



Potential Evaluation of Agroindustrial Waste from Three Passifloraces as a Source of Usable Biomass

Deivis Suárez Rivero^{*a}; Olga Marín Mahecha^a; Daniel Marin Torres^b; Maikel Suárez Rivero^c; Luis Carlos Ballesteros^d; Jannet Ortiz Aguilar^d

^a Fundación Universitaria Agraria de Colombia – UNIAGRARIA.

^b Universidad Nacional de Colombia – UN.

^c Instituto de Ciencia Animal – ICA.

^d Universidad Cooperativa de Colombia – UCC.
suarez.deivis@uniagraria.edu.co

Colombia is the country with the highest biodiversity of species of the Passifloraceae family in the world, to date possess an inventory of 167 species. Some of these have a high commercial importance derived from their consumption in fresh and processed; reason why they are widely cultivated in different regions of the country, but especially distributed throughout the Andean Region. Because of the above it is evident that a significant part of the fruits used in the consumption generate by-products that are going to give to the landfills, becoming a focus of environmental contamination. Therefore, this project evaluated the potential of the by-products generated from three *Passiflorae edulis* Sims, *Passiflorae mollissima* (Kunth) L.H. Bailey and *Passiflorae quadrangularis* L. for their potential use as biomass. For this purpose, fruits were used in consumption matures of the three-species forming a single batch and performing the analyzes of the variables in triplicate. Hence, the analysis of the content of holocellulose, cellulose, hemicellulose and lignin, as well as determination of the dry matter content and percentage of moisture by lyophilization and organic matter were carried out using a unifactorial design (residues of the studied fruit). Consequently, as a result of data processed in the Matlab statistical package through analysis of variance it was evidenced that the analyzed postharvest residues have a high potential in the three pasifloras evaluated as a source of usable biomass. Lastly, it is emphasized the pericarp of passion fruit (*Passiflorae edulis* Sims) because it showed higher percentage contents in the variables cellulose, hemicellulose and lignin what evidenced the potential of these residues (residual biomass) for the production of so-called " Biofuels - Second Generation".

1. Introduction

It is a fact that the generation of energy from fossil fuels is one of the main human activities that cause environmental problems. One of these problems is atmospheric pollution due to the emission of toxic gases and the consequent global warming, among other phenomena. For this reason, energy consumption has increased, due to the increment in industrial processes and direct consumption in households; thus, generating the need to reduce dependence on fossil fuels by other alternatives. Starting from this reality, the search for alternative energy sources that are renewable and friendly to the environment have been the subject of much research and participation in worldwide debates. It is then, that the use of residual biomass as a potential to produce alternative energy is a necessary aspect to deepen due to the different energy sources, the composition of these sources and the energy potential (CEPAL, 2011 and Suárez et al., 2017).

On the other hand, it is noteworthy that Colombia has been known as a country with a strong agricultural sector. In this area, more than 177 million tons of residual biomass are being generated per year (Ministry of Mines and Energy, 2011), which have a significant energy potential associated with it. This constitutes an opportunity in terms of the possibility of producing renewable alternative energy from its residual biomass, since as reported by Cadavid-Rodríguez and Bolaños-Valencia (2015), renewable resources currently play a crucial role in limiting CO₂ emissions. This is how the evaluation of the agroindustrial potential of the residual

biomass of the Passifloraceae, can open new alternatives for the biotechnological, chemical and biomass exploitation industry.

2. Materials and methods

2.1 Characteristics of vegetable material.

For the development of the different tests, it was used the fruit exocarp of *Passiflorae edulis* Sims (Maracuyá), *Passiflorae mollissima* (Kunth) L.H. Bailey (Curuba) and *Passiflorae quadrangularis* L. (Badea) in good quality condition, without apparent mechanical damage and fit for human consumption. Only the exocarp was used since it is the part of the fruit that is not of human consumption and that can be used for the generation of added value to the chain of the Passiflorae.

2.2 Preparation and lyophilization

The fruits were disinfected by immersion in water with sodium hypochlorite (3 %) with slight agitation. They were then washed with plenty of water and dried at room temperature. Subsequently, the pulp of the epicarp (peel) was separated, discarding the latter because it was not an object of study. For each of the tests described below, dried material was used in an oven at 105 °C until obtaining a constant weight, after drying and grinding, they were kept in a desiccator to avoid hydration of the tissue. Additionally, part of the raw material was dried by lyophilization at 0.01 °C and 4.5 mmHg in freeze dryer LABCONCO.

2.3 Determination of Lignin by the Klason method

The aforementioned method corresponds to the TAPPI 222 standard, which consists of carrying out a quantitative acid hydrolysis of two stages: the first with 72 % sulfuric acid that hydrolyzes the polysaccharides in oligosaccharides and a second 4 % that breaks the oligomers in monosaccharides. To this end, 1 g of sample (P1) was used, weighed on a precision scale of 0.0001 g, mixed with 15 ml of 72 % H₂SO₄ and left to stand for 24 hours. Subsequently, the contents of the beaker were transferred to a 1000 ml flask and 560 ml of deionized water was added to pass 72 % H₂SO₄ to 3 % H₂SO₄. The flask was connected to a refrigerant tube and kept boiling for 4 hours in a heating mantle. Once the previous time had ended, the solid could settle and filtered on a filter plate, previously dried in an oven at 105 °C and weighed (P2).

The filtered solid was washed with abundant hot deionized water until the pH of the wash water ceased to be acidic. The solid already washed was dried in an oven at 105 °C for 12 hours and weighed (P3), 100 mg of the dry solid was taken and calcined in muffle at 430 °C for 24 hours, thus obtaining its percentage in organic matter (MOlig). The lignin content was calculated with the following expression:

$$\text{Lignin (\%)} = \frac{[(P3 - P2) \times (\% \text{ MOlig}) \times 100]}{[P1 \times (100 - \% H)]} \quad (1)$$

Where:

% H is the percentage of water with respect to freeze-dried and milled sample.

2.4 Determination of Holocellulose

For the determination of this variable, acetic acid is used, a compound that acidifies the medium and becomes sodium dioxide. This allows to degrade the lignin, solubilizing it and clarifying the sample. For this, the ASTM D-1104 standard was followed, and 2 g of sample (P1) were weighed, with a precision of 0.0001 g, were weighed in an Erlenmeyer flask of 250 ml capacity and 63 ml of deionized water were added. To the suspension is added 0.2 ml of CH₃COOH and 0.6 g of NaClO₂. Said mixture is covered with a watch glass and it is introduced to the water bath (70-80 °C), shaking from time to time. This process is repeated twice more. At the end of the third hour, the Erlenmeyer flask is placed in an ice-water bath until the temperature drops to 10 °C. It is filtered on a pre-weighed filter plate (P2) and washed with deionized water until the yellow coloration is eliminated. It is then washed with deionized water, allowed to dry at 60 °C and weighed (P3). The percentages of humidity (% Hholo) and organic matter (% MOholo), respectively, are determined to the solid obtained. The content of holocellulose is calculated according to the expression (2).

$$\text{Holocellulose (\%)} = \frac{[(P3 - P2) \times (100 - \% \text{ Hholo}) \times (\% \text{ MOholo})]}{[P1 \times (100 - \% H)]} \quad (2)$$

Where:

% H is the percentage of water with respect to freeze-dried and milled sample.

2.5 Determination of cellulose content

It was determined according to the ANSI / ASTM standard (American National Standards Institute). To this end, 1 g of holocellulose (P1) was weighed on an analytical balance with a precision of 0.0001 g, and placed in a 100 ml Erlenmeyer flask. 5 ml of 17.5 % NaOH was added by mixing with a magnetic stirrer and adding 2.5 ml of 17.5 % NaOH every 5 minutes until a total of 12.5 ml was consumed; it was kept 30 minutes at room temperature. In the same way, 16,5 ml of deionized water was subsequently added at 20 °C to go from 17,5 % to 8.3 % NaOH; it was homogenized and kept for 1 hour at room temperature.

Then, it was filtered on filter plate a weight known (P2), and washed three times with 50 ml of 8.3 % NaOH and subsequently with deionized water. The suction is cut and 7.5 ml of 10 % CH₃COOH are added leaving 3 minutes in contact with the sample. The vacuum was again connected and washed with deionized water until the filtrate was neutral. It was dried at 105 °C in an oven for 12 hours and weighed (P3).

To determine the calculation of cellulose content is done according to the expression:

$$\text{Cellulose(\%)} = \frac{[(P3 - P2) \times (\% \text{ MOcel}) \times (\% \text{ Holocellulose}) \times 100]}{[P1 \times (\% \text{ MOholo}) \times (100 - \% \text{ Hholo})]} \quad (3)$$

2.6 Determination of hemicellulose

The content of hemicellulose was calculated by the difference between the content of holocellulose and that of cellulose.

2.7 Statistical analysis

A single analysis of variance (ANOVA) between the averages of the samples by treatment at a significance level of 95 % ($\alpha = 0.05$) was carried out to establish whether any differences exist for the variables under evaluation (Suárez, 2011). If there was no significant difference between the samples, a multiple range test was performed using the statistical package the statistical Matlab.

3. Results and discussion

3.1 Determination of the Dry Mass by lyophilization and preliminary indicators

Basically, drying by lyophilization was applied on 50 grams of each raw material in triplicate to evaporate the water through the surface of the product and transfer it to the surrounding air, that is, to sublimate the ice. In this sense, as shown in Figure 1, the highest results in terms of this indicator were presented for the pericarp (exocarp and mesocarp only) of Maracuyá (6.78 ± 1.07 g), although no difference was evidenced significant with respect to the Curuba (6.16 ± 0.31 g), they did present it when comparing their results with the Badea (3.73 ± 0.92 g).

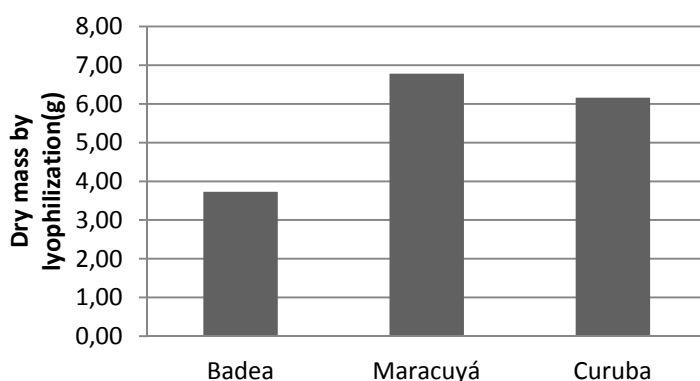


Figure 1. Dry mass of the three raw materials determined by lyophilization.

The previous difference can be given in large measure to that the Badea, although it has a pericarp (exocarp and mesocarp) of greater diameter than the Curuba and Maracuyá, it is spongier; that is, it has a greater number of spaces with large amounts of water in its structure, coinciding with what was expressed by Dorado et al. (2013) and by Ortiz et al. (2015) for other vegetables structures.

On the other hand, the Maracuyá, which presented the highest values of dry matter, has a leathery appearance; that is, its pericarp is drier. This result is consistent with those shown for the percentage (%) of humidity in the raw materials studied. When reviewing figure 2, the highest percentage of moisture (determined by lyophilization) was present in the pericarp (exocarp and mesocarp) of the badea (92.55 ± 1.85 %), differing significantly from the other two raw materials (Curuba with 87.67 ± 0.63 % and Maracuyá with 86.44 ± 2.14 % of Moisture) which do not differ from each other.

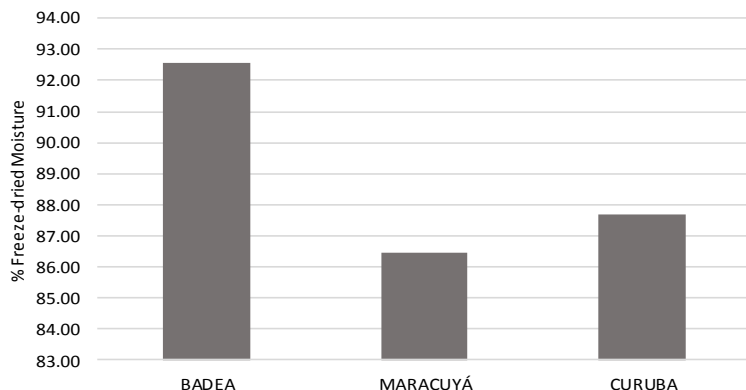


Figure 2. % humidity of the three raw materials determined by lyophilization.

Likewise, when performing the analysis of table 1, which reflects the variables Percentage (%) of Organic Matter and Percentage (%) of Holocellulose, for the two indicators, the three raw materials show values that differ significantly among them; but additionally, they are consistent with those shown in figures 1 and 2 of this article. It is then that the highest values for % of Organic Matter (% MO) and % of Holocellulose were presented in the Maracuyá.

Table 1. Quantification of preliminary variables

VARIABLE	RAW MATERIAL		
	Badea	Maracuyá	Curuba
% MO	0.28 ± 0.15^a	3.83 ± 0.99^c	0.99 ± 0.07^b
% Holocellulose	0.022 ± 0.01^a	0.68 ± 0.13^c	0.17 ± 0.03^b

(Equal letters show no significant differences between treatments and different letters show significant differences between treatments)

It is noteworthy, according to Prado-Martínez (2012) that the Holocellulose corresponds to the total of polymeric carbohydrates that are in the material; that is, the mixture of degraded cellulose, not degraded and hemicellulose, which would be a favorable feature of this raw material (Maracuyá) from the point of view of pulpable aptitude.

3.2 Determination of cellulose, hemicellulose and lignin.

The average values obtained from the content of lignocellulosic material (cellulose, hemicellulose and lignin) in the three raw materials used are shown in table 2. This table shows that for the cellulose content the three raw materials showed values that differed significantly from each other, being the Curuba peel the one that showed the highest values with 0.825 ± 0.006 %, with significant differences from the rest of the treatments. Different is what is appreciated when analyzing the results shown for hemicellulose and lignin, where the highest results are expressed for the pericarp of Maracuyá with 0.4965 ± 0.17 % and 1.026 ± 0.20 % respectively, differing significantly from the rest of the treatments.

For the three indicators analyzed, the lowest and eloquently unequal results to the rest of the raw materials under study, were presented for the Badea with a % Cellulose of 0.0034 ± 0.001 , a % Hemicellulose of 0.018 ± 0.009 and a % Lignin 0.133 ± 0.045 . All the results can be influenced by the structure of the tissues that make up the pericarp (epicarp plus the mesocarp) and by the drying method by lyophilization that was used, which is much more efficient than conventional methods in furnaces or forced conversion furnaces. By the

way, it should be noted that hemicellulose, cellulose and lignin according to Xiu and Ball (2012) and Suárez et al. (2016), are intertwined to form the cell wall of the cells that make up the different plant tissues. Its structure and chemical composition will determine the chemical, mechanical and physical properties of the structure and, therefore, the yields and the composition of the fractions obtained in the thermal decomposition processes (Sullivan and Ball, 2012; Rosso et al., 2015).

Table 2. Quantification of cellulose, hemicellulose and lignin variables.

VARIABLE	RAW MATERIAL		
	Badea	Maracuyá	Curuba
% Cellulose	0.0034±0.001 ^a	0.184±0.05 ^b	0.825±0.006 ^c
% Hemicellulose	0.018±0.009 ^a	0.4965±0.17 ^c	0.086±0.022 ^b
% Lignin	0.113±0.045 ^a	1.026±0.20 ^c	0.174±0.06 ^b

(Equal letters show no significant differences between treatments and different letters show significant differences between treatments)

It is then, that Nadh - Benarji (2016) notes that the production of bioethanol based on sugars and starches has been the subject of controversy for the generation of food competition, this is how more sustainable sources come from agricultural byproducts, forest residues or energy crops which are called lignocellulosic biomass or also called second generation.

Additionally, these authors point out that these raw materials have limitations given the high lignin and hemicellulose content of their structures as well as the high crystallinity of the cellulose and low surface area, so that in many cases it is essential to perform a physical pretreatment, chemical or biological with the purpose of improving the yield in biofuel production (Tejada et al., 2014). Since the composition of lignocellulosic materials depends on several factors (Muñoz - Muñoz et al., 2014), it is necessary to adapt certain parameters for each raw material, thus making structural sugars accessible to fermentation.

4. Conclusions

The amount of Passion Fruit bagasse (pericarp) that is produced in Colombia, not only at the industrial level, but also at the domestic level far exceeds the other two passionflower (Passifloraceae) under study, this together with the fact that this (Maracuyá) showed the highest values for all the evaluated indicators, thus becoming the waste that contributes the greatest amount of lignocellulosic compounds.

The agroindustrial residues of passifloraceae represent raw material with important agroindustrial potential, since not only does it contain a high percentage of cellulose, but it does not require strong pretreatments to decompose its lignin fraction given the structure of the tissues that make up the cell wall, where only a primary membrane rich in cellulose and hemicellulose is developed.

The values lower than 1% in Cellulose and other compounds for the three species, could be given to the degree of maturation of the fruits and to the readsoción of the elements that make up the exocarp during the maturation process.

References

- Cadavid-Rodríguez L. E., Bolaños-Valencia I. V., 2015, Use of organic waste for renewable energy production in a Colombian city, *Energética* 46, diciembre, pp.23-28.
- CEPAL, 2011, Análisis comparativo de patentes en la cadena de producción de biocombustibles entre América Latina y el resto del mundo, en *Diálogo de políticas sobre desarrollo institucional e innovación de biocombustibles en América Latina y el Caribe*, Santiago de Chile, 28 y 29 de marzo, 2011, Pp 1 – 43.
- Dorado D., Tafur H. H., Ríos R. L., 2013, Crop yield and fruit quality of yellow passion fruit (*Passiflora edulis* for Flavicarpa o Deg.) In response to the interaction between irrigation and fertilization, *Ingeniería de Recursos Naturales y del Ambiente*, No. 12, 109-117.
- Ministerio de Minas y Energía, 2011, Atlas del Potencial Energético de la Biomasa Residual en Colombia, <biblioteca.minminas.gov.co/pdf/ATLAS%20POTENCIAL%20ENERGETICO%20BIOMASA%20RESIDUAL%20COL.%20UPME.pdf> accessed 11.01.2017.

- Muñoz – Muñoz D., Pantoja - Matta A. and Cuantin – Guarín M., 2014, Aprovechamiento de residuos agroindustriales como biocombustible y biorefinería, *Biotecnología en el sector agropecuario y agroindustrial*, Vol. 12, No. 2, 10-19.
- Nadh – Benarji D., 2016, "Statistical Optimizations of Fermentation Factors on Bioethanol Production from Mahua Flower (*Madhuca indica*) with *Saccharomyces cerevisiae* by Response Surface Methodology in Batch Bioreactor", *Journal of Microbiology and Biomedical Research*, Vol. 2, No. 1, 1- 7.
- Ortiz J., Suarez D., Puentes A., Velásquez P., Santis Navarro A., 2015, Comparison of the effects in the germination and growth of corn seeds (*Zea mays* L.) by exposure to magnetic, electrical and electromagnetic fields, *Chemical Engineering Transactions*, 43, 169-174 DOI: 10.3303/CET1543029.
- Prado-Martínez M., Anzaldo-Hernández J., Becerra-Aguilar B., Palacios-Juárez H., Vargas-Radillo, J., Rentería-Urquiza M., 2012, Caracterización de hojas de mazorca de maíz y de bagazo de caña para la elaboración de una pulpa celulósica mixta, *Madera y Bosques*, Vol. 18, No. 3, 37-51.
- Rosso Ceron A.M., Weingartner S., Kafarov V., 2015, Generation of Electricity by Plant Biomass in Villages of the Colombian Provinces Chocó, Meta and Putumayo, *Chemical Engineering Transactions*, 43, 577-582 DOI:10.3303/CET1543097.
- Suárez D., 2011, *Estadística Inferencial*. Editorial EDUCC, 49 – 63.
- Sullivan, A. L., and Ball R., 2012, Thermal decomposition and combustion chemistry of cellulosic biomass. *Atmospheric Environment* 47: 133-141.
- Suárez D., Sua A., Marín O., Mejía A. Suárez M., Santis A., 2016, Evaluation of the Effect of Two Types of Fertilizer on the Growth, Development and Productivity of Hydroponic Green Forage Oat (*Avena sativa* L.) and Ryegrass (*Lolium multiflorum* Lam.) as a Biomass Source, *Chemical Engineering Transactions*, 50, 385-390. DOI: 10.3303/CET1650065.
- Suárez D., Marín O., Martínez J., Rodríguez J. V., Real X., Ortiz J., 2017, Evaluation of Potential Pseudostems *Musa* spp. for Biomass Production, *Chemical Engineering Transactions*, 57, 181-186. DOI: 10.3303/CET1757031.
- Tejada L., Marimón W., and Medina M., 2014, Evaluación del potencial de las cáscaras de frutas en la obtención del bioetanol, *Hechos Microbiol*, Vol. 5, No. 1, pp. 4-9.
- Xiu, S. and Ball, R., 2012, Thermal decomposition and combustion chemistry of cellulosic biomass. *Atmospheric Environment*, 47, 133-141.