MICROORGANISMS IN MACAUBA FRUITS (*Acrocomia aculeata*) AFTER DIFFERENT POST-HARVEST TREATMENTS AND STORAGE

L. A. L. QUEIROZ¹, C. S. NASCIMENTO², A. L. M. SILVEIRA³, V. L. SANTOS⁴, M. H. CAÑO ANDRADE⁵

¹ Universidade Federal de Minas Gerais, Departamento de Engenharia Química

² Universidade Federal de Minas Gerais, Departamento de Engenharia Química

³ Universidade Federal de Minas Gerais, Departamento de Engenharia Química

⁴ Universidade Federal de Minas Gerais, Departamento de Microbiologia

⁵ Universidade Federal de Minas Gerais, Departamento de Engenharia Química

E-mail: lorenaqueiroz84@yahoo.com.br

ABSTRACT: The Macauba fruits (*Acrocomia aculeata*) may be promising source of vegetable oil for food purposes, cosmetics and energy, due to the high productivity and obtainment of two oil types. However, the main constraint is associated with the rapid deterioration of the fruit, leading to production of oil with high acidity. The acidification process can be caused by microorganisms growing associated with fruits, by high pulp moisture and by the endogenous lipase activity. This study aimed to quantify microorganisms present in the husk and fruit pulp after different post-harvest treatments and after 30 days storage, comparing effectiveness of different conservation treatments by simple and inexpensive techniques. The storage period was determined according to preliminary tests where it was observed that at 30 days of harvest the fruits were already deteriorating. After storage there was a predominance of fungi in pulp and bacteria and yeasts in the husk.

KEYWORDS: *Acrocomia aculeata*; Macauba; Microorganism; Post-harvest conservation.

1. INTRODUCTION

Macauba palm (*Acrocomia aculeata* (Jacq.) Lodd. ex Mart) is a promising source of vegetable oil and biomass, which can be used for various purposes including human consumption, energy, pharmacological and cosmetics. This potential has attracted the interest of many researches. The pulp of the Macauba fruit contains oil with high monounsaturated fatty acid content, especially oleic acid, and this characteristic is desirable for food and biofuel industries due to its nutritional quality and oxidative stability (Nunes et al., 2015). The oil extracted from the kernel of the fruit is rich in lauric acid which is a valuable raw material in the cosmetic industry (Matsimbe et al., 2015; Coimbra and Jorge, 2011). Additionally to the oils, the endocarp and the residual cakes are also biomass with can be used for energy, food and feed applications (Silva and Caño Andrade, 2014; Cano Andrade et al., 2006).

Despite of this potential, the exploitation of the Macauba culture has been carried out in extractive way, which often provides low productivity and poor quality of fruits (Pires et al., 2013). Macauba fruit can degrade quickly after abscission of the bunch due to the high water content present in the pulp, which promotes microbial growth. As a result of this process, low-quality oil is obtained, with high acidity. Indeed, the acidification can be caused by high moisture and endogenous lipase activity in the pulp. Added to this, the harvesting system where the fruits are collected in the ground increases the microorganism contamination (Tilahun et al., 2013). According to the literature, the microbiota found in Macauba is largely represented by fungi, showing lipolytic activity that result in the hydrolysis of triglycerides present in the pulp leading to an increase in free fatty acids in the oil (Cavalcanti-Oliveira et al., 2015; Silva, 2007).

The post-harvest conservation of the Macauba fruits is still little known and oil extraction is performed by applying technologies adapted from other oils (Mota et al., 2011). To overcome the main difficulties in the processing of this oil crop and to get the real potential of the Macauba culture as a good quality vegetable crop, considering oils and residual biomass, it is necessary to establish a proper whole processing protocol, including post-harvest conservation methods and oil extraction (Nunes et al., 2015; Martins, 2013). Research on the post-harvest conservation of Macauba fruit has recently been published. Some physical and chemical methods of preservation have been studied as the use of solutions with different pH, autoclaving, pasteurization, drying for 4 and 8 days, gamma radiation and fungicidal. Although some of the treatments have aided to preserve the oil quality, they do not always have low operating costs and investment (Cavalcanti-Oliveira et al., 2015; Evaristo, 2015; Martins, 2013).

Different chemical and physical methods may be employed in order to reduce the metabolic activity and minimize the fruit decay process. Chemical methods act in physiology and control of microorganisms through the use of specific chemical compounds. Physical methods may act directly on pathogens, besides act on the physiology of fruit, delaying the ripening and thus maintaining fruit resistance (Martins, 2013). Some example of methods employed for this purpose are sanitizing with sodium hypochlorite and drying. The chlorine, in its various forms, is the sanitizer most commonly used for food and has a broad spectrum of antimicrobial action (Brecht, 1995). The sodium hypochlorite is widely used due to its fast acting, easy application and complete dissociation in water (Antoniolli, 2005). The preservation by drying is based on the fact that microorganisms and enzymes, as well as any metabolic mechanism, require water for their activities. By reducing the amount of available water is reduced the water activity, the growth of microorganisms and the rate of chemical reactions in the product (Goneli et al., 2007).

In this way, in order to reduce contamination by microorganism minimizing the fruit degradation process, simple and low cost techniques, which can be implemented both by cooperatives and family farmers, and larger industrial systems, must be applied. The aim of this study was to evaluate the efficacy of drying and the use of sodium hypochlorite solution as sanitizer through the quantifying of microorganisms present in the husk and pulp after different post-harvest treatments and after 30 days storage.

2. MATERIAL AND METHODS

2.1. Quantification of microorganisms

In this study were made two different harvests in January 2013. In each harvest, ripe fruits were collected directly from the ground on the campus of Federal University of Minas Gerais (UFMG) with a maximum of seven days after fall from the bunch. To evaluate the effect of different post-harvest treatments the fruits were divided into three lots that were submitted to three treatments: Lot 1: stored without cleaning (control); Lot 2: washed with water, quickly immersed in sodium hypochlorite (2% active chlorine) and dried at room temperature; and Lot 3: washed with water, quickly immersed in sodium hypochlorite (2% active chlorine) and dried in the oven at 60 °C for 24 h.

In each lot, six fruits were randomly selected, grouped in pairs and conditioned in plastic bags, totaling three plastic bags with two Macauba fruits on each.

For quantification of microorganisms present in the husk, 100 ml of saline solution (0.85% NaCl) were added in each plastic bag, and then these were placed in an ultrasonic bath for about five minutes, promoting dispersal of microorganisms present in the husk to saline solution. Sequentially, in a sterile environment, it was removed 1 ml of suspension to prepare dilutions of 10^{-1} , 10^{-2} and 10^{-3} , and transferred 100 μ L of each dilution to Petri dishes containing culture mediums TSA (bacteria quantification), PDA (fungi quantification) and YM (yeast quantification), previously sterilized. For the bacteria quantification was added to the culture medium one antifungal solution Benomyl 0.05 g/L and for the yeasts quantification and fungi was added an antibacterial chloramphenicol 0.2 g/L. The inoculated plates were incubated at 37 °C for 24 h for the growth of bacteria, 25-28 °C for 72 h for the growth of yeasts and at 25-28 °C for 5-7 days for the growth of fungi.

For the quantification of the microorganisms present in the pulp, the fruits immersed in saline solution in the previous steps were washed with a neutral detergent and dried with paper towel in order to decreasing the pulp contamination by husk. After, the husk was removed and approximately 1 gram of pulp was cut and transferred to 9 ml of 1% saline solution. Posteriorly, was followed the same procedure adopted in the husk.

The different morphotypes found in husk and pulp were listed and classified as following: bacteria (color, brightness, border, appearance); fungi (color, border, appearance, reverse); yeast (color, brightness, border, appearance).

Additionally, the husk moisture content and pulp were determined gravimetrically at 130 °C in an oven according to Ac 2-41 (AOCS, 2009). The titratable acidity of the pulp was determined by titrated with sodium hydroxide (0.1 mo/L) according to the technique described by Adolfo Lutz Institute (2008), method 310/IV. All the analyses were performed after the different post-harvest treatments (0 day) and after 30 days of storage (30 day). The study was not conducted over a longer period because preliminary tests showed that after 30 days the fruits already had some degree of degradation.

2.2. Statistical analysis

The analyses were performed in triplicate and the results were subjected to analysis of variance (ANOVA) and differences between the means were evaluated by the Tukey test at 5% probability using the software Sisvar 5.6.

3. RESULTS AND DISCUSSION

In this work have been numbered 25 bacteria morphotypes, 57 fungi and 26 yeasts. It is noteworthy that the classification by morphotype was done visually, and more research is needed, including phenotypes methods and genotypes for identifying each morphotype. Figure 1 show some morphotypes detected.

Figure 1 - Some morphotypes detected: (A) Yeast; (B) Fungi; (C) Bacteria

The quantity of bacteria, fungi and yeast (CFU/g_{dry weight}) and the total quantity of microorganisms are shown in Table 1 and Table 2. In general, at the initial time the quantity of microorganisms present in the pulp was lower than that found in the husk, being zero in some cases. This result indicates that the fruit pulp is initially protected by the husk. Exception to this rule was observed in lot 2 of the first harvest and lot 3 of the second harvest, which was attributed to the fact that the assessment was made in different fruits chosen at random and this fruits could be on the ground for longer and more degraded.

When assessing the effect of these treatments on each specific group of microorganisms, one cannot affirm that they were effective, since according to the statistical analysis to compare the means in most cases this difference was not significant. Note also, that the results show high standards deviation, probably due to the variation on the microbial quantity between fruits. This variation can be associated to the heterogeneity on the fruits maturation that results in differentiated microbial colonization. Considering all the stored fruits, for each analysis the fruits sampled was chosen at random and also during the experiments it was possible to visually observe that some samples were more deteriorated and with the presence of fungi while others do not.

Table 1 - The quantity of bacteria, fungi and yeast (CFU/g_{dry weight}) and the total quantity of microorganisms (first harvest).

		Total		Bacteria		Fungi		Yeast	
		0 day	30 days	0 day	30 days	0 day	30 days	0 day	30 days
Husk	Lot	5.9x10 ⁴ ±	9.8x10 ⁴ ±	1.0x10 ⁴ ±	4.6x10 ⁴ ±	1.2x10 ⁴ ±	2.9x10 ⁴ ±	3.7x10 ⁴ ±	2.3x10 ⁴ ±
	0	3.6x10 ⁴ aA	8.2x10 ⁴ aA	1.7x10 ⁴ aA	5.2x10 ⁴ aA	5.2x10 ³ aB	1.4x10 ⁴ aA	2.1x10 ⁴ aA	2.0x10 ⁴ aA
	Lot	8.5x10 ³ ±	2.2x10 ³ ±	3.0x10 ² ±	6.55x10 ² aA	3.0x10 ² ±	0 bA	7.9x10 ³ ±	1.5x10 ³ ±
	2	5.2x10 ³ aA	2.1x10 ³ bA	5.2x10 ² aA		5.2x10 ² aA		5.3x10 ³ bA	2.1x10 ³ aA
	Lot	1.2x10 ³ ±	6.3x10 ² ±	1.2x10 ³ ±	0 aA	0 aA	0 bA	0 bA	6.3x10 ² ±
	3	1.5x10 ³ aA	1.1x10 ³ bA	1.5x10 ³ aA					1.1x10 ³ aA
Pulp	Lot	3.2x10 ² ±	9.7x10 ² ±	3.2x10 ² ±	4.6x10 ¹ ±	0 B	9.2x10 ² ±	0 aA	0 aA
	0	5.5x10 ² aA	2.4x10 ² aA	5.5x10 ² aA	7.9x10 ¹ aA		2.9x10 ² aA		
	Lot	1.1x10 ⁴ ±	2.9x10 ³ ±	1.2x10 ² ±	0 aA	0 B	6.7x10 ² ±	1.1x10 ⁴ ±	2.3x10 ³ ±
	2	1.9x10 ⁴ aA	3.3x10 ³ aA	2.2x10 ² aA			5.8x10 ² aA	1.8x10 ⁴ aA	3.9x10 ⁴ aA
	Lot	5.4x10 ¹ ±	3.7x10 ² ±	5.4x10 ¹ ±	0 aA	0 A	3.3x10 ² ±	0 aA	4.1x10 ¹ ±
	3	9.4x10 ¹ aA	2.5x10 ² aA	9.4x10 ¹ aA			3.1x10 ² aA		7.1x10 ¹ aA

Mean ± standard deviation.

Means followed by the same letters in the same column do not differ by Tukey's test (p<0.05).

In each group of microorganisms, means followed by the same capital letters in the same row do not differ by Tukey's test (p<0.05).

Table 2 - The quantity of bacteria, fungi and yeast (CFU/g_{dry weight}) and the total quantity of microorganisms (second harvest).

		Total		Bacteria		Fungi		Yeast	
		0 day	30 days	0 day	30 days	0 day	30 days	0 day	30 days
Husk	Lot 0	5.0x10 ⁵ ± 1.3x10 ⁵ aA	1.8x10 ⁵ ± 1.2x10 ⁵ aB	1.6x10 ⁵ ± 1.3x10 ⁵ aA	1.4x10 ⁵ ± 1.2x10 ⁵ aA	1.5x10 ³ ± 8.7x10 ² aB	1.9x10 ⁴ ± 5.9x10 ³ aA	3.3x10 ⁵ ± 3.9x10 ⁴ aA	1.5x10 ⁴ ± 1.2x10 ⁴ aB
	Lot 2	3.3x10 ³ ± 5.0x10 ³ bA	3.8x10 ⁴ ± 4.6x10 ⁴ aA	0 bA	3.5x10 ³ ± 3.5x10 ⁴ aA	8.8x10 ¹ ± 1.5x10 ¹ aA	3.5 x10 ² ± 6.1x10 ² bA	3.2x10 ³ ± 4.8x10 ³ bA	3.4x10 ⁴ ± 4.7x10 ⁴ aA
	Lot 3	3.9x10 ³ ± 6.4x10 ³ bA	2.2x10 ⁴ ± 3.8x10 ⁴ aA	3.8x10 ³ ± 6.4x10 ⁴ abA	5.3x10 ³ ± 9.1x10 ⁴ aA	4.6x10 ¹ ± 3.9x10 ¹ aA	7.1x10 ² ± 1.2x10 ³ bA	0 bA	1.6x10 ⁴ ± 2.7x10 ⁴ aA
	Lot 0	0 aA	2.5x10 ³ ± 1.1x10 ³ aA	0 aA	1.1x10 ² ± 1.9x10 ² aA	0 B	2.4x10 ³ ± 1.0x10 ³ aA	0 aA	0 A
	Lot 2	0 aA	1.8x10 ³ ± 4.5x10 ² aA	0 aA	0 aA	0 B	1.8x10 ³ ± 4.5x10 ² aA	0 aA	0 A
	Lot 3	7.1x10 ³ ± 1.2x10 ⁴ aA	3.6x10 ² ± 4.5x10 ² aA	6.8x10 ³ ± 1.2x10 ⁴ aA	7.2x10 ¹ ± 1.2x10 ² aA	0 A	2.9x10 ² ± 4.9x10 ² bA	3.0x10 ² ± 4.5x10 ² aA	0 A

Mean ± standard deviation.

Means followed by the same letters in the same column do not differ by Tukey's test (p<0.05).

In each group of microorganisms, means followed by the same capital letters in the same row do not differ by Tukey's test (p<0.05).

After 30 days of storage presence of microorganisms was verified in all lots, both in the husk and in the pulp. According Brackett (1987), the increase in fruit decay rate is due to the transfer of the microorganisms of husk to the pulp, place where microorganisms find favorable conditions for their growth. Thus, pulp contamination may have occurred due to the husk deterioration, allowing access to the pulp. During storage occurs water loss and in accordance with Evaristo (2015) and Carvalho (2013), this causes the increased husk porosity. This pattern was also related by Silva et al. (2010) that evaluated the effectiveness of two sanitization methods: immersion in boiling water followed by drying in the sun and immersion in boiling water followed by drying in an oven at 120 °C. The authors concluded that contamination of Macauba fruits occurs gradually, with microorganism's infiltration into susceptible fruit areas, such as holes, and gradually infesting all the pulp, causing changes in structure, color, taste, smell and chemical composition.

The proportions between bacteria, fungi and yeast regarding to the total quantity of microorganisms are presented in Figure 2 and 3. According to Figure 2, at time 0, fruits without treatment (lot 1) presented bacteria, fungi and yeast in the husk. It was observed yeast predominance in the husks of fruits washed (lot 2) and bacteria in fruits washed and dried at 60 °C (lot 3).

Figure 2 - Microorganisms proportion in the husk regarding to the total quantity.

After 30 days, it was noted a percentage increase of fungi on husks fruits without treatment and of bacteria in fruits treated with sodium hypochlorite solution, but according to the Tables 1 and 2 only the increasing of fungi amount was significant.

According to Figure 3, in the first harvest, initially (0 days) there was bacteria predominance in the pulp of fruits that were not undergone treatment (lot 1) and of fruits washed and dried at 60 °C (lot 3). At fruits treated with sodium hypochlorite solution (lot 2) there were larger yeast amount. In the second harvest, at 0 day there were not microorganisms in the pulp both in the lot 1 as in the lot 2. This may be indication that the fruits randomly selected were very fresh, i.e., fallen a few days.

Figure 3 - Microorganisms proportion in the pulp regarding to the total quantity.

Furthermore, after 30 days, the fungi presence was predominant on pulp, except for the lot 2 of the first harvest, which initially contained a higher yeast percentage, keeping this predominance after storage. These results are indicative that the pulp has a tendency to increase fungi percentage with the storage time. According to the Tables 1 and 2 only in the lot 3 there was no significant increase of fungi in the pulp.

Pereira et al. (2013) also evaluated the presence of microorganisms in pulp of Macauba fruits sanitized with 400 ppm hypochlorite solution compared to untreated fruits. In both cases, this author observed a reduction in the bacteria number after storage and the filamentous fungi were the only microorganisms found by the end of the storage period (120 days). Antonioli et al. (2005) found an increase of two log cycles of molds and yeasts during the pineapple storage treated with sodium hypochlorite.

During storage occur changes in the physical-chemical characteristics of the husk and pulp that can affect the growth of microorganisms. In this work, were monitored two properties: the moisture and titratable acidity of the pulp, showed in Table 3 and 4.

Table 3 - Moisture (%) in Macauba fruit.

		Husk		Pulp	
		0 day	30 day	0 day	30 day
1st harvest	Lot 1	46.27± 1.76 a	29.99 ± 0.61 b	47.40 ± 0.54 a	27.54± 2.01 b
	Lot 2	45.93 ± 0.27 a	25.13 ± 0.26 b	46.20 ± 0.66 a	20.66 ± 0.45 b
	Lot 3	30.01 ± 1.80 a	21.97 ± 0.86 b	38.27 ± 1.20 a	18.50 ± 0.83 b
2nd harvest	Lot 1	45.00 ± 0.49 a	13.12 ± 0.25 b	40.27 ± 0.47 a	7.02 ± 0.32 b
	Lot 2	44.57 ± 0.45 a	12.23 ± 0.43 b	42.15 ± 1.43 a	7.66 ± 0.20 b
	Lot 3	28.45 ± 9.46 a	11.57 ± 0.1 b	31.76 ± 1.38a	7.43 ± 0.23 b

Mean ± standard deviation.

In each part of the fruit, means on the same row followed by the different letter differ significantly by Tukey's test ($p < 0.05$).

Table 4 - Titratable acidity (%) in pulp of Macauba fruit.

		0 day	30 day	Increase (%)
1st harvest	Lot 1	0.99 ± 0.11 b	26.36 ± 0.77 a	2562.63
	Lot 2	0.87 ± 0.19 b	19.51 ± 1.01 a	2142.53
	Lot 3	1.20 ± 0.02 b	17.72 ± 0.52 a	1326.67
2nd harvest	Lot 1	0.49 ± 0.03 b	34.59 ± 1.11 a	6959.18
	Lot 2	0.42 ± 0.11 b	21.23 ± 1.51 a	4954.76
	Lot 3	0.93 ± 0.09 b	16.87 ± 1.07 a	1713.98

Mean ± standard deviation.

In each part of the fruit, means on the same row followed by the different letter differ significantly by Tukey's test ($p < 0.05$).

After 30 days was observed a reduction of the moisture content in all lots in the husk and pulp, with the lowest values in lot 3. Despite the husk moisture content also to have decreased after 30 days, in all cases its final moisture content was greater than of the pulp. The titratable acidity of the pulp increased considerably in all cases after 30 days. It was observed that lot 3 showed the lowest increase in acidity, followed by lot 2. The fruits that were not undergone any treatment post-harvest (lot 1) despite having a lower initial acidity presented the highest acidity increases.

In order to evaluate the influence of the parameters moisture content and acidity on the microorganism growing, Table 5 presents the results of the Pearson analysis. To evaluate the significance of the correlations was performed hypothesis test (Ho: correlation is not significant and Ha: correlation is significant) using the Student's t table for comparison with the value of the test statistic calculated. In both harvests, there was only significant correlation between pulp acidity and amount of fungi, suggesting that the increase of pulp acidity favors the growth of fungi.

Table 5 - Pearson correlation between parameters of the pulp and husk and quantity of bacteria, fungi and yeasts.

	1 st harvest	2 nd harvest
Pulp moisture x bacteria	0.81	0.24 ns
Pulp moisture x fungi	-0.72 ns	-0.75 ns
Pulp moisture x yeast	0.41 ns	0.26 ns
Pulp acidity x bacteria	-0.63 ns	-0.39 ns
Pulp acidity x fungi	0.96	0.93
Pulp acidity x yeast	-0.37 ns	-0.39 ns
Husk moisture x bacteria	-0.02 ns	0.19 ns
Husk moisture x fungi	0.11 ns	-0.37 ns
Husk moisture x yeast	0.62 ns	0.53 ns

ns – not significant a 5%.

4. CONCLUSION

The results of this work indicates that after harvest, regardless of the treatment used, in both the husk and in the pulp was found major presence of bacteria and yeasts. After the storage, the yeasts and bacteria predominated in the husk, while the pulp showed greater contamination by the fungi.

It is important to highlight that the pulp is the most important raw material for industrial process application. The treatments applied in this work were not effective for non-proliferation of microorganisms, in the Macauba fruit in the 30 day storage period, especially considering the fungi growing in the pulp.

This work directs for some guidance when the objective is the preservation of the fruits of contamination by microorganisms. The first is the almost immediate removal of the fruit from the ground to another environment, may be search for places which minimize the microorganisms growth. The second is to perform the processing as soon as possible after harvest.

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MICRORGANISMOS EM FRUTOS DE MACAÚBA (*ACROCOMIA ACULEATA*) APÓS DIFERENTES TRATAMENTOS PÓS-COLHEITA E ARMAZENAMENTO

RESUMO: Os frutos da macaúba (*Acrocomia aculeata*) podem ser promissora fonte de óleo vegetal para fins alimentícios, cosméticos e energia, devido à elevada produtividade e a obtenção de dois tipos de óleo. Contudo, a principal restrição está associada com a rápida deterioração do fruto, levando à produção de um óleo com elevada acidez. O processo de acidificação pode ser causado pelos microrganismos que crescem associados aos frutos, pela alta umidade e pela atividade endógena de lipases na polpa. Este estudo teve como objetivo quantificar microrganismos presentes na polpa e casca após diferentes tratamentos pós-colheita e após 30 dias de armazenamento, comparando a eficácia de diferentes tratamentos por meio de técnicas simples e baratas. O período de armazenamento foi determinado de acordo com testes preliminares, onde se observou que com 30 dias de colheita os frutos já estavam se deteriorando. Após o armazenamento houve predominância de fungos na polpa e de bactérias e leveduras na casca.

PALAVRAS-CHAVE: *Acrocomia aculeata*; Macauba; Microrganismos; conservação pós-colheita.