Efeito do estádio de maturação na composição física, química e bioquímica de amora-preta

Effect of maturation stage on the physical, chemical and biochemical composition of black mulberry

Efecto de la etapa de maduración sobre la composición física, química y bioquímica de la mora

Rafael Carvalho do Lago
ORCID: https://orcid.org/0000-0002-8558-5217
Universidade Federal de Lavras, Brasil
E-mail: rcarvalholago@gmail.com

Jéssyca Santos Silva
ORCID: https://orcid.org/0000-0002-2414-3327
Universidade Federal de Lavras, Brasil
E-mail: jessycarv89@hotmail.com

Kelly Moreira Pinto
ORCID: https://orcid.org/0000-0002-1530-6761
Universidade Federal de Lavras, Brasil
E-mail: kellynhamorera@yahoo.com.br

Lucas Ferreira Rodrigues
ORCID: https://orcid.org/0000-0002-5717-9596
Universidade Federal de Lavras, Brasil
E-mail: llucasrodrigues@gmail.com

Eduardo Valério de Barros Vilas Boas
ORCID: https://orcid.org/0000-0002-0252-695X
Universidade Federal de Lavras, Brasil
E-mail: evbvboas@dca.ufa.br

Resumo

Este trabalho teve como objetivo avaliar as características físicas, químicas e bioquímicas da amoreira preta (*Morus nigra* L.) cultivada em Lavras, Minas Gerais, Brasil, em diferentes estádios de maturação. Os frutos foram colhidos e separados em cinco estádios diferentes, de

acordo com a cor da superfície. Posteriormente, os frutos foram submetidos às análises físico-químicas e análise da atividade da pectina metilesterase e poligalacturonase. Com o avanço dos estádios de maturação da amoreira preta, foi possível observar a degradação da clorofila com a síntese concomitante de antocianina. Houve aumento do teor de sólidos solúveis, açúcares e pH, diminuição da acidez total e da taxa respiratória, bem como diminuição da pectina total e aumento da pectina solúvel. Além disso, foi observado um aumento da atividade das enzimas pectina metilesterase e poligalacturonase e consequente amolecimento dos frutos.

Palavras-chave: *Morus nigra* L.; Antocianinas; Pectina; Pectina metilesterase; Poligalacturonase.

Abstract

This work aimed to evaluate the physical, chemical and biochemical characteristics of black mulberry (*Morus nigra L.*) cultivated in Lavras, Minas Gerais, Brazil, at different maturation stages. The fruits were harvested and separated into five different stages according to the color of the surface. Afterwards, the fruits were submitted to the physical and chemical analyses and analysis of pectin methylesterase and polygalacturonase activity. With the advancement in maturation stages of black mulberry, it was possible to observe chlorophyll degradation with concomitant anthocyanin synthesis. There was an increase in soluble solids contents, sugars and pH, decrease of total acidity and respiratory rate as well as decrease of total pectin and increase of the soluble pectin. Furthermore an increase of the activity of the enzymes pectin methylesterase and polygalacturonase and the consequent softening of the fruits was also noted.

Keywords: *Morus nigra* L.; Anthocyanins; Pectin; Pectin methylesterase; Polygalacturonase.

Resumen

Este trabajo tuvo como objetivo evaluar las características físicas, químicas y bioquímicas de la morera negra (*Morus nigra* L.) cultivada en Lavras, Minas Gerais, Brasil, en diferentes etapas de maduración. Los frutos fueron cosechados y separados en cinco etapas diferentes, de acuerdo con el color de la superficie. Posteriormente, los frutos fueron sometidos a análisis físico-químicos y análisis de la actividad de la pectina metilesterasa y la poligalacturonasa. Con el avance de las etapas de maduración de la morera negra, fue posible observar la degradación de la clorofila con la síntesis concomitante de antocianina. Hubo un aumento en el contenido de sólidos solubles, azúcares y pH, una disminución en la acidez total y la

frecuencia respiratoria, así como una disminución en la pectina total y un aumento en la pectina soluble. Además, se observó un aumento en la actividad de las enzimas pectina metilesterasa y poligalacturonasa y el consiguiente ablandamiento de los frutos.

Palabras clave: *Morus nigra* L.; Antocianinas; Pectina; Pectina metilesterasa; Poligalacturonasa.

1. Introduction

Black mulberry (*Morus nigra* L.) was domesticated over thousands of years and has adapted to the most diverse tropical, subtropical and temperate zones of Asia, Europe, North and South America and Africa (Ozgen, Serce, & Kaya, 2009). It is part of the genus Morus and family Moraceae. The best known species in the genus Morus are the white mulberry (*Morus alba* L.), the black mulberry (*Morus nigra* L.) and the red mulberry (*Morus rubra* L.) (Gundogdu, Muradoglu, Sensoy, & Yilmaz, 2011). The black mulberry berries weigh 4 to 7 g, have a black color and a flavor ranging from acid to sweet acid. The true fruit of black mulberry is called minidrupa or drupeta, in which there is a small seed, and its junction forms what is called an aggregate fruit (Poling, 1996).

The physical and chemical changes that occur during the development and maturation of the fruits are used as criteria to determine maturity, harvesting point and quality in several fruits. During development, the fruit goes through several stages, with well-defined characteristics, whose evaluation is important for the understanding of the changes that occur during development (Álvares et al., 2004).

During ripening, specific flavors and aromas develop, reducing acidity and astringency in most fruits. The fruits become softer, mainly due to degradation of the cell wall and starch, and more colored due to chlorophyll degradation and unmasking and/or development of carotenoid pigments and/or anthocyanins. The chemical and biochemical reactions are as diverse as possible, with variation between species, cultivars and among fruits of the same cultivar depending on the conditions of production and/or storage (Chitarra & Chitarra, 2005).

The physical, chemical and biochemical characterization of black mulberry at different maturation stages is important in the definition of the harvesting point and adoption of good production practices, such as type and timing of fertilization, as a function of fruit demand physical and chemical changes. In view of the above, this work had as objective to evaluate the physical, chemical and biochemical changes occurring in black mulberry cultivated in Lavras, Minas Gerais, Brazil, in different stages of maturation.

2. Material And Methods

The fruits of black mulberry (*Morus nigra* L.) were collected in the city of Lavras, Southern Minas Gerais. After collection, the fruits were transported to the Fruit and Vegetable Post-Harvest Laboratory of the Food Science Department of the Federal University of Lavras, where they were selected for the absence of injuries and defects and separated into 5 treatments according to the maturation stage based on the coloring of the surface, as shown in Figure 1. The maturation stage 1 corresponded to the mature green fruit and the 5 to the fully mature fruit. In the laboratory, part of the fruits was evaluated for color and firmness and part was frozen for further chemical and enzymatic analysis.

Figure 1. Degree of maturation of black mulberry according to surface coloration.

2.1 Coloring

The coloration was determined in three different points of the fruits, using the Minolta CR-400 colorimeter, with the determination in the CIE mode L * a * b *, and the chromaticity and hue angle variables.

2.2 Total Chlorophyll

Chlorophyll was determined in 1 g of crushed fruit in 10 mL of water, with the aid of a tissue homogenizer. The extract was transferred to a 50 mL volumetric flask, the volume was completed with acetone. After standing in the dark, filtration was carried out. The absorbance reading of the extract was carried out at 652 nm and the results expressed in $mg.100g^{-1}$. This was calculated using the equation adopted by Engel and Poggiani (1991).

2.3 Anthocyanins

The anthocyanins content was estimated, spectrophotometrically, according to the method of Lees and Francis (1972), with adaptations of Barcia, Pertuzatti, Jacques, Godoy, & Zambiazi (2012). For extraction of anthocyanin compounds, 1 g of sample was used, in which 50 mL of ethanol solution: 1.5 M HCL (85:15) was added and incubated for 1 h at room temperature. After this procedure, a spectrophotometer (Biospectro SP-220) at 535 nm wavelength, which represents the absorption spectrum of the anthocyanins, was used to read the blank with ethanol solution: 1.5 M HCL. The quantification of total anthocyanins was based on the molar extinction coefficient of cyanidin-3-glycoside (Equation 1), which represents the main anthocyanin present in fruits. The results were expressed in milligrams of cyanidin-3-glycoside per 100 grams of sample.

$$Abs = \varepsilon \cdot C \cdot l \text{ (Equation 1)}$$

In which, Abs is the absorbance read; ε is the molar absorption coefficient; C is the $mol.L^{-1}$ concentration and l is the optical path in cm.

2.4 Soluble Solids

The soluble solids content was determined using a digital refractometer, according to the AOAC (2012) method, the results were expressed in %.

2.5 Total Sugars

The determination of the total sugars was done using the Dische method (1962), read in a spectrophotometer, at 620 nm. The results were expressed in g.100g-1 of fruit.

2.6 pH

pH was determined using a Schott Handylab pH meter, according to AOAC technique (2012).

2.7 Titratable acidity

Determination of the titratable acidity was performed by titration with 0.1N sodium hydroxide solution (NaOH), using phenolphthalein as the indicator, according to the Adolfo Lutz Institute (1985). The results were expressed as percent of citric acid.

2.8 Respiratory Rate

Glass containers containing approximately 5 g of fruit were closed for 1 hour, after which aliquots of the internal sample were removed, with the aid of the PBI Dansensor gas analyzer. The results, expressed as % of CO_2, were converted to $mL.CO_2.kg^{-1}.h^{-1}$, taking into account the volume of the container, the mass and the volume of the fruit in each container and the time that it remained closed.

2.9 Total Pectin and Soluble Pectin

Pectins were extracted according to the technique of McCready and McColomb (1952) and determined, spectrophotometrically, at 520 nm, according to the technique of Blumenkrantz and Asboe-Hansen (1973). The results were expressed as mg of galacturonic acid per 100 g of fruit.

2.10 Pectin Methylesterase (PME)

The enzymatic extraction was performed according to Buescher and Furmanski (1978) technique, with modifications (Vilas Boas, Chitarra, & Chitarra, 1996). The determination of PME activity followed the techniques of Hultin et al. (1966) and Ratner (1969), with modifications (Vilas Boas et al., 1996). A PME unit was defined as the amount of enzyme capable of catalyzing the pectin demethylation corresponding to the consumption of 1 ηmol NaOH per gram of fruit per minute.

2.11 Polygalacturonase (PG)

The extraction of the PG enzyme was performed according to the technique of Buescher and Furmanski (1978), with modifications of Vilas Boas et al. (1996). The assay was performed according to Markovic, Heinrichová, and Lenkey (1975), with modifications of Vilas Boas et al. (1996). The enzymatic activity was expressed in ηmol of galacturonic acid per gram of fruit per minute. A measure of PG activity was defined as the amount of enzyme capable of catalyzing the formation of 1 ηmol of reducing sugar per gram of fruit per minute.

2.12 Firmness

The firmness was measured by the puncture test, using the Magness-Taylor penetrometer, with a 3 mm diameter probe. The evaluations were carried out in the center of the fruit surface. The results were expressed in newtons (N).

2.13 Experimental and statistical design

A completely randomized experimental design (DIC) was used, with 5 maturation stages and 3 replicates per analysis. Statistical analysis was performed using the SISVAR software (Ferreira, 2014). After the analysis of variance of the obtained results, the data were analyzed at the significance level of the F test. When significant at 5%, the means were submitted to the Tukey test at 5% probability.

3. Results and Discussion

The changes in the L, a*, b*, ºhue and Chroma variables (Table 1) objectively reflect the changes in coloration of the black mulberry, throughout the maturation, presented in Figure 1.

Table 1 Color parameters (L, a*, b*, ºHue and Chroma), total chlorophyll, anthocyanins, soluble solids content, total sugars, titratable acidity, pH, respiratory rate, total pectin, soluble pectin, of PG and black mulberry PME activity in different stages of maturation.

Parameters	Maturation Stage				
	1	2	3	4	5
L	52.67 a	51.00 a	38.21 b	29.27 c	22.49 d
a*	-0.26 e	15.35 c	27.59 a	20.49 b	4.35 d
b*	17.22 a	13.78 b	12.74 b	5.31 c	-1.38 d
ºHue	90.80 b	40.76 c	24.69 d	13.51 e	342.20 a
Chroma	17.22 b	21.23 b	31.02 a	16.62 b	4.60 c
Total Chlorophyll (mg.100g^{-1})	209.85 a	63.77 b	36.23 c	12.22 cd	0 d
Anthocyanins (mg.100g^{-1})	2.72 c	3.93 c	19.77 c	45.7 b	314.53 a
Soluble solids (%)	5.33 b	5.67 b	6 b	7 b	13 a
Total Sugars (g.100g^{-1})	0.96 c	0.68 c	1.77 bc	2.91 b	11.75 a
Titratable acidity (g.100g^{-1})	2.6a	2.43 a	1.92 b	1.62 c	0.81 d
pH	3.13 c	3.17 bc	3.27 bc	3.37 b	4.28 a
Respiratory rate (ml CO_2/Kg/h)	229.3 a	200 b	191.4 c	178.62 d	12.68 e
Total pectin (mg.100g^{-1})	787.73 a	410.81 b	291.47 c	273.81 c	260.96 c
Soluble pectin (mg.100g^{-1})	33.18 b	24.40 b	32.59 b	79.02 a	96.71 a
Firmness (N)	13.82 a	8.80 b	5.77 c	3.78 cd	1.80 d
PG (U.A.min^{-1}g^{-1})	388.35 b	385.84 bc	360.67 c	665.32 a	656.37 a
PME (U.A.min^{-1}g^{-1})	80 b	80 b	80 b	80 b	373.33 a

* Means followed by the same letter in the line do not differ statistically from each other by the Tukey test at 5% probability.

The L value, which varies from 0 to 100, measure how light or dark the fruit is, the lighter the fruit, the higher its value. Therefore, the decline in the L value indicates the browning of the fruit. The value a* represents the colors green (negative values) and red (positive values), while the value b*, the colors blue (negative values) and yellow (positive values). The isolated evaluation of a* suggests loss of green pigmentation between stages 1 and 2 and development of red pigmentation from stage 2. Red pigmentation reached its peak at stage 3, decreasing to stage 5. The evaluation isolated from the value b* suggests the loss of yellow pigmentation during maturation, with the appearance of blue traces in the last stage. Lin and Lay (2013) found lower values for the L of black mulberry of the *laevigata* variety in three different stages, presenting a result of 30.7 for green berry, 21.4 for intermediate berry and 13.5 for mature berry, these authors also observed a reduction of the L value, indicating the browning of the fruit. Ercisli and Orhan (2007) observed higher results for a* and b* parameters of black mulberry at the mature stage, being 7.02 and 1.72, respectively.

The hue angle, measured in degrees (0 to 360°), clearly represents the color of the fruit, since it joins the information obtained from a* and b*, from the equation ºh = tangent arc (b*/a*). The 2nd part is red (0º), passing through orange, yellow, green, blue, purple, returning to red (360º). The drop observed in ºhue, from stage 1 to 4, from 90.8 to 13.51, followed by increase to 342.2, in stage 5, correspond to the loss of the green coloration of the fruit and its reddening, the fruit tending towards purple in the last stage, as can be observed in Figure 1. The chroma represents the liveliness of color, the higher the vividness, the higher the chroma value. An increase in the chroma from stage 1 to stage 3 was observed, followed by a decrease to stage 5, suggesting increase and decrease in color liveliness throughout maturation. The decrease in the color vividness in the last two stages can also be associated with the darkening of the fruit. Lin and Lay (2013) observed for black mulberry atropurpurea at three different stages, a higher value of ºHue in the green stage (160) and similar values in the intermediate (26.6) and mature (323.8). The same authors found, for chroma, lower values to those found in this study, being 11.1 for black mulberry in the green stage, 23.4 for the intermediate stage and 1.9 for the mature stage.

Physically demonstrated changes in fruit staining can also be demonstrated chemically by the evaluation of the pigments (Table 1). Reduction of total chlorophyll content was observed during maturation, and in the last stage chlorophyll had been completely degraded. A decline of about 70% was observed comparing fruits in stage 1 and stage 2 of maturation, a period of more intense chlorophyll decrease. The decrease in chlorophyll is associated with a reduction in the green color of the fruit. At the same time, there was an

increase in anthocyanin levels, more intense from stage 3, when the fruit turns red. In fact, anthocyanins are associated with the reddish color of fruits, such as black mulberry and strawberry. The changes in chlorophyll and anthocyanin contents are consistent with the variations of a*, b* and ºhue, as well as the color changes observed in Figure 1. The total anthocyanins content of this study was higher that that found by Ramos-Solano et al. (2005) in black mulberry at mature maturation stage (254 mg.100g^{-1}).

The soluble solids content represents acids, salts, vitamins, some pectins and sugars present in plants (Gomes, Coneglian, Castricini, Medeiros, & Vital, 2004). According to Vilas Boas et al. (2004), the soluble solids are used as indicators of maturity and also determine the quality of the fruits, exerting an important role in the flavor. Normally, the higher the soluble solids content, the higher the sugar content and, consequently, the sweeter the fruit. Acosta-Montoya et al. (2010) observed an increase in soluble solids and total black mulberry sugars at three different maturation stages. Similar values were found for soluble solids contents for the green (5.03%) and intermediate (6.40%) and lower value for the mature stage (7.7%). For the total sugars contents Acosta-Montoya et al. (2010) observed values higher than those found in this study, being 5.5 g.100g^{-1} for the green stage, 13.1 g.100g^{-1} for the intermediate stage and 22.1 g.100g^{-1} for the stage mature.

The titratable acidity decreased and, in contrast, the pH increased over the maturation of black mulberry (Table 1). In general, during ripening, fruits tend to increase pH and decrease acidity. The reduction of acidity is mainly caused by the oxidation of organic acids in the Krebs cycle during respiration, which is more pronounced in the initial maturation stages (Rutz, Voss, & Zambiazi, 2012). Souza et al. (2014) found, for mature black mulberry, a titratable acidity value higher than that of this study (1.51) and a lower value for pH (2.99).

A reduction was observed in the respiratory rate of black mulberry with the maturation advance (Table 1), a respiratory peak not being noticed, characteristic of climacteric fruits. In fact, the black mulberry is considered a non-climacteric fruit (Borsatti, Mazaro, Danner, Nava, & Dalacosta, 2015). Wills, Mcglasson, Graham and Joyce (2007) state that in non-climacteric fruits respiration decreases during maturation and biochemical transformations, which make the fruit mature, occur more slowly. The ripening will only occur if the fruit is attached to the plant, unlike climacteric fruits that have the capacity to mature even after harvesting.

As for the total pectin content, there was a gradual decrease during maturation stages, with the inverse observed for soluble pectin as well as fruit softening, characterized by loss of firmness (Table 1). In the fruit maturation and maturation process, the loss of firmness

observed can be attributed to the degradation of protopectin in the middle lamella and the primary cell wall, with an increase of soluble pectin and loss of non-cellulose neutral sugars (Jacomino, Kluge, Brackmann, & Castro, 2002).

The total pectin contents are close to those observed by Antunes, Gonçalves, & Trevisan (2006) for freshly harvested fruits (600 mg galacturonic acid.100g^{-1}) and those found by Souza, Rodrigues, Gomes, Gomes, & Vieites (2015) for fully black (mature) fruits (182 mg galacturonic acid.100g^{-1}). The soluble pectin contents are below the values obtained by Souza et al. (2015) (182 mg galaconic acid.100g^{-1}) and Antunes et al. (2006) (135 to 220mg galacturonic acid.100g^{-1}), for black mulberry at the mature stage.

The firmness value found for mature fruits at stage 4 (3.85 N) is in agreement with Han, Gao, Chen, Fang, & Wu (2017) with a range of 3.5 to 5N, but disagrees with the results reported by Pires, Lima, Elias, Souza, & Lima (2016) of 0.35N. This variation can be attributed to differences in the cultivar, region, or even lack of standardization of the fruit ripening stage, which is based on visual evaluation. This assertion is validated by the fact that the majority of the studies on the development of black mulberry are based on fruit staining to define the maturation stage (Souza et al., 2015).

The degradation of pectin is usually accompanied by an increase in the activity of cell wall hydrolases, such as polygalacturonase (PG) and pectin methylesterase (PME) (Oliveira et al., 2006). PG catalyzes the hydrolysis of β-1,4 bonds between galacturonic acid residues within the pectin chain (Evangelista, Chitarra & Chitarra, 2000). This results in a decrease in the firmness of the fruits, accompanied by the solubilization of polyuronides (Bicalho, Chitarra, Chitarra & Coelho, 2000). However, the PME acts to promote the demethylation of polyuronides and should precede the PG activity in order to facilitate it, since PG has a higher affinity for the linear substrate, demethylated, after PME performance (Anthon, Sekine, Watanabe, & Barrett, 2002; Bicalho et al., 2000).

In this way, it is common to observe higher PME activity in still green fruits, an activity that is decreasing, while PG activity increases with fruit ripening (Antunes et al., 2006). Such behavior was not observed in this study, for PME activity, which remained constant, increasing only in the last stage of development. According to Resende et al. (2004), this type of behavior can be attributed to the possible presence of pectin with low degree of methoxylation, which would justify the low activity of the PME enzyme. The activity of PG showed a significant increase from the 4th stage of development, coinciding with the increase in the soluble pectin content and decrease of the firmness of the black mulberry, evidencing the role of this enzyme in the fruit softening process.

The results obtained for the pectinolytic enzyme activity in this work are close to those reported by Pires et al. (2016) of approximately 400 U.A./min./g tissue in mature fruits for PME activity and those observed by Antunes et al. (2006), for PG activity (between 400 and U.A./min./g tissue).

4. Conclusion

The progress in maturation of black mulberry is characterized by changes such as chlorophyll degradation and anthocyanin synthesis, increase in soluble solids and sugars and pH, decrease in titratable acidity and respiratory rate as well as decrease of total pectin and increase of soluble pectin, increased pectin methylesterase and polygalacturonase enzyme activity and the consequent softening of the fruits. It is suggested that future works evaluate other changes that may influence the ripening of blackberry not studied here, for example, production of ethylene and profile of volatile compounds.

Acknowledgments

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Porcentagem de contribuição de cada autor no manuscrito

Rafael Carvalho do Lago – 20%

Jéssyca Santos Silva – 20%

Kelly Moreira Pinto – 20%

Lucas Ferreira Rodrigues – 20%

Eduardo Valério de Barros Vilas Boas – 20%