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Ripeness stage at harvest influences postharvest life of cranberry fruit: physiological and anatomical changes

Mustafa Özgen^a, Jiwan P. Palta^{a,*}, Jonathan D. Palta^b

^a Department of Horticulture, University of Wisconsin, 1575 Linden Drive, Madison, WI 53706, USA

^b Northland Cranberries Inc., PO Box 8020, Wisconsin Rapids, WI 54495-8020, USA

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Abstract

We investigated the relationship between postharvest life of cranberry (*Vaccinium macrocarpon*) fruit and ripeness stage at harvest. Wet harvested, mature fruit were sorted into four ripeness stages based on color and quality after 4 and 7 weeks of cold storage at 3 °C. In addition, CO₂ and ethylene production and water loss content were measured. After 7 weeks of storage, the marketable fruit among dark-red, white, bluish, and light-red fruit were 82, 74, 63, and 44%, respectively. The ethylene production was nearly the same for all ripeness stages. However, white, bluish, and light-red fruit had significantly higher respiration rates than dark-red fruit. The cuticle thickness was significantly higher for red fruit as compared to other ripeness stages. The cuticle present at the calyx end of the fruit, which became impregnated with wax in red fruit. The wax layer in the calyx opening accumulated anthocyanins in red fruit only. Our studies suggest that longer postharvest life, possibly because (i) red fruit have lower respiration rates, (ii) thicker cuticle (especially at the calyx end) on these fruit may retard the entry of microorganisms into the fruit, and may mitigate mechanical injury by harvesting equipment. © 2002 Elsevier Science B.V.

Keywords: Anthocyanin; Ripeness stages; *Vaccinium macrocarpon*; Cranberry; Cuticle; Shelf life; Postharvest life

1. Introduction

Ripening is the terminal phase in the development of fleshy fruits that encompasses a range of physiological and biochemical changes in the tissue. It is frequently signaled by changes in color, firmness, and texture, and is associated with the degradation and

accumulation of sugars, degradation of organic acids, and destruction of chlorophyll (Giovani and Rhodes, 1980).

Association of picking time and ripeness stage is important for postharvest life. Fruits are generally divided into ripening stages according to their ripening

softening and thus, short shelf life. Therefore, such fruits are harvested at an earlier stage of ripening for long shelf life. The majority of tomatoes are harvested before they are able to withstand current long distance transportation, whereas strawberries are only good for local markets. To extend postharvest life (Kader and Steffensen, 1995). Fruits such as strawberries and raspberries are also picked before they are ripe to prevent postharvest losses (Kader et al., 1996; Sjulín and Kader, 1997). Studies aimed at determining the optimal time for harvest have utilized ethylene biosynthesis (Knee et al., 1989; Skrzynski, 1994), and ethylene production. Climacteric fruits are limited. Non-climacteric fruits and vegetables can be stored for long periods for designing storage systems to maximize their longevity. Exposure to high concentrations of O_2 is known to inhibit ethylene biosynthesis in climacteric fruits (Knee et al., 1989). Although not applicable for nonclimacteric fruits, ethylene production is generally thought to be a good indicator of postharvest life (Blanpied, 1995).

In the temperate zone cranberries ripen and develop color in the outer layers. Exposure to low temperatures slows ripening (Stang, 1983). The ripening process is accompanied with a distinct rise in ethylene production in the fruit (Abdallah and Kader, 1995). As a major cultivar that is grown in the fruit, resulting in one of the most popular berries, it suffers from poor postharvest life. Significant numbers of fruit are lost during ripening under Wisconsin growing conditions. Fruits intended for fresh market are harvested from flooded bogs. Wet storage conditions are stored for 1 or 2 months

lower incidence of physiological breakdown during storage than less colored fruit. This was reported to be true for fruit harvested at three times during the ripening period (early, middle and late). They suggested that highly colored cranberries should be used for cold storage (Ceponis and Stretch, 1983). These studies suggest that cranberries possibly do not behave like other climacteric fruits such as tomatoes and strawberries. Systematic information is not available on the relationship between ripeness stage at harvest and postharvest life in cranberries. We investigated the effect of ripeness stage (fruit color) on postharvest life. We also attempted to understand the physiological and anatomical basis for the influence of ripening stage on postharvest life.

2. Materials and methods

2.1. Plant materials

Cranberry (*Vaccinium macrocarpon* Ait. cv. Stevens) fruit were collected at the processing plant of Northland Cranberries Inc. (Wisconsin Rapids, WI). These fruit had been wet harvested using a Getsinger style fresh fruit harvester (Paul's Machine and Tool, Warrens, WI) on October 13, 1997 from a commercial bed established near Wisconsin Rapids. Fruit were sorted by visual inspection into four ripeness stages: dark-red, light-red, blush, and white. Fruit showing rot, mechanical injury, disease, or flesh softness were discarded. All fruit selected were physiologically mature and similar in size. Fruit lacking any visual chlorophyll are generally considered mature (Salunkhe and Desai, 1984). Maturity of the fruit was also determined using the seed color. As cranberry fruit mature, seed color gradually changes from light-green to light-brown and then dark-brown. Samples of 0.5 kg were placed into 2-l open ventilated plastic containers. Each of the

ing rot and/or flesh softness was designated as 'unmarketable' fruit. The remaining fruit were designated as 'marketable' fruit. In addition, fruit respiration (CO_2), ethylene production, and anthocyanin content were measured after 4 weeks of storage using subsamples of marketable fruit. Remaining fruit were kept in cold storage for an additional 3 weeks and again rated for fruit quality.

2.2. Fruit ethylene production and respiration

Fruit ethylene production and respiration were determined by using a procedure similar to the one used by Farag and Palta (1993). For this purpose, 0.05 kg of intact, healthy fruit were incubated in a 700-ml gas-tight glass jar for 24 h at room temperature (20 °C). All decaying and rotted fruit were removed prior to respiration and ethylene measurements. Headspace gas was sampled and ethylene concentration was determined by injecting 1-ml gas into a gas chromatograph (Shimadzu 9AM, Shimadzu Corporation, Kyoto, Japan) equipped with a flame ionization detector and a 1.2-m stainless steel packed column (80/100 Porapak KQ Supelco Inc., Bellefonte, PA). For quantification of respiration, 0.05 kg of fruit was incubated in the same gas-tight glass jars for 30 min. Headspace gas was sampled and carbon dioxide generated by the fruit was quantified by injecting 1 ml of gas into the same gas chromatograph (used for ethylene measurements). Attachment of a Methanizer (Shimadzu MTN-1) allowed the detection and quantification of carbon dioxide by reducing it to methane gas. Methanizer temperature was set at 450 °C. Five separate measurements were made for each ripeness stage.

2.3. Anthocyanin content

For quantification of total anthocyanins (TAcy) a procedure similar to that of Euleki and Francis

Delray Beach, FL). The extract (TEV) was 175 ml slurry (SV) were diluted into a 250-ml centrifuge tube with extraction solvent (total volume, DV = 27 ml). The extract was allowed to diffuse into the solvent. The samples were then stirred and centrifuged (Beckman J2-21, Beckman Inc., Irvine, CA) at 6800 rpm for 10 min. The supernatant was used to determine TAcy (OD) at 535 nm (Beckman DU-680 spectrometer; Beckman Instruments, Irvine, CA) calculated using the following equation (Euleki and Francis (1968):

TAcy (mg/kg)

$$= \text{OD} \times \text{DV} \times 100 \times \text{T}$$

2.4. Cuticle thickness

The equatorial portion of the fruit was used for measuring cuticle thickness. The fruit was cut perpendicular to the fruit surface. The sections were made with an Oxford Microtome (Oxford Co., San Mateo, CA). The microtome allows thin sectioning of the fruit. The section (Palta and Li, 1993) was measured by observation under a light microscope (Nikon Model SMU-1821) and television recording. The measurements were made using a 100x magnification stage and five different sections of the fruit.

2.5. Fruit resistance to

Cuticle strength (resistance) of the cranberry fruit was determined using a penetrometer equipped with a 2 mm diameter Synergie 200, MTS Computerized Test System cross-head drive into

Measurements were made
two sizes (ranges 1.0–1.3
ripeness stages (dark-red

s stage, eight, 0.05 kg
it were crushed, trans-
d squeezed by hand to
soluble solids content was
digital refractometer
Measurements Inc.,

Microscopy

y and accumulation of
etal) end were examined
microscopy (SEM). Obser-
white and dark-red stage
nd cross-sections were
ashed in distilled water,
s with silver paint, and
a thick gold–palladium
mens were viewed with
kyo) at 10 kV.

ion on the fruit calyx
ving thin cross-sections
cope (SZ-PT Olympus;
nts Inc., Minneapolis,
or video camera (Sony
on Instruments Inc.).
t 0.5 mm thick) were
e. Ten berries of each
ed.

e significant differences
ong the four ripeness

The storage quality of the fruit was significantly affected by the ripeness stage (Fig. 1(B), Table 1). At 4 weeks after storage, both dark-red and light-red fruit had higher percentages of good fruit than the blush and white fruit. At the end of 7 weeks of storage, the good berries were 82, 74, 63, and 44% in dark-red, light-red, blush, and white groups, respectively (Table 1). At 4 and 7 weeks after storage, the blush stage had a higher percentage of good fruit than the white stage. Between 4 and 7 weeks of storage, the percentage of good fruit declined by 17.5, 14.2, 11.5, and 6.6% in the white, blush, light-red, and dark-red ripeness stages, respectively.

There were large differences in respiration rates among the various ripeness stages (Fig. 1(C)). White berries had the highest respiration rate, which was 71% higher than the dark-red fruit. Dark-red fruit had the lowest respiration rate. The respiration rates of blush and light-red fruit were similar and their respiration rates were significantly higher than the dark-red fruit and significantly lower than the white fruit.

There were significant differences in total soluble solids (%) content among the four ripeness stages. The berries that had higher anthocyanin content also had the highest soluble solids (Fig. 1(D)). Rate of ethylene production by the fruit did not vary significantly among the four ripeness stages (Fig. 1(E)).

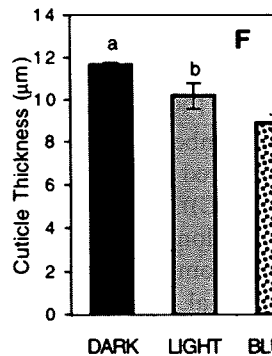
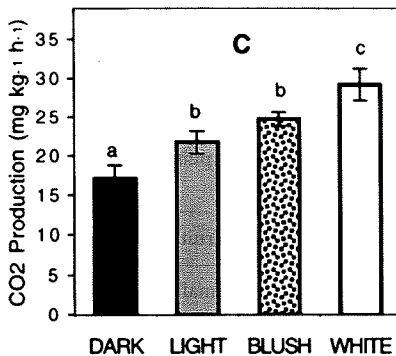
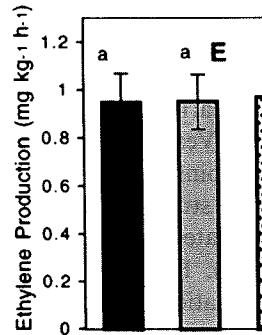
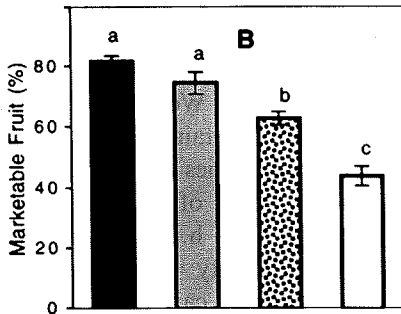
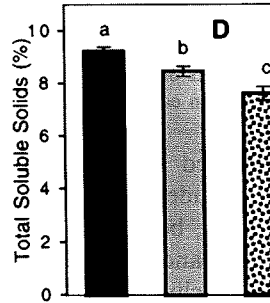
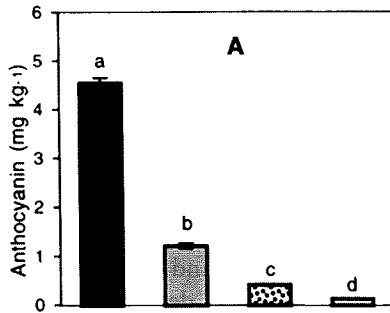
Dark-red and light-red berries had significantly thicker cuticles than blush and white berries (Fig. 1(F)). Cuticle thickness was 11.7 ± 0.6 , 10.2 ± 0.3 , 8.7 ± 0.45 , and 8.2 ± 0.5 μm in dark-red, light-red, blush, and white groups, respectively. In addition, resistance of the fruit to puncture was increased as fruit ripened. Dark-red fruit had higher resistance to puncture than white fruit (Table 2). This was true for both large and small size fruit.

Greater wax accumulation was seen in the dark-red fruit than the white fruit (Fig. 2). There were stomata present at the calyx end of the fruit

4. Discussion

The results of the present study (Fig. 1, Table 1) clearly show that, in contrast to other perishable fruits such as tomatoes, bananas, strawberries, and raspberries, ripe cranberries have a

longer shelf life than other fruits. These results also support the findings by Ceponis and Stretch (1997) that highly colored cranberries have a longer shelf life during ripening in classic climatic conditions.



Cranberry fruit on shelf life

Fruit (%) after storage at	Loss of good fruit (%) between 4 and 7 weeks of storage
7 weeks	
81.7a	6.6
74.4b	11.5
62.8c	14.2
44.0d	17.5

7 weeks of cold storage. The values represent mean of five replications. Means were compared by (5) within each column; means followed by the same letter do not differ significantly.

considered to be synonymous with a prelude to senescence. Studies have been conducted on various fruits and only limited information is available on nonclimacteric fruits (Guthrie et al., 1989; Skrzynski, 1996). Studies on reduced postharvest life and shelf life of climacteric fruits when managed in an early ripeness stage (Guthrie et al., 1989; Veazie et al., 1996; Robbins, 1987). In contrast, our study suggests that at late ripeness stages (Fig. 1). Furthermore, our explanation for this condition is that high respiration is generally correlated with short shelf life (Guthrie, 1969; Tucker, 1993) and reduced postharvest life (Guthrie, 1969). Our results show that dark-red cranberry fruit had a longer shelf life compared with light-red, (Fig. 1(C)). Thus, reduced respiration rate indicate longer postharvest life compared with less respiration (Guthrie et al. (1963) found that dark-red and poor shelf life of

showed that wax accumulation on the cranberry fruit surface as it ripens, and no stomata, lenticels, or trichomes were found on the fruit surface (Farag and Palta, 1989). In addition, the fruit cuticle was devoid of cracks. We confirmed these observations on the fruit morphology in our studies (data not shown). Furthermore, we found significant changes in the wax accumulation at the calyx end of the fruit during ripening (Figs. 2 and 3). We also found that this portion of the fruit contained stomata (Fig. 2). In Wisconsin, cranberries are wet harvested by flooding the fields. Fruit often remain submerged in water for over 24 h. During this time, there is a high probability that microorganisms (e.g., fungi, bacteria) can enter fruit. The main path of entry of these microorganisms would be through wounds or through stomata at the calyx end of the fruit. The presence of fungal hyphae on this part of the fruit (Fig. 2) supports

Table 2
Fruit resistance to puncture (N) of dark-red and white cranberry fruit

Ripeness stages	Fruit size	
	Small (1.0–1.3 g)	Large (1.7–2.0 g)
Dark-red	5.22a	5.33a

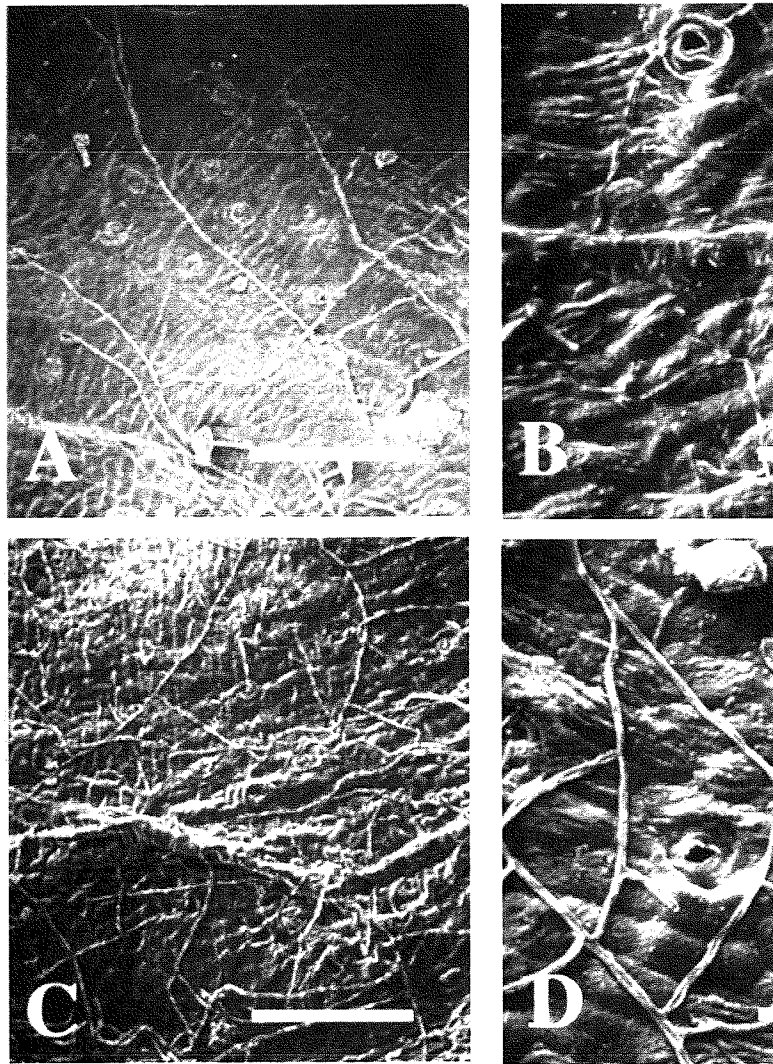
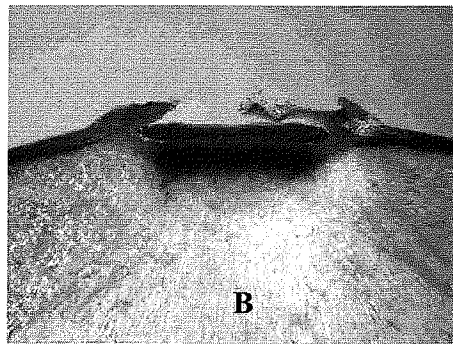
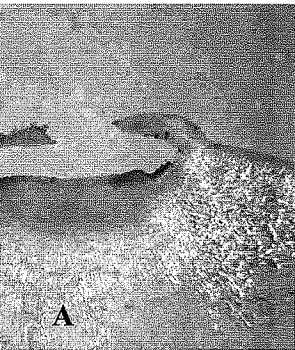


Fig. 2. Scanning electron micrographs of the calyx (distal) end of the cranberry fruit. Fruit ends at 10 kV. ((A) and (B)) White stage of fruit; ((C) and (D)) dark-red stage of fruit. Bars in (A) and (D), 30 μ m. Notice more wax accumulation in dark-red fruit ((C) and (D)) as compared to white fruit ((A) and (B)). Stomata are seen at this (distal) end of the fruit ((B) and (D)).

this idea. These observations suggest that ripe fruit may store better in part due to anatomical

calyx end of the red of microorganisms bet



ed under light microscope showing anthocyanin accumulation on calyx end of the fruit (A) white
notice accumulation of anthocyanin in the epidermal cells (B) in dark-red fruits (dark layer under
ot present in white fruit (A).

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