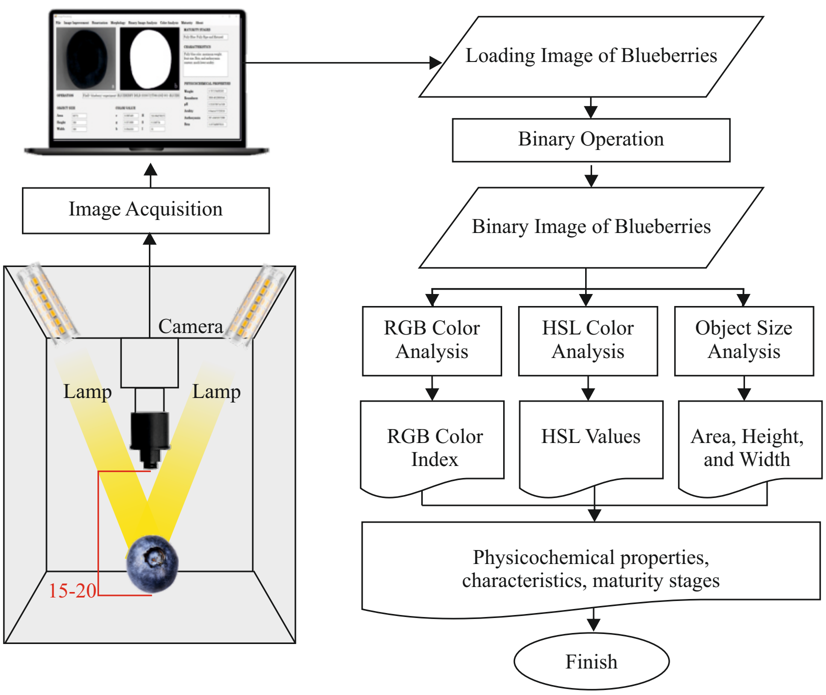

Figure 1. Image processing diagram along with the data stream and camera configuration.

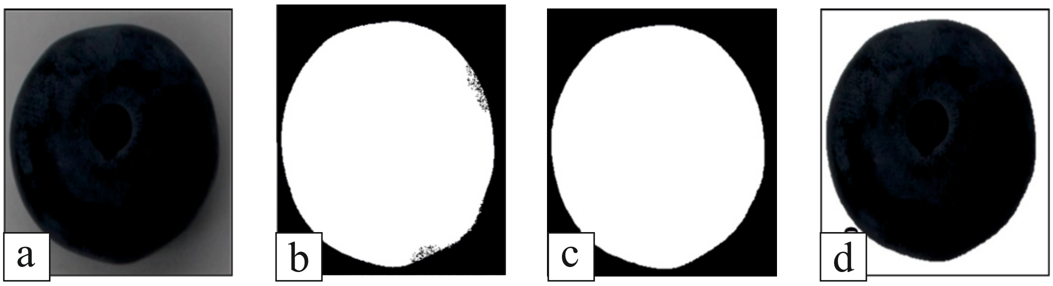

Figure 2. Image processing applied to extract chromatic and geometric of blueberries; (a) original image captured, (b) binary image obtained by grayscale thresholding process, (c) binary image after opening and closing, and (d) result of masking for background elimination before color extraction.

RESULT AND DISCUSSION

Fruit Size Changes on Different Maturity Stages

Significant changes could be detected ($P < 0.05$) on weight (g), roundness (cm2), areas (pixels), and roundness (pixels) at different maturity stages of the blueberries (Table 2 and Table 3). These changes indicated that the changes in the maturity stages were very closely related to fruit size.

Fruit sizes changes at different maturity stages are shown in Figure 3 and Figure 4. Rapid development in volume during fruit maturation was caused by cell enlargement. Moreover, berries continued to grow until they turned entirely ripe. Blueberries that grow on the same bush did not all ripen at the same time. Blueberries were harvested weekly over a period of four weeks. Blueberries are best harvested when the blue stage has a maximum fruit size (Eichholz et al., 2015).

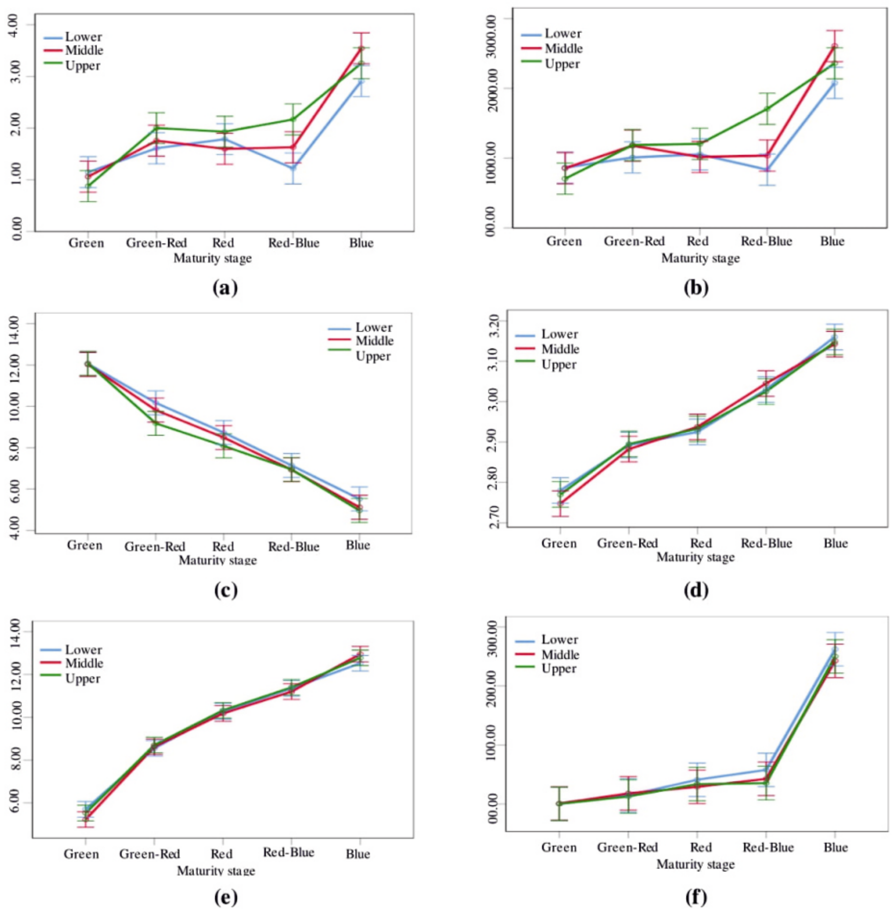

Figure 3. Physicochemical changes of blueberries at different maturity stages and fruit position; (a) weight in g, (b) cm2 of spherical volume, (c) g/L citric acid, (d) pH of the acidity of the juice, (e) Brix of soluble solid content, and (f) mg(100 g)-1 content of cyanidin-3-glucoside.

The size of the fruit on weight and roundness may differ due to the fruit position that has a different shading effect (Retamales et al., 2008). The lack of radiation had the most significant impact on physical attributes changes between the fruit set and the beginning of berry softening. Moreover, the deficiency of light during the last stage of highbush blueberry fruits may lead to a substantial delay (of about 10–16 days) in the harvest compared with well-illuminated fruits –(Godoy et al., 2018). In more cases on the middle and lower positions rather than the upper positions, the positive consequence of shading could occur in climates or regions with a high incidence of radiation. The blueberry tree can avoid super optimal light levels, plant heating, and photosynthesis inhibition by reducing stressful conditions during midday hours. Moreover, the developing fruit under shading benefited from having water and a significant supply of carbohydrates made available by young developing leaves.

These compounds being available to the fruit increased the amount of the fruit set, which influences the photosynthetic rate through a feedback mechanism. In addition, the shading effects on the different fruit positions led to changes in the quality of the transmitted light. Plants can detect the quality, quantity, and orientation of light and use light as a signal to optimize their growth and development in their particular environment(Retamales et al., 2008).

Color Changes on Different Maturity Stages

Significantly different ($P < 0.05$) changes can be detected from normalized RGB and HSI values at different maturity stages (Table 2). These changes indicated that the changes in the maturity stages were very closely related to the change in color. Color changes of normalized RGB and HSI on different maturity stages are shown in Figure 4.

Color changes on blueberries correlated to the diversity of pigment accumulation which implicates both synthesis and degradation of the plant pigments during fruit ripening. The unripe blueberry fruit has a high of polyphenols and tannins that give a green color. Therefore maturation of blueberry was accompanied by an increase in anthocyanin content and a decrease in proanthocyanidin, polyphenol, and flavonoid concentration which have a blue-purple color (Lin et al., 2020).

In this study, similar topographic features, soil characteristics, and management characteristics were used. Consequently, most of the environmental variability could be associated with the different availability of solar radiation by the specific courses of air temperature, both strongly related to fruit position. Another research reported that temperatures at the lower positions positively affected anthocyanin accumulation, mainly in the early ripening, when the berry started the pigmentation process (Spinardi et al., 2019). The fruit skin of blueberries grown at the lower positions showed more pigmentation than the higher position of the tree. Fruit belonging to the red stage and red-blue showed a shift from red-blue to blue. After this stage, the patterns of pigment accumulation were similar at all positions. The result suggested that the environmental conditions allowed the kinetic of pigment deposition coordinated to the ripening process to proceed faster in berries grown at the higher location(Spinardi et al., 2019).

Acidity, Anthocyanin, and Brix Changes on Different Maturity Stages

Significantly changes on total soluble solids content (TSS, Brix) was significantly changed ($P < 0.05$) at different maturity stages. However, titratable acidity (citric acid, g/L), anthocyanin content (mg/100 g)$^{-1}$ of cyanidin-3-glucoside), and the acidity of juice (pH) were significantly changed in each maturity stage only (Table 3).

Table 2. Object size and color from DSLR image processed changes on different maturity and fruit position

Properties	Green			Green-Red			Red			Red-Blue			Blue		
	Upper	Medium	Lower	Upper	Medium	Lower	Upper	Medium	Lower	Upper	Medium	Lower	Upper	Medium	Lower
R index	0.356 ± 0.004^{c}	0.351 ± 0.009^{cd}	0.357 ± 0.005^{bc}	0.402 ± 0.014^{a}	0.382 ± 0.010^{ab}	0.375 ± 0.012^{bc}	0.373 ± 0.019^{bc}	0.366 ± 0.008^{bc}	0.367 ± 0.018^{bc}	0.308 ± 0.006^{ef}	0.329 ± 0.012^{de}	0.316 ± 0.008^{ef}	0.292 ± 0.005^{f}	0.291 ± 0.003^{f}	0.296 ± 0.003^{f}
G index	0.408 ± 0.006^{a}	0.399 ± 0.007^{a}	0.402 ± 0.006^{a}	0.335 ± 0.028^{b}	0.318 ± 0.006^{bc}	0.323 ± 0.008^{b}	0.292 ± 0.010^{bc}	0.300 ± 0.002^{bcd}	0.298 ± 0.006^{bc}	0.319 ± 0.003^{d}	0.314 ± 0.001^{cd}	0.316 ± 0.003^{cd}	0.324 ± 0.00^{b}	0.330 ± 0.002^{b}	0.325 ± 0.002^{b}
B index	0.236 ± 0.008^{f}	0.250 ± 0.013^{ef}	0.240 ± 0.011^{ef}	0.263 ± 0.020^{e}	0.294 ± 0.006^{d}	0.308 ± 0.013^{d}	0.335 ± 0.009^{c}	0.335 ± 0.006^{c}	0.335 ± 0.013^{c}	0.372 ± 0.008^{ab}	0.357 ± 0.012^{bc}	0.368 ± 0.006^{ab}	0.384 ± 0.006^{a}	0.380 ± 0.003^{ab}	0.379 ± 0.002^{ab}
H	80.140 ± 2.285^{g}	82.782 ± 5.544^{g}	78.493 ± 3.154^{g}	123.266 ± 38.011^{f}	172.566 ± 7.093^{e}	180.605 ± 30.945^{de}	282.122 ± 6.055^{a}	270.364 ± 7.383^{ab}	275.532 ± 13.531^{a}	214.270 ± 15.317^{cd}	235.362 ± 4.695^{bc}	234.163 ± 9.942^{bc}	212.274 ± 6.169^{cd}	210.423 ± 1.881^{cd}	213.052 ± 3.900^{cd}
S	0.293 ± 0.024^{a}	0.252 ± 0.037^{a}	0.280 ± 0.032^{a}	0.236 ± 0.051^{a}	0.157 ± 0.012^{b}	0.144 ± 0.040^{bc}	0.129 ± 0.034^{bcd}	0.108 ± 0.008^{bcd}	0.114 ± 0.015^{bcd}	0.091 ± 0.018^{cd}	0.076 ± 0.008^{d}	0.086 ± 0.008^{cd}	0.128 ± 0.015^{bcd}	0.131 ± 0.007^{bcd}	0.116 ± 0.007^{bcd}
I	54.333 ± 1.656^{a}	49.000 ± 5.882^{ab}	47.667 ± 1.054^{abc}	40.667 ± 5.284^{cd}	40.667 ± 1.866^{cd}	45.500 ± 1.347^{bc}	35.250 ± 5.043^{de}	31.417 ± 2.644^{ef}	31.500 ± 1.503^{ef}	27.667 ± 3.209^{f}	30.167 ± 1.374^{ef}	33.917 ± 0.569^{def}	30.750 ± 1.134^{ef}	33.333 ± 0.981^{ef}	30.333 ± 1.414^{ef}
Roundness	17310673 ± 454374^{d}	17034414 ± 2404960^{d}	17496545 ± 17727^{cd}	19652079 ± 563807^{abc}	19056250 ± 496445^{abcd}	19007907 ± 608685^{abcd}	20306343 ± 663046^{ab}	18993586 ± 323360^{abcd}	19054614 ± 269526^{abcd}	18907184 ± 928236^{abcd}	18558038 ± 637197^{bcd}	17616039 ± 910128^{d}	21065666 ± 720461^{a}	19465578 ± 377220^{bcd}	20514458 ± 1203982^{ab}
Area	27610 ± 3892^{e}	33625 ± 3554de	33870 ± 1906de	46271 ± 4262^{abc}	42188 ± 2514^{cd}	40149 ± 7320^{cd}	51008 ± 5007^{abc}	45103 ± 4508	49094 ± 5410^{abc}	42477 ± 5460^{cd}	48236 ± 4369^{abc}	40828 ± 4420^{cd}	56221 ± 4453^{ab}	57515 ± 3849^{a}	54862 ± 5594^{ab}

a, b, c, d, e, f, g The mean value of twelve replication, numbers with different letters in each property show a significantly different effect ($\alpha = 0.05$) based on Duncan's multiple range test on both factors.

Table 3. Quality and fruit size changes on different maturity and fruit position

Properties	Green			Green-Red			Red			Red-Blue			Blue		
	Upper	Medium	Lower	Upper	Medium	Lower	Upper	Medium	Lower	Upper	Medium	Lower	Upper	Medium	Lower
Weight (g)	0.8773 ± 0.186^{f}	1.0607 ± 0.217^{ef}	1.1489 ± 0.075^{ef}	2.0014 ± 0.239^{c}	1.7569 ± 0.169^{cde}	1.6104 ± 0.421^{cdef}	1.9306 ± 0.283^{cd}	1.5992 ± 0.212^{cdef}	1.7866 ± 0.232^{cde}	2.1687 ± 0.366^{bc}	1.6301 ± 0.240^{cdef}	1.2193 ± 0.201^{def}	3.2566 ± 0.322^{a}	3.5462 ± 0.187^{a}	2.9109 ± 0.656^{ab}
Roundness (cm^2)	705.0 ± 127.0^{d}	855.0 ± 147.4^{d}	863.4 ± 61.1^{d}	1186.2 ± 225.7^{cd}	1178.4 ± 102.8^{cd}	1009.5 ± 280.8^{d}	1204.3 ± 148.8^{cd}	1059.1 ± 1016.0^{d}	1053.1 ± 122.6^{d}	1705.1 ± 240.3^{bc}	1036.3 ± 150.3^{d}	833.3 ± 267.8^{d}	2358.3 ± 267.2^{a}	2604.8 ± 244.4^{a}	2077.7 ± 473.5^{ab}
Titratable Acidity (g/L citric acid)	12.07 ± 0.40^{a}	12.02 ± 0.44^{a}	12.06 ± 0.49^{a}	9.17 ± 0.58^{bcd}	9.81 ± 0.34^{bc}	10.17 ± 0.45^{b}	8.09 ± 0.71^{def}	8.49 ± 0.41^{cde}	8.73 ± 0.31^{bcd}	6.95 ± 0.56^{fg}	6.93 ± 0.59^{fg}	7.14 ± 0.66^{ef}	4.96 ± 0.61^{h}	5.12 ± 1.17^{h}	5.52 ± 0.31^{gh}
pH of Juice	2.77 ± 0.03^{d}	2.75 ± 0.75^{d}	2.78 ± 0.01^{d}	2.90 ± 0.06^{c}	2.88 ± 0.01^{c}	2.89 ± 0.02^{c}	2.93 ± 0.03^{c}	2.94 ± 0.05^{c}	2.93 ± 0.02^{c}	3.03 ± 0.02^{b}	3.05 ± 0.04^{b}	3.03 ± 0.03^{b}	3.15 ± 0.04^{a}	3.14 ± 0.02^{a}	3.16 ± 0.03^{a}
°Brix	5.4 ± 0.2^{f}	5.2 ± 0.3^{f}	5.7 ± 0.1^{f}	8.7 ± 0.4^{e}	8.6 ± 0.3^{e}	8.6 ± 0.3^{e}	10.3 ± 0.4^{cd}	10.2 ± 0.3^{d}	10.3 ± 0.2^{d}	11.4 ± 0.4^{b}	11.2 ± 0.4^{bc}	11.4 ± 0.2^{b}	12.8 ± 0.4^{a}	13.0 ± 0.8^{a}	12.5 ± 0.4^{a}
Anthocyanin (mg(100 g)$^{-1}$ of cyanidin-3-glucoside)	0.146 ± 0.172^{b}	0.877 ± 0.958^{b}	0.167 ± 0.136^{b}	12.674 ± 1.263^{b}	17.927 ± 3.411^{b}	14.753 ± 8.682^{b}	33.662 ± 22.637^{b}	29.025 ± 20.344^{b}	41.069 ± 28.578^{b}	35.218 ± 8.183^{b}	42.532 ± 5.881^{b}	57.761 ± 18.790^{b}	250.158 ± 51.175^{a}	242.553 ± 60.914^{a}	262.156 ± 57.109^{a}

a, b, c, d, e, f, g, h The mean value of four replications denoted with different letters in each property show a significantly different effect ($\alpha = 0.05$) based on Duncan's multiple range test on both factors (except titratable acidity, anthocyanin content, and pH, which only show a significantly different for each factor).

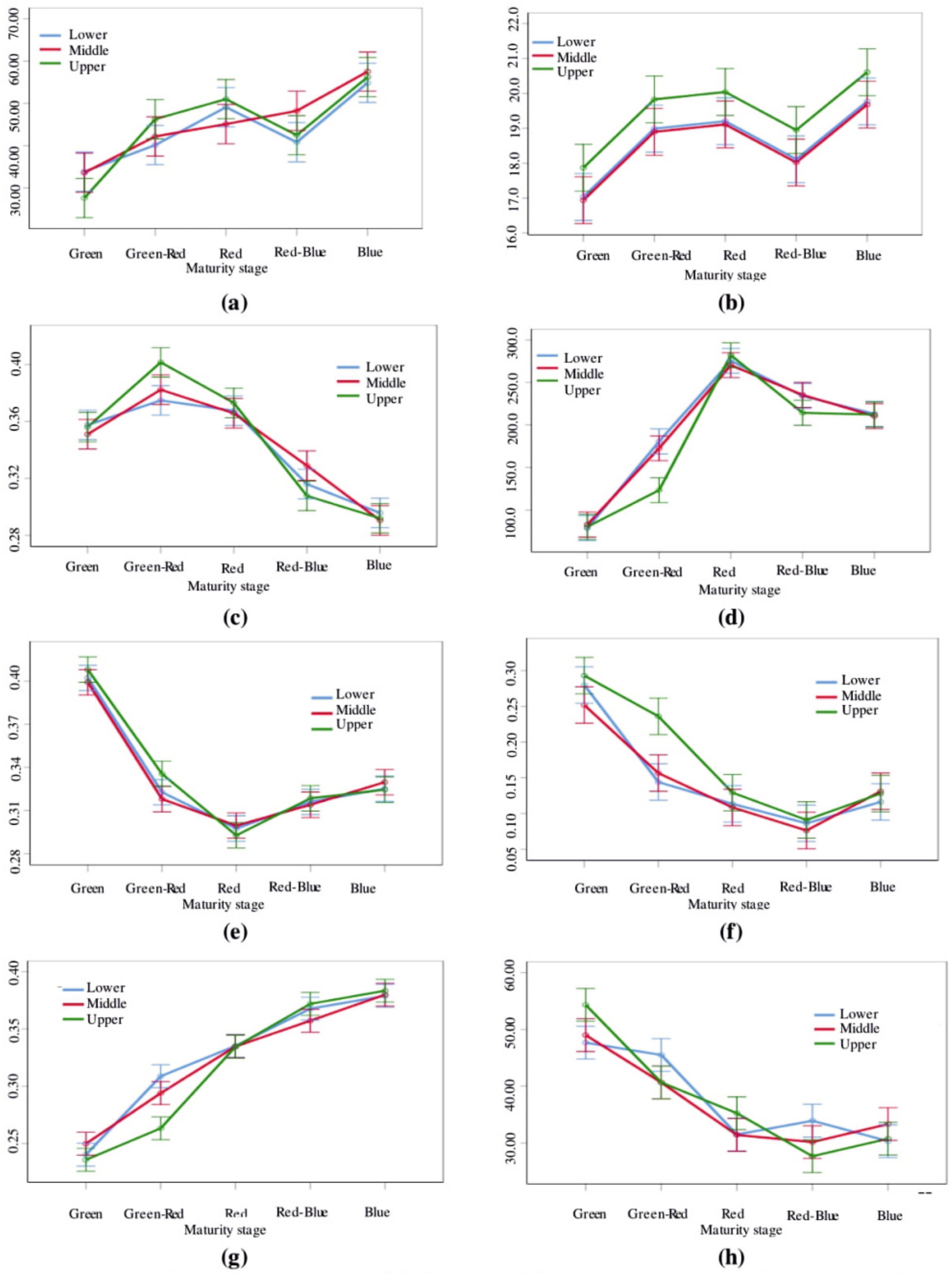

Figure 4. Image quality attributes changes of blueberries at different maturity stages and fruit position; (a) area of the image in 103 pixels, (b) roundness of image in 106 pixels, (c) Index R of the image, (d) H value of the image, (e) Index G of the image, and (f) S value of the image, (g) Index B of the image, (h) I value of the image.

As shown in Figure 3, blueberries have TSS, juice acidity, and titratable acidity that gradually change during maturation. However, anthocyanin content had significantly increased in the blue stages. Therefore, harvesting at the blue ripeness level becomes very important for blueberries in producing anthocyanin content optimally. The pigments in the blueberry fruit peels were almost exclusively present in the form of anthocyanins, which play a role in color changes of pink, blue, and purple. The accumulation of anthocyanins also leads to increased antioxidant activity.

The anthocyanins group from delphinidin, malvidin, peonidin and cyanidin individually increased during fruit maturation. Following their deep coloration, blue-purple anthocyanins pigments, especially the delphinidin, will beaccumulated dramatically. Other types of anthocyanin are hydrolyzed during the extraction process and changed into cyanidin-type. In a highly acidic environment (pH < 3), anthocyanins exist in their stable form of flavylium ion gives the matrix reddish coloration intensified and enables the anthocyanin to be highly soluble in water.

During berry development, anthocyanin proportions were always lower at a higher position at the early and turning stage of berry ripening and were likely associated with a slower ripening process. The changes of anthocyanins proportion occurred earlier at the lower positions characterized by higher temperatures, where blueberries initially ripened more quickly. However, the different positions did not affect total anthocyanin contents and did not alter their accumulation patterns.

Higher temperatures during day and night led to the initial difference in anthocyanin concentration. Nevertheless, these differences diminished along the ripening process. The final ripening stage counterbalanced the gap by accumulating pigments at a more rapid rate, resulting in equal levels at the blue stages. The berries are grown at a higher position compensated for initial disparity by accumulating pigments at a much more rapid pace as the temperature was closer to the optimum temperature. The compensatory mechanism suggests a feedback response resulting from the coordinated regulation of flavonoid pathway genes, mainly driven by developmental and genetic cues and specific environmental effects(Spinardi et al., 2019).

Decreases of proanthocyanidin and tannins improve sensory attributes by reducing astringency and bitterness (Lin et al., 2020). Moreover, sweetness is reported to be correlated with glucose, total sugars, oxalic acid, citric acid, anthocyanidins, and °Brix. Sour taste is correlated with total acids and citric acid. Blueberry flavor is correlated with oxalic acid and citric acid. The most organic acid in blueberry was citric acid, which makes up 77%–87% of the total acid, depending on the maturity stage at harvest. The organic acids consumption as a substrate for respiration during fruit maturation leads to the intensification of TSS and acidity of the juice, and decrease of the TA. Therefore, a lower citric acid was reported in ripening blueberry fruit(Hwang et al., 2020; Lin et al., 2020).

The sweetness of blueberries was also associated with late harvesting, as acids are converted into sugar contents (Bett-Garber et al., 2015). The increase of sugar accumulation in blueberries may be resulted from starch conversion to sugars due to the natural ripening process. Besides, titratable acidity during fruit ripening decreases due to organic acid conversion to phenolic compounds (Catrin and Sadler, 2019; Mallik and Hamilton, 2017). Moreover, the three substantial sugars of highbush blueberries are sucrose, fructose, and glucose, increased during fruit maturation (Hwang et al., 2020).

Relationship Between Physicochemical Properties with Image Quality Attributes

Based on the significant value in the F test, the image quality attributes of fruit were significantly correlated to physicochemical properties of fruit (fruit size, weight, the acidity of the juice, titratable acidity, anthocyanin content, and TSS) by 77–94%. The coefficient of determination (R - square), which was calculated from the correlation coefficient (R) squaring, could measure how much influence was given by image quality attributes of fruits simultaneously on the physicochemical properties.

The relationship interpretation (R) and percentage of variance explained (R square) was varied for all the image quality attributes with physicochemical properties. Generally, a significant correlation (79.9–94.0% of variance explained) was evaluated between image quality attributes and the acidity of the juice, titratable acidity, anthocyanin content, and TSS. Similarly, a significant correlation (77.1–77.9% of variance explained) was evaluated between image quality attributes with weight and roundness. The results have shown that image quality attributes changed at different maturity stages were related to physicochemical properties.

Based on the relative contribution in the image quality attribute, the index G had a more dominant influence on weight and acidity. The index B had a more dominant effect on the acidity of the juice, anthocyanins, and Brix. In addition, the index R had a more dominant influence on roundness. The relative contribution was able to identify the most important predictor variables in the regression model related to the beta coefficient and partial correlation coefficient.

Based on the significant value in the t-test, each image quality attributes significantly affected each physicochemical property differently. In the image quality attributes, the area was positively correlated with weight, roundness, and anthocyanins. The H value was negatively correlated with the weight, roundness, and acidity of the juice. The S value was positively correlated with weight, roundness, the acidity of the juice, anthocyanins, and Brix. The t-value indicated the predictor's correlation with the response. A positive correlation indicates that the higher the value of the variable predictor, the greater the response is. A negative correlation suggests that as the variable predictor increases, the response tends to decrease.

As color ratings of index R, index G, and S value decreased, acidity also decreased. Moreover, as color ratings of index B and H value increased, anthocyanins, weight, and Brix also increased. The result implied that blueberries with dark blue color had higher Brix, anthocyanin contents, and weight but lower acidity value. The colorimetric parameters in various studies showed correlations between anthocyanin biosynthesis and accumulation in grapes and strawberries as colored cultivars (Ferreira et al., 2017; Nunes, 2015). Anthocyanin biosynthesis and accumulation lead to red or blue color and a decrease in green during berry development(Ferreira et al., 2017).

However, there is no research detailing how fruit size is related to color. Other studies have found a positive correlation between berry diameter and berry weight with TSS in a bunch load of grapes (Somkuwar et al., 2020). The increased diameter and length of the berries contributed to the increase in berry weight (Somkuwar et al., 2020). TSS is highly correlated with sugar content, but it is also influenced by other soluble compounds found in blueberries, raspberries, and strawberries, such as organic acid and phenolics (Gerbrandt et al., 2020).

Other studies reported the significance of the correlation between the improvement of berry color and increased soluble solids and total anthocyanin content in red table grapes . The increase in color was associated with a greater abundance of anthocyanin and could be due to an increase in soluble solids content . Additionally, color changes were reported to be significantly correlated with strawberry acidity. As a result, as the color darkens with maturity, the acidity content decreases.

Maturity Stage Classification Based on Image Quality Attributes

All the samples were included in the discriminant function and model grouped according to different stages of maturity. The classification was based on the direct method, which was a group based on all independent variables. In the test of group equality, the image quality attribute as a predictor variable had a discriminating coefficient of less than zero based on a significant F-test. As statistical assumptions, the data passed linearity, normality, multilinearity, and equal variance tests. It means discriminant analysis could maximally separate groups within maturity stages.

Function 1 had the highest eigenvalues (30,803) and can explain 62.8% of the variance with the highest canonical correlation (0.984) on each image quality attribute. These results showed that function 1 had the best discrimination power. Moreover, function 1 had the smallest Wilks' lambda value (0) or equal to the highest chi-square value (439,635) for each image quality attribute. These results showed that Function 1 was statistically significant and provided an average discriminant score for each maturity stage.

Based on the standardized canonically discriminating function coefficient, index B and S values had significantly discriminating ability through function 1. Index R and H values had the highest discriminating ability through functions 2. The G index, H values, and I value had significantly discriminating ability through function 3. The S value was the only predictor variable that had the most significant discriminating ability through functions 4.

The image quality attributes of the structure matrix had shown that function 1 most closely correlated with index B, index G, H, and I value sequentially. The R index had the highest correlation with function 2. Furthermore, the S value, area, and height correlated most closely with function 4. Function three had not given any predictor variable the highest correlation value.

Canonical discriminant function coefficients had shown that the coefficients of all predictor variables in each function build the discriminant function. This function was used to validate predictions in the original validation section by calculating each function's discriminant score. Furthermore, discriminant models were built to classify the different maturity stages by maximizing lambda values using Fisher's linear discriminant method on classification function coefficients.

Five equations were developed and tested by inputting the values of index RGB, HSI value, area, and height extracted from each image. Where y_{green}, $y_{green\text{-}red}$, y_{red}, $y_{red\text{-}blue}$, and y_{blue} were the groups of maturity stages while indexing RGB, HSI value, area, and height were obtained from image processing.

$$y_{green} = -67670.804 + 11.043Height - 0.003Area + 6.773H - 2253.404S - 34.187I + 130039.773R + 144462.068G + 124544.258B \quad (5)$$

$$y_{green\text{-}red} = -67587.741 + 10.999Height - 0.002Area + 6.749H - 2159.109S - 33.995I + 130324.239R + 143620.113G + 124904.369B \quad (6)$$

$$y_{red} = -68272.415 + 11.146Height - 0.003Area + 7.324\mathrm{H} - 2039.343S - 35.634I + 130773.280R + 144387.767G + 125443.810B \quad (7)$$

$$y_{red\text{-}blue} = -68006.132 + 10.918Height - 0.002Area + 6.964H - 2061.500S - 35.858I + 130326.943R + 144290.935G + 125605.654B \quad (8)$$

$$y_{blue} = -68144.828 + 10.986Height - 0.002Area + 6.835H - 1907.607S - 35.678I + 130153.261R + 144291.789G + 125996.752B \quad (9)$$

This classification model was used to validate the cross-validation section by calculating the discriminant score using each model obtained at each maturity stage. Furthermore, the discriminant scores can classify the sample at a specific maturity stage. The results indicated that each maturity stage had different discriminant scores as critical or separator values.

The results showed the predicted maturity and percentage ofaccuracy after grouping. Most predictions for group membership were based on the initial data. An accuracy level of 98.3% was achieved in classifying the discriminant function into maturity stages (Table 4). The high accuracy showed that the model was suitable for all groups when using the original data to predict their maturity stage. Due to the high accuracy rate, the discriminant model could be helpful to predict different maturity stages based on image quality attributes.

Table 4. Maturity stage classification based on the image quality attributes using discriminant model

Real Maturity Stage*	Predicted Maturity Stage**					Total Sample	Accuracy (%)
	Green	Green-Red	Red	Red-Blue	Blue		
Green	12	0	0	0	0	12	100
Green-Red	0	12	0	0	0	12	100
Red	0	0	12	0	0	12	100
Red-Blue	0	0	0	11	0	12	91.7
Blue	0	0	0	0	12	12	100
Total	12	12	12	12	12	60	98.3

* The real maturity stage was classified based on color as human perception.
** Predicted maturity stage was classified based on image quality attribute using the discriminant model; 98.3% of original grouped cases correctly classified.

There was a continuous increase in weight, roundness, TSS, the acidity of the juice, and anthocyanin, and a subsequent decrease in acidity during fruit maturation. Previous studies showed that the fruit size, TSS, the acidity of the juice, and anthocyanin increased, and TA decreased with fruit maturation (Arena et al., 2013; Hwang et al., 2020; Mallik and Hamilton, 2017). Blueberry size increases during maturation due to cell enlargement, affecting total phenolics, total anthocyanins, and total antioxidant activity concentrated in blueberry epidermal tissues (Mallik and Hamilton, 2017). Moreover, total anthocyanin increases as blueberry mature and concludes that anthocyanin biosynthesis is highly related to the developmental stages of the fruit(Lin et al., 2020).

The accumulation of anthocyanin, deepening of color, and fruit maturation associates with high antioxidant capacity in blueberries and may assist commercial producers in harvest-

-ing blueberries with excellent antioxidant characteristics. A decrease in titratable acidity and an increase in fruit size, TSS, and juice acidity also contribute to a shift in blueberry fruit taste, with ripened blueberry fruits becoming more popular and edible directly (Hwang et al., 2020; Lin et al., 2020).

CONCLUSION

The quality changes of blueberries were affected by different maturity stages. The changes were also diverse within the fruit in the bushes. The image processing program was developed using algorithms by thresholding technique to classify the different maturity stages of blueberries based on single view fruit images and image quality attributes. The image quality attributes (normalized RGB, HIS, roundness, and area) have a relationship with physicochemical properties of blueberries weight, roundness, the acidity of the juice, titratable acidity, total anthocyanin content, and total soluble solid). The discriminant model was feasible to predict the different maturity stages based on image quality attributes. The image processing worked well to evaluate the visual quality, classify maturity levels, and predict physicochemical properties based on the image quality attributes at different maturity stages of blueberries. This research can be used by either industry or researchers, providing a reliable method for evaluating the visual quality of fruit.

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