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Brazilian fruit processing, wastes as a source of lipase and other biotechnological products: a review

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ABSTRACT

The global food loss and waste is the most urgent research area in food science to attend the current demand for more sustainable and profitable processes. Along the productive chain about 1/3 of the food is lost or wasted, this number reaches 1/2 for fruit and vegetable production in developing countries. Brazil has been investing in researches aiming to turn its wastes into byproducts, as biomolecules of high value such as lipases. These enzymes are found in a high diversity of plant sources and their researches are covered by promising market growth expectations due to the current demand for biofuels and bio-transformed food. Thus, the aim of the present study is to discuss the potential of wastes generated by the Brazilian fruit processing to become a source of lipases, by the analysis of the most recent studies on fruit lipases, as well as the inclusion of this process in the biorefinery concept. According to this concept, different products can be obtained from the same raw material. Considering the confirmation of the presence of lipases on fruit wastes, the annual fruit production and the percentage of residues, the assessed data showed that wastes from the processing of orange, mango, papaya and palm are promising for lipase obtainment.

Key words: fruit waste, biorefinery, plant lipase, orange waste, papaya peel and seed, mango peel.

INTRODUCTION

The global food loss and waste (FLW) is the most urgent research area in food science to attend the current demand for more sustainable and profitable processes, since, along the productive chain about 1/3 of the food is lost or wasted, which corresponds to around 1.3 billion tons (Eggersdorfer et al. 2016). This scenario is even more critical in relation to fruit and vegetable production in developing countries, wherein this loss reaches 1/2 (Kitinoja and Kader 2015), due to agricultural and technical problems (climate, transport, storage and post-harvest technology) but also due to the lower percentage of waste conversion into byproducts (Shafiee-Jood and Cai 2016, FAO 2015).

The definition of the term "food loss and waste" can vary in different studies, in this sense, the present study considered the following terms and respective definitions. Food loss is the decrease in edible food mass along the food supply chain, including production, post-harvest, processing, and transportation stages. Food waste is all material discarded or unused during the food processing,

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or any substance or object that the holder discards or intends or is required to discard. By-product is any substance resulting from a production process that can be used directly, without any processing other than that of normal industrial practice, and that don´t have overall adverse impacts from the environmental point of view or in human health, for a subsequent specific use (Baxter 2016, FAO 2015, MMA 2011).

In this context, Brazil is a country of large territorial extension and with different climatological conditions and plant cultivation systems. Moreover, Brazil stands out as the main producer and exporter of crops from different species and origins. Grain (cereals and oilseeds), fruits and vegetable yield in the 2014/15 harvest reached approximately 270 million tons. Overall, the estimates predict that this volume will keep on growing (CONAB 2015, IBGE 2003). Approximately 200 million tons of this production correspond to grains and cereals, which include soy, sunflower, corn, cotton, peanut, rice, castor beans, oat, rye, barley, wheat and triticale cultures. Most of the area for such production is located in the Southern and Midwestern regions (approximately 58 million hectares) and belongs to great agricultural producers (IBGE 2016).

Regarding fruits, Brazil ranks third among the world producers. The world fruit production reached 830 million tons in 2014, wherein, the three major producers, China, India, and Brazil, produced approximately 340 million tons of fruits in 2014 (FAO 2015, SEAB 2015). The Brazilian production profile differs from the world profile, i.e., the most produced fruits are, in descending order, orange, banana, coconut, pineapple, papaya, cashew nuts, cashew, and Brazil nuts; whereas the most produced fruits in global scale are banana, watermelon, apple, orange, and grapes (SEBRAE 2015, SEAB 2015). Production areas are spread throughout the country, and each specie is cultivated in the region that bests suits its climatic adaptation, most of these crops are managed by small producers (IBGE 2016, SEBRAE 2015).

Most of the fruits are subjected to some sort of processing before being traded, aiming to conserve their quality and the number of fruits available in the market, besides increasing their added value. Back in 2013, for instance, the fruits and vegetables processing industry processed approximately 136 million tons in China, 40 million tons in the USA, 4.8 million tons in India and 23 million tons in Brazil (SEBRAE 2015).

Fruit processing encompasses the production of whole and concentrated juice, frozen fruits and pulps, jams and syrup, dehydrated fruits, among other products (Sharma et al. 2016, Thassitou and Arvanitoyannis 2001). A high amount of wastes is generated during processing, and it represents high costs with proper destination, such as transportation and treatments applied to reduce environmental damages. However, this scenario has been changed by the inclusion of processes that use these wastes as raw material to obtain new products (Dhillon et al. 2016, Martínez et al. 2012). Once, the wastes of fruit processing represent a relevant quantity of FLW that means serious environmental risks, but also represent an expressive loss of nutrients with high bio-value (Patel et al. 2016).

Therefore, the conversion of wastes into byproducts is important and necessary to improve the sustainability and efficiency of the food supply chain (Baxter 2016). Since these wastes can be used to obtain pigments, flavors, fragrances, polysaccharides, biopesticides, phytohormones, biofuels, proteins and bioconverted oils (Panda and Ray 2015). Thus, the prospect is that the market for new bioproducts should move around USD 700 billion in 2018 (Panda et al. 2017, BBC 2015). Among the biomolecules that can be obtained from wastes, directly or indirectly (fermentative processes), are the enzymes lipases, capable of catalyzing hydrolysis reactions and triglyceride synthesis (Lopes et al. 2011). Lipases have been highly demanded by the enzyme market; therefore, new sources to obtain lipases have been currently studied.

Therefore, the goal of the current study was to assess the Brazilian fruit processing industry aiming at the inclusion of lipases and other biotechnological manufacturing processes based on wastes as raw material, as well as to discuss the inclusion of new products in the fruit production chain in Brazil.

PLANT LIPASES – BIOLOGICAL ROLE, FEATURES AND WASTES AS A LIPASE SOURCE

Enzymes are often proteins that act as biocatalyzers in living beings, accelerating biochemical reactions. This characteristic enables diverse biotechnological applications since they appear to be more efficient, more specific and less expensive than the chemical catalysis (Binod et al. 2013). More than 500 products and more than 150 industrial processes use enzymes in different production fields such as food, textile and detergent production, laundry, animal feeding, cosmetics, biofuel and fine chemicals production (Kumar et al. 2014). The enzyme market moved approximately 8 billion dollars in 2015 and estimates predict that this market will keep on exponentially growing until reaching 17 billion dollars in 2024 (Grand View Research 2016). Such increase is related to the growing demand for more sustainable products, such as biofuels produced by enzymatic catalysis; as well as to the demand for functional food with biological properties from catalytic processes (Prado et al. 2018, Markets and Markets 2015a, Kumar et al. 2014).

The enzyme activity is linked to the protein structure, which defines its function and specificity based on high diversity of functions. However, all described enzymes are divided by the *Enzyme Commission* in just 6 groups, according to the most common reaction catalyzed by the enzyme. It emphasizes the fact that biochemical reactions within different living beings are similarly catalyzed. Thus, most enzymes can be obtained from animals, plants, and microorganisms (Devasena 2010).

Commercial enzymes are mostly obtained through fermentation processes (Seth et al. 2014), however, plant enzymes are cheaper, because they can be obtained through simpler methods, without the need of applying genetic engineering, which is often used for microbial enzymes. It is estimated that plant lipases can cost 20 times less than those derived from fermentation processes, their value is expressed in enzyme activity units (Mounguengui et al. 2013).

Although the methods for plant enzymes production present lower costs, the majority of enzymes available on the market are isolated from microorganisms, which is justified by the fast growth (18 to 48 h for bacteria and 72 to 120 h for fungi, in general), using fermentative processes in reactors with controlled processes, while crops production demands longer development cycles, great territorial extensions and longer cultivation time. Moreover, enzymatic production through fermentation process can be carried out in reduced areas and its incubation time is limited to a few days (Lima et al. 2007). However, when the plant wastes are used to a direct enzyme obtainment, the barriers of agricultural production are neutralized, due to the use of already available plant sources (Okino-Delgado and Fleuri 2016).

Lipases are among the biocatalyzers with the most promising market growth expectations, since lipase production must generate approximately 600 million dollars per year up to 2020. It would mainly happen due to the increased demand in Asia and in Latin America concerning the fields of health, biofuel and food production (Markets and Markets 2015b).

The reaction typically catalyzed by lipases is the long-chain acyl glycerol hydrolysis, thus, lipases are grouped in the ester-carboxylic acid hydrolases subclass according to the Enzyme Commission.

They are codified by the number 3.1.1.3 and called triacylglycerol acyl hydrolase. The lipid hydrolysis reaction consists of breaking the ester bond from the glyceride to produce glycerol and fatty acids in presence of water (Castro and Sato 2014).

Lipases are also capable of catalyzing transesterification reactions under low water conditions. They may change the fatty acids composition and distribution, since the exchange of acyl radicals between compounds results in glycerides different from the initial ones. The name of the reaction changes according to the substance. Thus, it is called acidolysis, when it occurs between one ester and one acid; alcoholysis, when the ester is replaced by an alcohol; and interesterification, when it happens between two esters. One fatty acid, one alcohol or other ester react with the glyceride in transesterification, and it results in the rearrangement of fatty acids groups to produce a new glyceride, which has diverse physical and chemical properties (Carvalho et al. 2003, Pereira et al. 2003).

Lipases may have different characteristics depending on the source. Plant lipases vary from acid such as obtained from palm fruits (pH 4.5) (Abigor et al. 1985), pass through neutral such as those from orange wastes (pH from 6.0 to 7.0) (Okino-Delgado and Fleuri 2014), and reach the basic range like those deriving from wheat and soy grains (pH 8.0) (Kapranchikov et al. 2004). The optimum temperature of plant lipase lies between 20°C to 40°C (Seth et al. 2014). For the bacterial lipases the optimum conditions are usually alkaline pH (8.0) and temperature range from 45 to 50ºC (Chakraborty and Raj 2008, Panda et al. 2016). While for fungal lipases obtained from *Aspergillus niger* and *A. oryzae* the optimum conditions are usually acid to neutral pH (Ghosh et al. 1996) and temperatures from 40 to 60ºC (Contensini et al. 2010).

Plant lipases can be used in the interesterification of short and long triglycerides via papaya lipases to produce aromas, in the alcoholysis of plant oil for biodiesel production via castor bean and rapeseed lipases, and triglyceride hydrolysis via cereal lipases for food industry (Mounguengui et al. 2013, De Maria et al. 2006, Gandhi and Mukherjee 2000).

Despite the expressiveness of lipases market, until 2014 only 29 lipases were indexed to the UniProt database, among which, just a few were characterized. Table I lists the sources used in the commercial lipases and shows the reduced number of exploited species, among them, there are eight lipases isolated from fungi, two from yeasts, three from bacteria, one from animal and one from plant.

The price of commercial lipases varies from approximately US$ 0.87/100 activity units for pig pancreas lipase (Sigma-Aldrich®) to US$ 5,000.00/100 activity units for *Candida antartica* lipases (Sigma-Aldrich®) (Sigma-Aldrich 2017). This difference can be explained by the characteristics of the enzymes, the difficulty of obtaining, the process yield, the need of purification, and the inherent requirements of each application (BioCon 2017, Devasena 2010). Applications, such as in the fine chemicals and cosmetics production field, demand many specific reactions that need purified extracts of high enzymatic activity, restricting the number of lipases able to be used, as well as increasing the obtainment costs due to the need of purification. However, there are simpler applications, such as in the modification of emulsified olive oil, since most available lipases are capable of catalyzing less concentrated and crude extracts. Moreover, there are requirements inherent to the application area, as it occurs in the vegan food industry, which does not allow using genetically modified animal or microbial sources (Amano Enzymes 2017).

Thus, the low diversity of species used as lipase sources evidences the importance of studying new sources of these enzymes, mainly those resulting from plant wastes. Besides, plant lipases can have different characteristics from the

TABLE I
Species used as commercial lipases source.

Company	Species	Lipases source	Source
Sigma-Aldrich	*Aspergillus niger*	Fungi	www.sigmaaldrich.com
Novozymes	*Aspergillus oryzae*	Fungi	www.novozymes.com
Amano Enzymes	*Burkholderia cepacia*	Bacteria	www.amano-enzyme.co.jp
Sigma-Aldrich	*Candida antartica*	Yeast	www.sigmaaldrich.com
Novozymes	*Candida antartica* A	Yeast	www.novozymes.com
Novozymes	*Candida antartica* B	Yeast	www.novozymes.com
c-Lecta	*Candida antartica* (CalB)	Yeast	www.c-lecta.com
Sigma-Aldrich	*Candida rugosa*	Yeast	www.sigmaaldrich.com
Novozymes	*Humicola insolens*	Fungi	www.novozymes.com
Sigma-Aldrich	Pig (pancreas)	Animal	www.sigmaaldrich.com
Sigma-Aldrich	*Pseudomonas cepacia*	Bacteria	www.sigmaaldrich.com
Amano Enzymes	*Pseudomonas fluorescens*	Bacteria	www.amano-enzyme.co.jp
Sigma-Aldrich	*Rhizomucor miehei*	Fungi	www.sigmaaldrich.com
Novozymes	*Rhizomucour miehei*	Fungi	www.novozymes.com
Sigma-Aldrich	*Rhizopus oryzae*	Fungi	www.sigmaaldrich.com
Biocon	*Rhizopus oryzae*	Fungi	www.biocon.es
Novozymes	*Thermomyces lanuginosa*	Fungi	www.novozymes.com
Sigma-Aldrich	*Thermomyces lanuginosus*	Fungi	www.sigmaaldrich.com
Sigma-Aldrich	Wheat (germen)	Plant	www.sigmaaldrich.com

microbial lipases, enabling applications in distinct processes. Therefore, the next section describes the main fruits produced and processed in Brazil, the wastes from their processing, their biotechnological applications and the discussion about the potential of these wastes for lipases and for the obtainment of other biotechnological products based on the biorefinery concept.

ORANGE (*CITRUS SINENSIS* L. OSBECK)

Orange is the second most produced fruit in the world, it reaches 800 million tons per year, and Brazil is its major producer. Most of the Brazilian production is processed for the obtainment of concentrated juice, which corresponds to 80% of the total volume of produced fruits. Wastes generated during this process represent 50% of the fruit mass (SEBRAE 2015, IPEA 2012), and it is estimated that more than 15 million tons of citrus wastes are generated worldwide (Marin et al. 2007).

The main wastes generated from orange processing for juice production are bagasse (that corresponds to the fruit mesocarp) peel, (corresponds to the fruit epicarp), peel fragment or frit (that is constituted by the most external part of the epicarp), and the pulp (corresponds to the endocarp). Such fractioning is a result from the adoption of the *in line* equipment used by the company J.B.T. Food Tech, the most used equipment to extract citrus juice in the world (J. B. T. Food Tech 2018, Okino-Delgado and Fleuri 2016, Vidal and Vidal 2006).

Citrus wastes are often used to obtain dehydrated pulp, pectin, fibers and essential oils, and its extraction have been enhanced through techniques such as that described by Hosseini et al. (2016) to extract pectin; and by Boukrofa et al. (2015) to obtain pectin, polyphenols and essential oils. However, the volume used for this purpose is low in comparison to the total of produced wastes. Thus, it is worth searching for new applications of these wastes, such as their use for biotechnological purposes. This focus is possible and promising because the extraction process is mechanical, totally performed under mild temperature and pressure conditions, without the addition of chemical compounds, which could disrupt and/or diminish the biological properties of the compounds found in the wastes (Okino-Delgado and Fleuri 2016).

Orange wastes can also be used as a substrate in fermentation processes, as described by Madeira Jr. et al. (2014) about solid-state fermentation using *Paecilomyces variotii.* Their study aimed at increasing the antioxidant capacity and the yield of hesperidin, naringin and ellagic acid. The same was described by Mantzouridou et al. (2015), that used fermentation with the commercial yeast Vitilevure MT (*Saccharomyces cerevisiae*) to produce aromas. Another study used bagasse as the substrate in fermentation processes and focused on biogas and methane production (Erdogan et al. 2015). The peel was tested as solid fuel and as heavy metal bioadsorbent (Santos et al. 2015).

These wastes can also be used as direct lipase sources, as described by Okino-Delgado and Fleuri (2014). Orange lipase wastes have been applied in cooking oil bioremediation (Okino-Delgado et al. 2017) and in the modification of plant oils for biodiesel production. Moreover, other studies suggest the application of these enzymes for plant oil bioconversion in order to increase the biological activity for pharmaceutical purposes, which proves the diversity of these enzymes (Contensini et al. 2010).

MANGO (*MANGIFERA INDICA* L.)

Mango is among the most produced tropical fruits in the world, including in Brazil, which ranks seven among the biggest producers and is the second major exporter. Brazil has 66 thousand hectares in Southwestern and Northwestern regions dedicated to this crop (FAO 2015, EMBRAPA 2010).

Mango composition, as well as its wastes, changes depending on the soil and climate conditions, maturation point and variety. However, overall, pulp corresponds to 60% of the total fruit mass, and it is constituted by carbohydrates, lipids, proteins, vitamins, minerals, fibers, tannins, polyphenols, flavonoids, alkaloids, pigments and aromatic compounds (Jahurul et al. 2015, Ma et al. 2011). The peel corresponds to approximately 25% of the fruit mass, and it is constituted by fibers, minerals, mono- and diterpenes, and by antioxidant compounds such as the phenolic compounds mangiferin, kaempferol, quercetin, and anthocyanins. Seeds correspond to approximately 15% of the total fruit mass and are constituted by starch, fibers, lipids, and by tocopherols and sterols such as campesterol, stigmatesrol and β-sitosterol (Jahurul et al. 2015).

Mango wastes can be used as a substrate in fermentation processes such as in the study described by Fernando et al. (2014), in which pulp and wastes were used as a substrate for *Saccharomyces cerevisiae* Y2034 to produce bioethanol. They can also be used to extract pectin, as described by Pandit et al. (2015).

Lipases can also be obtained directly from mango wastes as described by Okino-Delgado (2014) that produced lipases using peel and pulp of mango fruits, variety Tommy Atkins. The enzymes present optimum activity in olive oil at acid to neutral pH and temperatures up to 51°C, showing that the mango wastes could be used as a direct lipase sources.

GRAPES (*VITIS VINIFERA* L. AND *VITIS LOBRUSCAS* L.)

Approximately 74 million tons of grapes were produced worldwide in the 2013/14 harvest. The production is concentrated in Europe, mainly in Italy, France and Spain which represents more than 50% of all grapes harvested in the world (UVIBRA 2014). Approximately 1.5 million tons of grapes were produced in Brazil in the 2014/15 harvest, and this production was concentrated in few regions, mainly in Serra Gaúcha – located in Rio Grande do Sul State, where most of the production is used in wine and juice manufacturing (EMBRAPA 2015).

Grape peel and seeds, wastes from wine processing, are rich in phenolic compounds, including flavonoids and phenolic acids, which have the ideal structure for free radical sequestration due to their high antioxidant activity. Therefore, these parts have been studied in order to treat and prevent degenerative and tumor diseases, conserve food, and produce cosmetics focused on preventing early aging (Nogales-Bueno et al. 2017, Riebel et al. 2017). Researches conducted by De Camargo et al. (2016) showed that phenolic compounds extracted from grape wastes were capable of inhibiting the action of α-glucosidase and lipases; therefore, they could be used to treat diseases such as diabetes and obesity.

Grape seeds have approximately 15% of lipid content and are used for oil obtainment, mainly in Europe. Oil extraction in Brazil remains low, but there is a high growing potential due to its versatile application in food, cosmetic and biodiesel industries (Freitas et al. 2008).

Grapes and its wastes are sources of chitinases, endoglycosyl hydrolases, which catalyze the hydrolysis of β-1,4 bonds of chitin. In turn, these bonds are among the most abundant carbohydrate sources; besides, they are found in the exoskeleton of insects and crustacean, as well as in the cell wall of fungal species. Plant chitins are associated with defense mechanisms since chitin, the main component of insect's exoskeleton, is degraded through chitinases. When plants are attacked, metabolic processes that induce ethylene and salicylic acid production are activated which, in turn, stimulates chitinases production and increases in immature fruit concentrations. These enzymes can correspond to 50% of the soluble protein concentration found in fruits under these conditions (Patel et al. 2016, Gomes et al. 2010).

Grape seed extracts have inhibitory action in pancreatic lipases and in cholesterol esterase; therefore, they have been studied to treat and prevent obesity hyperlipidemia (Adisakwattana et al. 2010, Moreno et al. 2003). However, data about the genus *Vitis vinifera* indicate the presence of lipases that would be involved in lipid catabolism (UniProt 2017); thus, by taking into account the presence of lipases in plants and the high concentrations of compounds with inhibitory action over this enzyme, it is possible assuming that lipases can be obtained from grapes and, consequently, from their wastes. However, further studies are necessary to assess the presence of active lipases, their features, and potential applications.

COCONUT (*COCO NUCIFERA* L.)

Coco nucifera L. palm tree is naturally distributed in all continents; approximately 90 countries cultivate it for commercial purposes. The commercial cultivations are concentrated in coastal regions, mainly in the Asian continent. Approximately 60 million coconut tons were produced in the world in 2008 (EMBRAPA 2011a).

In Brazil, coconuts are mainly consumed *in natura;* however, the processing of different fruit parts has been growing. The solid endosperm or albumen, which is commercially known as almond, corresponds to the white part of the pulp; it is dehydrated and used in grated coconut and coconut milk production. The liquid part corresponds to the endosperm, it is popularly known as coconut water, and has been processed and commercialized in bottles. The mesocarp is used as a fiber source in many processes (EMBRAPA 2011b).

Coconut oil is constituted by saturated triglycerides and has high market value; it is used in food, pharmaceutical and biodiesel industries (Sulaiman et al. 2013). Coconut biodiesel presents better performance than those produced from other plant oils, due to its specific and reduced heat (Kalam et al. 2016).

Lignocellulosic wastes deriving from the fruit mesocarp have also been assessed as substrate in fermentation processes focused on bioethanol manufacturing (Soares et al. 2016).

Ejedegba et al. (2007) aimed at showing that the coconut almond extract presents lipase activity in coconut oil, triolein, tripalmitin and olive oil at temperatures up to 35°C and pH range from 7.5 to 8.5. However, fruit processing includes the autoclaving stage, which is performed to make the almond separation from the fibrous peel easier (EMBRAPA 2010). This stage denatures the enzymes found in the wastes and, consequently, it is not possible exploring wastes for direct enzyme obtainment. Thus, the peel fraction of the coconut processing has been studied as a source of polymers and as support and substrate in fermentation processes focused on biofuel production (Kocaman et al. 2017).

PAPAYA (*CARICA PAPAYA* L.)

Brazil is the number one papaya producer in the world, more than 15 million tons were produced in 2013. Bahia State is responsible for most of this production, approximately 45% of the entire Brazilian production (IBGE 2014).

Immature papaya fruits, as well as the stem of papaya trees, have latex rich in enzymes such as proteases and lipases. Papaya trees in Asia and Africa are exclusively cultivated for latex obtainment, which is used in the pharmaceutical, food and cosmetic industries. Lipases from papaya latex are capable of catalyzing esterification, transesterification and interesterification reactions of glycerides (De Maria et al. 2006). Paques (2008) characterized the biochemistry of papaya waste lipases, which were capable of keeping their activity after concentration per precipitation with acetone and ammonium sulfate; they also presented optimum activity at pH ranging from acid to basic, at 55°C.

Thus, the agro-industrial wastes from papaya processing could be explored for lipases obtainment, since they have the differential of maintaining their activity within the reaction media at high solvent concentration.

CASTOR BEAN (*RICINUS COMMUNIS* L.)

Castor bean is distributed all around the world, and grows in climates from warm tropical to humid and temperate. The fruits are the commercially explored parts of the plant, which store smooth oval seeds with different sizes and colors (Malhotra et al. 2015).

The seeds correspond to 50% of their mass and are constituted by oil, which has biological activity associated with the protein ricin and with the alkaloid ricinin. Castor bean oil causes diarrhea, anorexia, weakness, apathy, and even death when it is ingested by animals and insects. It also presents other properties that are explored by the pharmaceutical industry such as convulsant activity and memory stimulant (Salimon et al. 2010).

Approximately 1.1 tons of wastes are generated during oil production. These wastes, in their turn, are used as biomass to produce power and in organic fertilization, since they cannot be directly used to feed animals due to their toxicity (Santos et al. 2016). However, it is possible to biodetoxify these wastes, allowing its use for animal feeding purposes (Godoy et al. 2009).

The presence of lipases in castor bean seeds was first recorded more than 50 years ago, and they present optimum activity at acid pH and stability at mild temperatures, as well as the capacity to catalyze the hydrolysis of plant oils rich in linoleic and linolenic acids (Avelar et al. 2013, Ory et al. 1960). However, they are not commercially explored for such means because they are active in dormant seeds, only, i.e., when the seeds are still immature and cannot be used for oil extraction (Avelar et al. 2013). Nevertheless, castor bean processing wastes can be used as substrate in fermentation processes focused on the production of different enzymes by microorganisms, such as cellulases by *Pseudomonas aeruginosa* (Amara and Salem 2009).

PALM (*ELAEIS GUINEENSES* JACQ.)

Palm or palm tree is natural to the African continent and cultivated all around the world at the Equator line. Palm production in Brazil is concentrated in the Northern region and covers approximately 65 thousand hectares (EMBRAPA 2001).

The fruit is the commercially explored part, which belongs to the spherical sessile drupe type (approximately 4 cm and weighs up to 30 g). The oil can be extracted from different parts of the fruit (Castro and Kluge 2001).

The oil from the mesocarp is called palm oil and represents approximately 50% of the fresh fruit mass; it is the second most produced oil in the world. It has reddish shade due to the presence of carotenoids, despite being rich in vitamin E precursors. On the other hand, the oil from the endocarp is commercially known as *palmist*, which is considered to be the byproduct from the palm oil production, thus corresponding to approximately 3% of the fruit mass (Silva, unpublished data).

The presence of active lipases was described in oil from the mesocarp, they present optimum activity at pH range from acid (4.5) to alkaline (9.0) and at mild temperatures ranging from 30°C to 35°C; these characteristics change according to the maturation stage and to the water concentration in the fruits (Ebongue et al. 2006, Abigor et al. 1985). Thus, lipases obtainment could be added to the palm oil cold extraction process, which is conducted through simple methods such as centrifugation, without damaging the final product.

Lipases can be obtained from other plant sources than from those currently used in the market. The summary of the plant waste potential shown in the present study as lipase sources is described in Table II. It is possible noticing that just few cultures, among the described ones, have high potential for the exploration of their wastes as direct lipase sources, due to characteristics inherent to the fruit; as the lipase activity only in dormant seeds (castor beans), as well as the presence of inhibitory lipases compounds (grape); or to processing, such as the use of high temperatures (coconut). On the other hand, the wastes generated by orange, mango, papaya and palm processing present high potential for direct lipase obtainment, due to the high lipase activity and to the large amount of wastes generated during different processing, which are appropriate to the maintenance of the enzyme activity. Wastes resulting from fruit processing can be used in different fields: peels from mechanical and physical processes are interesting for the direct obtainment of compounds to keep original fruit features. The pies deriving from heat extraction processes can be used as biomass in power production. Thus, further studies about the obtainment and application of lipases from these wastes could add new options of source for commercial exploration.

ANALYSIS OF THE FRUIT PROCESSING WASTES FOR USE IN BIOTECHNOLOGICAL APPLICATIONS AND THE BIOREFINERY PERSPECTIVE

The reuse of wastes for conversion into power, to feed animals and as source for the obtainment of

TABLE II
Potential of vegetal wastes for lipase obtainment.

Vegetal	Main product	Wastes	Lipase presence	Potential for lipase obtainment	Source
Orange	Juice	Frit, peel, bagasse and pulp	Confirmed and characterized	High, standardized processing	Okino-Delgado and Fleuri (2014a)
Mango	Pulp	Peel	Confirmed and characterized	High, high activity and production, however the processing is not standardized	Okino-Delgado and Fleuri (2014b)
Grape	Juice	Peel and seeds	Theoretically confirmed	Low, inhibitory action of lipase	Adisakwattana et al. (2010)
Coconut	Pulp	Peel	Confirmed	Low, high temperature during processing	EMBRAPA (2010)
Papaya	Pulp	Peel and seed	Confirmed and characterized	High, high activity and commercial use for the extraction of other enzymes	De Maria et al. (2006)
Castor bean	Oil	Peel and bagasse	Confirmed and characterized	Low, activity in dormant seeds, only	Avelar et al. (2013)
Palm	Oil	Peel and bagasse	Confirmed and characterized	High, high activity and commercial use for the extraction of other enzymes	Ebongue et al. (2006)

high added-value molecules, such as antioxidants and enzymes, has been considered as a feasible and sustainable option. However, the application type depends on the characteristics of processes adopted to transform the material *in natura* into final products, since different wastes with distinct biochemical features are generated. The main fruit processing types will be described next, with emphasis on the analysis of the generated wastes and on the discussion about their applicability for biotechnological purposes, as well as on the integrating perspectives of reusing wastes in existing processes to implement biorefineries.

Fruit processing is divided into primary and secondary transformation. The primary transformation encompasses processes able to prolong the shelf life of agricultural products by turning them into products that can be commercialized, but that are often used as raw material for secondary transformation, i.e., they constitute simple processes to extract pulp, essential oil, aromas and flavors. The secondary or final transformation, in its turn, generates more elaborated products, including beverages, sweets, jams, among others, to be consumed by final consumers (Cunha et al. 2008).

The type of product obtained from processing depends on the species, variety and physicochemical characteristics of each fruit to be processed; the main products are pulp, juice, nectar, beverage, oil, sweet past, jams and dehydrated fruits (Oliveira and Santos 2015). The fruit pulp and juice obtainment processes and the involved processes and the generated wastes are described below.

Fruit pulp is the non-fermented, non-concentrated, non-diluted product obtained from pulpy fruits through proper technological process. The pulp must have minimal total solid content, which must result from the edible part of the fruit obtained through primary transformation (MAPA 2000). Fruits are firstly sanitized and washed; it is possible adding chemical products such as hypochlorite or detergent to the process. The sanitized fruits are selected, and the fermented or immature ones are discarded. After selection, depending on the characteristics of each species, the fruits can be directly conducted to pulping, such as grapes, apples, *acerola* and *cajá;* or to be peeled, as it happens with bananas, mangos and papaya. Pulp, then, can be pasteurized or frozen in order to prolong the conservation time (Tolentino and Gomes, unpublished data).

Juice is the product obtained through the dissolution of the pulp from pulpy fruits in water through the appropriate, non-fermented technological process to keep the color, aroma and flavor characteristic of fruits subjected to a treatment that assures their conservation until consumption (MAPA 2003). Juice production includes the pulp processing stages and may include the dilution and addition of preservatives or other additives for standardization (Oliveira and Santos 2015).

The inadequate fruits for consumption are discarded during selection, i.e., those that are immature, fermented or rotten, those that overall present compromised organoleptic (aroma, flavor and texture) and microbiological characteristics (EMBRAPA 2004). The characteristics of wastes in this processing stage vary a lot since they are constituted by out-of-standard fruits (immature fruits and fruits in rotten stage are discarded together). The soluble solids content, for instance, faces high variation in different maturation stages; they can vary from the 14° BRIX, when the fruits are green, to 22° BRIX, when the fruits are yellow, as it happens with mangos belonging to variety Ataulfo. The total phenolic compounds also vary during mango maturation, the concentration is lower in green fruits, the peak is reached when fruits are in the ideal consumption stage and reduction is recorded when the fruits get close to the rotten stage (Aziz et al. 2012, Palafox-Carlos et al. 2012a). Thus, the use of wastes as direct source in the selection stage can be compromised due to low uniformity; therefore, they are more recommended for use as substrate in fermentation processes because of the high concentration of nutrients and humidity.

Peeling can be performed in equipment or by hand. Overall, the equipments are made out of stainless steel and include sets of knives that mechanically remove the peel to separate the edible parts. This stage is interesting for the use of wastes generated as direct compound sources since the peel is the part wherein the compounds related to fruit protection and of high biotechnological interest are often found. Mango peels, for example, show higher flavonoids concentration than the pulp (Palafox-Carlos et al. 2012b). The peeling and refining stages, though, generate promising wastes for use as raw material in biotechnological applications, because they are homogeneous and large amounts of bioactive compounds and enzymes are found in fruit's peel and seeds.

Heat pulping is the process used to separate the pulp from the fibers that present fast darkening such as apples, bananas and peaches. Heating inactivates the enzymes through disruption and immediately paralyze darkening (Oetterer et al. 2006). Moreover, they end up affecting other enzymes, which prevents wastes from these processes to be used in the direct obtainment of enzymes, vitamins, aromas and other thermal-sensitive substances (Castro and Sato 2014). Thus, it is assumed that waste use as direct source is possible for substances that are not altered by high temperatures such as flavonoids, polymers and fibers.

Throughout the pulping and refining processes, seed are often removed intact, since their disintegration can change the flavor of the product. Seeds are easily separable from the edible part, they are manually separated or even separated by mechanical equipment such as the case of papaya, star fruit and citrus. Therefore, it is assumed that these seeds can be used in the direct obtainment of bioactive compounds and enzymes.

The reuse of wastes can be maximized when the concept of biorefinery is implemented. The aim of such concept is to integrate biomass conversion processes to new products such as biofuel, chemical inputs, bioactive compounds, materials, food, feed and power, by optimizing resources and minimizing effluents and by maximizing benefits and profit (EMBRAPA 2011b).

Once biorefineries use different conversion routes, including biochemical, microbial, chemical and thermal-chemical paths, their concept is dynamic and is totally developed; however, they can be defined as *"the gathering of technologies between biological raw materials, industrial intermediates and final products"* (Kamm and Kamm 2004). Nowadays, there are different examples of installed biorefineries, such as sugarcane, ethanol and bioelectricity production plants based on sugarcane, as well as oil, feed, biodiesel, and plants to produce many other soy derivatives. However, the concept could be applied to many other cultures, mainly in Brazil, which presents high a diversity of cultures and processes (EMBRAPA 2011a). The flowchart shown in Figure 1 includes the most frequent fruit processing stages, the wastes generated in these stages and the suggestion for their use in biotechnological applications. The material discarded in the selection stage is less uniform, which is why they are less recommended for the obtainment of active compounds and enzymes through direct manners. On the other hand, wastes generated during peeling are, overall, uniform and concentrate bioactive compounds and enzymes. Therefore, new products would be included in already installed processing, increasing process profitability and sustainability.

The citrus industry is among the highest potential ones due to the size of the sector, standardized processing without denaturing stages, what enables the obtainment of new products without interfering in the production of the main product. Wastes such as frit, peel and bagasse from the orange processing for juice production can provide biocatalyzers, antioxidants and fibers. Depending on the waste, it can be used for essential oil, pectin and aroma obtainment after these biomolecules are extracted; subsequently, it can be used to feed animals, to produce bioenergy or it can be used in fermentation processes focused on the obtainment of new enzymes and bioactive compounds. On the other hand, orange processing for concentrated juice production can provide pulp, which is a source of antioxidants and aromas; after these compounds are extracted, the pulp can also be used to feed animals, to generate bioenergy or it can be used in fermentation processes (Okino-Delgado and Fleuri 2016).

Other types of processing that could include the fruit processing biorefinery concept are the pulp industries, which use the cold extraction process in pulp from different fruit species throughout the year. Therefore, processing would extract fruit pulp as main product, whereas fruit peel could be used in processes to extract antioxidant compounds and enzymes, the seeds could be used in oils and for enzyme obtainment processes and lignocellulosic wastes could be used for power generation or as substrate in fermentation processes.

CONCLUSIONS

Fruit processing has increased in order to maximize durability and add value to products; however, as consequence, the generation of wastes has also grown, mainly because the ratio between main products and wastes is 1:1 (m/m), on average. Thus, the use of these wastes as raw material in biotechnological processes, besides increasing profitability due to the inclusion of new products, reduces waste discard and makes the process more

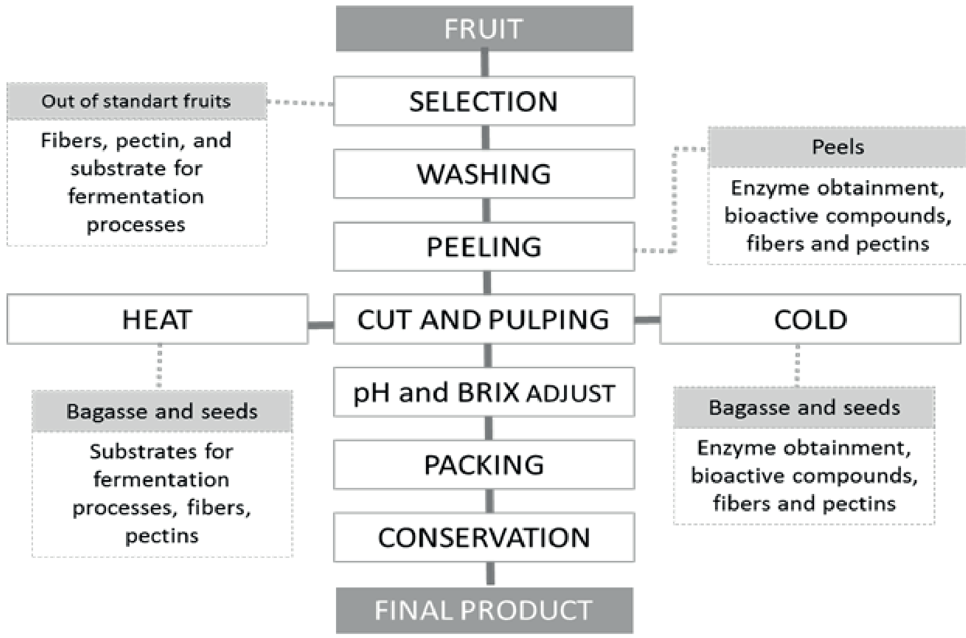

Figure 1 - Wastes generated during fruit processing and possible biotechnological applications.

sustainable. Lipases stand out among the new products with expressive place in the market and are extracted from a few sources for commercialization, although they are found in large amounts and in different species. Among the analyzed species, orange, mango, papaya and palm processing wastes present the highest potential to direct lipase obtainment. Moreover, the obtainment of lipases and other biotechnological products could be incorporated to the fruit processing, implementing biorefinery concept, in which a crop would enable the creation of different products, thus making the processing more profitable and sustainable.

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