Effect of the transport vibration on the generation of the color reversion in blackberry fruit

G.A. Pérez-Pérez[1], M.J. Fabela-Gallegos[2], M.E. Vázquez-Barrios[1], D.M. Rivera-Pastrana[1], L. Palma-Tirado[3], E. Mercado-Silva[1,a] and V. Escalona[4]

[1]Universidad Autónoma de Querétaro, Santiago de Querétaro, Querétaro, Mexico; [2]Instituto Mexicano del Transporte, Santiago de Querétaro, Querétaro, Mexico; [3]Instituto de Neurobiología UNAM Campus Juriquilla, Santiago de Querétaro, Querétaro, Mexico; [4]Centro de Estudios Poscosecha, Universidad de Chile, Santiago de Chile, Chile.

Abstract

Mexico provides 95% of the export market blackberry (*Rubus fruticosus*) worldwide. The characteristic black color of this fruit is an important quality factor. During its postharvest management, it can reverse its color from black to red (color reversion) this being a rejection factor in the market. Within 3-5% of the exported fruit can be rejected due to this problem. The origin of this change is not determined yet but can involve varietal aspects and postharvest handling. The objective of this study was to determine the effect of transport vibration on the color reversion. To identify the vibration conditions that generate the damage, exporting boxes with 12 clamshells of 6 oz with blackberry 'Tupy' were put under vibration patterns (5-30 Hz) with amplitude of 0.5 g. Under the identified damage conditions, different blackberry boxes were put at those conditions of vibration for 10 and 30 min, respectively, and later the fruit was stored at 3°C and 95% of HR for 2 days. The incidence of damage was evaluated and the black and red drupelets were separated from each fruit, frozen at -70°C and freeze dried for quantification of monomeric and polymeric anthocyanin. Fresh samples of each condition were prepared for its observation by optic microscopy and transmission electron microscopy (TEM). The frequency of 10 Hz and 0.5 g of amplitude generated color reversion, internal cellular damage and possible molecular changes in the anthocyanins structure. The monomeric anthocyanin content was lower on reverted drupelets (4.535 mg cyanidin-3-O-glucoside g^{-1} lyophilized sample) with respect to non-reverted (7.746 mg cyanidin-3-O-glucoside g^{-1} lyophilized sample), likewise the index of percentage of polymeric color was lower on the black drupelet (23%) in comparison with the red drupelets (37%). The tissue of the non-reverted drupelets showed a higher integrity and cellular order in comparison with the tissue of the drupelets with reversion of color. These results indicate that the frequency of 10 Hz should be avoided during the transportation of these fruits.

Keywords: *Rubus fruticosus*, blackberry, vibration, transport, anthocyanins

INTRODUCTION

In the last fifteen years, the blackberry production in Mexico has shown a strong growth, in 2014 the acreage reached 12,000 ha and the production was 153000 t, 10 times higher values than those recorded in 2000. The main producing area of Mexico is in Michoacán that generates 95% of the volume and 98% of the production value. On the other hand, Mexico has increased its export volumes of fresh berries (blackberry, raspberry, strawberry, blackcurrant among other) at a rate of 27% $year^{-1}$. The main export destinations are: United States as the main consumer, the Netherlands, United Kingdom, France, Canada, among others (Financiera Nacional de Desarrollo Agropecuario, Rural, Forestal y Pesquero, 2015).

In addition to high profitability and export opportunities, the interest generated

[a]E-mail: mercado501120@gmail.com

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towards the consumption of food with nutraceutical properties has been an important factor for the rapid growth of production and worldwide marketing of berries, which form part of foods containing high levels of antioxidants with nutritional and health benefits (De Carvalho et al., 2010). Fruit quality, is a combination of attributes that makes them desirable to the consumer. One aspect that plays an important role in the quality and that first captures the attention of the consumer is the visual appearance, which is evaluated in the first instance by the color (Ryall and Pentzer, 1982).

The color reversion in blackberry fruit during the commercialization process is a defect that has not been solved technically and it is one of the fundamental problems of rejection in the target markets. It has been noted that the conditions of transportation of perishable products can alter their quality by altering their physical and mechanical properties due to vibration patterns generated during road transportation (Fabela-Gallegos et al., 2002). Therefore, the aim of this study was to provide information regarding the conditions of vibration that generate the color reversion in blackberry fruits to establish control strategies.

MATERIALS AND METHODS

This study was conducted with 'Tupy' blackberry fruits, produced and harvested in Los Reyes, Michoacán, Mexico; provided by an exporting company in export boxes that contained 12 clamshells of 6 oz each. These were transported to the Laboratory of Vibrations of the Mexican Institute of Transport (IMT) to be subjected to vibration treatments and then taken to the Laboratory of Postharvest Physiology and Biochemistry of the Universidad Autónoma de Querétaro for preservation and analysis.

Vibration profile

Based on previous studies of vibration in transport of perishable (Fischer et al., 1992; Fabela-Gallegos et al., 2002) conditions that could generate mechanical damage during transport of the fruit in an articulated vehicle (semi-trailer) were identified. For example, the transport of strawberry and grape (Fischer et al., 1992) generated a vibration range into 2 to 30 Hz, with amplitudes near 0.5 g (4.9 m s^{-2}). Based on that information, a frequency scan was held into the range 5-30 Hz to determine the resonance or amplification frequency, which could bring damage during blackberry transportation.

With the frequencies identified in the first stage, the following study was submitted with another set of blackberry boxes to that frequency and amplitude during 10 and 30 min and to evaluate the color reversion after three days of storage at 3°C.

Equipment

The equipment used to study the effects of vibration was a vibration generator System brand LDS (Ling Dynamic Systems), integrated with a controller model COM200, an amplifier model PA2000 and an excitement table model V721. The system can support a vertical mass of up to 100 kg and generate sinusoidal vibration, scans and random vibration frequency in a range of 1 to 5000 Hz, with 25 mm peak-peak displacement, 1 m s^{-1} peak velocity; up to 97 g peak acceleration sinusoidal or 50 g random vibration acceleration.

For data recording, an accelerometer was placed on the vibration table in order to control the vibratory motion of the equipment and have registration reference of acceleration (Figure 1A). Another accelerometer was placed at the base of a box with blackberry clamshells that was placed on a first box (empty) placed directly on the vibration table (Figure 1B, C). The log data are captured through the amplifier system equipment.

Identification of conditions to induce color reversion and reproduce the damage

It was considered that the color reversion is generated when the fruit reaches a resonance frequency that makes the fruit knocked and vibrated mechanically. To identify this frequency, a frequency scan from 5 to 30 Hz was programmed to be performed by cycles up-down of 4 min. Several repetitions were performing with the same set of clamshells. The resonance frequency was identified, the clamshells were replaced with fresh fruit to be

subjected to the resonance frequency and amplitude identified. During this phase, the photographic records were obtained with the fruit inside the containers.

Figure 1. Experimental scheme to study the vibration profile. Accelerometer placed on the vibration table (A), accelerometer placed in the center of the bottom of the second box containing clamshells with blackberry 6 oz (B), an overview of all electro-hydraulic vibration system to simulate vibration in transit (C).

The samples subjected to vibration treatment or control were taken to the laboratory of Postharvest Physiology and Biochemistry of Fruit and Vegetables of the Universidad Autónoma de Querétaro (UAQ), for storage at 3°C and 95% RH, and its subsequent physicochemical analysis and preparation for its microscopic observation.

Extract preparation for analysis of anthocyanins

From damaged fruits, red and black drupelets were separated from their receptacles; then were frozen with liquid nitrogen and stored at -70°C. Subsequently the samples were freeze dried for quantification of monomeric anthocyanins and their percentage of polymeric color. One gram of freeze dried fruit, was ground in a mortar with 15 mL of 5% acetic acid/methanol (1:15 w/v), stirred for 24 h in darkness at 4°C, the mix was filtered (Whatman no. 4 filter), and the solid residue was extracted 3 times under the same conditions (Dufoo-Hurtado et al., 2013).

Quantification of monomeric and percentage of polymeric anthocyanins by differential pH method

The total content of monomeric and polymeric anthocyanins was determined by the method of differential pH (Giusti and Wrolstad, 2001). Two subsamples of 100 µL from the extracts were added one with 2.9 mL potassium chloride buffer, (0.025 M, pH 1.0), and the other with 2.9 mL of sodium acetate buffer, (0.025 M, pH 4.5). Both subsamples were equilibrated for 15 min at room temperature. The absorbance was read at 510 nm (maximum absorbance) and 700 nm (reading of the degradation degree of the compounds and correction by interfering substances) using a UV-Vis spectrophotometer Lambda 40 (Perkin Elmer Instrument). A blank with deionized water was used. The results were expressed as mg of cyanidin-3-O-glucoside g^{-1} (mg C3G g^{-1} freeze dried sample). For quantification of polymeric color; two subsamples of 100 µL from the extracts were prepared, with 2.9 mL distilled water. Then 2.9 mL of the sample was transferred and 100 µL of distilled water added for one sample and 100 µL of bisulfite solution to the second sample. Both subsamples were equilibrated for 15 min at room temperature, and the absorbance was read at 420, 510 (maximum absorbance) and 700 nm using a UV-Vis spectrophotometer Lambda 40 (Perkin Elmer Instrument). A blank with deionized water was used. The results were expressed as a percentage (%) of polymeric color, which was calculated by dividing the polymeric color (reading samples treated with bisulfite solution) between color density (reading samples treated with water).

The monomeric and percentage of polymeric color were calculated as follows:

$$\text{Absorbance of the diluted sample (A)} = (A_{\lambda vis\text{-}max} - A_{700nm})\ \text{pH 1.0} - (A_{\lambda vis\text{-}max} - A_{700nm})\ \text{pH 4.5}$$

$$\text{Monomeric anthocyanin content (mg L}^{-1}) = (A \times MW \times DF \times 1000)/(\varepsilon \times 1),$$

where MW is the molecular weight, DF is the dilution factor, and ε is the molar absorptivity.

$$\text{Color density (treated with water)} = [(A_{420nm} - A_{700nm}) + (A_{\lambda vis\text{-}max} - A_{700nm})] \times DF$$

$$\text{Polymeric color (bisulfite bleached sample)} = [(A_{420nm} - A_{700nm}) + (A_{\lambda vis\text{-}max} - A_{700nm})] \times DF$$

$$\text{Percent polymeric color} = (\text{polymeric color/color density}) \times 100$$

Changes in the microstructure of fruits with and without color reversion

1. Fixation of samples.

Drupelets with and without color reversion were separated from the whole fruit. These were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) at 4°C, for 24 h. After fixation, samples were washed twice in 0.2 M cacodylate buffer (pH 7.4); post-fixed with 0.1% osmium tetroxide in the same buffer for 1 h. Samples were dehydrated at 4°C in solutions of ethanol/water increasing its concentration (10, 20, 30, 40, 50, 60, 70, 80, 90, 96% and absolute ethanol), and changing the solution every 10 min.

Dehydrated samples in ethanol were placed in propylene oxide twice for 30 min, after which they were infiltrated with resin using 1:1 dilution of propylene oxide with a resin at room temperature. Finally, all samples were switched to a complete epoxy resin. Infiltrated samples were polymerized in epoxy resin for 36 h at 60°C. With the infiltrated and polymerized samples, sections 60-90 nm were obtained using a Reichert Ultracut or RMC ultramicrotome model MTx. These thin sections were placed on grids and contrasted with 1% aqueous uranyl acetate, pH 4.5 and 1% Reynold's lead citrate, to be observed at the transmission electron microscope JEM-1010 of the Brand JEOL a 80 Kv and by an optical microscope Zeiss Karl Mca using 100× objective (Martínez-Alfaro et al., 2014).

RESULTS AND DISCUSSION

Identification of frequency of damage

Figure 2 shows the relationship of vibration transfer, in terms of the amplitude of the acceleration in the second box regarding the frequency applied to the vibration table. The nominal value of acceleration introduced to the table was 0.5 g which was the reference value to estimate the amplification factor for all the frequency ranges analyzed. In both cycles, cycles of raising and lowering cycles, most amplification was observed in the frequency 10 Hz (Figure 2), in this condition the fruit entered to a state of movement and vibration (resonance frequency) which caused a continuous rebound of the fruit causing mechanical damage among them. This result agrees with the results of Fischer et al. (1992) in strawberries and grapes.

Figure 2 also shows that between 15 and 25 Hz the values of amplification factors were lower than the unit, indicating a slight attenuation of the vibration movement. This suggests that in this frequency range the product suffer less damage.

Induction of color reversion under damage conditions

Once identified the resonance frequency and damage (10 Hz), this remained constant as the amplitude in the vibration table (0.5 g), to perform a second experiment with a set of 12 clamshells per box, placed in the second box and induced to vibrate for 10 and 30 min. At the end of exposure to vibration, the fruit were visually analyzed to determine its color change or color reversion (Figure 3). Vibration during 10 and 30 min, induced strong color reversion in the fruits. The level of damage was directly proportional to the time of exposure to vibration conditions.

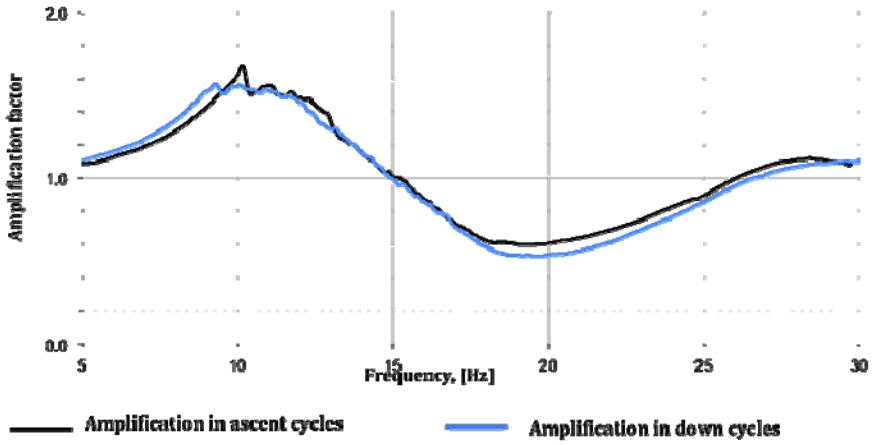

Figure 2. Amplification levels of the frequencies applied on vibration table, it was calculated as the ratio of the acceleration in the upper box over the acceleration in the vibration table.

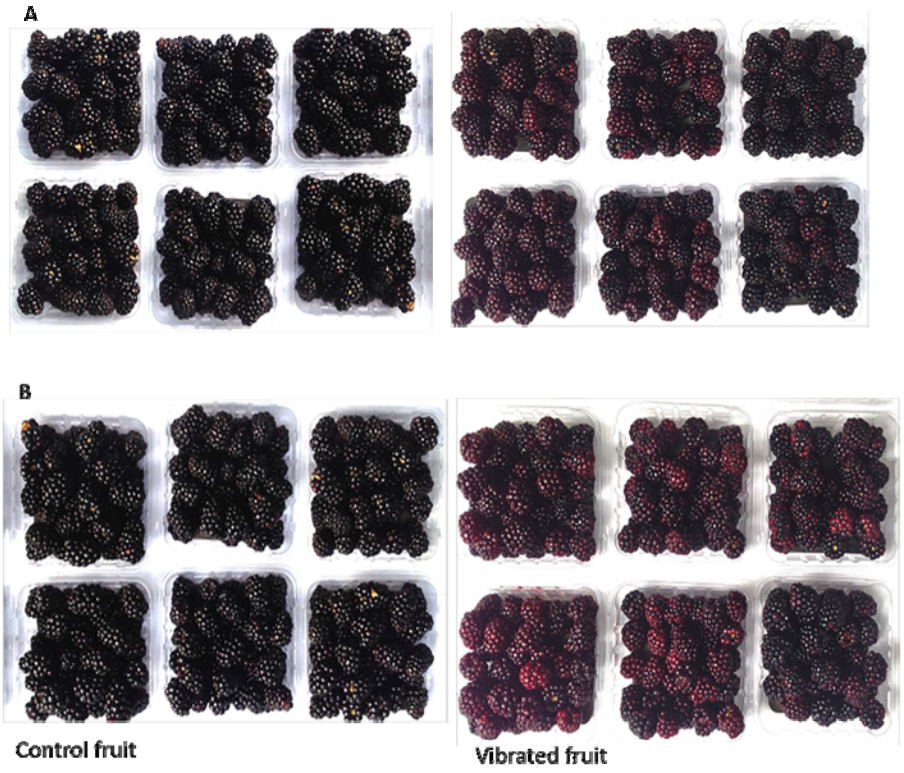

Figure 3. General view of the clamshells with blackBerry fruit subject to vibration at 10 Hz and 0.5 g for 10 min (A) and 30 min (B) and their respective fruits control.

Changes in the content of monomeric anthocyanins and percentage of polymeric color

Figure 4A shows the content of monomeric anthocyanins in black and red drupelets, while Figure 4B indicates the percentage of polymeric color in both types of fruits. The content of monomeric anthocyanins in reverted drupelets was lower (4.535 mg C3G g^{-1} freeze dried sample) with respect to non-reverted (7.746 mg C3G g^{-1} freeze dried sample). On the other hand, the rate of degradation and formation of complex anthocyanin-tannin or percentage of colored polymer was lower in black drupelet (23%) in comparison to red

drupelet (37%).

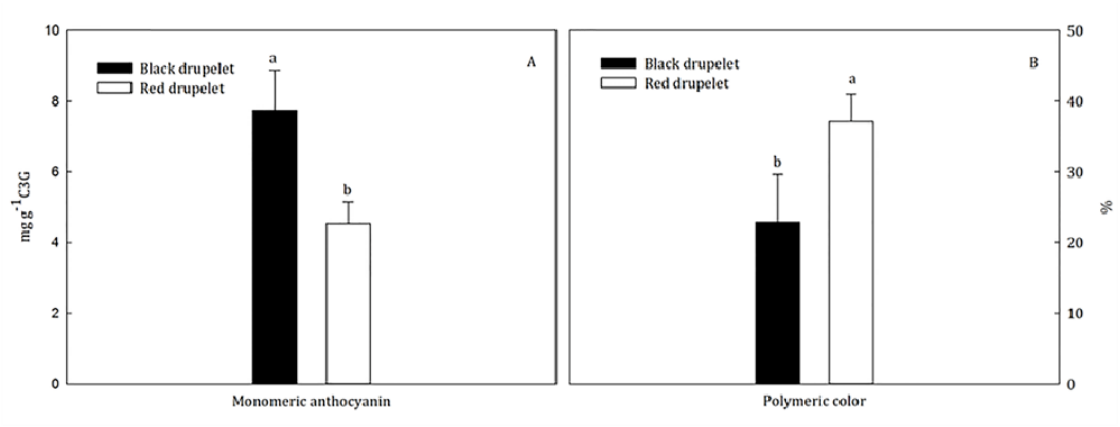

Figure 4. Content changes on monomeric anthocyanins (A) and percentage of polymeric color (B) in blackberry samples without color reversion (black bars) and with color reversion (white bars).

Perkins-Veazie and Clark (2011) indicated that the principal anthocyanin contained in blackberry is cyaniding 3-glucoside. It is possible that this anthocyanin is the one which shows the main changes during the color reversion. Considering the anthocyanins monomeric content and percentage of polymeric color; it could be noted that during the change process, the monomeric anthocyanins are lost and simultaneously the polymers of anthocyanins are being formed. However, the mechanism is not clear and requires more research to elucidate the chemical or biochemical mechanism that generates this regression.

Microstructural changes of reverted and non-reverted drupelets

Figure 5A and C show the microstructure of the internal tissue of blackberry fruits observed by TEM with color reversion and in control fruits, respectively. While Figures 5B and D show the microstructure through an optical microscope with color reversion and in control fruits, respectively. The cells of tissue without color reversion showed greater integrity and cellular order compared to tissues with color reversion, which showed further disintegration. These observations confirm that mechanical stress vibration altered the internal structure of the tissue, which probably facilitates the development of chemical or biochemical reactions leading to color reversion. Salgado et al. (2014) indicated low levels of color reversion in blackberry crispy selections.

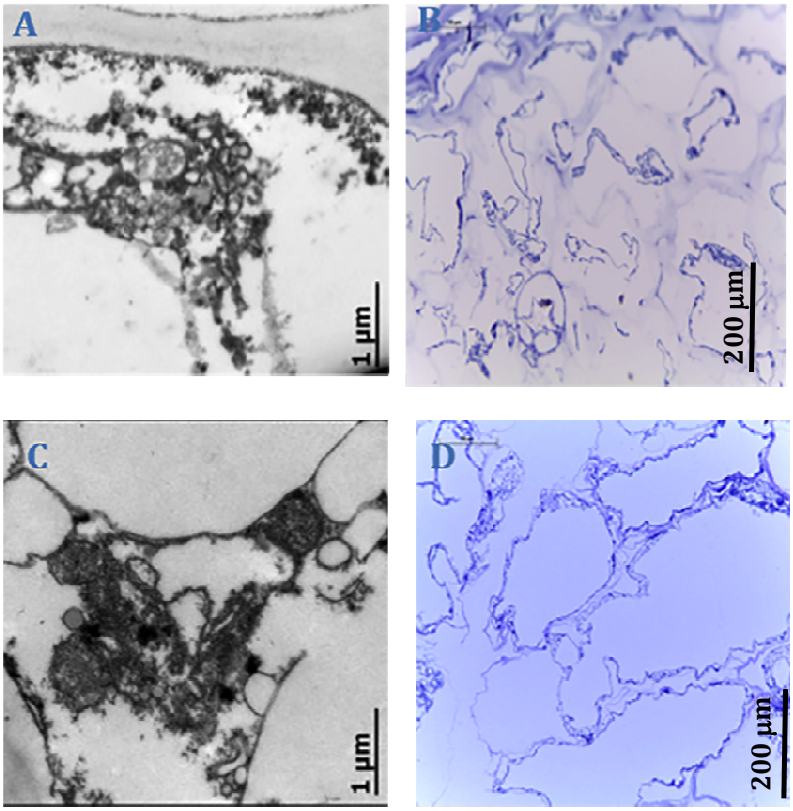

Figure 5. Cellular microstructure of control drupelet tissue and with color reversion observed under TEM (A and C) and by visible light optical microscope (B and D), respectively.

CONCLUSIONS

The results of this study indicated that patterns of vibration with frequencies of 10 Hz and an amplitude of 0.5g generates a resonance state in the fruit that makes it vibrate and leads to color reversion. The color reversion generated by the vibration, caused a decrease of monomeric anthocyanins and increased the polymeric color, which was correlated with a less integrity and order cellular of the tissue with color reversion. The damage caused by the vibration, probably facilitates chemical or biochemical reactions that lead to the color reversion.

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