

WOGAYEHU WORKU TILAHUN

**POSTHARVEST TREATMENTS OF MACAÚBA PALM (*Acrocomia aculeata*)  
FRUIT: STORAGE PERIOD, GAMMA RADIATION AND DRYING  
TEMPERATURE**

Thesis submitted to the Universidade Federal de Viçosa, as part of the requirements of the Graduate Program in Plant Sciences, to obtain the title of *Doctor Scientiae*.

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## **DEDICATION**

To my dear wife W/ro Atsede Tilahun and to my sweet sons Henok and Natnael for their affection, patience and compassion during the study periods.

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## **BIOGRAPHY**

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He then employed in the Ethiopian Institute of Agricultural Research in April 2006 as weed science researcher and served there until he joined the Graduate Program in the Department of Plant Sciences, Federal University of Viçosa, in August 2011. He submitted his thesis for defense in July 2015.

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## LIST OF ABBREVIATIONS AND SYMBOLS

AI:	Acidity Index
AOCS:	American Oil Chemists' Society
$a_w$ :	Water activity
CDTN:	Centro de Desenvolvimento da Tecnologia Nuclear (Nuclear Technology Development Center)
CETEC:	Fundação Centro Tecnológico de Minas Gerais (Technological Foundation Center of Minas Gerais)
DI:	Damage Index
$E_{a_w}$ :	Exocarp water activity
FFA:	Free Fatty Acids
g:	gram
IAL:	Instituto Adolfo Lutz (Adolfo Lutz Institute)
ICGFI:	International Consultative Group on Food Radiation
IUPAC:	International Union of Pure and Applied Chemistry
KGy:	Kilo Gray
$MA_{232}$ and $MA_{270}$ :	Molar absorptivity at 232 nm and 270 nm
$Ma_w$ :	Mesocarp water activity
MC:	Mesocarp Color
MLFF:	Moisture Loss in Fresh Fruit
MMC:	Mesocarp Moisture Content
MOC:	Mesocarp Oil Content
OS:	Oxidative Stability
PI:	Peroxide Index
POD:	Peroxidase
PORIM:	Palm Oil Research Institute of Malaysia
RDC/ANVISA:	Agência Nacional de Vigilância Sanitária. Resolução Colegiada da Diretoria (National Agency of Sanitary Surveillance /Board Resolution Collegiate)
SAL:	Specific Activity of Lipase
SAP:	Specific Activity of Peroxidase

SAS: ..... Statistical Analysis Software

TAG: ..... Triacylglycerol

TCC: ..... Total Carotene Content

U/mg: ..... Unit per milligram

UFMG: ..... Federal University of Minas Gerais

UFV: ..... Universidade Federal de Viçosa (Federal University of Viçosa)

WHO: ..... World Health Organization

$\beta$ : ..... Beta

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## ABSTRACT

TILAHUN, Wogayehu Worku, D.Sc. Universidade Federal de Viçosa, August, 2015. **Postharvest treatments of macaúba palm (*Acrocomia aculeata*) fruit: storage period, gamma radiation and drying temperature.** Adviser: José Antonio Saraiva Grossi. Co-advisers: Simone Palma Favaro and Carlos Siqueyuki Sedyama.

Macaúba is a multipurpose oleaginous palm distributed in tropical and subtropical America with high biomass productivity and high oil content. It is a promising vegetable oil that can be used either as a source of edible or raw material in the biodiesel industry. However, random and bulk harvest within few months of the year coupled with rudimentary processing technology is becoming a bottleneck to exploit the potential benefit of this fruit. Therefore, storage and maintenance of postharvest oil quality is a major challenge in the production chain of macaúba. Development of postharvest technology packages fills some of the gaps in the current scenario. This fruit is endowed with high water content that impairs the mesocarp oil quality. Moreover, harvesting is synchronized with hot and rainy season of the year that enhances hydrolysis and oxidation processes. This can be facilitated by the endogenous lipase or microorganisms growing in association that boost hydrolysis of triacylglycerol to free fatty acids. Moreover, the presence of peroxidase favored oil oxidation. Accordingly, three successive and independent experiments concerned to postharvest of macaúba fruits were conducted. The first experiment was aimed at assessing parameters related to oil quality, where ripe macaúba fruits were kept at room temperature for 0, 3, 6, 9, 12, 15, 20, 25, 30, 45 and 60 days. Hydrolysis and oxidation reactions were analyzed by biochemical activities (specific activity of lipase), physico-chemical properties of the mesocarp (water activity, moisture loss of fresh fruit, damage index) and physico-chemical properties of mesocarp crude oil (acidity index, peroxide index, molar absorptivity at 232 nm and 270 nm, total carotene content and oxidative stability) of the stored macaúba fruit. Increasing acidity and loss of oil stability along the storage was accompanied by reduction of moisture and increase in fruit decay. Lipase might not be related to increased acidification process. The stored crude mesocarp oil had oxidative stability of 31 days as per biodiesel standard. However, the overall quality of the oil maintained within the required standards up to 20 days. The second experiment was conducted to study the effect of gamma radiation in the mesocarp oil quality of macaúba fruit along the storage. Ripe fruits were treated with 3 gamma radiation doses (0, 4 and

8 KGy), stored at room conditions, and analyzed after 0, 10, 20 and 30 days. Mesocarp and exocarp water activity, mesocarp moisture and oil content, specific activity of lipase and peroxidase, and other physico-chemical properties of the crude mesocarp oil were studied. Gamma radiation decreased accumulation of oil as compared to control. The 8 KGy gamma dose resulted in oil oxidation. In general, decreased enzymatic activities and best oil quality was obtained with 4 KGy gamma dose along the 30 days of storage. The third experiment assessed the effect of drying temperature upon biochemical activity and physical-chemical properties of stored macaúba fruits. Ripe fruits, stored at room conditions, were dried with three drying temperatures (control, 60°C and 100°C) for 24 hours at four storage periods (0, 10, 20 and 30 days). Drying decreased water activities followed by enzymatic activities. Lipase was less stable to drying than peroxidase. Most oil quality indices, except acidity, were favored by drying after some time of storage. Macaúba fruits, dried at 100°C after 30 days stored, showed reduced enzymatic activities, increased oil content and suitable mesocarp oil quality. In general, direct fruit harvest from the plant conserved the desirable mesocarp oil quality of macaúba to be used as a raw material either in biodiesel or food industries with comparative advantage like oil palm. Macaúba fruits stored at ambient condition maintained the postharvest oil quality of the mesocarp for 20 days. This range extend to 30 days in 4 KGy gamma dose and for fruits dried after 30 day at 100°C with desirable mesocarp oil quality in storage. Despite the installation cost, these technological packages could be amplified either in subsistence or in large scale production schemes.

## RESUMO

TILAHUN, Wogayehu Worku, D.S., Universidade Federal de Viçosa, agosto de 2015. **Tratamentos pós-colheita do fruto da palmeira macaúba (*Acrocomia aculeata*): período de armazenamento, a radiação gama e temperatura de secagem.** Orientador: José Antonio Saraiva Grossi. Coorientadores: Simone Palma Favaro e Carlos Sigueyuki Sedyama.

Macaúba é uma palmeira oleaginosa com múltiplos usos distribuído na América tropical e sub tropical com elevada produtividade de biomassa e alto teor de óleo. É um óleo vegetal promissor para ser usado como óleo comestível e matéria-prima na indústria de biodiesel e biohidrocarbonetos. No entanto, a produção concentrada de frutos durante 4 meses do ano, a colheita aleatória e processamento rudimentar é um grande desafio para explorar o potencial desta planta. A ausência de tecnologia que mantenha a qualidade pós-colheita do óleo é um gargalo na cadeia produtiva da macaúba. Desenvolvimento do pacotes de tecnologia pós-colheita preenche algumas das lacunas no cenário atual. Elevado teor de água no fruto favorece degradação de qualidade do óleo do mesocarpo. Além disso, a colheita está sincronizada com a estação quente e chuvoso do ano que aumenta os processos de hidrólise e oxidação. Isso pode ser facilitado pela lipase endógena ou microrganismos que crescem em associação que aumentam a hidrólise de triacilgliceróis para ácidos graxos livres. Além disso, a presença de peroxidase favorecida o oxidação do óleo. Por isso, três experimentos independentes com foco de pós-colheita de frutos do macaúba foram realizados. O objetivo do primeiro experimento foi para avaliar os parâmetros relacionados à qualidade do óleo, onde os frutos maduros dos macaúba foram mantidos sob temperatura ambiente para 0, 3, 6, 9, 12, 15, 20, 25, 30, 45 e 60 dias. Reações de hidrólise e de oxidação foram determinada pela atividades bioquímica de fruto armazenado e propriedades físico-químicas do óleo bruto do mesocarpo. Atividades bioquímicas (atividade específica de lipase), as propriedades físico-químicas do mesocarpo (atividade da água, perda de umidade do fruto fresco, índice de dano) e propriedades físico-químicas do óleo bruto do mesocarpo (índices de acidez e peróxido, absorvibilidade molar a 232 nm e 270 nm, conteúdo de carotenóides totais e estabilidade oxidativa) foram estudaram. A perda de umidade e apodrecimento de fruto resultou aumenta de acidez e perda de estabilidade de óleo em armazenamento embora lipase não pode estar relacionado ao aumento processo de acidificação. O óleo bruto do mesocarpo armazenado tinha estabilidade oxidativa de 31 dias, como por padrão biodiesel. No entanto, a qualidade do óleo foi mantido dentro dos



padrões exigidos até 20 dias à temperatura ambiente. O segundo experimento foi conduzido para estudar o efeito da irradiação gama sobre a qualidade do óleo do mesocarpo durante o armazenamento dos frutos. Os frutos maduros foram tratados com 3 doses de radiação gama (0, 4 e 8 kGy), armazenados em condições ambiente, e analisados após 0, 10, 20 e 30 dias. Atividade de água no mesocarpo e epicarpo, conteúdo de umidade e teor do óleo do mesocarpo, atividade específica de lipase e peroxidase, as propriedades físico-químicas do óleo do mesocarpo foram estudado. A irradiação gama diminuiu o acúmulo de teor de óleo em comparação com o controle. A dose 8 KGy resultou na oxidação do óleo. Em geral, a 4 KGy dose diminuiu as atividades enzimáticas e mantém a qualidade do óleo durante 30 dias do armazenamento. O terceiro experimento foi conduzido para estudar o efeito da temperatura de secagem sobre a atividade bioquímica do fruto e propriedades físico-químicas do óleo bruto do mesocarpo durante o armazenamento. Os frutos maduros foram secos em três temperaturas de secagem (controle, 60° C e 100° C), em quatro períodos de armazenamento (0, 10, 20 e 30) para 24 horas, mantidos sob condições ambiente. Secagem diminuiu atividades de água juntos com atividades enzimáticas. A lipase foi menos estável do que peroxidase em secagem. A maioria dos índices de qualidade de óleo, exceto acidez foram favorecidos por secagem ao longo de armazenamento. A secagem do fruto da macaúba a 100°C, após 30 dias de armazenamento, mantém a qualidade e aumenta o teor de óleo do mesocarpo. Em geral, colheita dos frutos direta da planta conservada a qualidade do óleo do mesocarpo de macaúba desejável para ser usado como uma matéria-prima tanto no biodiesel ou indústrias alimentares com vantagem comparativa, como óleo de palma. Frutos de macaúba armazenados em condição ambiente manteve a qualidade pós-colheita do óleo do mesocarpo durante 20 dias. Na verdade, esta faixa aumentou para 30 dias, para a dose 4 KGy e para frutos secos após 30 dias a 100°C com boa qualidade do óleo do mesocarpo em armazenamento. Apesar do custo de instalação, pacotes tecnológicos poderia ser ampliado tanto na subsistência ou em produção de larga escala.

## 1. GENERAL INTRODUCTION

Energy is the driving force in our planet, though there is disparity in consumption between wealthy and poor countries (Grevé et al., 2011). The alarming population pressure in our globe is becoming imbalance with the ever increasing energy demand (Andrade-Tacca et al., 2014). This demand coupled with increasing oil prices, dwindling fossil reserves and issues of climate change scenario (Atabani et al., 2014; Andrade-Tacca et al., 2014; Singh et al., 2015). The petroleum price fluctuation is becoming not only an economic concern, but also is a political issue, where both petroleum producing and importing countries share the burden though in different magnitude. Therefore, looking for other alternative energy sources such as biofuels is a critical issue of this time. For instance, biodiesel that comes from vegetable oil processing is already a reality in the energy grids of many countries, and its broader production and consume depend on the availability and cost of the vegetable oils sources used as raw material.

A vegetable oil is a triglyceride extracted from plants (Voelker, 2011). Triacylglycerol is the chemical form in which oil is stored in almost all plant species (Ohlrogge and Browse, 1995). Globally, they are harvested from a few oil crops (Voelker, 2011) such as oil palm (35%), soybean (27%), rapeseed (16%) and sunflower (9%), (Poetsch et al., 2012). Most oils are used for human or animal consumption, although a minor fraction is derivatized to oleo chemicals (Voelker, 2011). Recently, vegetable oil is being diverted to the production of biodiesel (Durrett et al., 2008). Biodiesel is a renewable and sustainable source of energy that can satisfy energy security and reduce oil dependency in many countries (Andrade-Tacca et al., 2014; Singh et al., 2015). Nevertheless, factors such as geography, climate, and economics determine which vegetable oil is of greatest interest for potential use in biodiesel fuels (Knothe et al., 2005). That is why soybean oil is considered to be a prime feedstock in the United States, Brazil and Argentina; rapeseed (canola) oil in Europe; and palm oil in other tropical countries. However, satisfying energy demand in the expense of food security is a huge concern at this time (Grevé et al., 2011). Because food and non-food industries compete for limited crop options which are basically targeted for food production, so that introducing potential oleaginous plant with high energy density such as macaúba to non-edible industries like biofuel seems a sound solution (Pires et al., 2013).

Palms are the most abundant, wealthy and diverse plant families in the tropics occupying almost all habitats (Coimbra and Jorge, 2011). Macaúba or macaw palm is a single stemmed spiny palm widely distributed in sub-tropical or tropical America (Janick and Paull, 2008) that often grows on poor soils with remarkable tolerance to drought (Wandeck and Justo, 1982). It is a multipurpose palm that can be processed to provide food, medicine, feed, fiber and raw material source in biodiesel and cosmetics industry (Lorenzi and Negrelle, 2006; Poetsch et al., 2012). It has also a high potential to sequester carbon to the surroundings (Lanes et al., 2014) that might be an added advantage in the current climate change scenario. Macaúba has being assumed as a very high productive oleaginous source next to oil palm (Moura *et al.*, 2010) with a productivity potential of more than 20 metric tons (MT) fruit/ha/year and 6.2 ton oil /ha/year (Wandek and Justos, 1982), so that it became a candidate as a raw material source in biodiesel industry. Figure 1 shows the potential biomass productivity of this promising oleaginous plant. In line with the Brazilian National Biodiesel Production Program, the regional state of Minas Gerais enacted the legal framework to use macaúba as a renewable raw material source for biodiesel production in 2011 (Azevedo Filho et al., 2012).

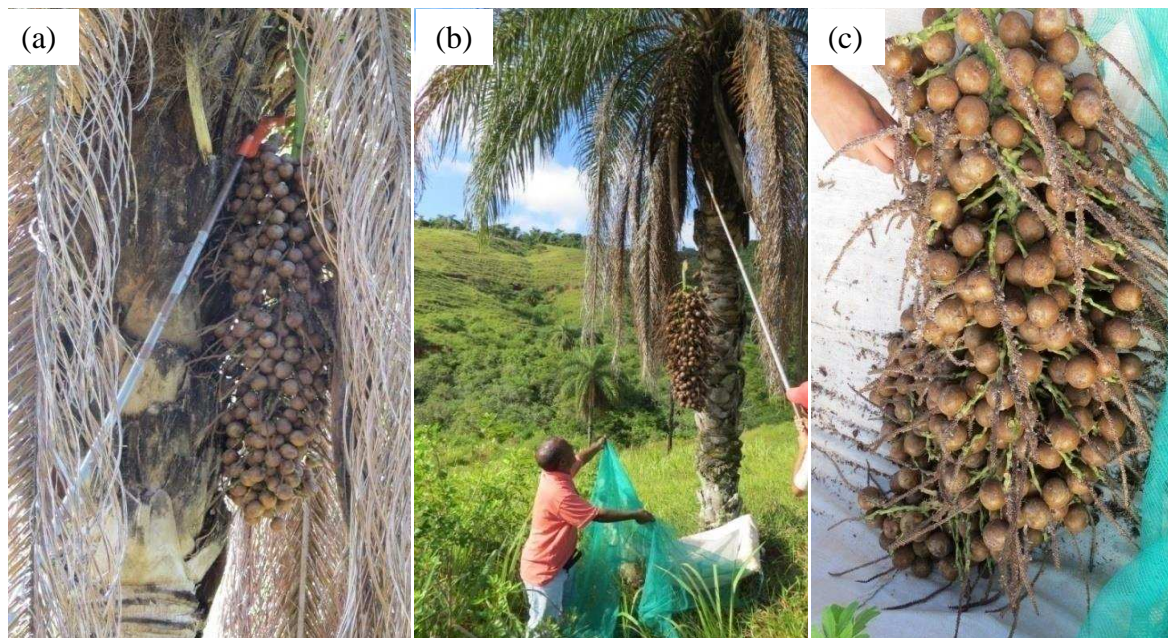


Figure 1 a. Harvesting macaúba bunch with sickle fitted cutter, b. Collecting cut macaúba bunch on spongy surfaces, c. Picking macaúba fruits from cut bunches at Acaiaca, Minas Gerais, Brazil (Source: W.W. Tilahun, 2014).

The program planned to minimize the socioeconomic disparity between regional states through income generation and employment opportunity for resource poor farmers. Currently, the Minas Gerais state in Brazil drafted a policy to use macaúba oil in the production of biokerosene (Lanes et al., 2014).

Macaúba mesocarp contains around 70% of the total oil content in the whole fruit (CETEC, 1983), and bears high water content, more than 60% in the ripe fruit, that favors degradation of the oil quality by hydrolysis. The amount of free fatty acid (FFA, acidity index) is a major quality parameter for vegetable oil processing (Pahoja and Sethar, 2002; Mariano et al., 2011). The free fatty acids are released due to the hydrolysis of the triacylglycerols catalyzed by lipases of its own fruit or coming from the associated microorganisms. Besides hydrolysis, oxidation reactions can compromise the oil quality. Several biochemical and physico-chemical parameters are used to measure changes in stored oleaginous plants. Besides, FAA, peroxides and hydroperoxies formed, and oxidative stability (OS) explained by induction time are some of the parameters considered. FAA and peroxides determined by titration while OS is determined by Rancimat apparatus in laboratory. OS is the resistance of oil to oxidation in exposure to heat and oxygen. The higher the OS value the better the oil either in food or biodiesel sectors.

The large scale biodiesel production requires standards for vegetable oils to assure the quality of the biofuel. Then, macaúba oil chain supply must develop practices to overcome the natural decay characteristics and provide high quality oil to be part of the biofuel industry. Hydrolysis and oxidation are the two basic processes that result in the deterioration of oils and fats (List et al., 2005). Recently, developing low lipase line in crop breeding is a suggested solution. A wide adoption of an elite low-lipase line in palm oil yielded an economic gain of up to one billion dollars per year for African smallholders (Morcillo et al., 2012). Moreover, the added value of products and coproducts in macaúba might be considered for the cost effectiveness of its value chain. Pires et al. (2013) reported that the economic feasibility of macaúba relays on its high oil productivity and integral use of the fruit to generate products and co-products upon processing. Studies in Paraguay showed that the sum of the heating values from the components from the industrialization of the fruits, it obtains 380.000 MJ/ha a value only comparable with the oil palm and sugar cane (Oberlaender and Bohn, info@acromiasolutions.com). At present market prices, production of macaúba oil in

silvo-pastoral systems is economically feasible while creating incomes above minimum wages with sustainable land use change (Averdunk et al., 2013).

Postharvest handling is an age old activity and it began soon after the start of agriculture. The transition from hunting-gathering society to sedentary agriculture leads to the start of storing surplus agricultural products which were a prerequisite for the development of complex technology and social stratification (Diamond and Bellwood, 2003). Under modern society, the philosophy of postharvest is by far complex to satisfy the basic and aesthetic values of human being. If suitable storage conditions are not employed for specific product varieties, qualitative and quantitative losses increase (Terigar et al., 2009).

Water plays a key role in physical, chemical and biochemical reactions of living things. The high water content impact in macaúba is therefore managed or at least reduced with proper postharvest management practices. Water content and water activity affect the physical, biochemical, and associated micro-organisms development in stored agricultural products. Water activity is the measure of free water in a system (Sethi, 2007) that could be more important to the quality and stability of food than the total amount of water present (Maltini et al., 2003). Some postharvest treatments such as refrigeration of fruits and vegetables (Kader et al., 1989), drying and radiation, modified and controlled atmosphere are used to keep the quality of agricultural products by decreasing humidity and deactivating enzymes. Nevertheless, the technological advancement and the economic feasibility of postharvest handling practices vary from place to place and country to country.

Irradiation is a physical treatment in which food is exposed to a defined dose of ionizing radiation (Arvanitoyannis and Tserkezou, 2010). Application of low gamma dose reduces postharvest losses caused by sprouting in potato and onion, extend shelf life by delaying maturity, control spoilage by microorganisms, disinfestations of insects in grains (Loaharanu, 1994). Depending on the size of operation, total operating costs for food radiation varies from 0.9 to 3.2 per cent of the value of the product for sprout inhibition in potatoes and onions, from 0.19 to 1.24 per cent for grain disinfestations and from 0.2 to 2.0 per cent for the prolongation of shelf life of fish (Lamm, IAEA Bulletin-Vol 21, No.2/3, p.34.). However, depending on the availability of oxygen and type of commodity/product, gamma radiation reduces the vitamin content of food such as C, E, and  $\beta$ -carotene (Aquino, 2012). Besides, storage, dose and time of exposure are

possible reasons of vitamin reductions. International Atomic Energy Agency claimed that for food and health safeness, radiation would require doses of less than 10 KGy (WHO, 1999). In a high water content oleaginous plant like macaúba, radiolysis of water during gamma radiation generates free radicals that can react with the polyunsaturated fatty acids. However, the positive effect of gamma radiation can be exploited with proper application in postharvest management of agricultural products.

Drying is one of the oldest methods of food preservation practice. During drying, heat is transferred from the hot air to the product and evaporated water is transported to the air (Nowak and Lewicki, 2004). In oleaginous fruit like macaúba, optimum drying temperature favor the reduction of water content and enzymatic activity and thereby prolong shelf life in storage. Moreover, it facilitates oil extraction during storage and processing. However, temperature has a vital role during drying process and should be accompanied with air circulation (PNW 397, 2009).

The bulk harvest of macaúba lasts a few months of the year. However, random collection of fruits and rudimentary oil extraction technology decrease the potential exploitation of this plant. Understanding the effect of storage periods on hydrolysis and oxidation process might help to devise proper postharvest management practices. However, there is no established gamma radiation dose and drying temperature that extends the shelf life in macaúba fruits and preserve its mesocarp oil quality in storage. That is why; gamma radiation and oven drying treatments were addressed to minimize spoilage and improve oil quality at storage in this study.

The potential of gamma radiation and drying temperature in postharvest management was explored briefly in the aforementioned discussion. However, there is no adequate and detail information on the cons and pros of these postharvest treatments on the physical, biochemical activities, and physico-chemical properties of the fruit and its mesocarp oil. Therefore, the general objective of this study was to determine the effect of postharvest treatments on the mesocarp oil quality of stored macaúba fruit and suggest possible technological recommendations. Accordingly, three independent experiments were conducted: (1) to measure physical analysis in the mesocarp (water activity, moisture loss in fresh fruit and damage index), (2) chemical analysis in the mesocarp (moisture and oil content), (3) biochemical analysis of the mesocarp (crude specific activity of lipase and peroxidase in the mesocarp, and (4) physico-chemical

analysis of the mesocarp oil (acidity and peroxide index, molar absorptivity at 232 nm and 270 nm, total carotene content, and oxidative stability).

## 1.1. REFERENCES

Andrade-Tacca, C.A., Chang, C-C.; Chen, Y-H.; Ji, D-R.; Wang, Y-Y. Yen, Y-Q.; Chang, C-Y. 2014. Reduction of FFA in *Jatropha curcas* oil via sequential direct-ultrasonic radiation and dosage of methanol/sulfuric acid catalyst mixture on esterification process. **Energy Conversion and Management** 88:1078–1085.

Arvanitoyannis, I.S; Tserkezou, P. 2010. Legislation on Food Radiation in European Union, United States, Canada, and Australia. In: Arvanitoyannis, I.S (Ed.). Radiation of Food Commodities: Techniques, Applications, Detection, Legislation, Safety and Consumer Opinion, U.S.A.

Atabani, A.E.; Mofijur, M.; Masjuki, H.H.; Badruddin, I.A.; W.T.; Cheng, S.F.; Gouk, S.W. 2014. A study of production and characterization of Manketti (*Ricinodendron rautonemii*) methyl ester and its blends as a potential biodiesel feedstock. **Biofuel Research Journal** 4:139-146.

Aquino, K.A.S. 2012. Sterilization by Gamma Radiation. Adrovic, F. (Ed.). ISBN: 978-953-51-0316-5, InTech. Available from: <http://www.intechopen.com/books/gamma-radiation-sterilization-by-gamma-radiation>. Retrieved on 22 July 2014.

Averdunk, K.; Zelt, T.; Golka, P.; Höpfner, M.; Müller, C. and Bettermann, I. 2013. Macaúba – Sustainable Palm Oil Results of the Feasibility Study of the Leuphana University of Lüneburg- Executive Summary. [www.leuphana.de/Summary-Macauba-Feasibility-Study](http://www.leuphana.de/Summary-Macauba-Feasibility-Study). Lüneburg University, June 26, 2013.

Azevedo Filho, J.A.; Colombo, C.A; Berton, L.H.C. 2012. Macaúba: palmeira nativa como opção bioenergética. **Pesquisa e Tecnologia** 9 (2), disponível em [www.aptaregional.sp.gov.br](http://www.aptaregional.sp.gov.br).

CETEC (Fundação Centro Tecnológico de Minas Gerais). 1983. Produção de combustíveis líquidos a partir de óleos vegetais: relatório final do Convênio STI- MIC / CETEC. Vol. 1 e 2. CETEC. Belo Horizonte.

Coimbra, M.C.; Jorge, N. 2011. Proximate composition of guariroba (*Syagrus oleracea*), jervá (*Syagrus romanzoffiana*) and macaúba (*Acrocomia aculeata*) palm fruits. **Food Research International** 44: 2139–2142.

Diamond, J.; Bellwood, P. 2003. Farmers and Their Languages: The First Expansions. **Science** 300: 597-603.

Durrett, T.P.; Benning, C.; Ohlrogge, J. 2008. Plant triacylglycerols as feedstocks for the production of biofuels. **Plant Journal** 54:593–607.

Grevé, A.; Barbanti, L.; Fazio, S. 2011. Handbook on biofuels and family agriculture in developing countries. Pàtron Editore, Bologna.

Janick, J.; Paull, R.E. 2008. The Encyclopedia of fruits and nuts. CABI, UK.

Kader, A.A., Zagoy, D.; Kerbel, E.L. 1989. Modified atmosphere packaging of fruits and vegetables. **Critical reviews in food science and nutrition** 28:1-30.

Lanes, É.C.M.; Costa, P.M.A; Motoike, S.Y. 2014. Brazil promotes aviation biofuels. Correspondence, Macmillan Publishers Limited, 3 July 2014, Vol. 511, **Nature** 31.

Lamm, C.G. Applications of Isotopes and Radiation in Agriculture, IAEA Bulletin-Vol 21, No.2/3.P.34. [http://www.iaea.org/Publications/Magazines/Bull212\\_3/212\\_305482935.pdf](http://www.iaea.org/Publications/Magazines/Bull212_3/212_305482935.pdf).

List, G.R.; Wang, T; Shukla, V.K.S. 2005. Storage, handling, and transport of oils and fats. Bailey's Industrial Oil and Fat Products, Sixth Edition, Six Volume Set. John Wiley and Sons, Inc.

Loaharanu, P. 1994. Food radiation in developing countries: A practical alternative. **IAEA Bulletin** :1/1994.

Lorenzi, G. M. A. C.; Negrelle, R. R. B. 2006. *Acrocomia aculeata* (Jacq.) Lodd. EX Mart.: Ecological aspects, uses and potentialities. Available from: <http://www.entabnabrasil.com.br>. Retrieved on 21/04/2014.

Maltini, E.; Torreggiani, D.; Vieira, E.; Bertolo, G. 2003. Water activity and the preservation of plant foods. **Food Chemistry** 82: 79-86.

Mariano, R.G.B.; Silva, C.M.; Couri, S.; Nogueira, R.I.; Freitas, S.P. 2011. Partition of Free Fatty Acids in Deacidification of Macaúba Pulp Oil by Liquid-Liquid Extraction using Ethanol/Water as Solvent. **Defect and Diffusion Forum** 312-315: 554-559.

Morcillo, F.; Cros, D.; Billotte, N.; Ngando-Ebongue, G.-F.; Domonhédou, H.; Pizot, M.; Cuéllar, T.; Espéout, S.; Dhouib, R.; Bourgis, F.; Claverol, S.; Tranbarger, T.J.; Nouy, B.; Arondel, V. 2012. Improving palm oil quality through identification and mapping of the lipase gene causing oil deterioration. DOI: 10.1038/ncomms 3160 /[www.nature.com/naturecommunications](http://www.nature.com/naturecommunications).

Moura, E.F.; Ventrella, M.C.; Motoike, S.Y. 2010. Anatomy, histochemistry and ultra structure of seed and somatic embryo of *Acrocomia aculeata* (Arecaceae). **Agricultural Sciences**, Piracicaba, 8: 399-407.

Nowak, D.; Lewicki, P.P. 2004. Infrared drying of apple slices. **Innovative Food Science and Emerging Technologies** 5: 353–360.

Oberlaender, D.; Bohn, E. [info@acrocomiasolutions.com](mailto:info@acrocomiasolutions.com). *Acrocomia aculeata*. Its potential cultivation as a multi purpose crop.



- Ohlrogge, J.; Browse, J. 1995. Lipid biosynthesis. **The Plant Cell** 7: 957-970.
- Pahoja, V.M.; Sethar, M.A. 2002. A review of enzymatic properties of lipases in plants, animals and microorganisms. **Pakistan Journal of Applied Sciences** 2 (4): 474-484.
- Pires, T.P.; Sousa, E.S.; Kuki, K.N.; Motoike, S.Y. 2013. Echophysiological traits of the macaw palm: A contribution towards the domestication of a novel oil crops. **Industrial Crops and Products** 44:200-210.
- PNW 397 (Pacific Northwest Extension Publication). 2009. Drying fruits and vegetables. Third edition. University of Idaho, USA.
- Poetsch, J., Hauptenthal, D.; Lewandowski, I.; Oberländer, D.; Hilger, T. 2012. *Acrocomia aculeata* – a sustainable oil crop. Rural 21 – 03/2012. **Science World** 41-44.
- Sethi, S. 2007. Horticulture postharvest technology: principles of food processing. Division of postharvest technology, Indian Agricultural Research Institute, New Delhi.
- Singh, B.; Guldhe, A., Rawat, I.; Bux, F. 2015. Towards a sustainable approach for development of biodiesel from plant and microalgae. **Renewable and Sustainable Energy Reviews** 29: 216–245.
- Terigar, B. G. Advanced microwave technology for biodiesel feedstock processing. M.Sc thesis, Louisiana State University, USA, 2009.
- Voelker, T. 2011. Secrets of palm oil biosynthesis revealed. [www.pnas.org/cgi/doi/10.1073/pnas.1109296108](http://www.pnas.org/cgi/doi/10.1073/pnas.1109296108).
- Wandeck, F.A.; Justo, P.G. 1982. Macaúba, fonte energetica e insumo industrial. Proceeding of the conference Vida Industrial. Sao Paulo, Brazil, p. 33-37.
- WHO (World Health Organization). 1999. High-dose radiation: wholesomeness of food irradiated with doses above 10 KGy. **WHO Technical Report Series 890**, Report of a Joint FAO/IAEA/WHO Study Group, World Health Organization, Geneva.

## CHAPTER 1

### STORAGE PERIOD EFFECTS ON THE MESOCARP OIL QUALITY OF MACAÚBA FRUIT

#### ABSTRACT

Macaúba palm tree has immense potential as a source of raw material for biofuel production, mostly the mesocarp oil, whereby quality can be affected by hydrolysis and oxidation reactions, in storage and processing. Understanding the postharvest biochemistry of lipids has paramount importance to drive technologies that can assure good standard oil. This work aimed to study parameters related to oil quality during storage. The experiment was conducted in a completely randomized design with 5 replications per treatment. The treatments were the 11 storage periods (0, 3, 6, 9, 12, 15, 20, 25, 30, 45, 60 days). Hydrolysis and oxidation reactions were investigated by biochemical activity (specific activity of lipase), physico-chemical properties of the mesocarp (water activity, moisture loss of fresh fruit, damage index) and physico-chemical properties of mesocarp crude oil (acidity index, peroxide index, molar absorptivity at 232 nm and 270 nm, total carotene content and oxidative stability) of the stored macaúba fruit. Moisture loss of fresh fruit was followed by fruit decay, change in mesocarp color and increased acidity after 20 days of storage. Specific activity of lipase correlated positively (0.74) with water activity. However, acidity seems not related to lipase. The peroxide index correlated negatively (-0.92) with oxidative stability. The oxidative stability was in line with the biodiesel standard for 31 days of storage. However, the overall quality of the oil was kept within the required standards upto 20 days in this study. This work confirmed that the mesocarp oil quality of macaúba could be achieved in both subsistence and large scale production with affordable technological package in the production chain of macaúba.

**Keywords:** *Acrocomia aculeata*, lipase, lipid hydrolysis, lipid oxidation, storage time.

# EFEITO DE PERÍODOS DE ARMAZENAMENTO NA QUALIDADE DO ÓLEO DO MESOCARPO DE FRUTOS DE MACAÚBA

## RESUMO

Macaúba tem alto potencial para produção de biocombustíveis, principalmente o óleo do mesocarpo, o que a qualidade pode ser afetada por reações de hidrólise e oxidação em armazenamento e processamento. Para fornecer o conhecimento sobre a bioquímica pós-colheita dos lipídios é uma questão fundamental que impulsionar tecnologias para assegurar um bom padrão do óleo. Objetivo de trabalho foram os parâmetros relacionado à qualidade do óleo durante armazenamento. O experimento foi conduzido em delineamento inteiramente casualizado, com cinco repetições por tratamento. Os tratamentos foram 11 (0, 3, 6, 9, 12, 15, 20, 25, 30, 45, 60 dias) períodos de armazenamento. Reações de hidrólise e do oxidação foram investigadas por atividade bioquímica (atividade específica de lipase), as propriedades físico-químicas do mesocarpo (atividade de água, perda de umidade do fruto fresco, índice de dano) e propriedades físico-químicas do óleo bruto do mesocarpo (índice de acidez, peróxido índice, absorvibilidade molar a 232 nm e 270 nm, teor de carotenóides totais e estabilidade oxidativa) do fruto de macaúba armazenado. A perda de umidade do fruto fresco foi seguido por por decadência do fruto, mudança de cor do mesocarpo e aumento de acidez após 20 dias do armazenamento. Atividade específica de lipase positivamente correlacionada (0.74) com atividade de água. No entanto, a acidez não parece estar relacionada com lipase. O índice de peróxido correlacionada negativamente (-0.92) com estabilidade oxidativa. A estabilidade oxidativa foi em conforme com biodiesel por 31 dias. No entanto, a qualidade do óleo foi mantido dentro dos padrões exigidos até 20 dias neste estudo. Este trabalho confirmou que a qualidade do óleo do mesocarpo de macaúba poderia ser alcançado em ambos produtores pequenas e produção em larga escala com pacote do tecnológico acessível.

**Palavras-chave:** *Acrocomia aculeata*, lipase, hidrólise de lípidos, oxidação de lípidos, tempo do armazenamento.

## 1. INTRODUCTION

Macaúba [*Acrocomia aculeata* (Jacq.) Lodd.ex.Mart] is a single stemmed spiny palm widely distributed in tropical and subtropical America (Janick and Paull, 2008). It can be used for human food, medicine, animal feed, fiber, biodiesel and cosmetics industry (Lorenzi and Negrelle, 2006; Poetsch et al., 2012). It has been assumed as a very high productive oleaginous source next to oil palm (Moura *et al.*, 2010) with a productivity potential of more than 20 metric tons fruit/ha/year (Poetsch et al., 2012) and 6.2 ton oil /ha/year (Wandek and Justos, 1982).

Bulk harvest of macaúba lasts a few months of the year. Mesocarp (pulp) portion provides the majority of the macaúba fruit oil and it is featured by a high content of water, what challenges to store and to process the fruits. Macaúba fresh fruit presents itself high quality mesocarp oil, even when it is dried immediately after harvesting, if good practices of harvest and processing are applied (Nunes et al, 2015). Nevertheless, deployment of quality can be expected during storage depending on the conditions, due to hydrolytic and oxidative reactions upon lipids. The oil acidification may be caused by endogenous and microbial lipase that boosts hydrolysis of triacylglycerol delivering free fatty acids and other related compounds into oil (Macare and Hammond, 1985; Mohankumar et al., 1990; Lopes and Neto, 2011). Acidity is one of the vital quality indices in fats and oil processing, and the deacidification process has economic implications in the value chain (Bhosle and Subramanian, 2005; Mariano et al., 2011). Adoption of the current or ingenious technologies to keep the natural good quality of macaúba mesocarp oil, though total production costs can be risen, ought to assure added value to macaúba exploitation. Pires et al. (2013) reported that the economic feasibility of this palm tree relays on its high oil yield and whole usage of the fruit to provide added value of the products and co-products upon processing. Macaúba fruit can render directly high oleic and carotene content oil and rich fiber cake from pulp oil; high lauric oil and rich proteic cake from kernel; biomass from husk, and hardy endocarp to be taken as solid fuel. Moreover, other high added value bioproducts could be developed from macaúba compounds. The overall quality of macaúba fruits, will allow a broad usage of its products and co-products.

Moisture content and storage conditions, like time and temperature, are usually related to the quality of stored fruits and grains, as reported for black-ripe olives (Agar et al., 1998) and soybean (Acklin, 1998). For instance, olive oil quality can be better

preserved when fruits are stored at 5°C, than at room temperature (Nabil et al., 2012). Crude palm oil quality is degraded by processing equipment and storage time that suggests direct processing of the fruits with no storage (Zu et al., 2012).

In tropical humid countries, the impact of storage period on the quality of agricultural product might be pronounced. The current practice of macaúba fruits exploitation, based on recollection of wild dropped fruits, and extraction of oil from dried fruits, which were kept losing water naturally without reasonable storage conditions, has rendered unsuitable mesocarp oil for food or biofuel purposes. To overcome this scenario and bring macaúba to the agribusiness, it is demanded to develop whole technological packages to settle processing pathways. Questions like “oil should be extracted from fresh or dried pulp”, are still to be answered, and depends on the cost-effectiveness of the technical approaches. This work aims to provide knowledge about postharvest behavior of macaúba fruits that can be useful to build up a successful macaúba processing. There is a lack of information about storage time and macaúba mesocarp oil quality. Therefore, this work assessed macaúba mesocarp oil quality, based on physical, chemical and biochemical activity and lipid physico-chemical properties, of fresh fruits stored at room conditions and increasing storage periods and recommended appropriate storage time as a technological package.

## **2. MATERIAL AND METHODS**

### **2.1. Study site and sample preparation**

A laboratory experiment was conducted at Macaúba Postharvest Laboratory, Department of Plant Sciences, Federal University of Viçosa (UFV), from February 2012 to November 2012. Fruits were collected from bunches that were cut from previously selected wild macaúba trees in Capela farm, Acaiaca municipality, Minas Gerais State, Brazil. Acaiaca is located at 20° 45'36" S latitude and 44° 15' W longitude at an altitude of 481 m above sea level with a humid subtropical climate CWa (Köppen and Geiger, 1928). The fruits harvested at full maturity and sorted for uniform size, were stored for 0,3,6,9,12,15,20,25,30,45,60 days in mesh net bag at room temperature (23 ±1°C). Each bag contained 20 fruits and this was considered as one experimental unit. All chemicals used were of analytical grade.

## **2.2. Physical, chemical and biochemical analysis of the mesocarp**

Macaúba fruits were manually pulped and the mesocarp used for the following measurements:

### **2.2.1. Physical analysis of the mesocarp**

Mesocarp slices were cut into small pieces to measure water activity ( $M_{a_w}$ ) using Aqua Lab 4TE water activity meter (Decagon, Inc., USA) at an accuracy of  $\pm 0.003 a_w$  at 25°C. Moisture loss in fresh fruit was measured in terms of fresh weight loss of the fruit taking the average of 10 fruits per replication along the storage periods. The result was expressed as the percentage of weight loss.

Damage index (%) was evaluated taking into account the number of fruits that showed decay symptom on the outer part of the peeled mesocarp per total number of fruits in each replication. It was scored 1 for symptom presence and 0 for absence.

### **2.2.2. Biochemical analysis of the mesocarp**

#### **2.2.2.1. Crude enzyme extraction of lipase**

An aliquot of 1g of mesocarp was homogenized with 20 mL of 0.1M Tris buffer at pH 8.0 using an electric hand blender. The homogenate was filtered through cotton gauze and centrifuged at 6000 rpm (Excelsa™ II Centrifuge, Mod.206 BL, Brazil) for 10 minutes. The supernatant, called crude enzyme extract, was collected and stored at -20°C (Iaderoza and Baldini, 1991) for further analysis.

#### **2.2.2.2. Enzyme incubation and specific activity measurement**

Lipase activity was measured as per the protocol of Iaderoza and Baldini (1991) with modifications. A mixture of 1 mL of crude enzyme extract, 5 mL of triacetin emulsion (25% triacetin/75% of 7% gum Arabic solution) and 5 mL of 0.1M Tris HCl buffer at pH 8.0, was incubated in water bath at 27°C/30 min. The reaction was stopped by adding 20 mL acetone: ethanol solution (1:1). The mixture, was added with 5 drops of 0.05% phenolphthalein, and titrated against 0.05N NaOH. The soluble protein content of the crude enzyme extract was measured by spectrophotometer at 260 nm and 280 nm (Thermo scientific, Genesys 10UV Scanning) as per the protocol of Iaderoza and Baldini (1991). The soluble protein content was considered to calculate the specific activity of the enzyme. One unit of activity (unit/mole) is defined as quantity of one

micromole of fatty acids released per minute. Specific activity (U/mg) is defined as the activity of enzyme per unit of protein.

### **2.2.3. Physico-chemical analysis of the mesocarp oil**

Mesocarp slices were dried in an oven with renewal and circulation of air (Tecnal, Model TE 394-3, Brazil) at a temperature of 65°C for 12 hours. Then, the oil was extracted using a manually operated hydraulic press (Prensa Ribeiro 30 Ton, Brazil). The extracted oil was stored at -20°C packed in amber glass vials wrapped with aluminum foil. Acidity and peroxide indices were determined as per AOCS (1983). Primary and secondary degree of oil oxidation was determined by molar absorptivity at 232 nm ( $MA_{232}$ ) and 270 nm ( $MA_{270}$ ), respectively, according to IUPAC (1979) by diluting the oil sample in 10 mL isooctane. The oxidative stability was measured by Rancimat method (873 Biodiesel Rancimat<sup>®</sup> - Metrohm) as per AOCS (1997). Total carotene content was determined by diluting the oil samples in 10 mL petroleum ether and taking the absorbance at 450 (Rodriguez-Amaya and Kimura, 2004). Recently peeled fruits had the opposite sides slightly cut to measure the mesocarp color by colorimeter (CR-10, Konica Minolta Sensing, Inc.). The result was interpreted as per Hunter color Lab (2001) scale into the corresponding color value. Accordingly, **L** is lightness (+**L**=light and -**L**=dark) ranging from 0 (white) to 100 (black), **A** is the red/green axis (+**A**=red, -**A**=green and **0** is neutral), and **B** is the blue/yellow axis (+**B**=yellow, -**B**=blue and 0 is neutral).

### **2.3. Experimental design and data analysis**

The experiment was conducted in a completely randomized design with 5 replications per treatment. The treatments were the 11 storage periods (0, 3, 6, 9, 12, 15, 20, 25, 30, 45, 60 days). Biochemical analyses were subjected to ten; whereas physical and physico-chemical analyses were done in three analytical replicates. Regression analysis was carried out considering storage period as independent variable using Sigma Plot 10 statistical software (Sigma Plot 10, 2006). Pearson's correlation coefficient was employed to compare the linear relationship of variables studied using SAS (SAS Institute, 2004).

### 3. RESULTS

#### 3.1. Physical, chemical and biochemical analysis of the mesocarp

Significant differences were observed among physical, chemical and biochemical characteristics of macaúba fruit. Mesocarp water activity ( $Ma_w$ ) was significantly ( $P < 0.0001$ ) different for periods of storage following a sigmoid trend with  $R^2$  of 98.2% (Figure 1-1). Freshly harvested fruits decreased from  $0.9742 \pm 0.0011$  to  $0.8341 \pm 0.0294$  at day 60.  $Ma_w$  decreased sharply around 23 days onwards.

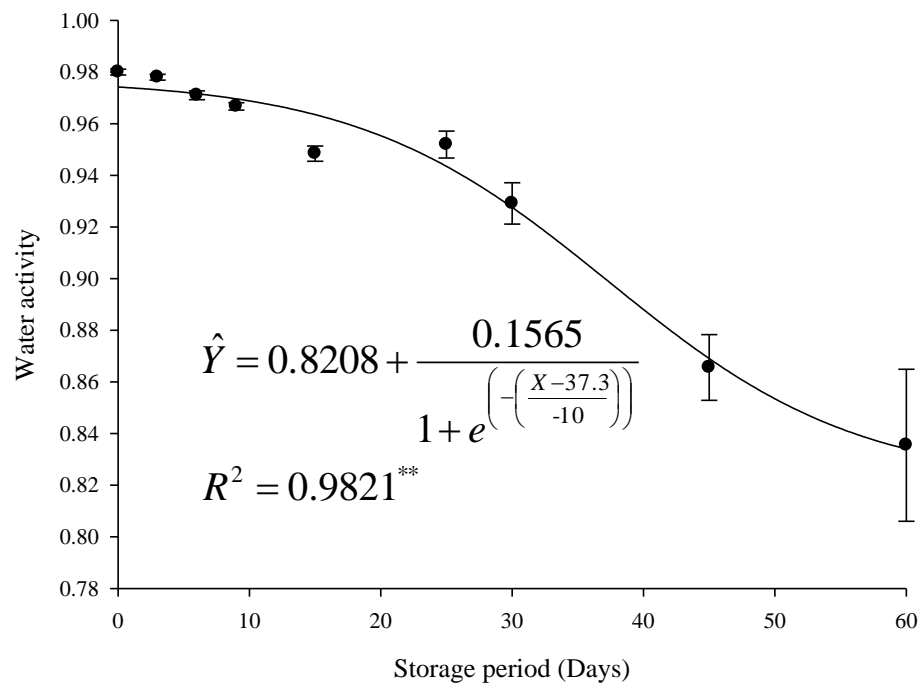


Figure 1-1. Mesocarp water activity ( $Ma_w$ ) along storage at room conditions. Each data point in the graph indicates mean  $\pm$  SE of 5 replications.

Moisture Loss in Fresh Fruit (MLFF, %) was significantly ( $P < 0.0001$ ) different for periods of storage following a quadratic trend with  $R^2$  of 99.9% (Figure 1-2). MLFF was increased from 0% from fresh fruits to  $41.1 \pm 0.2\%$  at the 60<sup>th</sup> day. MLFF was expressed in terms of mass loss of the stored fruit.



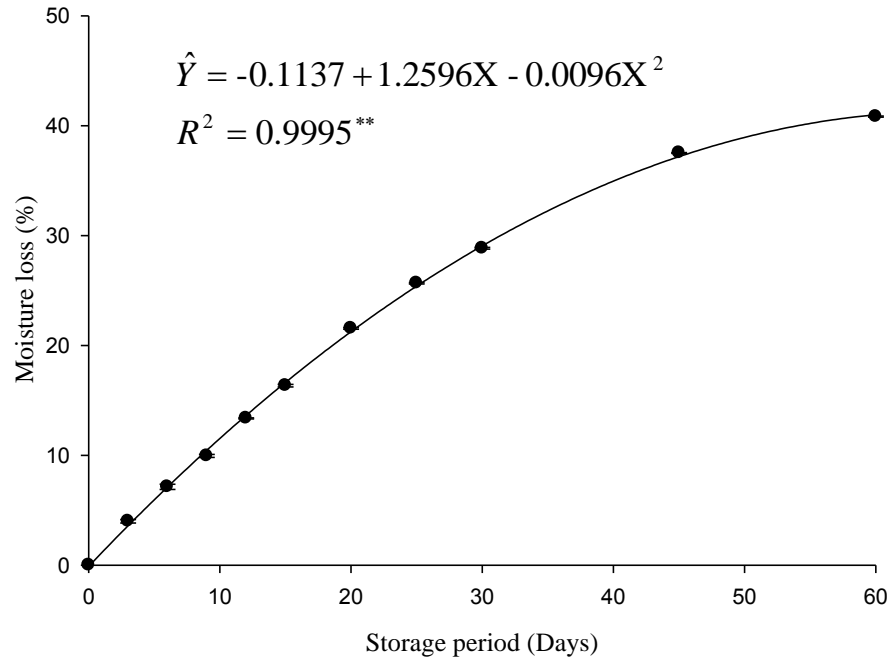


Figure 1-2. Moisture Loss in Fresh Fruit (MLFF, %) along storage at room conditions. Each data point in the graph indicates mean  $\pm$  SE of 5 replications.

Damage Index (DI) was significantly ( $P < 0.0001$ ) affected by periods of storage following a sigmoid trend with  $R^2$  of 98.2% (Figure 1-3).

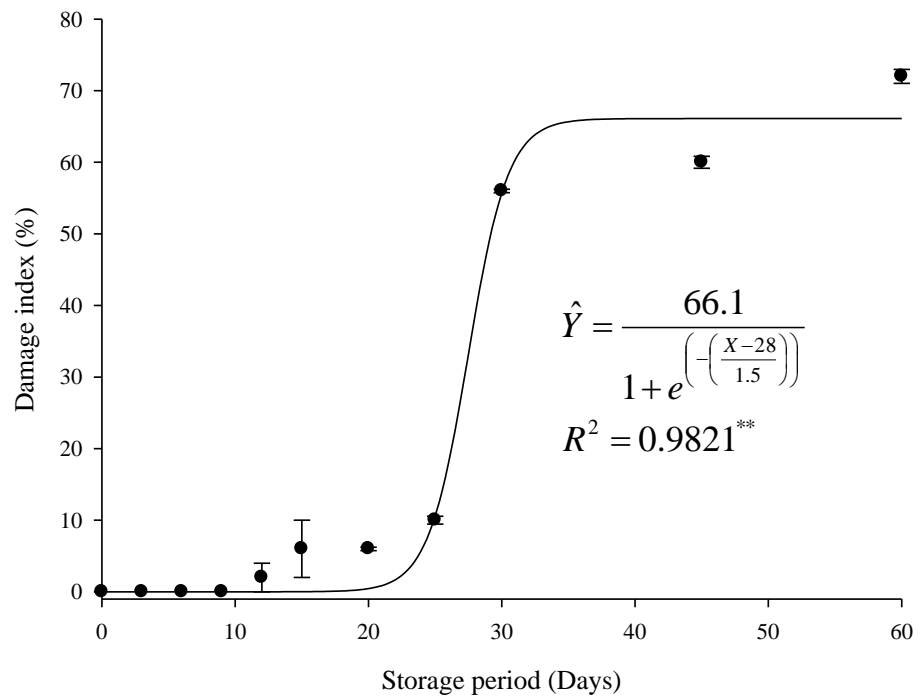


Figure 1-3. Damage Index (DI, %) along storage at room conditions. Each data point in the graph indicates mean  $\pm$  SE of 5 replications.

Fresh fruits had no symptoms of decay, but after 35 days  $66 \pm 0.3\%$  of the fruits were affected by some degree of damage index. It was then stabilized for the remaining periods of storage.

Specific activity of lipase (SAL) was significantly ( $P < 0.003$ ) affected by periods of storage following a sigmoid trend with  $R^2$  of 77.4% (Figure 1-4). SAL was attained its maximum value of  $19.3 \pm 4.1$  U/mg and started declining around 15 days onwards. Though the drastic reduction started after 21 days.

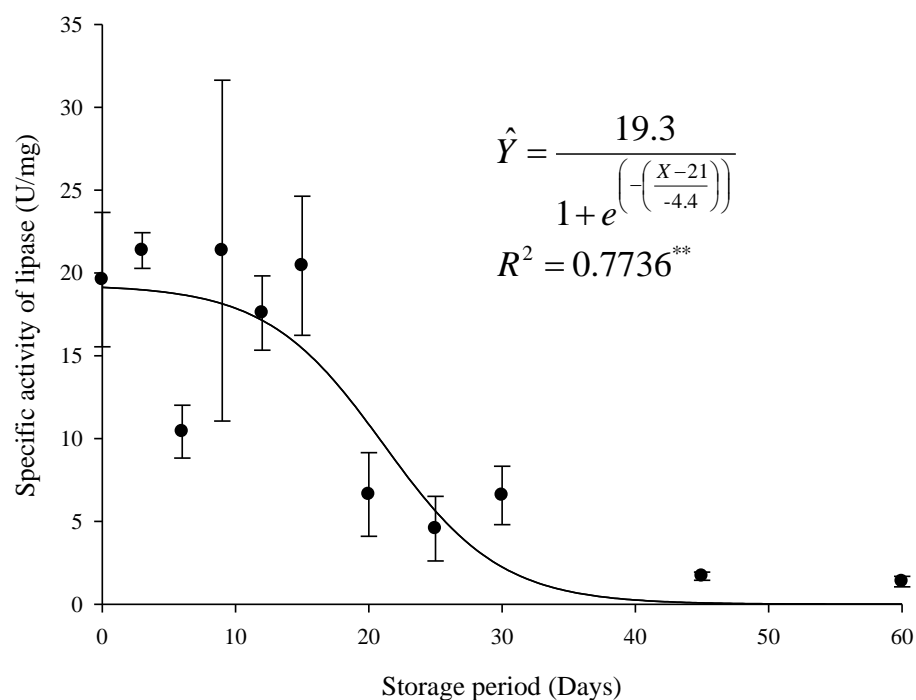


Figure 1-4. Specific Activity of Lipase (SAL, U/mg) along storage at room conditions. Each data point in the graph indicates mean  $\pm$  SE of 5 replications.

Correlation analysis of biochemical properties, total carotene content and acidity index was given in table 1-1. Pearson's correlation coefficient indicated that there was a significant ( $P < 0.01$ ) positive linear relationship between  $M_{w,x}SAL$  and  $MLFF \times DI$ . There was a negative correlation between  $M_{w,x}MLFF$ ,  $M_{w,x}DI$ , and  $SAL \times MLFF$ .

Table 1-1. Pearson's correlation matrix between mesocarp water activity ( $Ma_w$ ), specific activity of lipase (SAL), moisture loss in fresh fruit (MLFF), and damage index (DI) in stored macaúba fruits.

	$Ma_w$	SAL	MLFF
SAL	0.74 <sup>**</sup>		
MLFF	-0.91 <sup>**</sup>	-0.86 <sup>**</sup>	
DI	-0.92 <sup>**</sup>	-0.74 <sup>**</sup>	0.89 <sup>**</sup>

Pearson's correlation coefficient is significant at <sup>\*\*</sup> $P < 0.01$ .

### 3.2. Physico-chemical analysis of the mesocarp

Most of the evaluated mesocarp oil quality parameters were affected by periods of storage. Acidity Index (AI) was significantly ( $P < 0.0001$ ) different for periods of storage following a sigmoid trend with  $R^2$  of 99.4% (Figure 1-5). It was increased from  $1.72 \pm 0.06\%$  from freshly harvested fruits mesocarp crude oil to  $22.3 \pm 0.62\%$  at the 60<sup>th</sup> day. It presented a gradual increase after 20 days.

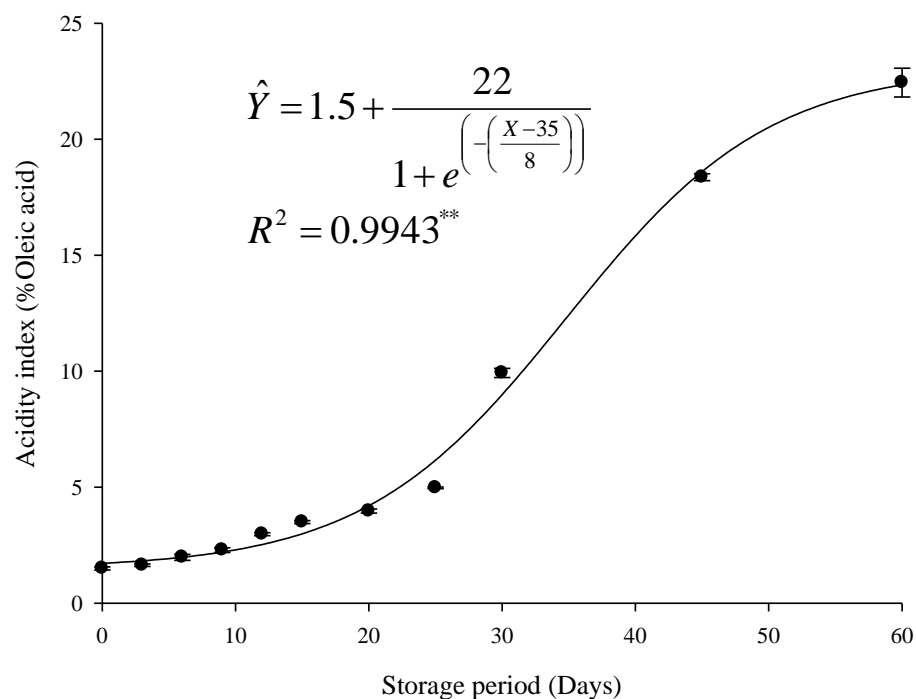


Figure 1-5. Acidity Index (AI, % oleic acid) of macaúba mesocarp oil along storage. Each data point in the graph indicates mean  $\pm$  SE of 5 replications.

Peroxide Index (PI) was significantly ( $P < 0.0001$ ) different for periods of storage following a sigmoid trend with  $R^2$  of 98.6% (Figure 1-6). It was increased from  $5 \pm 0.33$  from freshly harvested fruit mesocarp crude oil to  $12.4 \pm 0.51$  meq  $O_2$ /kg oil at the 60<sup>th</sup> day. Initial PI up to 18 days ranged within the limit of 10 meq  $O_2$ /kg oil settled for crude palm oil as per ANVISA (Brasil, 2005). A deep increase of PI was noticed afterwards.

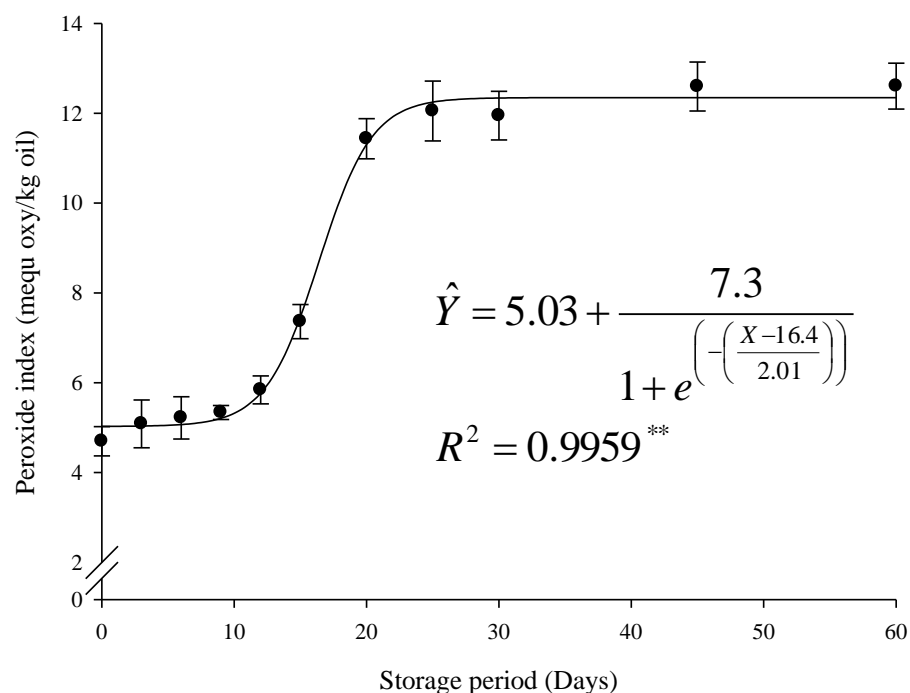


Figure 1-6. Peroxide Index (PI, meq  $O_2$ /kg oil) of macaúba mesocarp oil along storage. Each data point in the graph indicates mean  $\pm$  SE of 5 replications.

The molar absorptivity (MA) values were significantly ( $P < 0.0001$ ) affected by periods of storage.  $MA_{232}$  was increased from  $1.07 \pm 0.01$  from freshly harvested fruits mesocarp crude oil to  $2.60 \pm 0.13$  at day 60 ( $R^2=97.1$  %); while at 270 nm it was increased from  $0.10 \pm 0.002$  to  $0.81 \pm 0.046$  at day 60 ( $R^2=97.3$  %) as shown in Figure 1-7. There is no established Brazilian standard for MA. However, Nunes et al. (2015) reported MA values of 2.04 at 232 nm and 0.56 at 270 nm in the crude mesocarp oil of macaúba. Accordingly, the oil was stored for 17 days at  $MA_{232}$  and for 4 days at  $MA_{270}$  in this study.

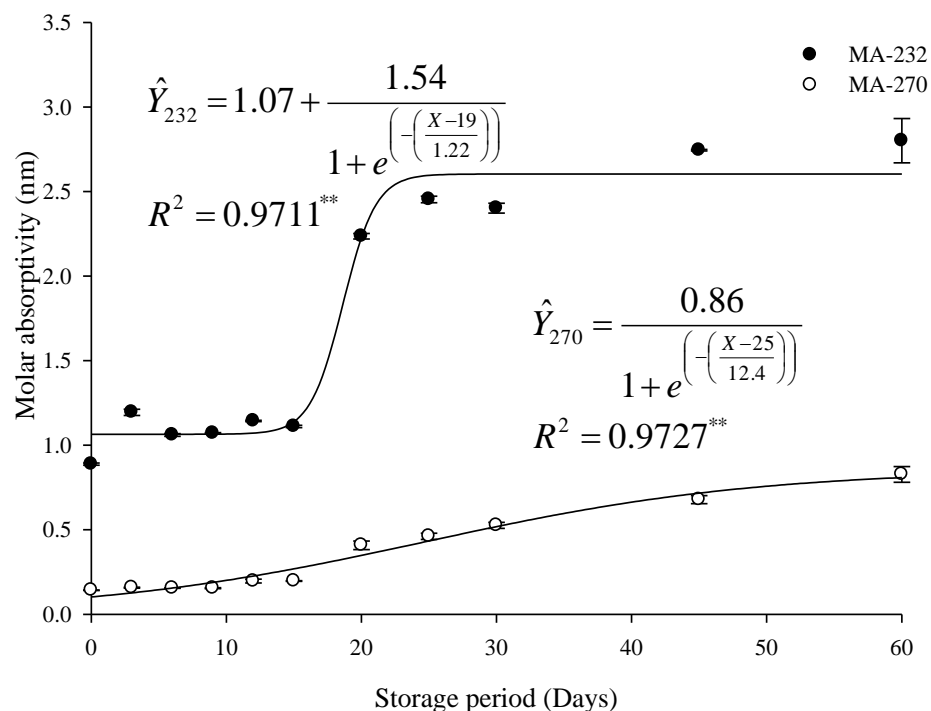


Figure 1-7. Molar Absorptivity (MA) value at 232 nm and 270 nm (MA<sub>232</sub> and MA<sub>270</sub>) of macaúba mesocarp oil along storage. Each data point in the graph indicates mean  $\pm$  SE of 5 replications.

Oxidative stability (OS) was significantly ( $P < 0.0002$ ) different for periods of storage following a sigmoid trend with  $R^2$  of 93.3% (Figure 1-8). OS was decreased from  $15.8 \pm 0.5$  h from freshly harvested fruits mesocarp crude oil to  $4.3 \pm 0.4$  h at the 60<sup>th</sup> day. It started declining after 31 days as per biodiesel standard of 6 h (Barabás and Todoruț, 2011).

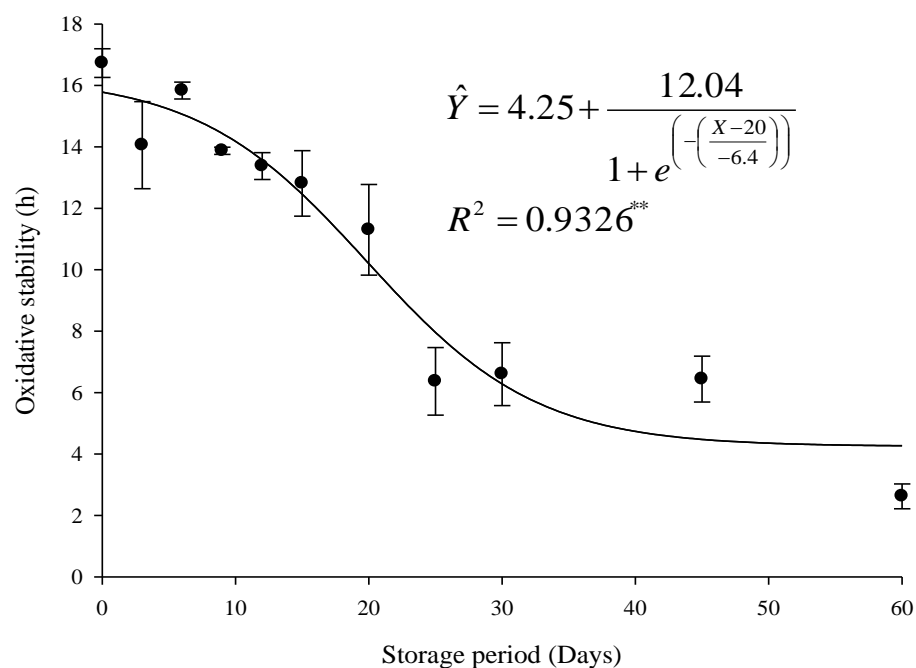


Figure 1-8. Oxidative Stability (OS, h) of macaúba mesocarp oil along storage. Each data point in the graph indicates mean  $\pm$  SE of 5 replications.

Mesocarp colour was significantly ( $P < 0.003$ ) different for **L** and **B** with  $R^2$  of 97.6% and 92.4%, respectively (Figure 1-9). On the other hand, **A** remained steady along periods of storage. Freshly harvested fruits presented **L** =  $75.2 \pm 0.7$  and **B** =  $53.8 \pm 1.2$ , what confers a yellowish colour to mesocarp. Nevertheless, at day 60 (**L** =  $50.9 \pm 1.9$  and **B** =  $21.8 \pm 2.7$ ), the yellow colour started decreasing. A sharp decline was started after 20 days for **B** value. Decrease in yellow color could be related to degradation of beta carotenes.

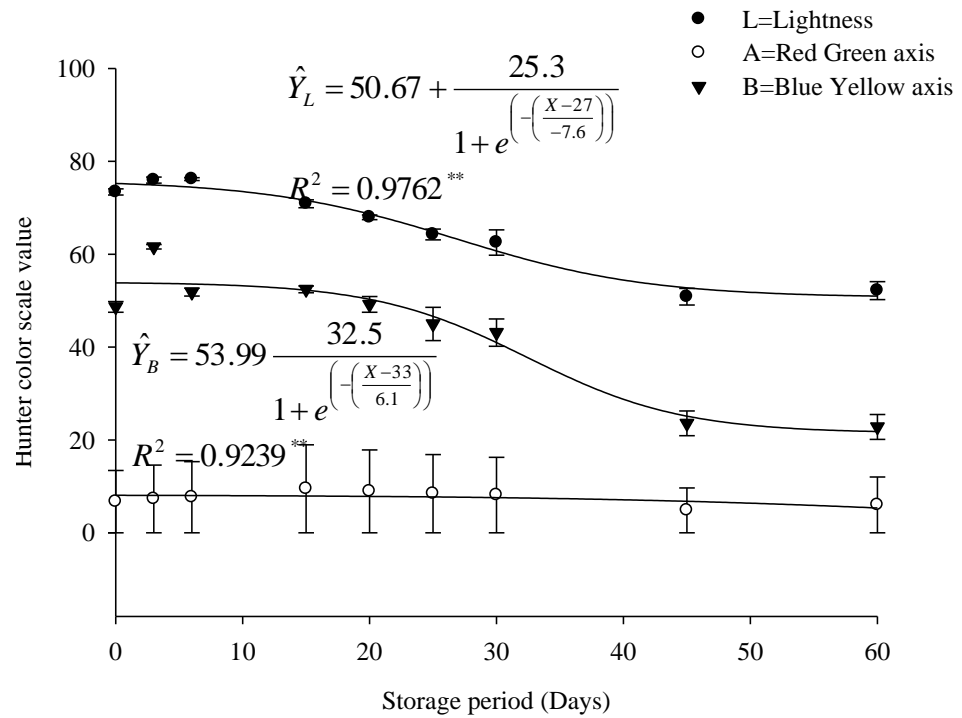


Figure 1-9. Mesocarp colour (MC) of macaúba mesocarp along storage. Each data point in the graph indicates mean  $\pm$  SE of 5 replications.

Total carotene (TCC) content was significantly ( $P < 0.001$ ) different along periods of storage following a piecewise bi-segmented linear regression with  $R^2$  of 95.3% (Figure 1-10). It was ranged from  $130 \pm 1.9$  from freshly harvested fruits mesocarp crude oil to  $152 \pm 2.9$  at the 26<sup>th</sup> day in region 1 (17 % increase) and then declined drastically to  $71.03 \pm 4.9$  mg/kg at the 60<sup>th</sup> day in region 2 (53 % decrease). There was a synthesis of TCC until 26<sup>th</sup> day then declined drastically after wards.

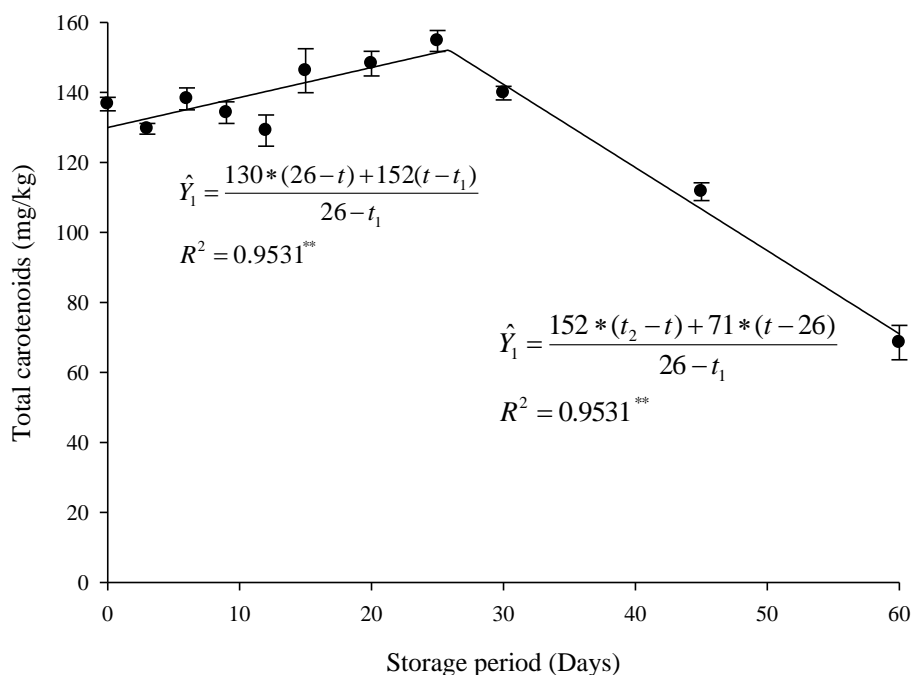


Figure 1-10. Total Carotene Content (TCC, mg/kg) of macaúba mesocarp oil along storage. Each data point in the graph indicates mean  $\pm$  SE of 5 replications.

Pearson's correlation coefficient among oxidative parameters indicated that there was a significant positive ( $P < 0.01$ ) linear correlation between  $PI \times MA_{232}$ ;  $PI \times MA_{270}$  nm and  $MA_{232} \times MA_{270}$  (Table 1-2). On the other hand, there was a strong negative linear correlation between  $PI \times OS$ ;  $MA_{232} \times OS$  and in  $MA_{270} \times OS$ . Formation of peroxides and hydroperoxides leads to oxidation of oils.

Table 1-2. Pearson's correlation matrix between peroxide index, molar absorptivity at 232 nm ( $MA_{232}$ ) and 270 nm ( $MA_{270}$ ) and oxidative stability in macaúba mesocarp oil.

	Peroxide index	$MA_{232}$	$MA_{270}$
$MA_{232}$	0.98 <sup>**</sup>		
$MA_{270}$	0.91 <sup>**</sup>	0.96 <sup>**</sup>	
Oxidative stability	-0.92 <sup>**</sup>	-0.94 <sup>**</sup>	-0.95 <sup>**</sup>

Pearson's correlation coefficient is significant at <sup>\*</sup> $P < 0.05$  and <sup>\*\*</sup> $P < 0.01$ , respectively.

Oil quality is related to the mesocarp properties. High positive correlations between  $AI \times DI$  and  $AI \times MLFF$  were observed (Table 1-3). The AI is also associated



with the decreasing of  $Ma_w$  and SAL. The rise in acidity could be explained by the high positive correlation between PI and AI. Carotene degradation seems to be related to the increasing acidity and fruits decay because they showed a negative correlation between them.

Table 1-3. Pearson's correlation matrix between mesocarp water activity ( $Ma_w$ ), specific activity of lipase (SAL), moisture loss in fresh fruit (MLFF), damage index (DI), acidity index (AI), and total carotene (TCC) content in stored macaúba fruits.

	$Ma_w$	SAL	MLFF	DI	AI	PI
AI	0.99*	0.75*	0.90*	0.96*		0.98**
TCC	0.41	0.37	-0.53	-0.70**	-0.81*	-0.27

Pearson's correlation coefficient is significant at \* $P < 0.01$  and \*\* $P < 0.05$ , respectively.

## 4. DISCUSSION

### 4.1. Effect of storage period upon biochemical activities of stored macaúba fruit

Many factors can contribute to the decrease in water activity ( $a_w$ ); among them is the sheer natural loss of water by a system. In biological units (fruit in this case), the loss can either occur due to the biological activity (transpiration/respiration) or physical process (evaporation). The small reduction in  $a_w$  along storage, observed in macaúba mesocarp (Figure 1-1), could be associated with the feature of its matrix which is mucilaginous and fibrous (Lorenzi and Negrelle, 2006), and adhered to endocarp endowed with sweet taste (Silva, 1994). The sweet taste could be related to the presence of sugars. In such kind of matrix the water is linked to the solutes, what prevent its release and, consequently, no decrease in  $a_w$ . On the 60<sup>th</sup> day of storage, the  $Ma_w$  was reduced only by 14.4% ( $0.8341a_w$ ), while the MLFF reached 41% (Figure 1-2). As a comparison, MLFF accounted for 26.08% and  $Ma_w$  of 0.9406 on the 26<sup>th</sup> day for macaúba in this study. However, at the same storage time, 23.75% moisture loss with  $a_w$  of 0.76 in oil palm mesocarp was reported (Tagoe et al., 2012). The intense water loss could be associated to the macaúba exocarp that is brittle at ripening (Lorenzi and Negrelle, 2006) with incomplete lignifications that leads to porosity (Reis et al., 2012), what facilitates moisture loss.

The high levels of  $Ma_w$  for about one month provided favorable conditions for several reactions taking place and compromise the oil quality. This is because the available water, measured by  $a_w$  (Sethi, 2007), favors microbial growth, degradation reactions of chemical, enzymatic and physical nature (Maltini et al., 2003). The effect of  $Ma_w$  on enzymatic reactions is well shown by the lipase activity in macaúba fruits. The correlation index between these variables was 0.74 (Table 1-1). Irrespective of the available substrate, SAL became inactive after 40 days of storage when  $Ma_w$  was less than 0.88. Previously, it was observed that small changes in  $Ma_w$  resulted in deeper reduction in SAL (Figure 1-1 and 1-4). This confirms the statement that small variability in  $a_w$  affected significantly catalytic activity of lipase (Pepin and Lortie, 1975). Despite this fact, the SAL in macaúba was lower as compared to oil palm (Figure 1-5). According to Desassis (1957) as much as 40% of the triglyceride present in the mesocarp could split in 15 min. This implied that lipase should be deactivated after harvest to minimize the increase in FFA content. Therefore, the shelf life of macaúba is better than oil palm in storage and processing.

The integrity of the exocarp is a barrier for microorganisms to penetrate the mesocarp and initiate the process of decaying. The bunch was cut and dropped inside a collector with spongy surfaces on the base during harvesting. This practice prevented the exocarp from mechanical damage and microbial contamination. Nevertheless, the exocarp of macaúba fruit is porous that can permit entrance of microorganisms, especially at ripening stage. The microorganisms' growth pattern, evaluated by DI in macaúba fruits, shown that there was no decay incidence up to the 10<sup>th</sup> day, and then it began increasing at a small rate up to the 25<sup>th</sup> day (Figure 1-4). A huge increase of DI was observed after 35 days of storage and about 60% of the fruits showed decay symptoms (Figure 1-4). Afterwards, the DI reached a plateau at the 35<sup>th</sup> day. In spite of the negative correlation between  $DI \times Ma_w$  (Table 1-1),  $Ma_w$  during the period of decay increase was higher than 0.94 what allows microbial growth. The absence of new spots of decay incidence does not mean the microbial growth ceased, as the measurement of the area under disease infection was not taken into account in this work. Therefore, further studies should be carried out to investigate thoroughly the relation between  $a_w$  and microbial growth in macaúba fruits in order to settle a safe range of storage.

#### **4.2. Effect of storage period upon physico-chemical properties of macaúba mesocarp oil**

The degradation of lipids explained by hydrolytic and oxidative reactions was more intense after the 20<sup>th</sup> day of storage in macaúba fruits that coincides with the increasing pattern of DI and decreasing trend of  $M_{a_w}$  (Figure 1-4 and 1-1).

The pattern of TAG hydrolysis reaction in macaúba mesocarp oil showed a small slope up to the 20<sup>th</sup> day of storage (Figure 1-5), and then increased gradually with steep slope, whereby the AI was increased from 5% to more than 20% on the 60<sup>th</sup> day. According to the adjusted regression model (Figure 1-5), until 22 days of storage, AI was within the range of standard established for crude palm oil, 5% at maximum as per ANVISA (Brasil, 2005) and (PORIM, 2011). It seems that the decrease in  $a_w$  does not prevent acidification in the mesocarp oil of macaúba. Actually, the results suggest the hydrolytic reaction rate was increased after some  $a_w$  reduction, reaching higher levels of the  $a_w$  within the range of 0.94 to 0.83. As discussed earlier, the increasing of DI was not avoided up to 0.94 of  $M_{a_w}$ . Free fatty acids can be released from TAG by catalysis of microbial and/or endogenous lipase, besides environmental conditions like temperature and light exposure. Then, it can be deduced that other factors rather than lipase activity are part of the increasing AI in macaúba oil in the later storage, once the former was reduced to a complete inactivation from the 30<sup>th</sup> day onwards. The lipase activity measurement protocol employed in this study encompasses both endogenous enzymes and micro-flora growing in association with the mesocarp, though it could be failed in a proper removal of them from the sampled tissue. However, the high positive correlation (0.98) between AIxPI could give a clue that decomposition of peroxides to hydroperoxides might contribute the rise in AI in this study.

The lipolysis either by autocatalytic and/or by microbial enzymes were reported for other oil bearing fruits during storage. The oil palm fruit is not protected by a husk like macaúba, its outer most layer is quite soft and susceptible to mechanical damage that can compromise the integrity of the cell structures. Therefore, microbial hydrolysis was reported in stored oil palm (Ngando Ebongue et al., 2006) and olive fruits (Clodoveo et al., 2007; Nabil et al., 2012).

Peroxide index (PI) is a measure of lipid oxidative degradation. It is characterized by the formation of peroxides and hydroperoxides as a primary stage of breakage at the double bonds of polyunsaturated fatty acids. The peroxide formation is a

typical chain reaction, that encompasses a small rate at the beginning and it will reach an exponential phase later on. Peroxides achieve a maximum at certain times, followed by their decomposition into degraded compounds (Wanasundara et al., 1995), and therefore, the reduction of PI. This pattern was almost complete for macaúba mesocarp oil within the storage period studied (Figure 1-6). The small peroxide formation rate lasts about the first 10 days of storage. PI was in line with the crude palm oil standard of 10 meq O<sub>2</sub>/kg oil (Brasil, 2005) at most up to 18 days of storage at room temperature, though reached the exponential increasing phase. This sharp increasing of PI lasts up to 20 days of storage. Afterwards, PI remained unchanged for the next 40 days of storage. Macaúba fruits that dropped straight into net mesh containers settled underneath the palm tree canopy, and were kept inside it under field conditions, shown increased PI after 16 days of storage (Souza et al., 2013), a time observed close to this work. The steady amount of peroxides from day 20 does not imply necessarily the cessation of lipids degradation. There could be formation of new peroxides at the same rate of their degradation into secondary compounds of oxidation.

Molar absorptivities (MA) are both identity and quality indexes of vegetable oils (IUPAC, 1979). In this work, MA<sub>232</sub> and MA<sub>270</sub> (Figure 1-7) are being measured as index of oxidation. The results of MA support the suggested reactions of peroxides in macaúba mesocarp oil. Looking at the similarity of the PI and MA<sub>230</sub> slopes (Figure 1-6; Figure 1-7), it can be seen the strong correlation between them (Table 1-2), because M<sub>232</sub> measures mostly peroxides. Nevertheless, it can be observed a different pattern to MA<sub>270</sub> that shows a trend of continuous increase with small slope in its values. It could mean peroxides are being decomposed into secondary compounds (conjugated trienes). The accumulation of MA<sub>232</sub> (conjugated dienes) was led to early detection of MA<sub>270</sub> in this study. Similarly, correlation of PI with MA<sub>232</sub> nm was reported in stored olive oil (Cinquanta et al., 2001; Nabil et al., 2012). Increased MA<sub>270</sub>, as an indicator of secondary oxidation compound, was reported in sunflower (Poiana, 2012) and in olive oil (Garcia et al., 1996). Therefore, MA<sub>232</sub> and MA<sub>270</sub> might be employed to evaluate further quality changes of stored macaúba oil, besides being an identity index and processing quality index (Nunes et al., 2015).

Oxidative stability (OS) is the oil resistance to oxidation; therefore, it also determines the oil quality and shelf life (Hamilton, 1994) and addresses the usage of the oil. Macaúba has high amount of monounsaturated fatty acid (oleic) in the mesocarp (Coimbra and Jorge, 2013) that offers resistance to oxidation. Since Rancimat method

of OS measures primary and secondary oxidation products, there could be a correlation between OS and PI. Correlation of OS, PI and MA<sub>232</sub> was reported in olive oil quality evaluation (Cinquanta et al., 2001; Negre et al., 2011). Similarly, Pearson's correlation coefficient indicated that there was a strong negative correlation between OSxPI (-0.92) and OSxMA<sub>232</sub> (-0.94) as in shown in table 1-2. Reduction in OS resulted in increased PI and MA<sub>232</sub> and their vice versa. OS reported decreasing along periods of storage in stored olive fruits (Nabil et al., 2012). The minimum OS is 6 hours as per EN 14214, European and Brazilian standard (Barabás and Todoruț, 2011) for vegetable oils used as raw material for biodiesel production. Macaúba mesocarp oil maintained this minimum OS standard for 31 days (Figure 1-8). The maximum OS using Rancimat test was 15.8 h at 0 day from the adjusted regression model. This value was smaller than the 25 h reported by Melo et al. (2014) in macaúba mesocarp oil. The higher the OS value, the better the oil to resist oxidation upon storage. Macaúba oil has high potential to achieve quality to industrial standard whenever storage and processing carried out in due time and conditions.

Though degradation reaction was expressed in terms of mesocarp colour change (occurrence of decay incidence) after 20 days, intense yellow colour was registered from Hunter colour Lab (2001) scale (Figure 1-9) in the end. This is due to the carotene content present in the mesocarp (Figure 1-10), especially beta carotene (CETEC, 1983; Coimbra and Jorge, 2012). Carotenoids are substances that retard or prevent the effects of free radicals (Coimbra and Jorge, 2013), therefore, provides antioxidant activity (Rodriguez-Amaya, 2001). Besides the fatty acid composition that favours the high oxidation stability of macaúba mesocarp oil, carotene probably plays an important role to this characteristic.

The color changes in mesocarp relied mostly on the reduction of the yellow intensity along the storage (**B**), followed by the reduction of lightness (**L**). The red color intensity (**A**) was kept unchanged along all the evaluated periods. The decline in yellow color is expressed by degradation of beta carotene.

The TCC concentration of the macaúba studied in this work, 130 mg/kg from the fresh fruits was smaller than reported before, 300 mg/kg by Coimbra and Jorge (2011). There was an increasing of TCC up to the 26<sup>th</sup> day, reaching 152 mg/kg, followed by a decreasing trend by approximately half of the amount after 60 days of storage. This slight increase in TCC could be related to oil synthesis that could be linked with

climacteric property of the fruit to accumulate more oil along storage. Exposure to heat, oxygen and high acidity imply structural change and reduction of carotene activity (Rodriguez-Amaya, 1993b). As shown in table 1-3, Pearson's correlation coefficient indicated that TCC was negatively correlated with AI. The sharp decline after 26 days could be related to oxidation of the oil as revealed by PI and MA<sub>232</sub> along periods of storage. This led to formation of free radicals that attack TCC and thereby resulted in rise of AI and oxidation of oil.

The FFA content of the raw material is an important aspect whether in biodiesel production or food industries. In oleaginous plant like macaúba, the FFA should be lower to 3% for industrial application (Nunes et al., 2015). This was equivalent to 13 days of storage to adjusted regression model. This is because in alkali based transesterification creates more FFA so that acid based catalyst could be used instead which is expensive in practical terms. A 5% FFA is a tolerable limit in alkali based transesterification of biodiesel production (Gerpen and Knothe, 2005). Therefore, the storage time extends to 22 days which is an additional merit in storage and processing. The reduction in oxidative stability after 31 days related oxidation status of the oil explained by peroxide index (after 18 days) and its decomposition to MA<sub>232</sub> (after 17 day). Early appearance of MA<sub>270</sub> was the result of MA<sub>232</sub> decomposition. The decline in OS was almost in line with total carotene content. The decline in carotene content linked with oxidation status of the oil associated PI and MA<sub>232</sub>. Direct harvest fruit from the plant and stored in a ventilated area at ambient conditions extend the shelf life and maintain the overall oil quality almost 20 days. So that further postharvest treatments prolong the shelf life and improve the oil quality in storage and processing.

## 5. CONCLUSIONS

The reduction in water activity led to decrease in specific activity of lipase expressed by correlation coefficient of 0.74. Although there was intense loss of water from the mesocarp, the Ma<sub>w</sub> was kept in a range that allowed microbial growth, acidification and oxidation after 20 days. The acidification seems not be related to lipase activity. However, the rise in acidity could be linked to decomposition of peroxides to hydroperoxides that hydrolyse triacylglycerol to free fatty acids. Although the reduction in total carotene content was occurred after 26 days, the oxidative stability was kept suitable for biodiesel production for 31 days of storage. The shelf life of macaúba is better than oil palm in storage. In general, macaúba fruits harvested directly from the

bunch and stored at ambient condition maintained oil quality up to 20 days in this study. This information could be implemented in the production chain of macaúba either in subsistence or large scale schemes.

## 6. REFERENCES

Acklin, T. 1998. Soybean handling and storage. <http://www.World-Grain.com>. Retrieved on 6/5/ 2014.

Agar, T.; Hess Pierce, B.; Sourour, M. M.; Kader, A. A. 1998. Quality of fruit and oil of black-ripe olives is influenced by cultivar and storage period. **Journal of Agriculture and Food Chemistry** 46: 3415-3421.

AOCS (American Oil Chemists' Society). 1983. Official and Tentative Methods. Champaign, Illinois.

AOCS. 1997. Official Method Cd 12b-92. American Oil Chemists' Society. Champaign, Illinois.

Barabás, I.; Todoruț, I.A. 2011. Biodiesel Quality, Standards and Properties. In: Biodiesel-quality-emissions-and-by-products.<http://www.intechopen.com/>. Retrieved in December 2013.

Bhosle, B.M.; Subramanian, R. 2005. New approaches in deacidification of edible oils: a review. **Journal of Food Engineering** 69: 481-494.

Brasil, 2005. Ministério da Saúde. Agência Nacional de Vigilância Sanitária. Resolução Diretoria da Colegiada, RDC/ANVIS/MS n° 270, 22 de setembro de 2005. Regulamento técnico para óleos vegetais, gorduras vegetais e creme vegetal. Diário Oficial da República Federativa do Brasil, Brasília, DF, 23 set. 2005. Seção 1. Disponível em: <http://www.anvisa.gov.br>. Retrieved on December 30, 2013.

CETEC (Fundação Centro Tecnológico de Minas Gerais).1983. Produção de combustíveis líquidos a partir de óleos vegetais: relatório final do Convênio STI- MIC / CETEC. Vol. 1 e 2. CETEC. Belo Horizonte.

Cinquanta, L.; Esti, M.; Matteo, M. D. 2001. Oxidative Stability of Virgin Olive Oils. **Journal of American Oil Chemists' Society** 78 (12): 1197-1202.

Clodoveo, M.; Delcuratolo, D.; Gómes, T.; Colelli, G. 2007. Effect of different temperatures and storage atmospheres on Croatian olive quality. **Food Chemistry** 102: 571-576.

Coimbra, M.C.; Jorge, N. 2011. Proximate composition of guariroba (*Syagrus oleracea*), jervá (*Syagrus romanzoffiana*) and macaúba (*Acrocomia aculeata*) palm fruits. **Food Research International** 44: 2139–2142.

Coimbra, M.C.; Jorge, N. 2012. Fatty acids and bioactive compounds of the mesocarps and kernels of Brazilian palm species, guariroba (*Syagrus oleraces*), jerivá (*Syagrus romanzoffiana*) and macaúba (*Acrocomia aculeata*). **Journal of Food Science and Agriculture** 92: 679–684.

Coimbra, M.C.; Jorge, N. 2013. Phenolic compounds, carotenoids, tocopherols and fatty acids present in oils extracted from palm fruits. CEPPA, Curitiba, 31 (2): 309-320.

Desassis, A. 1957. Palm oil acidification. **Oléagineux** 12:525-534.

García, J.M.; Gutierrez, F.; Castellano, J.M.; Perdiguero., S.; Morilla. A.; Alibi, M.A. 1996. Influence of storage temperature on fruit ripening and olive oil quality. **Journal of Agriculture and Food Chemistry** 44 (1): 264–267.

Gerpen, J.V.; Knothe, G..2005 Biodiesel Production:basics of the Transesterification Reaction. AOCS Press.

Hamilton, R. J. 1994. The chemistry of rancidity in foods. In: Allen, J.C. and Hamilton R.J. (Eds). **Rancidity in foods**. 3rd ed. London: Blackie Academic and Professional. p. 1–21.

Iaderoza, M.; Baldini, V. L. S. 1991. A importância da análise enzima em alimentos. In: Iaderoza, M; Baldini, V. L. S. **Ênzimose a qualidade de vegetais processados**. Campinas: ITAL, 1991. (Manual Técnico).

IUPAC (International Union of Pure and Applied Chemistry). 1979. Standard methods for the analysis of oils, fats and derivatives. Oxford, 6<sup>th</sup> ed., IUPAC, 1979.

Janick, J.; Paull, R.E. 2008. The Encyclopedia of fruits and nuts. CABI, UK.

Köppen, W. P.; Geiger, R. 1928. Die Klimate der Erde: Grundriss der Klimakunde. Berlin, Leipzig: Waltr de Gruyter.

Lopes, D.C.; Neto, A.J.S. 2011. Potential Crops for Biodiesel Production in Brazil: A Review. *World Journal of Agricultural Sciences* 7 (2): 206-217.

Lorenzi, G. M. A. C.; Negrelle, R. R. B. 2006. *Acrocomia aculeata* (Jacq.) Lodd. EX Mart.: Ecological aspects, uses and potentialities. <http://www.entabanbrasil.com.br>. Retrieved on 21/04/2014.

Macrae, A.R.; Hammond, R.C. 1985. Present and future applications of lipases. **Biotechnology and Genetic Engineering Reviews** 3: 193-217.

Maltini, E.; Torreggiani, D.; Vieira, E.; Bertolo, G. 2003. Water activity and the preservation of plant foods. **Food Chemistry** 82: 79-86.

Mariano, R.G.B.; Silva,C.M.; Couri, S.; Nogueira, R.I.; Freitas, S.P. 2011. Partition of Free Fatty Acids in Deacidification of Macaúba Mesocarp oil by Liquid-Liquid



Extraction using Ethanol/Water as Solvent. **Defect and Diffusion Forum** 312-315: 554-559.

Melo, M. A.R.; Melo, M.A.M.F.; Silva, E.V.; Filho, J.R.C; Souza, A.G. 2014. Study of the oxidative stability of vegetables oils for Biodiesel production. **Revista Verde** 9 (1): 84-88, Mossoró – RN – BRASIL.

Mohankumar, C.; Arumughan, C.; Kaleysa Raj, R. 1990. Histological localization of oil palm fruit lipase. **Journal of American Oil Chemists' Society** 67 (10): 665-669.

Moura, E.F.; Ventrella, M.C.; Motoike, S.Y. 2010. Anatomy, histochemistry and ultrastructure of seed and somatic embryo of *Acrocomia aculeata* (Arecaceae). **Agricultural Sciences** 8:399-407, Piracicaba.

Nabil, B. Y.; Youssef, O.; Nizar, D.; Bechir, B.; Chedly, A.; Mokhtar, Z. 2012. Effect of olive storage period at two different temperatures on oil quality of two Tunisian cultivars of *Olea europea*, Chemlali and Chétoui. **African Journal of Biotechnology** 11 (4): 888-895.

Negre, A.; Garau, M.C.; Luna, J.M.; March, J., Martorell, A.; Adrover, S.; Femenia, A. 2011. Influence of the fruit ripening degree on the virgin olive oil quality and conservation of Empeltre olives cultivated in a disadvantaged area. OLIVEBIOTEQ 2011 Chania, Crete, October 31<sup>st</sup> – November 4<sup>th</sup>, 2011.

Ngando-Ebonge, G.F.; Dhouib, R.; Carriere, F.; Amvam Zollo, P.H.; Arondel, V. 2006. Assaying lipase activity from oil palm fruit (*Elaeis guineensis* Jacq.) mesocarp. **Plant Physiology and Biochemistry** 44: 611-617.

Nunes, A. A.; Favaro, S.P.; Galvani, F.; Miranda, C.H.B. 2015. Good practices of harvest and processing provide high quality Macaúba mesocarp oil. [10.1002/ejlt.201400577].

Pepin, P.; Lortie, R. 1999. Influence of water activity on the enantio-selective esterification of (R, S)-Ibuprofen by *C. antarctica* lipase B in solvent free media. **Biotechnology and bioengineering** 63:502-505.

Pires, T.P.; Sousa, E.S.; Kuki, K.N.; Motoike, S.Y. 2013. Echophysiological traits of the macaw palm: A contribution towards the domestication of a novel oil crops. **Industrial Crops and ProduTCCs** 44:200-210.

Poetsch, J., Hauptenthal, D.; Lewandowski, I.; Oberländer, D.; Hilger, T. 2012. *Acrocomia aculeata* – a sustainable oil crop. Rural 21 – 03/2012. **Science World** 41-44.

Poiana, M.A. 2012. Enhancing oxidative stability of sunflower oil during convective and microwave heating using grape seed extract. **International Journal of Molecular Science** 13: 9240-9259.

PORIM. 2011. Standard Specifications for Processed Palm Oil. PORIM Handbook. <http://www.poram.org>. Retrieved on 29/4/2012.

- Reis S, B.; Mercadante-Simões, M.O.; Ribeiro, L.M. 2012. Pericarp development in the macaw palm *Acrocomia aculeata* (Arecaceae). *Rodriguésia* 63 (3): 541-549.
- Rodriguez-Amaya, D. B. 1993b. Stability of carotenoids during the storage of foods. In: Charalambous, F. (Ed.). Shelf life studies of foods and beverages: chemical, biological, physical and nutritional aspects. Amsterdam, Elsevier Science, pp. 591-624.
- Rodriguez-Amaya, D. B. 2001. A guide to carotenoid analysis in foods. ILSI press, Washington, D. C, U.S.A.
- Rodriguez-Amaya, D.B.; Kimura, M. 2004. Harvest plus Handbook for Carotenoid Analysis. Washington, DC and Cali, IFPRI and CIAT, p.58.
- SAS Institute, 2004. Base SAS 9.1.3: Procedures guide. Cary, NC.
- Sethi, S. 2007. Horticulture postharvest technology: principles of food processing. Division of postharvest technology, Indian Agricultural Research Institute, New Delhi.
- Sigma Plot 10. 2006. Sigma Plot for Windows, Version 10. Systat Software, Inc. Germany.
- Silva, J.C. 1994. Macaúba: fonte de matéria prima para os setores alimentício, energético e industrial. Viçosa: CEDAF/DEF/UFV, 41 p.
- Souza, C.F.T. 2013. Desenvolvimento, Maturação e Sistemas de Colheita de frutos da macaúba (*Acrocomia aculeata*). M.Sc thesis, Universidade Católica Dom Bosco, Campo Grande, Mato Grosso do Sul.
- Tagoe, S.M.A.; Dickinson, M. J.; Apetorgbor, M.M. 2012. Factors influencing quality of palm oil produced at the cottage industry level in Ghana. **International Food Research Journal** 19 (1): 271-278.
- Wanasundara, U.N.; Shahidi, F.; Jablonski, C.R. 1995. Comparison of standard and NMR methodologies for assessment of oxidative stability of canola and soybean oils. **Food Chemistry** 52: 249-253.
- Wandeck, F.A.; Justo, P.G. 1982. Macaúba, fonte energética e insumo industrial. Proceeding of the conference Vida Industrial. Sao Paulo, Brazil, p. 33-37.
- Zu, K.S.A.; Adjei-Nsiah, S.; Bani, R.J. 2012. Effect of processing equipment and duration of storage of palm fruits on palm oil yield and quality in the Kwaebibrem District, Ghana. **Agricultural Research and Reviews** 1(1):18 – 25.

## CHAPTER 2

### EFFECT OF GAMMA RADIATION IN THE MESOCARP OIL QUALITY OF STORED MACAÚBA FRUIT

#### ABSTRACT

Macaúba is a promising palm tree in Latin America with high biomass and oil content to be used as raw material source in biodiesel industry. However, high water content in the fruit favors conversion of its stored lipid into free fatty acids what compromises quality and market. Postharvest treatments could enlarge shelflife of macaúba fruits and allow staggered processing. This work aimed to evaluate the effects of gamma radiation treatment upon biochemical activities and physicochemical properties of stored macaúba fruits. The experiment was conducted in a completely randomized design with factorial arrangement of 3x4, 3 gamma radiation doses (0 as control, 4 KGy and 8 KGy) and 4 storage times (0,10, 20 and 30 days) with 5 replications. Physical, chemical and biochemical activity of the fruit (mesocarp and exocarp water activity, moisture content and oil content of mesocarp, specific activity of lipase and peroxidase) and physicochemical properties of the mesocarp crude oil (acidity index, peroxide index, molar absorptivity at 232 nm and 270 nm, total carotene content and oxidative stability) were studied. Water mediated parameters (SAP, SAL, MMC,  $Ma_w$  and  $Ea_w$ ) except acidity were affected by storage period while most of oil based parameters were (OS, MA at 232 and 270 nm, TCC and PI) influenced by gamma dose treatment. Gamma radiation decreased oil accumulation as compared to control. The 8 KGy gamma dose resulted in oil oxidation. Reduction of water activity followed by decreased enzymatic activity. Total carotene content was increased in control treatment. Application of 4 KGy gamma dose reduced enzymatic activities and showed best results in terms of oil quality during the 30 days of storage. Gamma radiation proved to be an effective technology to improve oil quality in macaúba. This implied that the high initial installation cost of gamma radiation facilities could be compensated by the net benefit gained from the postharvest oil quality in the production chain of macaúba, especially at the industrial level.

**Key words:** *Acrocomia aculeata*, acidity, lipase, oxidation, peroxidase.

## RESUMO

### EFEITO DA IRRADIAÇÃO GAMA NA QUALIDADE DO ÓLEO DO MESOCARPO DE FRUTOS DE MACAÚBA DURANTE O ARMAZENAMENTO

Macaúba é uma palmeira promissora na América Latina com elevada produção de biomassa e teor de óleo para ser usado como fonte de matéria-prima na indústria de biodiesel. No entanto, o elevado teor de água no fruto favorece a conversão do lípido armazenado em ácidos graxos livres. Foram avaliados os efeitos de irradiação gama sobre as atividades bioquímicas e propriedades físico-químicas dos frutos de macaúba durante o armazenamento. O experimento foi conduzido no delineamento inteiramente casualizado, num esquema fatorial duplo 3x4, 3 doses de radiação gama (0, 4 e 8 KGy) e 4 tempos do armazenamento (0, 10, 20 e 30 dias) com 5 repetições. Física, química e atividade bioquímica do fruto (atividade de água do mesocarpo e exocarpo, teor de umidade e teor de óleo do mesocarpo, atividade específica de lipase e peroxidase) e propriedades físico-químicas de óleo bruto do mesocarpo (índice de acidez e peróxido, absorvidade molar a 232 nm e 270 nm, teor de carotenóides totais e estabilidade oxidativa) foram avaliados. Parâmetros relacionados com água (SAP, SAL, MMC,  $M_{aw}$  and  $E_{aw}$ ) afetado pelo armazenamento enquanto a maioria dos a qualidade do óleo (OS, MA at 232 and 270 nm, TCC and PI) influenciado pelo a dose gama. A radiação gama diminuiu o acúmulo de óleo em comparação com o controle. A dose 8 KGy resultou na oxidação do óleo. A redução de atividade da água resultou em diminuição de atividade enzimática. Teor de carotenóides totais aumentou em controle. Em geral, a dose 4 KGy reduziu atividade de enzima e manteve a qualidade de óleo durante 30 dias do armazenamento. Aplicação de a dose 4 KGy reduziu atividades enzimáticas e mostrou melhores resultados em termos de qualidade do óleo durante os 30 dias de armazenamento. A radiação gama provou ser uma tecnologia eficaz para melhorar a qualidade do óleo em macaúba. Isto implicava que o custo de instalação inicial elevado para radiação gama poderia ser compensado pelo benefício líquido obtido a partir de óleo de qualidade pós-colheita na cadeia produtiva da macaúba, sobre todo ao nível de industrial.

**Palavras-chave:** *Acrocomia aculeata*, acidez, lipase, oxidação, peroxidase.

## 1. INTRODUCTION

Macaúba [*Acrocomia aculeata* (Jacq.) Lodd. ex. Mart] is a multipurpose thorny palm species, native of tropical forest and distributed from Mexico to Argentina (Lorenzi and Negrelle, 2006; Poetsch et al., 2012). It has great potential as a sustainable source for vegetable oils (Lorenzi and Negrelle, 2006) next to oil palm (Moura *et al.*, 2010) that could have a potential in the biodiesel industry. Its mesocarp encompasses around 70% of the total oil content in the whole fruit and features high water content that can be over than 60% in ripe fruits.

Degradation of the oil quality comes from several mechanisms, and most of them are leveraged by water availability (water activity). The amount of free fatty acid in oils, expressed as acidity index, is a major quality parameter for vegetable oils processing. The free fatty acids are released due to the hydrolysis of the triacylglycerols whose reaction can be catalyzed by endogenous lipases or coming from the associated microorganisms. Moreover, peroxidases (PODs) are involved in oxidative reactions of the oil (Sanchez-Moerno et al., 1998; Choe and Min, 2006). PODs are expressed when plants are exposed to stresses such as ultraviolet (UV) radiation, temperature extremes and other factors (Gill and Tuteja, 2010).

Irradiation is the process of exposing an object to managed level of ionizing radiation to protect stored products from spoilage (ICGFI, 1999). It can be applied to reduce post-harvest losses in fruits and vegetables such as delay ripening or senescence, prevent multiplication of pathogenic microorganisms and maintain nutritional and quality standards (Voisine et al., 1993; Loaharanu, 1994; ICGFI, 1999; Song et al., 2006). In 1980, International Atomic Energy Agency claimed that for food and health safeness radiation would require doses of less than 10 KGy (WHO, 1999). Nevertheless, negative effects of certain gamma doses on quality of agricultural products were mentioned. Fatty acid composition of soybean, peanut, and sesame were affected by higher gamma radiation dose (Afify et al., 2013). Cauliflower treated with higher dose than 10 KGy of gamma radiation showed deterioration in membrane lipids (Voisine et al., 1993), and it could be related to changes in the composition of polyunsaturated fatty acids (WHO, 1999). Peroxide and free fatty acid value in red palm increased with increasing gamma radiation dose in storage (Bangash et al., 2004). The first report on the effects of macaúba fruit gamma radiation indicated that oil content and oxidative

stability diminished, while acidity was kept steady in the mesocarp during storage of fresh fruits (Martins, 2013).

Although food radiation is currently permitted by over 50 countries and about 500,000 metric tons of food is treated annually worldwide (Farkas and Farkas, 2011), most of the people perceive radiation as risky and fancy business to be involved in. However, safety precautions and public awareness will grant confidence with the advent of modern technology. An industrial crop like macaúba, whatever improvement in quality, will have an added advantage in the value chain, though cost effectiveness is an imperative fact to be considered. Navarro-Díaz et al. (2013) reported that the actual price of crude macauba oil ranged from (US\$ 600 to US\$ 800 per ton) is lower than soybean oil (around US\$ 1100 per ton) in Brazil. The author suggested profitability of macaúba oil in biodiesel processing. In Brazil, the application of radiation technology is endorsed by National Agency of Sanitary Surveillance (ANVISA), in Board Resolution Collegiate (RDC) no. 21 in January 26, 2001 (Brasil, 2001). In view of the aforementioned success stories and legal frame work, application of gamma radiation might be an effective treatment in macaúba too. Therefore, the objective of this study was to assess the effect of different doses of gamma radiation upon the biochemical activities and physico-chemical properties of stored macaúba fruits.

## **2. MATERIAL AND METHODS**

### **2.1. Study site and sample preparation**

Fruits were collected from bunches cut from previously selected wild macaúba trees in Capela farm, Acaiaca municipality, Minas Gerais State, Brazil, in February 2014. Acaiaca is located at 20° 45'36" S latitude, 44° 15' W longitude, 481 m height above sea level, and climate is humid subtropical, CWa (Köppen and Geiger, 1928). Fruits were harvested at full maturity, sorted to uniform size and kept into mesh net bag. Each bag contained 20 fruits and this was considered as one experimental unit.

The fruits were treated with gamma radiation at Nuclear Technology Development Center (CDTN) located at the campus of Federal University of Minas Gerais (UFMG), Belo-Horizonte, Minas Gerais State, Brazil. The fruits were packed and sealed in cardboard boxes for gamma radiation treatment, before storage. The sealed boxes were settled on a rotating radiation bench and received 2 hours to reach 4 KGy and 4 hours to reach 8 KGy dose in a gamma cell chamber (Atomic Energy of Canada Limited, Canada) using Cobalt-60 as source of radiation. The control was left

untreated. Irradiated fruits were transferred to mesh net bag and transported back to Department of Plant Sciences, Federal University of Viçosa, Macaúba Post-harvest Laboratory, where they were stored for 0, 10, 20 and 30 days in open plastic box at  $23\pm 1^{\circ}\text{C}$ . Samples stored for 0 days were analyzed within 24 hour after radiation. All chemicals used were of analytical grade.

## **2.2. Physical, chemical and biochemical analysis of the mesocarp**

### **2.2.1. Physical analysis of the mesocarp and exocarp**

The fruits were manually peeled and pulped, and the mesocarp and exocarp were cut separately into pieces to measure water activity ( $a_w$ ) by using Pa<sub>w</sub>kit water activity meter (Decagon, Inc., USA) at an accuracy of  $\pm 0.02 a_w$ .

### **2.2.2. Chemical analysis of the mesocarp**

Mesocarp moisture content was determined as per the protocol developed for food and volatile materials (IAL, 1985). Five grams of mesocarp pieces were dried in an oven at  $105^{\circ}\text{C}$  until constant weight and moisture contents were determined by the difference in weight before and after drying. Mesocarp oil content was determined as per the protocol 032 /V (IAL, 1985). The oil was extracted from dried mesocarp using n-hexane as solvent in Soxhlet apparatus (for 4 h) and the hexane was recovered in a rotary evaporator.

### **2.2.3. Biochemical analysis of the mesocarp**

#### **2.2.3.1. Crude enzyme extract**

1g of mesocarp (for each enzyme) was homogenized with 20 mL of 0.1M Tris buffer at pH 8.0 (for lipase) or 6.5 (for peroxidase), using electric hand blender. The crude enzyme extract was filtered through cotton gauze and centrifuged at 6000 rpm (Excelsa<sup>TM</sup> II Centrifuge, Mod. 206 BL, Brazil) for 10 minutes. The supernatant, called crude enzyme extract, was collected and stored at  $-20^{\circ}\text{C}$  (Iaderoza and Baldini, 1991) for further analysis.

#### **2.2.3.2. Enzyme incubation and activity measurement**

Lipase activity was measured as per the protocol of Iaderoza and Baldini (1991) with modifications. A mixture of 1 mL of crude enzyme extract, 5 mL of triacetin emulsion (25% triacetin/75% of 7% gum Arabic solution) and 5 mL of 0.1M Tris HCl

buffer at pH 8.0, was incubated in water bath at 27°C/30 min. The reaction was stopped by adding 20 mL acetone: ethanol solution (1:1). The mixture, was added with 5 drops of 0.05% phenolphthalein, titrated against 0.05N NaOH. The soluble protein content of the crude enzyme extract was measured by spectrophotometer at 260 nm and 280 nm (Thermo scientific, Genesys 10UV Scanning) as per the protocol of Iaderoza and Baldini (1991). The soluble protein content was considered to calculate specific activity of the enzyme. One unit of activity (unit/mole) is defined as quantity of one micromole of fatty acids released per minute. Specific activity (U/mg) is defined as activity of enzyme per unit of protein.

Peroxidase activity was determined as per the protocol of Fatibello-Filho and Vieira (2002) with modifications. The activity was determined by tetraguaiacol formation in the blend solution of 1 mL of 50 mM Guaiacol, 1 mL of 15 mmol L<sup>-1</sup> hydrogen peroxide, 1 mL 0.1M TrisHCl and 0.5 mL of crude extract. The absorbance was measured at 470 nm after 1 minute. One unit activity (unit/mole) is defined as the increasing of 0.001 absorbance unit per minute. The soluble protein content was measured as cited above for lipase crude extract and used to calculate specific activity of the enzyme.

#### **2.2.4. Physico-chemical analysis of mesocarp oil**

Mesocarp slices were dried in oven with renewal and circulation of air (Tecnal, Model TE 394-3, Brazil) at 65°C for 12 hours. Then, oil was extracted using a manually operated hydraulic press (Prensa Ribeiro 30 Ton, Brazil). The extracted oil was stored at -20°C packed in amber glass vials wrapped with aluminum foil. Acidity and peroxide indices were determined as per AOCS (1983). Primary and secondary degree of oil oxidation was determined by molar absorptivity at 232 nm (MA<sub>232</sub>) and 270nm (MA<sub>270</sub>), respectively, according to IUPAC (1979) by diluting the oil sample in 10 mL isooctane. The oxidative stability was measured by Rancimat method (873 Biodiesel Rancimat<sup>®</sup>-Metrohm) as per AOCS (1997). Total carotene content was determined by diluting the oil samples in 10 mL petroleum ether and taking the absorbance at 450 (Rodriguez-Amaya and Kimura, 2004).

### **2.3. Experimental design and data analysis**

The experiment was conducted in completely randomized design with factorial arrangement of 3x4, 3 gamma radiation doses (0 as control, 4KGy and 8 KGy) and 4 periods of storage (0,10, 20 and 30 days) with 5 replications. Biochemical assays were



replicated eight times each, whereas physical and physico-chemical assays were replicated three times. Analysis of variance was performed by SAS (SAS institute, 2004) and difference between means was compared by using Tukey's test at  $P < 0.05$  significance level. The results are presented as mean values and data were compared with standard error of the means.

### **3. RESULTS**

The effects of gamma radiation and storage time can be seen upon each evaluated characteristic of macaúba fruit (Table 1, 2, 3, 4, 5 and 6).

Eta squared value was used to compare the magnitude of one influential factor over the other (Levine and Hullett, 2002; Blessington et al., 2007). The authors used eta squared value to explain the strength of two variables in an experiment where percent of the total variability including error term becomes 100%. Table 2-1 showed eta squared value and their percentage variation in the dependent variables. Accordingly, storage was found to be an influential factor in water mediated variables (mesocarp and exocarp water activity, mesocarp water content, specific activity of lipase and peroxidase) except mesocarp oil content and acidity index of the mesocarp oil. On the other hand, gamma dose had dominant effect on oil quality parameters (oxidative stability, molar absorptivity value at 232 nm and 270 nm, peroxide index and total carotene content) of the mesocarp oil.

Table 2-1. ANOVA components and percentage total variation in gamma irradiated macaúba fruits and its mesocarp oil during storage.

Evaluated parameters	Sum squares values of variance components			Error	Sum square total	Eta squared			Percentage total variation		
	Storage (S)	Gamma dose (D)	S x D			S	D	SxD	S	D	SxD
Specific activity of peroxidase	268241398***	74226729***	77407687**	176607487	596483301	0.450	0.124	0.130	45.0	12.4	13.0
Specific activity of lipase	483***	201*	411*	1301	2395	0.202	0.084	0.172	20.2	8.4	17.2
Mesocarp water activity	0.011***	0.003***	0.001ns	0.002	0.017	0.658	0.164	0.036	65.8	16.4	3.6
Exocarp water activity	0.018***	0.001**	0.002**	0.006	0.027	0.670	0.048	0.078	67.0	4.8	7.8
Mesocarp moisture content	5105***	83ns	206 ns	2007	7401	0.690	0.011	0.028	69.0	1.1	2.8
Mesocarp oil content	637***	286***	279**	704	1907	0.334	0.150	0.147	33.4	15.0	14.7
Oxidative stability	289***	1767***	760***	162	2979	0.097	0.593	0.255	9.7	59.3	25.5
Molar absorptivity at 232 nm	0.052**	4***	0.205***	0.210	5	0.012	0.896	0.046	1.2	89.6	4.6
Molar absorptivity at 270 nm	0.289***	0.766***	0.079***	0.067	1.2	0.240	0.637	0.066	24.0	63.7	6.6
Total carotene content	2806***	7963***	2918***	1407	15092	0.186	0.528	0.193	18.6	52.8	19.3
Peroxide index	497***	3323***	82***	141	4044	0.123	0.822	0.020	12.3	82.2	2.0
Acidity index	771***	100***	206***	21	1097	0.702	0.091	0.187	70.2	9.1	18.7

Eta squared ( $\eta^2$ ) is equal to sum of squares between groups divided by sum squares of total. Eta squared\*100= Percentage total variation. \*, \*\*, \*\*\* and ns is significant at  $P<0.05$ ,  $P<0.01$ ,  $P<0.001$  and non-significant, respectively. Variance components were taken from ANOVA table.

### 3.1. Physical, chemical and biochemical analysis

Mesocarp water activity ( $Ma_w$ ) and exocarp water activity ( $Ea_w$ ) were significantly ( $P<0.05$ ) affected by gamma radiations, storage periods and their interactions (Table 2-2). The reduction in  $Ma_w$  was significant at 10, 20 and 30 days of storage to applied gamma doses. The loss in  $Ma_w$  was higher in 4 KGy gamma dose after 20 days. The reduction in  $Ma_w$  to 4 KGy gamma dose was stabilized between 20 and 30 days of storage.

On the other hand, decrease in  $Ea_w$  was significant ( $P<0.05$ ) among all periods of storage. Although  $Ea_w$  reduced at 20 day with 4 KGy applied gamma dose, the difference was lower as compared to day 30. There is no significant difference between 4 and 8 KGy gamma dose at 0 and 10 days, but to all applied doses at day 30.

Table 2-2. Exocarp and exocarp water activity of gamma irradiated macaúba fruit with different doses after harvesting.

Gamma dose (KGy)	Mesocarp water activity (Ma <sub>w</sub> )				Exocarp water activity (Ea <sub>w</sub> )			
	Storage period (days)				Storage period (days)			
	0	10	20	30	0	10	20	30
<b>0 (control)</b>	0.9880Aa ±0.0020	0.9760Ab ±0.0024	0.9640Ac ±0.0024	0.9520Ad ±0.0020	0.9280Ba ±0.0037	0.9220Ba ±0.0037	0.9180Aa ±0.0020	0.8980Ab ±0.0039
<b>4</b>	0.9780Ba ±0.0049	0.9540Cb ±0.0040	0.9440Bc ±0.0040	0.9380Cc ±0.0020	0.9420Aa ±0.0020	0.9280ABa ±0.0037	0.8980Bb ±0.0037	0.8860Ac ±0.0024
<b>8</b>	0.9820Ba ±0.0037	0.9660Bb ±0.0024	0.9660Ab ±0.0024	0.9440Bc ±0.0038	0.9460Aa ±0.0024	0.9360Aa ±0.0024	0.9220Ab ±0.0020	0.8940Ac ±0.0131

Means in the same column followed by upper case letter, and in the same rows followed by lower case letters, are significantly different according to Tukey's test at  $*P < 0.05$ .

Mesocarp moisture content (MMC) was significantly ( $P<0.05$ ) affected by storage periods (Table 2-3). The reduction in MMC was severe at 30 days of storage. Gamma dose did not contribute reduction in MMC.

Table 2-3. Mesocarp moisture content of gamma irradiated macaúba fruit with different doses after harvesting.

<b>Treatment</b>	<b>Mesocarp moisture content (MMC)</b>
<b>Gamma radiation (KGy)</b>	
<b>0 (control)</b>	41.6a ± 2.62a
<b>4</b>	40.2a ± 3.26a
<b>8</b>	38.7a ± 2.22a
<b>Storage period (Days)</b>	
<b>0</b>	48.0a ± 1.96a
<b>10</b>	49.3a ± 3.14a
<b>20</b>	36.7b ± 2.99b
<b>30</b>	26.6c ± 2.70c

Means in the same column followed by the same letters are significantly different according to Tukey's test at  $*P<0.05$  for periods of storage in MMC.

Mesocarp oil content (MOC) was significantly ( $P<0.05$ ) different for gamma radiations, storage periods and their interactions (Table 2-4). It was increased at 10 and 30 days of storage for control treatment (32.5%). However, the fruit accumulate more oil at day 30 for all applied gamma doses except in 8 KGy gamma dose where it was stabilized between 20 and 30 days of storage.

Table 2-4. Mesocarp oil content of gamma irradiated macaúba fruit with different doses after harvesting.

Gamma dose (KGy)	Mesocarp oil content (MOC, % dry base)			
	Storage period (days)			
	0	10	20	30
0 (control)	47.1Ac ±0.6	58.6Ab±3.0	55.3Ab±4.0	62.4Aa±0.9
4	49.2Ab±1.0	50.5Bb±0.7	49.9Bb±1.2	54.9Ba±0.9
8	48.2Ab±2.0	48.5Bb±0.6	53.7Aa±0.8	54.7Ba±0.3

Means in the same column followed by upper case letter, and in the same rows followed by lower case letters, are significantly different according to Tukey's test at  $*P<0.05$  for MOC.

Both specific activity of lipase (SAL) and peroxidase (SAP) were significantly ( $P<0.05$ ) affected by gamma radiations, storage periods and their interactions (Table 2-5). Though tended to reduce at day 20 for SAL in 4 KGy gamma dose, the difference was non-significant along doses after day 10. Whereas in SAP tended to reduce at 20 and 30 days in 4 KGy gamma dose. SAL stabilized to all applied gamma doses after 0 day in 4 KGy gamma doses, following a reduction tendency.

Table 2-5. Specific activity of lipase and peroxidase in gamma irradiated macaúba fruit with different doses after harvesting.

Gamma dose (KGy)	Specific activity of lipase (U/mg)				Specific activity of peroxidase (U/mg)			
	Storage period (days)				Storage period (days)			
	0	10	20	30	0	10	20	30
0 (control)	13.14Aa ±2.2	9.17Ba±2.0	10.92Ba±1.8	8.68Aa±1.0	1238Ac±119	6893Aab±659	8572Aa±774	6275Ab±686
4	16.05Aa ±2.1	7.42Bb±0.9	4.44Cb±0.7	6.75Ab±2.5	1149Ac±120	6011ABa±634	3997Bb±1454	2385Bbc±294
8	15.37Aa ±2.2	14.89Aa±2.5	17.14Aa±5.4	5.08Ab±0.6	2353Ac±808	4837Bb±982	9376Aa±1452	6415Ab±985

Means in the same column followed by upper case letter, and in the same rows followed by lower case letters, are significantly different according to Tukey's test at  $*P<0.05$ .

### 3.2. Physico-chemical analysis of mesocarp oil

Acidity index (AI) was significantly ( $P<0.05$ ) different for gamma radiations, storage periods and their interactions (Table 2-6). The decrease in AI was significant ( $P<0.05$ ) to all applied gamma doses at 20 and 30 days. AI was reduced highly at 20 day in 4 KGy gamma dose. However, AI was below the maximum limit of 5% as per ANVISA (Brasil, 2005) and (PORIM, 2011) throughout the entire periods of storage in 4 KGy gamma dose.

Peroxide index (PI) was significantly ( $P<0.05$ ) different for gamma radiations, storage periods and their interactions (Table 2-6). The difference in PI was significant ( $P<0.05$ ) at 20 and 30 days to applied gamma doses. Though PI was below the maximum limit of 10 meq O<sub>2</sub>/kg as per ANVISA (Brasil, 2005) in control and 4 KGy gamma dose, it was lower in control treatment.

Molar absorptivity (MA) value for MA<sub>232</sub> and MA<sub>270</sub> was significantly ( $P<0.05$ ) different for gamma radiations, storage periods and their interactions (Table 2-6). The difference was significant ( $P<0.05$ ) to all applied gamma doses at 0, 20 and 30 days in MA<sub>232</sub>, but at day 30 for MA<sub>270</sub>. The increase in MA value was lower in 4 KGy gamma dose. There is no established Brazilian standard for MA. However, Nunes et al. (2015) reported MA values of 2.04 at 232 nm and 0.56 at 270 nm in the crude mesocarp oil of non-irradiated macaúba fruit. Accordingly, both MA<sub>232</sub> and MA<sub>270</sub> were below the value of this report.

Oxidative stability (OS) was significantly ( $P<0.05$ ) different for gamma radiations, storage periods and their interactions (Table 2-6). OS was significant ( $P<0.05$ ) across all periods of storage, but it fulfills the biodiesel standard of 6 h as per Barabás and Todoruț (2011) after 0 day in 4 KGy gamma dose, and until 20 day for control treatment.

Total carotene content (TCC) was significantly ( $P<0.05$ ) different for gamma radiations, storage periods and their interactions (Table 2-6). TCC was significant ( $P<0.05$ ) at 20 and 30 days to all applied gamma doses. The control treatment out smart the gamma treated fruits. It was increased until 20 day and then declined sharply until the 30<sup>th</sup> day.



Table 2-6. Physico-chemical analysis of mesocarp oil of gamma irradiated stored macaúba fruit along storage periods.

Gamma dose (KGy)	Acidity index (AI, % oleic acid)				Peroxide index (meq O <sub>2</sub> /kg oil)				Molar absorptivity at 232 nm			
	Storage period (days)				Storage period (days)				Storage period (days)			
	0	10	20	30	0	10	20	30	0	10	20	30
0	2.75Abc ± 0.07	2.41Ac ± 0.28	3.36Ab ± 0.24	11.80Ba ± 0.49	3.16Bb ± 0.14	2.94Bb ± 0.61	5.32Ca ± 0.38	6.68Ca ± 0.81	0.31Cb ± 0.00	0.32Bb ± 0.01	0.35Bb ± 0.00	0.50Ba ± 0.02
4	2.41Abb ± 0.16	1.52Bc ± 0.20	0.77Cd ± 0.00	4.36Ca ± 0.19	2.60Bc ± 0.29	2.49Bc ± 0.29	6.66Bb ± 0.60	8.96Ba ± 0.95	0.37Ba ± 0.02	0.29Bb ± 0.01	0.29Cb ± 0.01	0.39Ca ± 0.02
8	2.11Bb ± 0.15	1.25Bc ± 0.13	1.66Bbc ± 0.21	14.69Aa ± 0.68	16.74Ac ± 1.36	16.74Ac ± 0.30	21.74Ab ± 1.28	27.29Aa ± 0.93	0.92Ab ± 0.06	0.85Ac ± 0.05	1.01Aa ± 0.03	0.82Ac ± 0.05

Gamma dose (KGy)	Molar absorptivity at 270 nm				Oxidative stability (hour)				Total carotene content (mg/kg)			
	Storage period (days)				Storage period (days)				Storage period (days)			
	0	10	20	30	0	10	20	30	0	10	20	30
0	0.11Bc ± 0.01	0.12Bc ± 0.01	0.17Bb ± 0.01	0.34Ba ± 0.03	9.54Aa ± 0.63	9.40Ba ± 1.45	7.89Ba ± 0.87	5.51Bb ± 0.54	83.44Ac ± 1.46	101Ab ± 1.49	126Aa ± 1.38	105Ab ± 2.34
4	0.12Bb ± 0.00	0.13Bb ± 0.01	0.14Bb ± 0.01	0.21Ca ± 0.01	5.70Bd ± 0.49	15.45Ab ± 0.93	25.17Aa ± 1.25	11.92Ac ± 1.25	85.56Ac ± 1.95	99Aa ± 0.58	102Ba ± 1.54	92Bb ± 1.89
8	0.27Ad ± 0.01	0.39Ac ± 0.01	0.46Ab ± 0.02	0.50Aa ± 0.04	1.67Ca ± 0.25	1.03Ca ± 0.23	0.73Ca ± 0.22	1.66Ca ± 0.41	76.54Bb ± 2.69	82Ba ± 4.44	74Cb ± 2.91	72Cb ± 3.63

Means in the same column followed by upper case letter, and in the same rows followed by lower case letters, are significantly different according to Tukey's test at  $*P < 0.05$ .

## **4. DISCUSSION**

### **4.1. Magnitude of variability explained by Eta squared**

As indicated in table 2-1, the magnitude of variability explained by storage was higher in water mediated parameters. It includes physical, chemical and biochemical parameters. Physical parameters were higher in exocarp (67%) than mesocarp (65%) water activity. Chemical parameters were higher in mesocarp moisture content (69%) than mesocarp oil content (33.4%) to applied treatments. Among physico-chemical parameters, only acidity (70%) was affected by storage. Gamma dose affected oil mediated parameters such as oxidative stability (59%), peroxide index (82.3), molar absorptivity at 232 nm (89%) and 270 nm (64%), and total carotene content (53%). Proportion of total variance explained by interaction was less than gamma dose, except in, specific activity of lipase, exocarp water activity and acidity index. There was a large variability unexplained by storage, gamma dose and their interaction of the independent variables, except molar absorptivity at 232 nm followed by peroxide index, whereby most of the variability is accounted for treatment factors. This implied that elevated gamma dose beyond the optimum increase chance of oil oxidation. However, acidity was affected by storage time, which is a function of natural or biological water loss.

### **4.2. Effect of gamma radiation upon physical, chemical, biochemical and physico-chemical analysis**

The loss of water in storage is associated either due to biological process in the fruit or natural phenomena in the environment. The mesocarp moisture content (MMC) and water activity ( $Ma_w$ ) was reduced by 24.4% and 2.1%, respectively at the 30<sup>th</sup> day of storage. However, neither gamma dose nor the interaction was significant in MMC. Consequently, there is no reduction of moisture content. Nevertheless, gamma dose contributed to 4.1% reduction in  $Ma_w$ . The small reduction in  $Ma_w$  attributed to its fibrous and mucilaginous nature (Lorenzi and Negrelle, 2006) of the mesocarp. In such matrixes, water movement is impeded and thereby small change in  $a_w$ . Therefore, complete inactivation of the enzymes was impossible after 0 day due to applied gamma radiation.

Although lignified, the exocarp is brittle and porous (Reis et al., 2012) as compared to the mesocarp so that they respond differently to applied gamma dose. In this study, the average mesocarp moisture content of fresh fruits ranged from 47.1-

49.6%. Apart from variability in genotypes, ripening stage is another important factor for moisture content. Assaying moisture content in oil palm used as an indicator in oil quality parameters and predict changes during storage (Ngando-Ebongue et al., 2013). Moreover, presence of moisture has cost implications in oil extraction process.

The reduction in water activity and mesocarp moisture content contributed to corresponding decline in specific activity of lipase (SAL). Though SAL stabilized after 0 day following reduction, the difference was significant to all applied doses at day 20, in 4 KGy gamma dose. Therefore, application of this dose could come up with conformational changes in the enzyme. A 4 KGy gamma dose resulted in significant reduction in  $M_{a_w}$  and associated inactivation of the enzyme. On the other hand, the reduction of SAL in 8 KGy gamma dose might be linked to the high amount of water activity that led to consumption of the substrate. Though there is a decreasing tendency of SAL in 4 KGy gamma dose until 20 day, the difference was non-significant after 0 day. However, the reduction in SAL in 4 KGy gamma dose was significant as compared to 8 KGy dose at 20 day. This might linked to partial inactivation of lipase in lower dose. Similarly, partial inactivation of lipase using lower dose (>12 KGy) extend shelf life than higher dose (>35 KGy) in wheat germ was reported (Jha et al., 2013). While in oil palm, lipase is most active enzyme. So that microwave sterilization is suggested in oil palm (Sarah and Taib, 2013). This is because the oil is easily hydrolyzed to FFA as compared to macaúba in storage.

Unlike lipase, SAP was increased continuously until the 20<sup>th</sup> day in control and 8 KGy gamma dose along storage (Table 4). The availability of moisture sustained the enzyme along the applied doses. Since the availability of moisture leads to consumption of substrate in 8 KGy dose so that stress-induced production of SAP occurred in storage. Peroxidases are expressed when plants are exposed to stresses such as ultraviolet radiation (Gill and Tuteja, 2010). Therefore, increased dose to 8 KGy gamma dose resulted in oxidation (Table 3). This is because, gamma radiation remove electron from water to form reactive free radicals that can initiate auto oxidation (Kilcast, 1994). Free radicals are unstable and have a potential to attack lipids. High water content and oil content in amcaúba facilitate the oxidation process. This is because release of free radicals and radiolysis of water resulted in lipid oxidation during high gamma radiation (Afify et al., 2013). However, decline of SAP after 10 day in 4 KGy gamma dose was observed. This might be related to radiolysis of water during radiation come up with partial inactivation of peroxidase (Table 4).

In this study, the average mesocarp oil content in fresh fruits ranged from 47.1-49.2% (Table 2-4) which was closed to 51.7% (Ferrari and Azevedo-Filho, 2012) but less than 55.9-69.9% (CETEC, 1983). The variability might be linked to harvesting time, place and ripening stage. The oil content increased across all treatments with the higher being attributed to control (33% increase) as compared to gamma dose (12% increase at the end of storage periods (Table 2-5). A 22% difference offers an advantage to both producer and industry. The increase in oil associated with climacteric property of macaúba fruit (Goulart, 2014) that favored conversion of storage reserves to oil. In contrary to this study, an increase in gamma radiation dose from control to 10 KGy reduced the oil content in the 60 days of storage in macaúba fruits (Martins, 2013). This implied that higher gamma dose interfered in the metabolism process of the fruit that decreases oil accumulation. This result may be due to stage of ripening and applied gamma dose.

#### **4.2. Effect of gamma radiation upon physico-chemical analysis**

Oxidation of oil at 8 KGy gamma dose associated with formation of peroxides and its decomposition to conjugated dienes and trienes explained by  $MA_{232}$  nm and  $MA_{270}$  nm. Besides, the reduction in total carotene content and oxidative stability explain this phenomena at 8 KGy. However, synthesis of TCC was observed in control and 4 KGy gamma dose that attributed to higher OS.

Acidity index (AI) was in accordance with the crude palm oil standard limit of 5% at maximum as per ANVISA (Brasil, 2005) and (PORIM, 2011) in the entire duration of storage in 4 KGy gamma dose, but to all applied gamma doses until day 20. This might be associated with partial inactivation of lipase that led to reduced AI to applied gamma dose. This fact also accompanied with decreased  $Ma_w$  and  $Ea_w$  that impair SAL activity. Acidity is resulted from conversion of triacylglycerols (TAGs) to free fatty acids with the help of lipase in the presence of moisture. However, hydrolysis in macaúba was increased with associated reduction of  $a_w$  and SAL after day 20. The increase in AI observed at day 30 for control and 8 KGy gamma radiation dose, seems to be related to different mechanisms. Lipase stood in the highest activity for 8 KGy dose up to 20 days of storage, but it was not come along with higher AI. Instead, when SAL activity decreased to the lowest level, AI reached the maximum for this fruits. Non irradiated fruit presented increasing AI along storage, whereas SAL remained constant.

Maybe changes in membranes due to high dose of gamma radiation could promote a higher interaction between enzymes and substrates.

On the other hand, rise in AI with increasing gamma dose is due to the interaction of free radicals with the lipids. The oxidation of lipids explained by peroxides might be attributed to increased acidity. Formation Similar results were reported in soybean and sunflower (Lutfullah et al., 2003), in stored red palm oil (Bangash et al., 2004) and in black cumin (Arici et al., 2007). On the contrary, reduced AI was reported in the mesocarp oil of macaúba fruit when gamma radiation dose was increased from 0 (45.45% o.a) to 10 KGy (25.58% o.a) during the 60 day of storage (Martins, 2013). This may be related to difference in fruit ripening stage and harvesting time.

Oxidative reactions were also catalyzed by enzymes such as peroxidase or non enzymatic such as exposure to gamma radiation. The reaction of free radicals with lipids at their double bonds leads to oxidation. This may be exhibited by the formation of peroxides and hydro peroxides during lipid oxidation as observed in 8 KGy gamma dose in this study. Similarly, increased PI with increased gamma radiation dose was reported in stored red palm oil (Bangash et al., 2004) and in crude soybean oil (Mahrous, 2007). However, peroxide index (PI) was in line with the crude palm oil standard of 10 meq O<sub>2</sub>/kg oil at maximum as per ANVISA (Brasil, 2005) in control and 4 KGy gamma radiation dose (Table 5). Increased TCC content in control and 4 KGy gamma dose was attribute to reduced PI. Presence of high mono-unsaturated fatty acids in macaúba mesocarp oil led to low peroxide formation (Nunes et al., 2015).

Molar absorptivities (MA) are both identity and quality indexes of vegetable oils (IUPAC, 1979). They measure oxidation status of the oil. The result of this study was below the findings of Nunes et al. (2015) for both MA<sub>232</sub> and MA<sub>270</sub> in non gamma irradiated and stored macaúba fruits. The rise in peroxides was in line with MA<sub>232</sub>. Similarly, the low MA<sub>232</sub> value was led to small increase in MA<sub>270</sub> values though the degradation reaction continued. There is no expectation of secondary oxidative compounds measured by MA<sub>270</sub> in the absence of MA<sub>232</sub> (Nunes et al., 2015). A gamma dose above the recommended would create secondary compounds as a result of trienes. However, the optimum gamma dose reduces their formation. Therefore, they could be employed to assess further oil quality changes in gamma irradiated macaúba mesocarp oil.

Oxidative stability (OS) is the resistance of oils to oxidation during processing and storage (Guillen and Cabo, 2002), so that it determines oil quality and shelf life (Hamilton, 1994). OS can be measured by using accelerated test in biodiesel (Knothe, 2007). The OS value kept increasing until day 20 in control and 4 KGy gamma dose along periods of storage. This might be linked to synthesis of TCC that manage the amount of peroxides production and their associated decomposition to MA<sub>232</sub> and MA<sub>270</sub> during storage. However, increase in gamma dose from control to 10 KGy resulted in reduction of OS from 7.72 to 0.62 h in black cumin oil (Arici et al., 2007). That could be related to oxidation state of the oil beyond the optimum dose as indicated in 8 KGy gamma dose. Similarly, reduced OS was reported in the mesocarp oil of gamma irradiated macaúba fruit in the 60 days of storage (Martins, 2013). However, the maximum OS value of 25.2 h in this study was equivalent to the 25 h in the non-irradiated macauba fruit (Melo et al., 2014). This confirms the better oxidation stability of macaúba oil in storage and processing that offers additional advantage for further industrial applications such as biodiesel in gamma irradiated macaúba fruits.

Carotenoids are involved in protecting photo oxidative damage to photosynthesis apparatus. In oleaginous plant like macaúba, the climacteric property of the fruit leads to synthesis of TCC. Since gamma radiation interferes in metabolism of the fruit, reduction of TCC is expected. Although the fruit continue respiring, synthesis of TCC in control treatment might be related to increase in oil content. Macaúba mesocarp has ample  $\beta$ -carotene content (Coimbra and Jorge, 2012) that offers greater stability against rancidity. However, the free radicals generated during gamma radiation led to lipid oxidation. Moreover, storage conditions, activity of lipoxygenases and other enzymes coupled with lipid oxidation ended in degradation of carotenoids (Dutta et al., 2005). Although  $\beta$ -carotene content was not quantified in this study, it might be referred in relation to TCC content in this study. An increased dose from control to 20 KGy gamma radiation decreased  $\beta$ -carotene content in sunflower and soybean oils (Lutfullah et al., 2003), and reduced by half in red palm oil (Bangash et al., 2004). This in turn could reduce the antioxidant activity and the oil stability. However, stress related production of SAP until 20 day in control treatment might induce synthesis of carotene (TCC) to protect against oxidation in this study.

## 5. CONCLUSIONS

Water mediated parameters (SAP, SAL, MMC,  $M_{a_w}$  and  $E_{a_w}$ ) with the exception of MOC and AI were highly affected by storage time. On the other hand, oil quality related parameters (OS, MA at 232 and 270 nm, TCC and PI) were highly affected by gamma dose. Microwave sterilization is recommended for the active lipase of oil palm as compared to macaúba. The 8 KGy gamma dose led to oil oxidation in the entire periods of storage. Gamma radiation decreased oil accumulation as compared to control along storage periods. However, application of 4 KGy gamma dose decreased water activity, partially inactivated the enzymes, and kept the acidity, oxidative stability and other oil quality indexes within the satisfactory limits for 30 days of storage. Although the installation cost is high, comparative advantage of gamma radiation could be recommended as one possible option in postharvest technology of macaúba production.

## 6. REFERENCES

- Afify, A.M.R.; Rashed, M.M.; Ebtesam, A.M.; El-Beltagi, H.S. 2013. Effect of gamma radiation on the lipid profiles of soybean, peanut and sesame seed oils. doi: 10.3989/gya.119712.
- AOCS (American Oil Chemists' Society). 1983. Official and Tentative Methods. Champaign, Illinois.
- AOCS. 1997. Official Method Cd 12b-92. American Oil Chemists' Society. Champaign, Illinois.
- Aquino, K. A. S. 2012. Sterilization by Gamma Radiation. Adrovic, F. (Ed.). ISBN: 978-953-51-0316-5, InTech. Available from: <http://www.intechopen.com/books/gamma-radiation-sterilization-by-gamma-radiation>. Retrieved on 22 July 2014.
- Arici, M.; Colak, F.A.; Gecgel, Ü. 2007. Effect of gamma radiation on microbiological and oil properties of black cumin (*Nigella sativa* L.). **Grasas Aceites** 58 (4): 339-343.
- Bangash, F.K.; Ahmad, T.; Atta, S.; Zeb, A. 2004. Effects of radiation on the storage stability of red palm oil. **Journal of Chinese Chemical Society** 51: 991-995.
- Barabás, I.; Todoruț, I.A. 2011. Biodiesel Quality, Standards and Properties. In: Biodiesel-quality-emissions-and-by-products.<http://www.intechopen.com/>. Retrieved in December 2013.
- Blessington, T.; Miller, C.J. Jr.; Nzaramba, M.N.; Hale, A.L.; Redivari, L.; Scheuring, D.C.; Hallman, G. J. 2007. The effects of low-dose gamma radiation and storage time

on carotenoids, antioxidant activity, and phenolics in the potato cultivar Atlantic. **American Journal of Potato Research** 84:125-131.

Brasil. 2001. Resolução RDC nº 21, de 26 de janeiro de 2001. ANVISA - Agência Nacional de Vigilância Sanitária. D.O.U. Diário Oficial da União; Poder Executivo, de 29 de janeiro de 2001. Retrieved in 30 December 2013.

Brasil. 2005. Ministério da Saúde. Agência Nacional de Vigilância Sanitária. Resolução RDC/ANVIS/MS nº 270, de 22 setembro de 2005. Regulamento técnico para óleos vegetais, gorduras vegetais e creme vegetal. Diário Oficial da República Federativa do Brasil, Brasília, DF, 23 set. 2005. Seção1. Available in: <http://www.anvisa.gov.br>. Retrieved in 30 December 2013.

CETEC (Fundação Centro Tecnológico de Minas Gerais).1983. Produção de combustíveis líquidos a partir de óleos vegetais: relatório final do Convênio STI- MIC/ CETEC. Vol. 1 e 2. CETEC. Belo Horizonte.

Choe, E.; Min, D.B. 2006. Mechanisms and factors for edible oil oxidation: comprehensive reviews. doi: 10.1111/j.1750-3841.2006.00097.x

Ciconini,G.; Favaro,S.P.; Roscoe,R.; Miranda,C.H.B.; Tapeti,C.F.; Miyahira, M.A.M.; Bearari, L.; Galvani, F.; Borsato, A.V.; Colnago, L.A.; Naka, M.H. 2013. Biometry and oil contents of *Acrocomia aculeata* fruits from the Cerrados and Pantanal biomes in Mato Grosso do Sul, Brazil. **Industrial Crops and Products** 45: 208-214.

Cinquanta, L.; Esti, M.; Matteo, M. D. 2001. Oxidative Stability of Virgin Olive Oils. **Journal of American Oil Chemists' Society** 78 (12): 1197-1202.

Clodoveo, M.; Delcuratolo, D.; Gomes, T.; Colelli, G. 2007. Effect of different temperatures and storage atmospheres on Croatian olive quality. **Food Chemistry** 102: 571-576.

Coimbra, M.C.; Jorge, N. 2012. Fatty acids and bioactive compounds of the pulps and kernels of Brazilian palm species, guariroba (*Syagrus oleraces*), jervá (*Syagrus romanzoffiana*) and macaúba (*Acrocomia aculeata*). **Journal of Food Science and Agriculture** 92: 679–684.

Dutta, D.;Chaudhuri, U.R.; Chakraborty, R. 2005. Structure, health benefits, antioxidant property and processing and storage of carotenoids-review. **African Journal of Biotechnology** 4 (13): 1510-1520.

Farkas, J.; Farkas, C. M. 2011. History and future of food radiation. **Trends in Food Science and Technology** 22 (2-3): 121-126.

Fatibello-Filho, O.; Vieira, C. I. 2002. Uso analítico de tecidos e de extratos brutos vegetais como fonte enzimática. **Química Nova** 25 (3): 455-464.



- Ferrari, R.A.; Azevedo Filho, J. A. 2012. Macaúba as promising substrate for crude oil and biodiesel production. **Journal of Agricultural Science and Technology B2**: 1119-1126.
- Gill, S.S.; Tuteja, N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants: a review. **Plant Physiology and Biochemistry** 48: 909-930.
- Goulart, S.M. 2014. Amadurecimento pós-colheita de frutos de macaúba e qualidade do óleo para a produção de biodiesel. M.Sc thesis, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brasil.
- Guillen, M.D.; Cabo, N. 2002. Fourier transform infrared spectra data versus peroxide and anisidine values to determine oxidative stability of edible oils. **Food Chemistry** 77: 503–10.
- Hamilton, R. J. 1994. The chemistry of rancidity in foods. In: Allen, J.C. and Hamilton R.J. (eds). **Rancidity in foods**. 3rd ed. London: Blackie Academic and Professional. p. 1–21.
- Hartley, C.W.S. 1988. The oil palm (*Elaeis guineensis* Jacq.). Third edition, London: Longman.
- Iaderoza, M.; Baldini, V. L. S. 1991. A importância da análise enzima em alimentos. In: Iaderoza, M; Baldini, V. L. S. **Ênzimos e a qualidade de vegetais processados**. Campinas: ITAL, 1991. (Manual Técnico).
- IAL (Instituto Adolfo Lutz). 1985. Normas analíticas do Instituto Adolfo Lutz, Vol. 1: Métodos Químicos e Físicos para Análise de Alimentos, 3<sup>a</sup> ed., p.22. , IMESP, São Paulo.
- IAL (Instituto Adolfo Lutz). 1985. Normas analíticas do Instituto Adolfo Lutz, Vol. 1: Métodos Químicos e Físicos para Análise de Alimentos, 4<sup>a</sup> ed., p.116-118, IMESP, São Paulo.
- ICGFI (International Consultative Group on Food Radiation). 1999. Facts about food radiation. Vienna, Austria.
- IUPAC (International Union of Pure and Applied Chemistry). 1979. Standard methods for the analysis of oils, fats and derivatives. Oxford, 6<sup>th</sup> ed., IUPAC, 1979.
- Jha, P.K.; Kudachikar, V.B.; Kumar, S. 2013. Lipase inactivation in wheat germ by gamma radiation. **Radiation Physics and Chemistry** 86:136–139.
- Kilcast, D. 1994. Effect of radiation on vitamins. **Food Chemistry** 49: 157-164.
- Knothe, G. 2007. Some aspects of biodiesel oxidative stability: review. **Fuel Processing Technology** 88: 669–677.

Köppen, W. P.; Geiger, R. 1928. Die Klimate der Erde: Grundriss der Klimakunde. Berlin, Leipzig: Waltr de Gruyter.

Levine, T.R.; Hullet, C.R. 2002. Eta squared, partial eta squared, and misreporting the effects size in communication research. **Human Communication Research** 28: 612-625.

Loaharanu, P. 1994. Food radiation in developing countries: A practical alternative. IAEA Bulletin, 1/1994.

Lorenzi, G. M. A. C.; Negrelle, R. R. B. 2006. *Acrocomia aculeata* (Jacq.) Lodd. EX Mart.: Ecological aspects, uses and potentialities. Available from: [http:// www.entabanbrasil.com.br](http://www.entabanbrasil.com.br). Retrieved on 21/04/2014.

Lutfullah, G.; Zeb, A.; Ahmad, T.; Atta, S.; Bangash, F.K. 2003. Changes in the quality of sunflower and soybean oils induced by high doses of gamma radiations. **Journal of Chemistry Society of Pakistan** 25 (4): 269-275.

Mahrous, S.R. 2007. Chemical properties of *Aspergillus flavus* infected soybean seeds exposed to  $\gamma$ -radiation during storage. **International Journal of Agriculture and Biology** 9 (2): 231–238.

Maltini, E.; Torreggiani, D.; Vieira, E.; Bertolo, G. 2003. Water activity and the preservation of plant foods. **Food Chemistry** 82: 79-86.

Mariano, R.G.B.; Silva,C.M.; Couri, S.; Nogueira, R.I.; Freitas, S.P. 2011. Partition of Free Fatty Acids in Deacidification of Macaúba Pulp Oil by Liquid-Liquid Extraction using Ethanol/Water as Solvent. **Defect and Diffusion Forum** 312-315: 554-559.

Martins, A. D. 2013. Uso da radiação gama e secagem para a conservação da qualidade do óleo de frutos de macaúba. M.Sc thesis, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brasil.

Melo, M. A.R.; Melo, M.A.M.F.; Silva, E.V.; Filho, J.R.C; Souza, A.G. 2014. Study of the oxidative stability of vegetables oils for Biodiesel production. **Revista Verde** 9 (1): 84-88, Mossoró – RN – BRASIL.

Moura, E.F.; Ventrella, M.C.; Motoike, S.Y. 2010. Anatomy, histochemistry and ultrastructure of seed and somatic embryo of *Acrocomia aculeata* (Arecaceae). **Agricultural Sciences**, Piracicaba, 8: 399-407.

Nabil, B. Y.; Youssef, O.; Nizar , D.; Bechir , B.; Chedly, A.; Mokhtar, Z. 2012. Effect of olive storage period at two different temperatures on oil quality of two Tunisian cultivars of *Olea europea*, Chemlali and Chétoui. **African Journal of Biotechnology** 11 (4): 888-895.

Navarro-Díaz,H.; Gonzalez,S.L.; Irigaray,B.; Vieitez,I; Jachmanián,I; Carciofi,B.A.M; Oliveira,J.V. 2013. Macauba oil as an alternative feedstock for biodiesel: characterization and conversion to biodiesel by the supercritical method. III

Iberoamerican Conference on Supercritical Fluids Cartagena de Indias (Colombia), 2013.

Ngando-Ebongue, G.F.; Mpondo-Mpondo, E.A; Ewane, M.A. 2013. Some quality parameters of crude palm oil from major markets of Douala, Cameroon. **African Journal of Food Science** 7 (12): 473-478.

Nunes, A. A.; Favaro, S.P.; Galvani, F.; Miranda, C.H.B. 2015. Good practices of harvest and processing provide high quality Macaúba pulp oil. [10.1002/ejlt.201400577].

Oraei, M.; Motalebi, A. A.; Hoseini, E.; Javan, S.; Hemmasi, A. H. 2011. Effect of Gamma Radiation on Fatty Acid Composition of Rainbow Trout (*Oncorhynchus mykiss*) Fillets. **Iran Journal of Fish Science** 10 (2): 276-285.

Poetsch, J., Haupenthal, D.; Lewandowski, I.; Oberländer, D.; Hilger, T. 2012. *Acrocomia aculeata* – a sustainable oil crop. Rural 21 – 03/2012. **Science World** 41-44.

PORIM. 2011. Standard Specifications for Processed Palm Oil. PORIM Handbook. <http://www.poram.org>. Retrieved on 29/4/2012.

Reis S, B.; Mercadante-Simões, M.O.; Ribeiro, L.M. 2012. Pericarp development in the macaw palm *Acrocomia aculeata* (Arecaceae). **Rodriguésia** 63 (3): 541-549.

Rodriguez-Amaya, D.B.; Kimura, M. 2004. Harvest plus Handbook for Carotenoid Analysis. Washington, DC and Cali, IFPRI and CIAT, p.58.

Sanchez-Moreno, C.; Larrauri, J. A.; Saura-Calixto, F. 1998. A procedure to measure the antiradical efficiency of polyphenols. **Journal of Science and Food Agriculture** 76: 270-276.

Sarah, M; Taib, M.R.. 2013. Enzymatic Destruction Kinetics of Oil Palm Fruits by Microwave Sterilization. **International Journal of Chemical Engineering and Applications** (4):3129-133.

SAS Institute, 2004. Base SAS 9.1.3: Procedures guide. Cary, NC.

Song, H-Pa; Kima, D-Ho; Joa C.; Lee, C-Ho; Kimc, K-Soo; Byun, M-Woo. 2006. Effect of gamma radiation on the microbiological quality and antioxidant activity of fresh vegetable juice. **Food Microbiology** 23: 372–378.

Voisine, R.; Vézina, L.P.; Willemot, C. 1993. Modification of phospholipid catabolism in microsoma membranes of  $\gamma$ -Irradiated cauliflower (*Brassica oleracea* L.). **Plant Physiology** 102: 213-218.

Wang, Z.; Ma, Y.; Zhao, G.; Liao, X.; Fang, C.; Wu, J.; Chen, J.; Hiu, X. 2006. Influence of gamma radiation on enzyme, microorganism, and flavor of cantaloupe (*Cucumis melo* L.) juice. **Journal of Food Sciences** 71 (6): 215-220.

WHO (World Health Organization). 1999. High-dose radiation: wholesomeness of food irradiated with doses above 10 KGy. WHO Technical Report Series 890, Report of a Joint FAO/IAEA/WHO Study Group, World Health Organization, Geneva.

## CHAPTER 3

### EFFECT OF DRYING IN THE MESOCARP OIL QUALITY OF STORED MACAÚBA FRUIT

#### ABSTRACT

Macaúba fruit is a potential source of vegetable oil in biodiesel production. The high moisture content of the mesocarp is an important factor to be faced in order to prevent against oil quality degradation. This study assessed the effect of drying temperatures on biochemical activities and physico-chemical properties during periods of storage. The experiment was conducted in a completely randomized design with factorial arrangement of 3 drying temperatures by 4 periods of storage with 5 replications. Ripe fruits were stored under room conditions and after 0, 10, 20 and 30 days, groups of 20 fruits were separated and oven dried at 0, 60 and 100 °C for 24 hours. Physical, chemical and biochemical (water activity of the mesocarp and exocarp, moisture and oil content of the mesocarp, and specific activity of lipase and peroxidase) and oil quality parameters (acidity index, peroxide index, molar absorptivity at 232 nm and 270 nm, oxidative stability and total carotene content) were studied. Water mediated parameters were affected by temperature while most of oil based parameters were influenced by storage period. Reduction of water activity and mesocarp moisture content was followed by decreased enzymatic activities. Lipase was less stable than peroxidase to drying temperatures. Drying could not favor reduction in acidity. Oil content, oxidative stability and total carotene content were increased when fruits were dried at 100°C after some time of storage. Macaúba fruits dried at 100°C after 30 day presented reduced enzymatic activities and managed oil quality of the mesocarp in storage. This signifies that drying could be suggested as a postharvest treatment to improve oil quality in macaúba production.

**Key words:** *Acrocomia aculeata*, enzymes, oil content, oxidative stability, total carotene content, water activity.

## **EFEITO DE SECAGEM NA QUALIDADE DO ÓLEO DO MESOCARPO DE FRUTOS DE MACAÚBA DURANTE O ARMAZENAMENTO**

### **RESUMO**

Macaúba é uma fonte de óleo vegetal com alto potencial para produção de biodiesel. O alto teor de umidade do mesocarpo é uma questão importante a ser enfrentada, a fim de prevenir contra degradação de qualidade do óleo. Este estudo foi realizado para avaliar o efeito da temperatura de secagem nas atividades bioquímicas e propriedades físico-químicas, durante os períodos de armazenamento. O experimento foi conduzido no delineamento inteiramente casualizado, num esquema fatorial duplo 3x4, 3 temperaturas de secagem (23 °C controle, 60°C e 100°C) por 24 horas e 4 períodos de armazenamento (0, 10, 20 e 30 dias) com 5 repetições. Física, química e bioquímica (atividade de água do mesocarpo e epicarpo, umidade e teor de óleo do mesocarpo, atividade específica de lipase e peroxidase) e qualidade do óleo (índice de acidez, índice de peróxido, absorvibilidade molar a 232 nm e 270 nm, estabilidade oxidativa e conteúdo de carotenóides totais) foram avaliados. Parâmetros relacionados com água afetados pela temperatura enquanto a maioria dos a qualidade do óleo influenciados pelo tempo de armazenamento. Redução da atividade de água e teor de umidade do mesocarpo resultou diminuição de atividades enzimáticas. A lipase foi menos estável do que a peroxidase para temperatura de secagem. Redução de acidez não foi favorecido pela secagem. Teor de óleo, estabilidade oxidativa e teor de carotenóides totais foram aumentados para 100°C após algum tempo de armazenamento. A secagem do fruto da macaúba a 100°C após 30 dias de armazenamento, reduziu atividade das enzimas e manteve a qualidade do óleo do mesocarpo. Isto significa que secagem a uma tratamento pós-choleita para melhorar qualidade de óleo em produção de macaúba.

**Palavras-chave:** *Acrocomia aculeata*, atividade de água, enzima, estabilidade oxidativa, teor de óleo, teor de carotenóides totais.

## 1. INTRODUCTION

Depletion of natural fossil fuel reserves coupled with global warming in our planet challenges energy demand and energy sufficiency and, by large, the existence of mankind. Amending this challenge can be possible by using renewable bio-fuel crops that augment this deficit and maintain environmental balance. Macaúba [*Acrocomia aculeata* (Jacq.) Lodd.ex. Mart] is a multipurpose perennial palm tree with high biomass and oil productivity widely distributed in sub tropical or tropical America (Janick and Paull, 2008; Moura et al., 2010; Lanes et al., 2014). Under proper agronomic practices, it has a projected productivity potential of 16-25 ton/ha/yr fruits and 6.2 ton oil/ha/yr under plantation (Pires et al., 2013). Also, macaúba palm tree has a high potential to sequester carbon from the surroundings (Lanes et al., 2014). This might be an added advantage in carbon trading to the local community in the current climate change scenario. Moreover, it offers an advantage to be an alternative biodiesel feedstock as it maintains resource sustainability, environmental balance and social benefits in short and long terms. The regional state of Minas Gerais proclaimed macaúba as a renewable raw material source for biodiesel production in 2011 (Azevedo Filho et al., 2012). Currently, the region drafted a policy to use macaúba oil in biokerosene production (Lanes et al., 2014).

However, this productive palm tree has high water content in the mesocarp fruit, where most of the oil comes from, that impairs the oil quality during storage. Acidification and oxidation affects the oil quality, and can compromise its usage as a raw material for biofuel production. Hydrolysis that leads to the acidification can be facilitated by endogenous and microbial lipase (Lopes and Neto, 2011). Besides hydrolysis, peroxidase is involved in oxidation of the oil. Those degrading reactions are quite often observed in fruits that are collected by the traditional extractive practices of the wild macaúba palm trees. Moreover, the lack of a proper industrial processing plant for macaúba, contributes to the low productivity and poor quality products and coproducts (Pires et al., 2013). However, fruit harvested directly from the bunch at ripening stage coupled with immediate drying, mechanical pulping, extraction, and refining, maintained macaúba oil quality for further industrial applications (Nunes et al., 2015).

Microorganism growth and enzymes activity can be constrained by reduced water content (James and Kuipers, 2003). Drying is a thermo-physical action governed

by heat and mass transfer laws that might prolong the shelf life and quality of agricultural products (Sethi, 2007). Several drying methods are employed to ensure storability (Pawelzik et al., 1998). In processed foods, type of drying determines the quality and stability in storage (Maltini et al., 2003). Bankole et al. (2005) reported effect of drying methods on the oil content of stored melon seeds. They mentioned that oven dried seeds of melon increased the oil content and decreased disease infestation as compared to sun, smoke and solar drying. Besides the type of drying, temperature has a vital role during drying process and should be accompanied with air circulation (PNW 397, 2009).

The amount of water content determines the quality of products in storage. This might be managed by proper postharvest handling practices. Those practices include radiation, drying, cold storage, modified and controlled atmospheres that prolong the shelf life and quality of stored products. Out of this, drying is an old aged preservation practice that may be started with the on-set of agriculture such as sun drying.

So far, there is no established limit for minimum moisture content and drying temperature in macaúba that reduces hydrolytic and oxidative reactions in storage. This challenges quality assurance in postharvest handling. Therefore, the objective of this study was to assess the effect of drying macauba fruits after storage periods at increasing temperatures upon biochemical activity and physico-chemical properties and recommended optimum drying temperature in storage.

## **2. MATERIAL AND METHODS**

### **2.1. Study site and sample preparation**

A laboratory experiment was conducted at Macaúba Postharvest Laboratory, Department of Plant Sciences, Federal University of Viçosa (UFV), from February 2012 to November 2012. Fruits were collected from bunches that were cut from previously selected wild macaúba trees in Capela farm, Acaiaca municipality, Minas Gerais State, Brazil. Acaiaca is located at 20° 45'36" S latitude and 44° 15' W longitude at an altitude of 481 m above sea level with a humid subtropical climate CWa (Köppen and Geiger, 1928). Fruits were harvested at full maturity and were sorted to have uniform size and kept into mesh net bag. Each bag contained 20 fruits and this was considered as one experimental unit.

The fruits were dried in an oven with renewal and circulation of air (Tecnal, Model TE 394-3, Brazil) at 60°C and 100°C for 24 hours after 0, 10, 20 and 30 days of storage at open plastic box at a controlled temperature of 23±1°C for further analysis. The control was left untreated (23°C). Samples stored for 0 days were analyzed within 24 hour after drying. All chemicals used were of analytical grade.

## **2.2. Physical, chemical and biochemical analysis of the mesocarp**

### **2.2.1. Physical analysis of the mesocarp**

The fruits were manually peeled and pulped, and the mesocarp and exocarp were cut separately into pieces to measure water activity ( $a_w$ ) by using Pa<sub>w</sub>kit water activity meter (Decagon, Inc., USA) at an accuracy of ± 0.02  $a_w$ .

### **2.2.2. Chemical analysis of the mesocarp**

Mesocarp moisture content was determined as per the protocol developed for food and volatile materials (IAL, 1985). Five grams of mesocarp pieces were dried in an oven at 105°C until constant weight and moisture contents were determined by the difference in weight before and after drying. Mesocarp oil content (dry base) was determined as per the protocol of 032/V (IAL, 1985). The oil was extracted from dried mesocarp using n-hexane as solvent in Soxhlet apparatus (for 4 h) and the hexane was recovered in a rotary evaporator.

### **2.2.3. Biochemical analysis of the mesocarp**

#### **2.2.3.1. Crude enzyme extract**

1g of mesocarp (for each enzyme) was homogenized with 20 mL of 0.1M Tris buffer at pH 8.0 (for lipase) or 6.5 (for peroxidase), using electric hand blender. The crude enzyme extract was filtered through cotton gauze and centrifuged at 6000 rpm (Excelsa™ II Centrifuge, Mod. 206 BL, Brazil) for 10 minutes. The supernatant, called crude enzyme extract, was collected and stored at -20°C (Iaderoza and Baldini, 1991) for further analysis.

#### **2.2.3.2. Enzyme incubation and activity measurement**

Lipase activity was measured as per the protocol of Iaderoza and Baldini (1991) with modifications. A mixture of 1 mL of crude enzyme extract, 5 mL of triacetin emulsion (25% triacetin/75% of 7% gum Arabic solution) and 5 mL of 0.1M Tris HCl



buffer at pH 8.0, was incubated in water bath at 27°C/30 min. The reaction was stopped by adding 20 mL acetone: ethanol solution (1:1). The mixture was added with 5 drops of 0.05% phenolphthalein, titrated against 0.05N NaOH. The soluble protein content of the crude enzyme extract was measured by spectrophotometer at 260 nm and 280 nm (Thermo scientific, Genesys 10UV Scanning) as per the protocol of Iaderoza and Baldini (1991). The soluble protein content was considered to calculate specific activity of the enzyme. One unit of activity (unit/mole) is defined as quantity of one micromole of fatty acids released per minute. Specific activity (U/mg) is defined as activity of enzyme per unit of protein.

Peroxidase activity was determined as per the protocol of Fatibello-Filho and Vieira (2002) with modifications. The activity was determined by tetraguaiacol formation in the blend solution of 1 mL of 50 mM Guaiacol, 1 mL of 15 mmol L<sup>-1</sup> hydrogen peroxide, 1 mL of 0.1 M TrisHCl and 0.5 mL of crude extract. The absorbance was measured at 470 nm after 1 minute. One unit activity (unit/mole) is defined as the increasing of 0.001 absorbance unit per minute. The soluble protein content was measured as cited above for lipase crude extract and used to calculate specific activity of the enzyme.

#### **2.2.4. Physico-chemical analysis of the mesocarp oil**

Mesocarp slices were dried in an oven with renewal and circulation of air (Tecnal, Model TE 394-3, Brazil) at a temperature of 65°C for 12 hours. Then, the oil was extracted using a manually operated hydraulic press (Prensa Ribeiro 30 Ton, Brazil). The extracted oil was stored at -20°C packed in amber glass vials wrapped with aluminum foil. Acidity and peroxide indices were determined as per AOCS (1983). Primary and secondary degree of oil oxidation was determined by molar absorptivity at 232 nm (MA<sub>232</sub>) and 270 nm (MA<sub>270</sub>), respectively, according to IUPAC (1979) by diluting the oil sample in 10 mL isooctane. The oxidative stability was measured by Rancimat method (873 Biodiesel Rancimat<sup>®</sup> - Metrohm) as per AOCS (1997). Total carotene content was determined by diluting the oil samples in 10 mL petroleum ether and taking the absorbance at 450 (Rodriguez-Amaya and Kimura, 2004).

### **2.3. Experimental design and data analysis**

The experiment was conducted in a completely randomized design with factorial arrangement of 3x4, 3 drying temperatures (control, 60°C and 100°C) and 4 periods of

storage (0, 10, 20 and 30 days) with 5 replications. Biochemical sample was subjected to eight, whereas physical and physico-chemical samples were subjected to three analytical determinations. Analysis of variance was performed by SAS (SAS institute, 2004) and difference between means was compared by using Tukey's test at  $P < 0.05$  significance level. The results are presented as mean values and data were compared with standard error of the means.

### **3. RESULTS**

The parameters evaluated in oven dried macaúba at different storage periods (Table 1, 2, 3, 4 and 5), allowed to assess the effects of each variable and their interaction.

Storage and drying treatments are quantitative; so that the magnitude of one influential factor over the other was explained by using eta squared values (Levine and Hullett, 2002; Blessington et al., 2007). Treatment factors were compared with their level of significance between variables to explain the strength of variables in an experiment where percent of the total variability including error term becomes 100%. Table 3-1 showed eta squared value and percentage variation in the dependent variables. Accordingly, drying temperature was found to be an influential factor in water mediated variables (mesocarp and exocarp water activity, mesocarp moisture content and specific activity of peroxidase) except mesocarp oil content. On the other hand, storage period had dominant effect on oil mediated variables (molar absorptivity value at 232 nm and 270 nm, peroxide index and total carotene content and acidity index of the mesocarp oil) except specific activity of lipase. The variability on oxidative stability was dominated by interaction effects followed by storage.

Table 3-1. Components of ANOVA and total variation of quality parameter of macaúba fruits in relation to storage and drying temperature.

Evaluated parameters	Sum square values of variance components			Error	Sum square total	Eta squared			Percentage total variation		
	Storage (S)	Drying temp. (T)	S x T			S	T	SXT	S	T	SXT
Specific activity of peroxidase	6579315ns	633626444***	24252191**	48095563	712553513	0.009	0.889	0.034	0.9	88.9	3.4
Specific activity of lipase	1300***	564***	468***	419	2751	0.472	0.205	0.170	47.2	20.5	17.0
Mesocarp water activity	0.045***	0.305***	0.036**	0.070	0.455	0.098	0.670	0.078	9.8	67.0	7.8
Exocarp water activity	0.057***	0.296**	0.031ns	0.122	0.505	0.112	0.586	0.060	11.2	58.6	6.0
Mesocarp moisture content	3812***	15129***	1719***	2909	23570	0.162	0.642	0.073	16.2	64.2	7.3
Mesocarp oil content	1136***	1421***	35ns	912	3504	0.324	0.405	0.010	32.4	40.5	1.0
Oxidative stability	198***	127***	234***	249	809	0.245	0.157	0.290	24.5	15.7	29.0
Molar absorptivity at 232 nm	0.209***	0.026***	0.060***	0.039	0.334	0.625	0.078	0.180	62.5	7.8	18.0
Molar absorptivity at 270 nm	0.443***	0.034***	0.029***	0.048	0.554	0.800	0.061	0.052	80.0	6.1	5.2
Total carotene content	5481***	2866***	3600***	1094	13041	0.420	0.220	0.276	42.0	22.0	27.6
Peroxide index	77***	15***	57***	28	178	0.436	0.087	0.320	43.6	8.7	32.0
Acidity index	304***	0.369ns	9ns	39	352	0.863	0.001	0.024	86.3	0.1	2.4

Eta squared ( $\eta^2$ ) is equal to sum of squares between groups divided by sum squares of total. Eta squared\*100= Percentage total variation. \*, \*\*, \*\*\* and ns is significant at  $P<0.05$ ,  $P<0.01$ ,  $P<0.001$  and non-significant, respectively. Variance components were taken from ANOVA table.

### 3.1. Physical, chemical and biochemical analysis

Mesocarp water activity ( $Ma_w$ ) was significantly ( $P<0.05$ ) affected by drying temperature, periods of storage and their interactions (Table 3-2). Drying the fruits at 100°C brought up significant reduction in  $Ma_w$  along storage. Reduction in  $Ma_w$  was significant ( $P<0.05$ ) at 0, 10, 20 or 30 days in 100°C drying temperature.  $Ma_w$  reduced severely and stabilized in between 20 and 30 days of storage at 100°C drying temperature.

Exocarp water activity ( $Ea_w$ ) was significantly ( $P<0.05$ ) affected by drying temperature and periods of storage (Table 3-3). Drying was effective in reducing  $Ea_w$  after 10 day at 100°C. There was a steady loss in  $Ea_w$  in between 10 and 30 days at 100°C.

Mesocarp moisture content (MMC) was significantly ( $P<0.05$ ) affected by drying temperatures, periods of storage and their interactions (Table 3-2). Drying reduced highly MMC at 20 and 30 days in 100°C treatment. MMC was in equilibrium state (stabilized moisture content) in between 20 and 30 days at 100°C drying treatment.

Table 3-2. Water activity and moisture content of macaúba mesocarp dried with different temperatures and periods of storage after harvesting.

Drying temp. (°C)	Mesocarp water activity ( $M_{a_w}$ )				Mesocarp moisture content (%)			
	Storage periods (days)				Storage periods (days)			
	0	10	20	30	0	10	20	30
<b>23</b>	0.9880Aa ± 0.0020	0.9880Aa ± 0.0081	0.9700Aa ± 0.0415	0.9780Aa ± 0.0044	65.2Aa ± 5.1	47.3Ab ± 6.7	43.9Ab ± 3.1	34.8Ac ± 4.9
<b>60</b>	0.9900Aa ± 0.0037	0.9700Aa ± 0.0079	0.9820Aa ± 0.0114	0.9100Bb ± 0.0146	34.8Ba ± 1.5	41.3Aa ± 2.6	35.3Aa ± 5.2	10.9Bb ± 0.8
<b>100</b>	0.9080Ba ± 0.0270	0.8280Bb ± 0.0169	0.7740Bc ± 0.0128	0.7760Cc ± 0.0095	15.6Ca ± 1.5	10.7Bab ± 0.9	4.7Bbc ± 0.6	5.0Bc ± 0.2

Means in the same column and rows followed by upper and lower case letters, respectively are significantly ( $P < 0.05$ ) different according to Tukey's test.

Mesocarp oil content (MOC) was significantly ( $P < 0.05$ ) affected by drying temperatures and periods of storage (Table 3-3). MOC was increased highly after 30 day at 100°C drying temperature. Oil accumulation increased with drying temperature. MOC showed no change in between 10 and 20 days.

Acidity index (AI) of the mesocarp oil was significantly ( $P<0.05$ ) affected by periods of storage (Table 3-3). Drying brought up no change in AI. AI was below the maximum limit of 5% as per ANVISA (Brasil, 2005) and (PORIM, 2011) to 20 days of storage. There was a huge increase in AI after 30 days of storage.

Table 3-3. Exocarp water activity of macaúba fruit and its mesocarp oil content and acidity index at different periods of storage after drying.

<b>Treatment</b>	<b>Exocarp water activity (<math>E_{a_w}</math>)</b>	<b>Mesocarp oil content (MOC, % dry base)</b>	<b>Acidity index (AI, % oleic acid)</b>
<b>Drying temperature (°C)</b>			
<b>23 (control)</b>	0.9505a ± 0.0175	47.7c ± 2.41	2.18a ± 0.26
<b>60</b>	0.9085b ± 0.0156	51.1b ± 1.23	2.09a ± 0.24
<b>100</b>	0.7850c ± 0.0199	59.3a ± 1.09	1.99a ± 0.39
<b>Storage periods (days)</b>			
<b>0</b>	0.9240a ± 0.0153	46.1c ± 1.70	0.30c ± 0.04
<b>10</b>	0.8893ba ± 0.0101	53.6b ± 1.03	0.75cb ± 0.17
<b>20</b>	0.8733bc ± 0.0336	52.7b ± 2.73	1.37b ± 0.37
<b>30</b>	0.8387c ± 0.0116	58.3a ± 0.84	5.92a ± 0.61

Means in the same column followed by different letters are significantly ( $P<0.05$ ) different according to Tukey's test

Both specific activity of lipase (SAL) and peroxidase (SAP) were significantly ( $P<0.05$ ) affected by drying temperatures, periods of storage and their interactions (Table 3-4). Their activities were deeply reduced after drying at 100°C. Lipase was less stable than peroxidase to the moisture loss along the storage. SAP was stable at 0, 10 and 30 day for control treatment. It was also stable after 10 day for 60°C and in the entire periods of storage for 100°C treatment.

Table 3-4. Specific activity of lipase and peroxidase in dried macaúba fruit with different temperatures and periods of storage after harvesting.

Drying temp. (°C)	Specific activity of lipase (U/mg)				Specific activity of peroxidase			
	Storage periods (days)				Storage periods (days)			
	0	10	20	30	0	10	20	30
<b>23</b>	22.9Aa ± 2.8	8.1Ab ± 1.5	7.3Bbc ± 1.3	4.6Ac ± 0.9	5582Bb ± 578	5980Bb ± 256	7458Aa ± 531	6408Ab ± 542
<b>60</b>	20.8Aa ± 1.4	8.8Ac ± 0.9	12.2Ab ± 1.5	4.6Ad ± 0.3	9064Aa ± 288	6648Ab ± 467	7291Ab ± 790	6472Ab ± 710
<b>100</b>	6.5Ba ± 1.7	4.5Ba ± 0.5	3.9Ca ± 0.3	3.9Aa ± 0.7	75Ca ± 20	10Ca ± 4.3	11Ba ± 4.1	5.6Ba ± 1.5

Means in the same column and rows followed by upper and lower case letters, respectively are significantly ( $P<0.05$ ) different according to Tukey's test.

### 3.2. Physico-chemical analysis of mesocarp oil

Peroxide index (PI) was significantly ( $P<0.05$ ) different for drying temperatures, periods of storage and their interactions (Table 3-5). Peroxides came out with storage, and the drying process increased their amount when it took place at 0, 10 and 20 days after harvesting. Drying the fruits after 10 or 20 days at 100°C had a negative effect on oil quality because the PI measurements were higher than in other conditions. Nevertheless, fruits dried at 100°C after 30 days of storage, showed lower PI. The PI maximum limit of 10 meq O<sub>2</sub>/kg oil as per ANVISA (Brasil, 2005), was not reached in any condition.

The molar absorptivity (MA) at MA<sub>232</sub> and MA<sub>270</sub> were significantly ( $P<0.05$ ) affected by temperature, storage, and their interaction (Table 3-5). Likewise PI, MA<sub>232</sub> was reduced when fruits were dried at 100°C after 30 days of storage. It could be related to the loss of volatile compounds that comes from oil degradation, including peroxides, due to the high drying temperature. On the other hand, the formation of secondary degradation compounds, measured by MA<sub>270</sub>, were higher in the fruits dried at 100°C, unless when the fruits were dried after the 30<sup>th</sup> day of harvest, in comparison to the control.

Oxidative stability (OS) was significantly ( $P<0.05$ ) affected by drying temperatures, storage periods and their interactions (Table 3-5). The OS of macaúba oil tended to be reduced by half within 30 days of storage, when fruits were let down drying under natural conditions. But, OS was kept or increased with oven drying, and the best results were reached at 100°C.

Total carotene content (TCC) was significantly ( $P<0.05$ ) affected by drying temperatures, storage periods and their interactions (Table 3-5). It seems there is synthesis of carotenoids in the initial of storage, and the drying process did not avoid this metabolism.



Table 3-5. Physico-chemical analysis of mesocarp oil of dried macaúba fruit along storage periods.

Drying temp. (°C)	Peroxide index (meq O <sub>2</sub> /kg oil)				Molar absorptivity at 232 nm				Molar absorptivity at 270 nm			
	Storage periods (days)				Storage periods (days)				Storage periods (days)			
	0	10	20	30	0	10	20	30	0	10	20	30
23	2.15Ac ± 0.11	3.74Bb ± 0.29	4.64Ba ± 0.21	3.96Aab ± 0.47	0.33Bb ± 0.01	0.34Bb ± 0.01	0.35Bb ± 0.00	0.55Aa ± 0.01	0.10Bc ± 0.00	0.13Bc ± 0.00	0.17Cb ± 0.02	0.38Aa ± 0.02
	2.15Ac ± 0.12	4.07Ba ± 0.49	3.28Cb ± 0.11	4.52Aa ± 0.18	0.36Ac ± 0.02	0.38Abc ± 0.01	0.39Ab ± 0.01	0.54Aa ± 0.02	0.11Bd ± 0.00	0.15Bc ± 0.01	0.20Bb ± 0.02	0.32Ca ± 0.01
60	2.14Ac ± 0.11	7.46Aa ± 0.73	6.21Ab ± 0.43	2.72Bc ± 0.11	0.36Aab ± 0.01	0.34Bb ± 0.00	0.37Aba ± 0.02	0.39Ba ± 0.01	0.16Ad ± 0.01	0.20Ac ± 0.00	0.28Ab ± 0.02	0.35Ba ± 0.02

Drying temp. (°C)	Oxidative stability (hour)				Total carotene content (mg/kg)			
	Storage periods (days)				Storage periods (days)			
	0	10	20	30	0	10	20	30
23	11.7Aa ± 0.08	10.3Ca ± 0.86	7.8Cb ± 0.99	5.3Cc ± 1.11	65.5Ac ± 0.60	83.2Aa ± 0.78	82.6Ba ± 2.35	74.8Bb ± 3.35
	9.9Bc ± 0.17	13.5Ba ± 0.36	11.6Bab ± 1.18	10.5Bbc ± 1.81	57.8Bb ± 1.48	72.4Ba ± 2.60	71.3Ca ± 0.86	70.8Ca ± 2.59
60	11.8Ab ± 1.19	16.7Aa ± 0.47	14.8Aab ± 1.66	13.7Ab ± 1.05	60.0Bc ± 1.17	72.8Bb ± 0.75	101.9Aa ± 2.85	105.2Aa ± 3.25

Means in the same column and rows followed by upper and lower case letters, respectively are significantly ( $P < 0.05$ ) different according to Tukey's test.

## **4. DISCUSSION**

The final target of storage is to maintain quality and prolong shelf life of postharvest products. Hydrolytic and oxidative rancidity are the known cause of oil quality deterioration. Hydrolytic rancidity can be enzymatic (lipase) in the presence of moisture or non-enzymatic favored by heat or water (Hamilton, 1994). On the other hand, lipid oxidation can be favored by presence of oxygen, light, enzymes, high temperature and other related factors in unsaturated fatty acids. Because of their complex nature and instability, analysis of lipid oxidation is challenging (Wąsowicz et al., 2004).

### **4.1. Magnitude of variability explained by Eta squared**

As indicated in table 3-1, The magnitude of variability explained by eta squared indicated that water mediated parameters are influenced by temperature except MOC. This is associated with the loss in moisture due to applied temperature. The situation is aggravated with the rise in temperature. It includes physical, chemical and biochemical parameters. Physical parameters were higher in exocarp (67%) than mesocarp (59%) water activity. Chemical parameters were higher in mesocarp moisture content (64%) than mesocarp oil content (41%) to applied treatments. Among physico-chemical parameters, only oxidative stability (29%) was affected highly by interaction followed by storage. Accordingly storage time affected oil mediated parameters such as peroxide index (44%), molar absorptivity at 232 nm (63%) and 270 nm (80%), and total carotene content (42%). Magnitude of total variance explained by interaction was less than temperature, except in oxidative stability, total carotene content and peroxide index. There was a large variability unexplained by storage, drying temperature and their interaction of the independent variables, except acidity index followed by molar absorptivity at 270 nm, whereby most of the variability is accounted for treatment factors. This implied that drying temperature beyond the optimum increase chance of oil oxidation. However, acidity was affected by storage time, which is a function of natural or biological water loss.

### **4.2. Effect of drying upon physical, chemical and biochemical analysis**

Mesocarp moisture content was reduced by the interaction of storage period and drying temperature. It was reduced by 92% along periods of storage in 100°C drying

temperature. In this study, the average initial mesocarp moisture content over drying temperature and storage periods ranged from 5.0 (100°C) to 65.2% (control treatment) that might be in conformity with 27.9-47.7% (Ferrari and Azevedo-Filho., 2012), 48% (Mariano et al., 2011), 53% (Ramos et al., 2008) and 63% of Corumba region in Mato Grosso do Sul state of Brazil (Cinconni et al., 2013). However, the difference in MMC might be a result of ripening stage and harvesting time, genotypes variability, geographical area and other associated factors. High moisture in macaúba mesocarp can be a major concern because of the high cost implication during oil extraction (Cinconni et al., 2013). The higher the moisture content the more time required in drying and more difficult in extracting the oil.

Reduction in moisture generally implies extended shelf life of some agricultural products. Shelf life and oil quality of dried rape seed prolonged with reduced moisture content of 6-8% (Savić et al., 2009). Moreover, Ngando-Ebongue et al. (2013) reported that assaying moisture content in oil palm used as an indicator in oil quality parameters and predict changes during storage. The drastic reduction in MMC with increased temperature may be related to migration of water from the fruit to the environment that favored drying in macaúba. This implied that oven drying temperature of 100°C was efficient to reduce the MMC along periods of storage in this study.

Irrespective of the morphological and anatomical difference between macaúba mesocarp and exocarp (Lorenzi and Negrelle, 2006; Reis et al., 2012), water and volatile matters evaporated upon drying and therefore resulted in water activity reduction. Though varied in magnitude, reduction in mesocarp and exocarp water activity might lead to corresponding decrease of enzymatic activities. This is because; water activity ( $a_w$ ) has a key role in lipid oxidation, enzymatic and non enzymatic reactions and microbial growth (Barbosa-Cánovas, 2003). However, the interaction of water with solutes in the tissue of macaúba fruit might complicate prediction of ideal water activity during storage.

Specific activity of lipase was reduced by 40% along periods of storage for 100°C drying temperature which demonstrated its heat resistant features. At high drying temperature and reduced  $a_w$ , lipase became inactive even in the presence of substrate. This is because enzymes are protein and protein becomes denature at high temperature. There is unfolding of molecules at high temperature (LapanJe, 1978). In oil palm, presence of low amount of water during drying limits the reaction of lipase with the oil

(Tan et al., 2009). Similarly, at 100°C the enzyme became stable along periods of storage. However, its high resistance to applied temperature might complicate the management of this enzyme.

On the other hand, specific activity of peroxidase (SAP) as reduced by 93% (liable to heat) along periods of storage for 100°C drying temperature. The high heat stability nature of peroxidase was reported in earlier works (Burnette, 1977). However, this enzyme was stable at 60°C after 10 day and even for control (at 0, 10 and 30 day) treatments in this study. Peroxidase activity in date palm stored under ambient condition was deactivated at 100°C/14 min (Rashid, 1959) as compared to the reduced activity at 55°C/20 min heat treatment (Mustapha and Ghalem, 2007) in another study. Despite the drying time, reduction of SAP in this study contributed to the management of this enzyme in order to obtain good quality mesocarp oil of macaúba in storage.

Oil content is a crucial quantitative parameter either in production or processing perspectives. It is a salient feature in oleaginous plants (crops). The result showed that oil content was increased by 24.3% across drying and by 26.5% along periods of storage for increase in drying temperature from control to 100°C. Increase in oil content with increase in storage periods associated with climacteric property of macaúba fruit (Goulart, 2014) that converts stored reserves to oil and accumulate more oil. Fats and oils expressed more during heating. Therefore, at high temperature the extraction of oil from the oil reserves would be higher by the same token. In this study, the average initial mesocarp oil content over periods of storage and drying temperature was ranged from 40-53%. This result may be in the range of 46% by Coimbra and Jorge (2011), 52% by Ferrari and Azevedo-Filho (2012) and 53% by Ramos et al. (2008). A decrease in MOC and acidity was reported from macaúba fruits stored after drying at different temperature and times (Martins, 2013). The author reported drying reduced acidity and accumulation of mesocarp oil as compared to control. Therefore, compromising oil content with acceptable oil quality should be considered in macaúba production.

#### **4.3. Effect of drying upon physico-chemical analysis of mesocarp oil**

Acidity is an important oil quality indicator. Drying treatments did not contribute the reduction of acidity content in this study. However, AI was affected by storage periods. The longer the storage duration for harvested fruits, the higher the FFA content in the oil upon extraction was observed in oil palm (Hartley, 1988). AI was below the crude palm oil standard limit of 5% at maximum as per ANVISA (Brasil,

2005) and PORIM (2011) and as well as below the industrial and economical acceptable range of 3% (Nunes et al., 2015) until 20 days of storage. In all treatments, AI surpassed the established limit after 20 days of storage may be due to autocatalytic and/or other associated factors. Autocatalysis of free fatty acids was reported in stored palm oil with moisture content of greater than 0.13-0.15 and water activity of greater than 0.5 (Yuen et al., 2006). Since the amount of moisture content and water activity in the mesocarp was higher, autocatalytic hydrolysis reaction might be triggered after drying in this study. This is because lipase was not completely deactivated and hence increased AI could be expected in the return.

Drying increased the oil content, but not avoided increasing acidity along periods of storage in this study. Therefore, optimizing the appropriate drying temperature and storage duration fulfills the acceptable AI standards of the industries and economic benefit of producers in macaúba value chain.

Peroxide index (PI, meq O<sub>2</sub>/kg oil) measures the degree of oxidation in double bonds of unsaturated fatty acids. It is characterized by the formation of hydroperoxide which is considered as a primary oxidation product (Frankel, 1980) in oil quality evaluation. Although PI was increased with increased drying temperature, it was below the maximum crude palm oil limit of 10 meq O<sub>2</sub>/kg oil as per ANVISA (Brasil, 2005) in storage. Predominance of monounsaturated fatty acids (oleic) led to PI formation (Nunes et al., 2015) in macaúba. The synthesis of TCC content at drying might offer better oxidative stability potential of the oil. Therefore, the oil may have a capacity to absorb the release of volatile compounds or the applied temperature could not degrade the PI to other volatile compounds.

Molar absorptivity (MA) at MA<sub>232</sub> and at MA<sub>270</sub> related to primary (hydroperoxides, dienes) and secondary oxidation (aldehydes, ketones, trienes) products, respectively in polyunsaturated fatty acids (IUPAC, 1979; Wanasundara et al., 1995). There is no established Brazilian standard for MA. However, MA values of 2.04 at 232 nm and 0.56 at 270 nm were reported in the crude mesocarp oil of macaúba (Nunes et al., 2015) adopting from other oleaginous crops. The authors reported MA as quality parameter and identity characteristic of industrial processing. Early detection of MA<sub>270</sub> might not infer the oil was oxidized. This is because most of the evaluated quality indices were not affected by the applied treatments in this study. Once the

proper analysis protocol developed, issues pertaining to such type of problems can be resolved in macaúba.

Rancimat method is an accelerated aging test that measures the oxidative stability (OS) of edible fats and oils and fat-containing foods (Mendez et al., 1996). It is a resistance of oils to oxidation. High OS value recorded with increasing storage period and drying temperature. It was ranged from 11.8-16.7 hours in 100°C drying temperature along periods of storage in this study. This was greater than 4.87 hour (Coimbra and Jorge, 2011) but less than 25 hour (Melo et al., 2014) from non-dried macaúba mesocarp oil. This justified that heating macaúba oil did not incite the liberation of PI and MA<sub>232</sub> beyond their stated limit so that OS was increased. Moreover, increased TCC content contributes to higher OS value. This is a promising result either in food or biodiesel industries.

Macaúba mesocarp has ample  $\beta$ -carotene content (Coimbra and Jorge, 2012) that supposed to be resistant to oxidation. High TCC value recorded with increasing periods of storage and drying temperature. However, carotenoids can be degraded by enzymatic (lipoxygenase) and non-enzymatic oxidation (heat, light and oxygen exposure) during processing and storage of food (Dutta et al., 2005). However, at 100°C drying temperature, there was an increase in the TCC content of the fruits at 20 and 30 days. In these conditions the extracted oil showed a brownish color that might be due to the caramelization reaction at reduced moisture contents. The measurement of carotenoids could not be precise because of the influence of the caramelization compounds. That is why; increased TCC content may be masked by this caramelization process along periods of storage with increased temperature. Though TCC content might super estimate carotenoids content. Nevertheless, heating could not inhibited the the synthesis of carotenes that grants antioxidant potential of the oil along storage in the study.

## **5. CONCLUSIONS**

Storage periods, drying temperature and their interaction exerted an influence in both biochemical activities and physico-chemical properties of stored macaúba fruit. Moisture content of mesocarp was reduced to desirable pattern at 100°C, and similarly, to achieve a remarkable water activity reduction (mesocarp and exocarp). Both lipase and peroxidase activities were reduced at 100°C drying temperature. Oil content was increased along periods of storage and with increasing drying temperature. AI was not affected by drying process and after 20 days of storage it surpassed the standard limit.

Regard to oxidation parameters, drying fresh fruits does not lead to increase in PI. Though the higher PI values at 10<sup>th</sup> and 20<sup>th</sup> storage day, the final PI at 100°C was the lowest one, and MA<sub>232</sub> showed similar behaviour at this storage time. PI kept within the required standards in all treatments. Oxidative stability was greatly benefited by drying fruits immediately after harvest, mostly at 100°C. Carotenoids are synthesized in the first 10 days during storage and drying; however, drastic rise with drying temperature masked by caramelization process. Therefore, drying macaúba fruit after 30 days of harvest at 100°C is worth because it assured the highest oil content and an overall suitable oil quality. However, drying the fruit by different time and temperature can complement the findings of this study in future works.

## 6. REFERENCES

AOCS. 1983. Official and Tentative Methods. Champaign, Illinois.

AOCS. 1997. Official Method Cd 12b-92. American Oil Chemists' Society. Champaign, Illinois.

Azevedo Filho ,J.A.; Colombo , C.A; Berton,L.H.C.2012. Macaúba: palmeira nativa como opção bioenergética. **Pesquisa e Tecnologia** 9 (2), disponível em [www.aptaregional.sp.gov.br](http://www.aptaregional.sp.gov.br).

Bankole, S.A.; Osho, A.; Joda, A.O.; Enikuomihin, O.A. 2005. Effect of drying method on the quality and storability of 'egusi' melon seeds (*Colocynthiscitrullus* L.). **African Journal of Biotechnology** 4 (8): 799-803.

Barbosa-Cánovas, G.V.; Fernández-Molina, J.J.; Alzamora, S.M.;Tapia, M.S.; López-Malo A.; Chanes, J.W. 2003. Handling and preservation of fruits and vegetables by combined methods for rural areas. Technical Manual, FAO Agricultural Services Bulletin 149, Rome.

Blessington, T.; Miller, C.J.Jr.; Nzaramba, M.N.; Hale, A.L.; Redivari, L.; Scheuring, D.C.; Hallman, G. J. 2007. The effects of low-dose gamma radiation and storage time on carotenoids, antioxidant activity, and phenolics in the potato cultivar Atlantic. **American Journal of Potato Research** 84:125-131.

Brasil. 2005. Ministério da Saúde. Agência Nacional de Vigilância Sanitária. Resolução Diretoria da Colegiada, RDC/ANVISA/MS n° 270, 22 de setembro de 2005. Regulamento técnico para óleos vegetais, gorduras vegetais e creme vegetal. Diário Oficial da República Federativa do Brasil, Brasília, DF, 23 set. 2005. Seção 1. Disponível em: <http://www.anvisa.gov.br>. Retrieved on December 30, 2013.

Burnette, F.S. 1977. Peroxidase and its relationship to food flavor and quality: a review. **Food Science Journal** 42:1-6.

Ciconini,G.; Favaro,S.P.; Roscoe,R.; Miranda,C.H.B.; Tapeti,C.F.; Miyahira, M.A.M.; Bearari, L.; Galvani, F.; Borsato, A.V.; Colnago, L.A.; Naka, M.H. 2013. Biometry and oil contents of *Acrocomia aculeata* fruits from the Cerrados and Pantanal biomes in Mato Grosso do Sul, Brazil. **Industrial Crops and Products** 45: 208-214.

Coimbra, M.C.; Jorge, N. 2011. Proximate composition of guariroba (*Syagrus oleracea*), jerivá (*Syagrus romanzoffiana*) and macaúba (*Acrocomia aculeata*) palm fruits. **Food Research International** 44: 2139–2142.

Coimbra, M.C.; Jorge, N. 2012. Fatty acids and bioactive compounds of the mesocarps and kernels of Brazilian palm species, guariroba (*Syagrus oleraces*), jerivá (*Syagrus romanzoffiana*) and macaúba (*Acrocomia aculeata*). **Journal of Food Science and Agriculture** 92: 679–684.

Dutta, D.; Chaudhuri, U.R.; Chakraborty, R. 2005. Structure, health benefits, antioxidant property and processing and storage of carotenoids-review. **African Journal of Biotechnology** 4 (13): 1510-1520.

Fatibello-Filho, O.; Vieira, C. I. 2002. Uso analítico de tecidos e de extratos brutos vegetais como fonte enzimática. **Química Nova** 25 (3): 455-464.

Ferrari, R.A.; Azevedo Filho, J. A. 2012. Macaúba as promising substrate for crude oil and biodiesel production. **Journal of Agricultural Science and Technology B2**: 1119-1126.

Frankel, E.N.1980. Lipid oxidation. **Lipid Research** 19: 1-22.

Goulart, S.M. 2014. Amadurecimento pós-colheita de frutos de macaúba e qualidade do óleo para a produção de biodiesel.M.Sc thesis,Universidade Federal de Viçosa, Viçosa, Minas Gerais,Brasil.

Hamilton, R. J. 1994. The chemistry of rancidity in foods. In: Allen, J.C. and Hamilton R.J. (Eds). **Rancidity in foods**. 3<sup>rd</sup> ed. London: Blackie Academic and Professional. p. 1–21.

Hartley, C.W.S. 1988. The oil palm (*Elaeis guineensis* Jacq.). Third edition, London: Longman.

Iaderoza, M.; Baldini, V. L. S. 1991. A importância da análise enzima em alimentos. In: Iaderoza, M; Baldini, V. L. S. **Ênzimos e a qualidade de vegetais processados**. Campinas: ITAL, 1991. (Manual Técnico).

IAL (Instituto Adolfo Lutz). 1985. Normas analíticas do Instituto Adolfo Lutz, Vol. 1:Métodos Químicos e Físicos para Análise de Alimentos, 3<sup>a</sup> ed., p.22. , IMESP, São Paulo.



IAL (Instituto Adolfo Lutz). 1985. Normas analíticas do Instituto Adolfo Lutz, Vol. 1: Métodos Químicos e Físicos para Análise de Alimentos, 4<sup>a</sup> ed., p.116-118, IMESP, São Paulo.

IUPAC (International Union of Pure and Applied Chemistry). 1979. Standard methods for the analysis of oils, fats and derivatives. Oxford, 6<sup>th</sup> ed., IUPAC, 1979.

James, I. F.; Kuipers, B. 2003. Preservation of fruit and vegetables. Agromisa Foundation, Wageningen.

Janick, J.; Paull, R.E., 2008. The Encyclopedia of fruits and nuts. CABI, UK.

Köppen, W. P.; Geiger, R. 1928. Die Klimate der Erde: Grundriss der Klimakunde. Berlin, Leipzig: Waltr de Gruyter.

Lanes, É.C.M.; Costa, P.M.A; Motoike, S.Y. 2014. Brazil promotes aviation biofuels. **Nature correspondence** 511, 31.

Lapanje, S. 1978. Physicochemical aspects of protein denaturation. Watley, New York, 1-10.

Levine, T.R.; Hullet, C.R. 2002. Eta squared, partial eta squared, and misreporting the effects size in communication research. **Human Communication Research** 28: 612-625.

Lopes, D.C.; Neto, A.J.S. 2011. Potential Crops for Biodiesel Production in Brazil: A Review. **World Journal of Agricultural Sciences** 7 (2): 206-217.

Lorenzi, G. M. A. C.; Negrelle, R. R. B. 2006. *Acrocomia aculeata* (Jacq.) Lodd. EX Mart.: Ecological aspects, uses and potentialities. [http:// www. entabanbrasil. com. br](http://www.entabanbrasil.com.br). Retrieved on 21/04/2014.

Maltini, E.; Torreggiani, D.; Vieira, E.; Bertolo, G. 2003. Water activity and the preservation of plant foods. **Food Chemistry** 82: 79-86.

Mariano, R.G.B.; Silva, C.M.; Couri, S.; Nogueira, R.I.; Freitas, S.P. 2011. Partition of Free Fatty Acids in Deacidification of Macaúba Pulp Oil by Liquid-Liquid Extraction using Ethanol/Water as Solvent. **Defect and Diffusion Forum** 312-315: 554-559.

Martins, A. D. 2013. Uso da radiação gama e secagem para a conservação da qualidade do óleo de frutos de macaúba. M.Sc thesis, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brasil.

Melo, M. A. R., Melo, M.A.M.F., Silva, E.V., Filho, J.R.C, Souza, A.G., 2014. Study of the oxidative stability of oils vegetables for production of Biodiesel. **Revista Verde** 9 (1): 84-88, Mossoró – RN – BRASIL.

- Mendez, E.; Sanhueza, J.; Speisky, H.; Valenzuela, A. 1996. Validation of the Rancimat test for the assessment of the relative stability of fish oils. **Journal of American Oil Chemistry Society** 73: 1033–1037.
- Moura, E.F.; Ventrella, M.C.; Motoike, S.Y. 2010. Anatomy, histochemistry and ultrastructure of seed and somatic embryo of *Acrocomia aculeata* (Arecaceae). **Agricultural Sciences**, Piracicaba, 8: 399-407.
- Mustapha, K.; Ghalem, S.A. 2007. Effect of heat treatment on polyphenol oxidase and peroxidase activities in Algerian stored dates. **African Journal of Biotechnology** (6): 790-794.
- Ngando-Ebongue, G.F.; Mpondo-Mpondo, E.A; Ewane, M.A. 2013. Some quality parameters of crude palm oil from major markets of Douala, Cameroon. **African Journal of Food Science** 7 (12): 473-478.
- Nunes, A. A.; Favaro, S.P.; Galvani, F.; Miranda, C.H.B. 2015. Good practices of harvest and processing provide high quality Macaúba pulp oil. [10.1002/ejlt.201400577].
- Pahoja, V.M.; Sethar, M.A, 2002. A review of enzymatic properties of lipases in plants, animals and microorganisms. **Pakistan Journal of Applied Sciences** 2 (4): 474-484.
- Pawelzik, E.; Irfanl, I.; Luecke, W. 1998. Microwave treatment of rape to ensure seed quality during storage. In: **Proceedings of the 7<sup>th</sup> International Working Conference on Stored-product Protection**. Volume 2, 14-19 October 1998, Beijing, P.R.China.
- Pires, T.P.; Sousa, E.S.; Kuki, K.N.; Motoike, S.Y. 2013. Echophysiological traits of the macaw palm: A contribution towards the domestication of a novel oil crops. **Industrial Crops and Products** 44:200-210.
- PNW 397. 2009. Drying fruits and vegetables. Pacific Northwest Extension Publication, 3<sup>rd</sup> edition, University of Idaho, USA.
- PORIM. 2011. Standard Specifications for Processed Palm Oil. PORIM Handbook. <http://www.poram.org>. Retrieved on 29/4/2012.
- Ramos, M.I.L.; Ramos Filho, M. D.; Hiane, P.A.; Braga Neto, J.A.; Siqueira, E.M.A.; 2008. Nutritional quality of the pulp of bocaiuva *Acrocomia aculeata* (Jacq.) Lodd. **Ciência e Tecnologia de Alimentos, Campinas**, 28 (Supl.): 90-94.
- Rashid, I.M. 1959. Oxidizing enzymes in dates in relation to the darkening of fruit. Ph.D. Thesis, Massachusetts University, Amherst.
- Reis S, B.; Mercadante-Simões, M.O.; Ribeiro, L.M. 2012. Pericarp development in the macaw palm *Acrocomia aculeata* (Arecaceae). **Rodriguésia** 63 (3): 541-549.

Rodriguez-Amaya, D.B.; Kimura, M. 2004. Harvest plus Handbook for Carotenoid Analysis. Washington DC and Cali, IFPRI and CIAT, p.58.

Savić, T.B.; Krička, T.; Voća, N.; Jurišić, V.; Matin, A. 2009. Effect of storage temperature on rapeseed quality. *Agriculturae Conspectus Scientificus* 74 (3): 143-147.

Tan, C.H.; Ghazali, H.M.; Kuntomb, A.; Tan, C.P.; Ariffin, A.A. 2009. Extraction and physicochemical properties of low free fatty acid crude palm oil. **Food Chemistry** 113: 645–650.

Wanasundara, U.N.; Shahidi, F.; Jablonski, C.R. 1995. Comparison of standard and NMR methodologies for assessment of oxidative stability of canola and soybean oils. **Food Chemistry** 52: 249-253.

Wąsowicz, E. W.; Gramza, A.; Heś, M.; Jeleń, H.; Korczak, J.; Matecka, M.; Mildner-Szkudlarz, S.; Rudzińska, M.; Samotyja, M.; Samotyja, U.; Zawirska-Wojtasiak, R. 2004. Oxidation of lipids in food. **Polish Journal of Food and Nutrition Sciences** 13 (54): 87-100.

Yuen, C.S.; Fui, K.H.; Wren, W.G. 2006. Autocatalytic hydrolysis and autoxidation of crude palm oil under various constant humidities. **Journal Oil Palm Research** 18: 278-287.

## 5. GENERAL CONCLUSION

Macaúba is a multipurpose oleaginous palm in tropical America with high biomass productivity and oil content. The products and co-products offer huge advantage in the value chain of this plant. However, the bulk harvest of this plant lasts few months of the year and it overlaps with the hottest and rainy season. This is becoming a huge challenge in postharvest quality of the fruit with respect to the high moisture content and poor oil extraction technology. Hydrolysis and oxidation are the possible causes of oil quality loss. Lipase and peroxidase are associated with these processes impairing oil quality under storage. Oil content is a big concern to producers while oil quality has cost implication to industry. Therefore, there should be a compromise to balance the gap between these two stakeholders. Besides, complex process of oil oxidation and lack of established standards to some of oil quality parameters complicate the analysis result of macaúba oil.

The first experiment aimed at evaluating effects of storage periods on mesocarp oil quality of macaúba fruits under ambient condition and recommended appropriate storage time. Fresh fruits directly harvested from the plant, stored in a ventilated and ambient condition fulfilled the desirable oil quality. Slight reduction of water activity correlated with drastic change in lipase activity after 15 days. However, acidification might not be related to lipase activity. The stored fruit could be free of hydrolysis for 22 days. The oxidative stability was in line with the biodiesel standard for 31 days of storage. However, the overall quality of the stored oil was kept within the required standards up to 20 days at room temperature in this study.

In the second experiment, the total variability explained by storage period affected water related parameters highly; whereas gamma dose related to oil quality parameters. Gamma radiation decreased oil accumulation as compared to control. The 8 KGy gamma dose resulted in oil oxidation. The 4 KGy gamma dose partially inactivated the enzymes and maintained other oil quality indexes in almost the entire periods of storage.

In the third experiment, the total variability attributed to storage affected most of oil related parameters, while that of temperature to water related parameters. Lipase was less stable than peroxidase to higher drying temperature (100°C). Oxidative stability and oil content were benefited by drying along storage. On the other hand, drying could

not favor reduction in acidity. In general, drying macaúba fruit after 30 days of harvest at 100°C assures the highest oil content and an overall suitable oil quality.

Hydrolysis which is catalyzed by lipase increased in the entire experiments despite the applied gamma or drying treatments. This could confer the rise in peroxide attributed to the hydrolysis of triacyl glycerol to free fatty acids. Or else, small quantity of the enzyme might be remained in the substrate to trigger further hydrolysis. Oxidation might be accelerated by the rise in acidity in exposure to environmental factors such as light and heat during storage and processing. Therefore, those factors should be considered in the future work.

The quantity and post harvest oil quality of macaúba is comparable to oil palm. However, macaúba has extended shelf life in storage. The postharvest oil quality of macaúba mesocarp was improved even at room temperature in this study. Application of gamma dose and drying treatments offered additional advantage in both quality and quantity aspect of postharvest management. These technologies are tested and proved effective in other oleaginous crops. Though the initial cost of these technologies is elevated, the benefits gained from the postharvest oil quality of macaúba could complement the deficit in the long term.

## 6. APPENDICES

### ANOVA in Chapter 2

Table 2.1. ANOVA for mesocarp and exocarp water activity of gamma irradiated macaúba fruit with different doses after harvesting.

Sources of variation	Degrees of freedom	Mean square values	
		Mesocarp water activity	Exocarp water activity
Gamma dose (D)	2	0.00141167 <sup>***</sup>	0.00064667 <sup>***</sup>
Storage period (S)	3	0.00376444 <sup>***</sup>	0.00605500 <sup>***</sup>
D*S	6	0.00010278ns	0.00035333 <sup>**</sup>
Error	48	0.00005083	0.00011500
CV (%)		0.74	1.17

<sup>\*\*\*</sup>, <sup>\*\*</sup> and ns- significant at  $P < 0.0001$ ,  $P < 0.01$  and non-significant, respectively.

Table 2-2. ANOVA for mesocarp moisture and oil content of gamma irradiated macaúba fruit with different doses after harvesting.

Sources of variation	Degrees of freedom	Mean square values	
		Mesocarp moisture content (%)	Mesocarp oil content (%)
Gamma dose (D)	2	41.52ns	143.13***
Storage period (S)	3	1701.58***	212.44***
D*S	6	34.39ns	46.55**
Error	48	41.81	14.66
CV (%)		16.09	7.26

\*\*\*, \*\*, and ns- significant at  $P < 0.0001$ ,  $P < 0.01$  and non-significant, respectively.

Table 2-3. ANOVA for specific activity of lipase and peroxidase gamma irradiated macaúba fruit with different doses after harvesting.

Sources of variation	Degrees of freedom	Mean square values	
		Specific activity of lipase (U/mg)	Specific activity of peroxidase (U/mg)
Gamma dose (D)	2	100.48*	37113364.5***
Storage period (S)	3	160.94**	89413799.4***
D*S	6	68.47*	12901281.2**
Error	48	27.09	3679322.6
CV (%)		48.40	38.68

\*, \*\*, and \*\*\* significant at  $P < 0.05$ ,  $P < 0.01$ , and  $P < 0.0001$ , respectively.

Table 2-4. ANOVA for acidity and peroxide index and molar absorptivity at 232 nm of gamma irradiated stored macaúba fruit and mesocarp oil.

Sources of variation	Degrees of freedom	Mean square values		
		Acidity index ( % oleic acid)	Peroxide index (meq O2/kg oil)	Molar absorptivity at 232 nm
Gamma dose (D)	2	50.15***	1661.43***	2.004***
Storage period (S)	3	256.93***	165.70***	0.017**
D*S	6	34.28***	13.73***	0.034***
Error	48	0.427	2.942	0.004
CV (%)		15.97	16.97	12.3

\*\*\* significant at  $P < 0.0001$



Table 2-5. ANOVA for molar absorptivity at 270 nm, oxidative stability and Total carotene content of gamma irradiated stored macaúba fruit and mesocarp oil.

Sources of variation	Degrees of freedom	Mean square values		
		Molar absorptivity at 270 nm	Oxidative stability (hour)	Total carotene content (mg/kg)
Gamma dose (D)	2	0.383 <sup>***</sup>	883.50 <sup>***</sup>	3981.24 <sup>***</sup>
Storage period (S)	3	0.096 <sup>***</sup>	96.46 <sup>***</sup>	935.15 <sup>***</sup>
D*S	6	0.013 <sup>***</sup>	126.74 <sup>***</sup>	486.28 <sup>***</sup>
Error	48	0.0014	3.378	29.31
CV (%)		15.18	23.05	5.91

<sup>\*\*\*</sup> significant at  $P < 0.0001$

### ANOVA in Chapter 3

Table 3.1. ANOVA for mesocarp and exocarp water activity of macaúba fruits dried with different temperatures and storage periods after harvesting.

Sources of variation	Degrees of freedom	Mean square values	
		Mesocarp water activity	Exocarp water activity
Temperature (T)	2	0.15262167 <sup>***</sup>	0.148021670 <sup>***</sup>
Storage period (S)	3	0.01489056 <sup>***</sup>	0.018844440 <sup>***</sup>
T*S	6	0.00591056 <sup>**</sup>	0.005086110ns
Error	48	0.00145667	0.00254583
CV (%)		4.14	5.72

<sup>\*\*\*</sup>, <sup>\*\*</sup> and ns- significant at  $P < 0.0001$ ,  $P < 0.01$  and non-significant, respectively.

Table 3-2. ANOVA for mesocarp moisture and oil content of macaúba fruits dried with different temperatures and storage periods after harvesting.

Sources of variation	Degrees of freedom	Mean square values	
		Mesocarp moisture content (%)	Mesocarp oil content (%)
Temperature (T)	2	7564.48***	710.28***
Storage period (S)	3	1270.64***	378.78***
T*S	6	286.56***	5.78ns
Error	48	60.61	19.01
CV (%)		26.72	8.27

\*\*\* and ns- significant at  $P < 0.0001$  and non-significant, respectively.

Table 3-3. ANOVA for specific activity of lipase and peroxidase of macaúba fruits dried with different temperatures and storage periods after harvesting.

Sources of variation	Degrees of freedom	Mean square values	
		Specific activity of lipase (U/mg)	Specific activity of peroxidase (U/mg)
Temperature (T)	2	282.17***	316813222.1***
Storage period (S)	3	433.21***	2193105.1ns
T*S	6	77.99***	4042031.8**
Error	48	8.72	1001990.9
CV (%)		32.79	21.84

\*\*\*, \*\* and ns- significant at  $P < 0.0001$ ,  $P < 0.01$  and non-significant, respectively.

Table 3-4. ANOVA for acidity and peroxide index and molar absorptivity at 232 nm of dried macaúba fruits and mesocarp oil with different temperatures and storage periods after harvesting.

Sources of variation	Degrees of freedom	Mean square values		
		Acidity index ( % oleic acid)	Peroxide index (meq O2/kg oil)	Molar absorptivity at 232 nm
Temperature (T)	2	0.184ns	7.69***	0.01301167***
Storage period (S)	3	101.168***	25.81***	0.06953111***
T*S	6	1.417ns	9.49***	0.01006944***
Error	48	0.819	0.58	0.00081667
CV (%)		43.39	19.47	7.29

\*\*\* and ns- significant at  $P < 0.0001$  and non-significant, respectively.

Table 3-5. ANOVA for molar absorptivity at 270 nm, oxidative stability and Total carotene content of dried macaúba fruits and mesocarp oil with different temperatures and storage periods after harvesting.

Sources of variation	Degrees of freedom	Mean square values		
		Molar absorptivity at 270 nm	Oxidative stability (hour)	Total carotene content (mg/kg)
Temperature (T)	2	0.01702167***	63.61***	1433.12***
Storage period (S)	3	0.14765556***	66.11***	1826.89***
T*S	6	0.00475056***	39.04***	600.06***
Error	48	0.00099917	5.19	22.79
CV (%)		14.89	18.66	6.24

\*\*\* significant at  $P < 0.0001$