ABSTRACT: The present study aimed to evaluate the physical, physicochemical, and enzymatic characterization of bitch fruits (Brosimum gaudichaudii) during various stages of fruit development. A completely randomized design consisting of 8 stages was performed, totaling 108 days and 4 replications. The enhanced metabolism of the fruits was marked by color changes, higher soluble solids content, and higher levels of titratable acidity, respiratory rate, vitamin C, and enzyme activity (PPO) around the 63th day after anthesis, which characterized the fruit ripening, making it suitable for consumption with DT of 20.425 mm, and LD of 32.10 mm and 3.1475g. Vitamin C ranged from 6.29 to 35.73 mg ascorbic acid.100g⁻¹ of pulp, thus evidencing low vitamin C level. The seed heads of Brosimum gaudichaudii are slightly acidic, and the respiratory rate analysis suggested it is a climacteric fruit.

Keywords: Brosimum gaudichaudii, physicochemical, enzymatic activity

INTRODUCTION

Brazil is known worldwide as the "granary of the world ", due to its great
biodiversity in flora and fauna, great natural resources, fertile and bountiful land, plentiful freshwater and tropical climate with favorable conditions for cultivation of a variety of products, and much of this wealth benefits come from Brazilian cerrado. This biome has fruit trees of great potential use in commercial agriculture still to be explored in the fields of food science and technology, pharmaceutical, medicine, biology, among others.

However, this potential is threatened, once the non-compliance with environmental regulations, fires, expansion of agricultural frontiers and its exploitation, ignorance and irrational use of natural resources have caused irreversible impacts on this biome, compromising its sustainability and placing many animal and plant species at risk of extinction, especially the native fruits, which are holders of economic potential and human health benefits.

The consumption of native plant species, especially fruit trees economically promising in the cerrado region, is also limited to the local population by an essentially extractive process. Among these fruit trees, the *Brosimum gaudichaudii* species stands out, being very common in the Brazilian savana vegetation, where it is popularly known as “mamica-de-cadela”, “algodão-do-campo”, “amoreira-do-campo”, “mururerana”, “apé”, “conduru”, “inhoré” (Ceara State) and particularly “mama-cadela” or bitch fruit in the state of Minas Gerais and Goiás (SILVA et al., 2001).

As reported by Damiani (2009), native fruits can be consumed natural or processed in the form of candy, porridge, cake, bread, biscuits, jams, and liqueurs. However, not many people have access to these fruits, since they are found in a few areas in the country and in a few months. To solve this problem, the food technology industry adds value to these fruits through the manufacture of sweets, besides enabling their consumption throughout the year.

The bitch fruit is a tree species belonging to the *Moraceae* family, and plays an important role in the lives of the inhabitants of the cerrado region, due to the market of the fruit *in natura* for their own consumption or by inhibiting the disease vitiligo, a depigmenting disorder in humans. According to Pereira (2006), the *Brosimum gaudichaudii* Trec tree is widely used in folk medicine in the cerrado region, also used as a blood cleanser.

Few studies have focused on the development of native cerrado species and their uses in food science and technology, mainly related to growth stages, pre-maturation, maturation, ripening, senescence, post-harvest quality, as well as studies on shelf life and nutritional properties. The lack of information leads to alarming rates of post-harvest losses associated with this fruit, which range from 40 to 70%.

The fruit development should be investigated to establish the ideal harvest, its insertion in the consumer market, preserving their natural state, and implementation of future commercial crops (CHITARRA and CHITARRA, 2005) always aiming to obtain a high quality product, appreciated by consumers.

This study aimed to evaluate the different development stages of the bitch fruit (*Brosimum gaudichaudii*) to understand the pre- and postharvest behavior of the fruit and its life cycle from anthesis to senescence, by physical, physicochemical and enzymatic analyses, and to propose appropriate production and conservation techniques to minimize losses.

MATERIAL AND METHODS

**Plant Material**

Bitch fruits were harvested manually at various maturation stages from the native cerrado, at Matinha Farm located in the Triangulo Mineiro, Uberlândia-MG, latitude 18°56'38", and longitude 48 °18'39", and average altitude of 863 m (Uberlândia, 2014).

In the packing house, the fruits were selected and classified as Extra (free from mild or severe defects). Then the fruits were placed in plastic trays, which were later placed in insulated boxes and transported the
same day to the food analysis laboratory at IFTM - Campus Uberaba-MG.

The fruits were harvested from plants of 12 years old, throughout its development stages from anthesis to senescence, every 15 days, as follows: day 3, 18, 33, 48, 63, 78, 93 and 108 after anthesis. Flowering occurred in Uberlândia, Minas Gerais (Minas Triangle), in July 2012. The culmination of this event occurred in mid-August, when the initial fructification phase was also observed, with peak in September. Eight stages of fruit development were investigated, which totaled 108 days, characterized for changes in size, shape, fruit weight, and color from flowering (anthesis) at day 3 until harvest, defined by the ease of being detached from the bush and orange color.

Physical, physicochemical, and enzymatic analyses

Transverse diameter (TD) and longitudinal diameter (LD): the diameter measurements were carried out with a caliper (Mitutoyo®), and the results were expressed in millimeters (mm).

Average fruit weight: fruits were weighed using a semi-analytical scale Mettler®, model PC 2000, and the results expressed in grams (g).

Color parameters (L*, a*, and b*): the color was measured on the skin in the longitudinal central region of the fruit on two opposite sides. A Minolta® colorimeter, Model CR 400 was used, and the coordinates L*, a* and b* were determined. The L* coordinate is associated with the brightness or luminous intensity, ranging from 0 to 100, in which 0 corresponds to the absolute black and 100 corresponds to the absolute white color. The coordinate a* is related to the intensity of red/green with negative values of a* representing the color green and a positive a* representing red color. The coordinate b* is blue to yellow, with negative values representing the color blue and positive values for yellow. Standards of Y = 94.20, x = 0.3134 and y = 0.3207 were used for the calibration of the equipment in reflective white plate.

pH: pH measurements were performed in a pH meter Schott Handylab®, according to Instituto Adolfo Lutz - IAL (2008).

Total solids soluble (TSS): were determined by refractometry, using a digital refractometer with automatic temperature compensation (25°C), and expressed as °Brix, according Instituto Adolfo Lutz - IAL (2008).

Total Titratable acidity (TTA): the total titratable acidity was determined by titration with 0.1N NaOH solution, with phenolphthalein as an indicator in accordance with IAL (2008). The results were expressed as% of citric acid.

Respiratory rate: the CO2 produced by 10 fruits conditioned for one hour in hermetic glass were measured during the study period. A gas analyzer, Check Point O2 and CO2, PBI Dansensor® was used. The results were expressed as mLCO₂.kg⁻¹.h⁻¹.

Vitamin C: vitamin C content was determined according to Strohecker & Henning (1967), and the results were expressed as mg.100g⁻¹.

Enzymes polygalacturonase (PG), pectin methyl esterase (PME), polyphenol oxidase (PPO) and peroxidase (PER): the PG was extracted as described by Buescher&Furmanski (1978), and the quantitative determination was performed according to Markovic et al. (1975), with modifications by Vilas Boas et al. (1996). The PME extraction was performed as described by Buescher and Furmanski (1978), and the determination as reported by Hultin et al. (1966) and Ratner et al. (1969), with modifications by Vilas Boas (1995). The PG and PME activities were expressed as ηmol.g⁻¹.min⁻¹. The PPO extraction was performed according to Draetta and Lima (1976), and the enzyme activity was determined as reported by Ponting and Joslyn (1948) using a sample blank without 3,4-dihydroxyphenylalanine (DOPA). PER was determined according to Khan and Robinson (1994).
Experimental design

A completely randomized design (CRD) was used, consisting of 8 stages and 4 repetitions, and the experimental unit consisted of 500g of fruit. Eighty specimens were selected at random, with homogeneous size, in which the flowers were marked with pieces of yarns of different colors at the time of anthesis. The total collection period lasted until the berries on the bushes reached maturity, characterized by the intense orange color of the bark, which lasted 108 days.

Statistical analysis

Analysis of variance with at 5% significance level and regression analysis by F test were assessed using the software SISVAR (FERREIRA, 2000).

RESULTS AND DISCUSSION

Fruit ripening was enhanced characterized by the enhanced metabolism of the bitch fruits, marked by color changes, and high levels of soluble solids, titratable acidity, respiratory rate, vitamin C, and enzyme activity (PPO) around the 63th day after anthesis (Figures 3, 4 and 6), making the fruit suitable for consumption, with TD of 20.425 mm, LD of 32.10 mm and mean weight of 3.1475g (Figure 1). All variables were influenced interactively by development time throughout the study period. There were significant differences in the diameters (TD and LD) over the development of the Brosimum gaudichaudii fruit with increasing values of TD of 1.175 mm at day 3 to 21.300 mm at 93 days after anthesis. The LD ranged from 2.15mm at day 3 to 32.225 mm at 93 days after anthesis. After this period, a reduction of both TD and LD (Figure 1) was observed, probably due to the senescence period and low relative humidity during the study period.

The stage of fruit development starts normally with fertilization, followed by formation, growth and maturation, including the ripening stage and senescence (CHITARRA and CHITARRA, 2005). Knowledge about the development pattern and physiology of the fruit allows appropriate manipulation and storage, increasing shelf life and maintaining fruit quality (Figure 1). Bellé (2008) further reported that auxin and cytokinin in the seeds of the young fruits are responsible for the regulation of cell division. Subsequently, the auxins also regulate cell elongation and therefore are responsible for the increase in size. In the present study, the Brosimum gaudichaudii fruits growth was divided into three stages: Stage I, flowering characterized by an intense fruit growth, occurring at 48 days after anthesis; Stage II, slow growth and low dry matter accumulation, occurring from 48 to 63 days, due to core development (epicarp), as
described by Gomes et al. (2005); Stage III, intense growth, culminating with fruit ripening after 63 days (Figure 1).

As can be seen in Figure 2, there was an increase in $L^*$, $a^*$ and $b^*$ values along the fruit development. The increase in $L^*$ value indicated color changes from dark green ($L^* = 27.32$) to light green ($L^* = 69.17$). The $a^*$ value increased from $-1.78$ to $-6.42$, indicating that the fruits were unripe, unfit for consumption until 48 days after anthesis. From 48 to 63 days after anthesis, the normal ripening process started, but the peak occurred at day 63, as evidenced by the value of $a^* = 5.27$, and $b^* = 18.15$, thus being fit for consumption. According to Chitarra and Chitarra (2005), the decrease in total chlorophyll content throughout fruit development is characterized by a reduction in green, as in most fruits during ripening. The $b^*$ values of 1.35 and 2.48 were observed at day 18 after anthesis, and at day 33, respectively. These results indicate no ripeness, thus the fruits were unfit for consumption.

![Figure 2. $L^*$, $a^*$, $b^*$, and pH changes during bitchfruits development. IFTM, Uberaba, MG, 2015.](image)

Silva et al. (2009) studied the life cycle of Pequi fruit, and the characterization of gabiroba fruit (*Campomanesia pubescens*) during fruit development, respectively, and also observed an increase in $L^*$ and $a^*$ values indicating degreening of pequi and gabiroba, which is consistent with the data obtained in this study (Figure 2).

The pH is a measure of the acidity or alkalinity of the food. Acidity has an influence on the development of the microflora, particularly for low-pH foods. Foods are classified according to the degree of acidity in weak acid, with pH> 4.5 (milk, meat, fish and some vegetables); acid, pH 4.0 to 4.5 (fruits and vegetables) and very acidic, with pH <4.0 (fruit juice, soft drinks, etc.). Thus, the lowest pH value found in this study was 5.02 and the highest was 6.37, which evidenced that the *Brosimum gaudichaudii* fruits are weak acid (Figure 2). Significant differences in pH were observed throughout the *Brosimum gaudichaudii* fruit development, given that the pH was 5.17 at day 3, and 6.19 at day 48, a period characterized by higher pH values. After this period, a decrease in pH to 4.98 was observed, which remained until the day 108 (Figure 2).

According to Chitarra and Chitarra (2005), the loss of green color is due to structural decomposition of chlorophyll as a result of several factors acting together or separately. Among them, the changes in pH caused mainly by degradation of organic acids and other compounds in the vacuoles, and activation and presence of chlorophyllase oxidant systems (Figure 2).

The soluble solids (°Brix) are an indirect measure of the sugar content, which increases as the soluble solids accumulate in the fruit. The SST ranged from 1.21 ° Brix at...
day 3 to 3.26 °Brix at day 78 after anthesis, which included the ripening stage. After this period, lower values were observed (Figure 3), reaching 2.34 °Brix at day 108. According to Chitarra and Chitarra (2005), the soluble sugars present in the fruit in the free or combined form are responsible for sweetness, flavor, acids balance, attractive color as derivatives of anthocyanins (glycosides), and texture, when combined appropriately, compounding the structural polysaccharides. The low °Brix observed for bitch fruits can limit its consumption affecting the sensory acceptance of the product (Figure 3).

Figure 3. Changes in °brix and titratable acidity values of bitch fruits during fruit development. IFTM, Uberaba, MG, 2015.

The sugar content usually increases with the fruit ripening by biosynthetic processes or degradation of polysaccharides. The sugars are associated with fruit development and quality attributes. It is noticed that the SST levels of the bitch fruit are dependent on the development and ripening stage at time of harvest (Figures 1 and 3). Therefore, assessments of the fruit ripening are important because, when they are harvested at the appropriate time, i.e., with the proper degree of ripeness, the fruits have better edible quality and higher yield (Figure 3).

The content of organic acids, with few exceptions, decreases with fruit ripening, as these acids are used as substrate in the respiration process or are converted to sugars. This fact was not observed in this study, since the peak ripeness occurred around 63 days after anthesis. Figure 6 shows a slight increase in the ATT (% citric acid) of 1.73% at day 3 to 2.16% at day 63, a period characterized as the most significant, as demonstrated by the experimental conditions. This increase in titratable acidity is due to formation of starch or sugars, as observed in some banana cultivars, in which a significant increase of malic acid was observed during ripening. After 63 days after anthesis, there was a decrease in ATT, which reached 2.05% at day 108. According to Bellé (2008), all fruit breathes not only in the development stage (cell division and elongation - Figure 1), but also during ripening and senescence, even after harvest (Figure 4). After the harvest of any part of the plant, breathing becomes its main physiological process, since no longer depends on the absorption of water and minerals by the roots, the photosynthetic activity of the leaf, or other parts of one parent plant.
Therefore, the plant parts can live independently, using their own metabolic reserves accumulated during growing and ripening (CHITARRA and CHITARRA, 2005) (Figure 4). Significant differences in respiration rate of the bitch fruits were observed during the fruit development period, which increased from 5.36 mL CO₂·kg⁻¹·h⁻¹ at day 3 to 41.26 mL CO₂·kg⁻¹·h⁻¹ at day 63, with a reduction in respiratory rate after this period, reaching 22.36 mL CO₂·kg⁻¹·h⁻¹ after 108 days (Figure 4). The climacteric period occurred between 48-63 days after anthesis, a period coinciding with the beginning of ripening; with maximum value observed in around 63 days. According to Chitarra and Chitarra (2005) and Bellé (2008), the increase in respiratory rate in the climacteric period is attributed to an increase in the concentration of endogenous ethylene and may also be associated with an increase in the concentration of phosphorylated hexoses (fructose 1,6-bisphosphate) with a consequent increase in the glycolytic pathway (Figure 4).

As shown in Figure 4, the vitamin C content of the bitch fruits increased significantly from 6.285 mg·100g⁻¹ ascorbic acid, at day 3 to 35.730 mg·100g⁻¹ at day 63 after anthesis, thus demonstrating that the vitamin C levels in the bitch fruits were influenced by their life cycle (Figure 1), and that there was synthesis of this compound and a decline thereafter, understood as late maturation and early senescence, reaching up to 15.47 mg·100g⁻¹ ascorbic acid at day 108 (Figure 4).

According to Chitarra and Chitarra (2005), the vitamin C content tends to decrease with maturation and storage of many vegetables, due to the direct action of the enzyme ascorbic acid oxidase (Ascorbinase), or by the action of oxidizing enzymes such as peroxidase (Figure 6). Vilas Boas (2004) studied pequis in southern Minas Gerais, and found vitamin C values of 14.86 to 98.84 mg·100g⁻¹ and 105.00 mg·100g⁻¹, respectively.

As can be seen in Figure 4, the Brosimum gaudichaudii is not a Vitamin C rich fruit, since more significant levels are found in other fruits and vegetables. Fruit softening is one of the major transformations during ripening of fleshy fruits, with a marked influence on both fruit quality and shelf life. It is directly related to the chemical components of cell walls, especially with thepectins present in the lamella, which act as cementing material, maintaining cohesion between cells (WILLS et al. 1998). The protopectin predominates in immature plant tissues. Then throughout the ripening period, a calcium release and solubilization of protopectin occurs by the action of two specific enzymes, known respectively as pectinmetylesterase (SMEs) responsible for breaking the methyl ester bonds, and polygalacturonase (PG), which transforms galacturonic acid polymers into pectic acid, which is soluble in water. According to Chitarra and Chitarra (2005), the increase in
activity of hydrolases (PG) of the cell wall is an indicative of fruit softening and climacteric fruit ripening (Figure 5).

Figure 5. PG and PME activity of bitch fruits during fruit development. IFTM, Uberaba, MG, 2015.

The PG activity from day 3 to day 33 after anthesis was not significant, evidencing no loss of firmness and that fruits were still immature. After this period, a significant increase in PG activity was observed from day 48 to day 78, with values ranging from 3.198 to 25.540 ηmol.g⁻¹ min⁻¹, respectively (Figure 5). This period was characterized by loss of firmness, and consequently fruit softening. At 93 days after anthesis, there was a significant reduction in PG activity, demonstrating fruit softening for almost all samples, and thus fruit ripening (Figure 5).

The PME is involved in fruit ripening and is cleaved by the PG (Figure 5). Both PG and PME activities occurred more intensely around 48-78 days after anthesis. A synergistic effect of these two enzymes was observed, playing an important role in fruit softening process during the ripening stage (Figure 5).

The PME activity ranged from 2.48 ηmol.g⁻¹ min⁻¹ at day 3 to 34.39 ηmol.g⁻¹ min⁻¹ at day 108 after anthesis, and was observed in all stages of fruit development. However, an increase of PME activity occurred during ripening, from day 48 to 78 after anthesis and intensified at day 108, thus demonstrating an intense fruit ripening at that stage (Figure 5).

According to Chitarra and Chitarra (2005), the polyphenol act on phenolic compounds, causing their oxidation to quinones in the presence of O₂, with browning in tissues due to polymerization or reaction with amino acids and proteins. Usually, browning occurs due to injury in the product during harvest, storage, or processing. The usual PPO substrates include citric acids esters such as chlorogenic acid, catechins, 3,4-dihydroxyphenylalanine (DOPA) and tyrosine. They can also cause browning in anthocyanins-rich products, for acting together with the glycosidase, producing the corresponding quinones (Figure 6).
Although cultivars with low PPO activity are desirable for consumption in natura and for processing, the high PPO activity may provide greater resistance to pathogen attack (CHITARRA and CHITARRA, 2005). This fact was not verified in this study because some fruits have had improper pulp for consumption (rotten) after 80 days, even during the period of fruit development, which was characterized by the complete absence of rain and low humidity, which could cause fruit rot problems (Figure 6). The PPO activities of the present study increased from 8.62 ηmol.g⁻¹.min⁻¹ at day 3 to 110.58 ηmol.g⁻¹.min⁻¹ at day 63 after anthesis. After this period, a lower activity was observed, which reached 48.14 ηmol.g⁻¹.min⁻¹ at day 108. The PER has many physiological functions and acts on different substrates in the form of multiple anionic or cationic isoenzymes, in reactions such as oxidation of phenolics and carotenoids, degradation of auxins, chlorophyll, and ascorbic acid as well as in the biosynthesis of lignin. Therefore, PER activity is related to changes in the sensory attributes (darkening, hardening, off-flavors) and nutritional value (vitamin C losses) (Figures 4 and 6). It is also related to the development stages and senescence in tissues.

As shown in Figure 6, the PER activity increased significantly from 22.26 ηmol.g⁻¹.min⁻¹ at day 3 to 111.74 ηmol.g⁻¹.min⁻¹ at day 108 after anthesis. It is noticed that the PER activity is dependent on the life cycle of the Brosimum gaudichaudii fruit (Figure 1), with the peak at day 33 after anthesis. This peak in enzyme activity can act as a biochemical indicator to identify the degree of ripening of different varieties of this fruit (Figure 6).

The higher PER activity can also be associated with the biosynthesis of compounds of the cellular walls in response to injury of tissue damage. It has been used as an indicator of efficiency of thermal processing of fruits and vegetables due to their relative thermal stability. High PER levels are also associated with oxidative deterioration of many plants or fruits in advanced stage of ripening or senescence. In view of this, it can be stated that the deterioration of the bitch fruit occurred quickly from 78 to 108 days after anthesis, since many fruits were unfit for consumption in that period, once they were saggy, with dried pulp, and some fruits were fallen to the ground, which may be associated with PER activity, thus demonstrating the senescence of the bitch fruits of the present study.

CONCLUSION

The enhanced metabolism of the bitch fruits, marked by color changes and higher values of soluble solids, titratable acidity, respiratory rate, vitamin C, and enzyme activity (PPO) at day 63 after anthesis characterized the fruit ripening, making it...
suitable for consumption with TD 20.42mm, LD of 32.10mm, and average weight of 3.14g.

The *Brosimum gaudichaudii* fruit growth was divided into three stages: Stage I, flowering characterized by an intense fruit growth, occurring at 48 days after anthesis; Stage II, slow growth and low dry matter accumulation, occurring from 48 to 63 days, due to the core development (epicarp); Stage III, intense growth, culminating in fruit ripening after 63 days.

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