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The wax bloom on blueberry: Application of luster sensor technology to assess glossiness and the effect of polishing as a fruit quality parameter

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Summary

The wax bloom of the fruit is responsible for the visible quality of blueberries. This study aimed to investigate a new technology using the effect of polishing on micromorphology, wax content and weight loss of blueberries. Luster sensor (type CZ-H72, Keyence, Japan) technology was used to assess glossiness of polished blueberries compared with berries with a natural (unpolished) wax layer during 9 days after harvest. Blueberries were rubbed twice by hand within a soft microfibre tissue to obtain polished fruit. Unpolished blueberries contained ca. 120 µg wax cm^{-2}, which was reduced by ca. 22% to ca. 95 µg cm^{-2} by polishing. This reduction was associated with an increase in luster levels from ca. 65 to 80 a.u.. Weight loss was larger from polished than from unpolished blueberries with a concomitant 40% increase in luster levels from 60 to 85 a.u. in polished fruit. Luster levels sharply decreased from 85 a.u. in the first 5 days after harvest and then leveled off to remain almost constant at ca. 20 a.u. with significantly larger values for polished blueberries of ca. 30 a.u. with a larger magnitude of glossiness. Overall, luster sensor technology may offer a new effective, affordable, possibly portable, non-destructive technique to assess glossiness or other surface features in real time for classifying not only blueberry, but also other waxy fruit such as aubergine/eggplant, plum, *Juniperus*, blue grape berry etc..

Introduction

The quality of food encompasses both the inner and exterior quality parameters; the latter comprises the visual appearance, which is composed of the colour (e.g. ESTEBAN et al., 2014) and surface structure (BLANKE et al., 1986 and 1996) in both leaves and fruits. In a range of fruits such as blueberry (*Vaccinium* spp.), a conspicuous wax bloom develops on its surface during its ontogeny (FREEMAN et al., 1979) and strongly affects its fruit quality in that the wax bloom affects both transpiration rate, shrinkage, shriveling and water loss, as shown for plum (MUKHTAR et al, 2014) and possibly storability and, to a lesser extent, the visual appearance and hence possibly consumer appeal. Blueberry belongs to the *Ericaceae* family. Its increasing popularity is due to blueberry being recognized as a 'super fruit' or 'superfood' because of its positive health benefits including essential nutrients and antioxidants such as myricetin and quercetin glycosides (WOLFE et al., 2008; CHEN et al., 2015). However, the blueberry has a short postharvest life at room temperature because of moisture loss (PANIAGUA et al., 2013). MARTIN and JUNIPER (1970) reported that a wax bloom or layer could protect the underlying plant tissues from such desiccation, pathogen infection and insect attack. This wax bloom, the appearance of a whitish surface coating, occurs from the formation of a partial or continuous layer of amorphous or crystalline wax on leaves and fruits during their ontogeny (HOLLOWAY and JEFFREE, 1962; JEFFREE et al., 1975; BAKER, 1982). The wax bloom on fruit surfaces provides their visual attraction and acts as the water loss barrier (ALBRIGO et al. 1980; BAIN and MCBEAN, 1967; BLANKE, 1986; BLANKE et al., 1996; SAPERS, 1984; WISUTHIPHAET et al., 2014), and may be used as a good physical quality parameter for those fruits with a conspicuous wax bloom on the surface such as blueberry (FREEMAN et al., 1979). To our knowledge, no investigation on the appearance and measurement of glossiness of blueberries has been reported despite its conspicuous wax bloom and relevance described above. While wax removal has been postulated as a consequence of handling i.e. polishing fruit such as plum (MUKHTAR et al., 2014), there is no study of non-destructive detection of the wax surface, its relation to water loss as dependent on handling and no study on the amount of wax possibly removed by handling the fruit. Traditional gloss meters appear unsuitable for measurements on berry fruit due to their measurement restrictions to large areas and flat surface implying direct contact with the object. A new luster sensor is becoming available (MUKHTAR et al., 2014) and may be promising because of its compact size, low energy consumption, price and real time results.

Therefore, the objective of this work was to investigate the effect of polishing as part of postharvest handling on micromorphology, wax content, weight loss of blueberry and to apply the luster sensor to assess changes in glossiness during post-harvest weight loss of blueberries. These findings may be useful in establishing fruit quality assessment guidelines and help decision making processes such as whether to apply fruit coatings or assess keeping quality.

Materials and methods

Treatments and determination of weight loss

Highbush blueberries (*Vaccinium corymbosum* L.) were sourced locally. Half of the 700 blueberries were rubbed twice by hand within a soft microfiber tissue in order to modify the wax layer on the fruit's surface and served as 'polished berry sample', the other 350 blueberries with their natural wax layer (Fig. 1) served as control. The individual weight loss of 25 fruit, kept in a refrigerator at 6 ºC and 55% rh for 9 days, was calculated as the percentage difference between the initial and their final weight.

Examination of the blueberry surface and determination of epicuticular wax

Blueberry specimen were sputter-coated with gold and studied in a low vacuum in an ESEM microscope (Philips, Eindhoven, Netherlands) at a magnification of ×250 and ×500 to obtain images of the wax platelets before and after polishing.

Epicuticular wax of 25 blueberry fruit was extracted (HOLLOWAY and BAKER, 1962) in 300 mL chloroform at 35 °C and shaken for 10 seconds. The extract was filtered, evaporated to dryness and oven-dried overnight (BLANKE et al., 1996). The wax content was determined gravimetrically and expressed as μg total wax per cm^2 of berry surface area (645-800 mm^2 per berry), calculated as an

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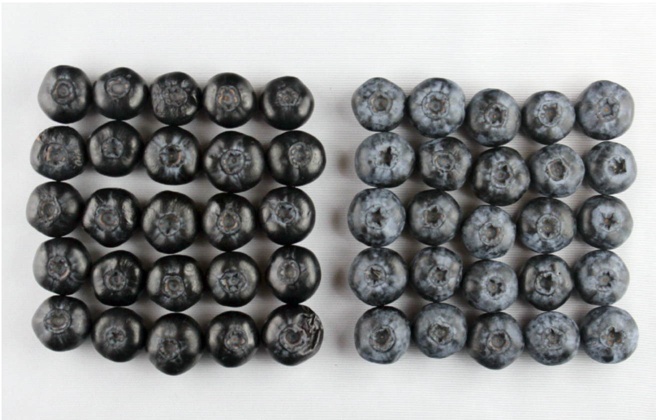

Fig. 1: Blueberries with their natural wax surface (right) and after polishing (left) as employed for the present study.

ellipsoid (formula 1) for the blueberry; all samples were analyzed in triplicate:

$$Area(berry\ surface) = 4 * \pi * (w / 2 * h / 2) \quad (1)$$

where
w – width of blueberry [mm]
h – height of blueberry [mm]

Glossiness measurement using the luster sensor

The glossiness or shininess of the blueberry was measured at five positions on the fruit, i.e. three around the fruit equator, one on the top and one on the bottom of the berry, daily for a period of 9 days after harvest using the luster sensor CZ-H72 (Keyence, Japan) with an amplifier CZ-V21AP, as described by MUKHTAR et al. (2014) with a small modification for the smaller fruit. The spot diameter was 3 mm. The distance between the luster sensor and the surface of the fruit sample was adjusted to a constant 15 mm using a purpose-designed micromanipulator for any variation in fruit size. After setting the micromanipulator to zero distance, it was then adjusted to 15 mm, using a digital integrated measuring device with a 0.1 mm resolution. The result obtained was expressed as luster level (MUKHTAR et al., 2014) and used to describe the degree of shininess or glossiness of the respective fruit surface (WITSUTHIPHAET et al., 2014).

Statistical analysis

Data of the effect of polishing on wax mass, weight loss, and luster level (Fig. 3) were analysed statistically using SPSS program version 25 (SPSS, Chicago, USA).

Results and discussions

Effects of polishing on micromorphology, epicuticular wax content and luster levels

On the blueberry surface, epicuticular wax crystals were detected mostly in the forms of platelets and, to a lesser extent, rodlets; polishing disturbed these wax crystal structures (Fig. 2).

The epicuticular wax content was reduced from ca. 120 to 95 µg cm^{-2} after polishing and from ca. 100 to ca 80 µg cm^{-2} after nine days refrigerated storage (Fig. 3a), equivalent to a 22% loss in wax content.

Polished blueberries had lost ca. 20% wax and transpired more rapidly than unpolished fruit with concomitant responses in the luster level (Fig. 3b).

Effects of polishing on weight loss and luster levels after harvest

Linear regression fitted with good coefficients of determination ($0.985 < R^2 < 0.99$) and showed an increase in weight loss over time for both types of berries with or without polishing. Unpolished blueberries with their natural wax layer had a significantly smaller weight loss with a slope coefficient resulting in 5-6% weight loss 9 days after harvest (Fig. 4a).

From plum studies, it is known that surfaces with larger luster levels indicate a higher degree of glossiness or shininess (WISUTHIPHAET et al., 2014). In the present blueberry study, the change with respect to the decrease in the glossy value (Fig. 4b) was similarly followed by a high regression coefficient ($0.95 < R^2 < 0.97$). The luster levels of polished and unpolished berries firstly decreased rapidly in the first 5 days after harvest, with a further slight decrease until they remained almost constant at 20-30 a.u. (Fig. 4b). Interestingly, the luster levels of all berries with polished (glossy) wax surfaces were greater than those of the berries with natural wax layer throughout the experiment ($P < 0.05$) (Fig. 4a and Fig. 4b).

Effects of polishing on epicuticular wax mass

For the first time to our knowledge, epicuticular wax was measured before and after polishing. MUKHTAR et al. (2014) identified the wax crystals as parallel platelets on the natural unpolished plum surface before and after polishing by ESEM, but without a comparable wax determination. The surface structures of the blueberries with a polished surface appeared smoother (Fig. 2b - right) and glossier (Fig. 1); ß-diketones, partly responsible for the crystalline structure (Fig. 2a - left), disappear towards maturity (FREEMAN et al., 1979; HOLLOWAY, JEFFREE and BAKER, 1982). As in plums (SKENE, 1967; MUKHTAR et al., 2014), polishing created the impression that the wax platelets melted together and also resulted in a small reduction of the physical thickness of the wax layer measured by carbon replica (SKENE, 1963) in line with the 22% less wax (Fig. 3a). BAKER (pers. comm., 2015) and MUKHTAR et al. (2014) postulated that wax on polished fruits was principally re-distributed rather than removed, but without giving evidence. The epicuticular wax crystals are responsible for the degree of wettability, reflection of UV and visible light (colour) and regulation of the uptake of molecules from the environment (SAPERS et al., 1984). On cabbage, the natural crystalline wax structure was associated with light reflection and scattering (BACHER et al., 1999).

The ca. 120 mg wax/cm^2 of the highbush blueberry (Fig. 3a) is slightly smaller than the 170 µg/cm^2 of the related *Vaccinium elliotti* Chapm. in the US (ALBRIGO et al., 1980). We suggest to classify blueberry as "waxy" following the Long Ashton wax classification by HOLLOWAY and JEFFREE (2005). BAKER (1975 and 1982) defined the category "exceptionally waxy" for apple and other fruit with 290-410 µg wax cm^{-2}.

Effect of polishing on weight loss

Moisture loss is the major cause of firmness and weight change during postharvest storage of blueberries (PANIAGUA et al., 2013). In blueberries with a mechanically polished wax layer, the weight loss was twice that of berries with their natural wax layer. This might be due to the berries with a natural unpolished surface had a wax layer thickness, which constitutes an effective barrier to moisture loss. The results are in agreement with the studies of MUKHTAR et al. (2014), who reported that European plums with their natural wax surfaces conserved more moisture content compared with those of a polished and chemically (chloroform) treated surface to remove wax. The relevance of the epicuticular wax on moisture conservation and its potential loss during postharvest handling was also the objective of

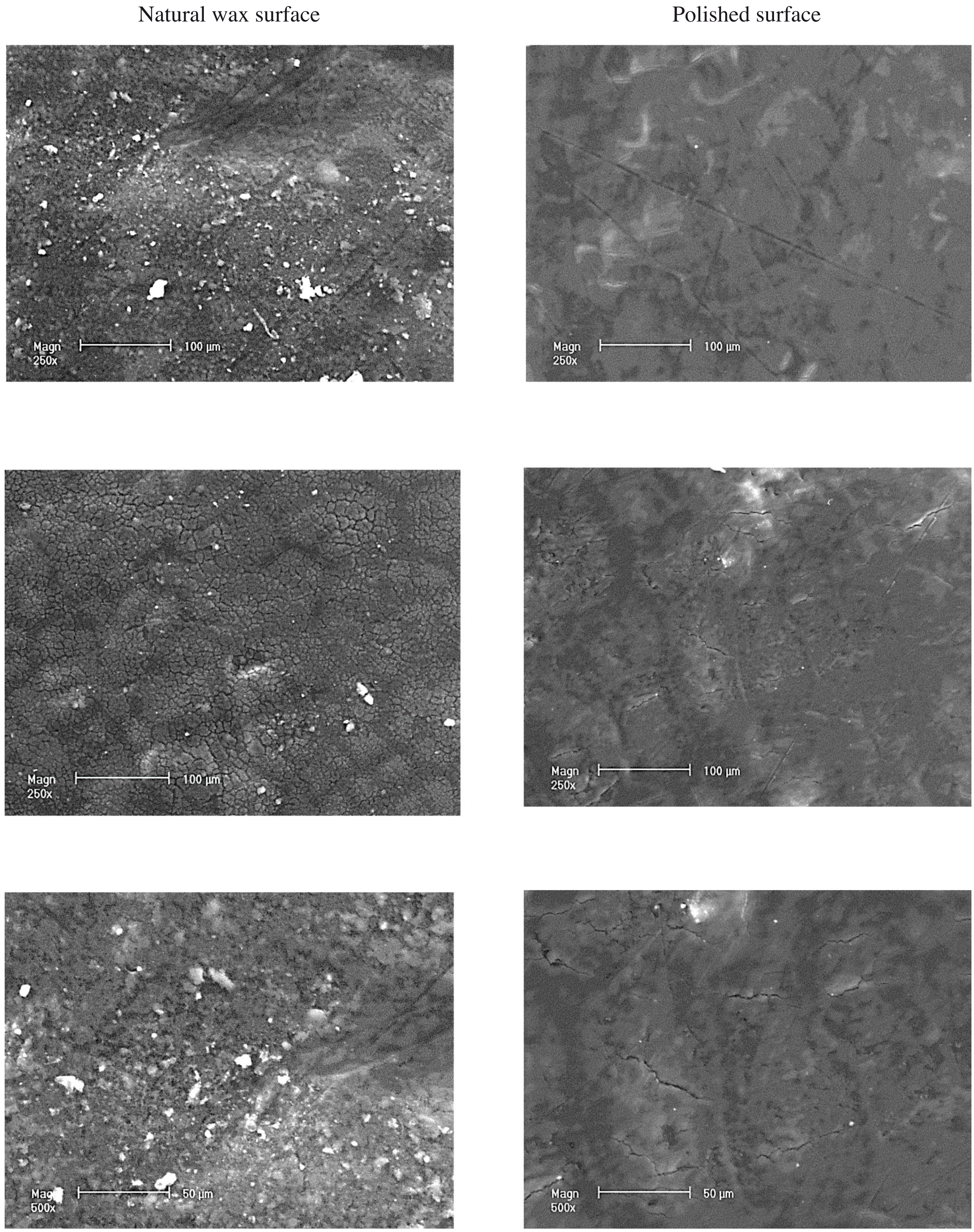

Fig. 2: Micrographs of the blueberry fruit surfaces with their natural wax surface (left) and after polishing (right hand column); scale bar indicates 100 µm (top) or 50 µm (bottom line).

coating for blueberry. PANIAGUA et al. (2013) found that weight loss of blueberries increased during storage, while firmness decreased. FORNEY et al. (1998) reported no loss in firmness of 'Burlington' blueberries simultaneously with 1 - 2% weight loss, but fruit softening when weight loss was 4 - 14%. These findings suggest that fruit with a thick natural wax surface have an efficient barrier against moisture loss highlighting its preservation as a prime target; failure to do so requires a decision process to apply edible coatings depending on the rate of wax removal – the luster sensor could aid this process.

Ecophysiology of glossiness and measurements with the luster sensor

In the present study, blueberries with their natural wax surface had lesser luster levels than those with a polished surface, possibly because polishing changed the surface characteristics of the blueberries (Fig. 2 and 3a), resulting in increased light reflection (BACHER and BLANKE 1999). MÜLLER and RIEDERER (2005) pointed out that a cover of epicuticular wax crystals is responsible for protection against large amounts of radiation or heat by reflecting incoming radiation.

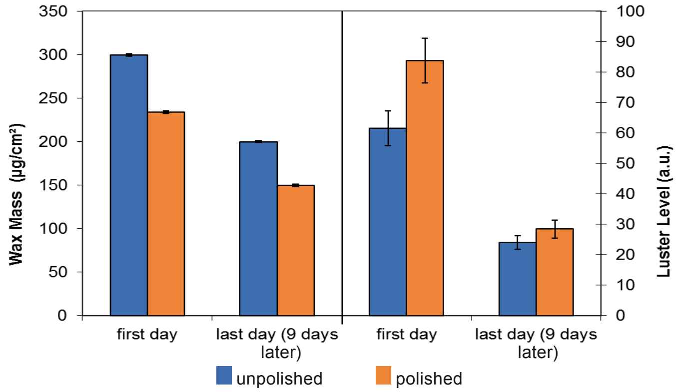

Fig. 3: Wax content (left; n = 3 of 25 berries each) and luster levels (right; n = 25) of polished (orange) and unpolished (blue) natural blueberries (mean and SDs – standard deviation).

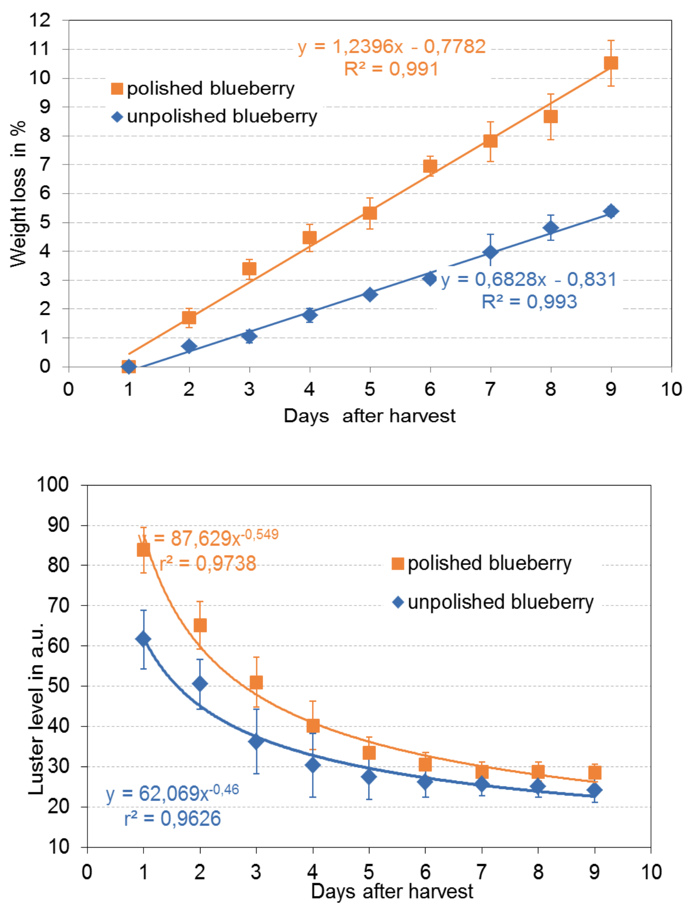

Fig. 4: Weight loss (a, top; mean of 25 berries) and luster levels (b, bottom) of polished (orange) or unpolished (blue) blueberries plotted over time during nine days of storage at 6 °C and 55% rh (n = 25) and SD (standard deviation); colour coding is identical in Fig. 3 and 4.

The positive correlation between the luster levels (Fig. 3) and polishing is in line with the work of MUKHTAR et al. (2014), who reported that the luster levels of the natural wax surface of plum fruit remained almost constant at 100-200 a.u. or with little *decrease* over time, but *increased* 2-3 fold after polishing.

Conclusions

1) Highbush blueberries (*Vaccinium corymbosum* L.) contained ca. 120 µg cm^{-2} wax per berry surface area, which classified them as "waxy" and which was reduced by 22% to 95 µg cm^{-2} by polishing, associated with a similar value of 20% removal of the epicuticular wax and increase in luster levels from ca. 65 to 80 a.u..
2) During storage, luster levels decreased; this decrease was pronounced during the first five days after harvest and then remained almost constant.
3) Luster levels, weight loss and epicuticular wax content seemed inversely correlated during storage.
4) Overall, application of the luster sensor to assess the glossiness of blueberries is promising for classifying them along with non-destructive determination of fruit quality not only for blueberry.

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