Valorisation of agricultural residues from Andean fruits: case studies

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Abstract

The growing demand for natural and healthy foods has boosted fruit production in Colombia and all around the world. In the links of the productive chains and in the stage of final consumption of fruits, large amounts of waste are produced, including stalks, stubble, foliage, husks, seeds, and non-commercial or unharvested fruit. These materials are wasted and are usually left in the field or make an important part of the waste that is deposited in municipal landfills with the consequent contaminating effects and risks to public health. Many alternatives have been proposed for the transformation of fruit residues into products of different levels of added value. In this article, a general and updated review of these proposals is initially made as a starting point to apply some of them in the use of the waste of five Andean fruit trees: blackberry, tamarillo, guava, passion fruit and banana. With the exception of these last two fruits, there is very little that has been researched about the use of the residues of the Andean fruit trees analyzed. In this article some innovative food and non-food processing options for the sustainable exploitation of agricultural and agroindustrial waste of these cultivars are suggested. They include the production of tisanes from seeds, the manufacture of supports for enzymes and the production of alcohols and other chemical products under the biorefinery scheme.

1. Introduction

Fruit consumption worldwide grew at 3% annually in the last decade. The Asian countries are the main producers that contribute almost 60% of the world volume of the fruit [1]. South America produces a quarter of Asian supply, and although Brazil is the first producer, Colombia is the country with the highest growth in fruit growing in the region between 2014 and 2017. More than 85% of agricultural production in Colombia comes from small producers. In the framework of a project to increase the productivity and competitiveness of the Andean fruit chains, the use of agricultural and agroindustrial waste of five fruits was analyzed, namely: blackberry fruit (*Rubus glaucus* Benth), passion fruit (*Passiflora edulis*), guava *(Psdium guajava)*, tamarillo *(Cyphomandra betacea* (Cav,) Sendtn*)* and banana *(Musa paradisiaca)*. Global annual production of these fruits, tropical fruits, banana, and the estimations of their agricultural residues are listed in Table 1.

Table 1. Global and Colombian production of tropical and five Andean fruits

Fruit	**Production (Metric tons)**		**Agricultural residues estimation (Metric tons)**	
	World (2016) [2]	Colombia (2017) [3]	World	Colombia
Passion fruit	24.184.510*	119.338	3.200.000 to 3.600.000*	14.000 to 17.000
Guajava		70.054		8.000 to 10.000
Tamarillo		186.032		26.000 to 31.000
Blackberry		140.504		18.000 to 23.000
Banana	113.280.302	2.040.497	550.000.000 to 600.000.000	9.000.000 to 11.000.000

*Tropical fruits

Studies from different countries showed that during production and harvesting approximately 20% is lost and in the industrial processing for 30 to 50% [4]. According to the Food and Agriculture Organization (FAO), annually around the world 1.3 billion tons of food is lost or wasted [5]. The five analyzed Andean fruits encompass a substantial amount of skin and seed which are underutilized or disposed as wastes after consumption or industrial processing (Table 2).

Table 2. Percent of flesh, skin, and seed in five Andean fruits.

Fruit	Flesh (%)	Peels (%)	Seed (%)
Passion fruit	44-56	45-55	1-8
Guajava	60-65	16-18	8-12
Tamarillo	53-58	14-16	20-24
Blackberry	88-96	-	4-12
Banana	63-70	30-37	-

Fruit and vegetables peels and seeds are generated in large quantities in big cities in which they become one of the main sources of c. Incorrect management of landfill will result in emissions of methane and carbon dioxide, and incineration releases pollutants and dioxins, furans, acid gases as well as particulates, which pose serious environmental and health risks [6]. Moreover, the long-term disposal of these wastes to the environment also facilitates a breeding ground for bacteria, pest and mice which lead to the spread of plague. The biomass of fruit plant cultivars, marketable and non-commercial fruits, and their agricultural, agro industrial and consumption wastes, contain a large number of molecules of interest. These materials are composed of cellulose, hemicellulose, lignin, fibers, vitamins and secondary metabolites (polyphenols, carotenoids, sterols, flavors, dyes, essential oils, flavonoids, alkaloids, tannins, coumarins, lactones, terpenes, saponins), among many others. One of the options evaluated for waste treatment are the development of products like enzymes, organic acids, flavoring compounds, food colorants, bio-ethanol, bio-methane via microbial applications

This paper describes some studies carried out for the use of the waste coming from the cultivation, harvest and processing of the blackberry, tamarillo, guava, passion fruit and banana Andean fruits. Through a technological surveillance of the potential uses of agricultural waste allowed the selection of processing alternatives. Under the biorefinery concept, the extraction of butanol, the preparation of biocomposite materials, the extraction of cellulose, the manufacture of catalytic supports and the production of food ingredients were considered.

2. General methodology and results

The technological surveillance of the potential uses of agricultural waste allowed the selection of processing alternatives. The raw materials, intermediates and products were elaborated with different methods and then characterized. The physical and chemical characterization included the use of the following techniques: antioxidant activity by measurement of DPPH radical inhibition [7]; total polyphenol content by Folin-Ciocalteu [8], scanning electron microscopy morphology. Fiber-filled biocomposite materials that were studied as enzyme supports, used foamed matrices of epoxidized pine oil resin. Tisanes and fruit fillings for confectionery based on these wastes were fabricated and subjected to sensory evaluation. Under the scheme of biorefineries the extraction of butanol from residues of the refining of fruit pulps was simulated. Hereinafter every part of the present work is separately described in terms of specific methodology and results.

3. Technological surveillance

The technological surveillance is an organized process to get information regarding science and technology, choosing, analyzing and recording it, in such a way this information becomes in knowledge. The technological surveillance has to be concentrated on both, current technologies and new ones, with the goal of selecting suitable and sustainable process to obtain valorization of fruit co-products, not only from a technological perspective but from market, socio-economic and regulatory points of view, to identify the best choices for each potential raw material.

The sources that was monitored included recent reviews, articles, books, theses, papers, reports, products, business and government websites, among others, dealing with studies on the beneficiation and application of fruit wastes. Different search engines were used in academic and patent databases, and different search equations were used with the keywords of the subject (i.e. ("*passiflora edulis*" OR "passion flower") AND (fiber

OR residues); ("*Psidium* guajava L") AND (Fiber OR residue); ("*solanum betaceum*" OR "cyphomandra betacea" OR tamarillo) AND (fiber OR residue); (banana AND (fiber OR residues))

3.1 Results

To take advantage of the low cost and high availability of fruit wastes, and to minimize their environmental impact, the recovery of health benefit compounds and transformation to other value-added products have become the focus of researchers recently. This trend is reflected in recent important reviews (Table 3).

Table 3. Relevant reviews on fruit wastes uses (2011-2018)

Applications	**Ref.**
Byproducts of the fruit processing industry (such as peels, seeds and unused flesh) are rich in bioactive compounds. Amongst the possible uses for these compounds that can be used as antioxidants, antimicrobials, flavoring, colorants and texturizer additives.	[9]
The antioxidant capacities, phenolic contents and their correlation, for water- and fat-soluble extracts of the residues of 50 fruits were studied in detail. .	[10]
Nutritional value, conservation methods and feeding management of some fruit wastes and by-products (Cashew apple meal, citrus pulp, grape and raspberry pomace, banana foliage, banana dried ripe peels, passion fruit seed meal and pineapple waste) are discussed.	[11]
This review focuses on the use of various agricultural waste peels as adsorbents for the water and wastewater treatment.	[12]
Fruit wastes and wastewaters, with a particular emphasis on research in South Africa, were reviewed. The characteristics and composition of each type of fruit waste are highlighted and their potential as feedstocks in the production of value-added products (mainly biofuels and enzymes) is identified.	[13]
The review is focused on polyphenolic compounds recovery from tropical fruit wastes (durian, mangosteen, rambutan, mango, jackfruit, papaya, passion fruit and pineapple.	[14]
The developments on microbial processing technologies for production of enzymes and organic acids from fruit and vegetable wastes and the concept of zero-waste economy are discussed.	[15]
The fruit and vegetable waste could be treated with different reduction, reuse and recycle strategies that are analyzed underlying their main advantages and pitfalls. To obtained valuable compounds the process should include waste characterization, output definition, process design and feasibility study.	[16]
Traditional (solid-liquid extraction, soxhlet extraction and liquid-liquid extraction) and novel processes of extraction (supercritical fluid extraction, microwave and ultrasonic assisted extraction, pressurized liquid extraction, pulse electric field extraction and ionic liquid extraction) methods used for the extraction of bioactives from solid and liquid biomass waste streams are analyzed.	[17]
It explores utilization of banana waste (fruit peels, pseudo-stem, trunks, and leaves) as precursor materials to produce an adsorbent of such as heavy metals, dyes, organics, pesticides, and various other gaseous pollutants.	[18]
The functionality of bioactives from fruit must have a market potential which in turn must be sufficient for its commercially competitive production. The difficulty of the demonstration of the health claims may explain why, despite the wide research and tons data available for bioactive extraction, few fruit and co-products are used at industrial scale for the production of bioactives.	[19]

Regarding the potential use, fruit wastes/co-products have been proposed as raw materials for: Bio-energy Enzymes, Direct soil application (fertiliser/carbon sequestration), Animal/fish feed, Composting, Pectin extraction, Source of bioactives such as polyphenolic and phenolic compounds (including antioxidants and pigments), Functional beverages, Incorporation into food products, Antioxidants, antimicrobials, flavoring, colorants and texturizer food additives. Sorbent for heavy metals dyes, organics, pesticides, and various other pollutants from aqueous solutions, Production of organic acids, Novel materials, bio-plastics, Immobilisation carrier in solid state fermentation, Substrate for growth of edible mushrooms, Substrate for microorganisms for the production of biopolymers.

4. Case study 1: Tisanes froms seeds of tomato tree and guava fruits

4.1 Tisane preparation

The peels and seeds of tamarillo and guava were washed and taken to a convective drying chamber (Vigitemp, Thermolab, model TH58) where the dehydration was carried out at a temperature of 30°C, between 12 and 15 hours. After the husks were ground and separated by dry sieving into the 40 to 60 mesh range. Tisane was made by mixing 25%, 50% and 75% of residue (selected from a previous chemical and sensory characterization). The tea or infusion was prepared immersing 1g of the tisane mix in 250 ml of boiling water for a period of 5 minutes [20].

4.2 Chemical characterization of residues

The total polyphenol content of the extract was measured by the Folin-Ciocalteu colorimetric method according to Singleton (1999) [8]. The total polyphenols content was expressed in mg of gallic acid equivalents / 100 g of dry fruit (mg GAE / 100 g fruit).

4.3 Determination of antioxidant activity (AA)

By measurement of DPPH (α, α-Diphenyl-β-picrylhydracil) radical inhibition was according to Morinova et. al (2011) [7] and ABTS radical cation decolorization assay according to Re *et al* .(1999) [21]. The results were expressed as µmol of Trolox®/100 g of dry fruit (TAA-µmol of Trolox®/100 g of fruit).

4.4 Sensory evaluation of tisanes

The sensory evaluation of the tisanes was made through an acceptance test of 9 points by an untrained panel of 27 people. In this test, the odor and flavor parameters of the formulations composed of 25%, 50% and 75% of fruit residues were evaluated. The data obtained from the surveys was evaluated by histograms. The significant difference ($p < 0.05$) between the tests was checked by a two-factor Anova with one sample per group.

4.5 Results

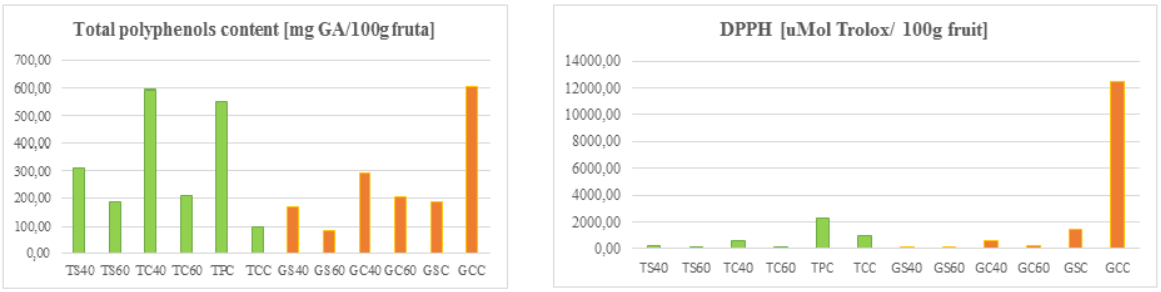
To determine the functional potential of the residues compared to the fresh fruit, the determination of the total polyphenols content and AA of the guava and tamarillo fruits was carried out. The results obtained are shown in table 4.

Table 4. Total polyphenols content and antioxidant activity of tamarillo and guava fresh fruits.

Fruit	**Total Polyphenols content [mg GA/100 g fruit]**	**DPPH [µMol trolox/ 100 g fruit]**	**ABTS [µMol trolox/ 100 g fruit]**
Tamarillo	26.24±3.44	1195.19±180.62	138.77±82.28
Guava	95.76±34.80	6019±1121.70	29.18±8.24

As a measure for the adequate management of the data obtained from the chemical analysis performed on the test, each of the tests carried out is coded. Table 5 shows the abbreviations used for each test.

Fig. 1 a. shows the total polyphenols content of the different fruit residues evaluated. The number of polyphenols found both in fresh residues and in infusion was greater than the content found in fresh fruit, except for the infusion test of guava seeds from 60 mesh. In the most successful cases (TC 40, TPC, GC 40 and GCC) an increase of the total polyphenols content of 2100% for tamarillo and 522% for guava was observed. Additionally, it is observed that the drying, grinding, sieving and infusion treatments of tamarillo peel concentrates its polyphenols content, while the seeds lose part of its antioxidant activity with the same treatment. In the case of guava, both the peel and the seed lose part of their polyphenol content after the infusion preparation. By means of this analysis it can be concluded that the residue whose processing generates a greater number of polyphenols is the tamarillo peel.



a. b.

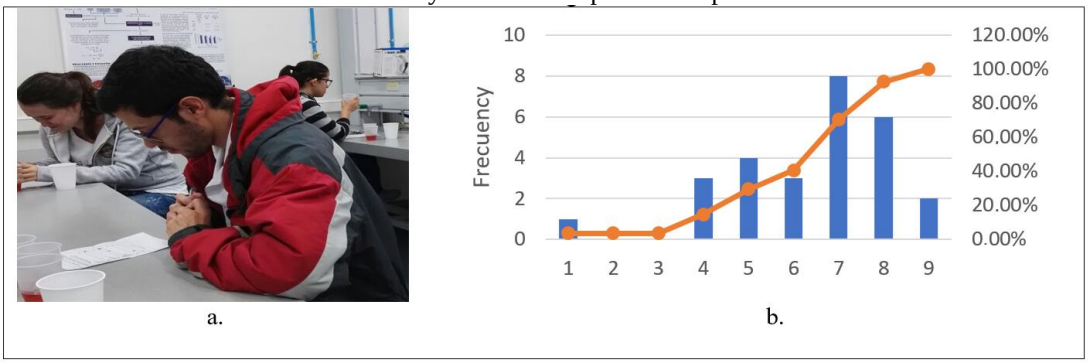
Fig. 1. a. Total polyphenols content of the different fruit residues. **b.** Antioxidant activity measured using DPPH of the different fruit residues evaluated. Tamarillo – infusions TS40: Tomato seed mesh 40, TS60: Tomato seed mesh 60, TC40: Tomato peel mesh 40, TC60: Tomato peel mesh 60; Tamarillo - Fresh TPC: Tomato fresh seed, TCC: Tomato fresh peel; Guava – Infusions GS40: Guava seed mesh 40, GS60: Guava seed mesh 60, GC40: Guava peel mesh 40, GC60: Guava peel mesh 60; Guava – fresh GSC: Guava fresh seed, GCC: Guava fresh peel.

Fig. 1. b. shows the antioxidant activity measured by DPPH of the different fruit residues evaluated. Before carrying out the analysis of the data, it should be clarified that the values reported for DPPH are inversely proportional to their antioxidant activity, in other words, a small value means that a smaller amount of the substance is needed to inhibit the radical, it is say it has a greater antioxidant capacity. As in the previous results the antioxidant activity increased compared to the fresh fruit, excluding the fresh guava skin, which presented 100% less activity. In both cases, it is appreciated that the drying, grinding, sieving and infusion procedures increase the antioxidant activity, suggesting that some of these components are concentrated and are not thermosensitive. In this test, the infusion prepared from guava seeds showed the highest antioxidant activity, reaching a value 65 times higher compared to fresh fruit. In the case of infusion with tamarillo skin, the antioxidant activity was close to twice the value for the fresh fruit.

From the initial chemical analysis, the tamarillo skin was selected as the residue for the preparation of the tisanes. First, because it had the highest polyphenols content and despite not being the one with the highest AA, it presented acceptable values. It should be noted that, when the infusion was prepared, the aroma and flavor contributed by the tamarillo peels was evident. On the other hand, guava seeds, despite showing the best results in terms of antioxidant activity, formed almost odorless infusions with a very mild flavor, not to mention that they presented an oily supernatant affecting the appearance of the sample.

4.5.1 Sensory evaluation of the tamarillo peel tisanes

The results obtained from the statistical analysis of the acceptance test performed are shown below.



a. b.

Fig. 2. Sensory evaluation of tisanes made with tamarillo peels. a. Cupping panel; b. Histogram of flavor evaluation by the panelists.

The tisanes with a content of 25% of tamarillo peel presented a good acceptance of their aroma and flavor, since more than 70% of the panelists gave a rating equal to or higher than the average (Figure 2).

In one cup of this beverage, the polyphenol content and antioxidant activity were 525% and 25% higher than those found in the fresh fruit, which makes the most attractive for the functional beverage market.

The sensory evaluation of the tamarillo peel formulated tisanes showed that as the concentration of residue in the formulation increases, the acceptance of both flavor and odor decreases. Some panelists that there was a slight bitter taste in the 50% and 75% formulations. Additionally, they identified a reduction in the odor of the infusion as the residue concentration increases. This may explain the observed trend. The tisanes with a content of 25% residues presented a good acceptance for the two parameters evaluated. It was also found, that in the case of flavor and odor more than 70% of those evaluated gave a rating equal to or higher than the average. Moreover, in the design of a product the functional characteristics of the products also influence the final decision of the consumer. In this formulation the total polyphenols content was 525% higher and had 25% more antioxidant activity respecting the fresh fruit.

5. Case study 2: Lipase immobilization on foamed epoxy resin support filled with activated milled stalks

5.2 Materials

The cultivar stalks of tamarillo and banana were dried, milled and sieved to obtain a controlled particle size (≤250 μm). After that, this powder was activated with glycidol-periodate, to use as filler of the composite. Unless otherwise stated all reagents were purchased from Sigma-Aldrich (St. Louis, MO, US). The bioepoxy matrix ingredients (epoxy resin and hardener, were purchased from Entropy Resins (Hayward, CA, US).

5.3 Preparation of pellets

Epoxy (66%), hardener (26%), foaming agent - polymethylhydrosiloxane- (4%) and activated filler (4%) were mixed and poured in a mold. The cured composite pellets (hollow cylinders with approximately 5.6 mm outer diameter, 3.8 mm inner diameter, and 8.1 mm height) of the porous composite were used as carriers for lipase immobilization.

5.3 Lipase immobilization

For immobilization 60 mg of Candida Rugosa lipase (CRL, Sigma L-1754) was dissolved in 50 mL of phosphate buffer (pH 7.2). The lipase solution was pre-incubated at 35°C under gentle stirring for 2 h. After that pellets were submerged into the enzyme solution for 20 h at 20°C under agitation (120 rpm). The amount of lipase adsorbed onto the cylindrical bioepoxy pellets was determined as the total protein quantity from the difference between the amount of protein in solution before and after adsorption and in washing solutions by the Biuret method [22]. Absorbance measurements were done in wavelength 550 nm, using a Perkin–Elmer Lambda 20 spectrophotometer. In a typical experiment, 2.0 mL of a protein or washing solution was mixed with the Biuret reagent and the protein concentration was determined spectrophotometrically using a calibration curve of bovine seroalbumin [23]. The efficiency of immobilization was evaluated in terms of protein coupling yield as follows:

$$Protein\ coupling\ yield\ (\%) = 100\ \times\ (Total\ protein\ coupled/Total\ protein\ introduced)$$

5.4 Characterization assays

The mechanical strength of the supports was measured with the fracture test, using a Texturometer TA-XT. plus Texture Analysis (TX.XT.Plus, Godalming, UK). Cylinders with a height of about 8.1 mm and 5.6 mm in diameter were evaluated. The test conditions were: pre-test speed of 1mm/s, test of 2mm/s and post-test of 10mm/s. The results were expressed as the force needed to fracture the support (Newton) [24]. For the measurement of chemical stability weighted catalyst pellets were immersed in olive oil, akaline and acidic water solutions (24 hr, 60°C). The final weight of the treated and free solvent pellets was used to estimate the stability (weight variability) according to the equation:

$$Weight\ variability\ (\%) = 100 \times ((Final\ weight - Initian\ weight)/Initial\ weight)$$

Lipase activity was determined by titrimetry using olive oil emulsion which was prepared by mixing 25 mL of olive oil and 75 mL of 7% Arabic gum solution in a homogenizer for 2 min. The reaction was initiated by adding various amounts of free or immobilized lipase into the reaction mixture. For the free lipase, the reaction mixture containing 5 mL of olive oil emulsion, 4 mL of 50 mM Tris-HCl buffer, pH 8.0, 1 mL of 110 mM $CaCl_2$ and 1 mL of enzyme (5 mg/mL) was incubated at 50ºC for 30 min under orbital shaking at 160 rpm. The reaction was immediately stopped after the incubation period by the addition of 15 mL acetone:ethanol mixture (1:1 v/v), and the released free fatty acids were titrated with 50 mM NaOH. One unit (U) of lipase activity was defined as the amount that released 1 μmol of fatty acid per min [25]. For the immobilized lipase the amount of enzyme in the reaction mixture was the same, estimated from the protein coupling yield. The acetone-ethanol stopped step was avoided and, instead, the immobilized enzyme was separated by filtration.
The operational stability of the immobilized enzyme was monitored by determining the residual activity of the immobilized enzyme after each cycle.

5.5 Results

Epoxidized plant oils have been used in many applications, such as plasticizers for plastics, stabilizers, or as a toughening agent for petroleum-based epoxy resins. They show superior potential as a renewable and an inexpensive material for some industrial applications and its addition to epoxy helps also to decrease the cost of the final product. However, biobased epoxy has some disadvantages such as low mechanical performance and heat deflection temperature compared to commercial synthetic epoxy resin. Fillers are required to overcome some of these hurdles and to fulfill certain industrial requirements. Biobased epoxy resin (bioepoxy) is composed of epoxy and hardener in liquid form. The bioepoxy contain biobased renewable materials extracted as a coproduct from waste streams of industrial processes, such as biofuel and wood pulp production. The epoxy is a mixture of epoxidized pine oils, bisphenol A/F type, and benzyl alcohol. The hardener is a mixture of cyclohexanemethanamine, 5-amino-1,3,3- trimethyl isophoronediamine (20–50%), polyoxy propylene diamine (30–60%), tris22,4,6-(dimethylaminomethyl) phenol (10– 20%), bis(dimethyl-amino-methyl)phenol (<10%), benzyl alcohol (<10%), piperazine (<10%), and aminoethyl piperazine (<5%). Bioepoxy is claimed to have up to 19% biobased content that could be increased by the use of natural fiber fillers. Fig. 3 shows three sets of photographs and scanning electron microscope images of the raw material, fibers and their biocomposites, respectively.

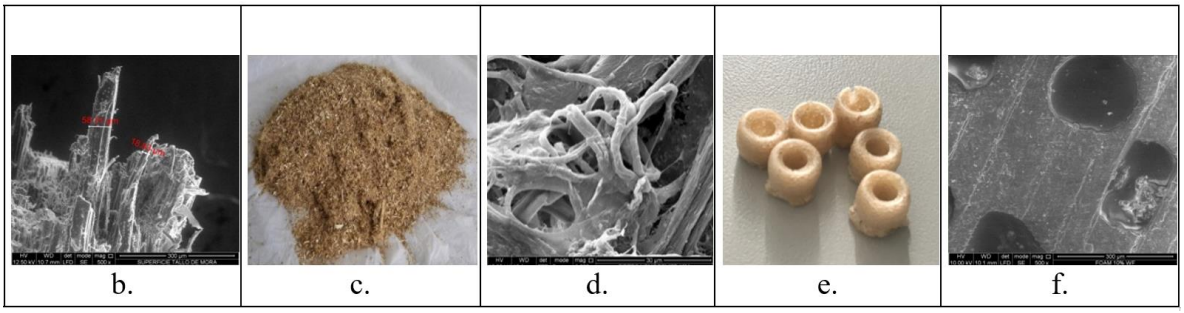


Fig. 3. Sequence of photographs and SEM micrographs of raw and intermediate materials in the preparation of biocomposites. a. and b. fruit stalks; c. and d. milled fibers; e. cylindrical fiber filled biocomposite pellets; f. surface SEM micrograph of pellet.

During the foaming process, two parallel reactions occur. A scheme of the same is shown in Fig. 4. In the first reaction, the blowing agent (siloxane) reacts with amino hardeners releasing hydrogen. The hydrogen gas acts as expanding agent and the system foams out. The second reaction is the typical addition reaction between the epoxy–amine groups.

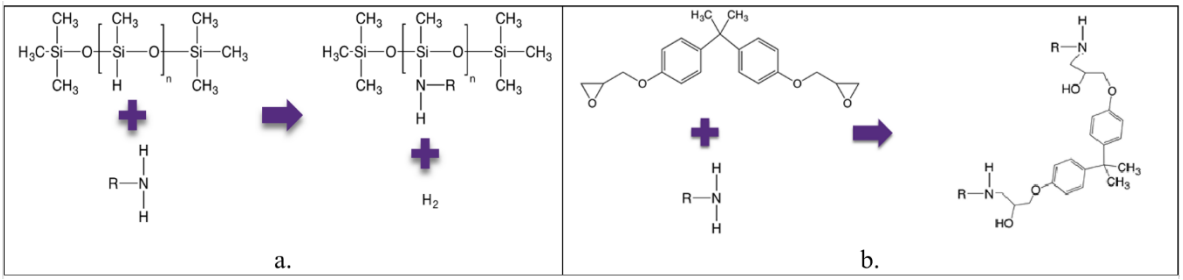


Fig. 4. Epoxy foam reactions. a. Hydrogen release reaction; b. Addition polymerization reaction.

There are proposed two ways of covalent binding of the CRL to the pellet. In both cases reactive groups are intended to react mainly with the amino groups of the enzyme. As fibers were activated using glycidol (forming a terminal diol as the epoxide ring opens) with subsequent oxidation with periodate (that creates an aldehyde at the end of each glycidol chain), the enzyme could be able to link to the fiber through Schiff base formation (steps 1-2, Fig. 5). The straightforward direct coupling to epoxy-activated support: A slow chemical reaction between the adsorbed enzyme and epoxy groups of the support via intermolecular reactions between the nucleophilic groups (such as amino or thiol groups) on the protein surface and the epoxy groups on the support surface. The reaction results in a covalent bond between the nitrogen of an amino group (of the enzyme) and the carbon of- methylenic group (of the epoxy- support) (step 3, Fig. 5).

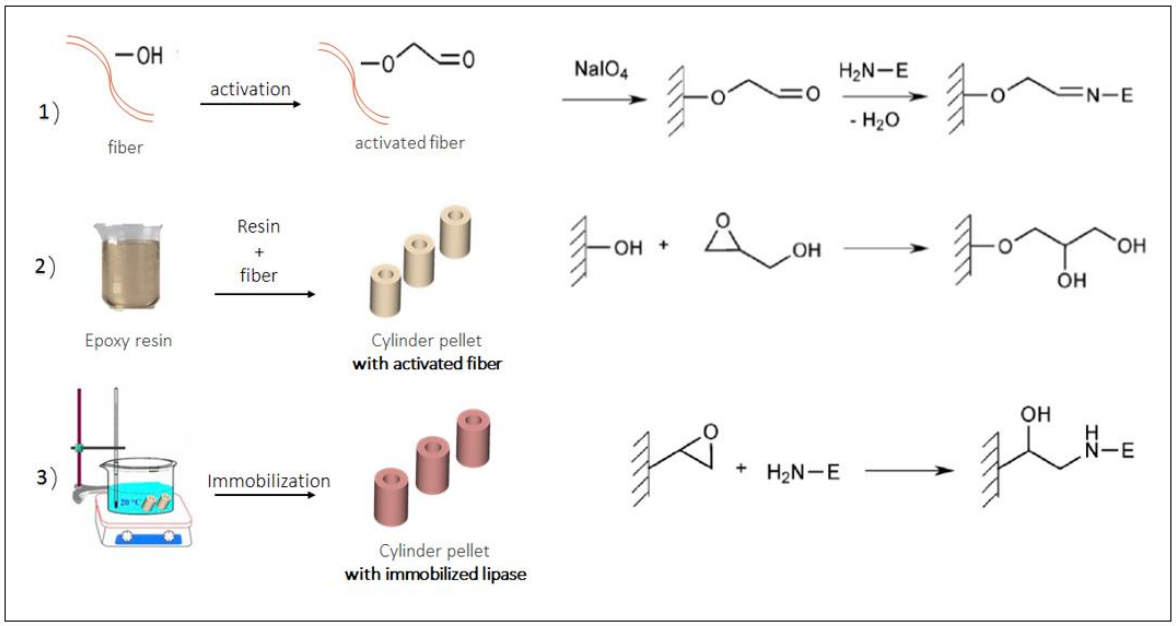


Fig. 5. Lipase immobilization. Step 1) Direct coupling to epoxy-activated support; Step 2) Glyoxyl activation of a hydroxyl group containing support and subsequent immobilization of the enzyme; Step 3) Glutaraldehyde-mediated immobilization on a support containing amino groups.

Fig. 6 depicts information about the structure of the pellets. The SEM micrographs show the porous structure by using two magnifications (90× and 250× for 1 mm and 500 micrometer scale bar, respectively). Fig. 6 a. is the micrograph of the cut surface and Fig. 6 b. is the SEM image taken on the fracture surface of a cracked pellet. It can be observed a macro pore structure with variable pore size and shape (50 -300 micrometer).

To know about the chemical stability of the pellets in regular lipase catalyzed reaction media, weighted pellets were immersed in extreme pH water solutions and in vegetable oil to measure their stability in these environments. The average weight variability is shown in the Fig. 7 pellets suffered slight losses in water solutions media and a positive gain of weight in the oil because the entrapped oil was not completely released from the porous matrix of the pellets. These results suggest apparent pellet inertness in oil media and slight solubility in extreme pH aqueous media. Lipase catalyzed reactions commonly require pH between 4-9 to proceed.

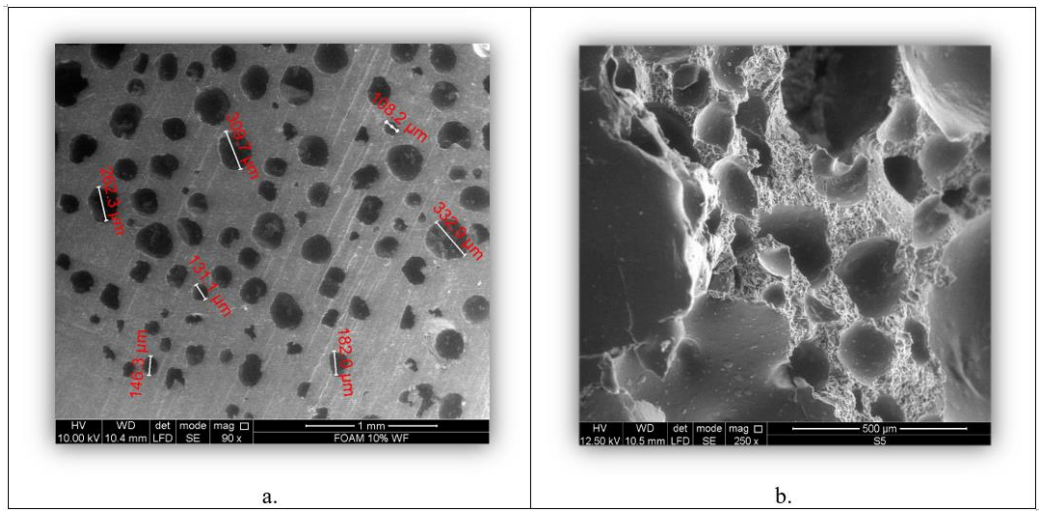


Fig. 6. Morphology of a.surface ; b. fractured b. pellets.

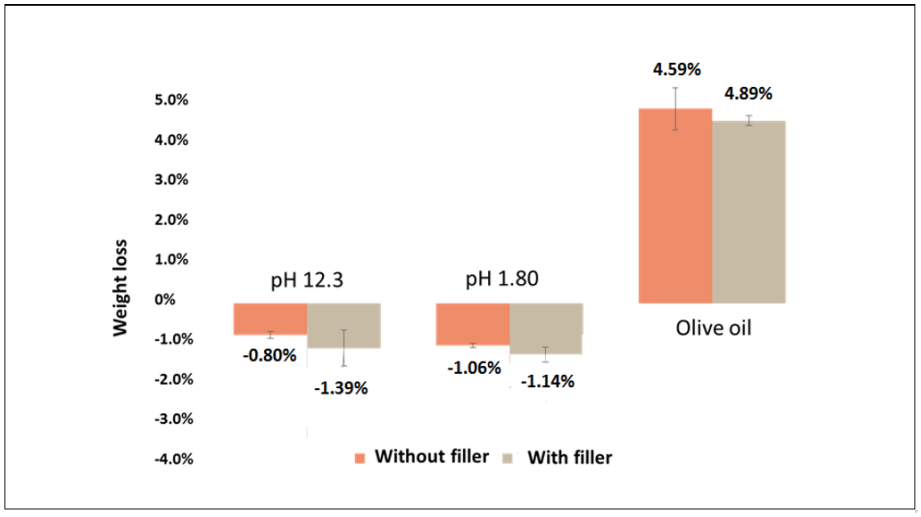


Fig. 7. Chemical stability of the pellets

The main mechanical test used for catalyst pellets the single pellet crushing strength test. This consists of the slow deformation of individual pellets by a platen moving against a static platen until the pellet fracture and a characteristic drop of the crushing load. The maximum applied force before the pellet fracture is recorded as the crushing strength. Table 6 shows the comparison between the radial crushing strength of the manufactured pellets the obtained in a study with solid cylinder pellets. The results were superior despite the cylinders used in this work were hollow.

Table 6. Results for the pellet crushing strength test

Size (mm)		**Radial Crushing Strength (N)**	
Diameter	**Heigth**	**Mean ± SD**	**Ref.**
9.1	5.17	27.50 ± 8.64	[24]
9.1	6.59	34.20 ± 11.20	
8.1	5.60	58.39 ± 11.17	This work (2)
8.1	5.60	40.41 ± 7.08	This work (3)

n = 3 (2)Cylinder pellet without lipase (3)Cylinder pellet with lipase

Table 7 summarizes the results of the catalytic behavior of the immobilized lipase. components. The data presented were replicated three times. The mean values were compared with the results of a similar work that immobilized CRL on Eupergit ® (an epoxide polymer bound matrix). Although the protein load was lower than the exhibited by the commercial support, the lipase activity was superior in the first use and similar at the third cycle of use. Apparently, the activity displayed by the CRL on natural-fiber bio-epoxy composite is similar than the observed for the lipase on recognized enzyme supports.

Table 7. Catalytic Parameters

SHAPE	**PROTEIN LOAD (mg/g support)**	**CYCLE**	**LIPASE ACTIVITY (U/g support)**	
			Mean ± SD	**Ref.**
Cylinder	10-27	1	33.93±3.17	This work
		2	19.13±3.34	This work
		3	13.32±1.08	This work
Sphere	80-90	1	17.13±0.87	[26]

6. Case study 3: Approaches to biorefineries from fruit waste

6.1. First approximation: Production of butanol from fruit waste: passion fruit and guava

1-Butanol (butyl alcohol or n-butanol) is an aliphatic alcohol of four carbons. It is an important chemical which is mainly used as a solvent in the cosmetic and pharmaceutical industry, chemical intermediate (i.e. in the production of butyl acrylate and methacrylate). Butanol has aroused interest in researchers as a good candidate for alternative biofuel because of its advantages over ethanol as its low volatility and higher energy content [27,28]. Butanol has traditionally been produced by the anaerobic fermentation of sugar substrates using several species of *Clostridia solventogenic* [29]. This process is often called the ABE fermentation, due to its main chemical products: Acetone, Butanol and Ethanol [30].

In this work the production of butanol is evaluated by a simulation of the ABE fermentation of passion fruit and guava seeds, two residues from the pulping of these fruits. Malacriada [31] reports a composition of 48.73% carbohydrates in the passion fruit seed while Uchôa-thomaz [32] reports a composition of carbohydrates for the guava seed of 3.08%. Based on the reactions reported by van der Merwe [33] presented in Table 8, and assuming the carbohydrates as glucose, the yields obtained for butanol are calculated for each one of the residues.

Table 8. Overall reactions in the ABE fermentation production of butanol.

Reaction	
1	$Glucose+H_2O \rightarrow Acetone+3CO_2+4H_2$
2	$Glucose \rightarrow Butanol+2CO_2+H_2O$
3	$Glucose \rightarrow 2Ethanol+2CO_2$
4	$Glucose \rightarrow Butiric\ acid+2CO_2+2H_2$
5	$Glucose \rightarrow 3Acetic\ acid$

Table 9 shows the results obtained for glucose conversion, butanol yield and inlet and outlet flows of butanol production from passion fruit and guava seeds.

Table 9. Results of butanol production from passion fruit and guava seeds

	Passion fruit seeds	**Guava sedes**	**Units**
Glucose conversion	79.55	80.22	%
Butanol yield	0.25	0.24	g butanol/g glucose
Feed flow	100	100	Kg
Bitanol flow	12.14	0.75	Kg

The seeds of passion fruit and guava have sugars that can be fermented for the production of butanol through the ABE process. In both processes, glucose conversion close to 80% is achieved, as well as a yield of 0.25 g butanol / g glucose for passion fruit seeds and 0.24 g butanol / g glucose for guava seeds. Although these values are similar, the butanol fluxes obtained for the same flow of raw material feed are quite different, this is due to the fact that the guava seed has a lower content of sugars. For this last reason it is concluded that of these two agroindustry residues passion fruit seeds present better yields.

6.2 Second approximation: Biorefinery from spent blackberry pulp (SBP)

The production of blackberry fruit in Colombia is well established. However, there is not enough research to find additional benefits of the fruit waste after processing. In addition, there is an excessive intermediation becomes an obstacle to enhance the competitiveness of the productive chain of this fruit.

In the biorefinery approach for the production of ethanol, xylitol, electricity, steam and phenolic compound extracts, the raw material chosen is the spent waste [34]. Generally, the blackberry is processed into concentrates, jams and juices and a considerable fraction of the fruit leaves the process as spent blackberry pulp (SBP). The flow of raw material in this case was 2000 kg/h.

The first stage of the process is aimed to obtain the phenolic compounds from the SBP. This should be the first stage to ensure the quality of the compounds and avoid degradation or contamination. These compounds are microencapsulated to prevent their degradation and ensure their use in cosmetics or pharmaceuticals. Next, the lignocellulosic residue is submitted to a pretreatment step which converts cellulose and hemicellulose into fermentable sugars, glucose and xylose, respectively. These sugars are used as substrates in two stages of fermentation to produce ethanol and xylitol. The remaining solid fraction is used in a cogeneration plant to produce electricity and steam.

After the respective simulation, the economic evaluation was carried out. Table 10 shows the respective results obtained in terms of depreciation costs, raw materials, inputs, public services and operating costs. The productivity obtained by the simulation was 452.2 kg/day, 6912 kg/day and 193.4 kg/day for xylitol, ethanol and phenolic compounds, respectively [34]. The ratio between the cost of sales and the total cost of production for the process with and without cogeneration is 19.36 and 23.69, respectively. This shows that the SBP is a promising raw material with potential uses to produce valuable compounds in a biorefinery concept.

Table 10. Costs of the proposed biorefinery from SBP. Adapted from [34].

Ítem	**Cost (USS/year)**	**Percentage**
Depreciation costs	3431641	28;90
Raw material	327903	2,76
Inputs	5137159	43,26
Gross profit	1661098	13,99
Operative cost	1316692	11,09
Total	**11874493**	**100**

Conclusions

The increase in consumers' demand not only to satisfy nutritional needs but a diet to sustain optimum human health has driven the market to naturalness and plant-based foods. This circumstance has sparked a renewed interest in fruit, their co-products and residues as alternative food source, food ingredients, bioactive and functional components. Conversely, these materials are also considered for non-food processing as feed, fertilizer and bio-energy resources.

Fruits and vegetable wastes are produced in considerable amounts in agricultural activities, agro industries, supermarkets and municipal wastes. In the industry their generation increases the operation costs of markets due to sales losses and transport and disposal costs. A huge experimental work has been devoted in the last 10–20 years to the development of technologies for the addition of value of the residues of plants, including fruit

and vegetables. However, few fruit wastes are used at industrial scale. Different waste management strategies could be efficaciously applied that should be economically sustainable for the companies and in agreement with law requirements.

Regarding the food options for use of fruit residues, and according to the sensorial study results, it was possible to obtain a formulation with acceptable characteristics, using tamarillo peel. In addition, this formulation had significantly higher nutritional characteristics compared to fresh fruit. As observed in the study, the different residues evaluated showed important contents of polyphenols and antioxidants. Future studies could include the study of methods of extraction and concentration of these components in order to use them to increase the properties of different food formulations. The use of this type of waste management techniques is presented as a promising solution to increase added value through the productive chain of the tamarillo, offering as an additional advantage the reduction of the pollution generated by the transformation of fresh fruits.

Fruit cultivar stalks could be used as fillers of bio-epoxy matrix for enzyme immobilization. A hybrid biobased: petrochemical (≈25:75) foamed hollow pellet enzyme carrier was manufactured and prelimilarly characterized. The SEM internal structure showed super macro pore size between 50-300 micrometers. Resistance responses to lipase catalysis conventional reaction environments were good and crushing strength measurements fulfill catalysis pellets standard. Although the protein load was low, the hydrolytic catalytic activity was comparable/superior than the observed in similar published works (for CRL on epoxy-based supports).

The final remark is that is technically possible to use fruit residues to produce energy, beverages, food ingredients and chemicals. These results promote further research in the field of biorefineries.

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