UNIVERSIDAD NACIONAL DE COLOMBIA

SEDE MANIZALES

Design of Biorefineries for High Value Added Products from Fruits

Javier Andrés Dávila Rincón

Universidad Nacional de Colombia

Facultad de Ingeniería y Arquitectura, Departamento de Ingeniería Eléctrica, Electrónica y Computación

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Design of Biorefineries for High Value Added Products from Fruits

Javier Andrés Dávila Rincón

Thesis submitted in partial fulfillment of the requirements for the degree of:
Doctor of Philosophy in Engineering

Advisor: Carlos Ariel Cardona Alzate, Ph.D.
Universidad Nacional de Colombia, sede Manizales
Co-advisor: Moshe Rosenberg, D.Sc.
University of California, Davis

Research line:
Chemical and Biotechnological Processes Engineering
Research group: Chemical, Catalytic and Biotechnological Processes

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Manizales, Colombia
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Gracias a mi esposa y mi hijo por su acompañamiento, paciencia y apoyo en este camino y por enseñarme a ser una mejor persona.

Thanks to my wife and my son for their accompaniment, patience and support in this way and for teaching me to be a best person.

Acknowledgment

I want to tank to all people that contribute in this research along of my Ph.D. studies, especially to my advisor professor Carlos Ariel Cardona from Universidad Nacional de Colombia at Manizales who guided me in these studies and to encourage on me the research, the teamwork and the responsibility on my activities as well as for guide and support the presentation of my ideas in international conferences. In the same way, I am gratefully with my co-advisor professor Moshe Rosenberg from University of California, Davis USA for your comments, suggestions and continue support in my research as well as for your valuable appreciations on my work that contributed to enhance not only my research but also my knowledge and products from this research.

Also, I want to thank to professor Eulogio Castro Galiano and your research group from Universidad de Jaén in Spain for your contributions during my doctoral stay and for your help in some experimental measurements as well as for your collaboration in some specific topics. Thanks to Dr. Ismael U. Nieves who is the project director and chief process engineer of the Stan Mayfield Biorefinery of the University of Florida for your collaboration in the doctoral stay for complimenting my research on biorefineries using your suggestions and comments as well as the data from the pilot plant. I want to thank to all my colleagues and partners for the scientific discussions that contributed in the develop of my research: Valentina Hernandez, Sergio Duque, Hector Forero, Valentina Aristizabal, Angela Idarraga, Juan C. Carvajal, Laura Daza, Miguel Rojas and Carlos Garcia. Finally, I am gratefully with Universidad Nacional de Colombia for the economic support and fellowship to carry out and finish my Ph.D. studies. Thanks to Asociación Universitaria Iberoamericana de Postgrado (AUIP) for the financial support in the doctoral stay in Spain. Thanks to the Department of Electricity, Electronic and Computational Engineering of Universidad Nacional de Colombia at Manizales for your collaboration and economic support in all the international events.

Resumen

Esta investigación está dirigida a evaluar el diseño de biorefinerias usando frutas y sus residuos. Compuestos con valor agregado como etanol, xylitol, aceites, y extractos con contenidos de compuestos fenolicos y capacidad antioxidante fueron obtenidos bajo el concepto de biorefinería. Por lo cual, dos materias primas fueron usadas, pulpa de mora gastada (SBP) (*Rubus glaucus benth*) y aguacate (*Persea americana mill*). Adicionalmente, dos casos de studio fueron abordados, residuo de lulo (*Solanum quitoense*) y borra de café (*SCG)*. La pulpa de mora y aguacate fueron quimicamente caracterizados estableciendo su contenido de celulosa, hemicelulosa, lignina, cenizas, extractives y componentes valiosos como compuestos fenolicos y aceites. Cuatro biorefinerias fueron diseñadas y evaluadas a partir de la pulpa de mora, aguacate, residuo de lulo y borra de café para obtener productos con valor agregado. Evaluaciones techno-economicas y ambientales fueron desarrolladas para las biorefineriesas planteadas. Finalmente, los resultados y analisis realizados permiten dar un enfoque de biorefinerias basadas en frutas y sus residuos.

Palabras clave: Biorefinerias, frutas, residuos de frutas, diseño conceptual de procesos, Compuestos valiosos, mora, aguacate.

Abstract

This research was aimed to evaluate the design of biorefineries from fruits and its wastes. Valuable compounds such as ethanol, xylitol, oil and extracts containing phenolic compounds with antioxidant capacity were obtained using the biorefinery concept. For this purpose, two feedstocks were used, Spent Blackberry Pulp (SBP) (*Rubus glaucus benth*) and avocado (*Persea Americana mill*). Additionally, two cases of studies were evaluated, naranjilla waste (*Solanum quitoense*) and Spent Coffee Grounds SCG. Both, SBP and avocado were chemically characterized found its content of cellulose, hemicellulose, lignin, ash, extractives and valuable compounds such as phenolic compounds and oil. Four biorefineries were designed and evaluated using SBP, avocado, naranjilla waste and SCG for obtaining value added products. and two cases studies. Techno-economic and environmental assessments were developed to the proposed biorefineries. Finally, the results and analyses made allow giving a biorefinery approach based on fruits and its wastes.

Keywords: Biorefineries, fruits, fruit wastes, conceptual process design, valuable compounds, blackberry, avocado

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Participation of this thesis in research projects

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52. Análisis de reactividad del líquido de desecho de decapaje de bobinas de acero laminado en caliente de la empresa Acesco S.A.S. Research project of Acesco S.A.

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53. Research stay in Stand Mayfield Biorefinery. University of Florida. Perry USA. November 2014.
54. Research stay in University of Jaen. Jaen Spain. June – July 2014.

Introduction

Fruits contain valuable biologically active compounds, such as vitamins, antioxidants, and minerals, which can positively impact human health and well-being. Some fruit-derived metabolites have applications in the pharmaceutical, cosmetic, chemical and food industries. Developing fruit extracts containing such compounds thus offer opportunities for developing value-added applications for fruits. Some important functional compounds can be found in peels and seeds of fruits, which have been traditionally considered waste in the fruit processing industry. Besides, fruit wastes have an attractive lignocellulosic content that allows obtaining value added products by means of several biochemical, mechanical and thermochemical processes. It is imperative to develop means that allow a complete utilization of all of the fruit constituents, in a sustainable manner.

The biorefinery concept allows accomplishing this goal. This research is aimed at developing the basic knowledge, applicable technological information and approaches that are necessary in order to introduce the biorefinery concept to Colombian fruit processing and to its derived waste streams. The objective of this research is aimed to evaluate fruits and its wastes as raw materials for biorefineries, using concepts of process engineering to enable design of such biorefineries. Different computational tools are utilized in designing and evaluating biorefineries for fruits utilization. Using techno-economic and environmental assessments, this research also develops knowledge and information about the feasibility of the biorefinery concept in obtaining fruit-derived value-added products.

This research also include bench activities where some models of value-added products from both, non-oil and oil feedstocks are prepared and studied in order to enhance the theoretical understanding and provide feedback data for the design of biorefineries. This research also can enhance the current knowledge about biorefineries, not only in the Colombian context, but also for other countries. At the same time, this research allows promoting the implementation of the biorefinery concept in fruit and fruit-derived byproducts processing. The latter represents a promising new approach to complete sustainable fruit processing and utilization.

Some challenges in the design of biorefineries are related with feedstocks (Availability, amounts, chemical composition, etc.), technologies, economic development and environmental issues that together, become in aspects that have to be evaluated for understanding the interaction and effects of the variables in the design of biorefineries based on fruit and its wastes. This research gives an approach of the economic development of biorefineries based on both, non-oil and oil feedstocks. At the same time, this research allows knowing a logical sequence in the design of biorefineries as well as aspects such as yields, energy consumption, costs associated to production, etc. Environmental point of view in this research allows understanding the environmental impact that has a biorefinery based on fruit and its wastes as well as the contribution with the greenhouse gases emissions.

Finally, all these results in this research allow giving a biorefinery approach based on fruits and its wastes in the Colombian context. Also, a logical way for designing biorefineries is highlighted as well as the potential of streams wastes from fruit processing.

HYPOTHESIS

There exists a way for using integrally the fruits for the production of value-added compounds applying the biorefinery concept by mean of the application of process engineering concepts and using computational tools for its evaluation.

OBJECTIVES

GENERAL OBJECTIVE

Perform the design of biorefineries from avocado and spent blackberry pulp in the Colombian context to obtain value-added products.

SPECIFIC OBJECTIVES

1. Characterization of the lignocellulosic biomass of avocado and spent blackberry pulp as raw materials for biorefineries.
2. To carry out special analyses of fatty acids, anthocyanins and phenolic compounds for avocado and spent blackberry pulp.
3. Modeling and simulating biorefineries based on fruits mentioned in objective 1.
4. Perform techno-economic and environmental assessments for the proposed biorefineries to the selected fruits.
5. To carry out experimental assays to obtain extracts and valuable compounds as products from the selected fruits with the biorefinery concept.
6. To carry out ultrafiltration processes to concentrate extracts obtained as products in objective 5.

PART I.

CONCEPTUAL APPROACH

1. Chapter 1. Fruits as source for valuable compounds

Overview

This chapter presents the justification of the use of fruits and its wastes after their processing for obtaining valuable compounds as well as the actual state of the art of the potential fruits in Colombia to be used with this purpose. Topics such as production, availability and composition of some fruits will show that the use of fruits and its residues can be considered as an interesting alternative to obtain added value compounds resulting in improvements of the productive chain of some fruits in Colombia.

Because Colombia is a tropical country, there is an important production and variety of fruits. There are exotic fruits that are not found in other countries and therefore, it is necessary to consider additional applications for some fruits, especially from its residues taking into account that the non-edible part of the fruits can represent a significant amount and that both seed and peel could contain important compounds of interest. Valuable compounds such as phenolic compounds, carotenoids, flavonoids, vitamins, antioxidants, enzymes and anthocyanins among others could be presented not only in the edible part of the fruits but also in their residues.

The valuable biologically active compounds present in fruits and its residues can positively impact human health and well-being therefore, some fruit-derived metabolites could have applications in the pharmaceutical, cosmetic, chemical and food industries therefore, fruits and its wastes become in an attractive source of valuable compounds that offer opportunities for developing value-added applications for fruits.

A wide variety of fruits contain valuable compounds that could be extracted or that could be obtained after thermochemical or biological processes. Despite that a lot of fruits have valuable biological active compounds not all of them can be extracted because different reasons such as the availability, the amounts presented or the required technology therefore, several variables should be taken into account to consider the production or extraction of these kinds of compounds.

All these compounds contained in fruits can be used for pharmaceutical and food applications, designing functional food or nutraceuticals that can contribute with the well-being and human health. Because Colombia has a wide biodiversity, there is a great potential for the production of functional food not only for national consumption but also for foreign countries that demand a consumption of natural compounds in all products.

With the last in mind, this chapter presents an introduction of the potential for obtaining valuable compounds from fruits and its residues and the possibility to use them in functional food. There is considering the composition of the fruits and the possible applications of the valuable compounds contained in the fruits. Also, there is showed the importance of the wastes generated in the fruit processing because the attractive chemical composition similar or better than the offered by the pulp. Finally, some general chemical compositions are showed for the most typical Colombian fruits including some exotic fruits.

1.1 Colombian fruits: Production, composition and valuable compounds

1.1.1 Production of Colombian fruits

Colombia has one of the most diverse varieties of fruits in the world, due to its thermal floors and its variety of soils that allow cultivating a broad spectrum of fruit species. Colombia leads in biodiversity per square kilometer and enjoys 433 species of native fruits. Additionally, Colombia has the second larger number of plant species, 51,220, some of which are exotic fruits containing important compounds (Contreras et al., 2011; Montaño and Toro, 2007).

The fruits cultivated in Colombia have a wide extension, more than 241,000 hectares that are extended along the country. Figure 1.1 shows the location of some important fruits cultivated in Colombia. For instance, blackberry (*Rubus glaucus benth*) is located in Santander and the grape (*Vitis vinifera*) is located in Timbio Cauca. Passion fruit (*Passiflora edulis*) is located in Cucuta, Pamplona and Ocaña Norte de Santander. Other fruits such as borojo (*Borojoa patinoi*), zapote (*Pouteria sapota*), apple (*Malus domestica*), pear (*Pyrus cummunis*) and apple guava (*Psidium guajava*) are cultivated in Medellin Antioquia (Contreras et al., 2011). Papaya (*Vasconcellea pubescens*), cashew (*Anacardium occidentale*) and carob tree (*Ceratonia siliqua*) are cultivated in Norte de Santander. Fruits such as araza (*Eugenia stipitata*), aguaje (*Mauritia flexuosa*), tree tomato, peach (*Prunus persica*), naranjilla (*Solanum quitoense*), spondias mombin (*Spondias purpurea*), cupuazu (*Theobroma grandiflorum*), abiu (*Pouteria caimito*) and umari (*Poraqueiba sericea*) are obtained from Leticia Amazonas (Sinchi, 2008).

On the other hand, the exotic fruits have special attention because they have important compounds that could be used for medicinal purposes. This is the case of Amazonian fruits because they belong to the wide biodiversity of the Amazonian region. The potential of the Amazonian fruits have been probed and the market of these fruits is growing not only in national but also in international markets (Gonzalez, 2014; Sinchi, 2008).

Figure 1.1. Location of some important fruits in Colombia. Number of the fruit is presented in Table 1.1 except for exotic fruits

The fruit processing industry in Colombia generates significant amounts of non-edible residues that can, potentially, be an important source for the extraction of valuable compounds. In Colombia, about of 70% (by weight) of fruits and vegetables become by-products or non-edible, due to the improper practices, challenges associated with the cold delivery chain and/or an inadequate transport that damage the fruits. Approximately 230,000 hectares are used to cultivate fruits (Maya, 2010b) and according to the "*Anuario Estadístico de Frutas y Hortalizas del Ministerio de Agricultura de Colombia*", around of 3.3 million tonnes of fruits were produced in 2011 (MinAgricultura, 2012). In this way and taking into account the percentage losses in the fruit processing industry, around 2.31 million of tonnes of fruits are lost annually. Table 1.1 and Figure 1.2 show the principal fruits cultivated in Colombia and the annual production of the 10 fruits with major production in the last years respectively.

Table 1.1. Principal fruits cultivated in Colombia (MinAgricultura, 2012)

No.	Fruit	Hectares	Production (Ton)
1	Avocado (*Persea Americana*)	24,514	215,095
2	Anon (*Annona squamosa*)	31	155
3	Badea (*Passiflora quadrangularis*)	55	794
4	Banana (*Musa paradisiaca*)	33,182	333,710
5	Borojo (*Borojoa patinoi*)	3,015	18,117
6	Brevo (*Ficus carica*)	316	1,991
7	Chirimoya (*Annona cherimola*)	201	1,222
8	Chontaduro (*Bactris gasipaes*)	8,872	70,471
9	Plum (*Prunus domestica*)	1,548	12,099
10	Passion fruit 1 (*Passiflora tarminiana*)	1,343	14,268
11	Peach (*Prunus persica*)	1,490	19,849
12	Feijoa (*Acca sellowiana*)	137	1,123
13	Strawberry (*Fragaria vesca*)	1,134	45,024
14	Passionflower (*Passiflora ligularis*)	3,727	38,914
15	Soursop (*Annona muricata*)	2,255	23,448
16	Guava (*Psidium guajava*)	12,196	121,773
17	Higo (*Ficus carica*)	104	1,823

18	Lime (*Citrus aurantifolia*)	436	9,280
19	Lemon (*Citrus limonum risso*)	6,192	83,421
20	Naranjilla (*Solanum quitoesne*)	6,810	57,712
21	Macadamia (*Macadamia integrifolia*)	261	767
22	Mamoncillo (*Melicoccus bijugatus*)	83	500
23	Mandarin (*Citrus tangerine tanaka*)	11,427	115,217
24	Mango (*Manguifera indica l*)	18,575	221,015
25	Mangostino (*Garcinia mangostana*)	7	116
26	Apple (*Pyrus malos l*)	121	1,219
27	Passion fruit 2 (*Passiflora edilus*)	5,335	79,678
28	Marañon (*Anacardium occidental*)	724	1,855
29	Melon (*Cucumis melo*)	2,454	43,751
30	Blackberry (*Rubus glaucus benth*)	11,673	94,303
31	Orange (*Citrus sinenses*)	16,245	260,034
32	Nispero (*Manilkara huberi*)	53	457
33	Papaya (*Carica papaya*)	4,968	153,120
34	Papayuela (*Carica pubescens*)	2	12
35	Patilla (*Citrullus lanatus*)	6,473	92,949
36	Pear (*Pyrus communis l*)	1,287	15,048
37	Pineapple (*Ananas comosus*)	12,984	512,316
38	Pitaya (*Stenocereus queretaroensis*)	691	6,579
39	Tamarindo (*Tamarindus indica*)	43	175
40	Tree tomato (*Solanum betaceu*)	8,371	129,492
41	Grapefruit (*Citrus paradise macf*)	2	20
42	Uchuva (*Physalis peruviana*)	745	10,770
43	Grape (*Vitis vinifera*)	2,253	24,153
	Total	**212,335**	**2,833,835**

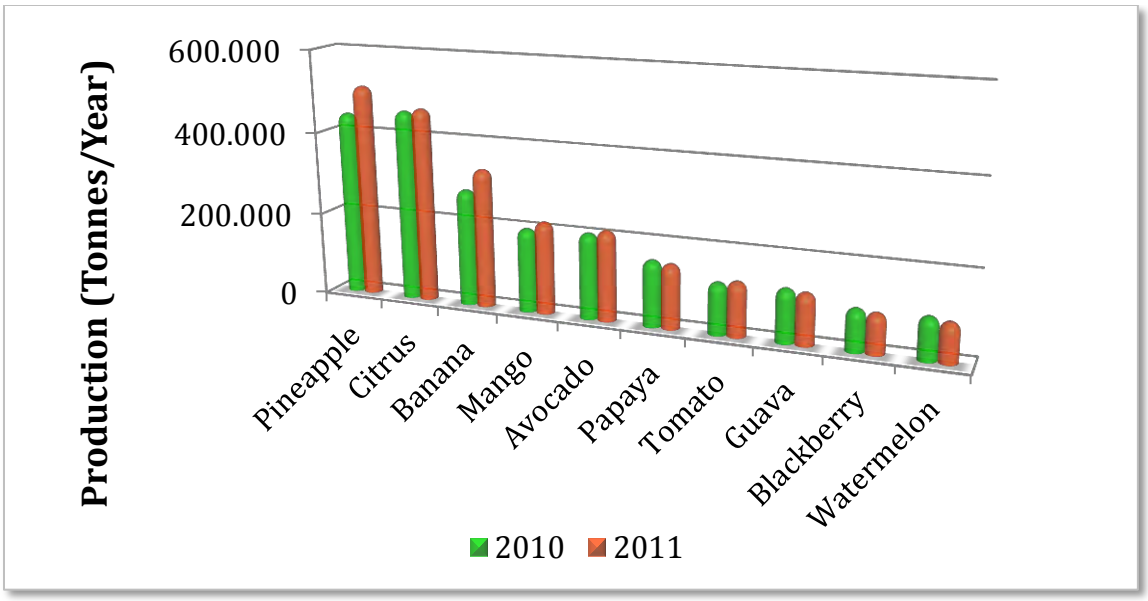


Figure 1.2. Annual production of fruits in Colombia in the last years. Adapted from (MinAgricultura, 2012)

Despite of the 230,000 hectares cultivated with fruits, Colombia has a high number of hectares available to cultivate them with more than 7,400,000 distributed along of the departments in Colombia such as is shown in Figure 1.3 (MADR, 2006). According to this only the 3.1% of the available hectares is used, this fact also increments the potential for producing fruits in Colombia.

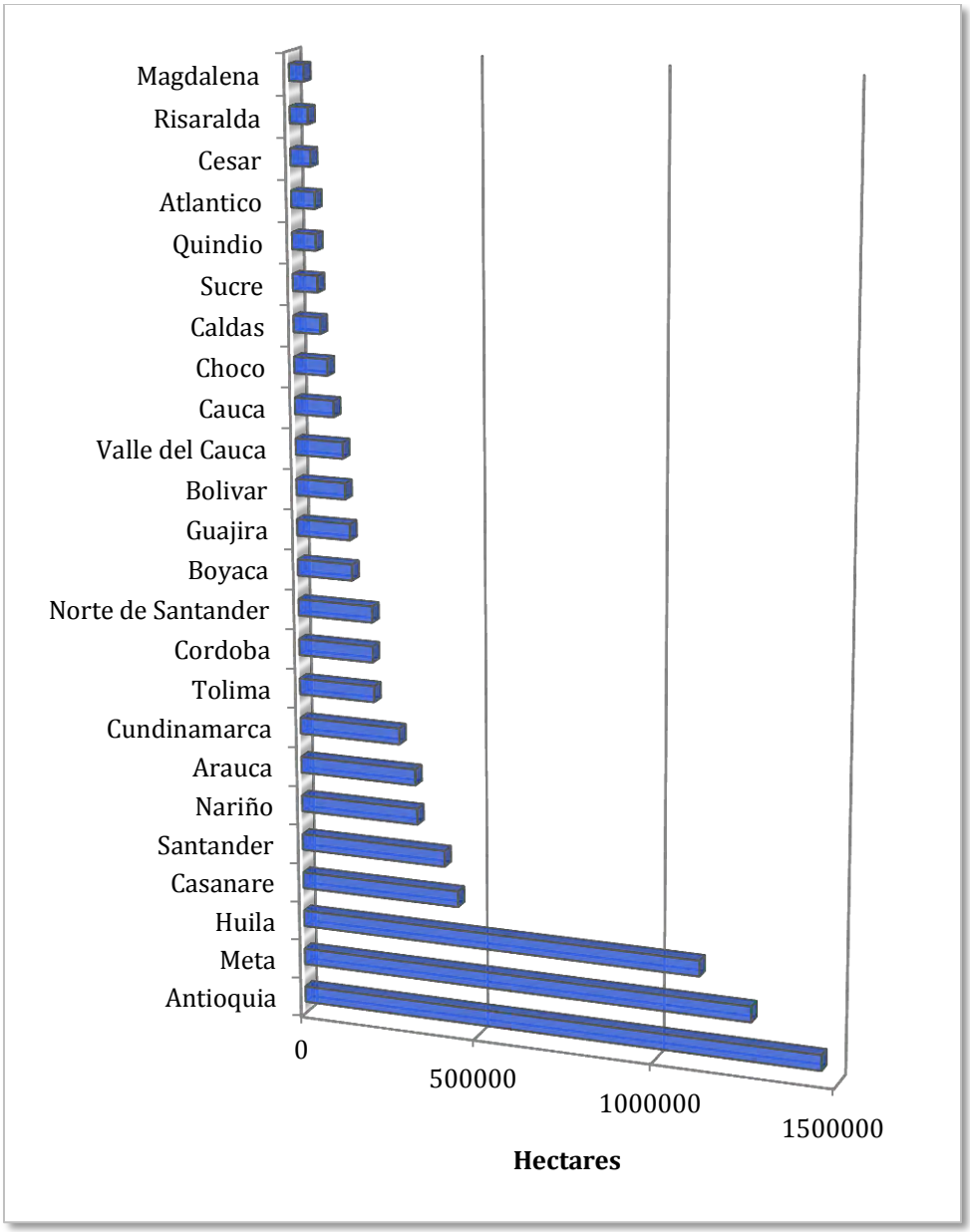


Figure 1.3. Hectares available for cultivate fruits. Adapted from (MADR, 2006)

Colombia occupies the ninth position at worldwide level regard to the production of tropical fruits as it is shown in Figure 1.4. The first country is India which produces more than 4,300 million of tonnes per year while Colombia produces a little bit more than 462,000 tonnes per year (FAO, 2013). In this regard, the Amazonian fruits play an important role because some of them are very attractive not only for its color and flavor but also because the valuable compounds contained in these fruits. Figure 1.5 shows the most available and commercialized fruits from Amazonian region.

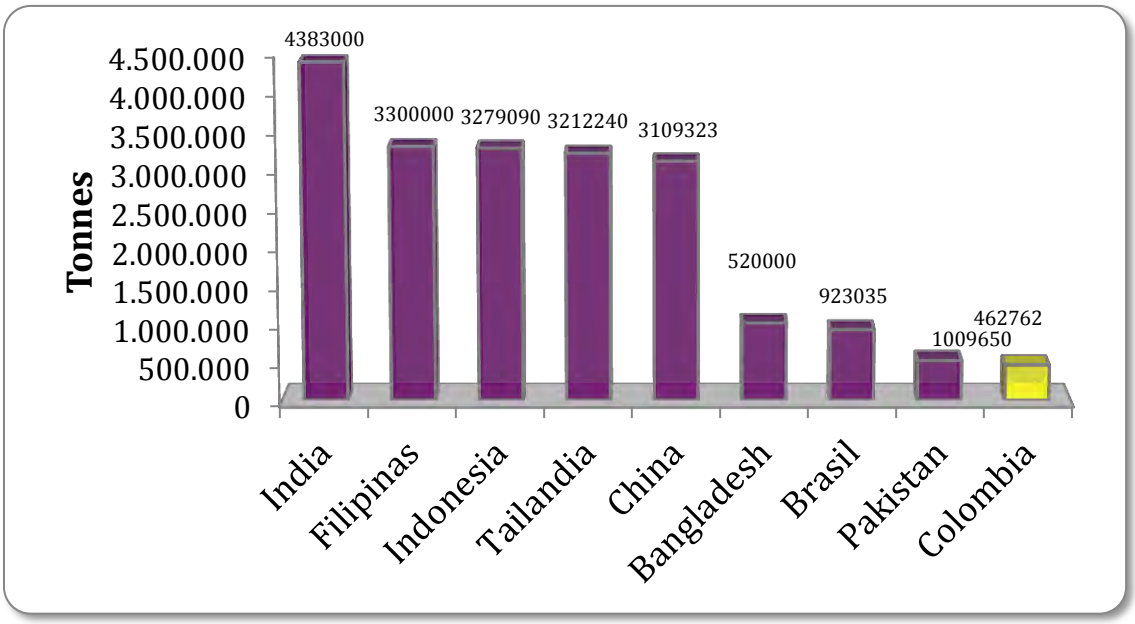


Figure 1.4. Tropical fruit production in the world

Figure 1.5. Available and commercialized fruits from Amazonian region

1.1.2 Benefits of the valuable compounds from Colombian fruits

Colombian fruits are very attractive not only for their flavors but also for the important compounds contained in its pulp, seed and peel. Some of these fruits are still unexplored (exotic fruits) and cannot be found in other countries. One of the principal uses of the fruits is their direct consumption and it is part of the food security. Some of the benefits that are offered by fruits to human health include prevention of both, micronutrient deficiencies and gastrointestinal cancer (Maya, 2010a). Consumption of fruits has been shown to allow modulating situations related to coronary heart diseases, bladder stones, biliary disorders, scurvy, cough and pulmonary tuberculosis (Einbond et al., 2004; Garzón et al., 2010).

Besides of the edible part of the fruits, the seed and peel are very important as well as the remaining residues after their processing because the wastes generated after fruit processing accounts important amounts that need special attention. Among the typical uses of residues from fruits are animal feed, fertilizers and biogas. However, due to the content of functional and/or bioactive compounds, these residues can be utilized as a source for essential oils, pectin, flavonoids, enzymes, carotenoids, fuels and an array of molecules for which pharmaceutical applications can be found (Yepes et al., 2011).

Recent studies have demonstrated that some important compounds contained in fruits such as flavonoids, phytochemicals, phenolic compounds, and anthocyanins can help reducing carcinogenesis, cancer of epithelial origin and digestive system cancer. Anthocyanins, in particular, can lower the level of cancer (Barrios et al., 2010; Cooke et al., 2005; Yi et al., 2005). Many of the valuable compounds of fruits can be found in the non-edible parts that are generated during fruit processing. Seed and peel of several fruits have significant amounts of valuable compounds such as the pectin and limonene that can be found in the peel of orange (*Citrus sinensis L.*) and citrus in general (Bicu and Mustata, 2011; Cerón and Cardona, 2011; Lagha and Madani, 2013; Lo Curto et al., 1992; Luengo et al., 2013). For instance, tree tomato (*Cyphomandra betacea*) contains lycopene, a compound that is found in the peel and has antioxidants properties (Ciurlia et al., 2009). Pineapple (*Ananas comosus*) contains bromelain in its peel; this is an enzyme which has uses in the cosmetic and food industries (Ketnawa et al., 2012). Avocado (*Persea americana*) contains noticeable amounts of phenolic compounds, with

antioxidant capacity in its seed as well as important content of lipids in the pulp (Soong and Barlow, 2004). Seed and peel of some Colombian exotic fruits, such as aguaje (*Mauritia flexuosa L.*), corozo (*Acronomia aculeata*), araza (*Eugenia stipitata*), passion fruit (*Passiflora tripartita*), borojo (*Borojoa patinoi*), cashew (*Anacardium occidentale*), zapote (*Matisia cordata*) and chontaduro (*Bactris gasipae*) contain extractable phenolic compounds (Contreras et al., 2011).

Compounds such as anthocyanins, antioxidants and phenolic compounds are found in fruits as grape (*Vitis vinifera*), coral (*Hyeronima macrocarpa Mull. Arg.*) and motilon (*Hyeronima sp*). The last fruit has important amounts of anthocyanins with around 240 mg per kg of fruit, this value is 10 times higher than values reported for other fruits (Santacruz et al., 2012). Other fruits with important amounts of compounds with antioxidant capacity are the blackberry (*Rubus glaucus benth*) and the passionflower (*Passiflora ligularis*), this last fruit has the majority of antioxidants compounds in its pulp (Contreras et al., 2011).

Other fruits such as cranberry (*Vaccinium myrtillus*) and the caimarona grape have significant amounts of anthocyanins with 329 mg and 95.3 mg per 100 gr respectively (Barrios et al., 2010; Cooke et al., 2005; Einbond et al., 2004). Gulupa (*Passiflora pinnatistipula*) is a fruit with high amounts of cyanidin 3-o- β glucopyranoside which is an anthocyanin, this fruit has around of 1.73 mg per kg of fruit (Jiménez et al., 2011). Even, sometimes, the agroindustrial wastes from fruit processing can be used as a source of anthocyanins for instance, using wastes from the wine industry around 26.2 mg of anthocyanins per 100 gr of wastes have been extracted (Clemente and Galli, 2011).

On the other hand, other fruits have special importance due to large production, which are accompanied with large quantities of wastes generated from the fruit processing industry. In this sense, fruits such as pineapple (*Anannas comosus*), citrus (*Citrus sinensis*), banana (*Musa paradisiaca*) and mango (*Manguifera indica L.*) have a potential to be used in the production of value-added products from their residues, not only due to the chemical composition but also because of the large amounts of wastes generated. Some of the potential fruits with important compounds are presented below.

Pineapple (*Ananas comosus*)

In this sense, Pineapple has between 30% and 42% of bromelain, which is an enzyme with applications in the food and cosmetic industries. The wastes generated from pineapple processing are a source of bioactive compounds and antioxidants. Pineapple wastes have been used to produce methane, ethanol and citric acid (Ketnawa et al., 2012; Umesh et al., 2008). These kinds of residues have been used to produce lactic acid reaching concentrations of 29 g/l in 72 hours of fermentation with *Lactobacillus delbrueckii* (Idris and Suzana, 2006). The cellulose content in pineapple wastes has enabled nanocrystals of cellulose to be obtained by means of acid hydrolysis processes (Santos et al., 2013). The cellulose content in the peel of the pineapple has been used to produce hydrogels with textures and mechanical properties with important applications (Hu et al., 2010). Pineapple wastes have also been used to produce hydrogen by means of fermentative processes (Wang et al., 2006). Table 1.2 shows a general composition of pineapple.

Table 1.2. Relevant composition of Pineapple

Pineapple (*Ananas comosus*)			
			
Component	**Value**	**Unit**	**Reference**

Minerals	72	mg/100 g	(Leterme et al., 2006)
Protein	6.27 ± 0.028	g/100 g	(Ramirez and Pacheco, 2011)
Fat	0.1 ± 0.014	g/100 g	
Sugars	77.17 ± 0.057	g/100 g	
Ash	1.14 ± 0.000	g/100 g	
Moisture	87.3	%	(Hemalatha and Anbuselvi, 2013)
Ascorbic acid	21.5	mg/100 gr	

Citrus (*Citrus sinensis*)

In the case of citrus, the peel in particular has essential oils with a high percentage of limonene, which can be used as intermediate compound for the production of other important products. These residues have been studied for the production of limonene and biofuels using the biorefinery concept with a capacity of 100,000 tonnes/year (Lohrasbi et al., 2010). Pectin is a product with high value in the market, this compound is found in the peel of the citrus and its extraction is carried out in acidic conditions (Pourbafrani et al., 2010). Organic acids such as p-cymene-2-sulfonic acid has been synthesized from the peel of citrus after essential oils recovery (Clark et al., 2012). The citrus peel has been used for the production of hydroxymethylfurfural (HMF) which is a key intermediate compound for the synthesis of other important products (Yi et al., 2013). Ethanol also has been produced from citrus wastes using *Saccharomyces cerevisiae* (Wilkins et al., 2007). Hesperidin has been isolated from the peel of citrus with assisted microwave technique (Inoue et al., 2010). The applications mentioned above suggest that citrus wastes, especially from oranges, should be used in integrated processes for the extraction of compounds of interest, to produce other products and to minimize the volumes of residues (Cerón and Cardona, 2011). Table 1.3 shows the most relevant composition of orange that is representative of citrus.

Table 1.3. Relevant composition of Orange

Orange (*Citrus sinensis*)

Component	Value	Unit	Reference
Calories	62	Kcal	(USDA, 2012)
Protein	1.23	g/131 g	
Fat	0.16	g/131 g	
Carbohydrates	15.39	g/131 g	
Fiber	3.10	g/131 g	
Sugars	12.25	g/131 g	
Vitamin C	69.7	mg/131 g	
Tiamine	0.114	mg/131 g	
Rivoflavine	0.052	mg/131 g	
Niacine	0.37	mg/131 g	
Vitamin B6	0.079	mg/131 g	
Vitamin E	0.24	mg/131 g	

Minerals	83.312	mg/131 g
Monounsaturated acids	0.03	g/131 g
Polyunsaturated acids	0.033	g/131 g

Banana (*Musa paradisiaca*)

For the case of banana (*Musa paradisiaca*), this fruit has been used with nutritional and therapeutic effects over human health because the phytochemicals with antioxidant properties that contribute to decrease cancer and cardiovascular diseases progress (Pereira and Maraschin, 2015). Banana peel has been used to isolate cellulose nanofibers using alkaline pretreatment methods for application in reinforcing elements and composites (Tibolla et al., 2014). Ripe pulp of banana has been used to obtain lipophilic extracts with content of fatty acids and sterols, this fact reveals that banana is a source of valuable phytochemicals with nutritional effects (Vilela et al., 2014). Banana leaves have been used as a potential energy source by means of combustion and value added products by pyrolysis because the hemicellulose and lignin contents close to other biomass fuels (Fernandes et al., 2013). Even, banana agricultural waste have been used for bioethanol production using acid and alkali pretreatments reaching 47.33% of reducing sugar yield (Gabhane et al., 2014). Table 1.4 shows the most relevant information of the general chemical composition for banana (USDA, 2012).

Table 1.4. Relevant composition of Banana

Banana (*Musa paradisiaca*)

Component	Value	Unit	Reference
Water	74.91	g/100 g	
Energy	89	Kcal	
Protein	1.09	g/100 g	
Fat	0.33	g/100 g	
Carbohydrate	22.84	g/100 g	
Fiber	2.6	g/100 g	(USDA, 2012)
Sugar	12.23	g/100 g	
Ca	5	mg/100 g	
Fe	0.26	mg/100 g	
Mg	27	mg/100 g	
P	22	mg/100 g	

K	358	mg/100 g
Na	1	mg/100 g
Zn	0.15	mg/100 g
Vitamin C	8.7	mg/100 g
Thiamin	0.031	mg/100 g
Riboflavin	0.073	mg/100 g
Niacin	0.665	mg/100 g
Vitamin B6	0.367	mg/100 g
Vitamin B12	0.0	µg/100 g
Vitamin A	3	µg/100 g
Vitamin E	0.1	mg/100 g
Vitamin D	0.0	µg/100 g
Vitamin K	0.5	µg/100 g

Mango (*Manguifera indica l*)

In the same way, mango has polyphenols, carotenoids and other bioactive compounds in its peel. The peel of the mango represents around of 20% of the fruit (Ajila et al., 2007). The peel of this fruit is an important source of enzymes such as protease and peroxidase, also this residue has vitamins C and E (Ribeiro and Schieber, 2010). Mango wastes have also been used to produce lactic acid using fermentation processes (Jawad et al., 2013). Additionally, the seed of mango has been used to extract nanocrystals of cellulose by means of acid hydrolysis (Henrique et al., 2013). The peel of the mango also has been used for the extraction of pectin in acidic conditions (Berardini et al., 2005; Koubala et al., 2008). The last indicates that mango and its wastes after fruit processing

can be used for extraction of valuable compounds because its important chemical composition. Table 1.5 shows the most relevant composition of mango.

Table 1.5. Relevant composition of Mango

Mango (*Manguifera indica l*)

Component	Value	Unit	Reference
Water	83.46	g/100 g	(USDA, 2012)
Energy	60	Kcal	
Protein	0.82	g/100 g	
Fat	0.38	g/100 g	
Carbohydrate	14.98	g/100 g	
Fiber	1.6	g/100 g	
Sugar	13.66	g/100 g	
Ca	11	mg/100 g	

Fe	0.16	mg/100 g
Mg	10	mg/100 g
P	14	mg/100 g
K	168	mg/100 g
Na	1	mg/100 g
Zn	0.09	mg/100 g
Vitamin C	36.4	mg/100 g
Thiamin	0.028	mg/100 g
Riboflavin	0.038	mg/100 g
Niacin	0.669	mg/100 g
Vitamin B6	0.119	mg/100 g
Vitamin B12	0.0	µg/100 g
Vitamin A	54	µg/100 g
Vitamin E	0.9	mg/100 g
Vitamin D	0.0	µg/100 g
Vitamin K	4.2	µg/100 g

In a similar way that the fruits presented above, other fruits have important and attractive chemical composition that could be used for other purposes. **Appendix A** presents the chemical composition of some selected fruits that correspond to the fruits with major production in Colombia as well as the most typical Amazonian fruits (Exotic fruits).

1.2 Promising fruits as a source of valuable compounds

Taken into account the wide variety of Colombian fruits and all possible compounds that could be obtained not only from the pulp but also from the peel and seed, there is important to highlight that a long list of compounds are present in the fruits. Figure 1.6 shows the most relevant constituents of a fruit that could be extracted (Lozano, 2006).

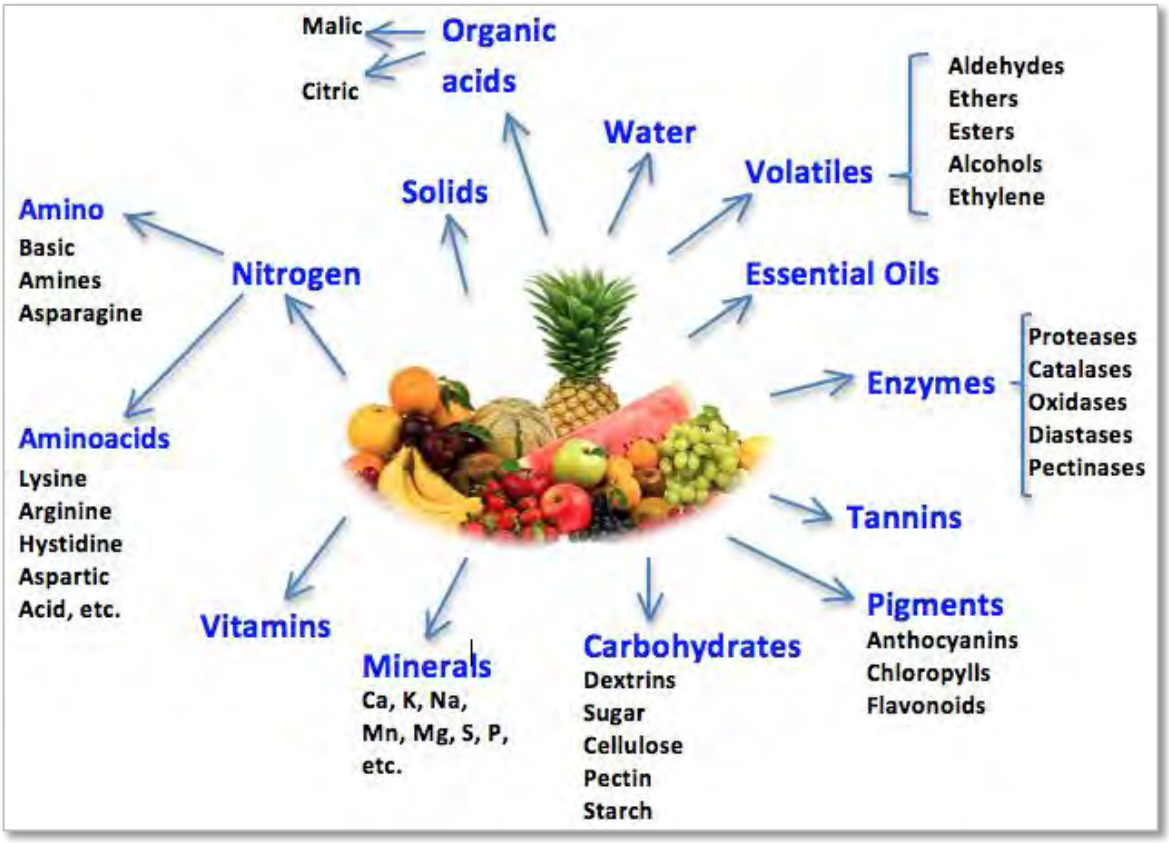


Figure 1.6. Relevant constituents of fruits. Adapted from (Lozano, 2006)

Organic acids such as malic, quinic, citric, pyruvic, fumaric, lactic, succinic, tartaric, formic, glyceric, glycolic, oxalic and aspartic among others are present in fruits not only in the pulp but also in the peel and seed. These acids play an important role on the organoleptic properties of fruits such as color, flavor and aroma. Besides, the organic acids contribute to the microbiological control and stability of the fruits (Flores et al., 2012).

Volatiles in fruits are represented as different substances that are an indicative of the fruit ripening. Volatiles are classified as primary or secondary compounds according to the presence of them in intact tissue or as a reaction of tissue disruption. These kinds of compounds influence strongly the aroma profiles of the fruits and sometimes, some aroma compounds are bound to sugars. On the other hand, flavor volatiles can be derived from an array of compounds phenols, terpenoids, amino acids, fatty acids and carotenoids. Other volatiles compounds such as esters, aldehydes, alcohols, lactones and ketones can be present in different fruits (El Hadi et al., 2013).

Essential oils act as natural antimicrobial substances and can be present in flowers, pulp, seed, peel, leaves and roots of the fruits. The essential oils can protect the plants from herbivores and play an important role as antibacterial, antiviral and antifungal: Essential oils have added value in the market and they are very attractive because they can be used as perfumes, sanitary products, as preservatives and to make-up products and flavor additives for food. Besides, essential oils can be used as industrial solvents and other applications (Bevilacqua, 2015).

A wide variety of enzymes can be found in fruits and they play an important role on the production of thousands of primary and secondary metabolites generated in the ripening state. Enzymes can define the metabolic pathway to obtain compounds that are characteristic of the flavor, color, taste and more in the fruit (Bayindirli, 2010). For instance, the oxidizing enzymes contribute to the rapid browning of cut fruits and produce changes in the appearance of the fruit manifested in color, flavor and even in the nutritional values. Some of the important enzymes for fruit quality are presented in Table 1.6 (Adel and Diane, 2004).

Table 1.6. Some important enzymes in fruits

Enzyme	Function
Polyphenoloxidase	Catalyzes oxidation of phenolics resulting in formation of brown polymers
Polygalacturonase	Catalyzes hydrolysis of glycosidic bonds between adjacent

	polygalacturonic acid residues in pectin, results in tissue softening
Pectinesterase	Catalyzes deesterification of galacturonans in pectin, may result in tissue firmig
Lipoxygenase	Catalyzes oxidation of lipids, results in off-odor and off-flavor production
Ascorbic acid oxidase	Catalyzes oxidation of ascorbic acid, results in loss of nutritional quality
Chlorophyllase	Catalyzes removal of phytol ring from chlorophyll, results in loss of green color

Pigments are present in all fruits and they define the color of the different parts of the fruit. These natural pigments are produced in all states of the ripening fruit and they are classified into chlorophyll, carotenoids, flavonoids (Anthocyanins and anthoanthins), melanoidins and caramels (Lozano, 2006). Many of these pigments have antioxidant properties such as the anthocyanins in the berries, grapes and in all fruits with purple, red and similar colors such it is shown in Figure 1.7. A lot of these compounds are phenolics that belong to a wide group of phytochemicals that include the flavonoids but also phenolic acids, lignans, coumarins, quinones, ketones, etc. Table 1.7 shows a classification of phenolic compounds based on the number of carbons (Vermerris and Nicholson, 2006).

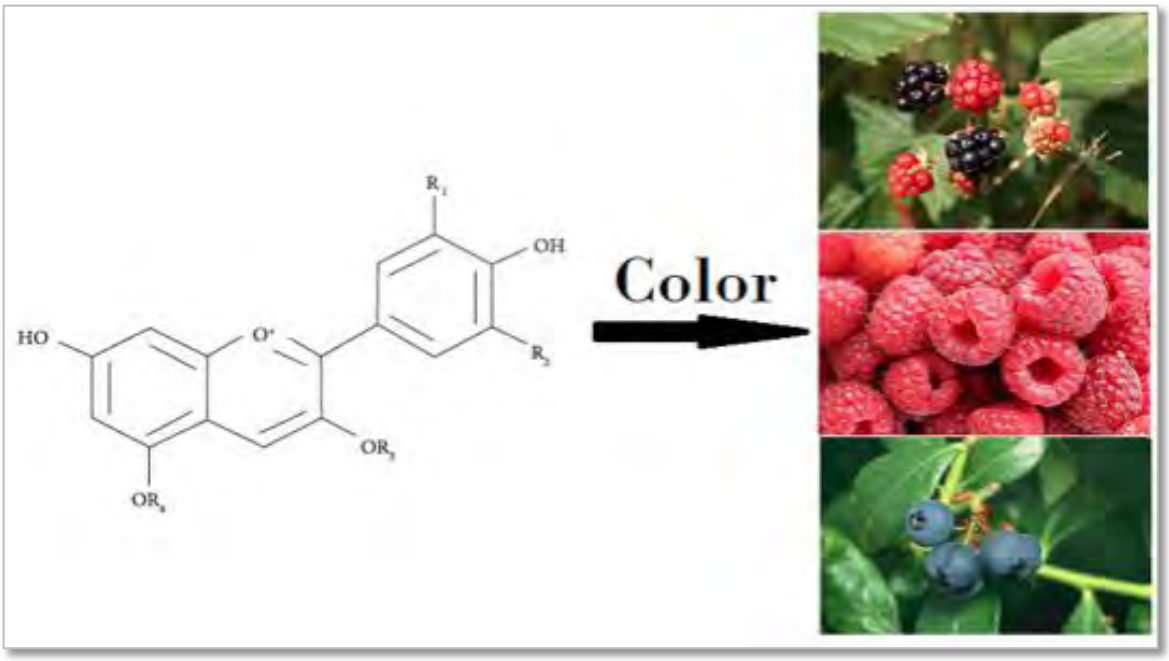
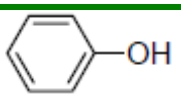
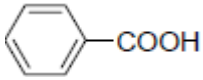
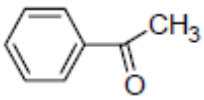
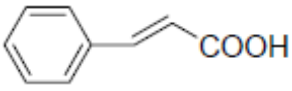
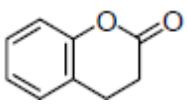
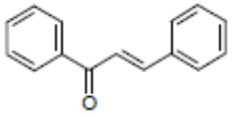
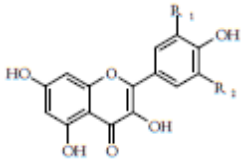
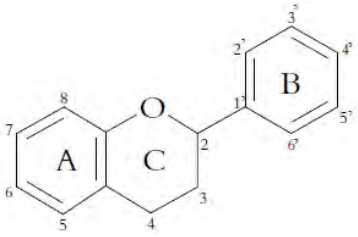
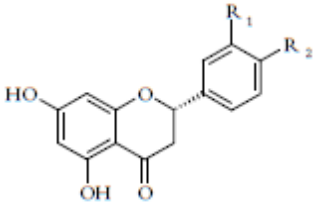
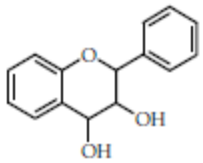
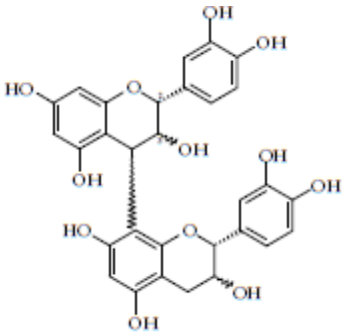
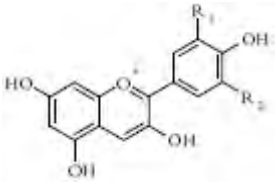
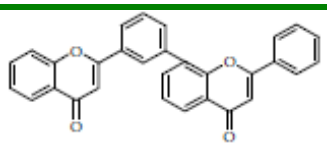
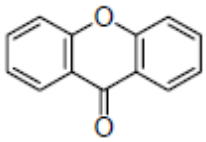
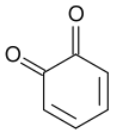
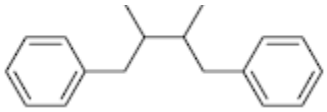


Figure 1.7. Basic structure of anthocyanins and its colors

Table 1.7. Classification of phenolic compounds

No. Carbons	Classification	Structure
C_6	Simple phenolics	
$C_6 – C_1$	Phenolic acids and related compounds	
$C_6 – C_2$	Acetophenones and phenylacetic acids	
$C_6 – C_3$	Cinnamic acids, cinnamyl aldehydes, cinnamyl alcohols	
$C_6 – C_3$	Coumarins, isocoumarins and chromones	

C_{15}	Chalcones, aurones, dihydrochalcones	
C_{15}	Flavans	
C_{15}	Flavones	
C_{15}	Flavanones	
C_{15}	Flavononols	
C_{15}	Anthocyanidins	
C_{15}	Anthocyanins	

C_{30}	Biflavonyls	
$C_6 – C_1$ - C_6, $C_6 – C_2$ - C_6	Benzophenones, xanthones and stilbenes	
C_6, C_{10}, C_{14}	Quinones	
Lignans, neolignans	Dimers or oligomers	
Lignin	Polymers	Complex structures
Tannins	Oligomers or polymers	Complex structures
Phlobaphenes	Polymers	Complex structures

On the other hand, carbohydrates are maybe the main component of fruits representing more than 90% of their dry matter. The structural framework, taste, texture and other characteristics of the fresh fruits are related with the carbohydrate content. The most relevant sugars presented in fruits are glucose, fructose and sucrose while maltose and minor percentage of other mono- and oligosaccharides are presented in the fruits. On the other hand, as starch, carbohydrates account for the energy reserves. Other polysaccharides presented in fruits are cellulose, hemicellulose, lignin and pectin that are presented even up to 50% (Adel and Diane, 2004; Lozano, 2006).

Minerals are very important and common in fruits, and its content becomes one of the most attractive contributions of fruits to human health. Typically, minerals are presented as salts of organic or inorganic acids or as complex organic combinations such as lecithin. Depending on the fruits, these substances can be dissolved in cellular juice. Potassium is the most relevant mineral presented in fruits and banana is the fruit with

the major content of potassium with 3,358 mg per 100 g of edible portion (Nirmal K. Sinha et al., 2012).

Vitamins are essential for normal function of the body therefore; the vitamins presented in fruits play an important role in this field. Vitamins A, B1, B2, B3, B5, B6, B9 as well as C, E and K are present in the majority of the fruits (Lozano, 2006; Nirmal K. Sinha et al., 2012). For instance, vitamin A contributes to cell reproduction and helps to the formation of some hormones also, vitamin A helps to vision and to protect the skin, hair and mucous membranes. The wide group of vitamins B (B1, B2, B3, B5, B6 and B9) helps to different functions such as conversion of carbohydrates in energy (B1 thiamine), vitamin B2 (Riboflavin) contributes with body growth while vitamin B3 (Niacin) helps to the correct function of digestive system. In the same way, vitamin B5 (Pantothenic acid) helps in the metabolism of food as well as in the formation of hormones. Vitamin B6 (Pryidoxine) helps to develop antibodies in the immune system as well as to maintain the normal nerve function, vitamin B9 (Folic acid) contributes to the proper cell growth and plays an important role in the pregnancy step in women. On the other hand, vitamin C is one of the most important vitamins and it is presented in citrus in large quantities. Vitamin C is an antioxidant and helps to protect the body against to the damage of oxidation because the free radicals. Vitamin D promotes the absorption of calcium and magnesium as well as to maintain the level of calcium and phosphorus in the blood. Vitamin E also protects against of oxidation damages and contributes to the protection of skin. Finally, vitamin K is very important for blood clotting and activates three proteins involved in bone health.

Organic acids obtainable in fruits contribute to the taste and give that acidity sensation in the fruits. Malic and citric acid are the most relevant and representative organic acids in fruits however, other organic acids such as glutamic, tartaric, quinic, malonic, shikimic, α-ketoglutaric, pyruvic, fumaric and succinic can be presented according to the fruit, ripening state and place (Flores et al., 2012). Some fruits are well known because its contribution of these kinds of acids, for instance, citrus is a good source of citric acids while apple contributes with malic acid however, a wide variety of fruits have these organic acids in different concentrations. These acids have an important role in several functions of the fruits, in this regard, malic acid contributes to the starch metabolism, and ripening and sugar changes and helps to the bacterial infections.

In these sense, some important fruits appear as promising fruits to obtain valuable compounds because their composition not only in the pulp but also in the peel and seed. Besides, the large quantities and attractive biological active compounds that can be extracted from different fruits are important reasons for exploring the possibility of use fruits and its wastes after their processing to obtain valuable compounds. The following fruits are taken into account because they are promising fruits to obtain valuable compounds on biorefinery concept and to enhance the productive chain of these fruits.

1.2.1 Blackberry (*Rubus glacus benth*)

The productive chain of blackberry or Andes Berry in Colombia is well established however, it lacking of research to find additional benefits of the residues after their processing. Besides, there is an excessive intermediation that contributes to decrease the effective of the production of blackberry and it becomes in a hurdle to enhance the productive chain of this fruit. The total production of blackberry in 2013 was 105,218 tonnes distributed in the entire national region as it is shown in Figure 1.8.

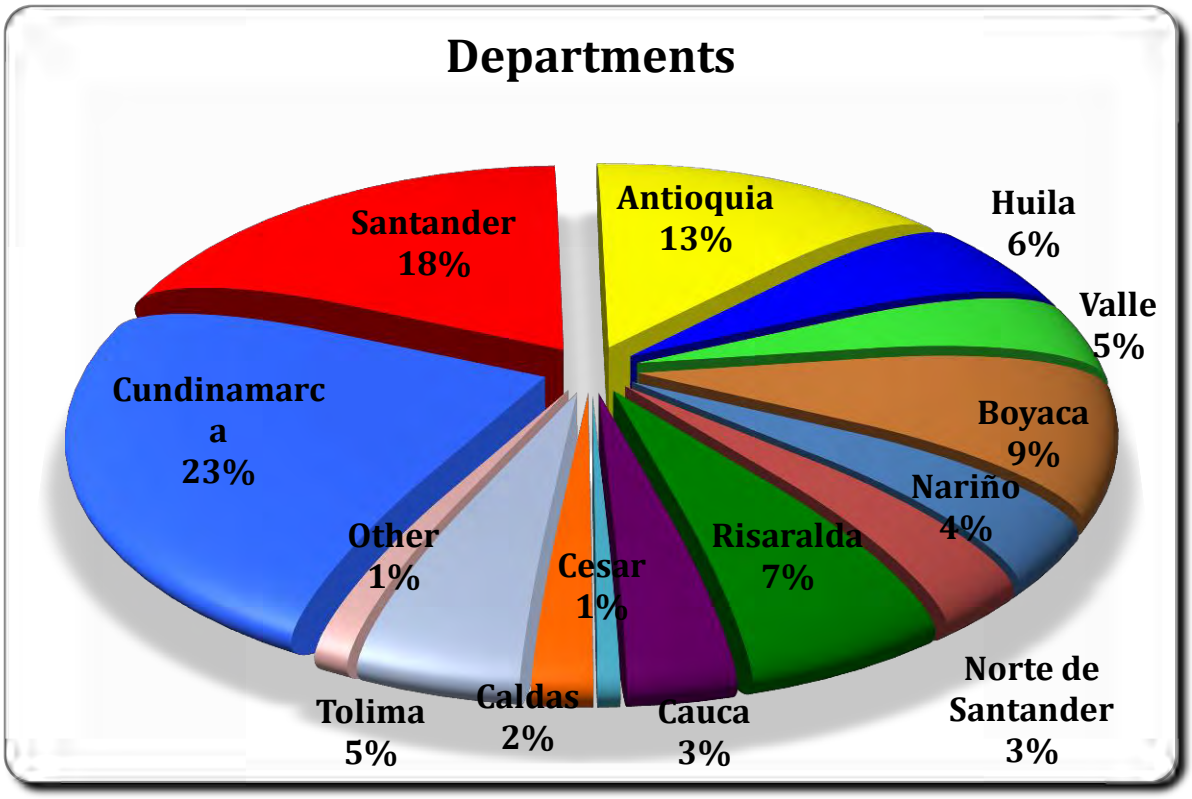


Figure 1.8. Production of blackberry in Colombia distributed by departments

The market for blackberry is wide expanded in all the national territory and part of this fruit is exported to USA, Panama, Germany, Spain, Chile, Australia, Costa Rica, Canada and United Kingdom among others. In 2013 the exportations accounted 19.33 tonnes of blackberry that represented more than 51.84 thousands USD. Blackberry is consumed and distribute with the route of marketing shown in Figure 1.9. Here, the major producers of blackberry are associated with small organizer producers that can distribute to industry, for exportation and wholesalers and at the same time, with gatherers that distribute to exportations and retailers.

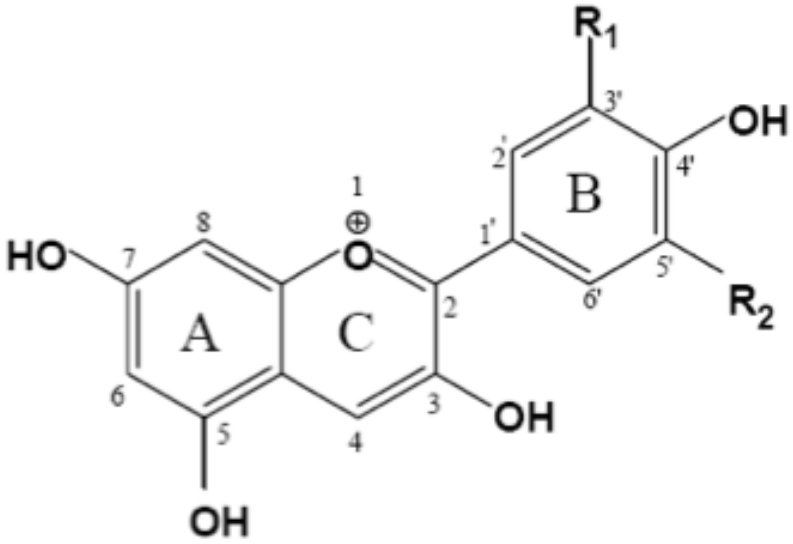
Figure 1.9. Marketing routes of blackberry in Colombia

Regard to chemical composition of blackberry, this fruit has antioxidant properties due to its phenolic compounds such as cafeic and chlorogenic acids as well as quercitin among others (Aybastıer et al., 2013). This fruit also has significant contents of ascorbic acid with 600 mg per each 100 gr of blackberry approximately (Patras et al., 2009). Blackberry can be used for the extraction of anthocyanins which have applications in the cancer field (Dai et al., 2009). Despite that there are more than 500 anthocyanins only 6 of them are found in the plants such it is shown in Table 1.8 (Warner, 2015).

The more characteristic anthocyanin presented in blackberry is cyanidin 3-O glucoside with more than 67% of the total anthocyanin content, this fact becomes to blackberry as a good source of anthocyanins which can be obtained as extracts (Osorio et al., 2012). The basic and general composition of valuable compounds of blackberry is presented in Table 1.9 (USDA, 2012). This fruit has been reported with a content of anthocyanins up to 180 mg/kg of fresh fruit and an antioxidant activity up to 1.08 mmol of Trolox/kg of fruit (Cerón et al., 2012).

Table 1.8. Basic chemical structure of anthocyanins

Anthocyanins



Anthocyanidin	R_1	R_2	Colour
Delphinidin	-OH	-OH	Violet
Cyanidin	-OH	-H	Red
Petunidin	$-OCH_3$	-OH	Violet
Pelargonidin	-H	-H	Orange
Peonidin	$-OCH_3$	-H	Red
Malvidin	$-OCH_3$	OCH_3	Red

Table 1.9. Relevant composition of Blackberry

Blackberry (*Rubus glaucus benth*)			
			
Component	**Value**	**Unit**	**Reference**
Water	83.15	g/100 g	(USDA, 2012)
Energy	43	Kcal	
Protein	1.39	g/100 g	
Fat	0.49	g/100 g	
Carbohydrate	9.61	g/100 g	
Fiber	5.3	g/100 g	
Sugar	4.88	g/100 g	
Ca	29	mg/100 g	
Fe	0.62	mg/100 g	
Mg	20	mg/100 g	

P	22	mg/100 g
K	162	mg/100 g
Na	1	mg/100 g
Zn	0.53	mg/100 g
Vitamin C	21	mg/100 g
Thiamin	0.02	mg/100 g
Riboflavin	0.026	mg/100 g
Niacin	0.646	mg/100 g
Vitamin B6	0.03	mg/100 g
Vitamin B12	0.0	µg/100 g
Vitamin A	11	µg/100 g
Vitamin E	1.17	mg/100 g
Vitamin D	0.0	µg/100 g
Vitamin K	19.8	µg/100 g

1.2.2 Avocado (Persea Americana mill)

Colombia occupies the fifth position in the production of avocado with more than 250 thousand tonnes per year after Mexico, Chile, Dominican Republic and Indonesia. This value corresponds to 5.7% of the total production at worldwide level. The total production is distributed among 10 Departments as it is shown in Figure 1.10.

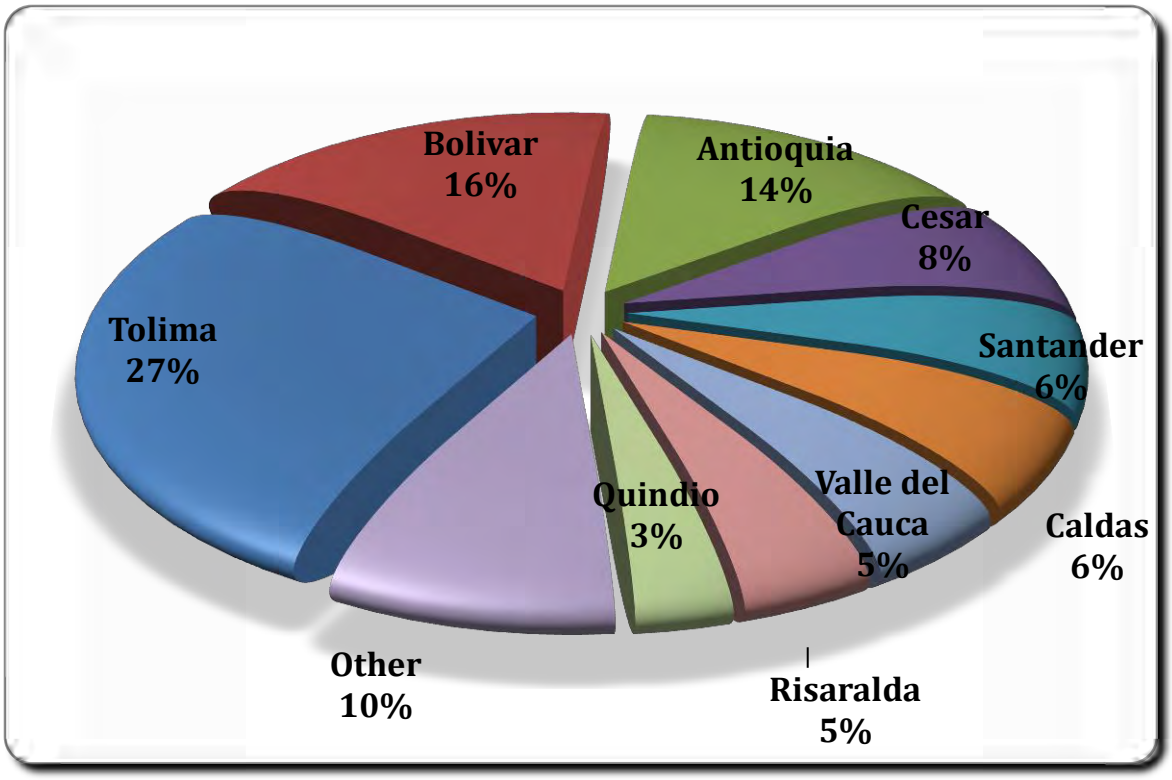


Figure 1.10. Production of avocado in Colombia distributed by departments

Colombia has several varieties of avocados among them Choquette, Santana, Lorena, Semil, Booth-8, Fuerte and Hass as it is shown in Figure 1.11 (Camero, 2012). However, the Hass variety is more attractive because it has the highest content of fatty acids and also, it is the variety that is cultivated in major proportion with 38% of the total production. The chemical composition of pulp avocado variety Hass is showed in Table 1.10 (USDA, 2012).

Figure 1.11. Varieties of avocados in Colombia

Table 1.10. Relevant composition of Avocado

Avocado (*Persea americana*)			
			
Component	**Value**	**Unit**	**Reference**
Water	73.23	g/100 g	(USDA, 2012)

Energy	160	Kcal
Protein	2.0	g/100 g
Fat	14.66	g/100 g
Carbohydrate	8.53	g/100 g
Fiber	6.7	g/100 g
Sugar	0.66	g/100 g
Ca	12	mg/100 g
Fe	0.55	mg/100 g
Mg	29	mg/100 g
P	52	mg/100 g
K	485	mg/100 g
Na	7	mg/100 g
Zn	0.64	mg/100 g
Vitamin C	10	mg/100 g
Thiamin	0.067	mg/100 g
Riboflavin	0.130	mg/100 g
Niacin	1.738	mg/100 g
Vitamin B6	0.257	mg/100 g
Vitamin B12	0.0	µg/100 g
Vitamin A	7	µg/100 g
Vitamin E	2.07	mg/100 g
Vitamin D	0.0	µg/100 g

Vitamin K	21	µg/100 g

Oil of avocado pulp is one of the most attractive products to be extracted because this oil contains monounsaturated fatty acids that becomes in an excellent component of a healthy diet. Avocado oil is a triacylglycerol with minor amounts of fatty acid and up to 1.5% unsaponifiable matter. These characteristics becomes the avocado oil similar to olive oil in many respects but has a higher betal-sitosterol content and lower level of squalene and polyphenols. Taken into account that the chemical composition of avocado pulp can vary with season, harvest and variety, Table 1.11 presents a general fatty acid profile of Colombian Avocado (Variety Hass) (Acosta, 2011).

Table 1.11. General fatty acid profile of Colombian avocado, variety Hass

Fatty acid	Percentage
Palmitic	15.60
Palmitoleic	13.44
Estearic	0.60
Oleic	54.70
Linoleic	13.20
Octadecanoic	1.12
Linolenic	1.03
Araquidic	0.38

Besides of the avocado pulp, both the peel and seed of avocado have important amounts of procyanidins and pigments. Avocado wastes have antioxidant capacities similar to other fruits (Wang et al., 2010). The peel of the avocado is a source of oil, which has industrial applications. This oil can be extracted with solvents such as hexane and anhydrous ethanol (Adama and Edoga, 2011). The seed of the avocado represents

a high percentage of the fruit; this seed is a residue of the fruit because it is not consumed. The seed has bioactive phytochemicals and phenolic compounds, which can be used in the medicinal field. Additionally, the seed has fungicide, insecticide and antimicrobial activity (Dabas et al., 2013). Both, seed and pulp of the avocado have important amounts of carotenoids that can be used with other applications (Gross et al., 1973). For all these characteristic mentioned above, avocado becomes in an attractive fruit to obtain valuable compounds.

1.2.3 Other fruits

The interest of other fruits and residues has been growing in the last decades because the large production of them and the attractive chemical composition. This is the case of Spent Coffee Grounds (SCG) obtained after beverage preparation using coffee and naranjilla waste obtained after juice extraction.

Coffee (*Caffea Arabica l*)

Coffee is one of the most important products for the Colombian economy, therefore is a product exported to other countries. Colombia is the third producer of coffee at worldwide level and has one of the most attractive coffees because its color, flavor and taste. Despite that the beverage is the most typical product from coffee, this industry uses only 9.5% of the total fresh fruit while the remaining 90.5% corresponds to residues. Figure 1.12 shows the principal parts of a fresh fruit of coffee (Colombia, 2015).

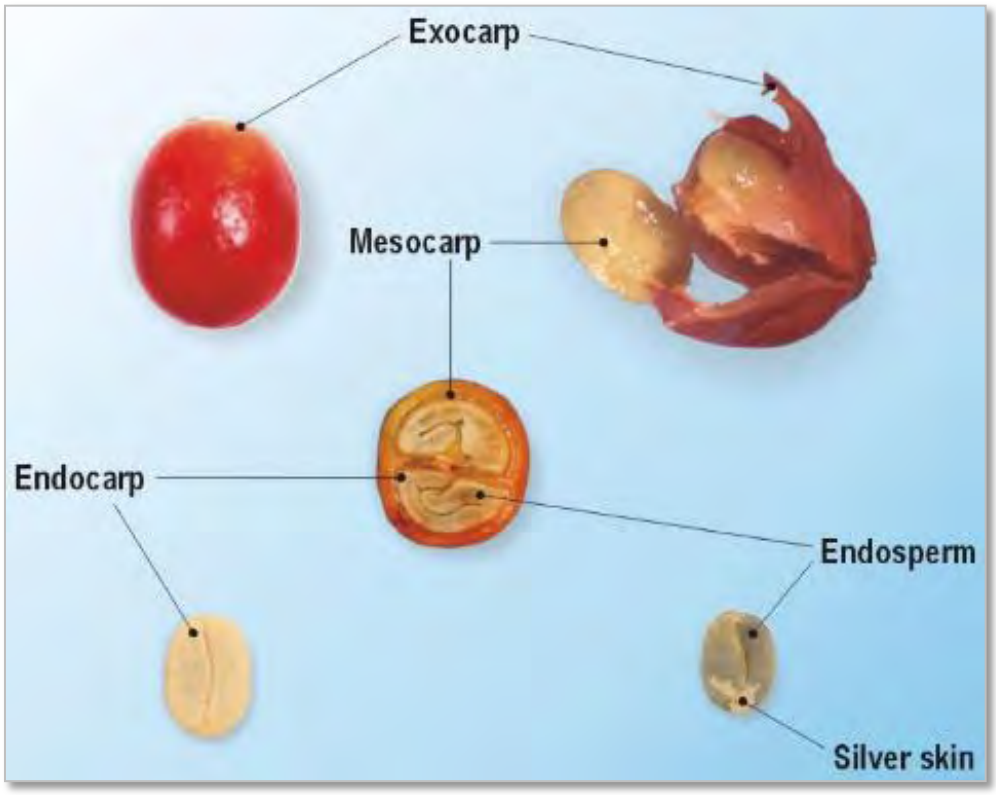


Figure 1.12. Parts of the fresh fruit of coffee

The grains or seeds of coffee obtain a red color in its ripening. The grain of coffee is a sweet pulp that is covered by an exterior skin. The grain of coffee is composed as it is shown in Figure 1.12. Despite of the high amount of coffee used for beverage preparation, other parts of the grain are available after this process. Table 1.12 shows the quantities obtained in each of the steps in the coffee process as well as the residue obtained per kg of coffee processed.

Table 1.12. Losses and residues in the coffee process per kg

Step	Losses (gr)	Residue
Pulping	394	Fresh pulp
Mucilage	216	Mucilage
Threshing	35	Pergamin

Drying	171	Water
Roasting	22	Volatiles
Beverage preparation	104	Spent Coffee Grounds
Total losses	942	

Some applications of the coffee residues are for the cultivation for edible mushrooms (*Pleurotus*) from the pulp (Rodríguez, 1998), also pulp has been used to organic fertilizer for the cultivation of worms (Dávila and Ramírez, 1996). Spent coffee grounds have been used as support for anaerobic microorganisms in the treatment of wastewater, the mucilage can be used as animal food especially swines as well as for obtaining pectin. From the pulp can be obtained yeast for food applications while spent coffee grounds can be used for obtaining manitol (Rodríguez, 1998). Some of the products that can be obtained from each part of the fresh fruit of the coffee are presented in Figure 1.13 (Salazar C., 1984).

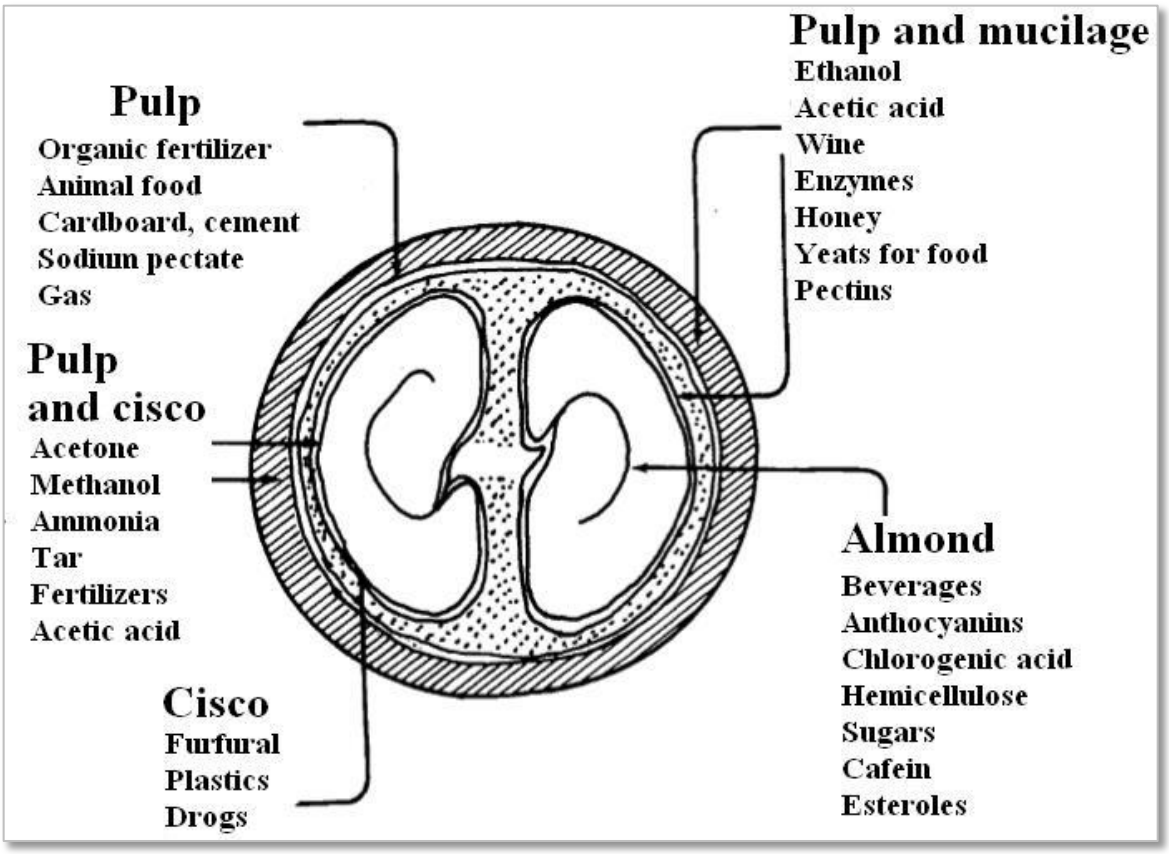


Figure 1.13. Possible products from coffee grounds. Adapted from (Salazar C., 1984)

The general chemical composition of fresh ground of coffee (*Caffea arabica l*) is presented in Table 1.13. The most common carbohydrate presented in coffee is in the form of cellulose and polysaccharides consisting of mannose, galactose and arabinose. Lipids appear as very stable compounds even, these lipids can be present in huge quantities after beverage preparation. There are very especial reactive amino acids such as arginine, aspartic, cystine, histidine and lysine among others. The predominate volatile acids are formic and acetic while lactic, tartaric, pyruvic and citric appear as nonvolatile acids. Caffeine forms part of a hydrophobic complex with chlorogenic acids in a molar ratio of 1:1. Other compounds such as melanoidins give the brown color in the soluble fraction of coffee.

Table 1.13. General chemical composition of coffee

Coffee (*Coffea Arabica l*)			
			
Component	**Value**	**Unit**	**Reference**
Caffeine	1.3	%	(Belitz et al., 2009)
Lipids	17	%	
Protein	10	%	
Carbohydrates	38	%	
Trigonelline, niacin	1.0	%	
Aliphatic acids	2.4	%	
Chlorogenic acids	2.7	%	
Volatile compounds	0.1	%	
Minerals	4.5	%	
Melanoidins	23	%	
Amino acids			
Alanine	4.75	%	

Arginine	3.61	%	
Aspartic	10.63	%	
Cystine	2.89	%	
Glutamic	19.80	%	
Glycine	6.40	%	
Histidine	2.79	%	
Isoleucine	4.64	%	(Belitz et al., 2009)
Leucine	8.77	%	
Lysine	6.81	%	
Methionine	1.44	%	
Phenylalanine	5.78	%	
Proline	6.60	%	
Serine	5.88	%	
Threonine	3.82	%	
Tyrosine	3.61	%	
Valine	8.05	%	
	Lipids		
Triacylglycerols	78.8	%	
Diterpene esters	15	%	
Diterpenes	0.12	%	(Belitz et al., 2009)
Triterpene esters	1.8	%	

Naranjilla (*Solanum quitoense*)

Naranjilla or lulo is a fruit native from Andes, cultivated and consumed in Ecuador, Colombia and tropical regions in America. This fruit is considered as tropical fruit and the annual production is more than 57,700 tonnes per year of which 28% is produced by the department of Huila as it is shown in Figure 1.14 (Huertas et al., 2011).

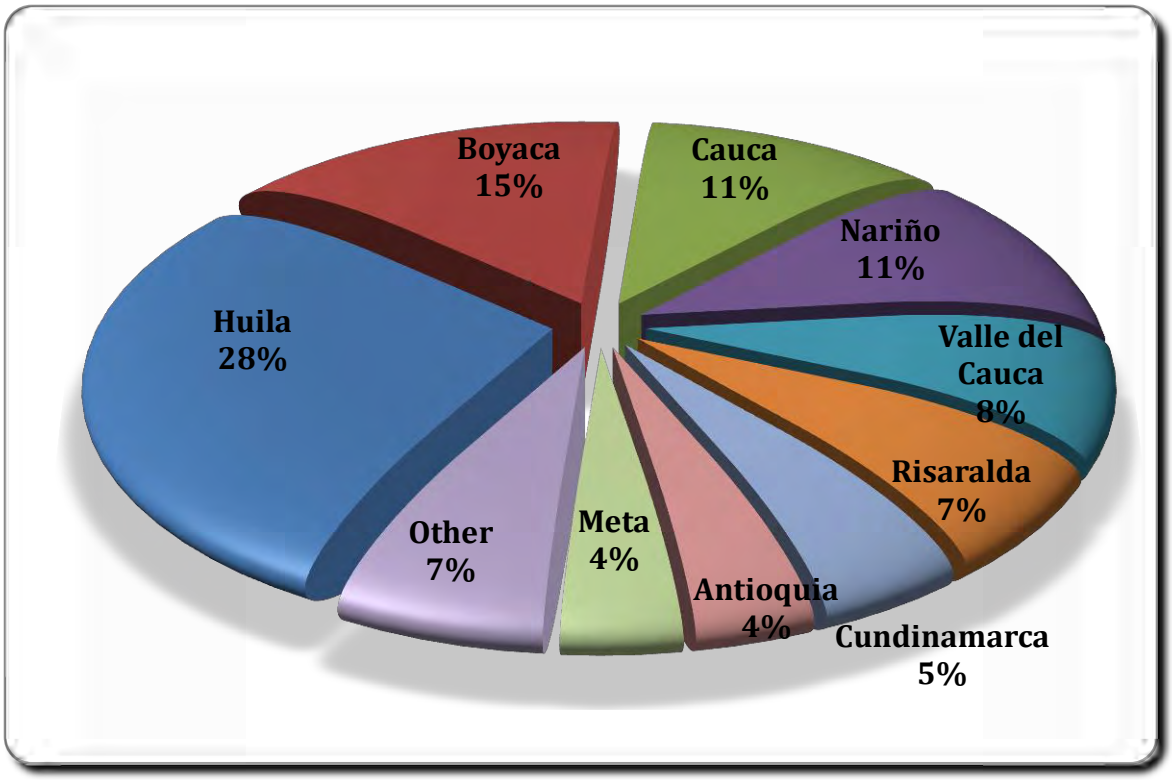


Figure 1.14. Production of naranjilla by departments

This fruit has a potential interest for international markets: Naranjilla is characterized by its high content of fiber and organic acids, minerals (Phosphorous, calcium, iron), vitamins (Niacin, thiamin, riboflavin, A and C). Other studies have been made to characterize the most important compounds of naranjilla such as volatiles (methyl and ethyl esters, aliphatic esters and alcohols). Despite of the high production of naranjilla in Colombia, this fruit is perishable therefore the postharvest becomes in a challenge

(Forero et al., 2015). The pulp of this fruit is rich in organic acids, minerals and sugars which the most representative corresponds to glucose such as it is shown in Figure 1.15.

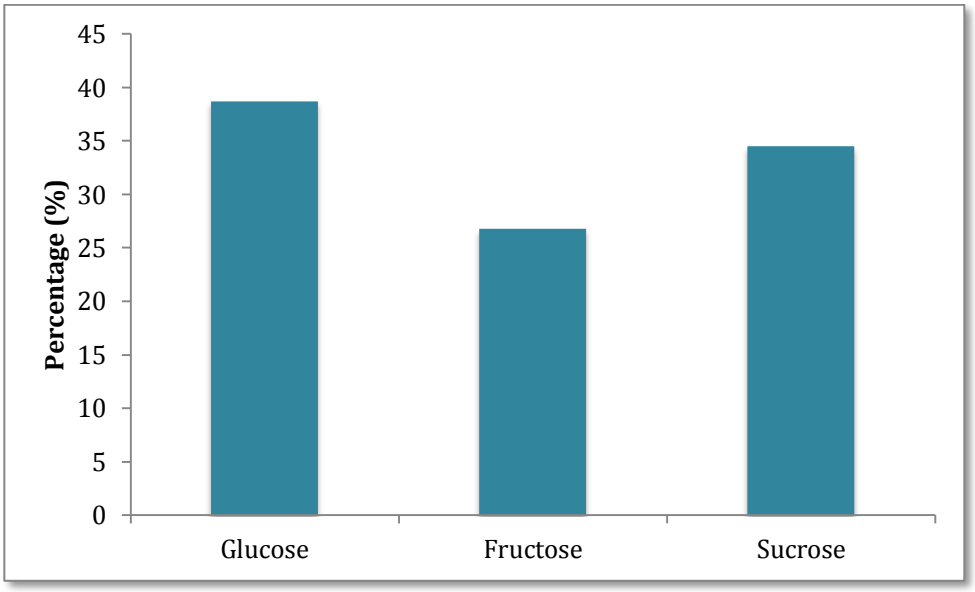


Figure 1.15. Sugar composition of the pulp of naranjilla

Naranjilla is formed by an exocarp (peel) that covers the whole fruit, a flesh (mesocarp and endocarp) and placental tissues as it is shown in Figure 1.16. This fruit has an interesting chemical composition not only in its pulp but also in its peel and seed such as it is shown in Figure 1.17 for Total Phenolic Compounds (TPC) and carotenoids. According to this, the peel is very interesting to obtain valuable compounds with antioxidant activity and in this way produce value added products (Gancel et al., 2008).

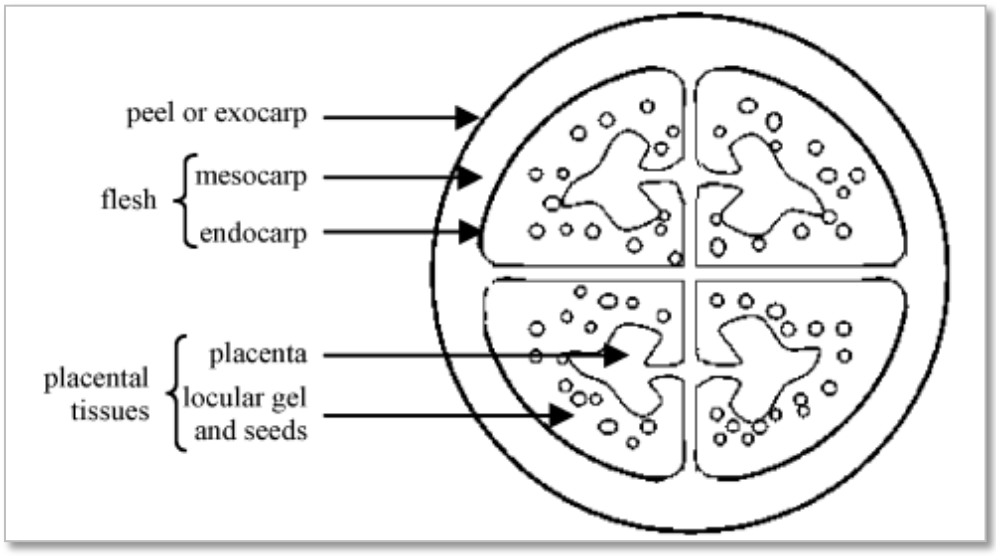


Figure 1.16. Parts of naranjilla fruit. Adapted from (Gancel et al., 2008)

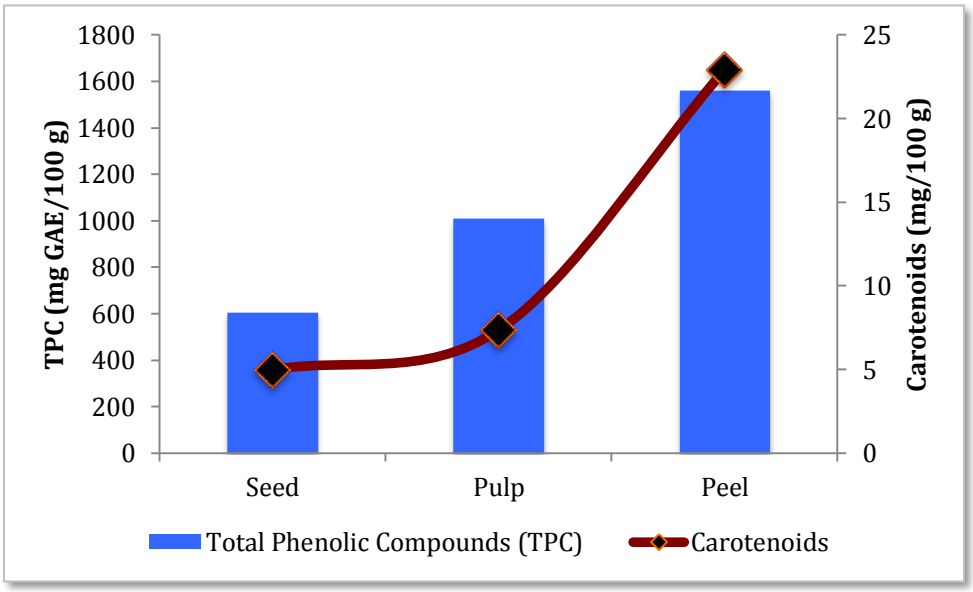


Figure 1.17. Phenolic compounds present in naranjilla

The chemical composition of this fruit offers a wide variety of compounds such as organic acids that can be extracted and that could be used for pharmaceutical and food applications. Table 1.14 shows the general chemical composition of naranjilla and its valuable compounds.

Table 1.14. General chemical composition of Naranjilla

Naranjilla (*Solanum quitoense*)			
			
Component	**Value**	**Unit**	**Reference**
Soluble solids	7.3	ºBrix	(Gancel et al., 2008)
pH	3.24		
Moisture	91.5	% DW	
		Sugars	
Glucose	14.9	% DW	(Gancel et al., 2008)
Fructose	10.3	% DW	
Sucrose	13.3	% DW	
		Organic acids	
Citrate	307	mg/g DW	
Isocitrate	5.8	mg/g DW	
Butyrate	1624	mg/g DW	

Succinate	610	mg/g DW	
Propionate	380	mg/g DW	(Gancel et al., 2008)
Oxalate	315	mg/g DW	
Galacturonate	157	mg/g DW	
Acetate	127	mg/g DW	
Cis-aconitrate	100	mg/g DW	
Citramalate	65	mg/g DW	
Malate	< 30	mg/g DW	

Conclusion

The chemical composition of some Colombian fruits has a promising potential for their use in the extraction of valuable compounds. The important content of phenolic compounds, carotenoids, flavonoids, vitamins, antioxidants, enzymes and anthocyanins among others that are presented not only in the pulp but also in the peel and seed of the fruits becomes in an attractive source for producing and extract these compounds to be used as functional food or nutraceuticals with applications in chemical, food and pharmaceutical industries. Besides of the chemical composition, the large quantities produced after fruit processing becomes to fruits and its wastes in a source to obtain value added products. Therefore, there is necessary to consider the use of some selected fruits for producing value added products. This approach allows the improvement of the productive chain of the fruits and at the same time, permits to obtain several benefits such as employment generate new products and expand the market of fruits. Off course, there is necessary to consider different challenges such as technologies, availability, transport and other hurdles that can to complicate the use of fruits for the purposes exposed above.

2. Chapter 2. Design of Biorefineries for fruits Processing

Overview

This chapter presents the biorefinery concept and the biorefinery approach for processing selected fruits. Taken into account the chemical composition and availability of some fruits that have been discussed in Chapter 1, this chapter presents the biorefinery approach for four raw materials, spent blackberry pulp (SBP) and avocado as well as the biorefineries for case study of spent coffee grounds (SCG) and naranjilla waste.

The biorefinery concept is explained as well as some important raw materials and related aspects that are relevant to biorefinery design (such as raw materials of first, second and third generation). The design of biorefineries of the selected raw materials is based on the chemical composition of them. The target products that could be obtained from the cited raw materials under biorefinery concept are a good contribution for expands the products from fruits. The conceptual design of biorefineries is explained in light of the value added products that can be obtained from the processed fruits and the possible constituent plants that are necessary in each biorefinery. Some of these constituent plants are needed in all of the biorefineries. An example for such cases is lignocellulosic biomass intended for sugar production (C_5 and C_6) that can be used for producing other important products such as acids, biofuels, biomaterials etc. Because valuable compounds such as phenolic compounds, anthocyanins and carotenoids can be found in these raw materials, their extraction is common to almost all biorefineries. Oil contained in some raw materials can also be considered a target for extraction as well as the use of sugar resulting from lignocellulosic biomass that can be used later for

producing organic acids, biomaterials or ethanol. Finally, in all cases, the use of a co-generation system for supplying part or all of the energy requirements of the biorefineries is considered.

The proposed biorefineries are based on the sequencing, hierarchy and integration concepts that have been developed for biorefinery design as well as the conceptual design of processes. Therefore, all cases are subjected to techno-economic and environmental assessments for biorefineries.

Four biorefineries are proposed for the processing of the selected raw materials. In each case the constituent plants of the biorefinery as well as the resulting products and their applications are explained and discussed. The proposed biorefineries are designed in a logical manner attempting to maximize the potential offered by the chemical composition of the specific fruit in question.

2.1 Biorefinery concept

A biorefinery is a structure that integrates biomass conversion processes in a given scheme for the production of different products such as biomolecules, biomaterials, bioenergy and biofuels (Moncada et al., 2013) such as shown in Figure 2.1. This concept is analogous to that of oil refineries, where crude oil is transformed into a family of fuels and byproducts (Moncada et al., 2013). The conceptual design of biorefineries is based on studying and assessing different types of biomass as raw materials. Raw materials can be classified as first, second and third generation (Moncada et al., 2013). The first generation type corresponds to biomass that should be used for food security, the second generation type corresponds to processable biomass that is generated as agroindustrial waste (lignocellulosic biomass) and the third generation type corresponds to algae (Moncada et al., 2013; Stoeglehner and Narodoslawsky, 2009).

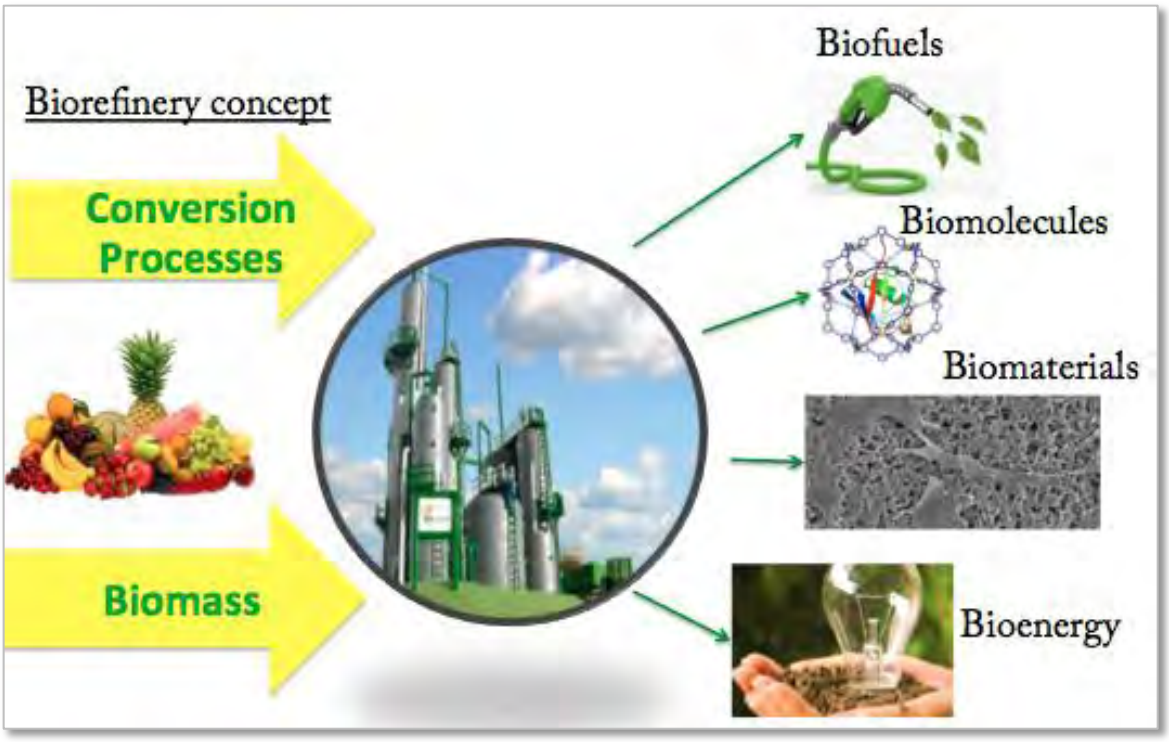


Figure 2.1. Biorefinery concept. Adapted from (Moncada et al., 2013)

Depending on the generation type of the biomass, it is possible to establish a sequence of processing to yield different types of products in a biorefinery, while preserving the food security when the first generation biomass is utilized. Depending on the chemical composition of the biomass, it is possible to obtain different products, which can be produced by means of integrated processes where all the components that are included in the biomass are utilized as raw material for the biorefinery.

When the selected biomass for a biorefinery is rich in cellulose, hemicellulose and lignin (lignocellulosic biomass) it is possible to propose cogeneration schemes capable of generating electricity and steam (at high, medium and low pressures) to meet all or part of the energy requirements of the biorefinery. Table 2.1 shows the cellulose, hemicellulose and lignin content of some common agroindustrial wastes.

Table 2.1. Composition of some lignocellulosic biomass

Material	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Reference
Hardwoods	40 - 55	24 - 40	18 - 25	(Sun and Cheng, 2002)
Softwoods	45 - 50	25 - 35	25 - 35	(Sun and Cheng, 2002)
Corncob	45	35	15	(Sun and Cheng, 2002)
Paper	85 - 99	0	0 - 15	(Kumar et al., 2009)
Wheat straw	30	50	15	(Kumar et al., 2009)
Agroindustrial wastes	37 - 50	25 - 50	5 - 15	(Limayem and Ricke, 2012)
Wastes from paper industry	50 - 70	12 - 20	6 - 10	(Limayem and Ricke, 2012)

The products obtained from a biorefinery can be produced at different ways such as, chemical and mechanical processes, by using thermochemical and biochemical routes or by using a combination of these approaches. In comparison with the raw materials of first generation, the second generation type raw materials enable the production of a broad array of products by different technological routes and techniques (Kokossis and Yang, 2010). Some of the typical routes used in a biorefinery are biochemical routes to obtain biomaterials and sugars, thermochemical routes to obtain energy and chemical routes to obtain other value added products (IEA, 2013).

Therefore, according to the specific raw material to be processing in a biorefinery, there is a need to consider different processing schemes or different configurations of biorefineries as well as different chemical, thermochemical, mechanical and/or biochemical processes that can be used in order to produce the target products.

Similarly, there is also a need to consider the most promising products that can be obtained from a given chemical composition of the raw material in a way that fully utilizes the potential offered by the raw material and allows obtaining the most appropriate products. The latter can be biofuels, biomaterials, bioenergy, biomolecules, and natural compounds. Figure 2.2 depicts the principal routes and products that can be obtained using the biorefinery concept, according to the characteristics of the raw material (IEA, 2013).

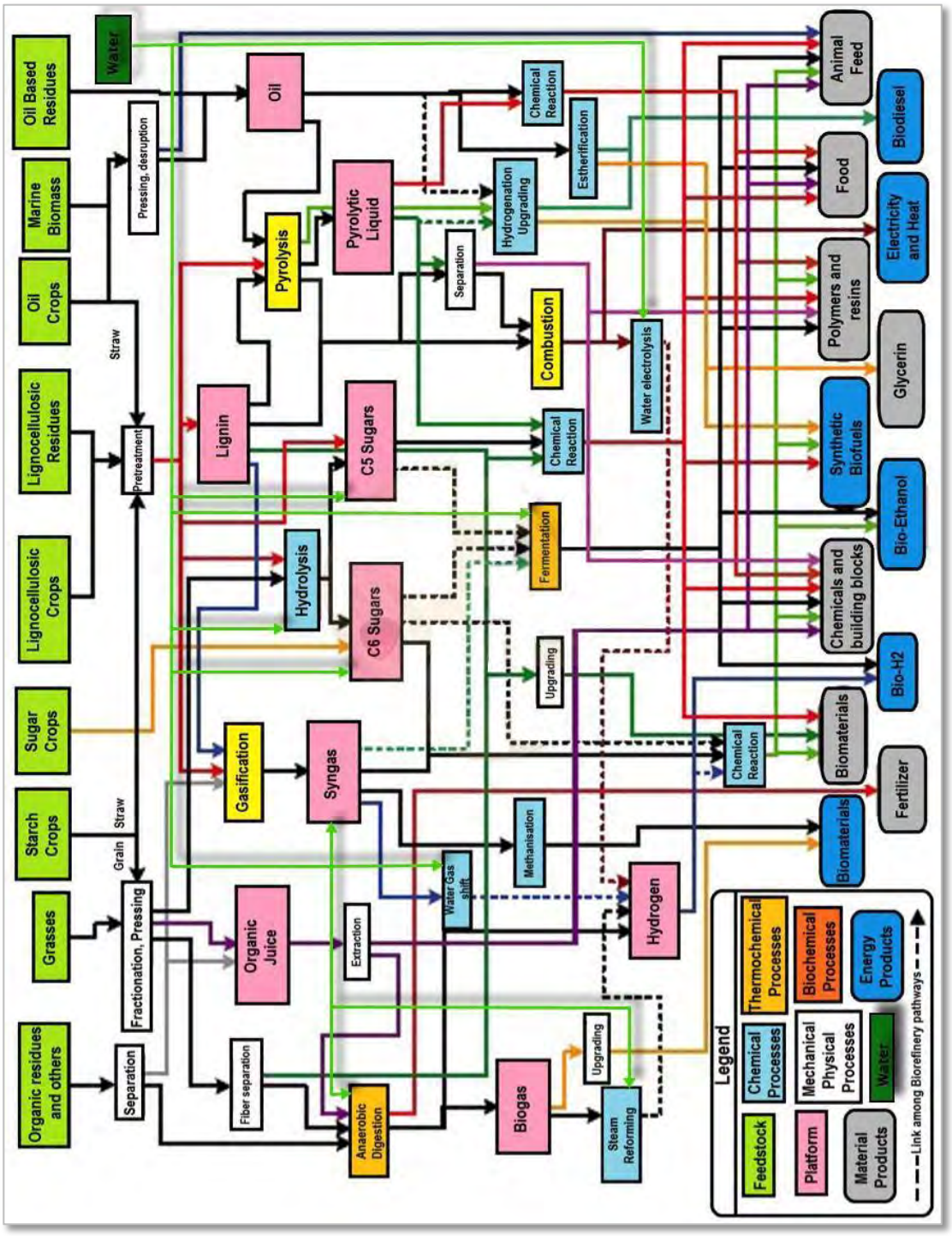


Figure 2.2. Pathways and products under biorefinery concept for several raw materials. Adapted from (IEA, 2013)

2.2 Raw materials for biorefineries

Different raw materials have been used to design biorefineries. These raw materials can be classified in two categories: crops and residues from crops processing (first and second generation respectively) (Moncada et al., 2013). Some principal crops for oil extraction are soy, sunflower, palm and jatropha (López-Bellido et al., 2014; Moncada et al., 2013). The crops for sugar production include sugarcane and beet (Maity, 2015). Other raw materials that have been used are starchy materials such as corn, potato, wheat, cassava and sorghum (López-Bellido et al., 2014; Maity, 2015). Hardwoods and softwoods (from forests and timber industry) also have a potential as raw material for biorefineries (Ghatak, 2011).

The wastes (or residues) that are generated from crops processing, such as those mentioned above can be used as raw materials for biorefineries. These residues are classified as lignocellulosics, lipids (oils and fats), etc. Some of the lignocellulosic materials are black liqueur, residues and sludge from paper industry, residues of woods, residues from wheat, rice and bagasse, etc. (Forster-Carneiro et al., 2013; García et al., 2014; Xu et al., 2012). With respect to residues from lipids, the most typical are fats from cattle processing and used oils (Forde et al., 2014; Tian et al., 2008). Other residues include wastes from vegetables and fruits, wastes from farms and municipal wastes (García et al., 2014; Maity, 2015; Tan et al., 2010).

The aforementioned raw materials are important because they contain proteins, fats, carbohydrates and other useful compounds that can be processed in a biorefinery and present a potential for producing value added products (Brehmer et al., 2009). Lignocellulosic biomass is an attractive raw material for biorefineries because it allows preparing fermentable sugars after a biomass-specific pretreatment using acid hydrolysis, biological processes, physical processes, chemical processes or a combination of these approaches (Alvira et al., 2010; Harmsen, 2010; Lenihan et al., 2010; Sun and Cheng, 2002; Yousuf, 2012). Other types of processes such as alkaline treatment, ozonolysis, thermal processes using solvents, ionic liquids, hot water and ammonia explosion have been investigated as means to improve the accessibility of the cellulose and minimize the generation of toxic compounds during the pretreatments of biomass (Alvira et al., 2010; Balat, 2011; Carvalheiro et al., 2008; Chiaramonti et al.,

2012; Esteghlalian et al., 1997; García et al., 2009; Hayes, 2009; Ingram et al., 2011; Karunanithy et al., 2013; Kim and Holtzapple, 2006; Kumar et al., 2009; Kumar and Wyman, 2009; Laureano et al., 2005; Lee et al., 2010; Lee et al., 1999; Lenihan et al., 2010; Mosier et al., 2005; Papatheofanous et al., 1995; Rogalinski et al., 2008; Sánchez, 2009; Silverstein, 2004; Sun and Cheng, 2002; Taherzadeh and Karimi, 2008; Travaini et al., 2013; Villalobos, 2010; Von Sivers and Zacchi, 1995; Wan and Li, 2012; Zhao et al., 2009).

Fruits and their wastes are currently less utilized in biorefineries design because of the availability of a more typical first and second generation biomass such as lignocellulosic materials. According to the Food Agricultural Organization (FAO), Colombia was positioned in 2011 as the ninth nation, before Mexico and after to Pakistan, in the production of tropical fruits with more than 462,000 tonnes per year (FAO, 2013). This fact supports the idea of using fruits pulp as well as peel and seed and other residues generated in fruits processing as raw materials for biorefineries.

Other studies have demonstrated that the fruits and their derived wastes are a promising raw material for the production of value added products such as limonene, pectin, essential oils and biofuels and also for the production of energy with cogeneration systems (Dávila et al., 2015; Dávila et al., 2014). Additionally, products such as succinic acids, mono and diglycerols can also be obtained from fruits (González et al., 2011; Lohrasbi et al., 2010; Lyko et al., 2009; Rezzadori et al., 2012).

Fruits and their derived waste streams or mass become attractive raw materials that can be used for obtaining value added products and thus increase the overall value of the fruits. Colombia has the agroindustrial potential to produce value added products from fruits, especially from the waste masses that are generated from their processing. Due to the fact that the volume of waste masses is proportional to the volume of production, the most processed fruits have a large potential to be used as raw materials for extraction of valuable compounds. The latter reflects both the high volume of generated biomass and the specific content of biologically active compounds (MinAgricultura, 2012).

Fruits, even with lower production volume such as naranjilla and blackberry contain biologically active compounds such as antioxidants, and phenolic compounds that can be extracted from their pulp, peel and seed of the fruits (Cerón et al., 2012; Forero et al., 2015). Some fruits have a chemical composition that can be used to the production and

extraction of other important products such as pectin, enzymes and vitamins (Berardini et al., 2005; Boukroufa et al., 2015; Cerón and Cardona, 2011; Contreras et al., 2011; Ketnawa et al., 2012). The lignocellulosic biomass of some fruits can be used to produce sugars such as xylose and glucose, which represent a broad base potential for production of a wide variety of products.

In this sense, it is clear that some fruits with high volumes of production or with an important content of biologically active compounds have to be investigated for their potential in offering unique opportunities in developing a complete, sustainable and attractive alternatives to the use of these fruits and fruits-derived products, including wastes and byproducts. The latter is possible with the biorefinery concept that integrates conversion processes of biomass of fruits to obtain value added products in the same scheme of production using pulp, seed and peel of the fruits in an integrate manner (Fava et al., 2015).

2.3 Design of biorefineries

The design of a biorefinery can be based on raw materials, type of compounds to be extracted or produced and the technologies available for transforming the biomass. The characteristics of the final products such as yield, concentration, amount etc. also define a design of a biorefinery. However, for fruits, there is a need to take into account that a specific scheme of a biorefinery depends on the detailed chemical composition of the selected fruit, considering the valuable compounds that are included in the selected fruit.

The processes or plants that constitute a biorefinery are selected according to the product that will be obtained. Because fruits can be composed of lignocellulosic, biomass, starch and other compounds with different structures and bonds that affect the yield of the process and the subsequent processes of the biorefinery, there is a need to consider all factors that can enhance the yield of the processes and maximize benefit from the chemical composition of the fruit. Therefore, the design of biorefineries should be based on the hierarchy, sequencing and integration concepts or strategies that permit

to follow a logical strategy for the design and evaluation of biorefineries (Moncada et al., 2014b).

2.3.1 Hierarchy, sequencing and integration strategies

Hierarchy concept

Hierarchy concept can be applied to feedstocks and technologies in the design of biorefineries (Moncada et al., 2014b). In the case of feedstocks, this concept is related to a logical hierarchal decomposition of feedstocks for obtaining the most promising products (Moncada et al., 2014b). The latter has to take into account that the wide range of raw materials can offer several feedstocks and that different feedstocks can yield different products. Because the feedstock is the base for several processing routes it is necessary to consider different sources of raw materials and the type of the feedstock used for the biorefinery (Moncada et al., 2014b).

As discussed above, the generation of the feedstock (first, second and third generation) is an important factor for biorefinery design because it defines the routes and products that can be obtained. For instance, the first generation feedstocks refer to crops and therefore are destined for food processing and preserve the food security. The second generation is related to the agroindustrial residues (waste) from the harvesting and processing of the first generation feedstocks such as lignocellulosic biomass and starch (Moncada et al., 2013). One of the most relevant characteristics of the second generation feedstocks is that it does not threaten food security. Finally, the third generation feedstocks is related to the use of algae for several metabolites production.

The hierarchy strategy for biorefinery design considers the type of feedstocks (first, second or third generation) to be utilized and the possible integration between these feedstocks. In this way, it is possible to propose different routes for obtaining value added products, based in the chemical composition to obtain the final products classified as biofuels, biomolecules, biomaterials, bioenergy, natural compounds among others. A biorefinery can be classified as biorefinery of first, second or third generation according to the type of the feedstock used. The integrated biorefineries can allow obtaining a

multiproduct biorefinery by means of the integration of the different feedstocks (Moncada et al., 2014b). For the case of integrated biorefineries, the hierarchy concept can also be applied for products because they can be used for other processes in the biorefinery or as a final product (Moncada et al., 2014b). Therefore, the hierarchy concept plays an important role in the design of biorefineries, following a logical hierarchal fractionation or disintegration of feedstocks.

From the technological point of view, the hierarchy concept is very important because it can define the performance of the entire facility. The latter is related to the fact that some parts of the biorefinery might need more attention than others. For instance, a biorefinery based on a second generation feedstock for producing sugars needs a special attention directed at the technology used for pretreated the biomass. The conditions, equipment, duration and additional variables associated with the pretreatment affect the yield of sugars, which in turn, become the feedstock for other processes of the biorefinery. Therefore, the hierarchial treatment is based on the process that has the highest implicant of the entire facility.

Feedstocks, products and technologies need a hierarchal treatment to ensure high feasiblity of the biorefinery. This fact permits to take into account different raw materials, technologies and possible products and the correct way to obtain them in the most coherent manner.

Sequencing concept

This concept is related to the logical order in which the products should be obtained by the biorefinery concept. For biorefineries based on fruits, it is necessary to consider the sequencing strategy with special attention because the type of valuable compounds contained and their correct extraction. For instance, the first products that should be obtained are phenolic compounds, anthocyanins, carotenoids and similar compounds that are degraded at high temperatures (more than 45 ºC) therefore, it is not possible to obtain pectin first and then extracts containing these valuable compounds. High temperature causes degradation of these compounds, thus leading to a loss of physicochemical and/or biological activity of compounds such as phenolic compounds,

antioxidants, etc. Thus, it is clear that both sequencing strategy and hierarchy for products are of critical importance to the design of biorefineries (Moncada et al., 2014b).

As a consequence of the relationship between sequencing and hierarchy strategies, it is necessary to integrate these concepts in a logical manner. It has to be realized that a given feedstock can allow producing different products by applying different processing sequences and technologies (Moncada et al., 2014b). Therefore, different biorefinery concepts and scenarios should be assessed for a given raw material. For instance, a biorefinery that uses lignocellulosic biomass for cogeneration systems, there is a possibility to suggest the utilization of this lignocellulosic biomass, if it is maybe rich in cellulose and hemicellulose, to obtain sugars (C_5 and C_6) that can be then converted into other important products, such as biofuels (Ethanol), acids (Citric, lactic, etc.) or biomaterials (Polyhydroxybutirate).

Integration concept

The integration strategy can be applied at different levels with the purpose of using it in the optimal way aimed at maximum yield and throughput, highest purity and lowest energy consumption. The first level of integration is energy integration that refers to the most efficient of all the energy resources that are available in the biorefinery. The latter is based on the Pinch methodology (Shenoy and Shenoy, 2014) that uses the composite curves approach to recover part of the energy requirements and to calculate the target requirements for heating and cooling. The second level of integration is related to mass integration that refers to the use and recycle of all mass streams available in the biorefinery (Martinez-Hernandez et al., 2013). For instance, water streams can be used again after some treatments that permit obtaining water at the quality level that is needed in several plants of the biorefinery. Also, in the case of third generation biorefineries, CO_2 from the process can be captured and recycled. It is possible to have a mass integration for products that permits distributing some of them from some plants of the biorefinery as feedstocks for other plants, For example, ethanol can be a product from lignocellulosic biomass processing and can be used as feedstock in other plants; it can be used as solvent in supercritical extraction of valuable compounds or as reagent in polyhydroxybutitare production.

In the same way, the mass and energy integrations keep a relationship when a mass integration is made for produce energy. For instance the remaining solids (biomass, lignin and other) can be used for cogeneration systems to produce energy (steam at low, medium and high pressure as well as electricity).

The final level of integration is related to the type of biorefineries; it is possible to integrate first, second and third generation biorefineries for obtaining a wide variety of products and to take advantages of the products generated in the different plants of the biorefinery. This fact permits to introduce the green engineering principles for design biorefineries (Moncada et al., 2014b). In this way, it is possible to integrate hierarchy, sequencing and integration concepts in a logical manner for designing biorefineries that could be named “Green Biorefineries” as it is shown in Figure 2.3.

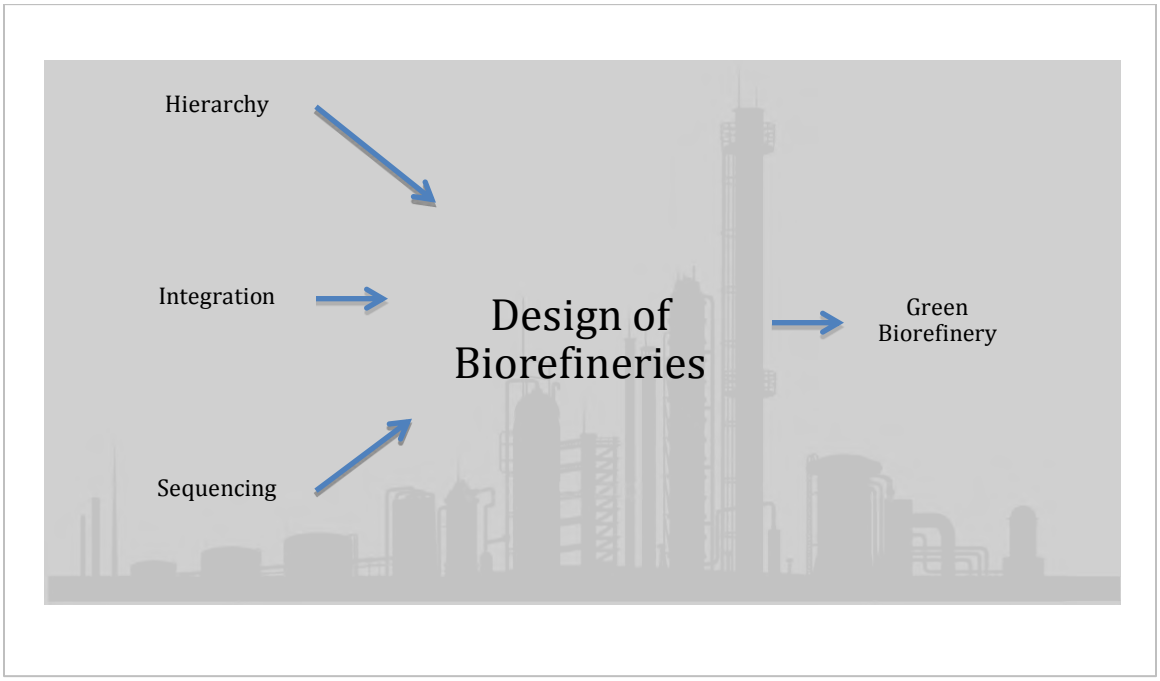


Figure 2.3. Hierarchy, sequencing, integration concepts for design biorefineries

2.3.2 Conceptual design of processes

The design of a process depends on several variables including: raw materials, available technologies, efficiencies, environmental impact, energy consumption and more. However, one of the most important challenges is related to the sequencing of the constituent unit operations (that compose pretreatment, reaction and separation sections) of a given process configuration. Here, the three afore-discussed concepts (Hierarchy, sequencing and integration) play an important role because they define the order of constituent units in the process.

Taken into account the hierarchy, sequencing and integration, the design of a biorefinery can be approached using the well known Onion diagram for design of processes (Smith, 2005). This diagram describes the order at which the design should be made, as it is shown in Figure 2.4. This strategy suggests that the design should begin with the reactor zone followed by separation and recycling, then, a heat integration strategy can be established and finally, an analysis of the utilities of the process is carried out (Smith, 2005). In each of the steps, it is necessary to calculate mass and energy balances and therefore, there is an interaction between all steps in the design of processes. Hence, the hierarchy concept is very important because it permits to have a feedback for all the calculated parameters and allows the attaining and enhancing the targets of the process, such as throughput, conversion ratio, energy consumption yield and more (Moncada et al., 2014b).

When several plants constitute a process such as in the case of biorefineries, and when the heat integration should be carried out for the complete process, the “Rubix cube” strategy (Gundersen, 2000) is very useful to follow a hierarchy strategy for the design of processes applying the Pinch methodology as it is shown in Figure 2.5 (Gundersen, 2000). According to this strategy, the heat integration is expanded in three dimensions. These dimensions are related to energy consumption, capital cost associated to the heat integration and Heat Exchanger Network (HEN).

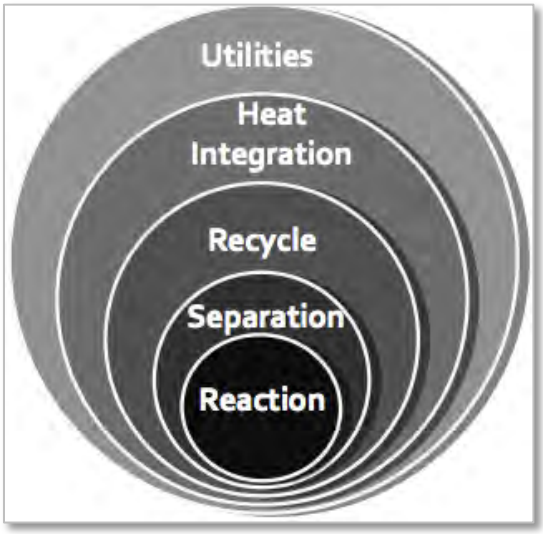


Figure 2.4. Onion model for conceptual design of processes. Adapted from (Smith, 2005)

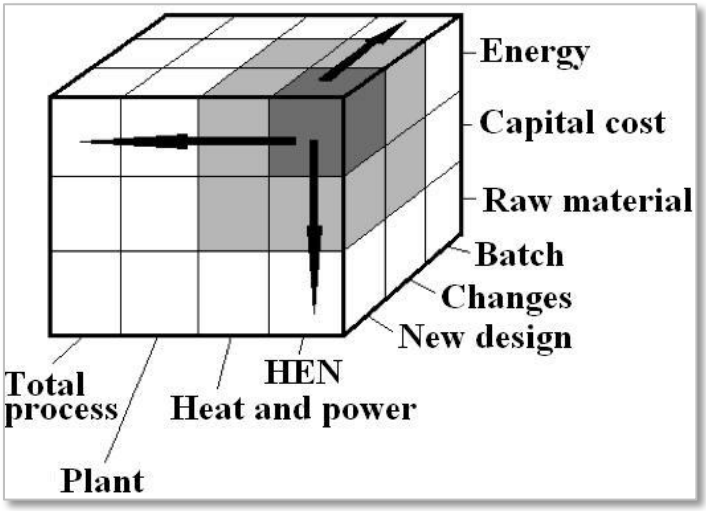


Figure 2.5. Hierarchy for design of process applying the Pinch methodology. Adapted from (Gundersen, 2000)

It is clear that the design of biorefineries should be carried out taken into account more rules and strategies than in the classical conceptual design of processes. This reflects the complexity of the raw materials, the wide range of products that can be obtained and

the fact that biorefineries should be supported by pillars based on environmental, economic and even social developments to ensure a sustainable biorefinery.

Some environmental considerations should be taken in the design of biorefineries (Moncada et al., 2014b). Some of the environmental impacts associated with biorefineries are those obtained as a result of the energy consumption of the process, which is according to the fuel used (carbon, gas or another fuel). Also, the effluent streams are associated with an environmental impact of the biorefinery, due to the byproducts and wastes that are generated (Rincón et al., 2014). Therefore, the conceptual design of biorefineries should consider the inclusion of a strategy aimed at minimization of residues, obtaining clean production and at establishing a process that is environmentally friendly. This strategy could be consider aspects such as:

- Definition of the boundary system
- Define environmental objective functions
- Define the environmental constraints of the process
- Assessment of the pathways of the process (Reactions, raw materials, fuels, etc.)
- Generate alternative process flow diagrams for minimization of residues
- Selection of optimization methods
- Sensibility analysis and feedback to the process

Process flow diagram for Biorefineries

The process flow diagram (PFD) is defined according to different considerations of operability, targets and constrictions over the process. The PFD can be as complex as the designer considers therefore, for the case of biorefineries, it depends on raw material, products, available technologies and more. Nevertheless, a general and simple structure of the PFD of a process even for biorefineries can be considered as it is shown in Figure 2.6.

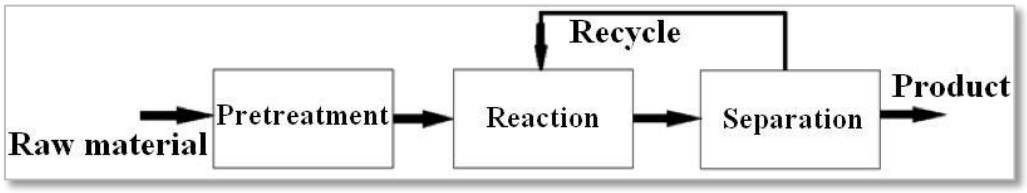


Figure 2.6. General PFD of a process. Adapted from (Smith, 2005)

Reaction system selection

Taken in to account the hierarchy strategy and the onion diagram, the design of processes should begin with the reaction zone selection, which is based on different parameters that determine the performance of the complete biorefinery. The reaction zone is considered as the “heart of the process” because, according to the development of the reaction zone, the separation schemes are selected. Some of the most important parameters in the selection of the reaction zone are presented in Figure 2.7 (Smith, 2005).

The type of process (batch or continues) should be selected according to several factors such as: the production volume, conversion, yield and additional parameters that have to be attained. Reagents are also an important factor because some of them are corrosive and need special materials. The purity of reagents also defines the conversion efficiency, yield, and side reactions that might be undesirable. Kinetic is a determinant factor in the design of the reaction zone because it permits to evaluate the effect of pressure, temperature, flow rate and other parameters that influence the reaction system (Douglas, 1988; Smith, 2005).

Safety considerations also affect the selection of the reaction system because the nature of the reagents and products as well as the conditions needed such as high pressures or temperatures. Finally, the cost and environmental performance define the selection of the reaction system because although all factors (conversion, yield, flows and more) can be reached, both, the capital cost and environmental impact associated with the reaction system define the final selection of the technology for the reaction zone.

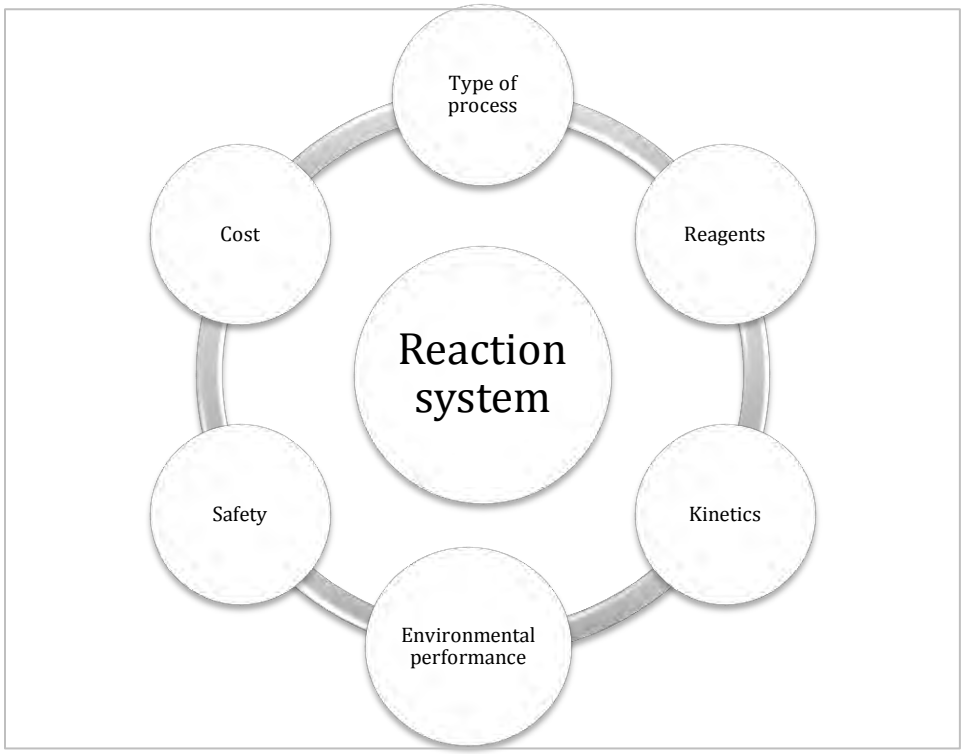


Figure 2.7. Factors that affect the reaction system selection. Adapted from (Douglas, 1988; Smith, 2005)

Separation system selection

The separation system depends strongly on the reaction performance because the separation system will be selected according to the conversion efficiency of the reaction, yields and operational conditions (Temperature, pressure, flow, etc.). The first aspect that has to be considered is related to the phase of the streams. If the streams contain solids, liquid and gas it is necessary to consider different alternatives for its separation. In the case of solids, it is possible to use filters, membranes and other units, taken into account the particle size distribution, moisture content etc. Operations such as sedimentation, filtration, centrifugation and drying can be used according to the characteristics of the streams (Dimian and Bildea, 2008).

However, the separation systems for gases and liquids might be more complex than for solids. For gases, operations such as condensation, cryogenic distillation, absorption, molecular sieving, permeation etc. can be used, according to the thermodynamical aspects of the system. Here, the azeotropes and other constrains should be considered as well as the operational conditions such as temperature, pressure and flows (Dimian and Bildea, 2008). For liquid, wider options are available because it is the most common phase after reactions. In these cases, the thermodynamic is a determinant factor affecting the selection of separation system (Dimian and Bildea, 2008; Douglas, 1988). Parameters such as boiling point, azeotropes, liquid phases that exist in the mixture are very important for the selection of the correct separation approach. In addition to the thermodynamic considerations other factors are also important such as maintenance, capacity, safety and flexibility of the separation system (Douglas, 1988; Smith, 2005). Separation should be design thinking on the capacity requirements as well as ease maintenance and the flexibility to separate other streams with different chemical composition (Douglas, 1988; Smith, 2005). Figure 2.8 shows the principal factors that need to take into account in the selection of the separation system.

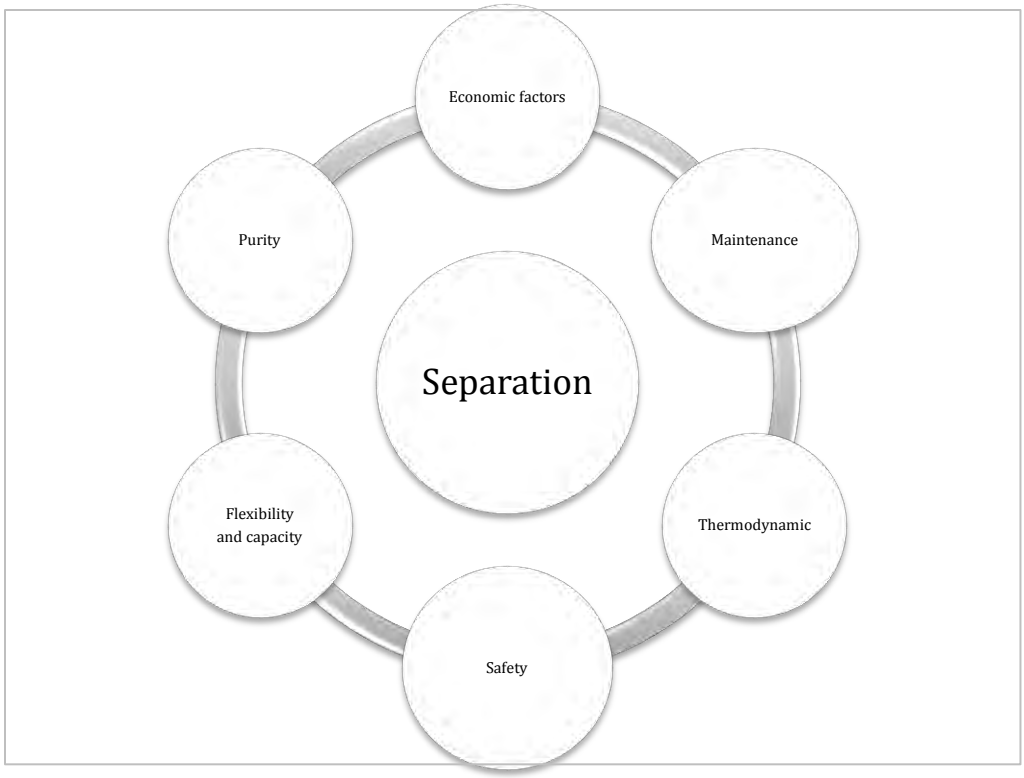


Figure 2.8. Factors affecting the separation system. Adapted from (Douglas, 1988; Smith, 2005)

After the selection of the principal sections (pretreatment, reaction and separation) it is necessary to consider the use of recycling for cases where the separation generates solvents or reagents that are expensive and can be re-used in the process. The selection of these sections in a biorefinery has to be based on the concepts of hierarchy, sequencing and integration as well as on the basic conceptual design of processes based on onion diagram. Also, it is necessary to complement with environmental factors for obtaining a best configuration or design of a biorefinery. More detailed information about the design of processes can be found in the book entitle "**Conceptual Process Design**" in Spanish which is a result of this research and that is under revision in the editorial of the Universidad Nacional de Colombia.

2.3.3 Techno-economic analysis of biorefineries

Techno-economic analysis of a biorefinery could be carried out with several computational tools however, for this research, Aspen Plus V8.0 was used because it permits to calculate mass and energy balances of the biorefinery involving non-conventional compounds (Moncada et al., 2013). This computational tool is licensed software for Universidad Nacional de Colombia, at Manizales provided by Aspen Tech. After propose a PFD based on the hierarchy, sequencing and integration concepts, it is necessary to develop the mass and energy balances of the biorefinery and define the following:

- **Topology of the process:** It is necessary to define the operation units of the biorefinery as well as streams and all necessary recycables.
- **Entrance of compounds:** From the data base of Aspen Plus, should be added all components involved in the simulation of the biorefinery. In the case of non-conventional compounds such as cellulose, hemicellulose, lignin and so on, the compounds are added from the National Institute of Standard and Technology (NIST).
- **Thermodynamic models:** According to the characteristics of the mixtures in the streams, the thermodynamic model for liquid and vapor phases are selected from the wide options of Aspen Plus.
- **Specifications on the PFD:** All operational conditions for each of the units as well as for each of the streams have to be specified. Temperature, pressure, flow rates, efficiencies etc are required for the simulation.
- **Simulation:** After define all parameters necessary; the mass and energy balances can be obtained from the simulation.

As was mentioned above, for biorefineries it is necessary to select and calculate the physicochemical properties for non-conventional compounds because biorefineries utilize substances of biological origin such as cells, lignocellulosic biomass and more (Mussatto et al., 2013).

According to these steps, it is possible to calculate the requirements for a raw material, consumables, utilities and energy needed for the flowsheet of the biorefinery by means of the mass and energy balances of the process. The economic assessment for biorefineries is carrying out using the computational tool Aspen Process Economic Analyzer that is a module of Aspen Plus (The use of this computational tool as well as it use is showed in **Appendix B1**). With this computational tool is possible to get the basic dimension of the units involved in the biorefinery and then according to the investment parameters give by the user (Tax rate, internal rate of return, life of the project among others) there is possible to calculate the capital cost, operating costs, utilities and more (Moncada et al., 2013; Moncada et al., 2014b; Mussatto et al., 2013). According to this, the economic evaluation follows the sequence showed here:

- Knowledge of mass and energy balances of the biorefinery
- Dimension of the units
- Estimation of capital cost
- Estimation of operating costs
- Evaluation of the process

It is possible to assess different scenarios according to the flowsheet of the biorefineries, evaluating volumes of feedstock, mass and energy integrations, cost of raw materials, products, etc (Dávila et al., 2015; Moncada et al., 2013; Moncada et al., 2014b; Mussatto et al., 2013). More detailed information about the economic assessment using Aspen Process Economic Analyzer is given in **Appendix B1**.

For the case of evaluation of several scenarios, it is necessary to take a base case that corresponds to a biorefinery with single characteristics and then to evaluate other scenarios, based on the same methodology used for the base case. The scenarios are cases of interest for the user and it is possible to evaluate scenarios such as the use of a cogeneration system for supply the energy requirements of the biorefinery and the integration of this system in the production cost (Rincón et al., 2014). Therefore, it is necessary to evaluate all possible alternatives to enhance the biorefinery. Some scenarios of interest in the design and evaluation of biorefineries are:

- Evaluation of different raw materials
- Evaluation of different schemes of production
- Evaluation of several operational conditions
- Evaluation of the final characteristics of product (Purity, volume, etc.)
- Evaluation of heat integration
- Evaluation of mass integration
- Evaluation of a cogeneration system
- Evaluation of environmental impacts
- Other of interest of the user

Heat integration of biorefineries

The heat integration of biorefineries is one of the most important aspects because it allows evaluating the level of energy integration in the biorefinery and it defines the targets for heating and cooling as well as the associated costs. Because biorefineries can consist of many plants, especially for multiproduct biorefineries, the heat integration allows recovering the maximum heat generated in the biorefinery using it in other parts (plants) of the biorefinery (Fatih Demirbas, 2009).

The heat integration belongs to the integration strategy of the design of processes (Moncada et al., 2014b), this kind of integration calls for a special attention because it is necessary the use of a base case without heat integration such as it is shown in Figure 2.9. For this base case, all energy requirements (heating and cooling) have to be purchased, in this way, when heat integration is carried out then all possible heat from the process is recovered by means of a heat transfer between cold and hot streams. This fact permits to decrease the net consumption of utilities and at the same time decreases the requirements for heating and cooling. Several strategies can be used to develop heat integration however, the Pinch methodology is the most common used (Ng, 2010; Oliveira et al., 2015; Shenoy and Shenoy, 2014).

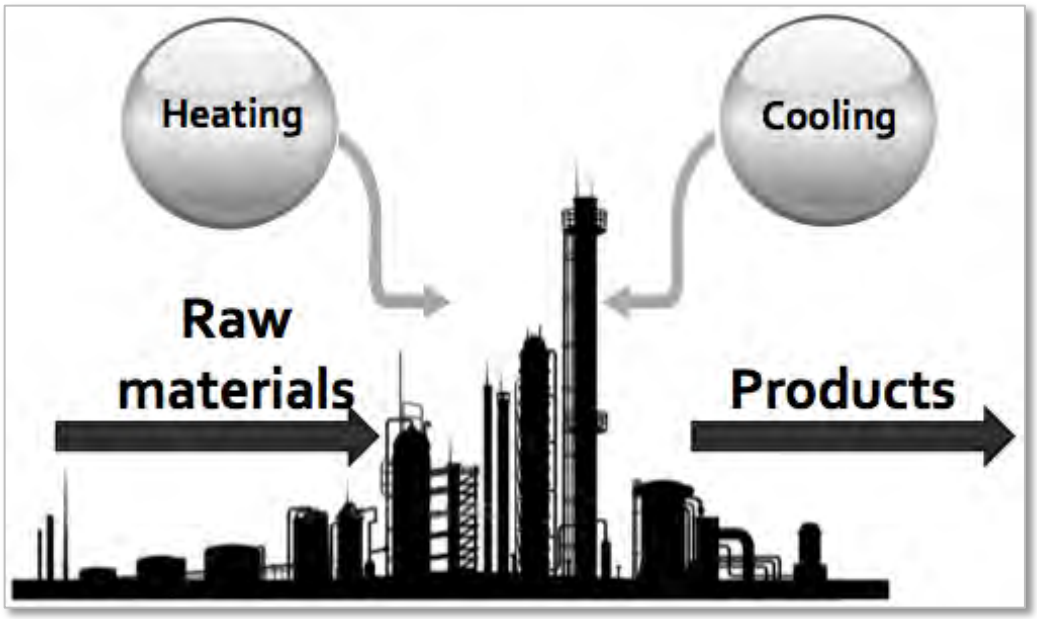


Figure 2.9. Heat integration for a base case of a biorefinery. Adapted from (Moncada et al., 2014b)

Pinch methodology (Gundersen, 2013; Kemp, 2007; Martín and Mato, 2008) is based on the composite curves of the process (or biorefinery) and allows designing the Heat Exchanger Network (HEN) of the process and guarantees the minimum use of utilities for heating and cooling because the targets calculated correspond to the minimum energy requirements. This methodology is based on the thermodynamic of the streams involved in the process and using some graphics based on temperatures, enthalpies, heat capacities and number of streams it is possible to calculate the targets related to utilities consumption (Gundersen, 2013; Kemp, 2007; Martín and Mato, 2008). Once the targets (cooling and heating requirements) are known then it is possible to evaluate the possibility of the use of a cogeneration system for supply the energy requirements in the biorefinery. The steps for Pinch methodology are represented in Figure 2.10.

For biorefineries, the heat integration could be more complex because of the number of plants involved. The heat available from some plants can be used for heating purposes in other plants therefore; sometimes the heat integration can meet all energy requirements of the biorefinery and generate a surplus to be sold to the electrical grid, as it is shown in Figure 2.11.

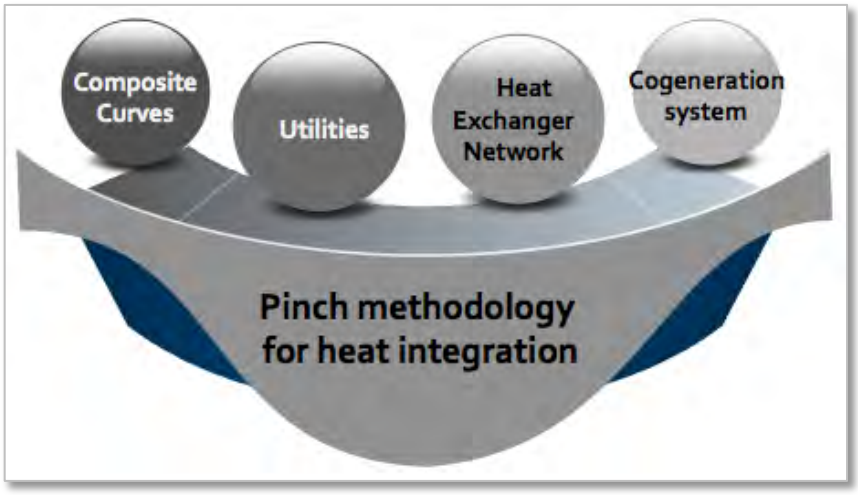


Figure 2.10. Steps for Pinch methodology for heat integration. Adapted from (Gundersen, 2013)

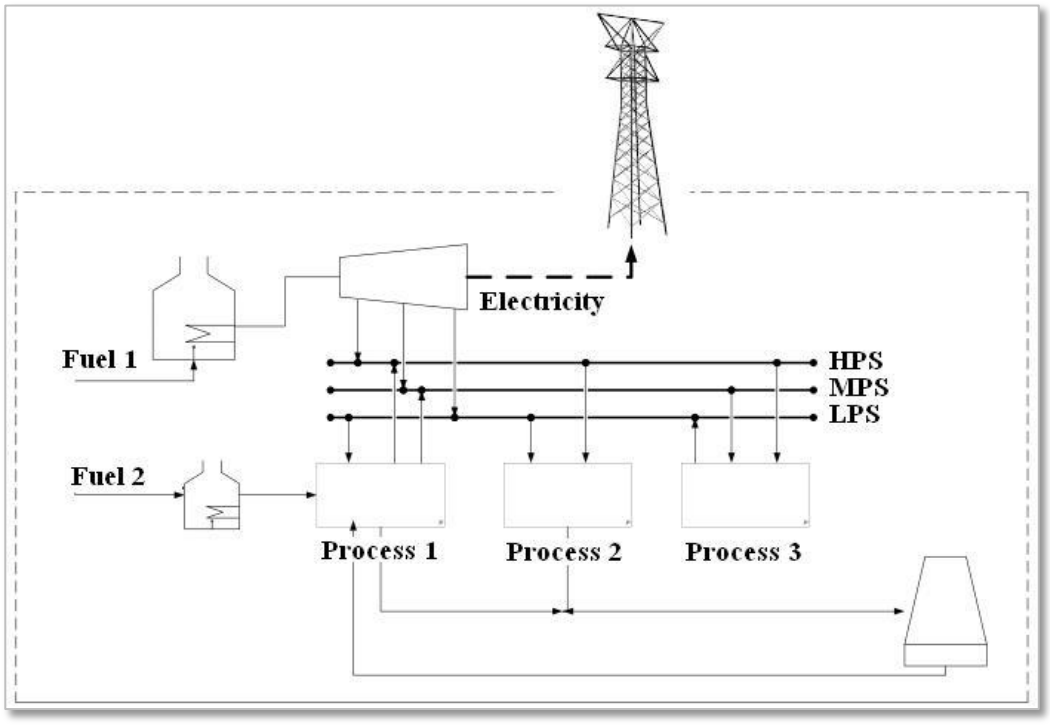


Figure 2.11. Heat integration of a biorefinery including a cogeneration system

The Pinch methodology for heat integration consists of several steps. The first one is related to the composite curves based on the thermodynamic of the streams (Temperatures, enthalpies, etc.). From this information the possible extent of integration as well as the targets for heating and cooling are calculated (Martín and Mato, 2008). Then, a grand composite curve is created in order to know the possibilities of the use of different utilities. Then, a Heat Exchanger Network (HEN) is constructed based on the possibilities of the heat transfer between cold and hot streams (Kemp, 2007; Martín and Mato, 2008). Finally, the cost of the complete heat exchanger network is calculated using the economic parameters for heat exchanger based on index costs. This procedure is showed in more detail in **Appendix B2**. As a final result, the heat integration strategy can reduce the energy consumption according to different levels of integration such as it is shown in Figure 2.12.

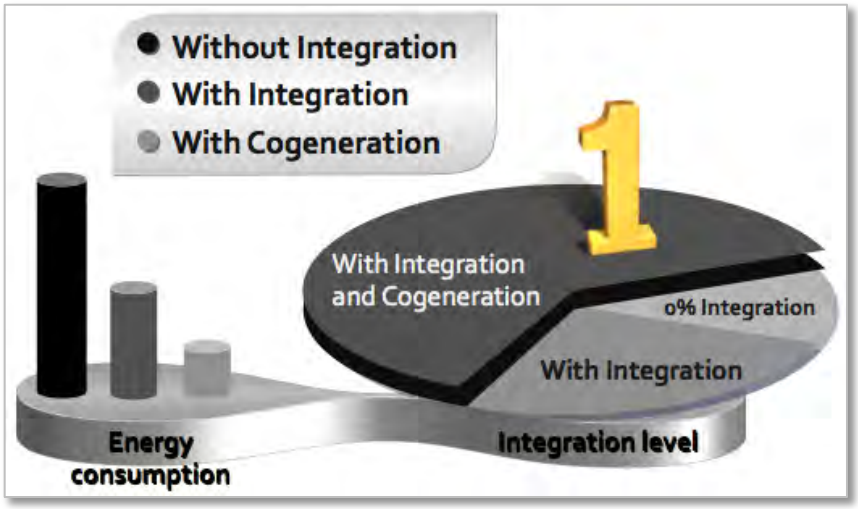


Figure 2.12. Heat integration at different levels. Adapted from (Moncada et al., 2014b)

2.3.4 Environmental analysis of biorefineries

Environmental analysis of biorefineries is a very important component of their design because it can define if the biorefinery is environmentally friendly or not and becomes in

a vital decision for evaluate the performance of the biorefinery (Moncada et al., 2014b; Renó et al., 2014; Schebek and Mrani, 2014). By means of this analysis in the conceptual design of biorefineries it is possible to propose strategies for minimize the residues generated in the process as well as to decrease the pollution and the level of contamination from biorefineries (Moncada et al., 2014b).

All streams leaving the biorefinery are potential pollutants and according to the composition, temperature, pH and other factors, these streams can contribute strongly to the contamination produced by the biorefinery (Moncada et al., 2014a). The environmental impact is measured not only on the environment but also on the human health, especially for operators and all personnel near the process. Because a biorefinery needs a plant to supply all energy requirements, it is necessary to take into account the emissions from this plant that uses any fuel such as carbon, gas and others (Young and Cabezas, 1999). Therefore, an environmental evaluation should consider a general balance around the entire process, including a plant for energy requirements. This is carried out by taken into account all streams of the biorefinery as shown in Figure 2.13. In this way, all emissions, including the energy process (plant for energy requirements) are considered.

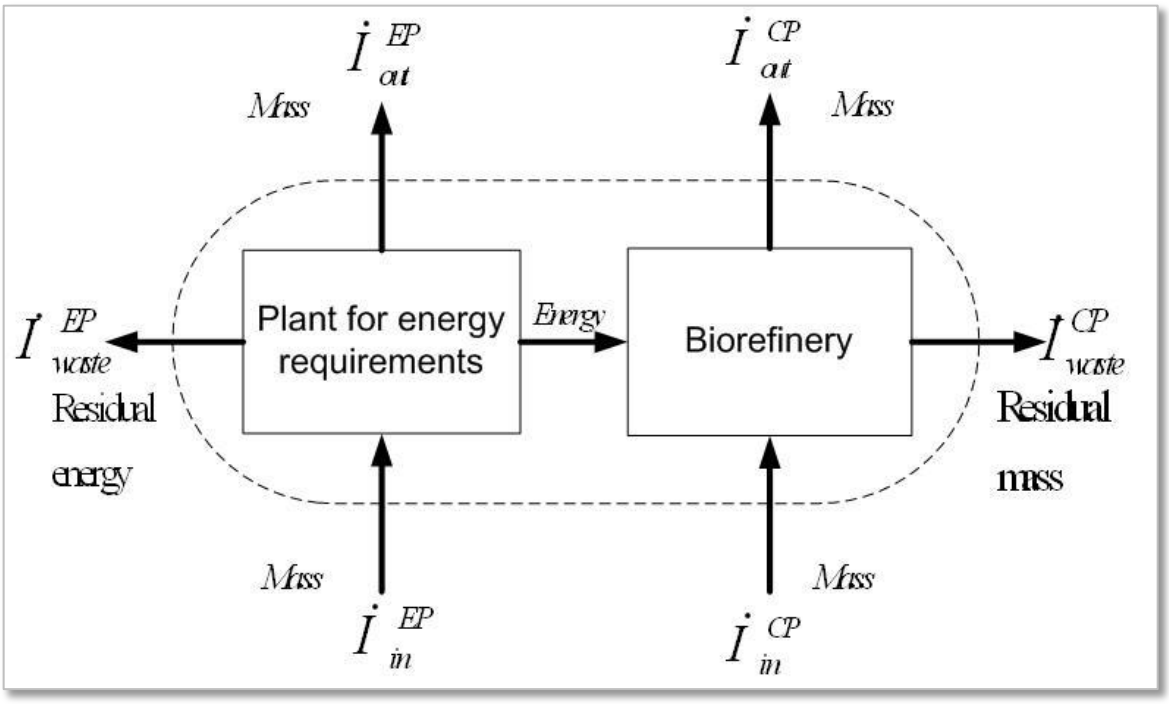


Figure 2.13. Streams involved in an environmental assessment of biorefineries. Adapted from (Young and Cabezas, 1999)

For this evaluation all streams associated with the biorefinery (I^{CP}_{waste}) and plant for energy requirements (I^{EP}_{waste}) are taken. These streams are related to the wastes from the Chemical Process (superindex CP) or biorefinery and to the Energy Plant (superindex EP) or plant for energy requirements. Both, incoming and leaving streams are taken for environmental analysis.

The polluting characteristics of the streams depend on chemical composition and concentration of the components that are a result of the type of process, technology used, mode of operation, units involved and all other considerations in the design of the biorefinery. Therefore, according to the polluting characteristics of the leaving streams of the biorefinery, the environmental impact of the process could affect significantly the environment (Cabezas et al., 1999; Young and Cabezas, 1999).

The environmental evaluation of a process can be based on the Potential Environmental Impact (PEI) (Barrett Jr et al., 2011; Cabezas et al., 1999). This parameter permits quantifying the PEI of a process based on the pollutant characteristics of the streams of

mass and energy that leave the process. Although PEI is a well established approach for assessing the environmental effect of a process; this conceptual parameter cannot be measured exactly. However, it is possible to establish an estimated theoretical value based on mass and energy balances for obtaining a PEI and integrate it into the conceptual process design (Barrett Jr et al., 2011; Cabezas et al., 1999; Young and Cabezas, 1999).

The inclusion of the PEI in the conceptual design of processes allows accomplishing environmental objectives that are reflected in reduction of residues and identification of the treatments for the associated pollutants. Therefore, the PEI evaluation allows to better design processes with a minimal level of the pollutants leaving biorefinery. The environmental evaluation should be made after all other steps in the process design such as heat integration, mass integration, and evaluation of scenarios as well as consideration of a cogeneration system (Moncada et al., 2014b).

For PEI assessment, different techniques and approaches have been used to obtain quantitative and qualitative environmental evaluations of the process. PEI establishes several impact categories that are affected by the emissions of a chemical process to the environment. Commonly four impact categories are used as well as eight subcategories of environmental impact (Barrett Jr et al., 2011; Castillo and Mora, 2000). These categories as well as the parameters used for measuring them are showed in Table 2.2.

Table 2.2. Environmental categories for measuring PEI (Barrett Jr et al., 2011; Castillo and Mora, 2000)

General Impact Category	Impact Category	Measure of Impact
Human toxicity	Ingestion	LD_{50}
	Inhalation/dermal	OSHA PEL
Ecological toxicity	Aquatic toxicity	LC_{50}
	Terrestrial toxicity	LD_{50}

Global atmospheric impacts	Global warming potential	GWP
	Ozone depletion potential	ODP
Regional atmospheric impacts	Acidification potential	AP
	Photochemical oxidation potential	PCOP

For evaluating the last environmental impact categories the WAste Reduction Algorithm (WAR) (Barrett Jr et al., 2011; Cabezas et al., 1999; Young and Cabezas, 1999) is commonly used because it is a free software of the Environmental Protection Agency (EPA) of the United States of America (Barrett Jr et al., 2011). This software is used in this research for the evaluation of the PEI of the biorefineries. This method uses the direct sum of environmental data based on the information of mass and energy streams of the process and the compounds involved in these streams. WAR evaluates eight impact categories as follow:

- HTPI: Human Toxicity Potential Impact
- HTPE: Human Toxicity Potential by Either
- TTP: Terrestrial Toxicity Potential
- ATP: Aquatic Toxicity Potential
- GWP: Global Warming Potential
- ODP: Ozone Depletion Potential
- PCOP: Photo Chemical Oxidation Potential
- AP: Acidification Potential

The measuring of each one of the categories is based on parameters of environmental impact such as LD_{50} (Lethal Doses for death 50% of the population), LC_{50} (Lethal Concentration to death 50% of the population) and OSHA PEL (Permissible Exposition Level of a substance for a human). Both, LC_{50} and LD_{50} are measured on animals like rats. WAR develops the environmental evaluation for continuous processes because there is necessary to take the stationary state of the process. According to this, the PEI

is measured for the non-products that are considered, as pollutants and then the PEI for these streams will be different to zero while for the products PEI takes a zero value (Barrett Jr et al., 2011; Cabezas et al., 1999; Castillo and Mora, 2000; Young and Cabezas, 1999). More detailed information of the PEI measurement using WAR as well as equations and calculations are showed in **Appendix B3**.

Another approach for evaluating the environmental impact of a biorefinery is the greenhouse gases (GHG's) emissions that are related with the balance of CO_2-eq per kg of compound that enters and leaves form the process. This balance take into account all inputs and outputs streams related with mass and energy of the biorefinery. It is a need to calculate the activities and factor emissions for all compounds involved in each stream of the biorefinery. After that, the CO_2-eq per kg of product can be calculated for both, inlet and outlet stream of the biorefinery and thus, calculated the generated GHG emissions. **Appendix B3** presents the GHG emissions methodology for biorefineries.

2.4 Proposed biorefineries for Fruits

The proposed biorefineries are based on the chemical composition of each one of the fruits or waste selected. For instance, spent blackberry pulp (SBP) has an attractive chemical composition because its phenolic compounds, specially anthocyanins, have important applications in cancer prevention and because its processing generate a significant amount of residues containing anthocyanins (Cerón et al., 2012). Thus SBP is a possible source of such compounds that contribute to the human health as it was discussed in Chapter 1.

Avocado has a potential use for oil extraction. In addition to the amount produced in Colombia (more than 215,000 tonnes/year), the avocado seed contains important compounds that can be recovered using the biorefinery concept. Avocado oil has important properties, similar to olive oil and thus this oil can be used for food purposes (Adama and Edoga, 2011).

Two cases of study were evaluated. The first one is for spent coffee grounds (SCG) that correspond to a residue after coffee beverage preparation. This residue is rich in oil that can be extracted and the remaining solids used for other purposes also under biorefinery

concept. The second case is for naranjilla waste obtained after juice extraction. This fruit is rich in valuable compounds as was discussed in chapter 1. From the biorefinery point of view, these two cases of study give possible products using a biorefinery to obtain valuable compounds.

2.4.1 Biorefinery based on spent blackberry pulp

The biorefinery is based on SBP because this residue becomes in an interesting raw material due to its content of anthocyanins. The SBP contains a lignocellulosic biomass that can be used for other purposes (Machado et al., 2015). The proposed biorefinery is based on the sequencing concept that permits give a priority in the extraction of valuable compounds to avoid its degradation in other processes due to the high temperature reached. Therefore, an extraction plant is used for obtaining anthocyanins and phenolic compounds extracts. Because the high selectivity, low viscosity and high diffusivity of the supercritical fluid extraction (Veggi et al., 2014), this technique is used for this purpose. Then, a microencapsulation plant is proposed to obtain a value added for the extracts. After, the remaining solid mass is used in a sugar plant to obtain xylose and glucose by means of acid and enzymatic hydrolysis. These sugars are the basis of a biomolecules plant that produces ethanol and xylitol. Finally, the remaining solids (riches in lignin) from sugar plant are used in a cogeneration system that can provide part of the energy requirements of the biorefinery. Figure 2.14 shows the biorefinery based on SBP.

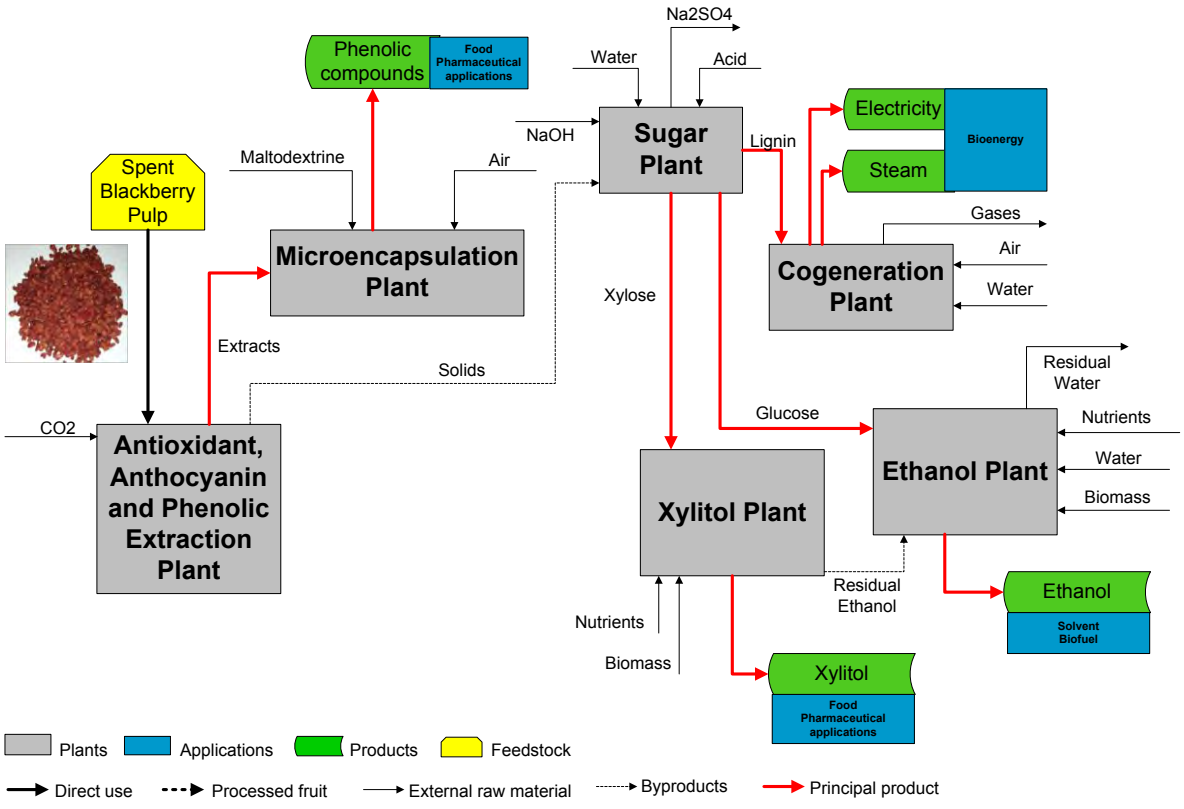


Figure 2.14. Biorefinery based on SBP

2.4.2 Biorefinery based on avocado

Because the wide range of compounds that avocado offers, this biorefinery uses not only the pulp but also the peel and seed. The first plant is to obtain oil from the pulp using a thermo mechanical extraction technique. Both, seed and peel are used in a supercritical extraction plant to obtain extracts with phenolic compounds. The exhausted solids from this plant are used to produce xylose and glucose in a sugar plant. The xylose and glucose produced in this plant are used for xylitol and ethanol production respectively. Finally, the remaining solids (reach in lignin) from sugar plant are used in a cogeneration system to cover part of the energy requirements of the biorefinery. In Figure 2.15 is presented the biorefinery based on avocado.

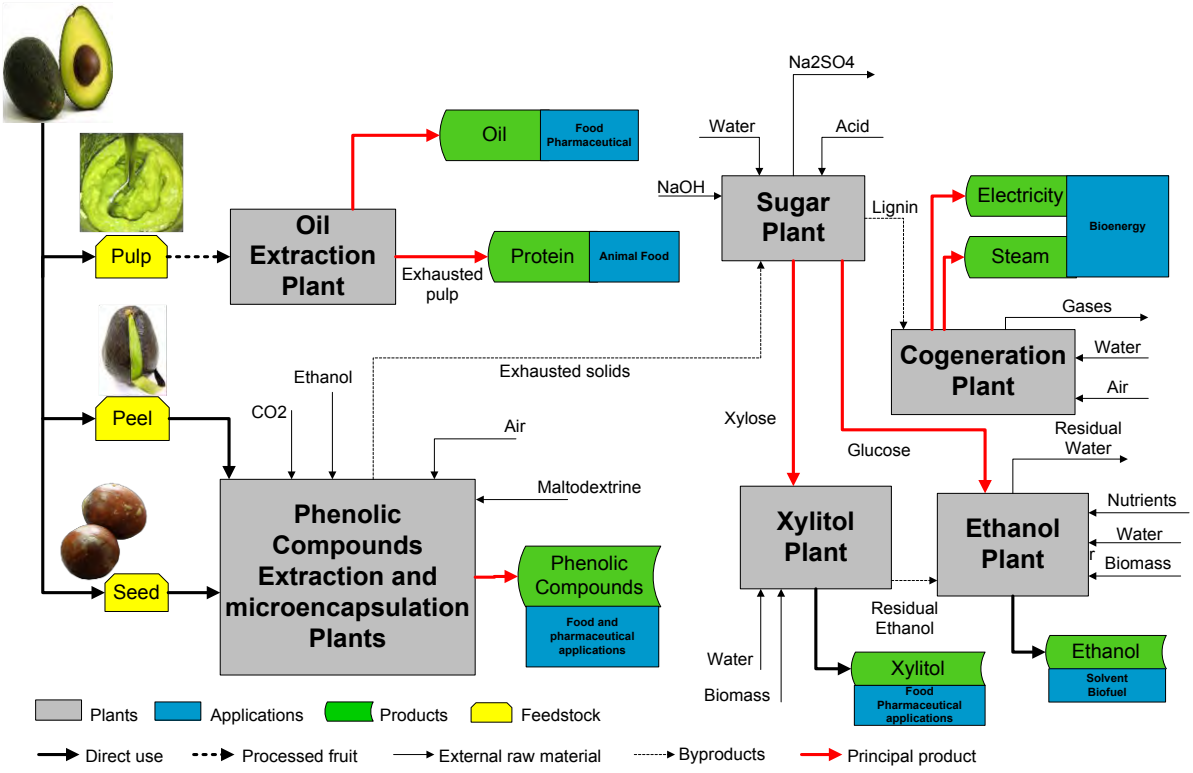


Figure 2.15. Biorefinery based on avocado

2.4.3 Case studies

Two case studies are presented. The first one for spent coffee grounds (SCG) and the second one for residues obtained after juice extraction from naranjilla. These two cases of studies were not the subject of this thesis nevertheless, biorefineries are proposed using chemical characterizations found by other colleagues in the revised literature.

Biorefinery based on spent coffee grounds

Spent coffee grounds (SCG) has a high content of oil (around 25% as was discussed in chapter 1) besides, SCG also has valuable compounds such as chlorogenic acids that have food and pharmaceutical applications. This residue has a high content of

hemicellulose that could be used for sugar production. Therefore, taken into account the sequencing concept, the biorefinery begins with a plant for phenolic compounds extraction. After that, the solids are used in a plant for oil extraction by means of a solvent technique. The exhausted grounds are then used in a sugar plant involving acid and enzymatic hydrolysis for producing xylose and glucose. The sugars produced are used for ethanol and xylitol production. Finally, the remaining solid (based on lignin) is used for cogeneration to provide part of the energy requirements. Figure 2.16 presents the biorefinery based on SCG.

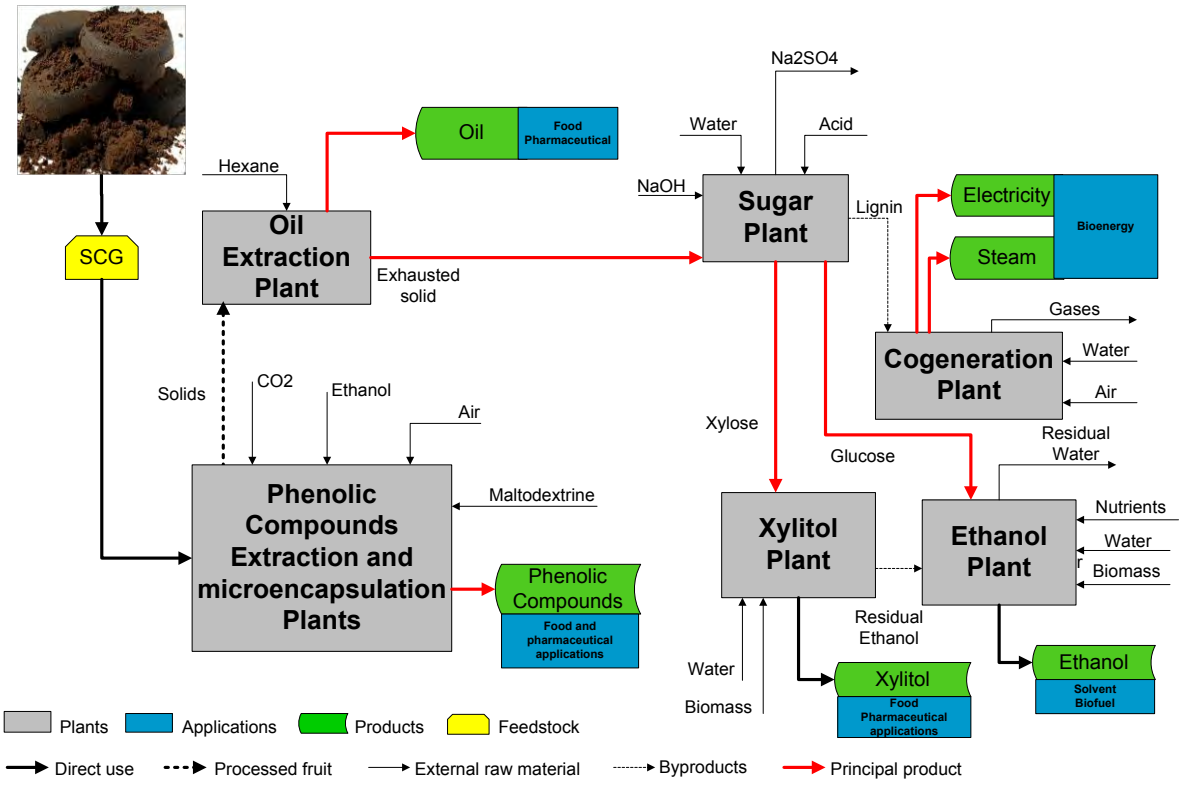


Figure 2.16. Biorefinery based on SCG

Biorefinery based on naranjilla waste

Because naranjilla waste has valuable compounds such as phenolic compounds, carotenoids among others, the first plant is for extraction of phenolic compounds using

supercritical extraction. After that, the extracts are microencapsulated to added value to this product. Then, the exhausted waste is used in a sugar plant to produce xylitol and glucose. Then, these sugars are used for production ethanol and xylitol. Finally, the remaining solids from sugar plant (lignin) and biomass from biomolecules plant are used in a cogeneration system for supply part of the energy requirements of the biorefinery. Figure 2.17 shows the biorefinery based on naranjilla waste.

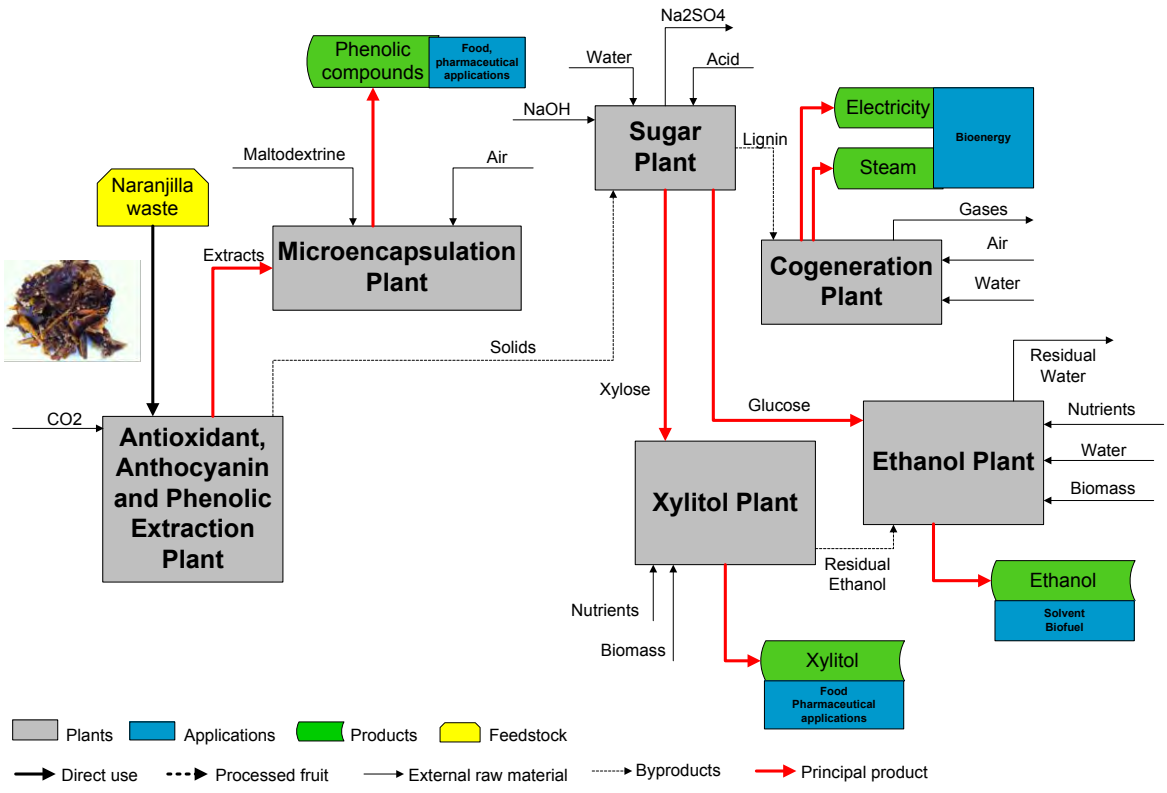


Figure 2.17. Biorefinery based on naranjilla waste

Conclusions

The design of biorefineries is more complex than common chemical processes because the number of the plants involved. The types of biorefineries involved (first, second and third generation) can be integrated; this fact also makes that the design of biorefineries become more complex. In the light of the aforementioned, the design of biorefineries

should be carried out taken into account additional rules and strategies than the classical conceptual design of processes because the complexity of the raw materials and the wide range of products. Additionally, aspects such as environmental, economic and even social should support the design to ensure a sustainable biorefinery.

For biorefinery design it is necessary to consider the raw materials, type of compounds to be extracted or produced, the available technologies for transforming the biomass and other relevant factors that affect directly the performance of the biorefinery. Additionally, to ensure the best design of a biorefinery it is necessary to consider additional concepts, which are hierarchy, sequencing and integration. The last concepts together with rules for conceptual process design permit to design and evaluate a biorefinery in a logical way. According to this, a base case of biorefinery should be proposed and then evaluate scenarios based on levels of integration begin with energy integration, mass integration, inclusion of a cogeneration system and finally, integration of several types of biorefineries (first, second and third generation). These levels of integrations should be analyzed as scenarios to compare techno-economic and environmental points of view.

Biorefineries based on fruits and its residues, should be designed taking into account the concepts described above but also based on the chemical composition of the selected raw material because the valuable compounds contained. The design of a biorefinery based on fruits and its residues can be focused in the extraction of valuable compounds such as vitamins, antioxidants, carotenoids, flavonoids, enzymes and phenolic compounds among others, Because the non-edible parts of the fruits (as peel and seed) have valuable compounds similar to pulp, it is necessary to consider the entire fruit including peel and seed as well as the residues generated after fruit.

Thus, spent blackberry pulp and avocado are attractive raw materials to obtain valuable compounds under biorefinery concept. For instance, spent blackberry pulp offers phenolic compounds and extracts with antioxidant capacity as well as a source of anthocyanins, for which there are applications in medicine for cancer prevention. In the case of avocado, this fruit becomes in a good source of oil for food applications while its seed and peel could be used for obtaining extracts with antioxidant capacity. Similar to these fruits, two additional feedstocks could be considered to obtain value added products under biorefinery concept. The first one corresponds to spent coffee grounds that offer a high content of oil that could be used for biodiesel production or for other

purposes. Additionally, spent coffee grounds have a potential use for sugar production because its content of cellulose and hemicellulose. Finally, naranjilla waste could be considered for design a biorefinery because its valuable compounds such as phenolic compounds, carotenoid and similar compounds that confer an antioxidant activity to its extracts.

3. Chapter 3. Methods and Materials

Overview

This chapter presents the analytical procedures that were used in order to analyze and determine the composition of spent blackberry pulp (SBP) and avocado. The compositional variables that were investigated included: moisture, extractives, ash, holocellulose (cellulose and hemicellulose) and lignin. The investigated biomasses were analyzed for their content of valuable components. The Lignocelulosic content of SBP, avocado peel and avocado seed was determined. Total phenolic compounds (TPC), antioxidant activity (AA), anthocyanins content and volatiles constituents were determined for SBP. Oil content from avocado pulp was extracted and the fatty acids composition of this oil was analyzed obtaining the fatty acids profile. Volatiles constituents of both, SBP and avocado were quantified.

Experimental approaches for preparing value added products, using the biorefinery concept are discussed. The latter includes supercritical fluid extraction (SFE) to obtain extracts with antioxidant activity as well as solvent and mechanical extractions for obtaining oil from avocado pulp and Spent Coffee Grounds (SCG), a case study in this research. Acid and enzymatic hydrolysis for sugar production from lignocellulosic biomass are discussed as well as the application of Ultrafiltration (UF) for obtaining concentrated extracts.

This chapter also describes simulation procedures as well as methodology for techno-economic and environmental assessment of suggested biorefinery platforms. Thermodynamic models for calculating physical properties for all compounds involved in the simulation are explained as well as models for the principal unit operations involved in the biorefineries. Technical parameters, evaluation criteria and methodology used for evaluating biorefineries are explained.

3.1 Materials

3.1.1 Spent Blackberry Pulp (SBP) (*Rubus glaucus benth*)

The Spent Blackberry Pulp (SBP) was obtained from Frugy S.A. (Manizales Colombia), a local producer of fruit juices and concentrates. Figure 3.1 shows the SBP as was received from Frugy.

Figure 3.1. Spent Blackberry Pulp (SBP) obtained from Frugy S.A.

The SBP was delivered to our laboratory and was stored at -20ºC pending analysis and processing.

3.1.2 Avocado (Persea Americana mill)

In this research only Hass avocado variety was investigated. Fully ripened avocado (Figure 3.2) was purchased at a local market in Manizales Colombia.

Figure 3.2. Avocado (Hass variety) used for this research

In all cases, avocado was thoroughly washed with distilled water prior to chemical characterization and the washed fruit was then stored at -20ºC pending analysis and processing.

3.2 Characterization of raw materials

3.2.1 Sample preparation

The frozen SBP was, thawed at room temperature for 5 hours and then dried at 40ºC (to avoid degradation of phenolic compounds) to a constant weight, using an atmospheric

drying oven (Thermolab, Maharashtra, India). Then, the dry SBP (DSBP) was ground using a manual mill Moldel Rey1 (Toolcraft, Medellin, Colombia) and the powder was sieved using Standard USA sieves (mesh 20 to 80) (ASTM E 11-09) provided by Pinzuar LTDA (Bogota Colombia). Following size reduction and sieving, the dry powders were stored at -20ºC pending utilization.

For avocado, the fruit was manually cut and the pulp, peel and seed were separated. The peel and seed were each milled to obtain particles that pass mesh 20 (850 µm) sieve and be retained on mesh 80 (180 µm) sieve (Hames et al., 2008). Avocado fractions (peel and seed) that had been obtained and treated in this way were then stored at -20ºC pending analysis while the pulp was immediately used for oil extraction, as described later in this chapter.

The methods were adopted to determine the moisture (Hames et al., 2008), extractives (Han and Rowell, 1997), lignin (Han and Rowell, 1997), holocellulose (cellulose and hemicellulose) (Han and Rowell, 1997) and ash (Sluiter et al., 2008) content of the samples (SBP, avocado peel and seed). Each experiment (N) for moisture, extractives, cellulose, hemicellulose, lignin and ash were made with three replicates and one analysis (n) was made for each one of them, thus (Nxn) was developed.

3.2.2 Moisture content

Moisture content of the samples was determined, in triplicate, according to Technical Report NREL/TP 510-42620 (Hames et al., 2008). In all cases, accurately weighed samples of about 7g were placed in aluminum weighing dishes and were then dried at 105 ºC to a constant weight using a convection oven (Thermolab, Maharashtra, India). After being dried for 4 h, samples were removed from the oven and placed in a desiccator (Todoquimica, profinas, Bogota Colombia) for 30 minutes in order for them to reach room temperature. Then, samples were weighted, using an analytical balance (Precisa XR 205SM-DR, Moosmattstrasse Switzerland). Samples were then dried for an additional 1 hour (as described above) and the change in mass was determined as described above. This procedure was repeated until samples reached a constant weight. The moisture was calculated according to equation (3.1)

$$Moisture\ (\%) = 100 - \left(\frac{Weight_{dish+sample} - Weight_{dish}}{Weight_{initial\ sample}}\right)*100 \quad (3.1)$$

3.2.3 Extractives content

The term extractives refers to the non-structural constituent compounds of a lignocellulosic biomass. In general, extractives of lignocellulosic biomass mainly consist of fats, fatty acids, fatty alcohols, phenols, terpenes, steroids, resin acids, rosin, waxes, etc (Zayed et al., 2008). These compounds can account for more than 30% of the total mass. These compounds can be soluble in water (organic material, sugars and nitrogenous materials) and ethanol (Chlorophyll, natural pigments and more) (Han and Rowell, 1997). Therefore, the term extractives in this research refers to the total proportion of extractives that was extracted in water and ethanol (Han and Rowell, 1997).

In all cases, 8 g of the sample was placed in thimble and introduced in a soxhlet extraction unit (Schott Duran, Main, Germany) and extracted by 250 ml of water as solvent. Several boiling chips were added to the water to prevent bumping. The solvent was heated using a heating mantle (RC F20710174, Medellin Colombia) at 100 ºC. In all cases, the soxhlet unit was covered with an aluminum foil to protect the samples against light effects. Extractions were carried out for 24 hours keeping the liquid boiling. Following extraction, the extract was removed and the solid phase was dried to a constant weight, in a convection oven at 105 ºC (Thermolab, Maharashtra, India). Changes in mass during drying were determined using an analytical balance (Precisa XR 205SM-DR, Moosmattstrasse Switzerland). The proportion of extractives that could be extracted by ethanol from the sample was determined similar to what has been described for the aqueous extraction procedure, In all cases, ethanol (99.95%, Merck, Darmstadt, Germany) was utilized as a solvent and extraction temperature was 80 ºC. The total extractives was defined as the sum of aqueous and ethanol extractives. In all cases, the proportion of extractives that could be extracted (by each of the solvents) was

calculated as the difference between mass of the sample prior and following extraction and drying. Total extractives content was determined in triplicate and the proportion of extractives that could be extracted by a given solvent was calculated according to equation (3.2). Figure 3.3 shows the picture of extractives measurements and extracts obtained.

$$Extractives\ (\%) = 100 - \left(\frac{Weight_{sample\ after\ extraction}}{Weight_{initial\ sample}}\right) * 100 \quad (3.2)$$

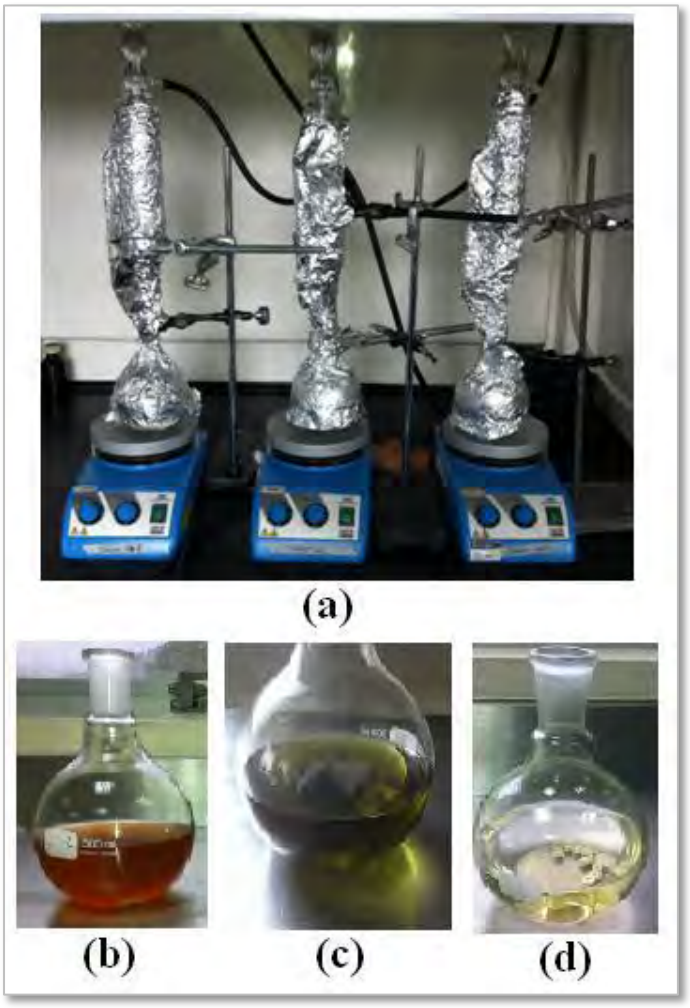


Figure 3.3. Extractives measurements. (a) Soxhlet unit. (b) Extractives obtained for SBP. (c) Extractives obtained for avocado peel. (d) Extractives obtained for avocado seed.

3.2.4 Ash content

The ash content was determined according to Technical Report NREL/TP 510-42622 (Sluiter et al., 2008). The ash is defined as the remaining residue obtained after incineration at 575 ºC for 3 hours. Thus, 0.5 g of moisture-free sample was placed in a clean empty crucible. The sample was then incinerated in a muffle oven (Thermo scientific BF51828C-1, Equipos y laboratorio de Colombia S.A.S, Medellin Colombia) at 575 ºC for 3 hours. Following incineration, the sample was placed in a desiccator (Todoquimica, profinas, Bogota Colombia) for 30 minutes and then its weighted was determined using an analytical balance (Precisa XR 205SM-DR, Moosmattstrasse Switzerland). All measurements were carried out in triplicates and ahs content was calculated according to equation (3.3).

$$Ash\ (\%) = \left(\frac{Weight_{incinerated\ sample}}{Weight_{initial\ sample}}\right) * 100 \quad (3.3)$$

3.2.5 Holocellulose content

Holocellulose was determined, in triplicates, according to ASTM Standard D-1104 (Han and Rowell, 1997). This is a chlorination method that requires dry and extractives-free samples. Thus, 2.5 g of sample was placed in a 250 ml erlenmeyer flask. Then, 80 ml of distilled water at 70 ºC and 0.5 ml of acetic acid (96%, Chemi) was added along with 1 g of sodium chloride (80%, Carlo Erba). The neck of each 250 ml Erlenmeyer was then covered with an inverted 25 ml erlenmeyer flasks. The erlenmeyers were placed in a water bath (Thermo Fisher Scientific model 2870, MA USA) at 70 ºC for 60 minutes. Then, 1 g of sodium chloride (80%, Carlo Erba) and 0.5 ml of acetic acid (96%, Chemi)

were added to the heat-treated mixture while shaking the receiving erlenmeyer. The latter was repeated every hour for a total of 7 hours. This reaction was carried out until the fibers were completely separated from lignin. It usually require 6 hours for delignification of fibers and the reaction can continue under shaking without further addition of sodium chloride and acetic acid until complete 24 hours. This delignification process degrades some of the polysaccharides and the application of sodium chlorite should be avoided (Rowell, 2012). Then, the samples were cooled to room temperature and then filtered at 350 mmbar using a buchner funnel (Glassco 1000 ml, Vardhmansoft, India) and a filter paper (10 µm, Boeco Germany). The filtration process was carried out until the color of the sample changed from yellow to white and the odor of chlorine dioxide disappeared. Finally, the sample was washed with acetone (99%, Merck), dried at 105 ºC and cooled in a desiccator (Todoquimica, profinas, Bogota Colombia). Then, the sample was weighted and the holocellulose content was determined according to equation (3.4). Figure 3.4 shows the picture of delignification process for holocellulose measurement.

$$Holocellulose\ (\%) = \left(\frac{Weight_{final\ sample}}{Weight_{initial\ sample}}\right) * 100 \qquad (3.4)$$

Figure 3.4. Delignification process for holocellulose measurement

3.2.6 Cellulose and hemicellulose content

The cellulose is defined as the insoluble residual part obtained after treating the biomass with NaOH (17.5% w/w) (Han and Rowell, 1997). An amount of 1 g of extractives-free holocellulose sample was weighted using an analytical balance (Precisa XR 205SM-DR, Moosmattstrasse Switzerland) and placed in a 250 ml glass beaker connected to a condenser (Schott Duran, Main, Germany) to allow refluxing. Then, 10 ml of NaOH solution at 17.5% (supply by Pareac) was added and the mixture was kept at 20ºC while being mixed with a glass rod until all solid sample became soaked with the NaOH solution. After 5 minutes, 5 ml of NaOH solution (17.5%) was added and thoroughly stirred with the glass rod. 5 ml of NaOH solution (17.5%) were added under mixing in intervals of 5 minutes until complete 45 minutes at 20ºC. Then, 33 ml of distilled water at 20ºC were added and mixed into the mixture that was then allowed to stand by 1 hour at 20 ºC. Then, the mixture was filtered at 350 mmbar using a buchner funnel equipment (Glassco 1000 ml, Vardhmansoft, India) and a filter paper (10 µm, Boeco Germany). The separated solid phase was washed, first with 100 ml of a NaOH solution at 8.3% and then with 100 ml of distilled water. Finally, the samples were dried at 40ºC in a convection oven (Thermolab, Maharashtra, India) then, placed in a desiccator

(Todoquimica, profinas, Bogota Colombia) for 30 minutes. Then, the samples were weighted and the cellulose content was calculated according to equation (3.5).

$$Cellulose\ (\%) = \left(\frac{Weight_{final\ sample}}{Weight_{initial\ sample}}\right) * 100 \quad (3.5)$$

The hemicelluloses content was determined from difference between holocellulose and cellulose content measured.

3.2.7 Lignin content

Lignin content of the samples was determined according to TAPPI T-222 (Han and Rowell, 1997). The lignin isolated using this procedure is also called sulfuric acid lignin (Han and Rowell, 1997). The sample should be free of extractives as well as moisture. Thus, 200 mg of sample were placed into a 100 ml glass centrifuge tube. Then, 1 ml of H_2SO_4 (72% w/w, Mallinckrodt, Damastown Ireland) was added to each 100 mg of sample. The mixture was stirred and dispersed thoroughly with a glass rod twice. Then, the tubes were incubated in a water bath (Thermo Fisher Scientific model 2870, MA USA) at 30 ºC for 60 minutes. After that, 56 ml of distilled water was added to obtain a 4% solution. The sample was treated in an autoclave (Sanyo MLS-3781L, Wolflabs, Pocklington, UK) at 121°C, 15 psi, for 60 min. Then, samples were removed from the autoclave and the lignin was filtered off, through glass fiber filters (filters were rinsed and dried) under reduced pressure (350 mmbar), keeping the solution at 70 ºC. Residues were washed with hot water (70ºC) and dried at 105°C overnight in an oven (Thermolab, Maharashtra, India). Then, the sample was placed in a desiccator (Todoquimica, profinas, Bogota Colombia), for 1 hour and weighed. Lignin content was calculated according to equation (3.6). Figure 3.5 shows the final step for lignin measurements.

$$Lignin\ (\%) = \left(\frac{Weight_{final\ sample}}{Weight_{initial\ sample}}\right) * 100 \quad (3.6)$$

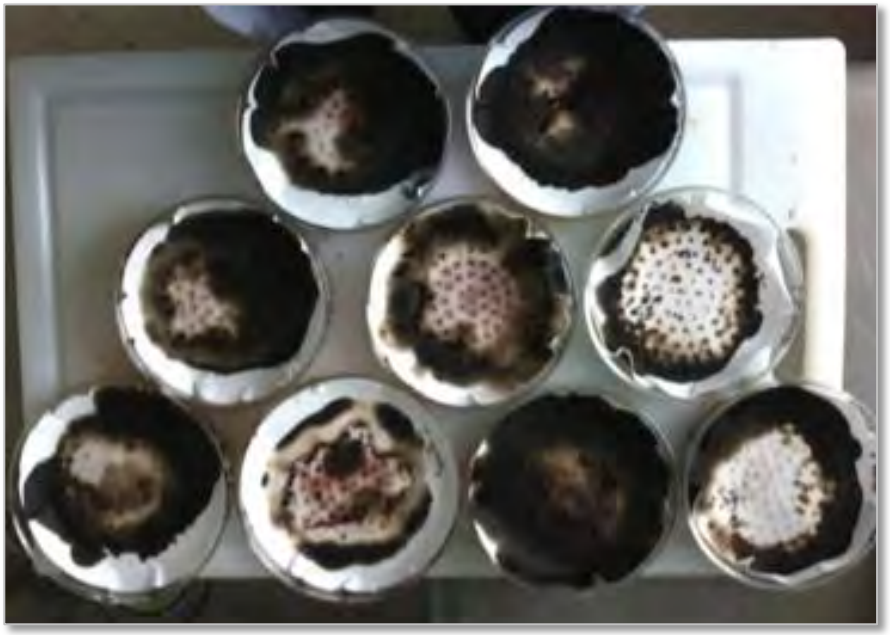


Figure 3.5. Final step for lignin measurements

3.2.8 Total phenolic compounds

Total phenolic content of the samples was measured using a modified colorimetric Folin-Ciocalteu method (Singleton and Rossi, 1965). The Folin-Ciocalteu method is commonly used to determine the total phenolic content of foods (Cicco et al., 2009). It involves oxidation in alkaline solution of phenols by the yellow molybdotungstophosphoric heteropolyanion reagent and colorimetric measurement of the resultant blue molybdotungstophosphate. These blue pigments have a maximum optical absbance that depends on the composition of phenolic mixtures and on the pH of solution, which is usually obtained by adding sodium carbonate (20% w/v) (Cicco et al., 2009).

Thus, according to (Cicco et al., 2009), for Folin-Ciocalteu method, 100 µl of properly diluted samples were taken, calibration solutions or blank were pipetted into separate test tubes and 1600 µl of distilled water and 100 µl of Folin-Ciocalteu 1 N reagent (2N,

supply by Sigma Aldrich) were added to each. The mixture was mixed well and allowed to equilibrate at room temperature for 8 min. Then, 200 µl of a sodium carbonate solution (20% w/v, Sigma Aldrich) was added. The mixture was swirled and allowed to stand 60 minutes at room temperature in the dark. Gallic acid (GA) (99% supply by Sigma Aldrich) was used as standard solution in a calibration curve (0 – 500 mg GA/l) for determining the total phenolic compounds (TPC) content and the blank was prepared with distilled water. Optical absorbance was measured at 765 nm, using a JENWAY 6405 UV-Vis spectrophotometer (Keyson products, UK). The TPC was expressed as milligrams of Gallic Acid Equivalent (GAE) per unit mass of dry weight of sample (mg GAE/g dw).

3.2.9 Antioxidant activity

The DPPH (di(phenyl)-(2,4,6-trinitrophenyl)iminoazanium) method was used. The total Antioxidant Activity (AA) was measured as DPPH free radical scavenging using 1,1 diphenyl 2-picryl hydrazyl (DPPH) reagent which is a stable free radical. The analysis is based on the fact that when reacting with antioxidants, DPPH reagent is converted to 1,1 diphenyl 2-picryl hydrazine and a change in color from violet to pale yellow occurs. Therefore, DPPH scavenging assays have been used to study the antioxidant activity (Gawron et al., 2012). Analysis mixture contained 2850 µl of 100 ppm DPPH solution (Sigma Aldrich, St Louis, USA) and 150 µl of sample. For blank, 2850 µl of DPPH solution and 150 µl of ethanol were used. In all cases, optical absorbance at 515 nm was measured using a JENWAY 6405 UV-Vis spectrophotometer (Keyson products, UK) after 1 hour of incubation at room temperature in the dark (Thaipong et al., 2006). The percentage of scavenging activity was calculated according to equation (3.7).

$$\%\ Scavenging\ Activity = \left(1 - \frac{Abs_{extract}}{Abs_{Reference}}\right) 100 \quad (3.7)$$

The IC_{50} is defined as the amount of antioxidant necessary to reduce the initial DPPH concentration by 50%, this term is used to express the AA of a sample. The coefficient $1/IC_{50}$ was calculated and used to determine the correlation between antioxidant activity

and the total phenolic content of a sample that contains phenolic compounds with antioxidant activity (such those obtained from SBP using any technique) (Gawron et al., 2012). The antioxidant activities were estimated from the decrease in absorbance from three replicates for each sample. The results are expressed as IC_{50} value.

3.2.10 Anthocyanins content

The content of anthocyanins is represented as pigments and it is measured according to color change of the anthocyanins as a function of pH using optical spectroscopy (Giusti and Wrolstad, 2001). The pH differential method allows accurate and rapid measurement of the total anthocyanin of a sample even in the presence of polymerized degraded pigments and other interfering compounds (Giusti and Wrolstad, 2001).

The differential pH method is based on the reaction from the colored oxonium form (which predominates at pH 1.0) to the colorless hemiketal form (which predominates at pH 4.5) (Giusti and Wrolstad, 2001). This change in color is presented at different absorbance spectra.

The anthocyanins content was measured as cyanidin-3-glucoside, which is the most common anthocyanin pigment in fruits (Lee et al., 2005). Two buffer solutions were prepared. The first was a potassium chloride at pH 1.0 (99%, Carlo Erba, Barcelona, Spain) and the second was sodium acetate solution at 4.5 pH (99%, Carlo Erba, Barcelona Spain). An appropriate dilution factor (DF) was determined with buffer solution 1 (Potassium chloride at pH 1.0) to guarantee that the absorbance of the sample at the $\lambda_{vis\text{-}max}$ was within the linear range of the spectrophotometer (Less than 1.2). Then, two solutions were prepared taking 300 µl of the sample and mixing with 1700 µl of buffer solutions 1 and 2 respectively. This corresponds to a ratio 5.6 buffer to sample (1700 µl/ 300 µl), this was needed in order to not exceed the buffer's capacity (the sample should not exceed the 20% of the total volume). Then, the solutions were incubated for 15 minutes at room temperature in the dark. Then, the absorbance was measured using a spectrophotometer (JENWAY 6405 UV-Vis. Keyson products, UK). All measurements were made for triplicate and the anthocyanins content was calculated according to equation (3.8).

$$Abs = (A_{vis-max} - A_{700})_{pH=1.0} - (A_{vis-max} - A_{700})_{pH=4.5} \quad (3.8)$$

Where $A_{vis\text{-}max}$ is the maximum absorbance measured between 300 and 700 nm and A_{700} is the absorbance measured at 700 nm. The latter was made for both solutions, buffers at pH 1.0 and 4.5. Then, the Anthocyanin content was calculated according to equation (3.9).

$$A(mg/l) = \frac{Abs * MW * DF * 1000}{\varepsilon * 1} \quad (3.9)$$

MW is the molecular weight (of cyanidin-3-glucoside), DF is the dilution factor (of the samples), ε is the molar absorptivity (of cyanidin-3-glucoside) and 1000 is in order to obtain the anthocyanins per liter of sample. For cyanidin-3-glucoside, the MW is 449.2 and ε is 26,900 (Giusti and Wrolstad, 2001).

3.2.11 Fatty acid profile of avocado pulp

A thermo-mechanical extraction procedure (similar to olive oil extraction) was used (Wong et al., 2010). The skin and seed were removed, and the pulp was macerated to a paste using a porcelain maceration unit and then mixed at 250 rpm for 60 minutes at 50°C in a beaker (Schott Duran, Germany) with a mechanical stirrer rod (Model AX686/1 AUXILAB S.L.). The elevated temperature promotes the extraction of the oil from the oil-containing cells and does not affect the quality of the oil (Wong et al., 2010). The oil phase was separated from the pulp in two steps of centrifugations. First, using a high-speed decanting centrifuge (Hermle Z 206 A, Germany) at 6000 rpm and then, the oil was extracted with a syringe and subjected to second centrifugation using a high-speed decanting centrifuge (Hermle Z 32 HK, Germany) at 12000 rpm. After centrifugation, the

oil phase (supernatant) was collected using a syringe. Figure 3.6 shows the thermo-mechanical extraction and avocado oil obtained.

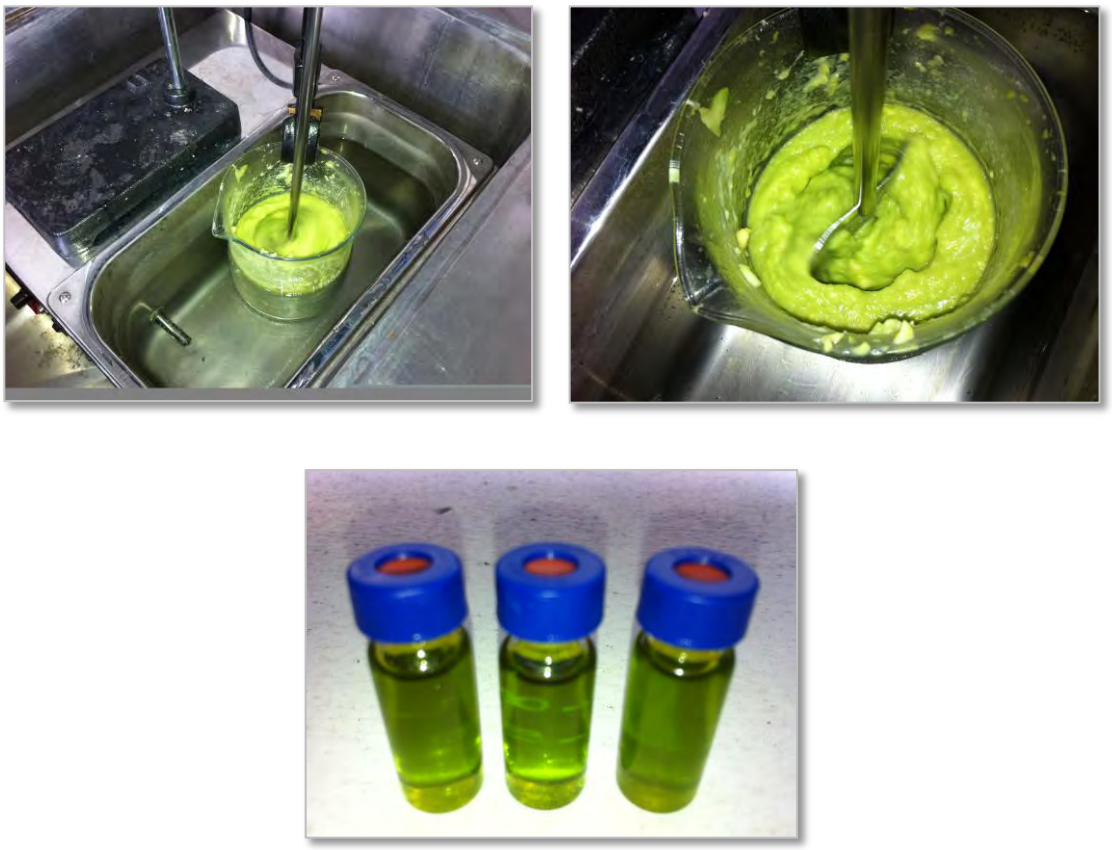


Figure 3.6. Thermo-mechanical extraction and avocado oil

The Fatty Acids profile was obtained from the *Lab Unaproliva* University of Jaén Spain. Gas Chromatography (Hewlet Packard 5890 A, Minnesota USA) with a flame-ionization detector was used. The fatty acid composition was based on the regulation "Regl2568/91 (Salvador et al., 1999) while the isomers were based on the regulation "Regl1429/92" (Salvador et al., 1999). Methyl esters were prepared by derivatization of oil (Salvador et al., 1999). Then, 1 ml of boron trifluoride-methanol solution (14%, Sigma Aldrich, St Louis, USA) was added to 50 µl of oil, then the mixture was mixed for 15 seconds placed in a water bath (Thermo Fisher Scientific model 2870, MA USA) at 80 ºC

for 40 minutes. Then, 2 ml of hexane (98%, Sigma Aldrich, St Louis, USA) were added and the mixture was mixed for 30 seconds. Then, the system was allowed to separate into 2 phases over a period of 2 hours at room temperature. A 1 ml sample of the supernatant, containing methyl esters, was used for fatty acid profile determination. Fatty acids were determined using a total retention time of 40 min as well as a fused silica column (50 m of length x 0.25 mm ID). Helium was used as carrier gas at a flow of 1 ml/min. The temperature of the injector and detector was 250ºC. The oven temperature was 210ºC and 1 µl of the sample was injected. The results were reported as concentration of each one of the fatty acids contained in the sample of avocado oil according to a methyl esters pattern previously measured in the lab Unaproliva.

3.2.12 Volatiles profile

The headspace solid phase microextraction (SPME) method was used for extracting the volatiles (Beaulieu and Grimm, 2001). Volatiles extraction from samples of SBP as well as avocado peel and seed was carried out at 50 °C. The SPME fiber was polydimethylsiloxane (PDMS 100 µm), obtained from Supelco (Sigma-Aldrich, Bellefonte, PA, USA). The fiber was preconditioned at 250°C for 30 min and was manually inserted into the headspace of the sample's recipient. Thus, 3.5 g of sample was placed in a glass vial of 20 ml with a headspace volume of 10 ml and capped with a screwcap for 20 minutes at 50ºC using a heating mantle (RC F20710174, Medellin Colombia) to deliver the volatiles as it is shown in Figure 3.7. After that, the sampler was inserted to the injection port of the Gas Chromatograph (Hewlett Packard 6890) equipped with a detector of microelectron capture (µECD) and a AT1701 capillary column (Supply by Agilent, Santa Clara, USA) (30 m of length, 0.53 mm ID, 1 µm thick stationary phase of 7% of cyanopropyl, 7% phenyl methyl siloxane) was used. The carrier gas was Nitrogen (99.99%, Praxair, Manizales Colombia) with a flow rate of 8.8 ml/min. The injection was manual and a split mode 5:1 was used. The oven temperature was 60ºC during 1 min to 100ºC at 2ºC/min. After 3 min, temperature increases to 150ºC at 6ºC/min. After 3 min, temperature was increased to 230ºC at 15ºC/min for 2 min. (Run time of 42 min). The volatiles were identified using the base data of the Mass Spectrophotometric and using 1-octanol as internal standard.

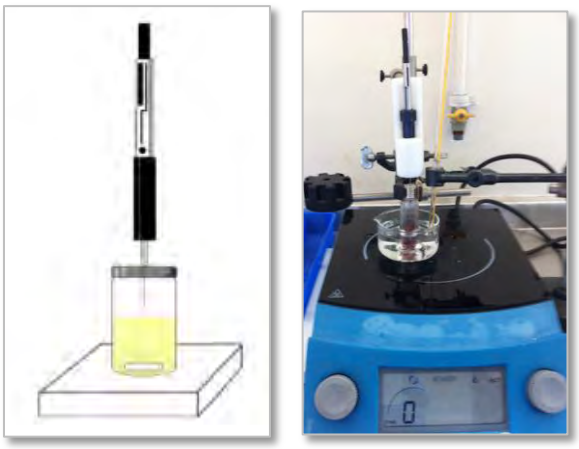


Figure 3.7. SPME method for volatiles analysis

Summarizing, for SBP was determined lignocellulosic biomass (Moisture, ash, holocellulose and lignin) as well as valuable compounds (phenolic compounds and anthocyanins). For avocado peel and seed, the lignocellulosic biomass (Moisture, ash, holocellulose and lignin) was determined while for avocado pulp, valuable compounds (fatty acids) were determined. For both, (SBP and avocado), the volatiles constituents were identified. Thus, Figure 3.8 depicts the chemical characterization route for SBP and avocado.

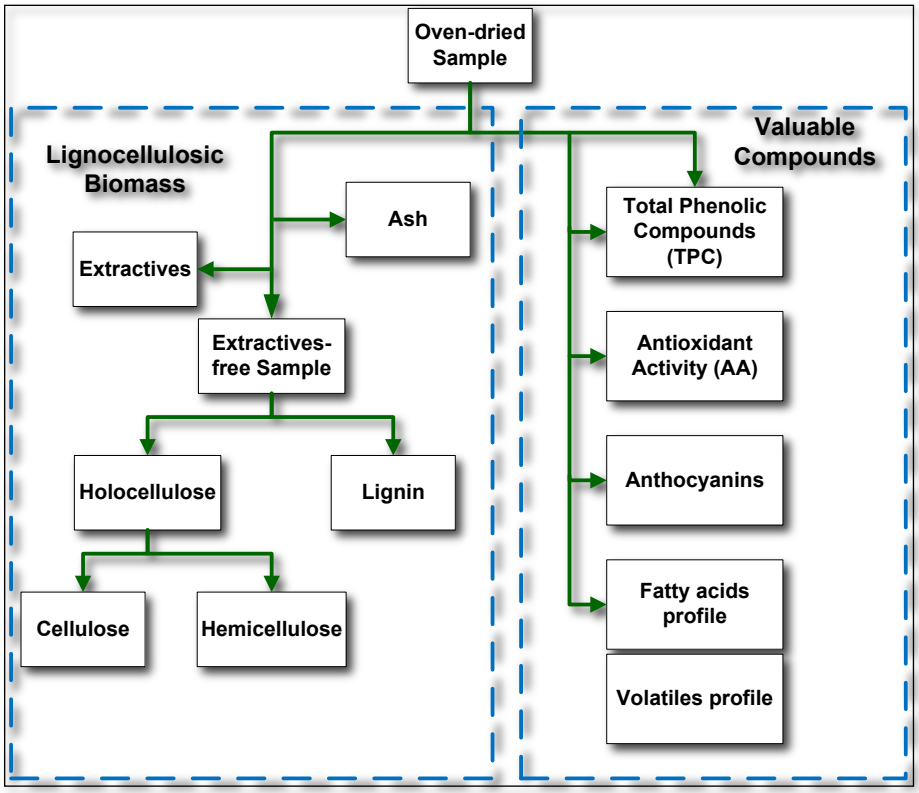


Figure 3.8. Chemical characterization route for selected biomasses

3.3 Experimental procedures for obtaining extracts and valuable compounds

Several assays were carried out for obtaining valuable compounds using the biorefinery concept. These valuable compounds included extracts with antioxidant activity, oil, reducing sugars and concentrated extracts containing phenolic compounds. Extracts from SBP and naranjilla waste (that is a case study) using supercritical carbon dioxide were obtained. Oil from avocado pulp and spent coffee grounds (that is a case study) using both, solvent and mechanical extractions was obtained. Sugars (represented as reducing sugars) from lignocellulosic biomass by means of both, acid and enzymatic hydrolysis were obtained from SBP, peel and seed of avocado as well as for other residues for comparison purposes (peel of orange, mandarin, pineapple and naranjilla

waste). Finally, concentrated extracts containing phenolic compounds from SBP using ultrafiltration membranes were obtaining. Below are explained each one of the techniques used for obtaining valuable compounds.

3.3.1 Extraction of phenolic compounds from SBP

To determine the initial content of total phenolic compounds (TPC) and anthocyanins as well as antioxidant activity (AA) of SBP, the following procedure was done. The extracts were obtained after sample preparation described above using ethanol at (99.95%, Merck, Darmstadt, Germany) during 8 hours in a soxhlet unit (Schott Duran, Main, Germany). The solid fraction was subjected to other 3 re-extractions (a total of four extractions) to ensure the extraction of all phenolic compounds. At the end, TPC was measured according to Folin Ciocalteu method for all extractions as well as for final volume obtained which is the sum of all extractions. The essays were carried out in triplicates. Figure 3.9 shows the extracts obtained for each one of the four extractions. The procedure for the extractions was as follow:

1. 32 gr of sample were put in a soxhlet with 250 ml of ethanol (for triplicate)
2. The extraction of phenolic compounds was made at 80 ºC for 6 hours
3. Then, the extracts were stored in the dark at -20 ºC
4. The remaining solid was used for subsequent extractions with new solvent (250 ml of ethanol) for three more times (4 times in total)
5. Each extract was stored at the same conditions that the first one
6. TPC was measured for each one of the extractions as well as for total volume which is the sum of all extractions.

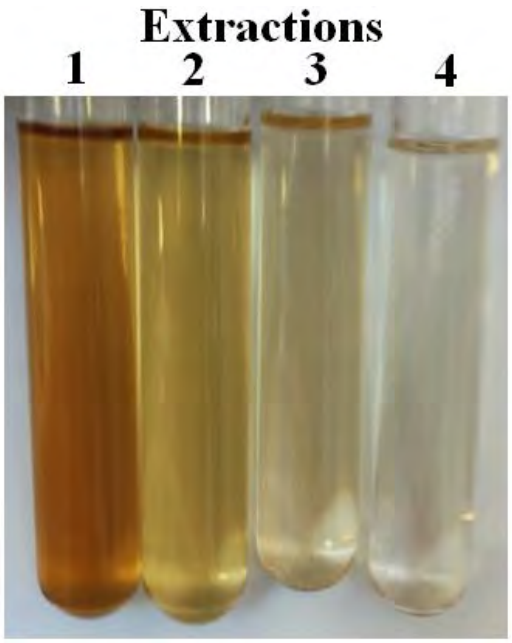


Figure 3.9. Consecutive extractions for phenolic compounds from SBP

3.3.2 Supercritical fluid extraction

The Supercritical Fluid Extraction (SFE) unit is a home-made equipment, consisting of a line to conditioning CO_2, an extractor chamber and a vessel (Collector) to separate CO_2 from extracts (All the equipment uses stainless steel of 10,000 psi supply by Swagelok, Barranquilla Colombia) as it is shown in Figure 3.10. The maximum pressure of the SFE unit is 350 bar and the extractor capacity is 254 ml. All the extractions were carried out at static extraction mode (No flow of CO_2 through the camber extraction) and at 40ºC to avoid the possible degradation of the phenolic compounds because some of them are susceptible to high temperatures. Thus, the sample was introduced in the extraction chamber along with ethanol as co-solvent. Then, CO_2 from a gas cylinder was routed to a heat exchanger (that uses ethylene glycol as refrigerant) its temperature can be lowered to up to -25 ºC and then the cold CO_2 was pumped into the extraction chamber until the desired pressure has been reached. After 65 min of extraction (Cerón et al., 2012), the extract was transferred to the collector in order to remove the solvent (CO_2) and co-solvent (Ethanol). This kind of extraction is also named enhanced supercritical fluid extraction due to the use of ethanol as co-solvent.

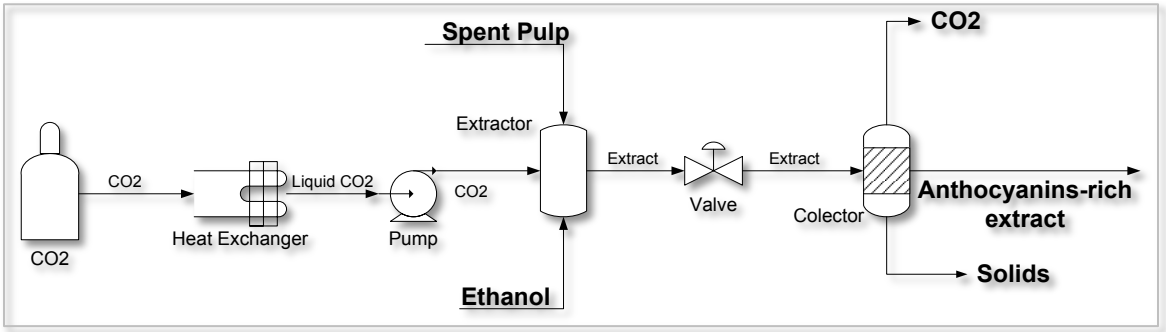


Figure 3.10. SFE scheme for obtaining extracts

The matrix of extraction experiments with SBP was carried out in the following manner. First a series of experiments aimed at determining the optimal particle size of SBP for maximal extraction of Total Phenolic Compounds (TPC) was carried out using SBP of different particle sizes as it is shown in Table 3.1.

Table 3.1. Particle sizes used for supercritical extractions of SBP

Mesh	Size (μm)	Powders
No. 10	2000	
No. 14	1400	
No. 20	850	

No. 30	600	
No. 40	425	
No. 60	250	

Then based on the results of the first stage, a second series of experiments aimed at identifying the effect of the pressure and co-solvent-to-solid ratio on TPC extraction was carried out and finally, using the results obtained in the first two stages, a third series of experiments were carried out in order to establish extraction curve (Sovová, 2005). Table 3.2 shows the operational conditions and purposes of each stage.

Table 3.2. Steps, operational conditions and purpose in the SFE of SBP

Step	Conditions	Purpose
1	Temperature: 40 ºC Pressure: 300 bar Time: 90 min Particle size: from 1400 µm to 180 µm	Evaluate the effect of the particle size over the Total Phenolic Compounds (TPC) and total Antioxidant Activity (AA) of the extracts.

2	Temperature: 40 ºC Particle size: 850 µm Pressure: 150 - 300 bar Co-solvent to solid ratio: 2, 4 and 6 (64, 128 and 192 ml ethanol/32 g solid respectively) Time: 90 min	Evaluate the effect of the pressure and co-solvent to solid ratio over TPC
3	Temperature: 40 ºC Particle size: 850 µm Pressure: 300 bar Co-solvent to solid ratio: 4 (128 ml ethanol/32 g solid) Time: 30 to 240 min	Evaluate the extraction curve for solubility and mass transfer coefficient calculation

3.3.3 Oil extraction techniques

Oil was extracted from avocado pulp and spent coffee grounds (SCG) (case study). The avocado oil was extracted from the pulp using a mechanical method (Wong et al., 2010). This mechanical extraction was carried out at 60 ºC for 60 minutes in an extractor (Extractor IBG, Monforts, Germany). Then, the sample was centrifuged in two steps: 6000 rpm (Hermle Z 206 A, Germany) and then 12000 rpm (Hermle Z 32 HK, Germany). Then, oil was separated from the remaining solid by decanting and stored at -20ºC pending for analysis. Figure 3.11 depicts the steps followed for oil extraction from avocado pulp. For SCG, two types of extractions were carried out. First, a mechanical

extraction was carried out using home-made equipment which is a hydraulic piston (carbon steel, stainless steel and bronze) with 500 ml of capacity and 60 bar of pressure. Three temperatures were evaluated for mechanical extraction, 50 ºC, 70 ºC and 90 ºC. The second extraction was using hexane (98%, supply by Sigma Aldrich) as solvent for 24 hours (Caetano et al., 2012; Haile, 2014). This extraction was carried out using a soxhlet unit (Schott Duran, Main, Germany) of 500 ml. Also three temperatures were evaluated, 90 ºC, 120 ºC and 160 ºC. Figures 3.12 and 3.13 show the steps for oil extraction from SCG by mechanical and solvent extractions respectively. Additionally, the acid value was measured for oil extracted from SCG according to official method NTC-218 (Montoya et al., 2013). This measure characterizes the presence of free fatty acids in the oil and it is a good indicative for biodiesel production. For this method, 0.35 g of oil were taken and 10 ml of ethanol (99.95%, Merck, Darmstadt, Germany) were added along with some drops of phenoltaleine (solution at 1%, Panreac, Barcelona, Spain) as indicator. Then, a titulation was carried out using a KOH (Panreac, Barcelona, Spain) solution 0.12 N. When the pink color was reached then, the test is done. The acid value (known as acid index) was calculated according to equation (3.10).

$$Acid\ index = \frac{(ml\ KOH)(N\ KOH)(25.6)}{Sample\ weighted} \tag{3.10}$$

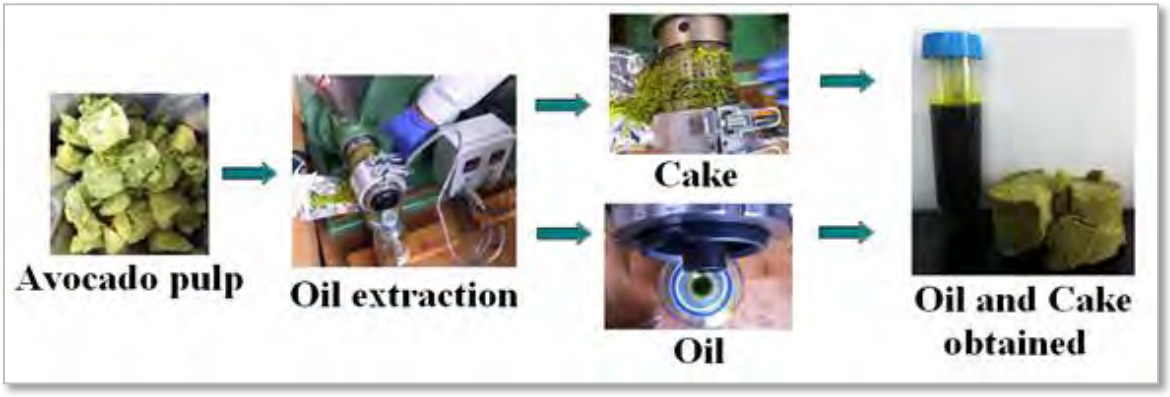


Figure 3.11. Steps for avocado oil extraction for mechanical method

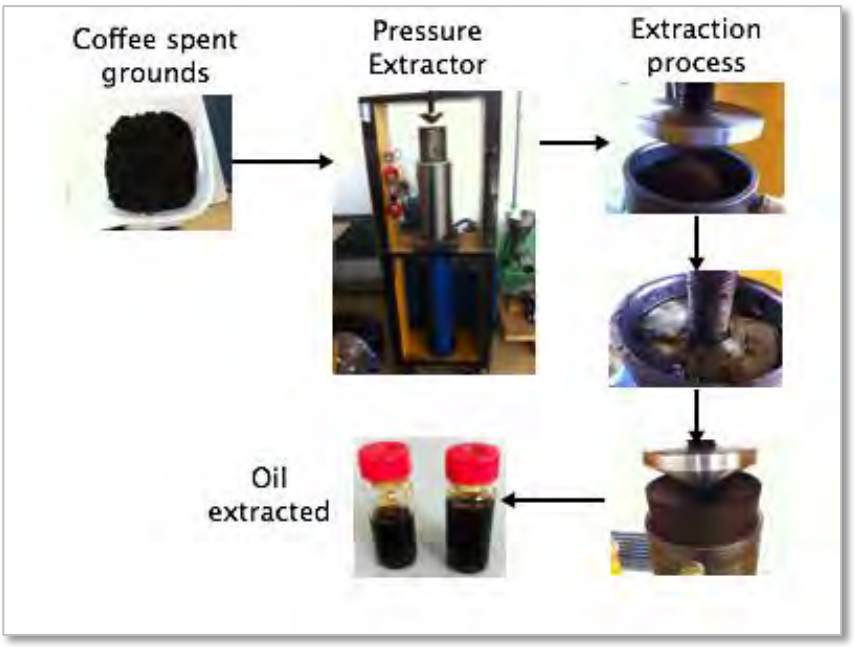


Figure 3.12. Mechanical extraction of oil from SCG

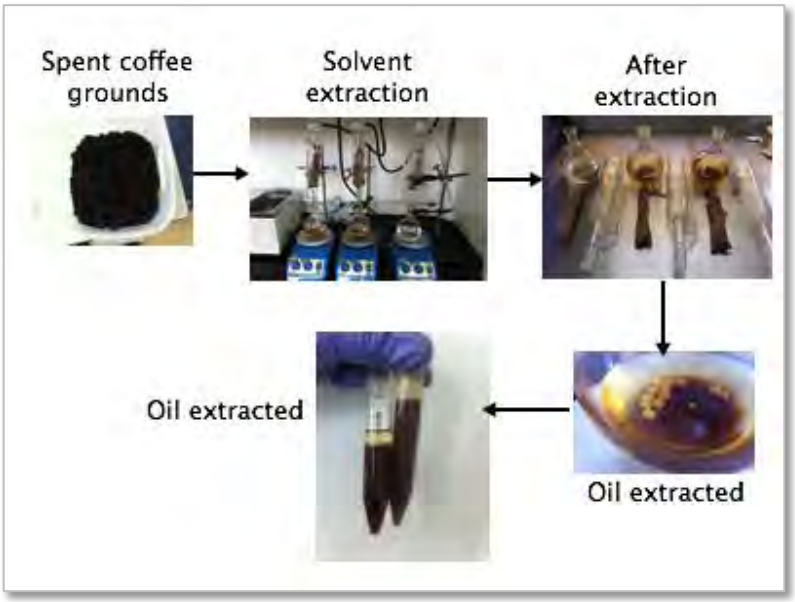


Figure 3.13. Solvent extraction for SCG

3.3.4 Sugar production

Sugar production was carried out for SBP, avocado peel and avocado seed as well as for naranjilla waste (which is case study) and other agroindustrial residues (pineapple, orange and mandarin) for comparison purposes. Naranjilla waste corresponds to a solid residue obtained after juice extraction (supply by Frugy S.A., Manizales Colombia) while pineapple, orange and mandarin waste are the peel of these fruits that were acquired in a local market in Manizales Colombia. For all these residues, the samples were first milled using a domestic mill (model rey1 Toolcraft, Medellin, Colombia) for obtaining particles that pass mesh 20 (850 µm) sieve and are retained on mesh 80 (180 µm). Then, the samples were dried at 40ºC using an atmospheric drying oven (Thermolab, Maharashtra, India).

For sugar production 10 g of the samples were used, acid hydrolysis was carried out with 2% (w/w) of H_2SO_4 (90% (w/v) supply by Mallinckrodt, Damastown Ireland) at 121 ºC in an autoclave (Sanyo MLS-3781L, Wolflabs, Pocklington, UK) at 15 psi, for 90 min. After that, the sugar content was determined by DNS method. For raw materials that had the highest sugar production, they were used for an enzymatic hydrolysis to increase the sugar yield. Enzymatic hydrolysis was carried out according to Technical Report NREL/TP-510-42629 of the National Renewable Energy Laboratory (Selig et al., 2008). The substrate to liquid ratio was 1/10 and 60 FPU/g of sample (FPU is defined as the amount of enzyme required to liberate 1 µmol of glucose from filter paper per minute at 50 ºC) was used at 50 ºC using cellulose as enzyme (Celluclast 1.5L from Novozymes) for 72 hours. After that, the reducing sugars were measured by DNS (3,5-Dinitrosalicylic acid) method.

The sugars were expressed as reducing sugars using DNS (3,5-Dinitrosalicylic acid) method (Wood and Bhat, 1988). For this method, 0.5 ml of liquid sample after hydrolysis was placed in a test tube and 0.5 ml of the DNS reagent (98%, supply by Sigma Aldrich) was added. Tubes were placed in boiling water bath (Thermo Fisher Scientific model 2870, MA USA) at 90 ºC for 9 min. Then, samples were transferred to ice to rapidly cool down (room temperature). Then, 5 ml of distilled water was added. The mixture was mixed and allowed to stand 15 min at room temperature. Then, absorbance was

measured at 540 nm (in a JENWAY 6405 UV-Vis spectrophotometer, Keyson products, UK), against a blank consisting of DNS with distilled water. A calibration curve was prepared using an anhydrous glucose standard solution (Glucose anhydrous supply by Panreac, Brugera Spain) at concentration of 1, 2, 3, 4 and 5 g/l. Figure 3.14 shows the raw materials for sugar production.

Figure 3.14. Raw materials for acid hydrolysis

3.3.5 Concentration of extracts from SBP

After determine the best conditions for extracts obtained from supercritical extractions, the extract of SBP was subjected to an ultrafiltration process to concentrate the extract taken into account that ultrafiltration (UF) process is suitable technology to concentrate polyphenols from extracts due to the high retention of these compounds in the retentate (Liu et al., 2011). An ultrafiltration device Lab Scale TFF (Merck Millipore, Billerica MA, USA) system using a membrane Pellicon XL Biomax 5 (Millipore, Bedford, MA, USA) was used. The UF device has a peristaltic pump and agitation system. A polyethersulphone membrane with a molecular weight cut-off (MWCO) of 5 kDa and a total filtration surface of 50 cm^2 was used (supplied by Merck Millipore, Darmstadt, Germany). The feed flow was fixed at 15 ml/min and a feed pressure of 30 psig was used. A volume of 277 ml of extract was subjected to a UF process that was carried out

at room temperature in dark to avoid degradation of phenolic compounds. The UF process was carried out for 18 hours and samples were taken each 4.5 hours. Both, retentate and permeate were tested and characterized according to their phenolic compounds content using Folin-Ciocalteu method. Figure 3.15 shows the UF unit used for concentrate extracts.

Figure 3.15. Ultrafiltration process for concentrated extracts

3.4 Process Simulation

Process simulation was carried out according to the procedure described in chapter 2. It includes hierarchy, sequencing and integration concepts for designing biorefineries (Moncada et al., 2014b). Four biorefineries (for SBP, avocado, SCG and naranjilla waste described in chapter 2) were simulated. The biorefineries were based on the chemical composition and potential products that can be obtained from the feedstocks. For SBP

and avocado, the chemical composition was defined by the chemical characterization made at laboratory according to procedure described in section 3.2. For case studies, SCG and naranjilla waste, the chemical characterization was taken from literature (Gancel et al., 2008; Riaño, 2008). All evaluations were developed for Colombian context (it includes techno-economic and environmental assessments).

Simulations were carried out to obtain the mass and energy balances of the biorefineries. From these results, were evaluated and analyzed some features that include yields, energy consumption, total production cost and potential environmental impact for each biorefinery. These features become in evaluation criteria that provide information about performance of the biorefineries (Posada et al., 2012; Rincón et al., 2014). For comparison purposes, these features were compared and analyzed by means of scenarios that include several levels of integration and volumes of production.

Yields were calculated from the mass balance according to the products obtained from a specific feedstock. Energy consumption was calculated from the energy balance of the biorefinery and reveals the energy requirements of the biorefinery according to the desired products. Total production cost was calculated per kg of products after economic evaluation. Finally, environmental assessment was carried out from the mass and energy balances of the biorefineries taken into account both, incoming and leaving streams.

Aspen Plus V8.0 (Aspentech. Cambridge MA, USA) was employed as computational tool for obtaining mass and energy balances for each biorefinery. For environmental analysis, WAste Reduction Algorithm (WAR) (Free software from Environmental Protection Agency (EPA)) was used as computational tool for obtaining the potential environmental impact of the biorefineries. These two computational tools were described in chapter 2 and **Appendix B**. Evaluation of biorefineries was made according to following steps:

1. Base case for each biorefinery (such proposed in chapter 2)
2. Evaluation of a scenario considering heat integration (Applying the Pinch methodology)
3. Evaluation of a scenario considering mass integration (specially water)

4. Evaluation of a cogeneration system
5. Evaluation of some allocation of products for biorefineries

Finally, the features mentioned above were analyzed for all scenarios and biorefineries from techno-economic and environmental points of view.

3.4.1 Simulation procedure

The simulation was carried out in three steps using different computational tools. The first step consisted of process simulation and was aimed at obtaining the energy and mass balances of the biorefineries, using Aspen Plus V.8.0 (AspenTech: Cambridge, MA, USA). The physicochemical properties of all of the compounds involved in the simulation step were obtained from the data base of Aspen Plus and from National Institute of Standards of Technology (NIST, 2013). To calculate the properties in the liquid phase, the Non Random Two Liquids (NRTL) model and Unifac were used while for vapor phases, the Hayden O'Conell (HOC) equation was used. NRTL and Unifac models were used to estimate the activity coefficients of all the compounds from its mole fractions in the liquid phase. The HOC equation provides a method for predicting a second virial coefficient for multi-compounds vapor mixtures. These methods are used to calculate successfully phase equilibria in the type of mixtures with non-conventional compounds (Cardona Alzate and Sánchez Toro, 2006; Jaramillo et al., 2012; Quintero et al., 2008).

The second step of the simulation consisted of economic analysis and was carried out using Aspen Process Economic Analyzer (AspenTech: Cambridge, MA, USA). Finally, the third step consisted of environmental analysis which was carried out using WAste Reduction Algorithm (WAR) developed by the US Environmental Protection Agency (Young and Cabezas, 1999).

The simulations for each biorefinery were based on the schemes proposed in chapter 2. Once the flow diagram and compounds were defined, the thermodynamic models were selected according to the characteristics of each compound and units as well as operational conditions. Then, the simulation was carried out to obtain the mass and

energy balances of the biorefineries. Table 3.3 depicts the model calculation to carry out mass and energy balances (first step). The model calculation scheme involves the mathematical models for the principal units used in the simulation for all biorefineries as well as the algorithms for them, including the sequence of information obtained in each step.

Table 3.3. Models calculation of the process simulation

First step: Mass and energy balances
1. Introduction of compounds (From databases of Aspen plus and NIST)
2. Selection of the thermodynamic model (Unifac Dortmund)

$$Ln\gamma_i^c = Ln\left(\frac{\Phi_i'}{x_i}\right) + 1 - \frac{\Phi_i}{x_i} - \frac{z}{2}q_i\left(Ln\frac{\Phi_i}{\Theta_i} + 1 - \frac{\Phi_i}{\Theta_i}\right)$$

$$\frac{\Phi_i'}{x_i} = \frac{r_i^{3/4}}{\sum_j x_j r_j^{3/4}}; \Phi_i = \frac{x_i r_i}{\sum_j^{nc} x_j r_j};\ \Theta_i = \frac{x_i \frac{z}{2} q_i}{\sum_j^{nc} x_j \frac{z}{2} q_j}$$

$$r_i = \sum_k^{ng} \nu_{ki} R_k\,; q_i = \sum_k^{ng} \nu_{ki} Q_k$$

Indices i and j refer to components, k is the type of group, nc is the number of components, ng is the number of groups in the mixture, z is the coordination number set to 10, x is the molar composition, R and Q are the volume and area parameters, respectively, ϕ_i is the fugacity coefficient of component i, Υ_i is activity coefficient Θ_i is molecular surface area fraction, q_i and r_i are molecular surface area and molecular Van der Waals volume respectively and v_{ki} is the number of groups of type k in molecule i.

3. Definition of operational conditions (From the literature and experimental essays)
4. Models for units involved in the biorefineries

1. **Pumps:** (According to power consumption)

For power requirements: $P = m\left(\Delta\left(\frac{u^2}{2\alpha}\right) + g\Delta z + \int_{p1}^{p2} \nu dP + F\right)$

Where P is the total work, m is the masic flow, u refers to the velocity, g is gravity, Δz is the static head, vdP is the pressure head, F is the dynamic head, α is a coefficient used to take into account the velocity profile inside the pipe (0.5 and 1 for laminar and turbulent flow respectively)

2. **Crusher:** (Cumulative Analysis)

$$D_{P,n} = Particle\ diameter$$

$$A_{Solids} = \frac{6\lambda}{\rho_P}\int_0^1 \frac{d\phi}{D_{P,n}}$$

$$N_P = \frac{1}{a\rho_P}\int_0^1 \frac{d\phi}{D_{P,n}^{\ 3}}$$

Where Dp,n is the particle diameter, λ is the form factor, ϕ is cumulative fraction and ρ is the particle density.

3. **Heat exchangers:** (Heat and mass balances)

$$Q = UA\Delta T = \dot{m}C_P\Delta T$$

$$\frac{1}{U} = \frac{1}{h_0} + \frac{2.3D_0}{2K}\left(log\frac{D_1}{D_0}\right) + \frac{1}{h_1(D_1/D_0)}$$

Where Q is heat, U is total coefficient of heat transfer, h_1 and h_2 are the convective heat transfer coefficients calculated for each of the fluids, respectively, D_0 and D_1 are the internal and external diameters of the tube in the heat exchanger, respectively, m is the mass flow and Cp corresponds to the heat capacity of the fluid.

4. **Flash:** (Radchford-Rice equation)

$$F = L + V, \quad Fz_i = Lx_i + Vy_i, \quad y_i = K_i x_i, \quad \psi = V/F$$

$$z_i = (1-\psi)x_i + \psi K_i x_i, \quad x_i = \frac{z_i K_i}{(1-\psi)+\psi K_i}, \quad y_i = \frac{z_i K_i}{(1-\psi)+\psi K_i}$$

$$\sum_{i=1}^{M} x_i = 1, \quad \sum_{i=1}^{M} y_i = 1, \quad f(\psi) = \sum_{i=1}^{M}\left(\frac{z_i(1-K_i)}{1+\psi(K_i-1)}\right)$$

Where F, L and V are feed, liquid and vapor, respectively, x_i, y_i, and z_i are the compositions for liquid, vapor and feed, respectively. Ψ is the vapor fraction.

5. **Distillation:** (Radfrac model using the MESH equations)
Mass and energy balances of component i around plate j

$$M_{i,j} = L_{j-1}x_{i,j-1} + V_{j+1}y_{i,j+1} + F_j z_{i,j} - (L_j + U_j)x_{i,j} - \left(V_j - W_j\right)y_{i,j} = 0$$

$$E_{i,j} = y_{i,j} - K_{i,j}x_{i,j} = 0$$

$$(S_y)_j = \sum_{i=0}^{c} y_{i,j} - 1 = 0$$

$$(S_x)_j = \sum_{i=0}^{c} x_{i,j} - 1 = 0$$

$$H_j = L_{j-1}h_{L,j-1} + V_{j+1}h_{V_{j+1}} + F_j h_{F_j} - (L_j + U_j)h_{L_j} - \left(V_j - W_j\right)h_{V_j} - Q_j = 0$$

Where F, L, V, U and W are the flows for feed, liquid, vapor, lateral liquid and lateral vapor, respectively. x, y and z represent the mol compositions in the liquid, vapor and feed respectively. H and h are the enthalpies in the plate and flows, respectively. Finally, i and j represent the component and the plate, respectively.

6. **Turbine:** (Mollier method)

$$\eta \Delta h = \int_{p1}^{p2} V dP$$

Where η is the efficiency, h is the enthalpy, P_1 and P_2 are the pressure and V is the volume.

7. **Units of hydrolysis, reactor and extractor:** (Yields from experimental essays and literature)

3.4.2 Techno-economic assessment

The economic assessment was made from the mass and energy balances of the biorefineries obtained from simulations. The total production cost was calculated considering the total raw material, utilities, operating, labor and maintenance costs as well as the operating charges, plant overhead and general and administrative costs. Additionally, tax rate was taken according to the laws in Colombia (25%). The labor and utilities costs are shown in Table 3.4.

Table 3.4. Operational costs for biorefineries

Item	Price	Unit
Operator [a]	2.14	(USD/h)
Supervisor [a]	4.29	(USD/h)
Electricity [a]	0.10	(USD/KWh)
Potable Water [a]	1.25	(USD/m^3)
Fuel [b]	7.21	(USD/MMBTU)

[a] Typical prices in Colombia.

[b] Estimated cost of Gas to a period range of 2015 – 2035 (NME, 2013b).

After, a basic dimension of the units involved in the biorefinery was made and then, according to the investment parameters given by the user (Tax rate, internal rate of return, life of the project among others) the capital cost, operating costs, utilities and other relevant costs associated to the biorefinery are calculated (Moncada et al., 2013; Moncada et al., 2014a; Mussatto et al., 2013).

The straight-line depreciation method was used for depreciation expense and 8000 hours of work per period as well. Once the equipment sizing was made then, it was possible to calculate the cash flow and Net Present Value (NPV) that permit to evaluate the final earnings of the biorefinery. All this evaluation was made according to procedures explained in **Appendix B1**.

Heat integration of biorefineries was carried out using Pinch methodology as was described in **Appendix B2**. This integration was based on the composite curves that permit to obtain the highest energy recovered by the biorefinery as well as the targets for heating and cooling requirements (Ng, 2010; Oliveira et al., 2015; Shenoy and Shenoy, 2014).

3.4.3 Environmental assessment

For the environmental analysis was evaluated eight environmental impact categories such are: human toxicity potential by ingestion (HTPI), human toxicity potential by dermal and inhalation exposure (HTPE), terrestrial toxicity potential (TTP), aquatic toxicity potential (ATP), global warming potential (GWP), ozone depletion potential (ODP), photochemical oxidation potential (PCOP) and acidification potential (AP). The Potential Environmental Impact (PEI) of the process was calculated per kilogram of products. Natural Gas was used as fuel to cover the heat requirements for biorefineries (Cabezas et al., 1999; Young and Cabezas, 1999).

Potential Environmental Impact (PEI) was evaluated per kg of products for each biorefinery. PEI was calculated according to procedures described in **Appendix B3**. A comparison between scenarios was considered according to the level of integration of the biorefineries. It permitted to analyze the effect of the integration level on the

designed biorefineries and offered evaluation criteria to decide the best design of a biorefinery in terms of environmental performance.

The greenhouse gas (GHG) emissions associated with the biorefineries was calculated according to emissions factors for each gaseous pollutant (CH_4, N_2O, etc.) that contribute to the GHG emissions (Mussatto et al., 2013). This measurement was made based on the equivalent carbon dioxide that permits to establish a base line for comparison purposes between all scenarios evaluated for all biorefineries. Finally, environmental assessment provided evaluation criteria that along with techno-economic analysis permitted suggest the best design of a biorefinery according to feedstock and products involved.

Conclusions

The procedures used for chemical characterization established a reference composition for each feedstock and thus provided information that is critically needed for the design of the biorefineries. These methods of characterization are based on standards that permit to obtain both, lignocellulosic biomass and valuable compounds content in a sample. The proposed valuable compounds to be obtained using the biorefinery concept are based on the chemical composition of each feedstock thereby extracts, oil, reducing sugars and concentrated extracts are proposed as valuable compounds from the selected feedstocks.

The technologies and methodologies used for obtaining value added products from selected feedstock are explained. These results will support the design of the biorefineries. For the design, the yields, final concentrations and more results will be used but also operational conditions such as flows, temperature and pressures.

Process simulation was carried out according to procedures described in Apendix B. This procedure was made in three steps. The first one step corresponds to the mass and energy balances for each biorefinery obtained from simulations. The second step is the techno-economic assessment that includes analysis of several scenarios according to

the level of integration. The final step is the environmental assessment for obtaining the potential environmental impact of the biorefineries as well as GHG emissions.

PART II.

EXPERIMENTAL RESULTS

4. Chapter 4. Chemical characterization results

Overview

This chapter presents the results of the chemical characterization of spent blackberry pulp (SBP) and avocado (pulp, peel and seed). The characterization was carried out according to the procedures described in chapter 3. Lignocellulosic biomass of SBP as well as peel and seed of avocado was characterized for its moisture, extractives, cellulose, hemicellulose, lignin and ash contents. Total phenolic compounds (TPC) and anthocyanins content as well as the antioxidant activity (AA) associated with these compounds were characterized for SBP. Oil obtained from avocado pulp was characterized for its fatty acid profile. The volatile constituents of both, SBP and avocado were characterized.

This chapter also highlights the great potential for obtaining valuable compounds from SBP and avocado. These include extracts with antioxidant activity, and significant amount of anthocyanins that can be used for pharmaceutical, food and/or chemical applications. Similarly, the lignocellulosic biomass of SBP can be used as source for valuable compounds such as reducing sugars that can be obtained after hydrolysis processes.

Similarly, characterization of avocado revealed that this fruit has a potential chemical composition to obtain different value added products. The non-edible parts of avocado (peel and seed) contain significant amount of holocellulose to be used for reducing sugar production. The fatty acid profile of oil obtained from avocado pulp indicated that the most representative fatty acid is oleic acid.

4.1 Chemical characterization for spent blackberry pulp

4.1.1 Lignocellulosic biomass

Characterization of SBP for moisture, extractives, cellulose, hemicellulose, lignin and ash is presented in Table 4.1.

Table 4.1. Chemical characterization for spent blackberry pulp

Component	Repetition			Content (%)	St. Dev.	% Error
	1	2	3			
Moisture	5.01	4.95	5.41	5.12	0.20	4.00
Extractives	11.64	12.79	14.51	12.98	1.18	9.09
Holocellulose*	60.67	62.84	61.10	61.54	0.94	1.52
Cellulose	42.25	41.13	41.82	41.74	0.46	1.11
Hemicellulose	18.42	21.71	19.28	19.80	1.39	7.03
Lignin	21.54	18.30	17.82	19.22	1.65	8.60
Ash	1.14	1.12	1.16	1.14	0.01	1.31
Total	**100**	**100**	**100**	**100**		

* Holocellulose corresponds to a cellulose plus hemicellulose

The moisture content (5.12%) is similar to that reported by (Pasquel Reátegui et al., 2014) for blackberry bagasse (5.24%). The holocellulose content of SBP was more than 61%. This content of holocellulose suggests that it can be used for reducing sugars (xylose and glucose) production. The use of cellulose and hemicellulose for reducing sugars production has been reported for other fruits wastes (Davila et al., 2014a). This amount of holocellulose in SBP is comparable to what has been reported for mango peel and other similar agroindustrial wastes (sugarcane bagasse, rice husk, etc.) that has been used to produce reducing sugars (Dávila et al., 2014). Production of reducing sugar from fruit processing waste streams has been investigated due to the content of cellulose and hemicellulose and yield of up to 70% has been reported (Visioli et al., 2014). SBP can be used similar to other fruit wastes (orange bagasse, orange peel, banana peel and mango peel) that have significant contents of cellulose and hemicellulose that allow to obtain reducing sugars which in turn, become in a platform of other valuable products such as ethanol and organic acids (Dávila et al., 2014; Sánchez Orozco et al., 2014).

Lignin content (19.22%) is very similar to that found in spent mass that is derived from apple, chokeberry and pear processing with 20.4%, 24.1% and 33.5% respectively (Nawirska and Kwaśniewska, 2005). The significant lignin content of SBP supports the concept of utilizing SBP for developing value added products that can be used for liquid fuels from biomass as well as components of polymer composites (Doherty et al., 2011). Moreover, this content of lignin along with holocellulose of SBP can be used for producing bioenergy by means of hydrothermal gasification (Kang et al., 2013).

Results (Table 4.1) indicated that extractive content of SBP was 12.98%. Similar values of extractives (ranging from 10.77% to 33.53%) from fruits have been reported when ethanol was used as solvent (Seal et al., 2013). Extractives of blackberry pulp contain water-soluble pigments (Jiao et al., 2005). Moreover, extractives in ethanol contain not only pigments but also other compounds such as resins, tannins and sugars (ASTM, 2005). Some of the substances obtained in extractives of SBP are volatiles that confer the characteristic smell of blackberry (Meret et al., 2011). Besides, some of these extractives substances that can be found in fruits are phenolic compounds that have antioxidant capacity (Seal et al., 2013).

The ash content of SBP is low, 1.14% of the total biomass. This value is in agreement with results reported for milled and dried blackberry bagasse which is 1.62% (Pasquel Reátegui et al., 2014). However, higher values have been found for other fruit wastes such as orange bagasse, orange peel, banana peel and mango peel with 2.87%, 2.82%, 3.90% and 11.19% respectively (Sánchez Orozco et al., 2014).

The content of holocellulose overcomes 60% of the total biomass of SBP. This amount suggests that SBP would be used for sugars production purposes such as it has been done for other fruit wastes (Davila et al., 2014a). The lignocellulosic biomass content of SBP suggests that this waste has some potential (depending on the scale) for obtaining valuable compounds (reducing sugars, bioenergy as was discussed before).

4.1.2 Total Phenolic Compounds

Total phenolic compounds (TPC) of SBP were measured according to procedures described in chapter 3 using the Folin-Ciocalteu method. **Appendix C1** presents the calibration curve used for TPC measurements. Table 4.2 shows the TPC content made for all the extractions (four extractions) for triplicate as well as the absorbance, concentration and standard deviation for each one of the extractions and its replicates.

Table 4.2. TPC content of SBP

Extraction	Repetition	Absorbance (nm)	Concentration (mg GA/l)	Concentration (mg GA/g)	Average concentration (mg GA/g)	St. Deviation
Extraction 1	Sample 1	0,205	186,077	15,478	15,358	0,084
	Sample 2	0,207	187,615	15,293		
	Sample 3	0,214	193	15,303		
Extraction 2	Sample 1	0,21	189,923	7,899	8,092	0,160
	Sample 2	0,221	198,385	8,086		
	Sample 3	0,235	209,154	8,292		
Extraction 3	Sample 1	0,551	452,231	3,762	3,281	0,272
	Sample 2	0,418	349,923	2,852		
	Sample 3	0,494	408,385	3,238		
Extraction 4	Sample 1	0,008	34,538	0,287	0,268	0,013
	Sample 2	0,004	31,461	0,256		
	Sample 3	0,006	33	0,262		

The TPC content of SBP was 27.003 mg GA/g of SPB (2700 mg GA/100 g of SPB) which is the sum of all TPC extracted in all extractions. After four extractions, the TPC are not present in appreciable amounts because the content of TPC in the fourth extraction (26.85 GA/100 g of SPB) represents 0.994% of the TPC content of the SBP. Figure 4.1 shows the TPC extracted in each one of the extractions.

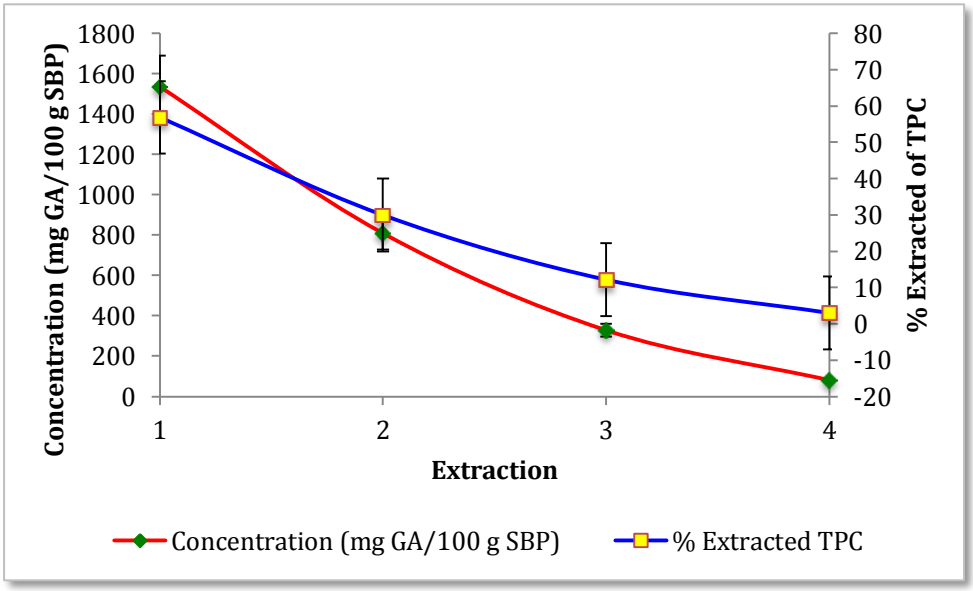


Figure 4.1. TPC profile for each one of the extractions

This value of TPC (2700 mg GA/100 g of SBP) is lower than those found for extracts obtained from fresh blackberry which is around 4250 mg GA/100 g of fresh fruit (Mertz et al., 2009). However, similar results have been obtained for other berry species such as strawberry, red currant, raspberry and black currant with TPC content of 1303, 1379, 1108 and 2813 mg GA/100 g of fruit respectively (Balogh et al., 2010). TPC content of SBP is comparable with that found for blueberry residues which is 2550 mg GA/100 g of residue obtained by soxhlet extration using ethanol (Paes et al., 2014). The phenolic compounds in berries include mainly phenolic acids, flavonoids (flavonols, anthocyanins, catechins), stilbenes, hydrolysable and condensed tannins (proanthocyanidins) and lignans (Nile and Park, 2014).

4.1.3 Antioxidant Activity

Antioxidant Activity (AA) was measured according to procedure described in chapter 3 using the DPPH method. **Appendix C2** presents the IC_{50} values as well as the Trolox standard curve. Table 4.3 shows the total Antioxidant Activity (AA) ontained for each of the extracts (Table 4.2) and its repetitions (as for TPC measurements).

Table 4.3. AA measurements for all extractions and its replicates

Extraction	Repetition	IC_{50} (ml sln DPPH/ml extract)	IC_{50} (mg SBP/ml DPPH)	Average IC_{50} (mg SBP/ml DPPH)	St. Deviation
	Sample 1	26,98	4,46		
Extraction 1	Sample 2	28,40	4,32	4,41	0,064
	Sample 3	28,33	4,45		
	Sample 1	20,19	5,95		
Extraction 2	Sample 2	21,89	5,60	5,68	0,19
	Sample 3	22,99	5,48		
	Sample 1	7,92	15,18		
Extraction 3	Sample 2	6,85	17,92	16,35	1,15
	Sample 3	7,91	15,94		
	Sample 1	1,83	65,55		
Extraction 4	Sample 2	1,74	69,20	67,38	1,83
	Sample 3	Lost	Lost		

After four extractions, the total AA is reduced (such it happens with TPC) because the content of TPC also is reduced. Because the IC_{50} means the amount of antioxidant necessary to reduce at 50% the initial DPPH concentration (Gawron et al., 2012), highest values of IC_{50} indicate low AA while smallest indicate high AA. The $1/IC_{50}$ coefficient was calculated in order to determine the change in AA with successive extractions and to determine the correlation between antioxidant activity and TPC (Gawron et al., 2012). Thus, the total $1/IC_{50}$ of SBP was 0.479 ml solution of DPPH/mg of SBP that corresponds to an IC_{50} value of 2.09 mg of SBP/ml of DPPH solution as it is shown in Table 4.4.

Table 4.4. Initial AA for SBP expressed as IC50 value

Extraction	Average IC_{50} (mg SBP/ml DPPH)	S. Dev.	% Error (%)	$1/IC_{50}$ (ml sln DPPH/mg SBP)	Initial IC_{50} (mg SBP/ml DPPH)
1	4.41	0.063	1.43	0.227	2.09
2	5.68	0.19	3.51	0.176	
3	16.35	1.15	7.06	0.061	
4	67.38	1.82	2.71	0.014	
Total				**0.478**	

Figure 4.2 depicts the AA for each one of the extractions indicating that AA decreased with successive extractions until reach an exhausted SBP. This AA can also be expressed as mg of a reference antioxidant (Trolox) per g of SBP. Using the Trolox standard curves from **Appendix C2**, the AA of SBP was 174.8 μmol TE/g of SBP. This value is higher than that obtained for blackberry residues using ethanol as solvent by means of soxhlet extraction at 100 ºC which is 76.03 μmol TE/g of SBP (Machado et al., 2015).

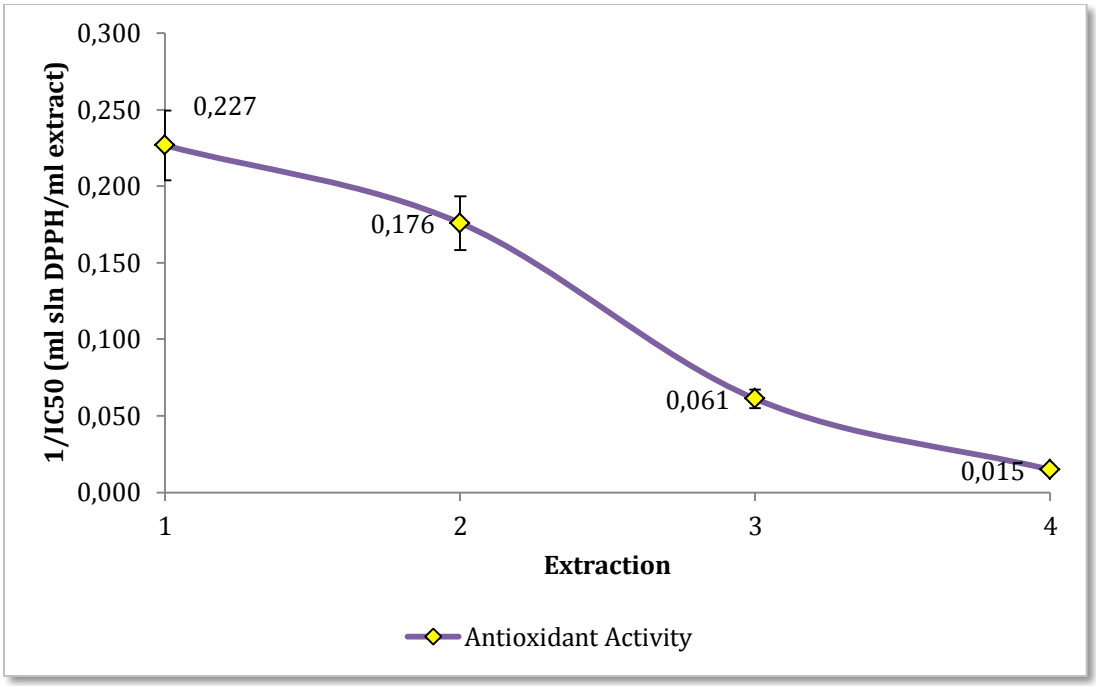


Figure 4.2. 1/IC50 profile for each one of the extractions

The contribution of TPC over AA can be explained by a linear relationship of AA as a function of TPC (Gawron et al., 2012). Figure 4.3 depicts the correlation between $1/IC_{50}$ and TPC of SBP indicating that AA is due in high percentage of TPC. This result suggests that 93.58% of the total antioxidant activity is due to the contribution of phenolic compounds present in SBP. This relationship between AA and TPC is very common for fruits especially for berries. For instance, other authors found a relationship between AA and TPC of 96.5% for blackberry residues (Machado et al., 2015). Other studies have been reported a contribution of 81.1% of TPC on AA for other blackberry species (Gawron et al., 2012).

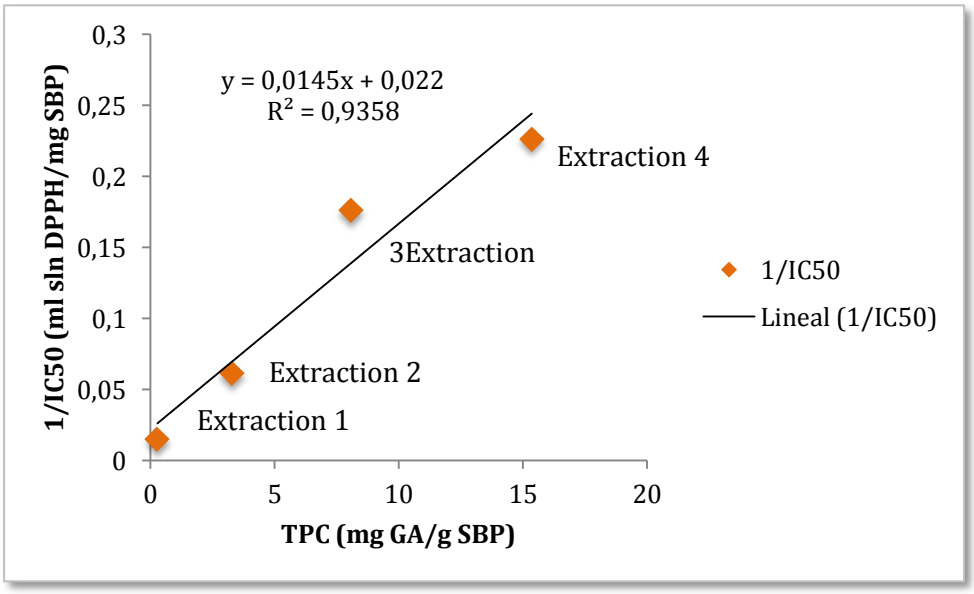


Figure 4.3. Correlation between 1/IC50 and TPC for each one of the extractions

4.1.4 Anthocyanins

Anthocyanins content was determined according to procedure described in chapter 3 using the differential pH method. Table 4.5 shows the measurements for the initial content of anthocyanins which corresponds to the sum of four successive extractions (Similar for TPC and AA). The extractions were made in triplicate. Absorbance, concentration and standard deviation for each one of the extractions and its replicates are also presented in Table 4.5.

Table 4.5. Anthocyanins content (AC) for all extractions and its replicates

Extraction	Repetition	Buffer solution 1 (Potassium Chloride at pH=1.0)		Buffer solution 2 (Sodium Acetate at pH=4.5)		AC (mg/l)	AC (mg/kg SBP)	St. Deviation (in mg/kg SBP)
		$\lambda_{Vis-Max}$	λ_{700}	$\lambda_{Vis-Max}$	λ_{700}			
Extraction 1	Sample 1	0,09	0,103	0,07	0,099	6,68	55,56	2,95
	Sample 2	0,09	0,102	0,08	0,109			
	Sample 3	0,08	0,098	0,05	0,083			
Extraction 2	Sample 1	0,122	0,114	0,130	0,135	5,43	45,14	0,60
	Sample 2	0,120	0,116	0,130	0,138			
	Sample 3	0,120	0,113	0,130	0,137			
Extraction 3	Sample 1	0,040	0,128	0,180	0,275	2,71	22,36	1,94
	Sample 2	0,045	0,189	0,223	0,373			
	Sample 3	0,040	0,109	0,052*	0,1*			
Extraction 4	Sample 1	0,020	0,104	0,250*	0,336*	0,41	3,35	0,06
	Sample 2	0,023	0,109	0,200	0,287			
	Sample 3	0,023	0,139	0,180	0,297			

* This corresponds to values not taken into account for bad measurements

The total content of anthocyanins was 126,41 mg/kg of SBP. The content of anthocyanins in the fourth extraction was found to be insignificant (2.6% of the total anthocyanin content) thus indicating an exhaust SBP without significant amount of anthocyanins. Figure 4.4 depicts the anthocyanins content for each one of the four successive extraction steps.

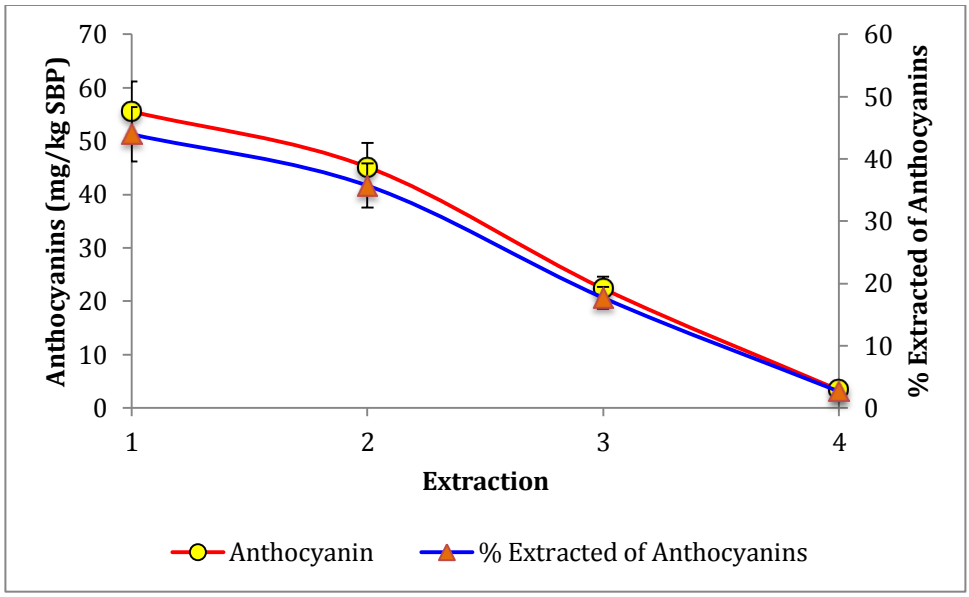


Figure 4.4. Anthocyanins content for each one of the successive extractions

The anthocyanins content is in agreement with data reported for Colombian blackberry. For example, (Garzón et al., 2009) reported a value of 180 mg/kg of fresh fruit from blackberry, (Osorio et al., 2007) found 62.81 mg/kg of fresh fruit of blackberry and (Tamayo et al., 2011) obtained 130.8 mg/kg of fresh fruit of blackberry.

Therefore, SBP becomes in an interesting source of anthocyanins and TPC that can be used for pharmaceutical and food purposes. Thereby, anthocyanins contained in SBP are valuable compounds that can be extracted for further applications like those discussed in chapter 1.

4.1.5 Volatiles profile

Figure 4.5 shows the chromatogram obtained for volatiles profile of SBP using SPME method according to procedure described in chapter 3 (using 1-octanol as internal standard). 2-heptanone, 2-heptanol and benzoic acid were the most significant compounds present in SBP with 1064, 707 and 542 µg/kg of SBP. Despite that a volatiles profile for SBP has not been reported in the available literature, these kinds of compounds also have been found in fresh fruit of blackberry. For instance, 2-heptanol

was reported as the most important compound present in blackberry (*Rubus glaucus benth*) with 5,912 µg/kg of fruit (Ramos et al., 2005). 2-heptanol has also been identified as aroma potential in blackberry (*Rubus glaucus benth*) with 5,597 µg/kg of fruit using the SPME method for volatiles (Meret et al., 2011).

Table 4.6 shows the concentration for each one of the compounds found in the volatiles profile for SBP. The total concentration of volatiles present in SBP was 7,938.87 µg/kg of SBP. This amount is lowest than that reported by (Ramos et al., 2005) with 13,274 µg/kg of blackberry. However, it is expected a lowest amount of volatiles in SBP as in fresh fruit because the possible losses in the fruit processing. **Appendix C3** presents the volatiles profile for naranjilla waste, which was a case of study.

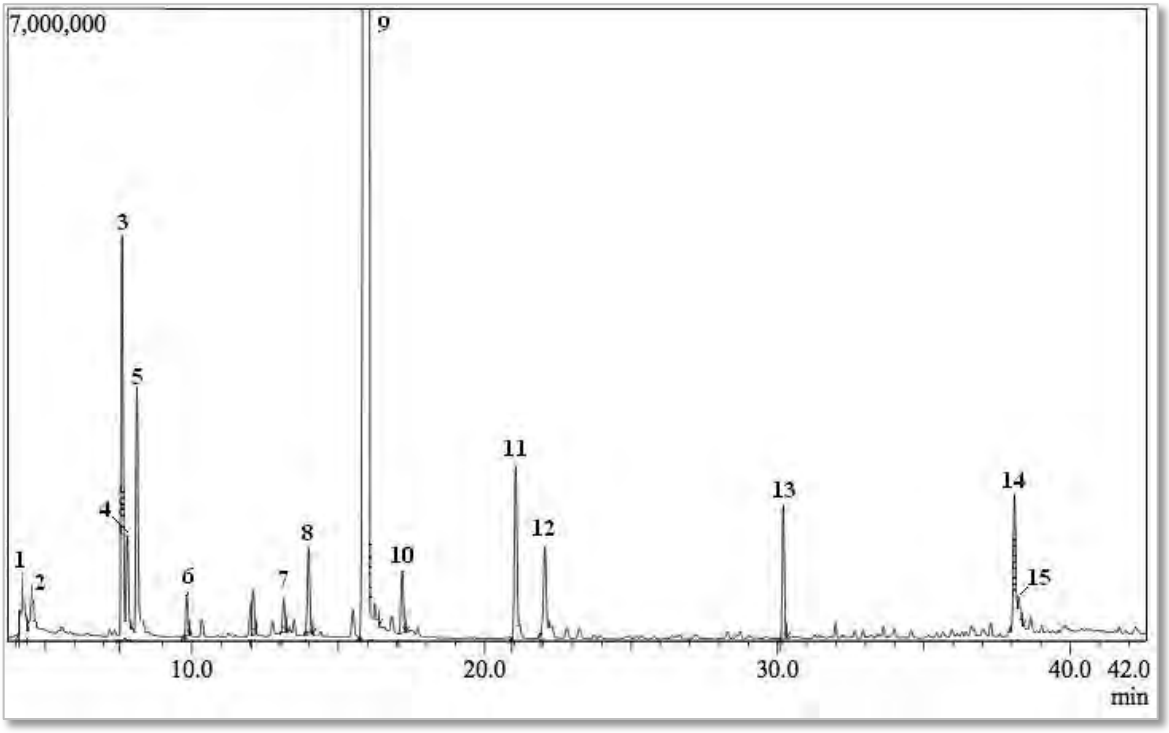


Figure 4.5. Volatiles profile for SBP using the SPME method

Table 4.6. Volatile compounds present in SBP

Peak	Compound	Concentration (µg/kg SBP)
1	Silanediol, dimethyl	249.16
2	1-Butanol, 3-methyl	166.05
3	2-Heptanone	1,064.50
4	Bicyclo[4.2.0]octa-1,3,5-triene	303.80
5	2-Heptanol	706.93
6	Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl	101.40
7	1,3,6-Octatriene, 3,7-dimethyl	91.28
8	Limonene	250.03
9	1-Octanol	19,373.14
10	Cyclohexene, 4-methyl-3-(1-methylethylidene)	184.84
11	Benzoic acid, ethyl ester	542.32
12	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)	301.56
13	Tridecane	345.48
14	Diethyl Phthalate	405.43
	TOTAL *	**7,938.87**

* Total concentration of volatiles is reported avoid the internal standard (1-octanol)

4.2 Chemical characterization for avocado

The chemical characterization of avocado was made according to procedures described in chapter 3. The lignocellulosic biomass of both, avocado peel and seed were characterized while from avocado pulp was obtained both, fatty acid profile and volatiles constituents.

4.2.1 Lignocellulosic biomass

The lignocellulosic biomass of peel and seed of avocado was characterized according to procedures described in chapter 3 based on NREL technical reports (Hames et al., 2008; Han and Rowell, 1997; Sluiter et al., 2008). Tables 4.7 and 4.8 show the chemical composition of avocado peel and seed, respectively.

Table 4.7. Chemical characterization for avocado peel

Compound	Repetition			Content (%)	St. Dev.	% Error
	1	2	3			
Moisture	7,16	7,32	7,52	7,33	0,15	1,98
Extractives	34,12	34,86	34,16	34,38	0,34	0,99
Holocellulose*	53,34	52,56	52,74	52,88	0,33	0,63
Cellulose	27,86	28,86	26,02	27,58	1,18	4,27
Hemicellulose	25,48	23,71	26,72	25,30	1,24	4,90
Lignin	4,41	4,19	4,50	4,37	0,13	2,96
Ash	0,97	1,07	1,08	1,04	0,05	4,58
Total	100	100	100	100		

* Holocellulose corresponds to a cellulose plus hemicellulose

Table 4.8. Chemical characterization for avocado seed

Compound	Repetition			Content (%)	St. Dev.	% Error
	1	2	3			
Moisture	7,21	7,07	6,77	7,02	0,18	2,61
Extractives	33,45	38,21	36,20	35,95	1,95	5,43
Holocellulose*	56,62	52,00	54,48	54,37	1,89	3,48
Cellulose	6,44	6,97	6,04	6,48	0,38	5,91
Hemicellulose	50,18	45,02	48,45	47,88	2,14	4,47
Lignin	1,77	1,84	1,75	1,79	0,04	2,01
Ash	0,95	0,88	0,79	0,87	0,06	7,12
Total	100	100	100	100		

* Holocellulose corresponds to a cellulose plus hemicellulose

The moisture content for both, peel and seed are similar to other reported values of 5.33% and 9.22% respectively (Arukwe et al., 2012). For avocado Hass variety, extractives represent 34.38% and 35.95% (in dry basis) of the total peel and seed, respectively. Similar methanolic extractions (by soxhlet method) of avocado seed were reported to contain flavonoids, steroids, terpenoids, saponins and tanins (Idris et al., 2009). Other compounds such as chlorophyll and carotenoids have been found in avocado peel, it explains the green color of the extracts (Ashton et al., 2006). Both, peel and seed of avocado are attractive sources for obtaining extracts containing phenolic compounds (Osborne, 2014; Warner, 2015).

Results (Tables 4.7 and 4.8) indicated that holocellulose content was 52.88% and 54.37% (in dry basis) of the total biomass of peel and seed of avocado, respectively. Similar content have been reported for avocado peel and seed with 45.74% and 51.21%

respectively (Arukwe et al., 2012). This holocellulose (cellulose and hemicellulose) content suggests some possibilities to be used for reducing sugars production as it has been proposed for other authors (Davila et al., 2014a). The ash content of avocado peel and seed was found to be 1.04% and 0.87% respectively, and was similar to what has been previously reported: 1.50% and 1.29% for peel and seed, respectively (Vinha et al., 2013).

In light of the results, the non-edible parts of avocado (peel and seed) are interesting sources of holocellulose (cellulose and hemicellulose) and they have a potential to be used for obtaining value added products such as reducing sugars. Both, peel and seed of avocado instead of being considered waste can be used as source of important products with added value such as extracts with antioxidant capacity (Davila et al., 2014a; Osborne, 2014; Warner, 2015).

4.2.2 Fatty acids profile

Figure 4.6 depicts a typical chromatogram obtained for fatty acids constituents of avocado pulp. Several fatty acids are contained in the oil extracted from the pulp of avocado and they include acids from C14 to C18. The most common fatty acids of avocado oil were oleic acid (C18:1), palmitic acid (C16:0), linoleic acid (C18:2), palmitoleic acid (C16:1) and linolenic acid (C16:3) with 50.96%, 24.74%, 15.07%, 5.97% and 1.66% of the total fatty acids respectively. Other fatty acids such as myristic, margaric, margaroleic, estearic, arachidic, gadoleic, behenic, lignoceric and other isomers trans of C18 were present only in low proportions.

These results reveal that oleic acid is the main fatty acid present in avocado oil with more than 50% of the total fatty acids such as it is shown in Table 4.9. This composition of fatty acids in avocado pulp is very similar to others reported in literature where oleic acid as the main fatty acid (Acosta, 2011; Pérez-Monterroza et al., 2014; Salesa et al., 2011). Additionally, these authors showed the same distribution of fatty acids that has been found in this research (Figure 4.7).

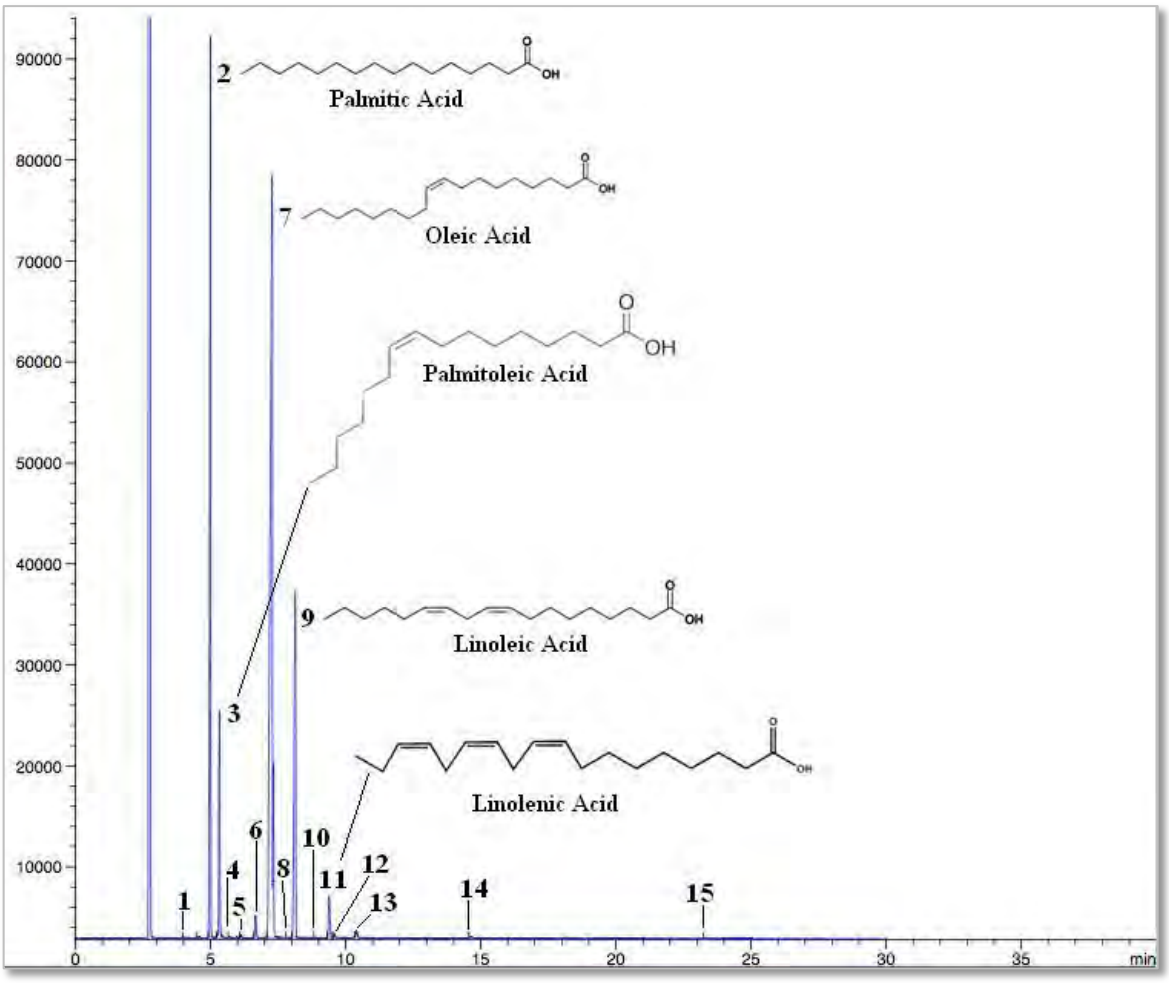


Figure 4.6. Fatty acid profile for avocado pulp

Table 4.9. Chemical composition of fatty acids in avocado oil

No.	Acid	Percentage (%)
1	Myristic Acid (C14:0)	0.05
2	Palmitic Acid (C16:0)	24.74
3	Palmitoleic Acid (C16:1)	5.97
4	Margaric Acid (C17:0)	0.03

5	Margaroleic Acid (C17:1)	0.08
6	Estearic Acid (C18:0)	0.84
7	Oleic Acid (C-C18:1)	50.96
8	Oleic acid (C18:2 CT)	0.02
9	Linoleic Acid (CC-C18:2)	15.07
10	Linoleic acid (C18:3 CCT)	0.04
11	Linolenic Acid (CCC-C18:3)	1.66
12	Arachidic Acid (C20:0)	0.14
13	Gadoleic Acid (C20:1)	0.27
14	Behenic Acid (C22:0)	0.07
15	Lignoceric Acid (C24:0)	0.06

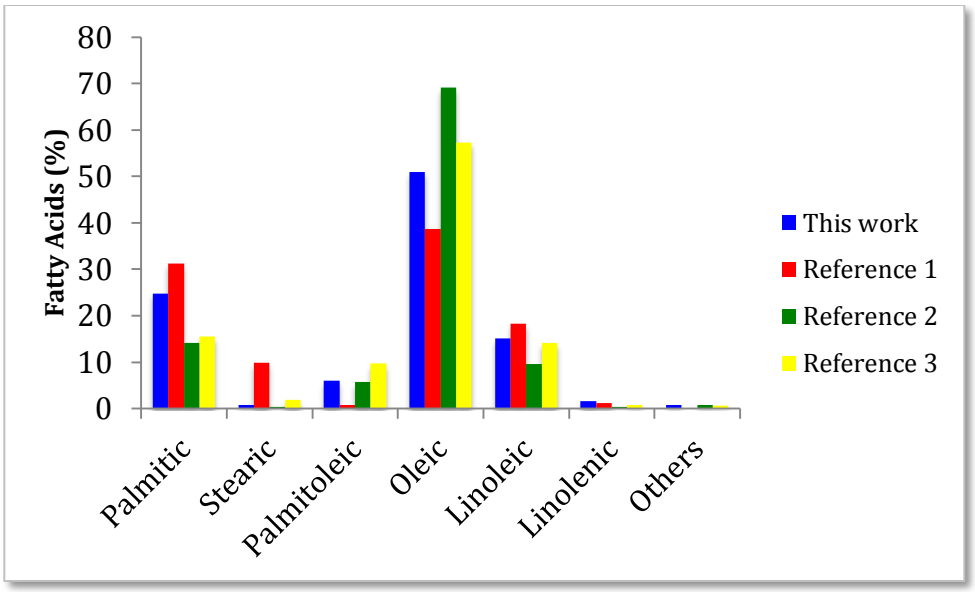


Figure 4.7. Comparison of fatty acids for avocado oil. Reference 1 is (Salesa et al., 2011), reference 1 is (Acosta, 2011) and reference 3 is (Pérez-Monterroza et al., 2014)

This fatty acid composition for oil from avocado is very interesting and agrees with what has been previosely reported. Oil from avocado is considered a valuable product (Adama and Edoga, 2011) therefore, the fatty acid profile confirms that oil from avocado can be of great interest for food industry. However, it is important to note that the fatty acid profile of avocado pulp depends on variables such as variety, season, etc., but the qualitative composiiton does not change significantly showing similar distribution of fatty acids as it is shown in Figure 4.7 (Acosta, 2011; Pérez-Monterroza et al., 2014; Salesa et al., 2011).

4.2.3 Volatiles profile

Figure 4.8 shows the chromatogram obtained for volatiles profile of avocado pulp using SPME method according to procedure described in chapter 3 (using 1-octanol as internal standard). Phthalic acid, propanedioic acid and aromadendren were the most significant compounds present in avocado pulp with 387, 183 and 116 µg/kg of avocado pulp respectively. However, other compounds such as alcohols are present in minor

amounts such as 3-hexenol and 1-hexanol that have been reported as volatiles in avocado mesocarp by other authors (López et al., 2004; Obenland et al., 2012).

Table 4.10 shows the concentration for each one of the compounds found in the volatiles profile for avocado pulp. It was found few volatile compounds in the avocado pulp (809 µg/kg of avocado pulp) in comparison with other reports that found 9.6 mg/kg of fruit pulp. However, other peaks were not identified in this research (See Table 4.10). **Appendix C4** presents the volatile compounds found in peel and seed of avocado.

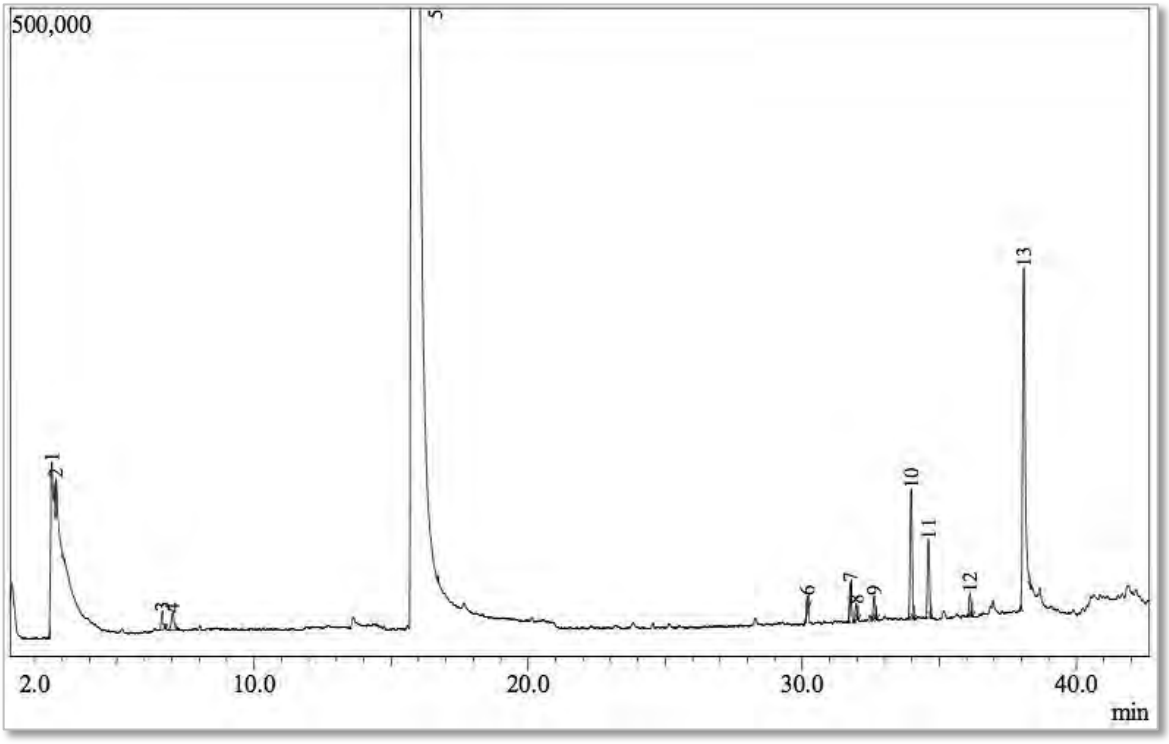


Figure 4.8. Volatiles profile for avocado pulp using the SPME method

Table 4.10. Volatile compounds present in avocado pulp

Peak	Compound	Concentration (µg/kg Avocado pulp)
1	Propanedioic acid, dihydroxy	183.14

3	3-Hexen-1-ol	18.76
4	1-Hexanol	21.40
5	1-Octanol	19,373.14
8	Cyclopropyl carbinol	10.85
10	Aromadendrene	116.36
11	trans-.alpha.-Bergamotene	71.16
13	Phthalic acid, di-(1-hexen-5-yl) ester	387.26
	TOTAL *	**808,94**

Peaks 2, 6, 7, 9 and 12 were not detected

* Total concentration of volatiles is reported avoid the internal standard (1-octanol)

Conclusions

In light of the results from chemical characterization for spent blackberry pulp and avocado (including pulp, peel and seed), it is clear that these fruits can be used as feedstocks for obtaining valuable compounds. Spent blackberry pulp has a potential chemical composition for obtaining extracts containing phenolic compounds and anthocyanins that have antioxidant activity. Besides, spent blackberry pulp has more than 60% of holocellulose (cellulose and hemicellulose) that can be used for sugar production by means of hydrolysis processes. Moreover, simulations developed in later chapters will define the feasibility of spent blackberry pulp as feedstock based not only on chemical characterization but also in the production, availability and more.

Avocado has an important composition of fatty acids the attractive properties of this oil for food applications. On the other hand, the non-edible parts of avocado (peel and seed) have more than 50% of holocellulose therefore both, peel and seed of avocado become in source of valuable compounds using hydrolysis processes.

Thereby, according to the chemical characterization made for spent blackberry pulp and avocado, it is possible to consider them as attractive candidates for obtaining valuable compounds using the biorefinery concept.

5. Chapter 5. Experimental assessment of main valuable compounds production from avocado and spent blackberry pulp

Overview

This chapter presents the results obtained from several essays for obtaining value added products from avocado and Spent Blackberry Pulp (SBP). Avocado pulp was used for obtaining oil using thermo-mechanical method. Oil extracted from avocado pulp presented high content of oleic acid (more than 50%). Extracts using Supercritical Fluid Extractions (SFE) from SBP were obtained. These extracts were characterized obtaining its total phenolic compounds (TPC) content. The effect of variables such as particle size of SBP, pressure and co-solvent (ethanol) to solid ratio in the SFE were evaluated over TPC of the extracts. The simplified Sovova's model was used to correlate the experimental yields of SFE and thus, calculate solubility and the mass transfer coefficient. To validate the experimental results for solubility of TPC in CO_2-ethanol mixture at supercritical conditions, the Peng Robinson Equation of State (PREOS) was used. Finally, extracts from SBP obtained using SFE were concentrated using Ultrafiltration (UF) process. The extract containing 2.47 g GA/l was concentrated up to final concentration of 6.6 g GA/l. On the other hand, the membrane separation efficiency was 52.87%.

The holocellulose content of SBP as well as peel and seed of avocado were used for reducing sugar production by means of both, acid and enzymatic hydrolysis. The results demonstrated that the hydrolysis in two steps (acid and enzymatic) allows producing reducing sugars from fruit wastes reaching high yields (until 15.1 g/l).

Finally, the results presented in this chapter suggest that it is possible to obtain value added products under biorefinery concept using the entire fruit including peel and seed as well as the fruit wastes. Value added products such as extracts (dilute and

concentrated) containing phenolic compounds, oil free of solvents and reducing sugars are some of the possible products with value added that can be obtained from fruit wastes.

5.1 Oil from avocado pulp

The avocado oil was extracted from the pulp using a thermo-mechanical method as it was explained in chapter 3. For this extraction was obtained 40 ml of oil from 264.43 g of pulp. The latter corresponds to a yield of 14.28% (w/w). Similar results have been obtained for similar techniques reporting yields from 1.8% to 11.6% using hot water and manual pressing (Kameni and Tchamo, 2003). Other authors have reported 41 ml of oil per avocado by mechanical pressure (IDDS, 2014). The slightly higher temperature used aids the extraction of the oil from the oil-containing cells and does not affect the quality of the oil (Wong et al., 2010). Thermo-mechanical method has the advantage that it is not necessary to remove solvents. This fact (oil free of solvents) allows a cheaper method in comparison with soxhlet method in which the solvent recovery can be expensive. Thus, solvent extraction is not attractive for oil extraction from avocado pulp taken into account that this method can modify the physical and chemical characteristics of the final product in addition to the separation of the solvent used and its cost associated (Moreno et al., 2003).

The yield obtained from avocado using thermo-mechanical method (14.28%) in this research is lower in comparison with other reported for Colombian avocado (variety Hass) using other methods such it is shown in Figure 5.1. Supercritical fluid extraction (SFE) method can recover a high percentage of oil from avocado pulp but it is necessary the use of CO_2 and a special equipment that have to support high temperatures and pressures (Restrepo et al., 2012). Solvent extraction allows obtaining similar yields to those for SFE but it is necessary the separation and recovery of solvent (Restrepo et al., 2012). Enzymatic treatment for oil extraction of avocado oil has the highest yields however, the use of an enzyme becomes in a disadvantage because the cost associated to this (Buelvas et al., 2012). The cold pressing method allows obtaining similar yields to other techniques but it is necessary to have special equipment that works at 2000 psi

(136 bar) of pressure as well as a previous lyophilization of the avocado pulp (Serpa et al., 2014).

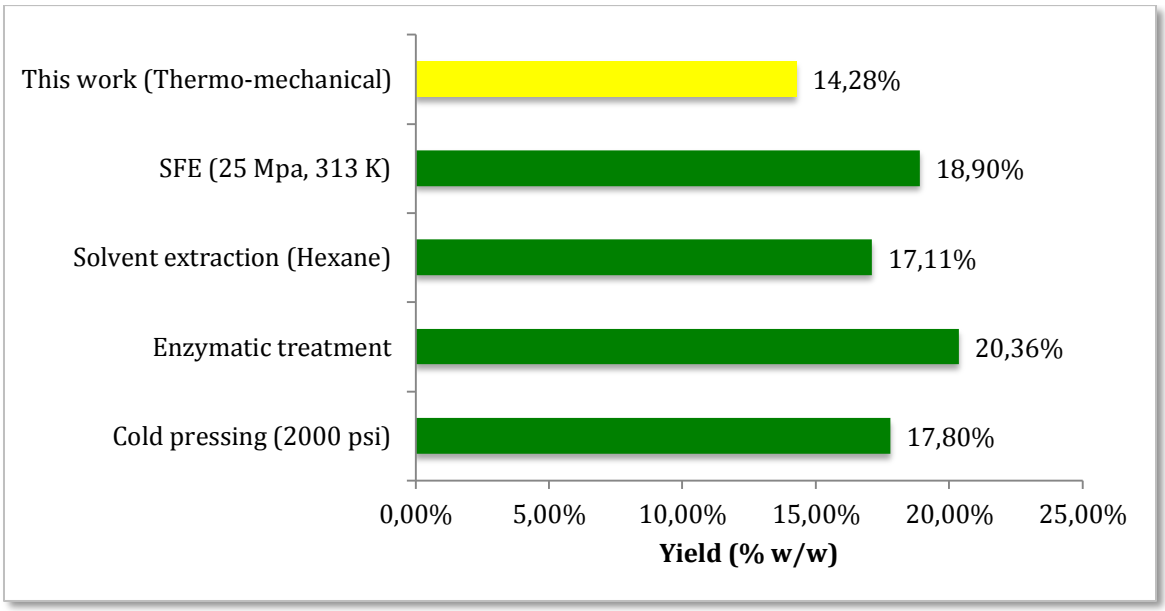


Figure 5.1. Extraction yields of avocado oil for several methods. Supercritical fluid extraction (SFE) and solvent extraction (Restrepo et al., 2012). Enzymatic extraction (Buelvas et al., 2012). Cold pressing (Serpa et al., 2014).

Despite that thermo-mechanical method has lowest yield than other reported, this method permits to obtain unrefined and extra-virgin pressed oil from avocado pulp that allows maintaining the bioactive phytochemicals present in the fruit (Berasategi et al., 2012). Therefore, as other authors have been reported, thermo-mechanical method becomes in a promising way to obtain oil from avocado (Basuni, 2013).

5.2 Sugar production from lignocellulosic biomass of spent blackberry pulp, peel and seed of avocado

Sugar production was carried out according to procedure described in chapter 3. Table 5.1 shows the reducing sugar concentration produced after acid hydrolysis (**Appendix C4** shows the calibration curves for reducing sugar).

Table 5.1. Reducing sugar concentration after acid hydrolysis

Sample	Absorbance	Concentration (g/l)
SBP	0.425	0.925
Avocado peel	0.578	1.313
Avocado seed	1.709	4.184

Avocado seed presented the highest sugar production from all the fruit wastes evaluated while SBP leads to the lowest sugar production. This can be attributed to the fact that avocado peel has a higher content of hemicellulose (47.88%) than SBP (19.80%). Besides, because in an acid hydrolysis, the hemicellulose gives rise to several pentoses and hexoses (Taherzadeh and Karimi, 2008) then, a high content of holocellulose resulting in increased production of reducing sugars. Similar results have been reported for banana peels, obtaining 2.3 g/l of glucose using 1.5% of H_2SO_4 at 110 ºC for 2 hours (Chongkhong and Doromae, 2012). In another report, 9.9 g/l of xylose was obtained from oil palm empty fruit bunches, using 2% of H_2SO_4, 100 ºC and 90 minutes (Rahman et al., 2007). Glucose concentration ranging from 0.09 to 0.15 g/l was obtained using acid hydrolysis of apple pomace at 90ºC and 1.5% of H_2SO_4 (Parmar and Rupasinghe, 2012). Residues from coconut milk production (coconut dregs) have been used for glucose production using dilute acid hydrolysis at 130 ºC, 60 minutes and 1% of H_2SO_4

(Bujang et al., 2013). Despite that the reducing sugars production using SBP as well as avocado peel and seed are similar to those reported in the literature for fruit wastes, these amounts are lower than those obtained using lignocellulosic biomass from crops such as sugarcane bagasse, corncob, olive tree pruning and corn stover which are higher than 20 g/l (Behera et al., 2014). However, dilute acid hydrolysis enhances the cellulose accessibility to enzymes and substantially increases the yield due to the removal of hemicellulose from lignocellulose (Jung and Kim, 2015).

The sugars released after dilute acid hydrolysis of agroindustrial residues depend on the type of waste (lignocellulosic material), composition of substrates and operational conditions such as temperature, acid concentration and time (Chandel et al., 2012). Because dilute acid hydrolysis was carried out to obtain the maximum sugar production to be used in fermentations, high temperatures and acid concentrations (concentrate acid hydrolysis) were avoided. Besides, dilute acid hydrolysis can reduce the inhibitors production, which in turn, becomes in advantage for fermentation processes (Duque et al., 2015). Therefore, dilute acid hydrolysis becomes in an attractive option for pretreating lignocellulosic biomass of fruit wastes before enzymatic hydrolysis.

After dilute acid hydrolysis, enzymatic hydrolysis was carried out in order to enhance the production of reducing sugar as it was explained in chapter 3. Table 5.2 presents the results obtained from enzymatic hydrolysis.

Table 5.2. Reducing sugar concentration after enzymatic hydrolysis

Sample	Absorbance	Concentration (g/l)
SBP	0.306	13.41
Avocado peel	0.348	15.10
Avocado seed	0.318	13.90

From these results, it is evident that for fruit wastes it is necessary the use of two steps hydrolysis for increasing the reducing sugar yield. The first step of hydrolysis (dilute acid

hydrolysis) contributes from 6.89% to 30.1% of the total reducing sugars produced while enzymatic contributes with the remaining such as it is shown in Figure 5.2.

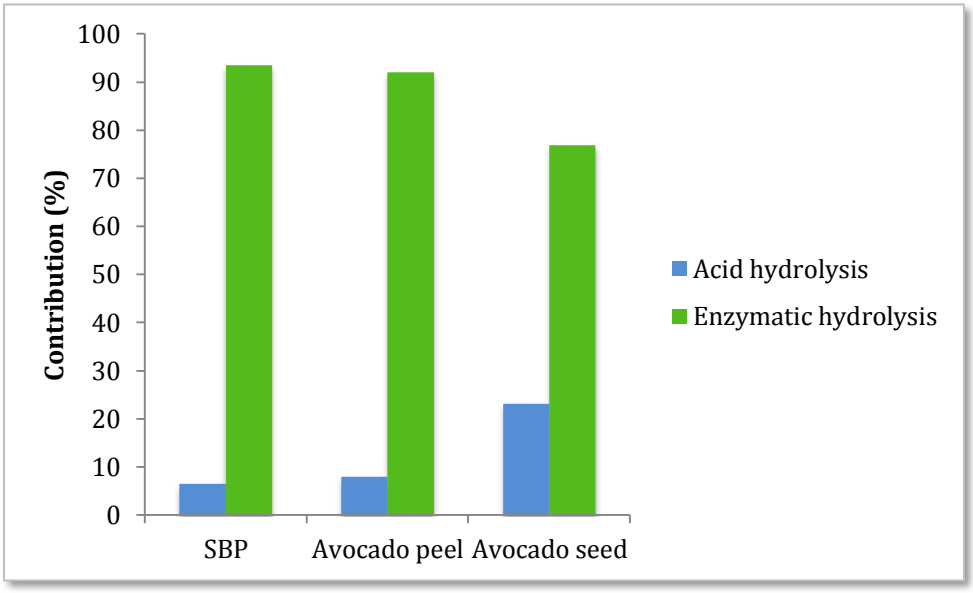


Figure 5.2. Contribution of acid and enzymatic hydrolysis over total reducing sugars production

The first step (dilute acid hydrolysis) allows obtaining more accessible cellulose for the second step (enzymatic hydrolysis) because the hemicellulose is hydrolyzed (Jung and Kim, 2015; Taherzadeh and Karimi, 2008). The second step (enzymatic hydrolysis) releases more reducing sugars and increases the total yield of the hydrolysis processes up to 70% regard to first step. These two steps are very common when hydrolyze cellulosic materials for obtaining reducing sugars for other applications such as bioethanol production is considered (Idrees et al., 2014). Therefore, when agroindustrial wastes (such as fruit wastes) have to be hydrolyzed it is necessary to consider two steps hydrolysis, acid and enzymatic to obtain the highest yield from the biomass (Quintero et al., 2011).

According to the results obtained for sugar production, fruit wastes become a low cost agroindustrial residue that is available for reducing sugar production (Davila et al., 2014a; Dávila et al., 2014). The use of these kinds of fruit wastes allows obtaining value added products such as reducing sugars that, in turn, become a platform for producing other products such as organic acids, ethanol, biomaterials (Polyhydroxybutirate) and other compounds with several applications (Dávila et al., 2014).

5.3 Extracts of phenolic compounds from spent blackberry pulp

Extracts containing phenolic compounds were obtained by means of supercritical fluid extractions (SFE) using supercritical CO_2 as it was exposed in chapter 3. Spent Blackberry Pulp (SBP) was subjected to SFE for obtaining extracts containing phenolic compounds with antioxidant activity.

The initial content of total phenolic compounds (TPC), anthocyanins (A) and antioxidant activity (AA) associated to these compounds were exposed and analyzed in chapter 4. Table 5.3 shows the initial content of TPC as well as A and AA for SBP found by chemical characterization.

Table 5.3. Chemical characterization for spent blackberry pulp

Property	Value	Standard Deviation
TPC	2700 mg GA/100 g of SBP	±0.16
TAA (IC_{50})	2.09 mg SBP/ml DPPH solution	±0.81
Anthocyanins	126.41 mg/kg of SBP	±1.39

As was discussed in chapter 4, TPC content contributes with more than 93% on AA of the SBP. However, the yield of SFE of these compounds (phenolic compounds) depends strongly on the solubility of they in the solvent used (For this case, a mixture of supercritical CO_2 and ethanol as co-solvent). Below is explained the effect of particle size of SBP, pressure and co-solvent to solid ratio over TPC extraction.

5.3.1 Effect of particle size of SBP over extraction of TPC

Particle size affected not only the TPC but also the AA of the extracts. Figures 5.3 and 5.4 show the effect of particle size of SBP on the TPC and AA of the extracts respectively. For TPC, the best particle size was 600 µm (Mesh 30) while for AA was 850 µm (Mesh 20). Despite that the extraction from fine particles could be easy because the large area per unit volume that represents a reduction in resistance to internal mass transference by diffusion, these results suggest that the mass losses increase with the reduction of particle size such it was reported for the extraction of oil from microalgae using SFE (Mouahid et al., 2013). It was expected that smallest particle sizes of SBP lead to a highest extraction yield. However for particle size below of 600 µm the extraction yield of TPC is decreased. This is due to the fact that the particle size has to be limited because an exceedingly small particle tend to agglomerate leading to a decrease of solvent penetration in the solid matrix and therefore, negatively affecting the mass transfer process such it happened for extraction of total phenolic content from olive leaves (Stamatopoulos et al., 2014).

On the other hand, the maximum AA was reached at 850 µm and smallest particle sizes reduce a little bit the AA. Because 93% of the AA of the extracts is attributed to the TPC as it was found and explained in chapter 4, then, the same behavior is expected for AA that for TPC. Thereby, possible agglomerations formed with small particle sizes affect in the same way to both, TPC and AA.

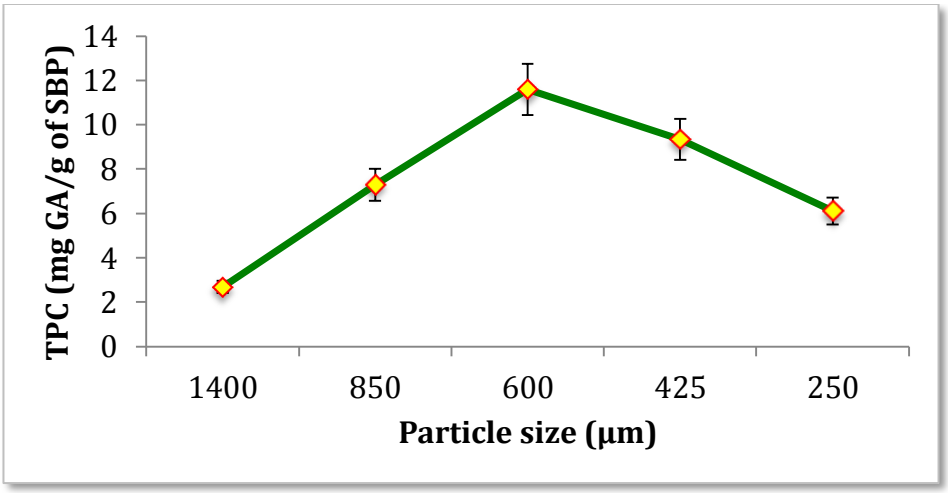


Figure 5.3. Effect of particle size of SBP over TPC of the extracts

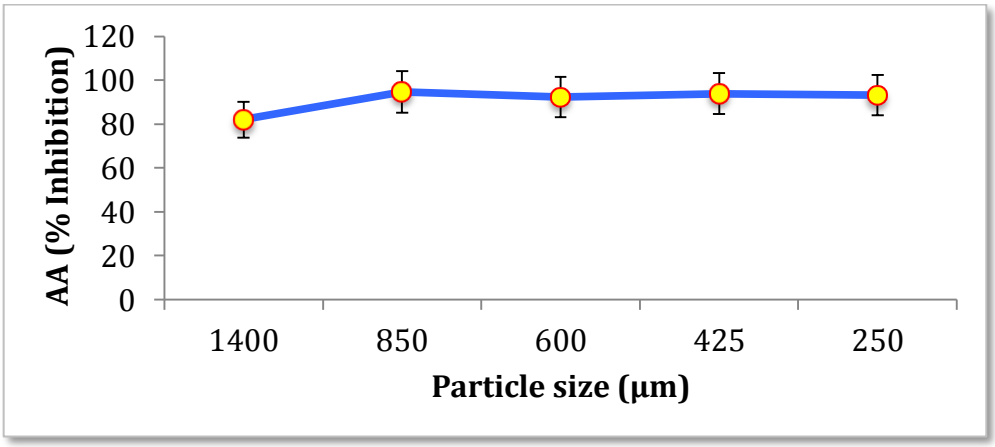


Figure 5.4. Effect of particle size of SBP over AA of the extracts

5.3.2 Effect of pressure and co-solvent to solid ratio over extraction of TPC

Once the particle size effect was evaluated, 850 µm (Mesh 20) was chosen as the suitable size for the remaining extractions. Figure 5.5 depicts the effect of pressure and co-solvent to solid ratio over TPC extraction.

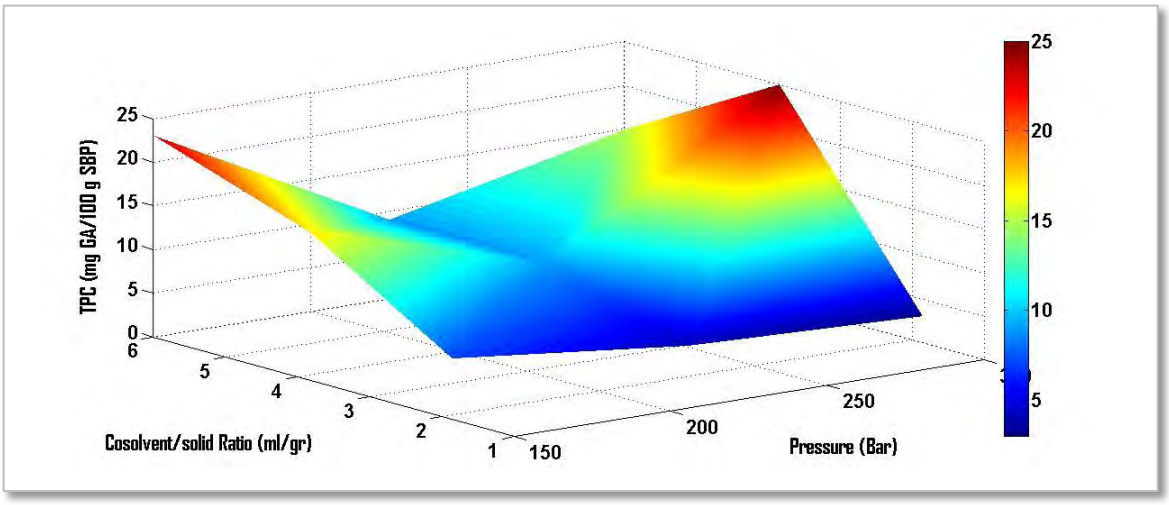


Figure 5.5. Effect of pressure and co-solvent to solid ratio on SFE of TPC

At high pressures (300 bar), the extraction of TPC increased from 3 mg GA/100 g of SBP to 2500 mg GA/100 g of SBP when co-solvent to solid ratio increases from 2 to 4. However, at low pressures (150 bar), it is a need to increase the co-solvent to solid ratio because it increases significantly the phenolics extraction. The maximum yield according to initial content of TPC of SBP (2700 mg GA/100 g of SBP) is 92.6%. Therefore, the CO_2 modified with ethanol becomes one of the most suitable and promising solvents for SFE of phenolic compounds (Santos et al., 2012). For SFE, the extraction yield of high molecular weight compounds (such as phenolic compounds) increases when pressure increases (Zulkafli et al., 2014). Table 5.4 shows the analysis of variance ANOVA developed by the tool of analysis of data of Excel using a significance level of 0.05.

Table 5.4. ANOVA for co-solvent to solid ratioand pressure over TPC extraction

Source of variation	SS	DF	MS	F	P	F_c
Co-solvent to solid ratio	274.889	2	137.444	4.7760	0.087	6.9442
Pressure	120.222	2	60.111	2.0888	0.239	6.9442
Error	115.111	4	28.777			
Total	510.222	8				

SS is sum of squares, DF is degrees of freedom, MS is mean sum of squares, F is F value, P is p value and Fc is critical F value.

According to the ANOVA results, it is not a significant difference between co-solvent to solid ratio as well as for pressures measurements (Because F calculated is highest than Fc). In other words, the different levels of both, co-solvent to solid ratio and pressure behave similarly over TPC of the extracts.

Taking into account that a high pressure could be more economic to reach than the cost of ethanol (for a high co-solvent to solid ratio), the use of high pressure (300 bar) and intermediate co-solvent to solid ratio (4) were defined as the most convenient conditions for the extraction of TPC from SBP.

5.3.3 Experimental solubility and mass transfer coefficient for SFE of SBP

For solubility and mass transfer coefficient calculation, a simplified Sovova's model (Sovová, 2005) was used. This model adopts a simplification of the mass balance to obtain an analytical solution and assumes that the extractable solute is partitioned into accessible solute that corresponds to the solute contained in the broken solid particles and hardly accessible solute that corresponds to the amount of solute, in the intact solid particles, that was not broken after mechanical pre-treatment, such as size reduction operation (Dos Santos et al., 2013; Hrncic et al., 2010; Sovová, 2005). This model describes the extraction curve by means of three segments; the first is related to the extraction of accessible solute and is represented by a linear behavior where the slope can be taken as the solubility of the solute in the solvent. The second segment is a transition where the rate of extraction drops substantially, and the third segment represents the extraction of hardly extractable solute where the extraction rate is small (Sovová, 2005). The simplified Sovova's model allows obtaining the solubility and the total mass transport coefficient from a set of experimental data that are represented in two periods of extraction. The first and second periods are represented by equations (5.1) and (5.2) respectively.

$$e = qy_s \quad for \quad 0 < q < q_c \tag{5.1}$$

$$e = x_u[1 - C_1\exp(-C_2 q)] \quad for \quad q > q_c \tag{5.2}$$

Where e is the extraction yield (kg extract/kg insoluble solid), q is the relative amount of the passed solvent (kg solvent/Kg insoluble solid), y_s is the solubility of the phenolic compounds in the solvent which corresponds to the slope of the first period of the extraction, x_u is the concentration of TPC in the initial solid (kg solute/kg insoluble solid), q_c is the relative amount of solvent that represents the transition between the two periods of extraction and C_1 and C_2 are constants to be estimated and used for the mass transfer coefficient ($k_s a_s$) and grinding efficiency (r) or fraction of broken particles according to equations (5.3) and (5.4) respectively.

$$k_s a_s = (1 - r)(1 - \varepsilon)\dot{Q}C_2/N_m \tag{5.3}$$

$$r = 1 - C_1\exp(-C_2 q_c/2) \tag{5.4}$$

Where ε is the bed void fraction (0.42 measured with a picnometer), Q is the solvent flow rate (kg/s) and N_m is the mass of insoluble solid (kg). From the experimental data of e (yield) and q (solvent used) after the extractions, the extraction curve was plotted and the solubility and mass transfer coefficient were calculated.

Figure 5.6 depicts the extraction curve of TPC from SBP. The slope (y_s) of the first extraction period corresponds to the solubility of phenolic compounds in the solvent, which was 0.00812 kg solute/kg solvent. The solubility in mol fraction is 4.69×10^{-4} (Taken GA as reference component). This value keeps up the trend of the solubility of GA at other pressures reported in the literature for SFE using CO_2 at supercritical conditions. Figure 5.7 depicts the solubility of TPC (expressed as GA) in CO_2 at different pressures. The solubility calculated in this research is compared with those reported by (Sanjaya et al., 2014) at different pressures.

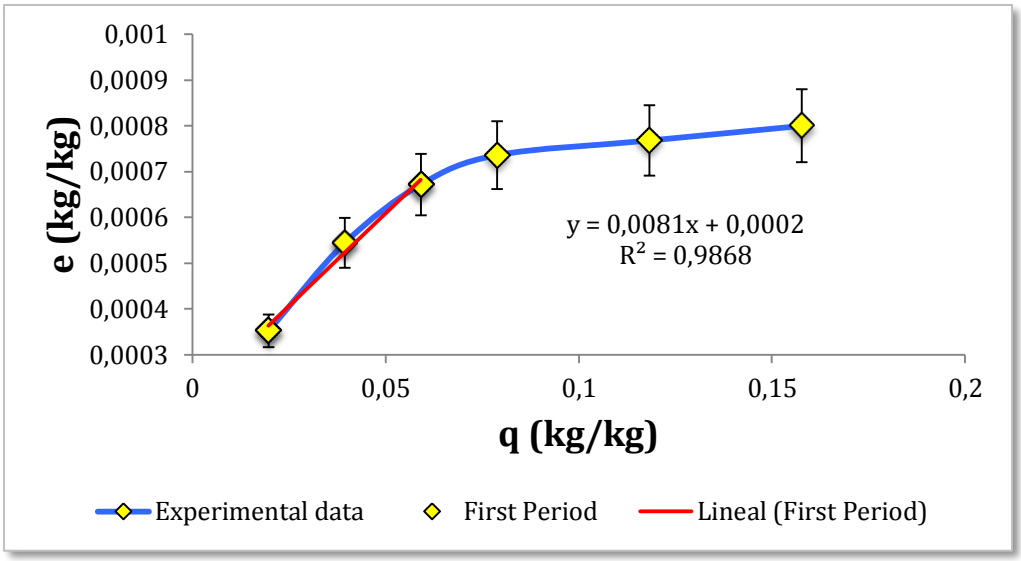


Figure 5.6. Extraction curve for TPC from SBP

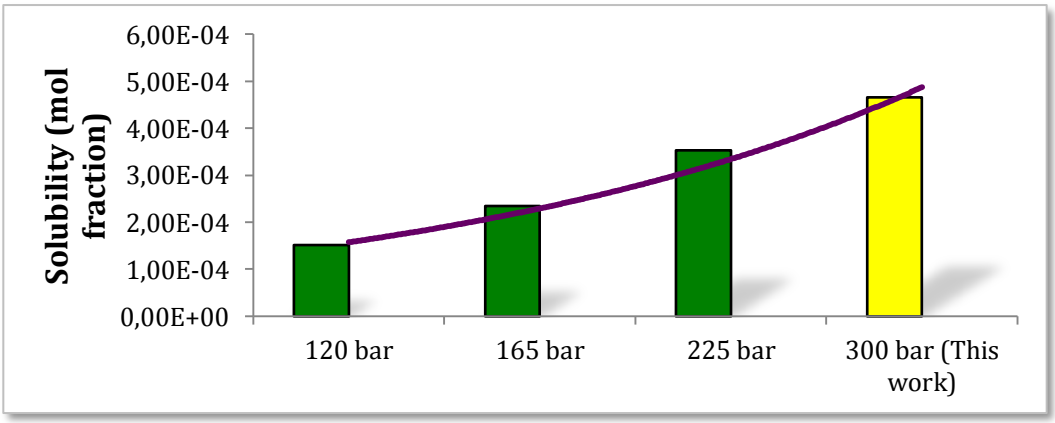


Figure 5.7. Solubility of TPC represented as GA in a mixture of supercritical CO2 and ethanol

From the second period equation, the mass transfer coefficient ($k_s a_s$) and the fraction of broken particles (r) were calculated. Table 5.5 shows the mass transfer coefficients and fraction of broken particles reported for some extracts obtained using SFE as well as for this research. The mass transfer coefficient found for SBP extracts in this research is similar to other reported for similar extracts however the fraction of broken particles was

poor. This fact suggests that the milling pretreatment of the SBP before SFE is poorly efficient therefore the phenolic compounds delivered (solute contained in the broken solid particles) after milling process are not enough and the highest amount of TPC keep up in the intact solid particles. This is proved by the mass transfer coefficient calculated which was very low in comparison with other values reported for similar extracts (as it is shown in Table 5.5) therefore the extraction of TPC is more difficult and it is reflected in a low amount of TPC extracted.

Table 5.5. Mass transfer coefficients and fraction of broken particles for some SFE. N.R. means Not Reported

Extracts	Conditions	$K_s a_s$ (s^{-1})	r	Reference
Bioactive enriched extracts	300 bar, 45ºC, CO_2-Ethanol	$1.11x10^{-6}$ – $1.73x10^{-6}$	0.17-0.22	(Solana et al., 2014)
Bioactive compounds	200 bar, 40ºC, CO_2-Ethanol	$1.25x10^{-7}$	0.23	(Martín et al., 2011)
Extracts with antioxidant activity	300 bar, 40 ºC, CO_2	$3x10^{-5}$	N.R.	(García-Risco et al., 2011)
Propolis	250 bar, 40 ºC, CO_2-Ethanol	$1x10^{-4}$	N.R.	(Biscaia and Ferreira, 2009)
Extracts with antioxidants	300 bar, 35 ºC, CO_2-Ethanol	$4.34x10^{-5}$	N.R.	(Leitão et al., 2013)
Extracts with phenolic compounds	300 bar, 40 ºC, CO_2-Ethanol	$2.769x10^{-7}$	0.0235	This work

5.4 Concentrated extracts of phenolic compounds from spent blackberry pulp

Concentrated extracts from SBP were obtained according to procedure explained in chapter 3. After supercritical fluid extraction (SFE) conducted at the best conditions found in section 5.3 (300 bar, 40ºC and co-solvent to solid ratio of 4), SBP extract was subjected to an ultrafiltration (UF) process to concentrate the extract. The extract obtained from SFE had a volume of 277 ml and TPC content of 6950 mg GA/100 g of SBP (2474.61 mg GA/l). The samples of retentate and permeate were characterized according to their content of total phenolic compounds (TPC). Each 4.5 hours was tested the TPC of both, retentate and permeate as well as the volume obtained for each one of them. Figure 5.8 depicts the TPC of both, retentate and permeate tested each 4.5 h until complete 18 h and Figure 5.9 shows pictures of both, permeate and retentate obtained for each 4.5 h of UF.

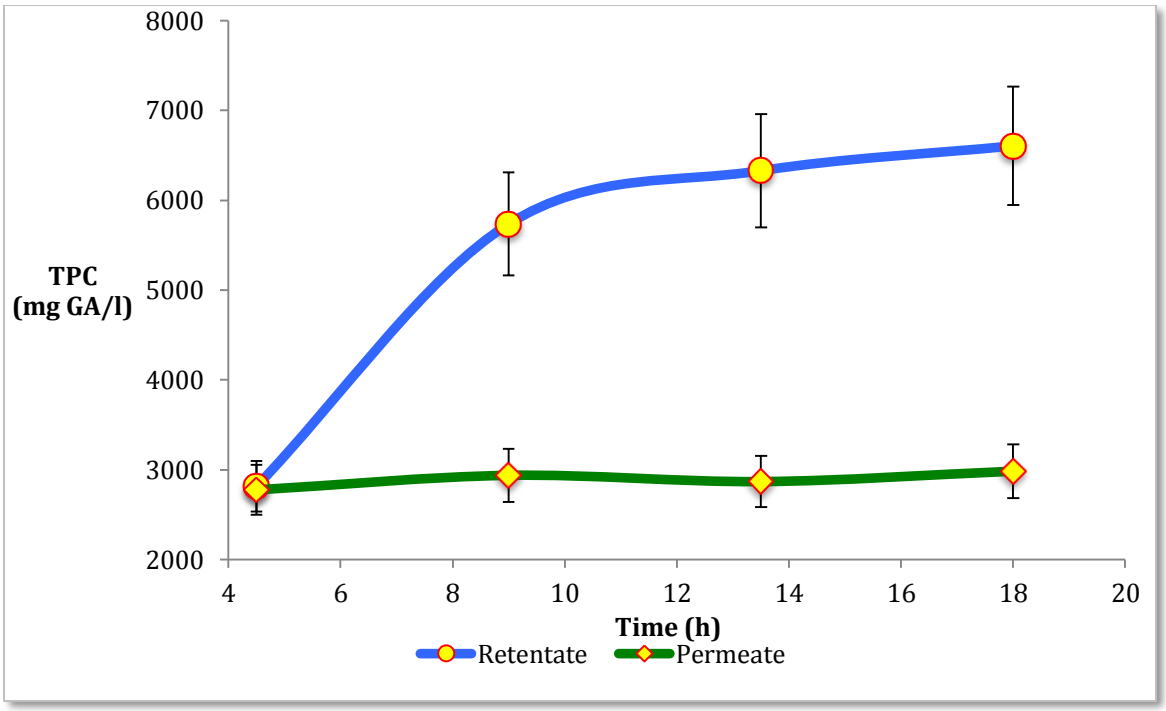


Figure 5.8. TPC of retentate and permeate for UF process

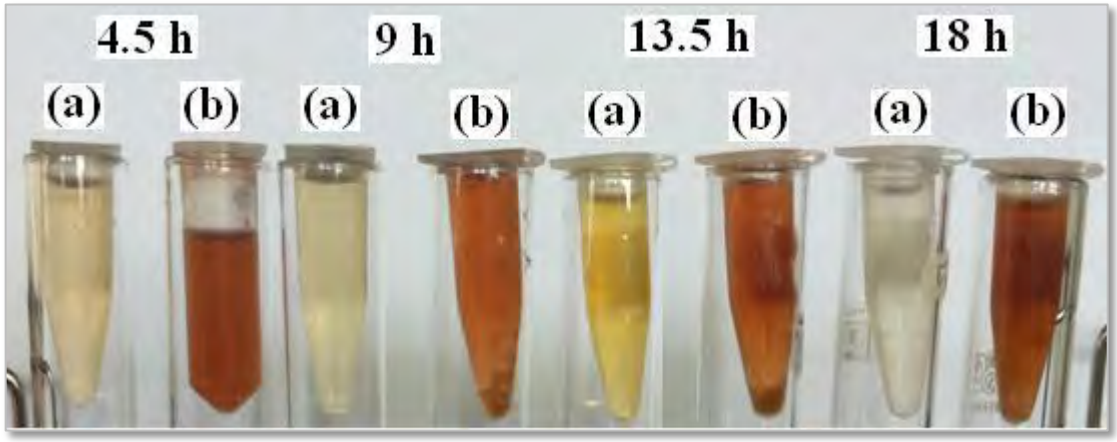


Figure 5.9. Pictures of retentate and permeate obtained for UF process. (a) is permeate and (b) is retentate

The phenolic compounds of the extract were concentrated in the retentate as it was expected because the retention of polyphenols in the retentate is high (Liu et al., 2011; Schafer, 2001). According to this, the extract was concentrated in the retentate and the

highest concentration reached was 6605.38 mg GA/l that corresponds to a 62.54% of concentration regard to initial content (after SFE) of TPC (2474.61 mg GA/l). However, after 9 h of UF, was reached 56.86% of concentration from the initial content of TPC therefore, subsequent ultrafiltrations may be not considered because only 4.32% of concentration will be reached as it is shown in Figure 5.10.

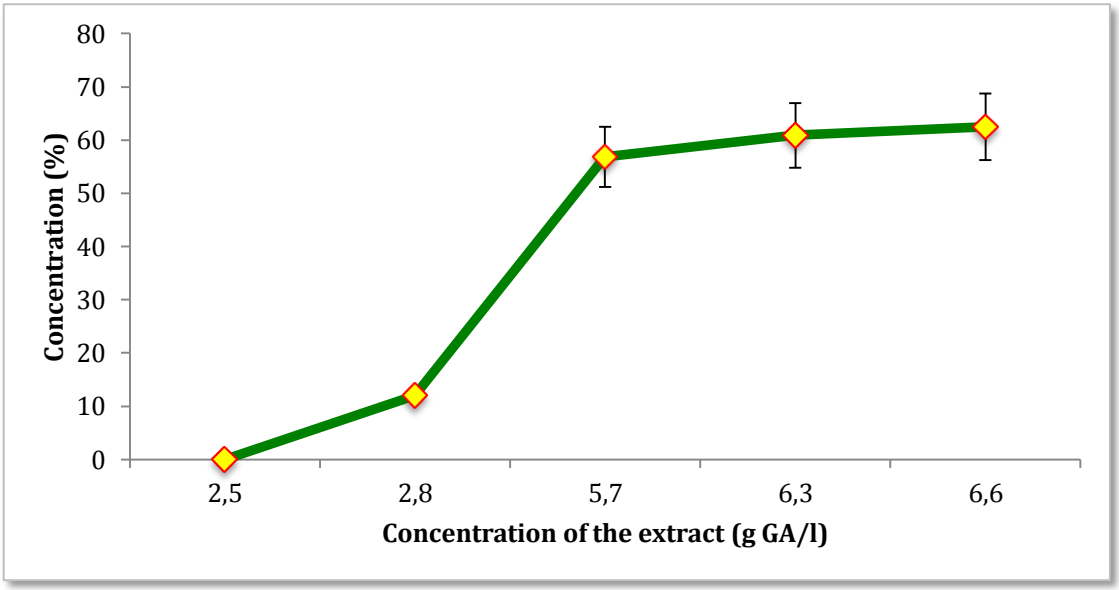


Figure 5.10. Percentage of concentration reached in each 4.5 h of UF

The Volume Retention Factor (VRF) was a criteria for stop UF processes and it was calculated as initial feed volume (V_0) divided by retentate volume (V_R) by equation (5.5) (El-Abbassi et al., 2014).

$$VRF = \frac{V_0}{V_R} \quad (5.5)$$

The VRF reached was 1.87 that is very similar to that used for bioactive compounds concentration using UF processes (Cassano et al., 2008; Cassano et al., 2009; Dawale

and Jawade, 2014; Pap et al., 2010; Pinto et al., 2014), Figure 5.11 depicts the VRF along the UF process.

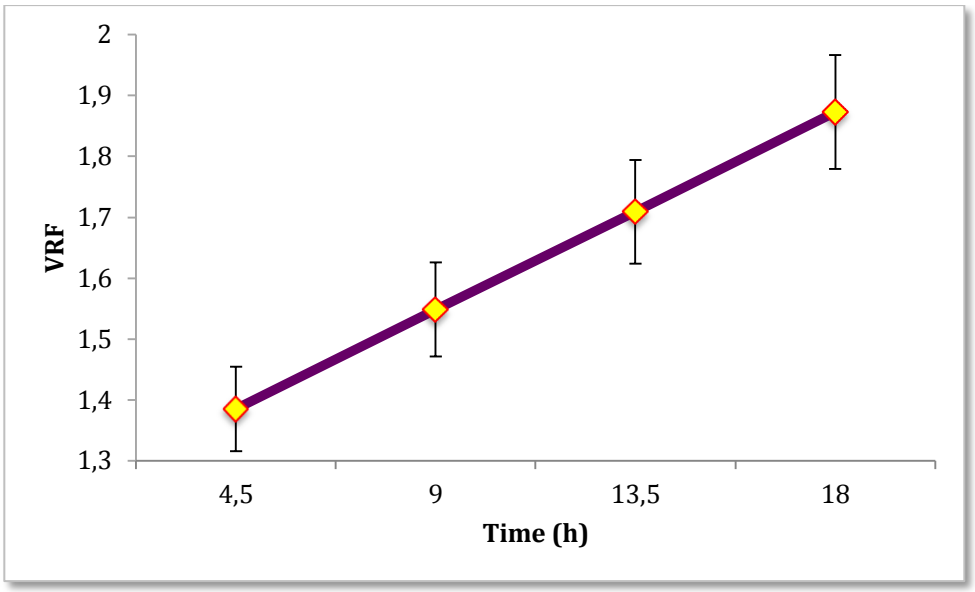


Figure 5.11. VRF along of UF process

The membrane separation efficiency (R) of UF that is also known as rejection of the UF membrane towards specific compounds was calculated by equation (5.6) where C_P and C_F are the concentrations in the permeate and feed respectively (Cassano et al., 2009).

$$R = \left(1 - \frac{C_P}{C_F}\right) * 100 \quad (5.6)$$

The R value reached for UF for SBP extract was 52.87%. This value is low in comparison to those reported for concentration of similar extracts. For instance, 98% and 90% of retention have been obtained for concentration of ellagitannins and anthocyanins respectively from blackberry extracts using UF processes and membrane of 5 kDa (Acosta et al., 2014). 85% of retention has been obtained for TPC concentration from ethanol/water extracts using a membrane of 5 kDa (Pinto et al.,

2014). However, for ethanol/water extracts from Eucalyptus globulus bark, retentions from 61.7% to 72.7% have been obtained for TPC (Baptista et al., 2015). The reason of low membrane separation efficiencies is because ethanol/water mixtures affect significantly the performance of the membrane. As it was demonstrated for (Baptista et al., 2015), the presence of organic solvents (such ethanol) decreases the retention of TPC, therefore, the low retention of TPC in UF process for SBP is due to the presence of ethanol in the extract. This fact also explains why from juice of blackberry it is possible to obtain more than 90% of retention (Acosta et al., 2014).

The permeate flux is an indicative of the performance of the UF process (Destani et al., 2013). The permeate flux should decrease gradually with the operating time due to the accumulation of components (TPC) in the pores of the membrane (membrane fouling). Figure 5.12 depicts the flux of both, permeate and retentate of the UF process for SBP extract.

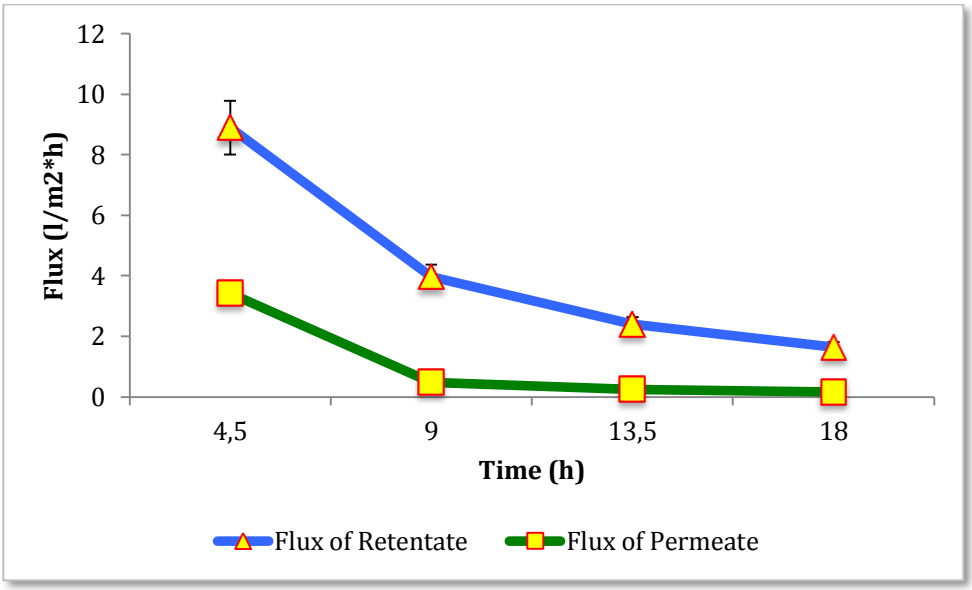


Figure 5.12. Permeate and retentate flux along of UF process

In the first 9 hours, the permeate flux was reduced by 86.3%. This fact strengthens the idea that after this time of UF, subsequent ultrafiltrations may be not considered. The behavior obtained for permeate flux is very similar to those reported for concentration of

TPC by UF process. For instance, for UF process for orange juice, a reduction of 78.6% of the permeate flux has been obtained in 8.33 h (Destani et al., 2013). For concentration of bioactive compounds from kiwifruit juice using UF, a reduction of 75.7% of the permeate flux has been obtained after 10.25 h (Cassano et al., 2008).

In light of the afore-mentioned results, it is clear that UF process applied for concentrating extracts from SBP is an attractive option to added value of these extracts. After 9 h of UF process, it is not attractive to follow with subsequent ultrafiltrations for the SBP extracts because at this point, it is reached 56.86% of concentration regard to the initial concentration. Figure 5.13 depicts the volume obtained along the time for UF process. The retentate volume is reduced gradually (almost in a linear manner) while the significant reduction of permeate volume is obtained at 9 h. The latter suggests that after 9 h the concentration is not significantly important as was discussed above.

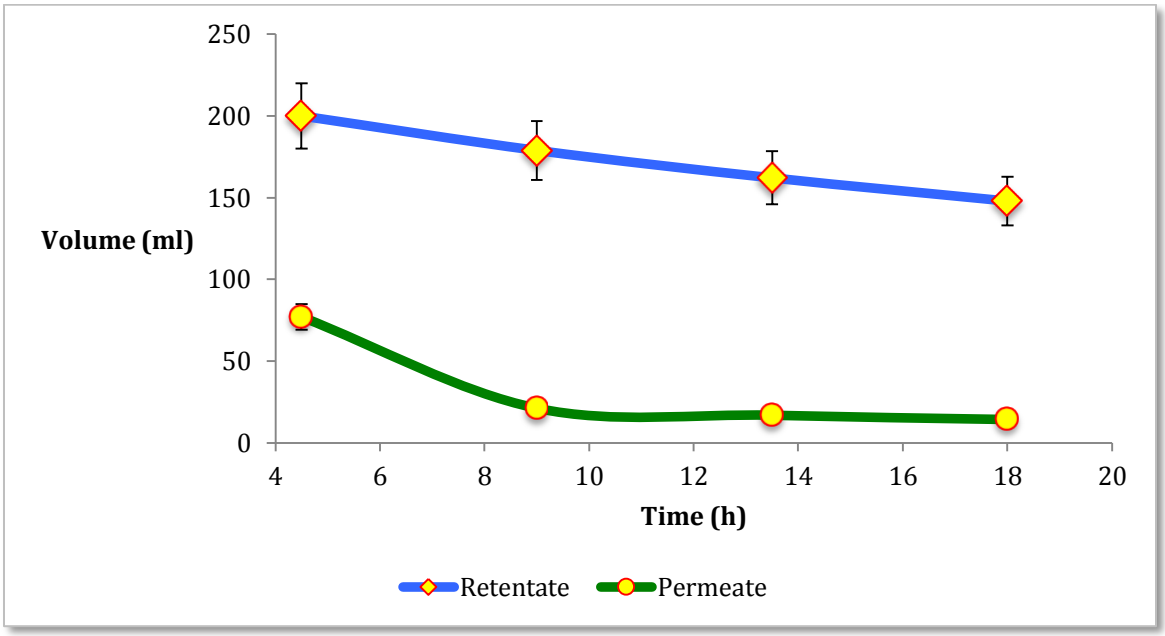


Figure 5.13. Volumes of retentate and permeate for UF process

5.5 Validation of experimental results for solubility of phenolic compounds in supercritical CO_2 using a thermodynamic approach

For comparison purposes, it is calculated the solubility of phenolic compounds (represented as GA) of SBP in supercritical CO_2 using a thermodynamic approach. The Peng-Robinson Equation of State (PREOS) (Peng and Robinson, 1976) was used for calculating solubility. This equation has been used to describe the thermodynamic behavior of complex systems at high pressures and is suitable for cases involving small molecules (Peng and Robinson, 1976). The PREOS is widely used as a good approximation tool for calculating phase equilibrium in CO_2 systems (Murga et al., 2002). The PREOS and its variables are presented in Table 5.6 as well as the equations that were used to calculate the fugacity of a solid component (GA) in a mixture (CO_2 and ethanol). The equations 5.7 through 13 were used to calculate the volume of the mixture while equations 5.14 and 5.15 were used as a polynomial expression to solve the densities of both liquid and vapor phases and the factor compressibility (Z). The term a(T) represents the intermolecular attractive forces while the term b is the volume parameter related to molecular size. These terms were calculated from the pure component properties and the mixing rules of Wong Sandler (Peng and Robinson, 1976). After obtaining the Z factor, it was necessary to calculate the fugacity coefficient in order to calculate the phase equilibrium and thus equation 5.16 was used to calculate the fugacity coefficient of a solid component in a solvent.

Table 5.6. PREOS and its parameters for solubility calculation

Equations	
$P = \frac{RT}{V-b} - \frac{a(T)}{V(V+b)+b(V-b)}$	(5.7)
$a_i(T) = a_i(T_{Ci})\alpha_i(T_{ri}, \omega_i)$	(5.8)
$a_i(T_{Ci}) = 0.45724\frac{R^2T_{Ci}^2}{P_{Ci}}$	(5.9)

$$\alpha_i(T_{ri},\omega_i) = \left[1 + \left(0.37464 - 1.54226\omega_i - 0.26992\omega_i^2\right)\left(1 - T_{ri}^{1/2}\right)\right] \quad (5.10)$$

$$b_i = 0.0788\frac{RT_{Ci}}{P_{Ci}} \quad (5.11)$$

$$a(T) = \sum_{i=1}^{n}\sum_{j=1}^{n} x_i x_j (1 - k_{ij})\sqrt{a_i(T)a_j(T)} \quad (5.12)$$

$$b = \sum_{i=1}^{n}\sum_{j=1}^{n} x_i x_j (1 - l_{ij})\left(\frac{b_i + b_j}{2}\right) \quad (5.13)$$

$$Z^3 - (1-B)Z^2 + (A - 3B^2 - 2B)Z - (AB - B^2 - B^3) = 0 \quad (5.14)$$

$$A = \frac{aP}{R^2T^2}; \quad B = \frac{bP}{RT}; \quad Z = \frac{PV}{RT} \quad (5.15)$$

$$\ln(\phi_i) = \frac{b_i}{b}(Z-1) - Ln(Z-B) - \frac{A}{2b\sqrt{2}}\left(\frac{2\sum_j x_j a_{ij}}{a} - \frac{b_i}{b}\right) Ln\left(\frac{Z+(1+\sqrt{2})B}{Z+(1-\sqrt{2})B}\right) \quad (5.16)$$

$$\phi_S^P \cong \frac{F_S^P}{x_S P} \quad (5.17)$$

$$\phi_S^P \cong \frac{f_S^P}{x_S^* P} \quad (5.18)$$

$$\frac{f_S^P}{f_S^{P0}} = exp\left(\int_{P^0}^{P} \frac{V_S^{Sol}}{RT} dP\right) \quad (5.19)$$

$$x_S^* = \frac{f_S^{P^0}}{\phi_S^P P} exp\left(\int_{P^0}^{P} \frac{V_S^{Sol}}{RT} dP\right) = \frac{K(T)}{\phi_S^P P} exp\left(\int_{P^0}^{P} \frac{V_S^{Sol}}{RT} dP\right) \quad (5.20)$$

In order to model the solubility of a solute in a compressible solvent mixture, the fugacity coefficient of the solute in the solution (ϕ_s^P), which is defined with its fugacity in the solution (F_s^P), mol fraction (x_s) and pressure (P) by equation 5.17 are needed. At the equilibrium mole fraction (x_s^*), the fugacity in the solution can be substitute for the fugacity of the pure solid solute (f_s^P) as is shown in equation 5.18. However, the effect of pressure on the fugacity of the solid could be significant and thus it is necessary to introduce the Poynting factor, expressed by equation 5.19. Where V_s^{sol} is the molar volume of the solid, which was taken as 199 cm^3/mol and P^0, is the atmospheric pressure (Cháfer et al., 2006a; Wubbolts et al., 2004). With the substitution of the Poynting factor it is possible to obtain an expression for the solubility of the solid solute in a solvent mixture as is described in equation 5.20. When modeling the solubility of a solid in a dense gas, the fugacity of a pure solid (f_S^{Po}) or K(T) can be approximated by its

vapor pressure (Wubbolts et al., 2004), therefore, K(T) was taken as $1.2x10^{-7}$ mm Hg (GuideChem., 2015). Table 5.7 depicts the physical constants: molecular weight (MW), critical temperature (Tc), critical pressure (Pc) and acentric factor (ω) that were used in PREOS to model phase behavior and to calculate the solubility (Cháfer et al., 2006a; Cortesi et al., 1999; Kordikowski et al., 1995b).

Table 5.7. Physical constants for phase behavior. Taken from (Cháfer et al., 2006b; Cortesi et al., 1999; Kordikowski et al., 1995a)

Component	Formula	M.W. (g/mol)	Tc (K)	Pc (MPa)	ω
Carbon Dioxide	CO_2	44.01	304.19	7.38	0.225
Ethanol	C_2H_5OH	46.07	516.25	6.38	0.637
Gallic Acid	$C_7H_6O_5$	170.12	881.28	8.87	1.14

Figure 5.14 shows the solubility of GA as a function of pressure at different molar fraction of ethanol (co-solvent) in the mixture, according to the PREOS. The solubility increases with the molar fraction of ethanol in the modified solvent (CO_2 - Ethanol) and as could be anticipated, the solubility increases with the pressure. Although the solubility calculated according to Sovova's model was higher than the solubility calculated by PREOS, they are in the same range ($1x10^{-4}$ in molar fraction). It is clear that the use of a modified solvent (CO_2 - Ethanol) is attractive in the extraction of phenolic compounds by SFE because ethanol has an important role in affecting the solubility of the solute and therefore on the overall extraction efficiency.

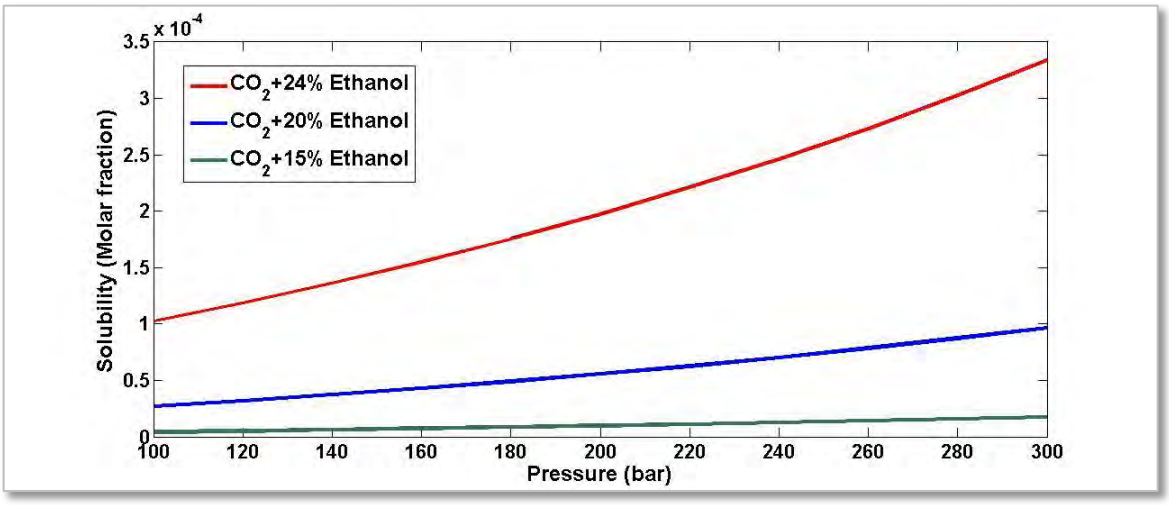


Figure 5.14. Solubility of GA in CO2-Ethanol mixture at high pressures

Conclusions

This chapter demonstrated that it is possible to obtain value added products from SBP and avocado. From SBP, the promising value added products are related with the extracts that contain phenolic compounds with antioxidant activity. However, the holocellulose content (cellulose and hemicellulose) is also a potential feedstock to be used for reducing sugar production for other purposes such as for ethanol production. The extracts from SBP are attractive as value added product using supercritical fluid extractions (SFE) because in this way, it is possible to obtain extracts free of solvent thereby, SFE are also a promising technique for its use for obtaining extracts from fruit wastes.

On the other hand, avocado oil obtained by thermo-mechanical method becomes in an attractive way because it presented similar yields (14.28 w/w) to those reported in the literature for similar techniques. Additionally, this method offers free solvent oil that can be used for food applications due to its content of oleic acid that confers similar properties that olive oil as was exposed in chapter 1.

Regard to the holocellulose content of SBP as well as avocado peel and seed, they should be used for sugar production because its yields found. Sugar production essays demonstrated that it is necessary two steps hydrolysis for obtaining reducing sugars. The first step (acid hydrolysis) is used for hydrolyze the hemicellulose and to provide a best accessibility to a cellulose content. The second step (enzymatic hydrolysis) increases significantly the reducing sugar production.

Finally, UF processes also proved that it is possible to concentrate extracts from SBP giving value added to these extracts. UF essays showed that after 9 h it is possible to obtain a good concentration of TPC in the retentate achieving 56.86% of the concentration regard to initial content of TPC of the extract. The membrane separation efficiency was affected significantly by the ethanol/water mixture and suggests that maybe the UF process should be carried out using a volume retention factor highest to 1.87.

In the light of all the exposed in this chapter, the results suggest that SBP and avocado peel and seed can be used as feedstock under biorefinery concept for obtaining value added products that permit to obtain an integral use of the feedstock. Products such as extracts containing phenolic compounds, oils for food and chemical applications, reducing sugars as platform for producing other important compounds and concentrated extracts can be obtain from SBP and avocado.

6. Chapter 6. Experimental assessment of main valuable compounds production from naranjilla waste and spent coffee grounds

Overview

This chapter presents the results obtained from several essays for obtaining value added products from naranjilla waste and Spent Coffee grounds (SCG). Extracts obtained using Supercritical Fluid Extractions (SFE) from naranjilla wastes were obtained. These extracts were characterized for total phenolic compounds (TPC) content and antioxidant activity (AA).

SCG was used for obtaining oil using both, pressing and solvent methods. For oil extraction from SCG, solvent (hexane) extraction was the most promising technique because its high yields (16.46 ml of oil/g of SCG). The acid Index for oil obtained from SCG and from both methods (pressing and solvent) indicates that this oil is an attractive candidate to be used as feedstock in the biodiesel production.

The holocellulose content of naranjilla waste as well as other peel residues (orange, mandarin and pineapple for comparison purposes) was used for reducing sugar production by means of both, acid and enzymatic hydrolysis. The results demonstrated that the hydrolysis in two steps (acid and enzymatic) allow producing reducing sugars from fruit wastes reaching high yields (until 18.85 g/l).

Finally, the results presented in this chapter suggest that it is possible to obtain value added products under biorefinery concept using naranjilla wastes and SCG. Value added products such as extracts containing phenolic compounds, oil for biodiesel production and reducing sugars for obtaining other important products are some of the possible products with value added that can be obtained from naranjilla wastes and SCG.

6.1 Extracts of phenolic compounds from naranjilla waste

Extracts containing phenolic compounds were obtained by means of supercritical fluid extractions (SFE) using supercritical CO_2 as discussed in chapter 3. For obtaining extracts from naranjilla waste (which is a case study) the best conditions found for SBP (300 bar, 40ºC and co-solvent to solid ratio of 4 and 65 min of extraction in static mode) were used. This residue was milled to obtain particles that pass mesh 20 (850 µm) sieve and are retained on mesh 80 (180 µm). These particles were subjected to a SFE to obtain extracts with phenolic compounds with antioxidant activity.

Figure 6.1 depicts the total phenolic compounds (TPC) content of the extract obtained from naranjilla waste, which corresponds to 252 mg GA/100 g of naranjilla waste compared with other reported in the literature. This value is higher than that reported (83.6 mg GA/100 g of naranjilla peel) for naranjilla peel using soxhlet extraction with methanol-water mixture (1:1) (Cerón et al., 2010). Other authors have reported 505 mg GA/100 g of residue of naranjilla using solvent (ethanol:hexane) extraction (Gancel et al., 2008). Similar yields have been obtained for extracts obtained from apple peels using static SFE obtaining total phenolics of 550 mg/100 g of apple peel (Massias et al., 2015).

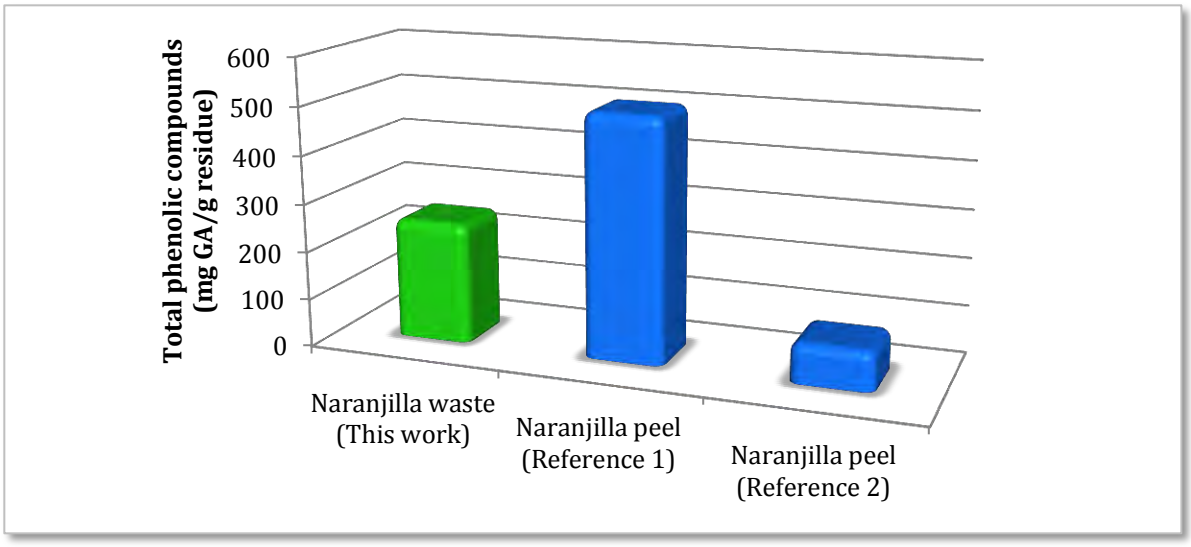


Figure 6.1. Total phenolic compounds in the extract. Reference 1 is (Gancel et al., 2008). Reference 2 is (Cerón et al., 2010)

From these results, it is evident that the technology used affects significantly the TPC extracted. Solvent extraction using methanol results in a higher extraction of TPC but with the disadvantage that it is necessary separate the solvent. Figure 6.2 depicts the AA of the extract obtained from naranjilla waste for this research and other reported in the literature. The AA was 0.4855 μmol Trolox/g of naranjilla waste. This amount is high in comparison to that reported for extracts from Colombian naranjilla using solvent (hexane) extraction (0.15 μmol Trolox/g of naranjilla peel) (Mertz et al., 2009).

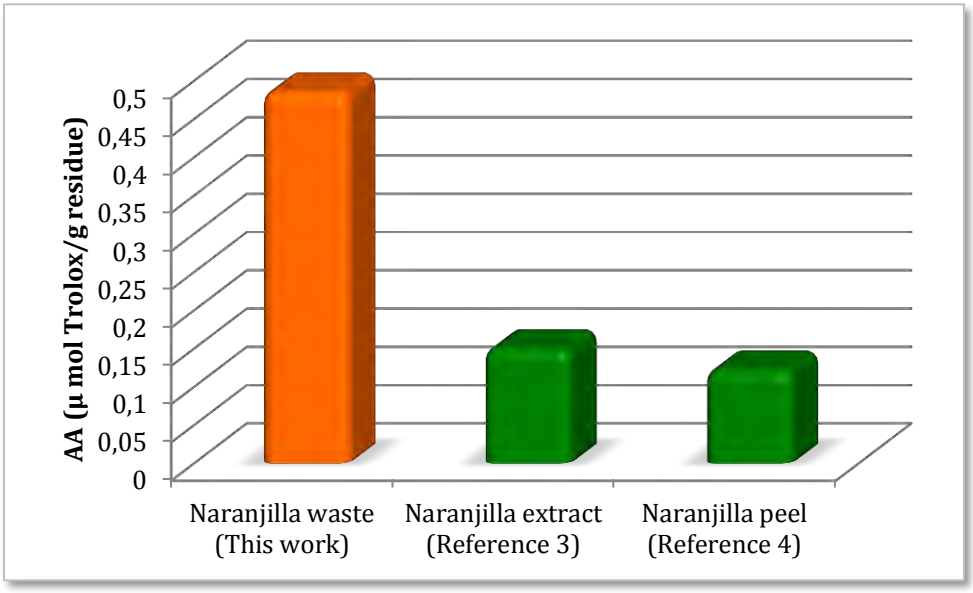


Figure 6.2. Total antioxidant activity of the extract. Reference 3 is (Mertz et al., 2009). Reference 4 is (Contreras et al., 2011).

Antioxidant capacity obtained of naranjilla waste from supercritical fluid extraction was higher than other reported in the literature, which was obtained using solvent extractions. As it was found by (Acosta et al., 2009), naranjilla extracts have highest antioxidant capacities in comparison with other fruits such as banana, pineapple. However, the low value of TPC of extracts from naranjilla wastes (252 mgGA/100 g of naranjilla waste) is lower in comparison with that obtained for SBP (2700 mg GA/100 g SBP) discussed in chapter 5. The latter can be attributed to the small particles sizes (a mix of particles that pass mesh 20 (850 µm) sieve and are retained on mesh 80 (180 µm)) because as it was discussed for extracts from SBP (in chapter 5), smallest particles sizes contribute to clumping phenomena that decrease the solvent penetration in the solid matrix and therefore, it becomes in a resistance in the mass transfers in SFE (Stamatopoulos et al., 2014).

These kinds of extracts from naranjilla wastes have been evaluated for producing valuable compounds such as extracts containing phenolic compounds reporting a total production cost of 4.43 USD/kg of extract (Davila et al., 2014b). This fact suggests that here is a need of search new applications of naranjilla waste that permit to use those valuable compounds such as phenolic compounds for interesting applications.

6.2 Oil from spent coffee grounds

The extraction of oil from spent coffee grounds (SCG) was made according to procedures explained in chapter 3. Oil was extracted using both, solvent (at 90, 120 and 160ºC) and pressing (at 50, 70 and 90ºC) methods. Figure 6.3 shows the oils obtained for both methods at different temperatures.

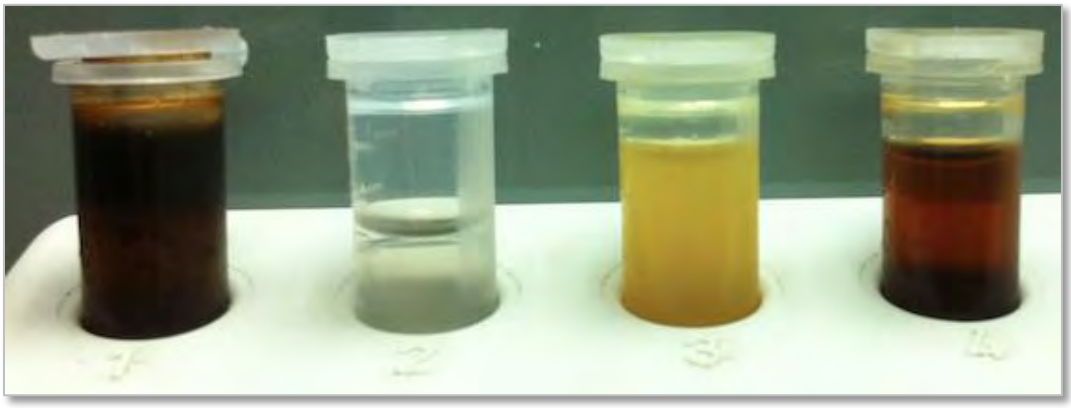


Figure 6.3. Types of oils obtained for several temperatures using pressing and solvent extractions. 1 was obtained by pressing at 60 bar at any of the temperatures evaluated (50, 70 or 90ºC). 2 was obtained using hexane at 90ºC. 3 was obtained using hexane at 120 or 160ºC after decanting for 2 hours. 4 was obtained using hexane at 120 or 160ºC evaporating immediately the solvent.

It is clear that the type of extraction method leads to a several kinds of oils. Therefore, according to the possible final application, it is the need of determines a specific extraction method. For pressing method, the oil has the same appearance (brown color) at any temperature however; the high temperatures increase the extraction yield from 0.86 ml of oil/100 g of SCG (at 50ºC) to 2.25 ml of oil/100 g of SCG (at 90ºC). In the case of solvent extraction, different colors of oil can be obtained according to the temperature used and treatment after extraction. When 90ºC is used for solvent

extraction, clear oil is obtained while highest temperatures are used (120 and 160ºC) oils have yellow and brown color respectively (See Figure 6.3). However, for clear oil, the yield is the lowest (0.21 ml of oil/100 g of SCG) while for yellow and brown colors, the yields are 11.17 and 16.46 ml of oil/100 g of SCG respectively. Figure 6.4 depicts the behavior of extraction yield (ml of oil/g SCG) vs. temperature for both, pressing and solvent methods. Table 6.1 presents the yields in (w/w) for both, pressing and solvent extractions.

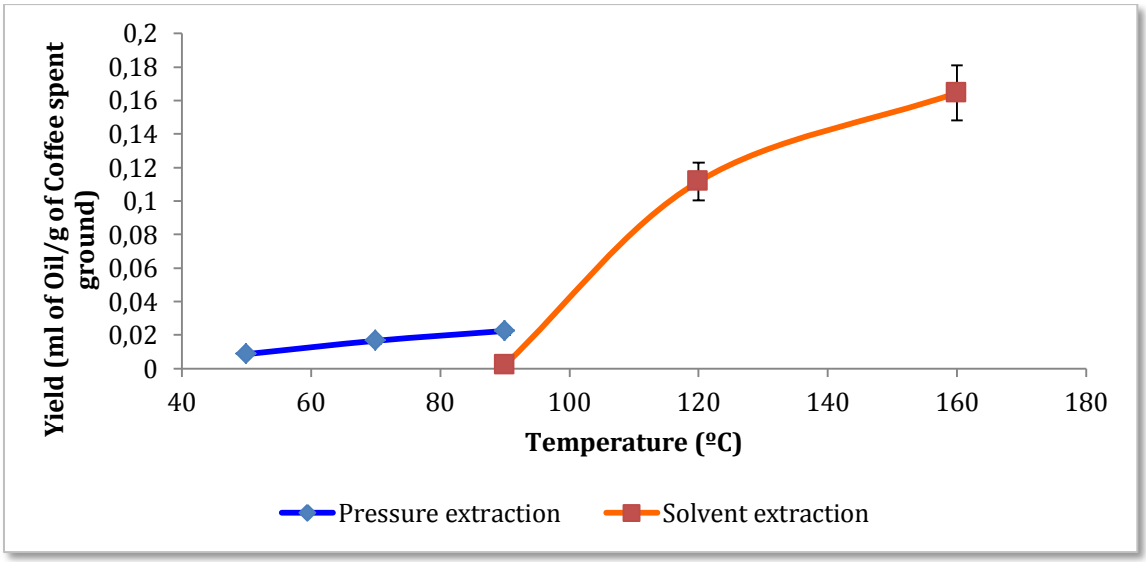


Figure 6.4. Yields (ml of oil/g of SCG) for oil extraction methods

Table 6.1. Yields (w/w) of pressing and solvent extractions

Temperature (ºC)	Pressing extraction		
	Initial mass (g)	Oil (g)	Yield (% w/w)
50	436.15	4.29	0.98
70	440.41	8.32	1.89
90	489.42	12.54	2.56

	Solvent extraction		
	Initial mass (g)	**Oil (g)**	**Yield (% w/w)**
90	46.08	0.11	0.25
120	51.03	6.50	12.73
160	60.75	11.40	18.76

Solvent extraction method leads to the highest yields in comparison to those obtained using pressing method however; pressing method permits to obtain oil free of solvents therefore, from an economic point of view, pressing method is more attractive. Similar yields have been reported by other authors for oil extraction from SCG, for instance, (Kondamudi et al., 2008) reported a yield between 10 to 15% using a solvent extraction (hexane and ether). Yields from 6.3% to 28.3% (w/w) have been reported by (Caetano et al., 2012) using solvent extractions. A yield of 15.6% (w/w) has been obtained using hexane under reflux conditions (Haile, 2014). The most promising application for oil obtained from SCG is to produce biodiesel because the biodiesel derived from SCG have proved to be stable for more than 1 month under ambient conditions and besides, because 100% conversion of oil to biodiesel have been found (Kondamudi et al., 2008).

In this way, the Acid Index (% free fatty acids) which is used for characterize the free fatty acids of oils for biodiesel production was measured for oil obtained from SCG as is was explained in chapter 3. Acid indexes of 3.08 and 3.26 mg of KOH/g of SCG were found for pressing and solvent extractions respectively. This value is similar to that reported by (Ramirez, 2008) for oil extracted from coffee which was 2.99 mg of KOH/g of oil. These values of acid index found for oil extracted from SCG are in the range of those found for palm oil of use in Colombia that ranges from 1.69 to 3.57 mg of KOH/g of oil (Rincon and Martinez, 2009). This fact strengthens the idea of use oil extracted from SCG for biodiesel production.

The extraction of oil from SCG has been evaluated from an economic point of view found that solvent extraction becomes an attractive alternative for extract this oil even, more attractive than pressing and supercritical fluid extractions (Davila et al., 2014c). However, solvent extraction can be combined with other techniques for enhance the yield extraction such as ultrasonication that permits to extract 98% of the total oil contained in SCG in only 30 minutes (Abdullah and Bulent Koc, 2013). The above discussion suggests that oil from SCG can be used as low cost and good quality feedstock for biodiesel production.

6.3 Sugar production from lignocellulosic biomass of naranjilla waste and other residues

Sugar production was carried out according to procedure described in chapter 3. Table 6.2 shows the reducing sugar concentration produced after acid hydrolysis.

Table 6.2. Reducing sugar concentration after acid hydrolysis

Sample	Absorbance	Concentration (g/l)
Naranjilla waste	0.901	2.133
Pineapple peel	0.799	1.874
Orange peel	1.576	3.846
Mandarin peel	1.273	3.077

Orange peel presented the highest reducing sugar production in comparison with other hydrolyzed fruit wastes however; this value (3.846 g/l) is low compared to other reported in the literature. For instance, 6.5 g/l of reducing sugars has been obtained from acid hydrolysis of orange peel using 3% of H_2SO_4 (Alvear et al., 2009). The reducing sugar

production from pineapple peel was 1.87 g/l. This production of sugars is low in comparison with 45 g/l of sugars using acid hydrolysis at 5% of H_2SO_4 reported by (Tejeda et al., 2010). However, the low reducing sugar production obtained in this research is due to the low acid concentration (2%) compared with those used in the literature (3% and 5%) that can increase the inhibitors production such as furfural and hydroxymethylfurfural (HMF) (Charles et al., 2004).

Because, the acid hydrolysis allows that the hemicellulose gives rise to several pentoses and hexoses (Taherzadeh and Karimi, 2008) then, a high content of holocellulose resulting in increased production of reducing sugars. In the same way that other lignocellulosic biomass (such as fruit wastes), the hydrolysis enhances the cellulose accessibility to enzymes and substantially increases the yield due to the removal of hemicellulose from lignocellulose (Jung and Kim, 2015).

Following the dilute acid hydrolysis, enzymatic hydrolysis was carried out to enhance the reducing sugar production, as explained in chapter 3. Table 6.3 presents the results obtained from enzymatic hydrolysis.

Table 6.3. Reducing sugar concentration after enzymatic hydrolysis

Sample	Absorbance	Concentration (g/l)
Naranjilla waste	0.350	15.19
Pineapple peel	0.282	12.44
Orange peel	0.411	17.64
Mandarin peel	0.441	18.85

In this case, the contribution of the first step of hydrolysis (acid hydrolysis) ranges from 12.3% to 17.9% of the total reducing sugars produced. Figure 6.5 depicts the contribution of both, acid and enzymatic hydrolysis to the total reducing sugars production. These results confirm that for fruit wastes it is necessary to use of two steps

hydrolysis in order to obtains the maximum reducing sugar production and to reach more accessible cellulose as well as hydrolyzed hemicellulose (Jung and Kim, 2015; Taherzadeh and Karimi, 2008).

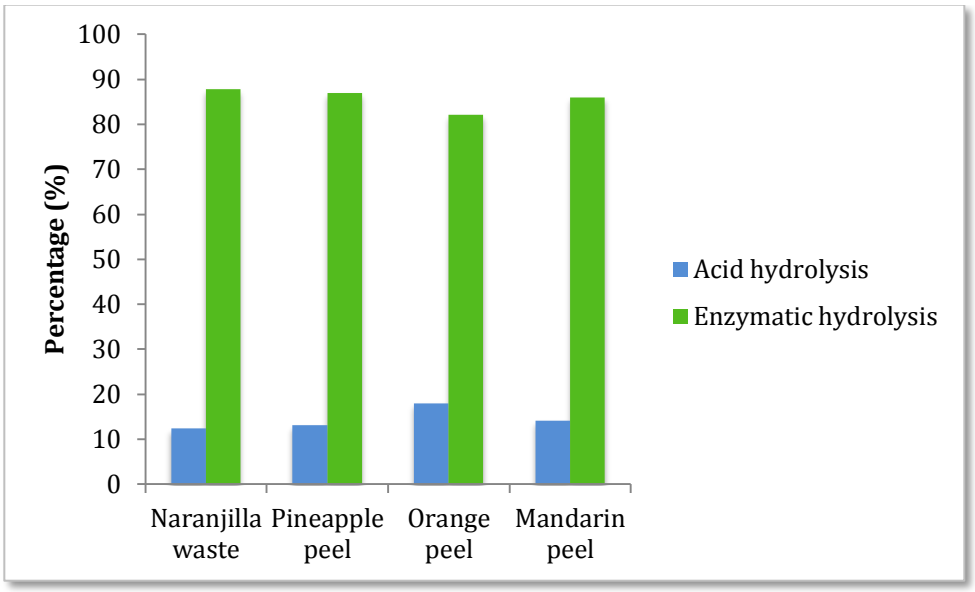


Figure 6.5. Contribution of acid and enzymatic hydrolysis over total reducing sugars production

Similar to results obtained from SBP and avocado peel and seed (in chapter 5), the above mentioned fruit wastes (naranjilla waste and peel of pineapple, orange and mandarin), allow to obtain value added products such as sugar that becomes a platform for producing other products such as organic acids, ethanol and other alcohols, biomaterials (Polyhydroxybutirate) and other compounds with several applications (Dávila et al., 2014).

Conclusions

This chapter demonstrated that it is possible to obtain value added products from naranjilla waste and SCG. The most promising value added products are related with the extracts from naranjilla waste that contain phenolic compounds as well as antioxidant

activity. These extracts presented a value added using supercritical fluid extractions (SFE) because in this way, it is possible to obtain extracts free of solvent thereby, SFE are also a promising technique for its use for obtaining extracts.

For oil from SCG, the techniques evaluated demonstrated that solvent extraction is a method that allows highest yields (until 18.76% w/w) and because this oil is a good feedstock for biodiesel production, it should be used for that purpose according to the highest yields obtained with solvent extraction method.

On the other hand, the holocellulose content (cellulose and hemicellulose) of naranjilla waste is also a potential feedstock to be used for reducing sugar production for other purposes such as for ethanol and organic acids production. Sugar production essays demonstrated that it is necessary two steps hydrolysis for obtaining reducing sugars. The first step is (acid hydrolysis) allows hydrolyze the hemicellulose and provide a best accessibility to cellulose content. The second step (enzymatic hydrolysis) demonstrated that this final step is necessary to increase significantly the yield of reducing sugars.

In the light of all the exposed in this chapter, the results suggest that naranjilla waste and SCG should be used as feedstock under biorefinery concept for obtaining value added products that permit to obtain an integral use of the feedstock. Products such as extract containing phenolic compounds; oils and reducing sugars can be used for obtaining valuable compounds.

PART III.

DESIGN AND ANALYSIS OF BIOREFINERIES

7. Chapter 7. Techno-economic assessment of the biorefineries

Overview

This chapter presents the results of techno-economic assessment of biorefineries based on non-oil feedstocks (SBP and naranjilla wste) and based on oil feedstocks (avocado and SCG). The level of integration was evaluated taken into account four scenarios: scenario 1 (base case) without mass and energy integrations and without cogeneration system, scenario 2 with energy integration and without mass integration and without cogeneration system, scenario 3 with mass and energy integrations but without cogeneration system and scenario 4 considering mass and energy integrations as well as cogeneration system. Total production cost and energy requirements were calculated for the two types of biorefineries (based on non-oil and oil feedstocks). Three products were obtained from biorefineries based on non-oil feedstocks, ethanol, xylitol and phenolic compounds. The last product was the most promising product from these biorefineries. Four products were obtained from biorefineries based on oil feedstocks, xylitol, ethanol, phenolic compounds and oil. The last product was the most promising from these biorefineries. Raw materials, inputs and utilities were the most important economic factors for the evaluated biorefineries. Heat integration was the most significant level of integration over total production cost. Finally, a sensibility analysis based on chemical composition of the feedstocks was developed demonstrating that both, yield and production capacities for all biorefineries can change significantly.

7.1 Biorefineries based on non-oil feedstocks (SBP and naranjilla waste)

The design of biorefineries based on non-oil feedstocks was made according to the chemical compositions for SBP and naranjilla waste. The chemical composition for SBP and naranjilla waste was obtained from the experimental results (chapter 4) and literature (Gancel et al., 2008) respectively. The inlet flow rates for both biorefineries were fixed at 2,000 kg/h taken into account that the Colombian production for SBP and naranjilla is 105,218 and 57,712 tonnes per year respectively (MinAgricultura, 2012). Because the biorefinery operates 8,000 hours per year, the available amounts for SBP and naranjilla waste correspond to 6.57 and 3.6 ton/h respectively. However, from these values only 2 ton/h were taken for each biorefinery because of the possible logistic problems associated with gathering the residues. The descriptions of the constituent plants of these biorefineries as well as chemical characterization of the feedstocks are presented in **Appendix D1**.

The process flow diagrams for biorefineries based on SBP and naranjilla waste are showed in Figures 7.1 and 7.2 respectively.

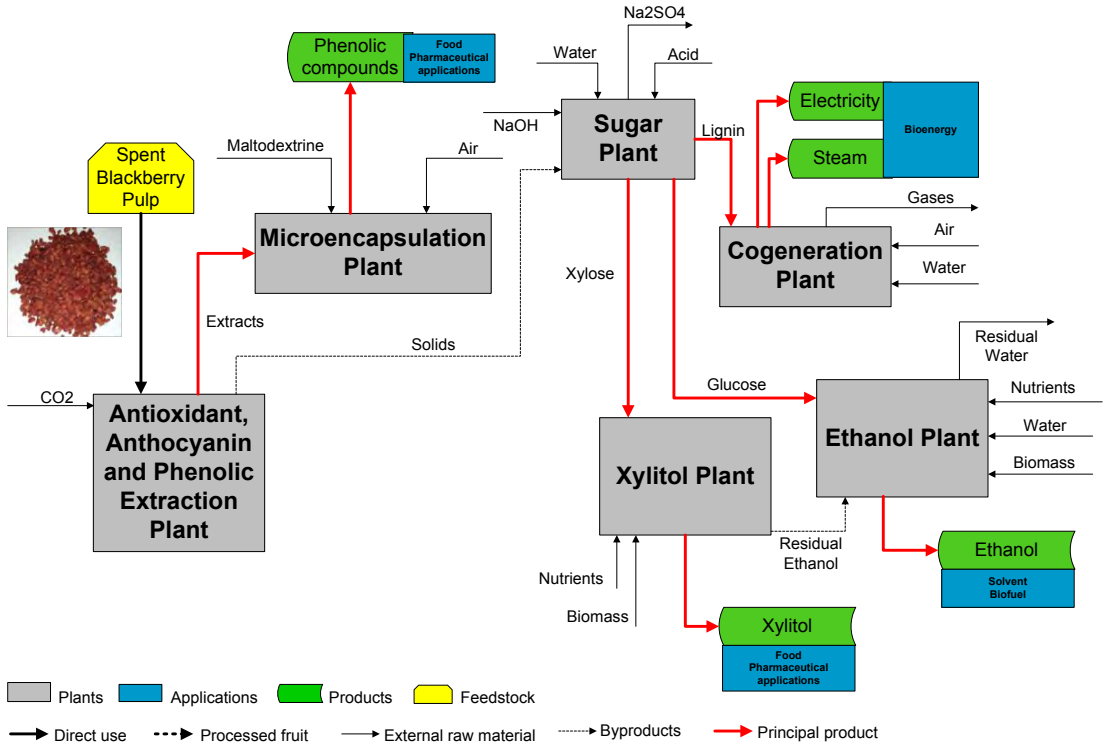


Figure 7.1. Biorefinery based on SBP

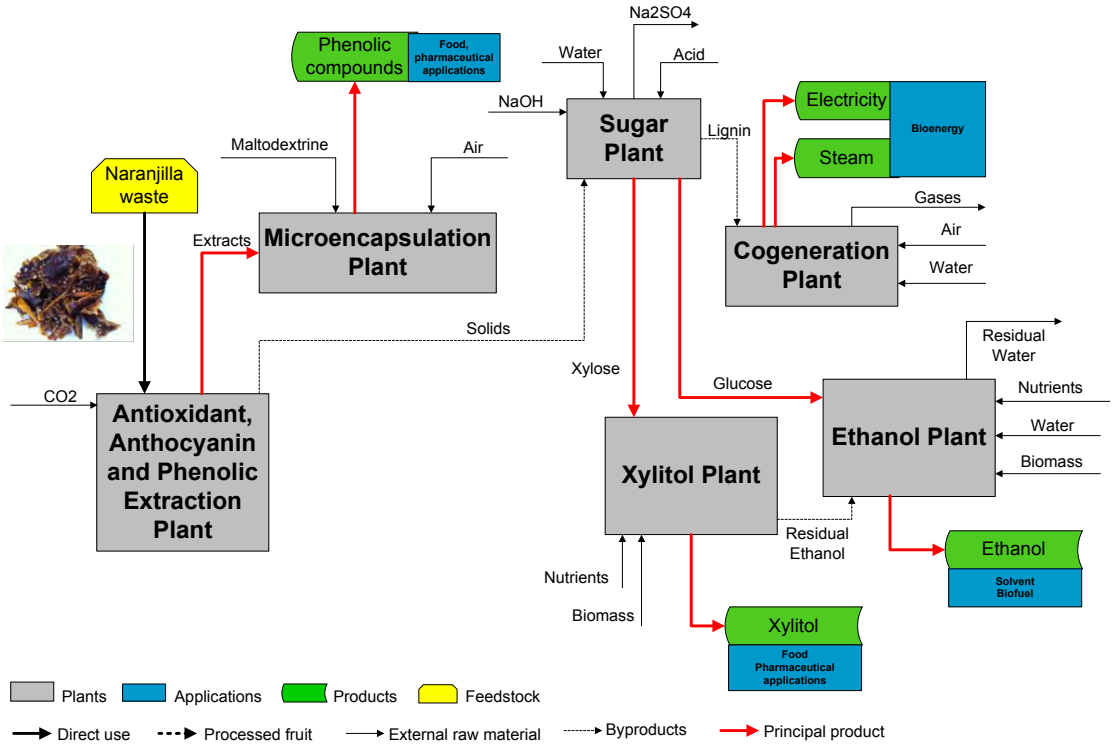


Figure 7.2. Biorefinery based on naranjilla waste

The constituent plants of biorefineries based on non-oil feedstocks are aimed to obtain phenolic compounds (by means of a plant of extraction and microencapsulation), xylitol (by means of a plant for xylitol production) and ethanol (by means of a ethanol plant). A plant for sugars (xylose and glucose) production is required for obtaining the feedstock for both, xylitol and ethanol plants. Each biorefinery was evaluated considering an additional plant for cogeneration. The scenarios considered for both biorefineries are described in Table 7.1.

Table 7.1. Scenarios evaluated for biorefineries

Scenario	Description of the scenarios
1	Evaluates the base case (Biorefinery without heat and mass integration and without cogeneration system)
2	Biorefinery with heat integration but without mass integration and without cogeneration system
3	Biorefinery with heat and mass integrations and without cogeneration system
4	Biorefinery with heat and mass integrations and with cogeneration system

For economic assessment, the total production cost per kg of products was calculated. Thus, phenolic compounds, xylitol and ethanol were taken as products. The cost associated to sugar plant was distributed between xylitol and ethanol plants (for scenarios 1, 2 and 3) because xylose and glucose are the feedstock for xylitol and ethanol plants respectively. The cost of cogeneration plant (for scenario 4) was distributed between xylitol, ethanol and phenolic compounds plants. Table 7.2 shows the distribution of the costs associated to biorefineries. Table 7.3 shows the distribution cost of each one of the economic factors taken into account for economic assessment.

Table 7.2. Distribution of the costs associated to sugar and cogeneration plants

Plant	Plant that assume the cost	% Assumed	Reason
Sugar	Xylitol plant	40	For hemicellulose fraction used for xylose production and subsequent xylitol production
	Ethanol plant	60	For cellulose fraction that is processed for glucose production and later for the ethanol plant
Gasification	Xylitol plant	39	Because this plants consumes around 39% of the total energy requirements
	Ethanol plant	58	Because this plant consumes around 58% of the total energy requirements
	Phenolic compounds plant	3	Because this plant consumes around 3% of energy requirements

Table 7.3. Distribution of the costs associated to each economic factor

Economic factor	Purpose
Depreciation expense	This cost was distributed as 40%, 40% and 20% for xylitol, ethanol and phenolic compounds plants respectively. Taken into account the number of units involved for each plant.
Raw material and inputs	This cost was distributed as 30%, 65% and 5% for xylitol, ethanol and phenolic compounds plants respectively. Taken into account that enzyme and H_2SO_4 are used for xylose and glucose production respectively.
Utilities	This cost was distributed as 39%, 58% and 3% for xylitol, ethanol and phenolic compounds plants respectively. Taken into account the energy requirements for each plant

Operating cost *	This cost was distributed in the same way that depreciation expense because it has defined by the number of units in each plant.

* This economic factor is composed by operating labor cost, maintenance cost, operating charges, plant overhead and general and administrative costs

Finally, the cost of raw material, inputs and services used for economic assessment of the biorefineries are presented in **Appendix D2**.

7.1.1 Results of production costs for biorefineries based on non-oil feedstocks

Table 7.4 presents the mass balance for each biorefinery and Table 7.5 shows the capacity and yield for each product and biorefinery. As it was expected according to chemical composition, SBP had the highest yield for xylitol and phenolic compounds because the content of hemicellulose and phenolic compounds respectively. However, naranjilla waste presented the highest yield for ethanol because this feedstock has the highest cellulose content.

Table 7.4. Mass balance for SBP and naranjilla wastes biorefineries

Stream	**Flow (kg/h)**	
	SBP biorefinery	**Naranjilla waste biorefinery**
Feedstock	2000	2000
Ethanol	463.24	450.27
Maltodextrine solution	10	10

Water	9315	7464.4
CO_2	11.58	11.58
NaOH	58	48.88
H_2SO_4	140	185.3
Enzyme	194	133
Biomass	1.56	1.1
Air	40.42	40.42
Total Inlet	**12233.8**	**10344.95**
Ethanol	27.22	19.03
Xylitol	18.85	13
Phenolic compounds	8.06	5.76
Residual water	9377.49	7571.94
Residual CO_2	35.29	26.29
Lignin	136.08	95.11
Residual biomass	13.21	9.21
Residual gases from SFE	334.58	334.58
Residual ethanol	260.82	276.58
Na_2SO_4	289.3	246.82
Air	1739.46	1746.2
Total outlet	**12233.4**	**10344.52**

Table 7.5. Productivity and yields for biorefineries based on non-oil feedstocks (SBP and naranjilla waste (NW))

Product	Productivity (kg/day)		Processing Yield (kg/tonne feedstock)	
	SBP	NW	SBP	NW
Xylitol	452.4	312	9.43	6.5
*Ethanol	6912	7095	144.02	147.8
Phenolic compounds	193.4	138.2	4.03	2.88

* Both, productivity and yield of ethanol are referred after mass integration of ethanol.

For the extraction of phenolic compounds, highest yields have been reported in the literature for SBP. For instance, for blackberry residues using pressurized liquid extraction, 7.36 mg of phenolic compounds per g of residue were extracted (Machado et al., 2015). A yield of 9.87 mg of phenolic compounds per g of blackberry bagasse was reported by (Pasquel Reátegui et al., 2014) using supercritical fluid extraction assisted by ultrasound. In this research, the yields obtained after the extraction of phenolic compounds were 4.31 and 3.08 mg/g of SBP and naranjilla waste respectively while after microencapsulation were 4.03 and 2.88 mg/g of SBP and naranjilla waste respectively. These values lead phenolic compounds retention of 93.4% and 94.4% for SBP and naranjilla waste respectively. Similar values of retention have been reported for phenolic compounds and anthocyanins with 85% and 87% respectively (Tonon et al., 2010; Yousefi et al., 2015). The ethanol (99.95%, wt) production corresponds to 27.22 kg (34.50 litters) and 19.03 kg (24.12 litters) per 2 tonnes of SBP and naranjilla waste respectively (See Table 7.4). These values lead 17.25 and 12.06 litters per tonne of feedstock for SBP and naranjilla waste respectively. However, when residual ethanol is recycled to be distilled for recovering part of ethanol, the productivity and yield increase to 144 and 147.8 kg/ton of SBP and naranjilla waste respectively (that correspond to 182.5 and 187.3 l/ton respectively). These productions are similar to those reported by

(Do et al., 2015), where 150 l/ton of empty fruit bunches was obtained in a plant working 8000 h/year. Other authors have reported lower values of yield from fruit wastes, this is the case of lemon peel that was used for ethanol production reaching a yield of 60 l/ton of waste (Boluda-Aguilar and López-Gómez, 2013). However, higher production yields of ethanol from other lignocellulosic materials have been reported by (Quintero et al., 2013), where 250 litters of ethanol per tonne of feedstock (biomass that ranges from 26.45% to 37.35% of cellulose) were obtained. This also confirm that residual biomass such as fruit wastes that contain lower cellulose content lead to lower ethanol production than the commonly feedstocks used (rice husk, sugarcane bagasse, corncob, etc.). However, the content of cellulose in fruit wastes can be used for obtaining ethanol, which can be used as feedstock for other purposes in the biorefinery. On the other hand, xylitol yield obtained in this research is lower than that reported in the literature. For instance, xylitol was produced from oil palm empty fruit bunch with yield ranging from 14 to 33 kg of xylitol per tonne of feedstock (Mohamad et al., 2009). However, the yield reported in the literature did not include downstream processes that can reduce the final yield, as in this research. Although other researches did not report yield, different fruit wastes have been used for producing xylitol. For example, banana peel was used for producing xylitol reaching a concentration that range from 9 to 24.7 g/l after fermentation (Rehman et al., 2013). Cashew apple bagasse was used for producing xylitol reaching a concentration of 2.95 g/l and a productivity of 0.118 g/l*h (Delgado and Barbosa de Lima, 2014).

The costs associated with all plants of the biorefineries were calculated taking into account depreciation expense, raw material, inputs (such as reagents for processing), utilities and operating costs. Tables 7.6 and 7.7 show the cost and share of each scenario for SBP and naranjilla waste biorefineries respectively.

Table 7.6. Cost and share of SBP biorefinery

Item	Scenario 1		Scenario 2		Scenario 3		Scenario 4	
	Cost (USD/Year)	Share (%)	Cost (USD/Year)	Share (%)	Cost (USD/Year)	Share (%)	Cost (USD/Year)	Share (%)
Depreciation expense	1,231,340	8.74	1,231,340	11.03	1,231,340	12.68	3,431,641	28.90

Raw material	336,205	2.37	336,205	3.01	327,903	3.38	327,903	2.76
Inputs	6,581,595	46.72	6,581,595	58.95	5,137,159	52.89	5,137,159	43.26
Utilities	4,621,990	32.81	1,699,505	15.22	1,699,505	17.50	1,661,098	13.99
Operating cost	1,316,692	9.35	1,316,692	11.79	1,316,692	13.55	1,316,692	11.09
Total	**14,087,822**	**100**	**11,165,337**	**100**	**9,712,599**	**100**	**11,874,493**	**100**

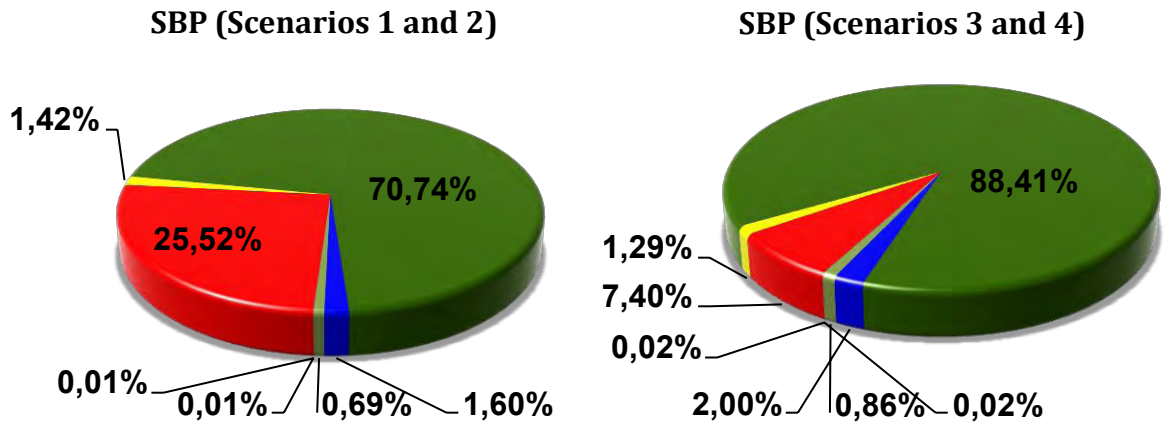
Table 7.7. Cost and share of naranjilla waste biorefinery

Item	Scenario 1		Scenario 2		Scenario 3		Scenario 4	
	Cost (USD/Year)	Share (%)	Cost (USD/Year)	Share (%)	Cost (USD/Year)	Share (%)	Cost (USD/Year)	Share (%)
Depreciation expense	1,242,240	7.43	1,231,340	11.45	1.231,340	12.89	1,451,641	15.16
Raw material	336,057	2.01	336,057	3.12	334,255	3.50	334,255	3.49
Inputs	5,438,123	32.55	5,438,123	50.55	4,238,318	44.35	4,238,318	44.26
Utilities	8,203,150	49.10	2,263,249	21.04	2,263,249	23.68	2,234,461	23.34
Operating cost	1,489,060	8.91	1,489,060	13.84	1,489,060	15.58	1,489,060	13.75
Total	**16,708,630**	**100**	**10,757,829**	**100**	**9,556,222**	**100**	**9,575,367**	**100**

For all scenarios, inputs represent the highest cost. This is due to the consumption of enzyme for enzymatic hydrolysis and ethanol for extraction and xylitol plants. Both, enzyme and ethanol represent more than 90% of the total cost of inputs. These results are in agreement with those reported for biorefineries based on sugarcane in which the raw material including inputs for all plants represented 70% of the total production cost (Moncada et al., 2014b). Other cases of biorefineries based on residues (brewer's spent grains) have been reported where raw materials including inputs accounted for 48% of the total production cost (Mussatto et al., 2013). For both biorefineries (SBP and naranjilla waste), ethanol plant is the highest consumer of inputs taken into account that

the enzyme is used for obtaining glucose from sugar production that becomes in a raw material for ethanol plant. This fact is common for bioethanol production from lignocellulosic biomass in which the enzyme is a challenge for obtaining feasible processes. Similar results were reported by (Moncada et al., 2013) where raw materials including inputs account more than 72% of the total production cost.

However after mass integration, 21% of saving over total cost of raw materials was achieved for both, SBP and naranjilla waste biorefineries. Figure 7.3 depicts the share of each input over total cost of inputs for scenarios without (scenarios 1 and 2) and with (scenarios 3 and 4) mass integration for SBP and naranjilla waste. Cost of Inputs contributes from 43.26% (scenario 4) to 58.95% (scenario 2) over total production cost in SBP biorefinery and from 32.55% (scenario 1) to 50.55% (scenario 2) for naranjilla waste biorefinery. This fact suggests that mass integration is necessary to reduce the consumption of inputs for biorefineries.



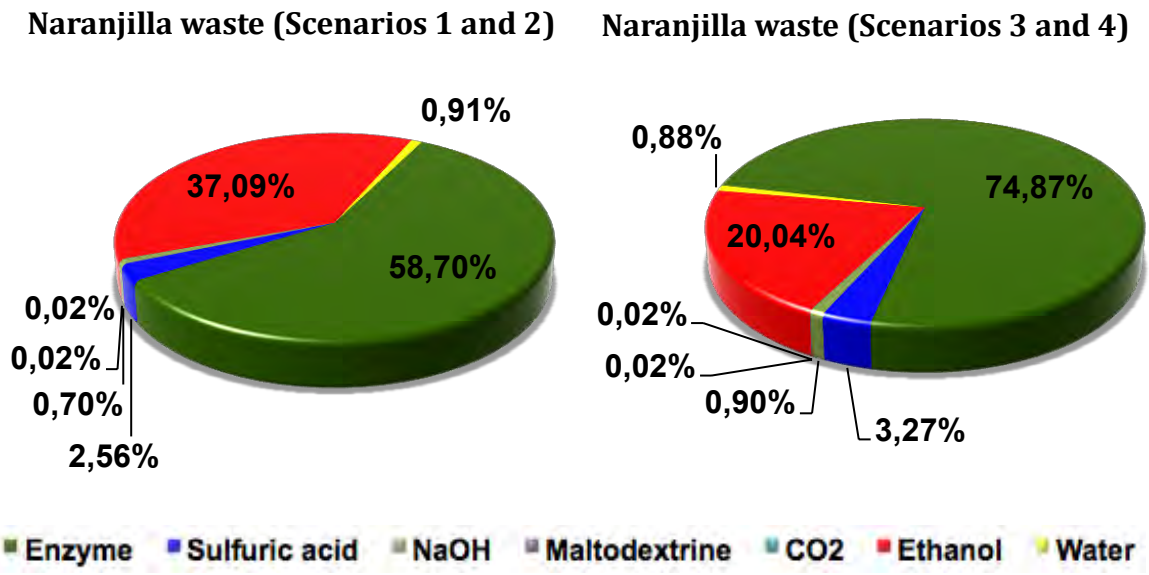


Figure 7.3. Cost of inputs for SBP and naranjilla waste biorefineries with and without mass integration

Figure 7.4 depicts the saving of water and ethanol with mass integration for each biorefinery. These values of savings for ethanol and water highlight that mass integration is very useful and necessary to reduce inputs consumption. Besides, ethanol recycling allows increase the ethanol yield such as it is shown in Table 7.5. **Appendix D3** presents both, the share and cost for each plant for SBP and naranjilla waste biorefineries.

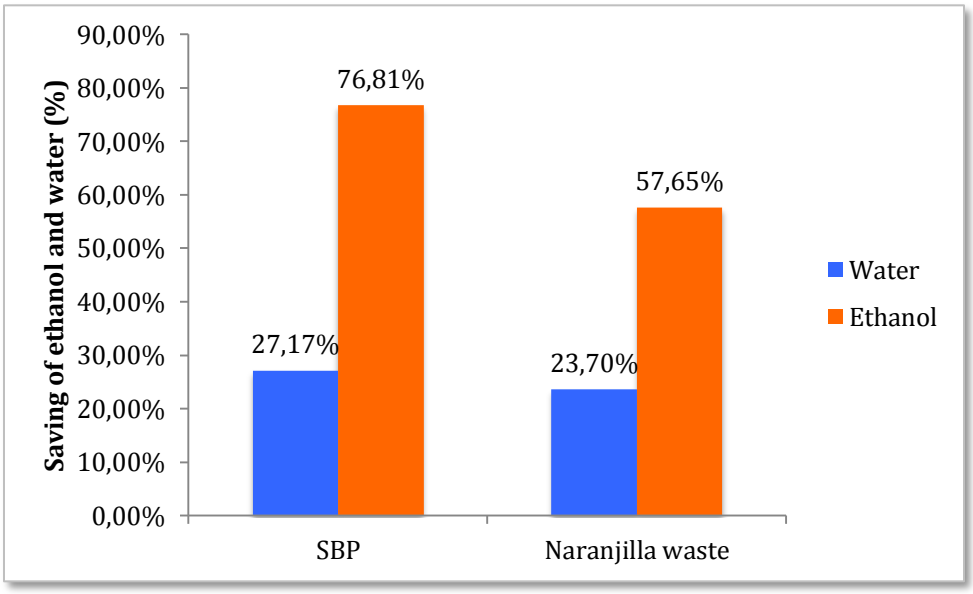


Figure 7.4. Saving of water and ethanol after mass integration

For both biorefineries, inputs and utilities are the most important economic factors that affect the total production cost. Therefore, mass and energy integrations are very useful for reducing total production cost. Figures 7.5 and 7.6 depict the total production cost for each one of the products in each scenario for SBP and naranjilla waste biorefineries respectively.

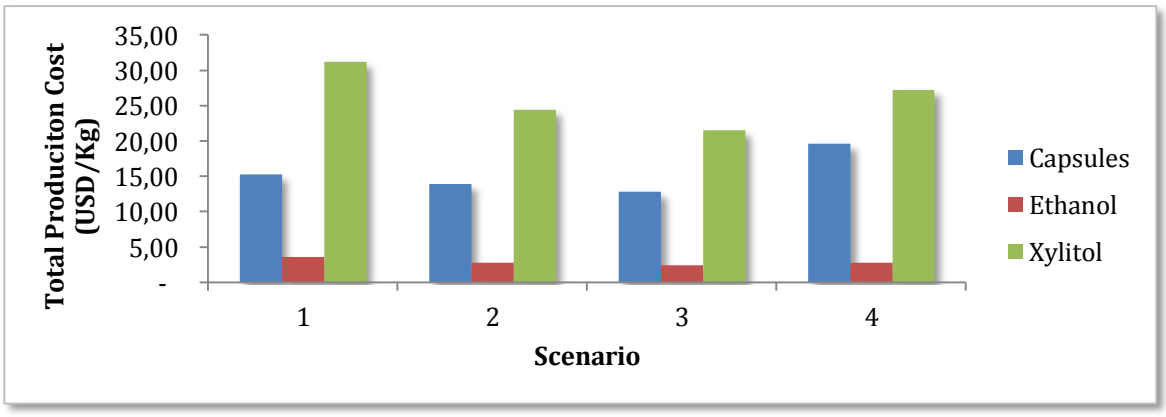


Figure 7.5. Total production cost for SBP biorefinery

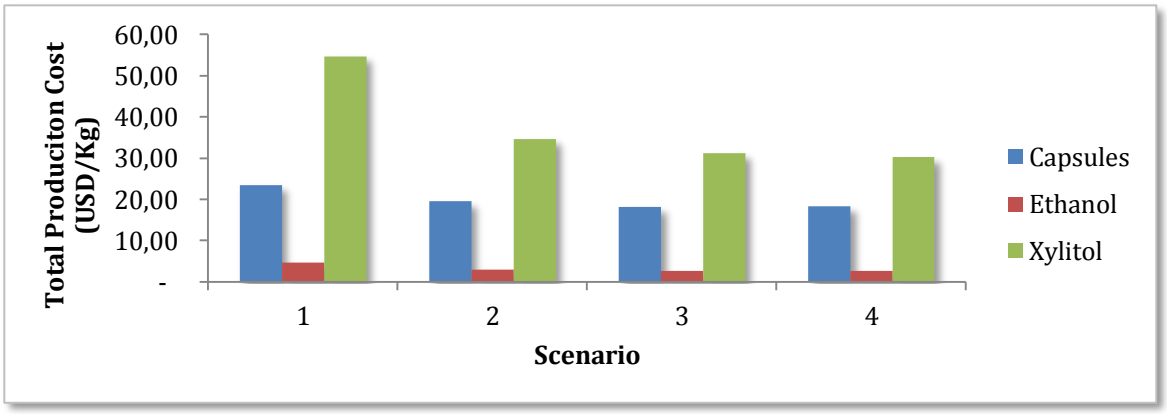


Figure 7.6. Total production cost for naranjilla waste biorefinery

According to the level of integration, the total production cost was reduced except for xylitol and ethanol in scenario 4 in SBP biorefinery where, the addition of a cogeneration plant increased the capital cost which in turn increased the total production cost for each product. For naranjilla waste biorefinery, a cogeneration system did not affect significantly the total production cost of all products.

In order to determine the effect of the level of integration on economic evaluation, the ratio sale price to total production cost (Mussatto et al., 2013) was calculated using the prices reported in **Appendix D2** and mass balance of Table 7.4. Figure 7.7 shows the sale price to total production cost ratio. Thus, scenario 3 has the highest sale price to total production cost ratio indicating that mass and energy integrations can reduce significantly the total production cost while the addition of a cogeneration system is not attractive because it increases the total production cost which, in turn, reduces the sale price to total production cost ratio.

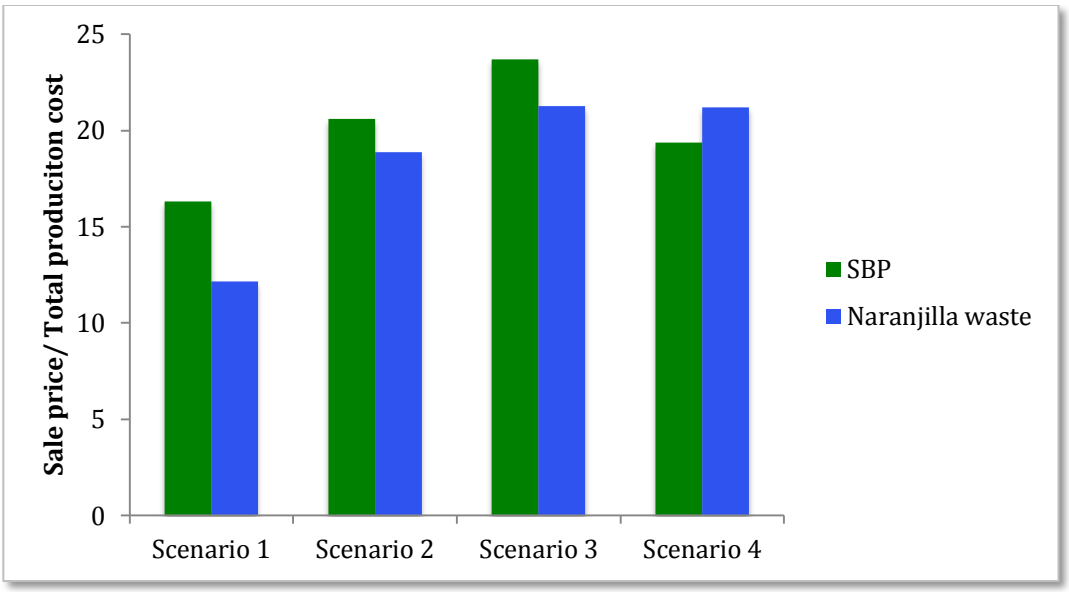


Figure 7.7. Sale price to total production cost ratio for biorefineries

7.1.2 Results of energy consumption of biorefineries based on non-oil feedstocks

Ethanol plant is the higher consumer of utilities in both biorefineries. This result is in agreement with what is reported in the literature for biorefineries in the Colombian context where ethanol plant can reach up to 63% of the energy requirements of the biorefinery (Moncada et al., 2013). Figures 7.8 and 7.9 depict the energy consumption for scenario 1 (base case) for of each plant per tonne of SBP and naranjilla waste respectively.

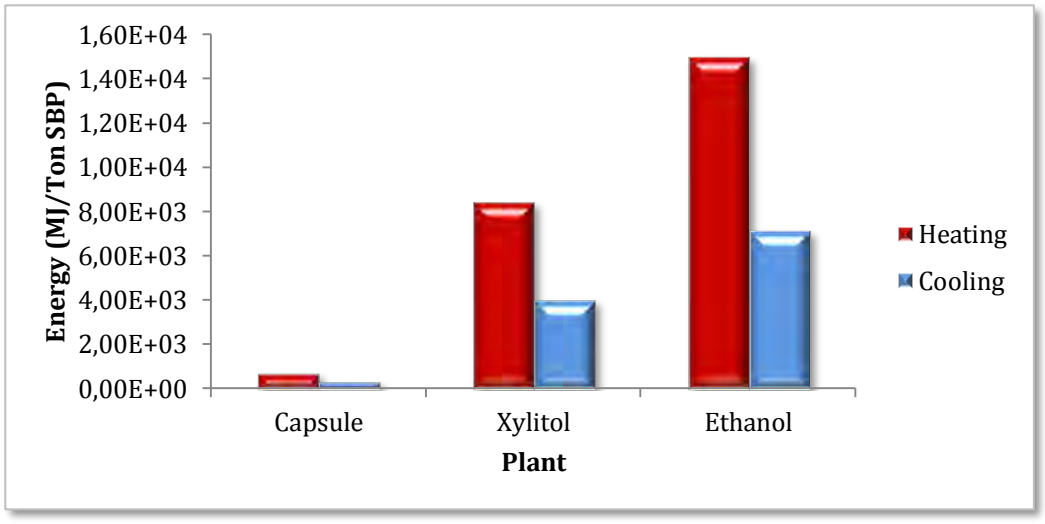


Figure 7.8. Energy consumption of each plant for SBP biorefinery in the case of base case (scenario 1)

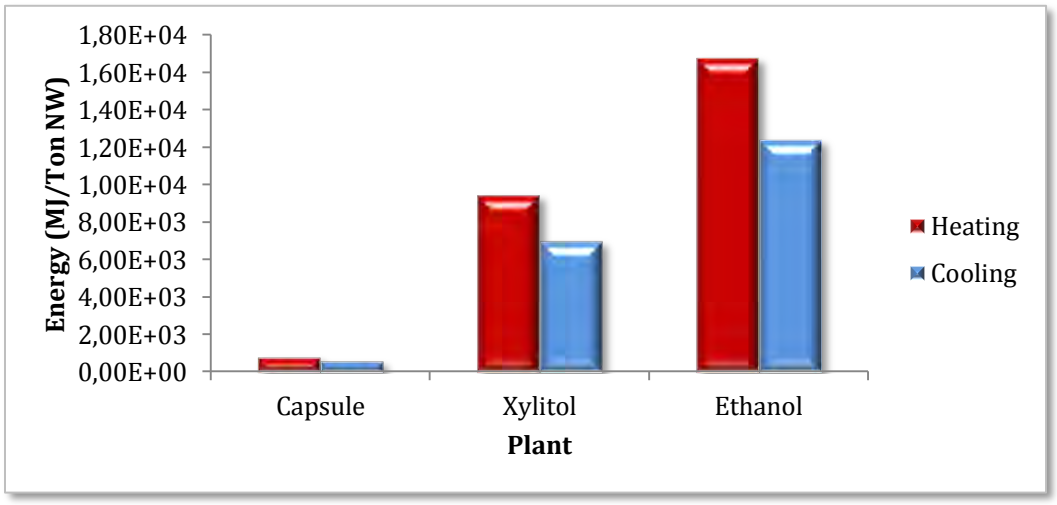


Figure 7.9. Energy consumption of each plant for naranjilla waste biorefinery in the base case (scenario 1). NW means naranjilla waste

The total energy consumption in the base case (Taken into account heating and cooling) is 35,655 and 47,120 MJ/ton of SBP and naranjilla waste respectively. However, according to the level of integration, the energy requirements can be reduced

significantly. Figure 7.10 depicts the reduction of utilities for SBP and naranjilla waste biorefineries.

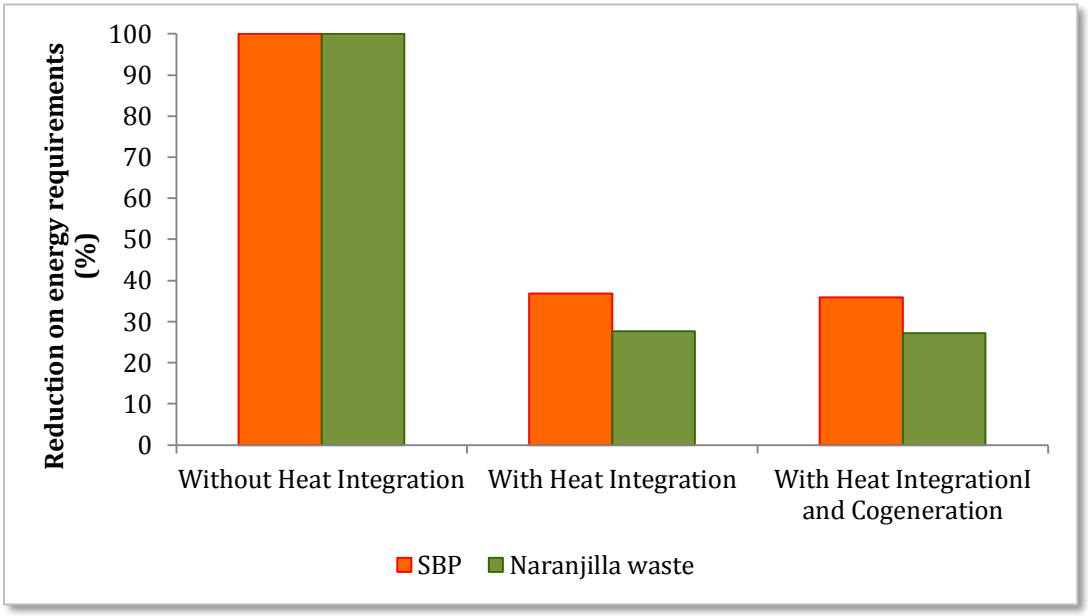


Figure 7.10. Reduction of utilities consumption according to the level of integration

After heat integration and the addition of a cogeneration system, the energy requirements can be reduced to 27.24% and 35.94% of the energy requirements needed for the base case (without heat and mass integrations and without cogeneration system) for SBP and naranjilla waste respectively. This reduction allows obtaining 9,712 and 16,934 MJ/ton of SBP and naranjilla waste respectively. Similar results were obtained for biorefineries based on sugarcane with 8,370 MJ/ton of feedstock including both, cooling and heating services (Moncada et al., 2014b). A total energy consumption of 8,332 MJ/Ton has been reported by (Moncada et al., 2013) for a biorefinery based on sugarcane in the Colombian context. The most important level of integration is heat integration because it allows reducing the energy requirements significantly in comparison with the addition of a cogeneration system. The latter is because the Pinch methodology strategy allows recovering 47% and 53% (common area between cool and heat composite curves) of the energy needed for SPB and naranjilla waste respectively according to the composite curves obtained and presented in **Appendix D4**.

In light of the results found in this research both, mass and heat integrations are needed for biorefineries based fruit wastes while the addition of a cogeneration system is not attractive because it does not contribute significantly to the reduction of energy requirements.

7.2 Biorefineries based on oil feedstocks (Avocado and SCG)

The design of these biorefineries was based on the chemical compositions of avocado (peel, seed and pulp) and spent coffee ground (SCG). The chemical composition for avocado and SCG was obtained from the experimental results (chapter 4) and literature respectively (Urribarr. et al., 2014). Similarly to SBP and naranjilla waste, the inlet flow for both biorefineries was fixed at 10000 and 2000 kg/h, respectively, taken into account that the Colombian production for avocado and SCG is 215,095 and 22,300 tonnes per year respectively. Because the biorefinery operates 8,000 hours per year, the available amounts for avocado and SCG correspond to 26.88 and 2.78 ton/h respectively. From these values only 10 and 2 ton/h for avocado and SCG biorefineries were taken because of potential problems associated with logistic in acquiring these feedstocks. The descriptions of the plants that compose these biorefineries as well as chemical characterization of the feedstocks are presented in **Appendix D5**. The process flow diagrams for biorefineries based on avocado and SCG are showen in Figures 7.11 and 7.12 respectively.

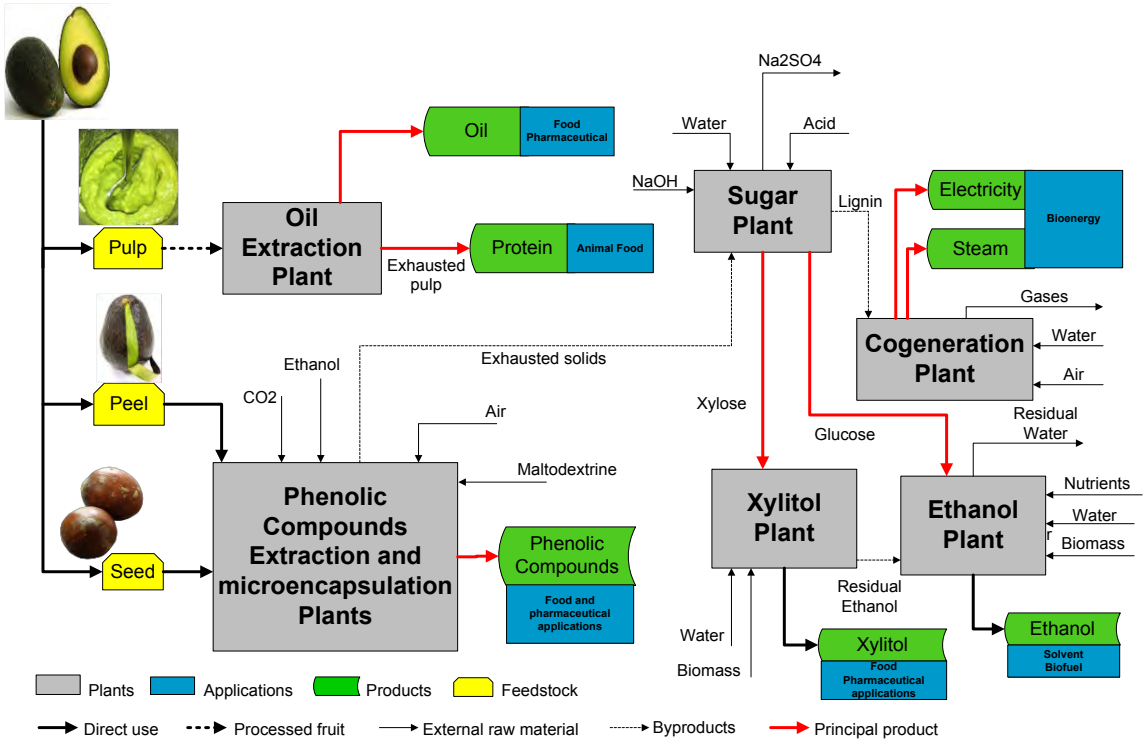


Figure 7.11. Biorefinery based on avocado

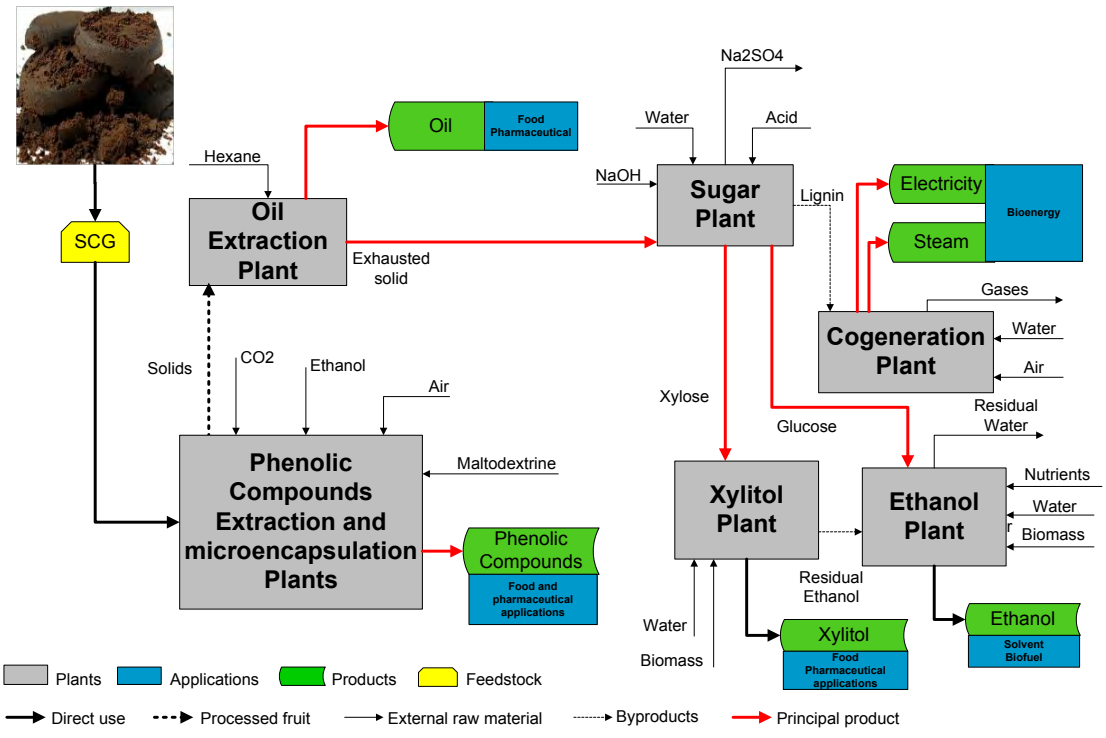


Figure 7.12. Biorefinery based on SCG

For economic analysis, the prices reported in **Appendix D2** were used. The same four scenarios analyzed for biorefineries based on non-oil feedstocks (SBP and naranjilla waste) were assessed for oil feedstocks (avocado and SCG). The cost associated with sugar and cogeneration plants are showed in Table 7.2. The distribution cost of each economic factor is showed in Table 7.8.

Table 7.8. Distribution of the costs associated to each economic factor for avocado and SCG biorefineries

Economic factor	Purpose
Depreciation expense	This cost was distributed as 40%, 40%, 10% and 10% for xylitol, ethanol, phenolic compounds and oil plants respectively. Taken into account the units involved for each plant.
Raw material and inputs	This cost was distributed as 19%, 28%, 17% and 36% for xylitol, ethanol, phenolic compounds and oil plants respectively. Taken into account that enzyme and H_2SO_4 are used for xylose and glucose production respectively and hexane for oil plant.
Utilities	This cost was distributed as 10%, 80%, 6% and 4% for xylitol, ethanol, phenolic compounds and oil plants respectively. Taken into account the energy requirements for each plant
Operating cost	This cost was distributed in the same way that depreciation expense because it has defined by the number of units in each plant.

7.2.1 Results of production costs for biorefineries based on oil feedstocks

Table 7.9 presents the mass balance for each biorefinery and Table 7.10 shows the capacity and yield for each product and biorefinery. As it was expected according to chemical composition, avocado had the highest yield for xylitol because the content of

hemicellulose. However, SCG presented the highest yield for ethanol because this feedstock has the highest cellulose content.

Table 7.9. Mass balance for avocado and SCG biorefineries. N.A. means Not Apply

Stream	Flow (kg/h)	
	Avocado biorefinery	**SCG biorefinery**
Feedstock	10000	2000
Ethanol	1767.7	781.51
Maltodextrine solution	10	10
Water	35417.26	19421.3
CO_2	44.04	24.14
NaOH	220.52	190.8
H_2SO_4	1047.67	304.6
Enzyme	320.26	298.3
Biomass	5.72	3.25
Hexane	N.A.	19.79
Air	67.65	23.26
Total Inlet	**48900,9**	**23076.9**
Ethanol	94.80	55.82
Xylitol	51.02	36.73
Phenolic compounds	6.27	7.75
Oil	1420.4	106.7

Residual water	40074.8	20422.5
Residual CO_2	99.97	72.14
Lignin	101.2	279
Residual biomass	47.87	26.76
Residual gases from SFE	1246.95	697.3
Residual ethanol	1008.7	650
Na_2SO_4	246.82	153.9
Spent pulp	1579.6	N.A.
Hexane	N.A.	19.79
Air	2922.48	548.9
Total outlet	**48900.2**	**23077.4**

Table 7.10. Productivity and yields for avocado and SCG biorefineries

Product	**Productivity (kg/day)**		**Processing Yield (kg/tonne feedstock)**	
	Avocado	**SCG**	**Avocado**	**SCG**
Xylitol	1224.5	881.5	5.10	18.37
* Ethanol	26472	16939.7	110.35	352.91
Capsules	150.5	186	0.63	3.88
Oil	34089.6	2560.8	142	56.35

* Both, productivity and yield of ethanol are referred after mass integration of ethanol.

The ethanol productions correspond to 94.80 kg (120.15 litters) and 55.82 kg (70.75 litters) per 2 tonnes. These values lead 60.08 and 35.38 litters per tonne of feedstock for avocado and SCG respectively. Highest yields have been reported for ethanol production from Colombian SCG (Rodríguez and Zambrano, 2010) found 27.85 g of ethanol per 56.98 g of cellulose (that corresponds to 204.23 g of SCG), this amount corresponds to 140 kg (177 litters) per tonne of SCG. However, when residual ethanol is recycled to be distilled and recovered, the productivity and yield increase as shown in Table 7.10. Other authors (Hernández et al., 2014) reported 50 kg (63.37 litters) of ethanol per tonne of feedstock (olive tree pruning) under biorefinery concept. On the other hand, xylitol yield from avocado and SCG are lowest than other reported from lignocellulosic biomasses. For instance, from a lignocellulosic feedstock (brewer's spent grains), 103 kg per tonne of feedstock has been obtained (Mussatto et al., 2013). For oil extraction in this research, avocado and SCG lead 142 and 56.35 kg per tonne of avocado and SCG respectively. For the case of SCG, highest yield was reported by (Al-Hamamre et al., 2012) found 152.8 kg/ton of SCG using a mix of hexane and methanol as solvents. While for avocado oil, extraction yield of 100 kg of oil per tonne of avocado has been reported in a pilot scale level (Martinez et al., 1992).

The costs associated to all plants of the biorefineries were calculated in the same way that was used for biorefineries based on non-oil feedstocks (SBP and naranjilla waste), taking into account depreciation expense, raw material, inputs, utilities and operating costs. Tables 7.11 and 7.12 show the cost and share of each scenario for avocado and SCG biorefineries respectively.

Table 7.11. Cost and share of avocado biorefinery

Item	Scenario 1		Scenario 2		Scenario 3		Scenario 4	
	Cost (USD/Year)	Share (%)	Cost (USD/Year)	Share (%)	Cost (USD/Year)	Share (%)	Cost (USD/Year)	Share (%)
Depreciation expense	1,968,970	1.41	1,231,340	1.35	1,231,340	1.42	1,451,641	1.88
Raw material	41,315,739	29.56	41,315,739	45.26	41,313,010	47.69	41,313,010	53.52
Inputs	16,924,661	12.11	16,924,661	18.54	12,277,612	14.17	12,277,612	15.90

Utilities	68,712,100	49.17	20,970,933	22.97	20,970,933	24.21	20,838,659	26.99
Operating cost	10,836,500	7.75	10,836,500	11.88	10,836,500	12.51	10,836,500	1.71
Total	**139,757,970**	**100**	**91,279,173**	**100**	**86,629,395**	**100**	**77,197,615**	**100**

Table 7.12. Cost and share of SCG biorefinery

Item	Scenario 1		Scenario 2		Scenario 3		Scenario 4	
	Cost (USD/Year)	Share (%)	Cost (USD/Year)	Share (%)	Cost (USD/Year)	Share (%)	Cost (USD/Year)	Share (%)
Depreciation expense	1,876,866	0.32	1,876,866	0.66	1,876,866	0.66	2,097,167	0.87
Raw material	149,419	0.03	149,419	0.05	148,987	0.05	148,987	0.06
Inputs	11,256,581	1.90	11,256,581	3.96	10,197,375	3.60	10,197,375	4.24
Utilities	534,777,777	90.28	226,745,777	79.76	226,745,777	80.06	226,551,265	94.27
Operating cost	44,267,755	7.47	44,267,755	15.57	44,267,755	15.63	44,267,755	0.55
Total	**592,328,422**	**100**	**284,296,422**	**100**	**283,236,784**	**100**	**240,311,487**	**100**

For avocado biorefinery without heat and mass integrations (scenario 1), the most important economic factor of the total production cost was utilities due to the high energy consumption that is necessary without heat integration. However, after mass and energy integrations (scenarios 2, 3 and 4), raw material becomes in the most important economic factor over total production cost because the high cost of avocado (fresh fruit). Indeed, integration strategies are very useful for designing multi-product biorefineries (Moncada et al., 2013). In this sense, mass and energy integrations allow reducing the total production cost of products obtained from avocado biorefinery.

Similar to biorefineries based on non-oil feedstocks (SBP and naranjilla waste), for those based on oil feedstocks (avocado and SCG) enzyme is still the most expensive input that contributes to more than 50% of the total cost of inputs in all scenarios. Figure 7.13 depicts the share of each input over total input costs for scenarios without (scenarios 1 and 2) and with (scenarios 3 and 4) mass integration for avocado and SCG.

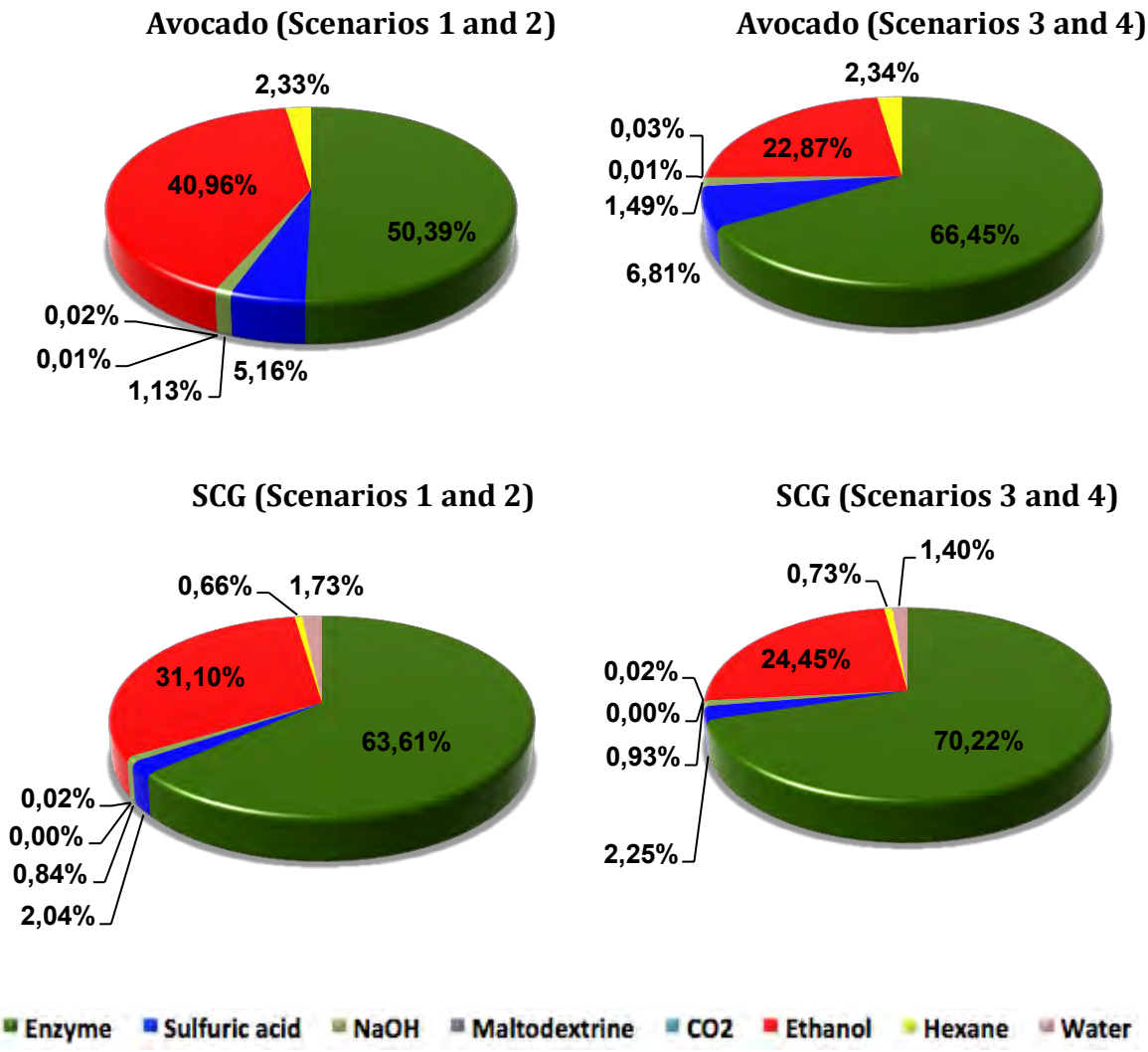


Figure 7.13. Total raw material cost for avocado and SCG biorefineries with and without mass integration

Input costs contribute from 29.56% (scenario 1) to 53.52% (scenario 4) of total production cost in avocado biorefinery and from 3% (scenario 1) to 6% (scenario 4) for SCG biorefinery. On the other hand, raw material for avocado biorefinery is very expensive because it is a fresh fruit while SCG is a residue obtained after coffee brew preparation. This fact suggests that mass integration is necessary to reduce the consumption of inputs for biorefineries. Figure 7.14 depicts the saving of water and ethanol with mass integration for biorefineries based on oil feedstocks. **Appendix D6** presents the contribution of each plant involved in the biorefineries based on oil feedstocks.

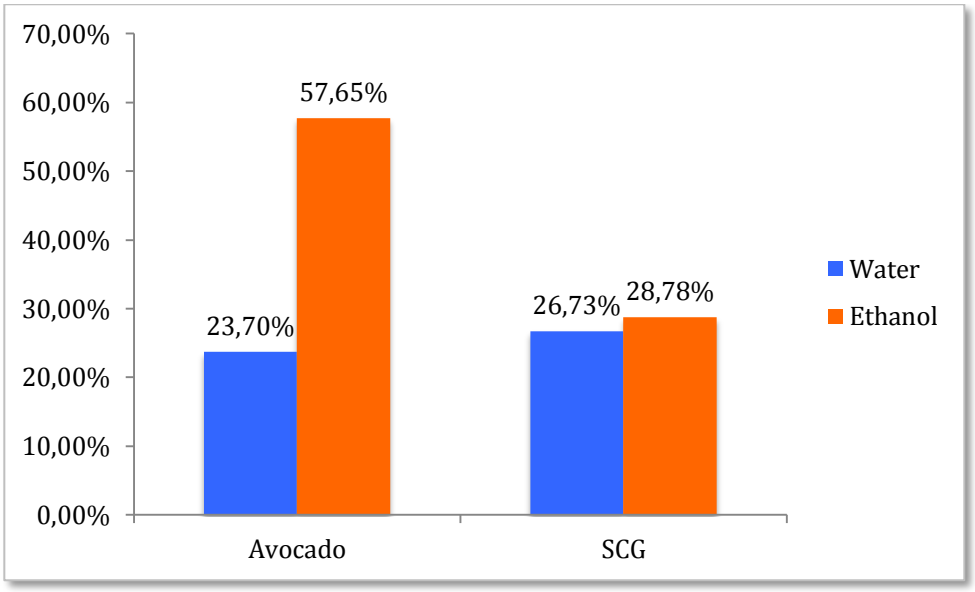


Figure 7.14. Saving of water and ethanol after mass integration

For both biorefineries, raw materials, inputs and utilities are the most important economic factors over total production cost. Therefore, mass and energy integrations are very useful for reducing total production cost. Figures 7.15 and 7.16 depict the total production cost for each one of the products in each scenario for avocado and SCG biorefineries respectively.

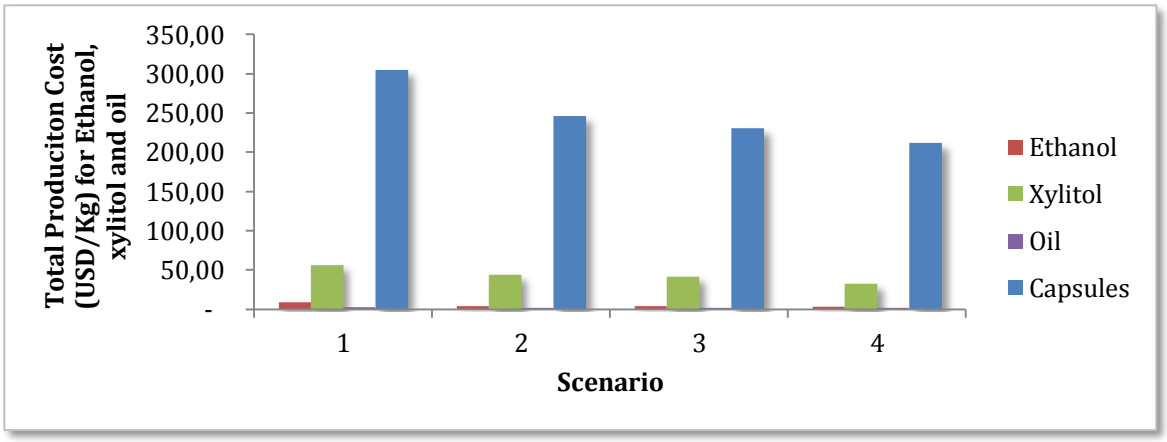


Figure 7.15. Total production cost for avocado biorefinery

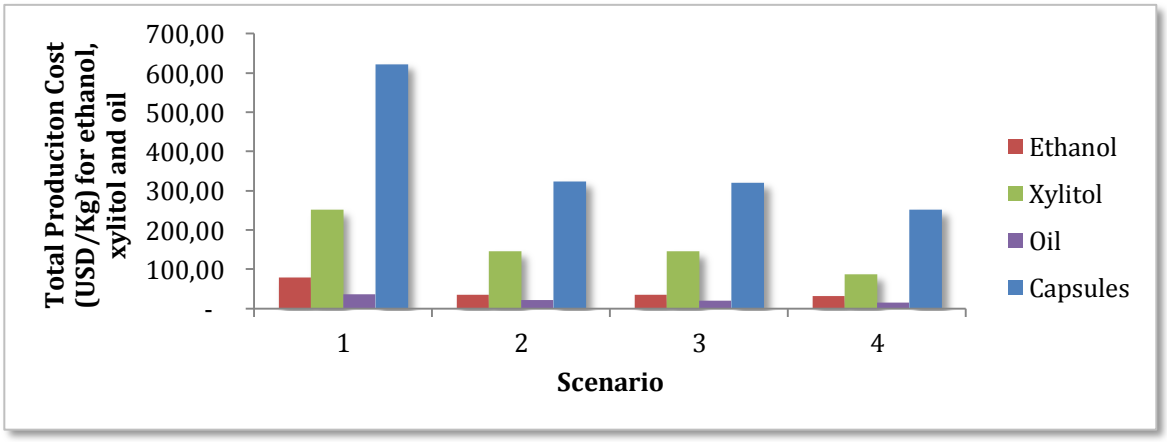


Figure 7.16. Total production cost for avocado biorefinery

Similar to biorefineries based on non-oil feedstocks (SBP and naranjilla waste), the ratio of sale price to total production cost (Mussatto et al., 2013), was calculated using the prices reported in **Appendix D2** and mass balance of Table 7.9. Figure 7.17 shows the sale price to total production cost ratio. Thus, scenario 4 has the highest sale price to total production cost ratio indicating that mass and energy integrations can reduce significantly the total production cost as well as the addition of a cogeneration system.

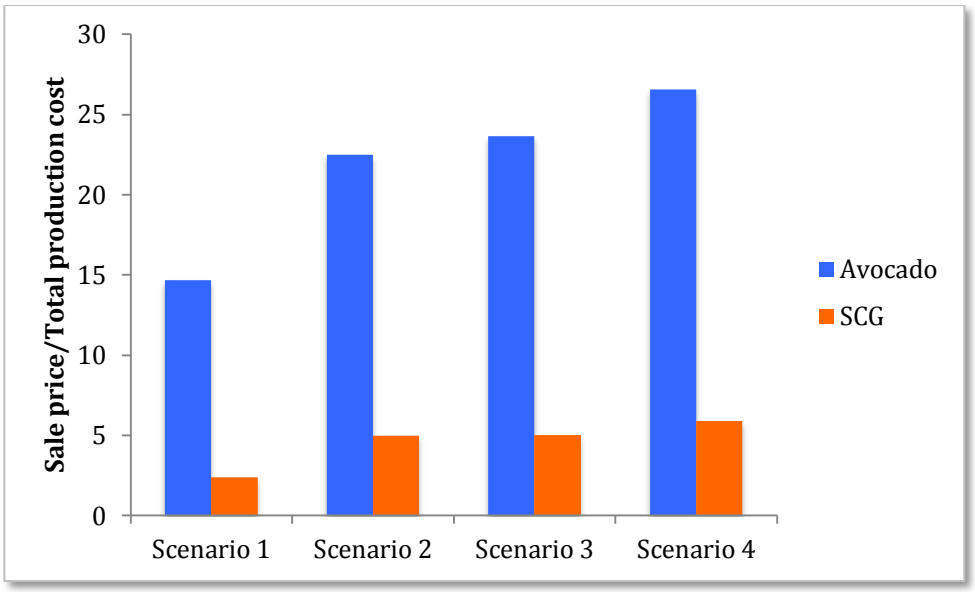


Figure 7.17. Sale price to total production cost ratio for biorefineries

7.2.2 Results of energy consumption of biorefineries based on oil feedstocks

Figures 7.18 and 7.19 depict the energy consumption of each plant for avocado and SCG biorefineries respectively. As it was expected, ethanol plant is the highest consumer of utilities in avocado and SCG biorefineries. This result is similar to that obtained for biorefineries based on non-oil feedstocks (SBP and naranjilla waste). The most important economic factor over total production cost for ethanol plant was utilities. This fact also was found by (Quintero et al., 2013), where using rice husk as feedstock they found for both, standalone ethanol plant and coupled with cogeneration system, that utilities represented the highest contribution with 48.89% and 45.40% respectively.

According to the level of integration, the energy requirements can be reduced significantly. Figure 7.20 depicts the reduction of utilities for avocado and SCG biorefineries. Similarly to results of biorefineries based on non-oil feedstocks (SBP and naranjilla waste), the heat integration is the most important level of integration because with this level it is possible to reach 30.33% and 42.36% of reduction of utilities regard to base case (scenario 1) for avocado and SCG biorefineries respectively. This fact

suggests that in the design of biorefineries, heat integration should be considered to reduce significantly the energy consumption of the biorefineries. On the other hand, a cogeneration system does not contribute significantly over utilities reduction, therefore this level of integration could not be considered in the design of biorefineries based on fruit wastes.

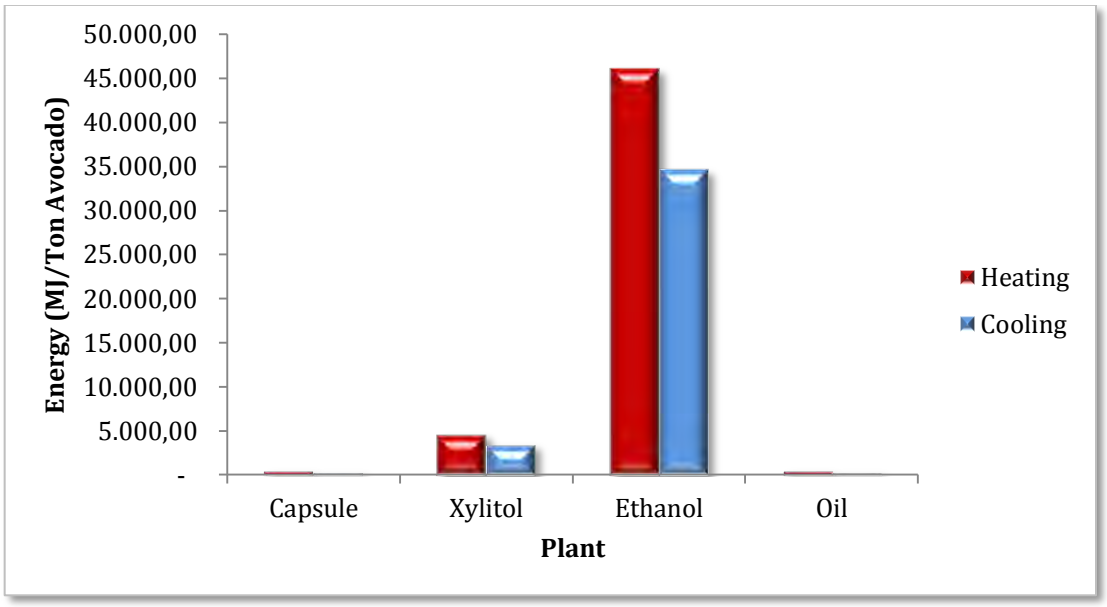


Figure 7.18. Energy consumption of each plant for avocado biorefinery

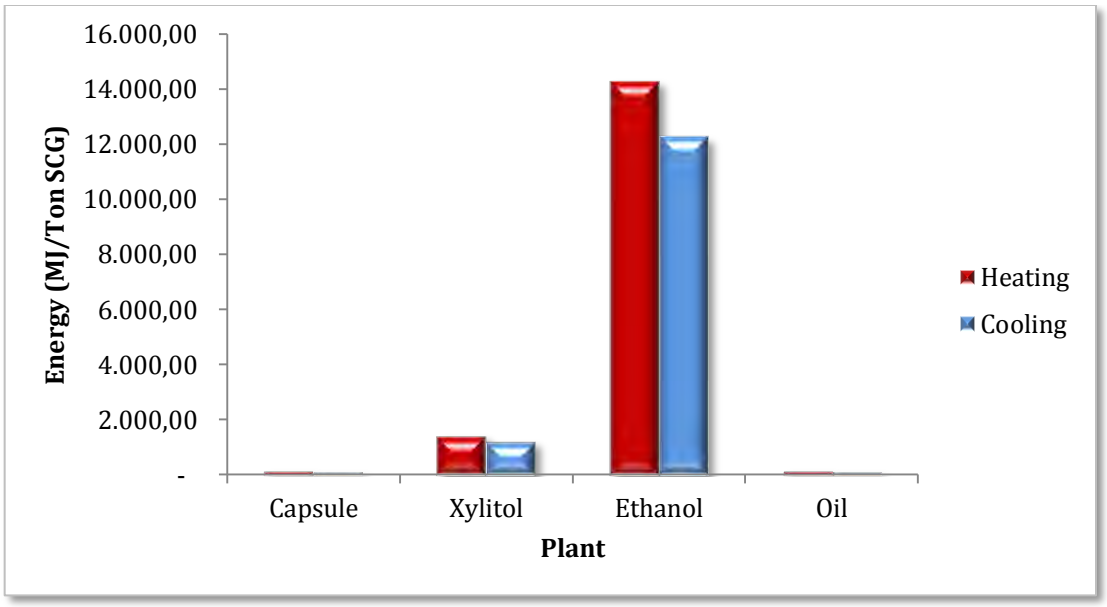


Figure 7.19. Energy consumption of each plant for SCG biorefinery

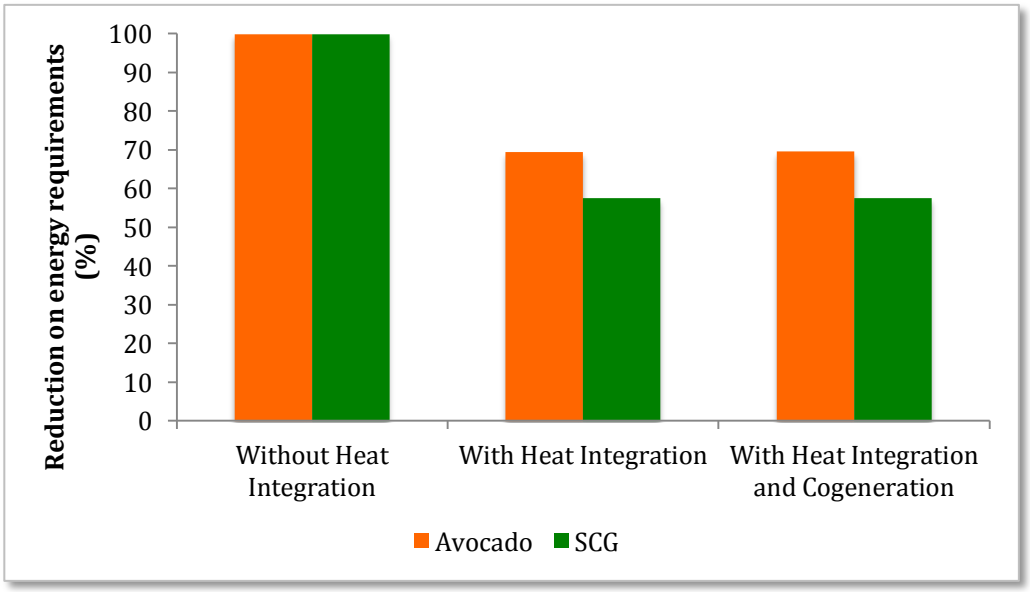


Figure 7.20. Reduction of utilities consumption according to the level of integration

Heat integration has a significant effect on utilities reduction; this is due to the percentage of recovering that Pinch methodology permits. This strategy of heat integration allows recover 50.18% and 37.26% (common area between cool and heat

composite curves) of the energy needed for avocado and SCG biorefineries respectively according to the composite curves presented in **Appendix D7**.

7.3 Sensibility analysis on chemical composition of feedstocks of the biorefineries

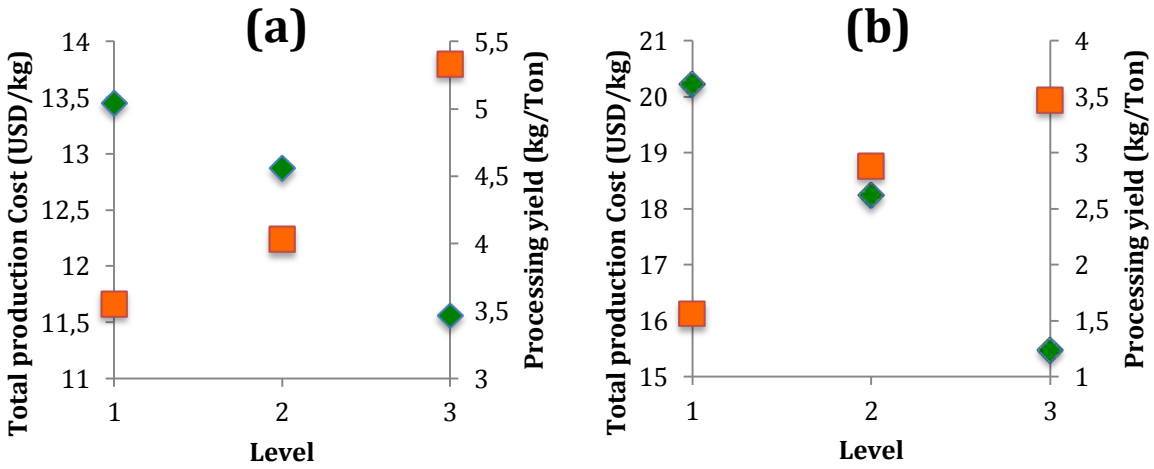
Here, a sensibility analysis over both types of biorefineries (based on non-oil and oil feedstocks) was developed taken into account a change on the chemical composition of the feedstocks. For this, the chemical composition of a target compound of the best scenario was changed 10% toward up and down regard to the base composition used in previous sections. Table 7.13 shows the information of compounds and changes for each feedstock.

Table 7.13. Parameters for sensibility analysis of biorefineries

Biorefinery	Scenario	Target product	*Initial composition (%). Level 2	Chemical composition (10% down). Level 1	Chemical composition (10% up). Level 3
SBP	3	Capsules	0.03	0.027	0.033
Naranjilla waste	3	Capsules	0.01	0.009	0.011
Avocado	4	Oil	14.28	12.85	15.71
SCG	4	Oil	5.96	5.36	6.56

* Percentage of the feedstock in previous sections that corresponds to that obtained form laboratory analysis (SBP and avocado) and literature (naranjilla waste and SCG).

Figure 7.21 depicts the total production cost and yields obtained for each one of the levels evaluated regard to chemical composition for biorefineries based on non-oil feedstocks (SBP and naranjilla waste). As it was expected, with higher content of the target compound, it is possible to obtain highest yields and lowest total production costs. For all products, the total production cost is reduced if higher amount of the target compound is present in the feedstock. For example, if the concentration of phenolic compounds in SBP and naranjilla waste is 10% higher than that determined experimentally and, used in the base case (that used in previous sections) then, 10.18% and 15.19% reduction in the total production cost is reached for phenolic compounds in the SBP and naranjilla waste biorefineries respectively. On the other hand, yield increases when the target compound increases in the chemical composition as it was expected. Table 7.14 shows the percentage of reduction on total production cost as well as the percentage of increasing on yields when the chemical composition changes due to the variations on the target product for SBP and naranjilla waste.



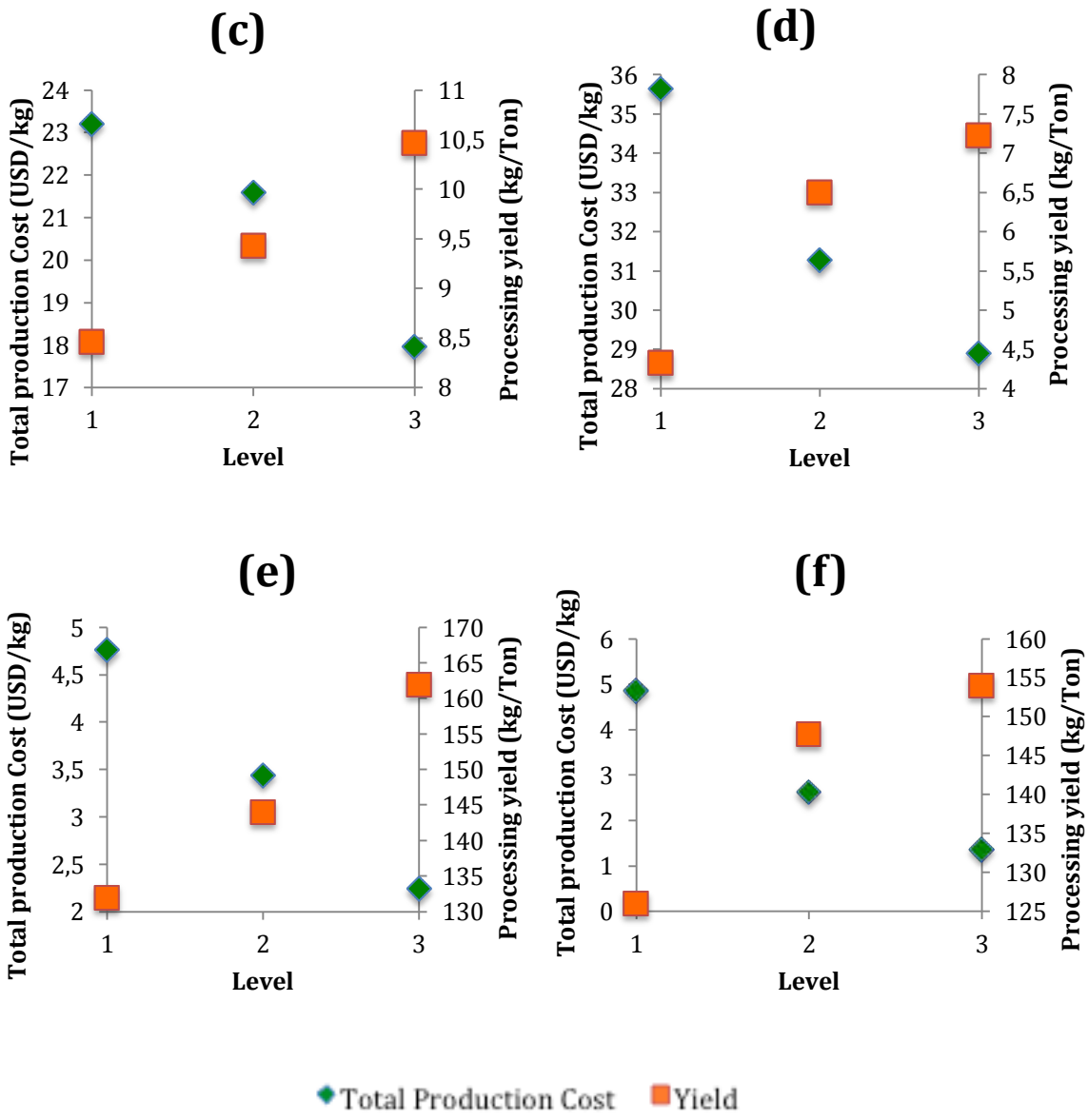


Figure 7.21. Total production costs and yields for the best scenarios for biorefineries based on non-oil feedstocks (SBP and naranjilla waste). (a), (c) and (e) are phenolic compounds, xylitol and ethanol for SBP biorefinery. (b), (d) and (f) are phenolic compounds, xylitol and ethanol for naranjilla waste biorefinery

Table 7.14. Changes on total production cost and yields due to variations of chemical composition of biorefineries based on non-oil feedstocks (SBP and naranjilla waste). NW means naranjilla waste

Product	Total production cost (%)		Yield (%)	
	SBP	NW	SBP	NW
Phenolic compounds	10.18	15.19	32.25	20.49
Xylitol	16.77	7.62	11.03	11.23
Ethanol	34.88	47.91	12.5	4.19

Similar results were obtained for biorefineries based on oil feedstocks (avocado and SCG). Figures 7.22 and 7.23 depict the total production cost and yields obtained for each one of the levels evaluated regard to basic chemical composition for avocado and SCG biorefineries respectively. Reduction of total production cost for all products (phenolic compounds, xylitol, ethanol and oil) in avocado biorefinery was similar to those obtained for SBP and naranjilla waste biorefineries. Reduction of total production cost ranges from 4.68% to 29.44% when 10% of the oil increases in the chemical composition of avocado. Yields for products increase from 3.62% to 19.05% according to the product obtained. For SCG, total production costs have a reduction from 2.54% to 9.10% while yields have an increasing from 2.8% to 18.3%. Table 7.15 shows the percentage of reduction on total production cost as well as the increasing on yields when the chemical composition changes due to the variations on target product for avocado and SCG.

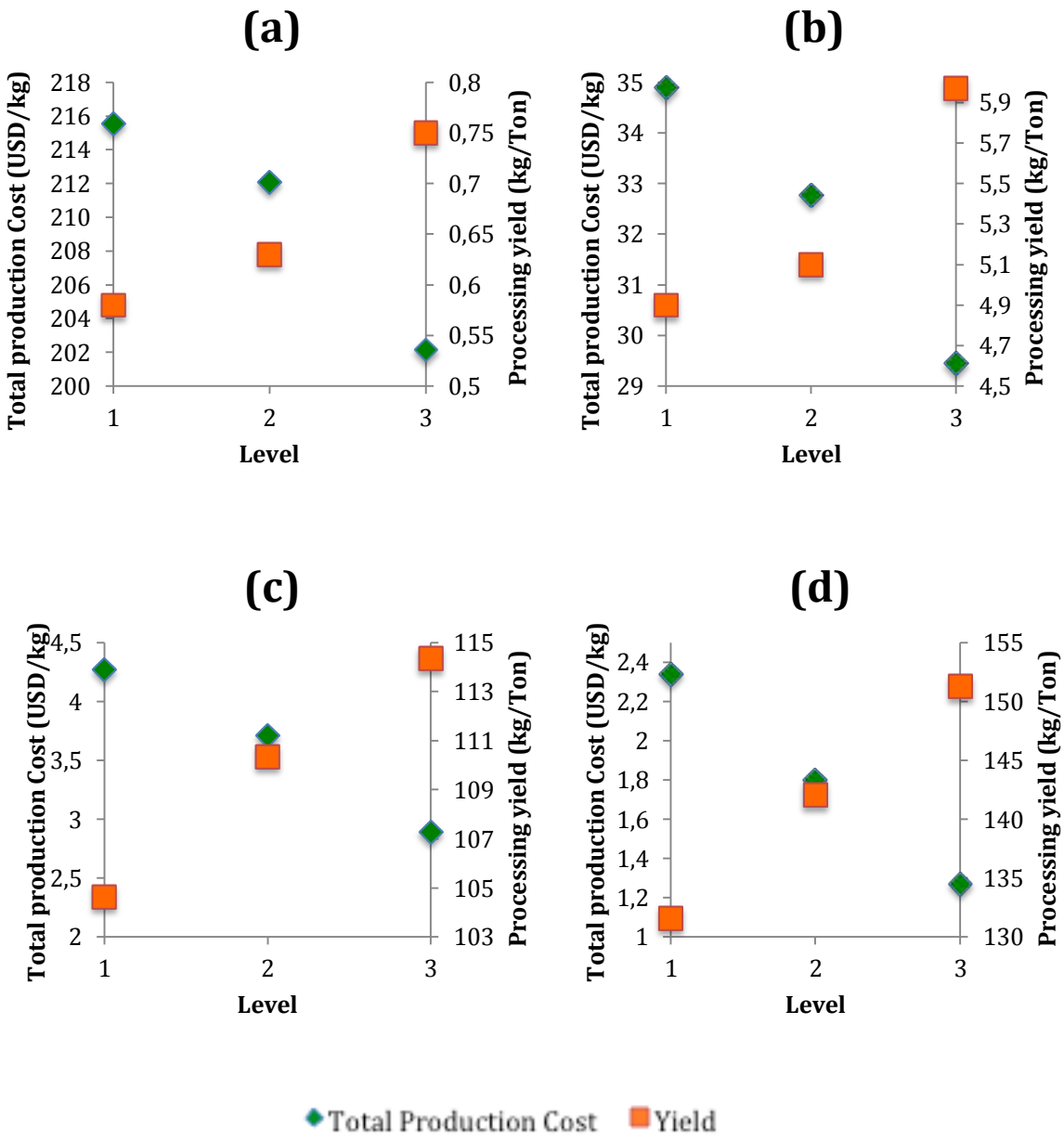


Figure 7.22. Total production costs and yields for the best scenarios for avocado biorefinery. (a), (b), (c) and (d) are phenolic compounds, xylitol, ethanol and oil respectively

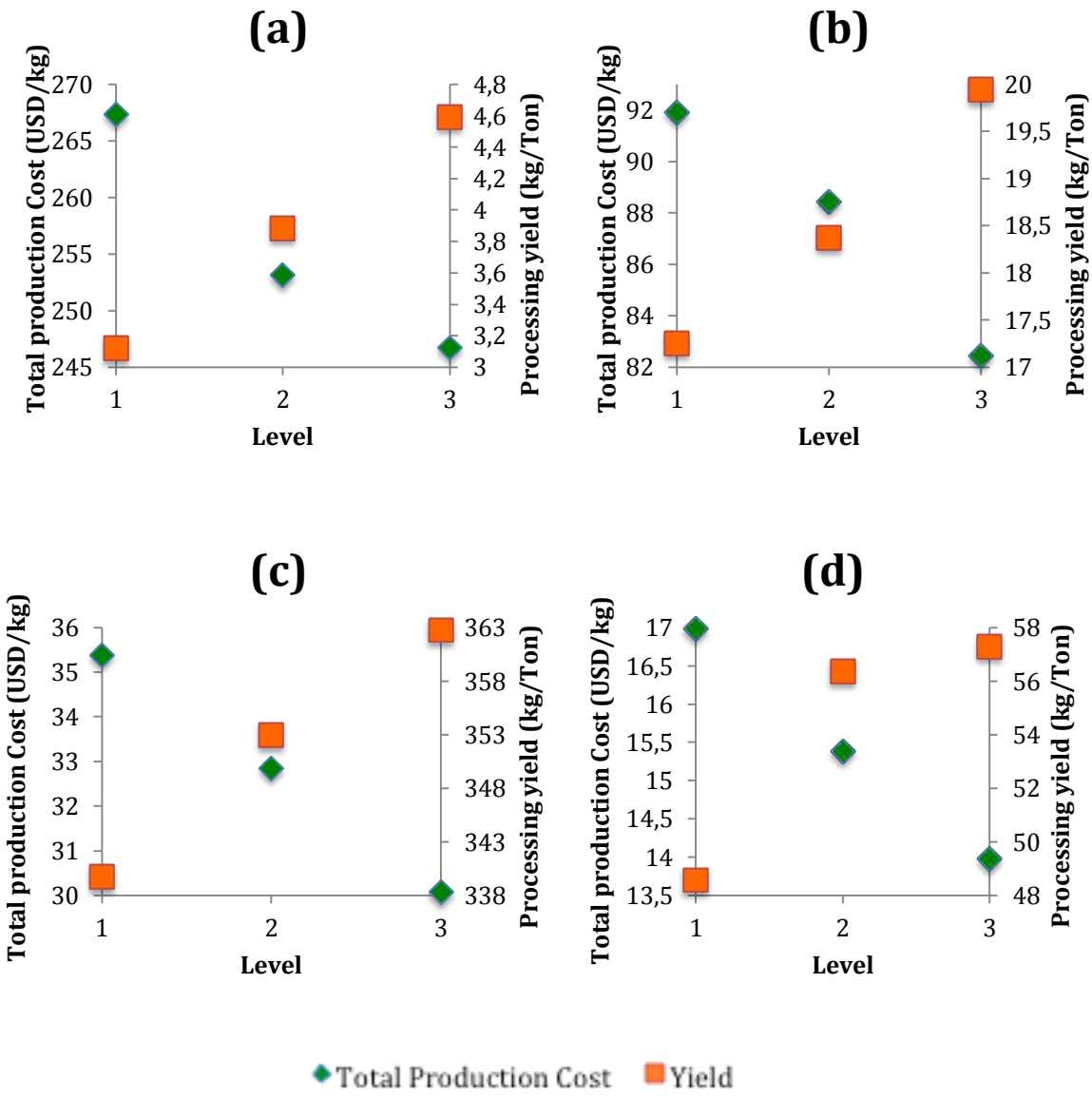


Figure 7.23. Total production costs and yields for the best scenarios for SCG biorefinery. (a), (b), (c) and (d) are phenolic compounds, xylitol, ethanol and oil respectively

Table 7.15. Changes on total production cost and yields due to variations on chemical composition of avocado and SCG

Product	Total production cost (%)		Yield (%)	
	Avocado	SCG	Avocado	SCG
Phenolic compounds	4.68	2.54	19.05	18.3
Xylitol	10.13	6.77	17.06	8.55
Ethanol	22.10	8.49	3.62	2.8
Oil	29.44	9.10	6.5	1.67

These results demonstrate that variations on chemical composition of the feedstock (SBP, naranjilla waste, avocado and SCG) lead to changes on total production costs and yields. Both, heat and mass integrations become in the most important levels of integration that have a significant effect over total production cost for entire biorefinery.

Conclusions

In light of the results obtained after techno-economic analysis, both types of biorefineries, based on non-oil and oil feedstocks (based on SBP, naranjilla waste, avocado and SCG) are attractive because the ratio of sale price to total production cost is higher to 1. Both, heat and mass integrations are necessary to reduce considerably the utilities consumption and inputs. However, heat integration was the most important integration level for biorefineries based on the feedstocks evaluated. Raw material also

had an important contribution in the design of biorefineries, especially for avocado biorefinery because avocado (the fresh fruit) is an expensive raw material in comparison with SBP, naranjilla waste and SCG, which are residues from fruit processing. Enzyme also has an important effect over total production cost because it becomes in an increasing of the inputs for ethanol production. The most promising products were phenolic compounds from SBP and naranjilla waste biorefineries and oil from avocado and SCG biorefineries.

A change on the chemical composition also has important variations on the yield and productions of the biorefineries. An increasing of the target compound in the feedstock resulted in an increasing of the yield. SCG is maybe, the most promising feedstock for biorefinery because its high content of holocellulose that allows obtaining high yields as well as sale to total production cost ratio. Despite that ethanol plant has the highest energy consumption in the biorefineries, the production of ethanol integrated in a biorefinery is important because it allows obtaining part of the ethanol necessary to other plants.

8. Chapter 8. Environmental assessment of the biorefineries

Overview

This chapter presents the results for environmental assessment for both types of biorefineries, based on non-oil feedstocks (SBP and naranjilla waste) and based on oil feedstocks (avocado and SCG). The environmental performance of the biorefineries was evaluated by two ways. The first way using the leaving and generated Potential Environmental Impact (PEI) of the biorefineries by means of the WAste Reduction Algorithm (WAR) and the second way corresponds to the quantification of greenhouse gases (GHG) emissions of the entire biorefinery.

For PEI assessment, eight environmental categories were used and the same scenarios evaluated in chapter 7 were also assessed. For the quantification of GHG emissions, the emission factors for all the involved compounds were used. The results showed that mass integration has an important role in reducing the leaving PEI in the biorefineries. For generated PEI, heat integration becomes the most important level of integration for reducing generated PEI. The quantification of GHG emissions confirmed that despite that all scenarios are environmentally friendly; heat integration is very effective in reducing significantly the GHG emissions from biorefineries.

GHG emissions differed among the products obtained in each biorefinery however, according to the scenario evaluated, the contribution of each product to the GHG emissions increased proportionally to the level of integration.

8.1 Potential Environmental Impact (PEI) for biorefineries based on non-oil feedstocks (SBP and naranjilla waste)

Potential Environmental Impact (PEI) of each one of the biorefineries as well as for each scenario evaluated was calculated according to procedures described in chapters 2 and 3 as well as in **Appendix B3**. For PEI, eight environmental impact categories were evaluated (Cabezas et al., 1999; Young and Cabezas, 1999): human toxicity potential by ingestion (HTPI), human toxicity potential by dermal and inhalation exposure (HTPE), terrestrial toxicity potential (TTP), aquatic toxicity potential (ATP), global warming potential (GWP), ozone depletion potential (ODP), photochemical oxidation potential (PCOP) and acidification potential (AP). The Potential Environmental Impact (PEI) of the process was calculated per kilogram of products (ethanol, xylitol and phenolic compounds for SBP and naranjilla waste biorefineries and oil as additional product for avocado and SCG biorefineries). Natural Gas was used as fuel to cover the heat requirements for the biorefineries.

For all biorefineries, both, leaving and generated PEI were calculated. Leaving PEI corresponds to that impact due to the outlet streams from the biorefinery while generated PEI is that impact resulted from the difference between outlet and inlet streams in the biorefinery. Total leaving and total generated PEI corresponds to the sum of the eight environmental impact categories.

8.1.1 PEI for SBP biorefinery

Figures 8.1 and 8.2 depict the leaving and generated PEI for SBP biorefinery respectively. For leaving PEI, the most affected environmental categories correspond to HTPI, TTP and PCOP. The latter is based on the organic waste present in the liquid streams leaving from the biorefinery, for instance, the discharge of stillage that contains organic matter. Similar behavior was reported by (Moncada et al., 2014b) for a

biorefinery based on sugarcane where HTPI and TTP are affected by organic matter. On the other hand, PCOP is associated with the production of CO_2 (Montoya R et al., 2006). Therefore, the CO_2 leaving the xylitol and ethanol plants as well as the CO_2 losses from SFE in phenolic compounds plant contribute to PCOP. Similar results have been reported by (Hernández et al., 2014) where PCOP was the most important environmental category that contributes to the PEI of a biorefinery based on olive stone. Mass integration (scenarios 3 and 4) reduces significantly the PEI of the SBP biorefinery in comparison with energy integration (scenario 2). The decrease in outlet streams such as residual ethanol and water results in lower level of possible pollutants that increase the PEI. Therefore, mass integration has an important role from an environmental point of view. For total leaving PEI, scenario 4 was the most environmentally friendly because the reduction of possible pollutants in the outlet streams which are recycled to the process.

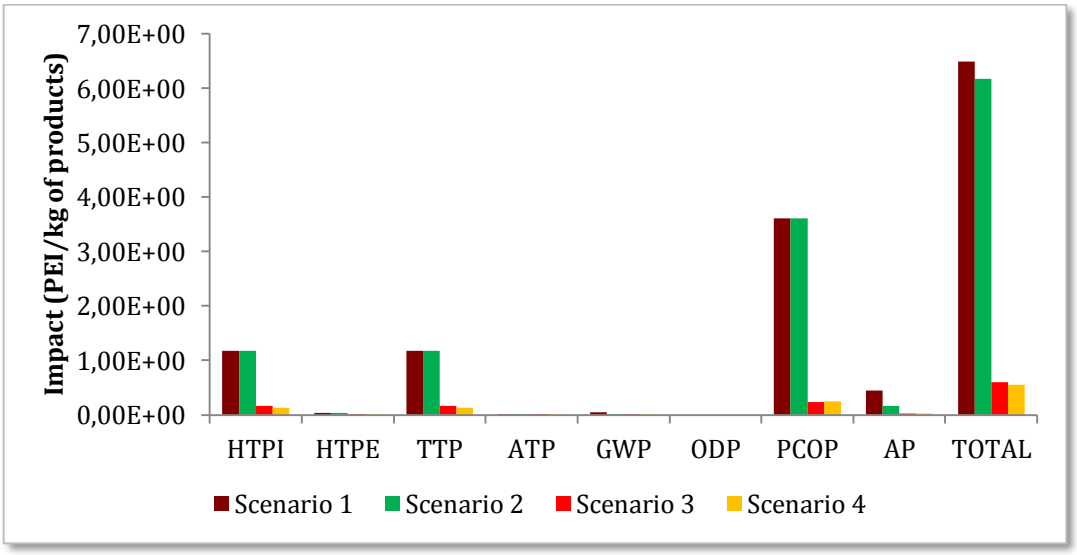


Figure 8.1. Leaving PEI for SBP biorefinery

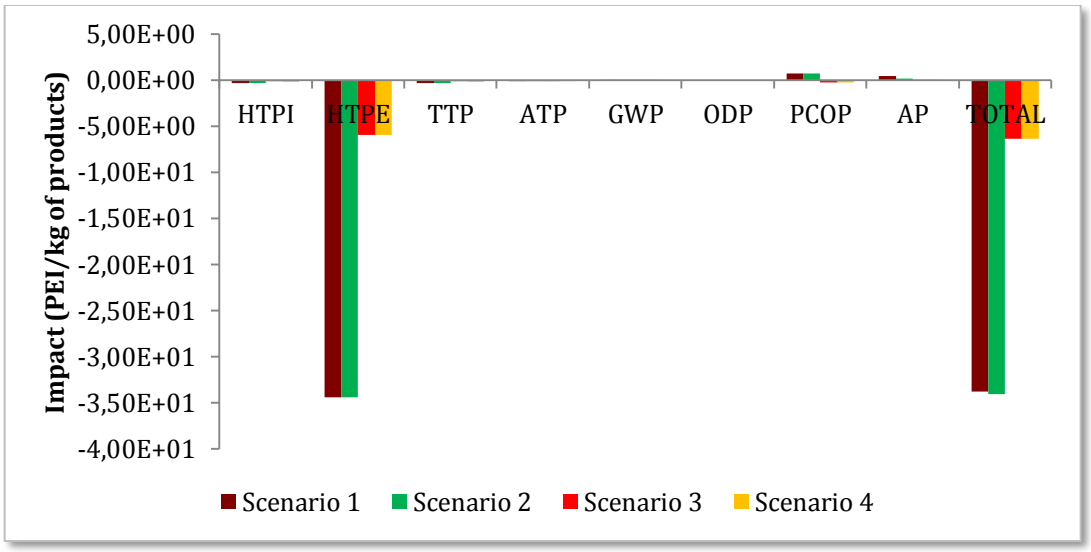


Figure 8.2. Generated PEI for SBP biorefinery

For generated PEI, the total PEI was reduced (negative bars) in all scenarios. For generated PEI, scenario 2 becomes the most attractive alternative from an environmental point of view because this scenario has the lowest value for total PEI, thus indicating that effluents are less contaminant that inlet streams. The latter is because with heat integration (for scenario 2) a lower consumption of utilities is needed and therefore, a low production of gases is obtained. Scenarios 3 (with mass integration) and 4 (with cogeneration system) are less environmentally friendly because the mass integration allows reducing the impact of inlet streams and a cogeneration system produces gases that affect the PEI in a negative manner.

8.1.2 PEI for Naranjilla waste biorefinery

Figures 8.3 and 8.4 depict the leaving and generated PEI for Naranjilla waste biorefinery respectively. Leaving PEI of naranjilla waste biorefinery has tendency similar to that of SBP biorefinery because the products and plants are the same for both however; naranjilla waste biorefinery has total leaving PEI values a little bit highest than SBP biorefinery. This is because the holocellulose content of naranjilla waste is a bit higher that SBP and therefore, naranjilla waste biorefinery consumes more ethanol and H_2SO_4

that influence the environmental impact. Total generated PEI is reduced and scenario 2 (with heat integration) reduces significantly the total generated PEI such as it happened for SBP biorefinery.

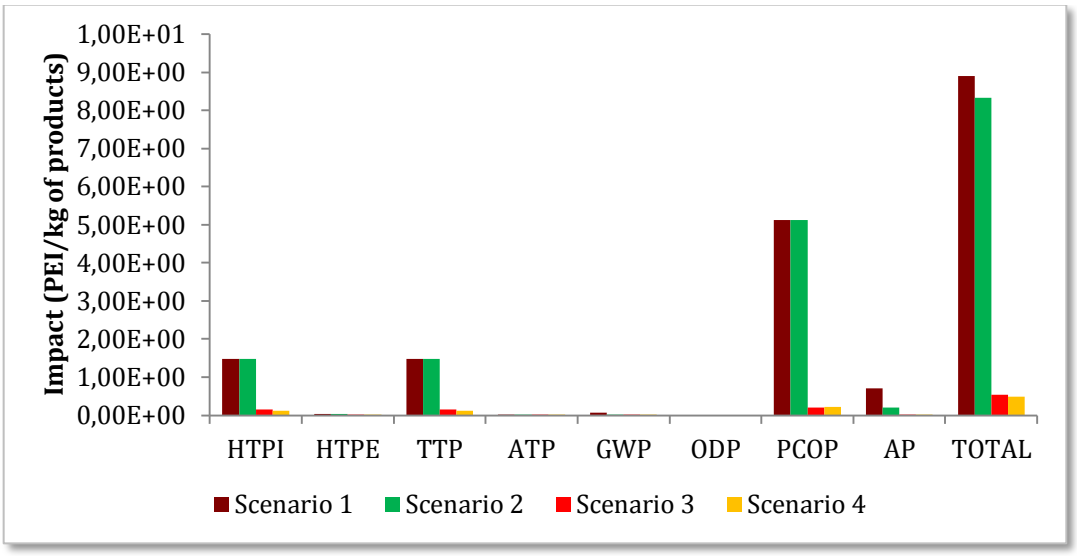


Figure 8.3. Leaving PEI for naranjilla waste biorefinery

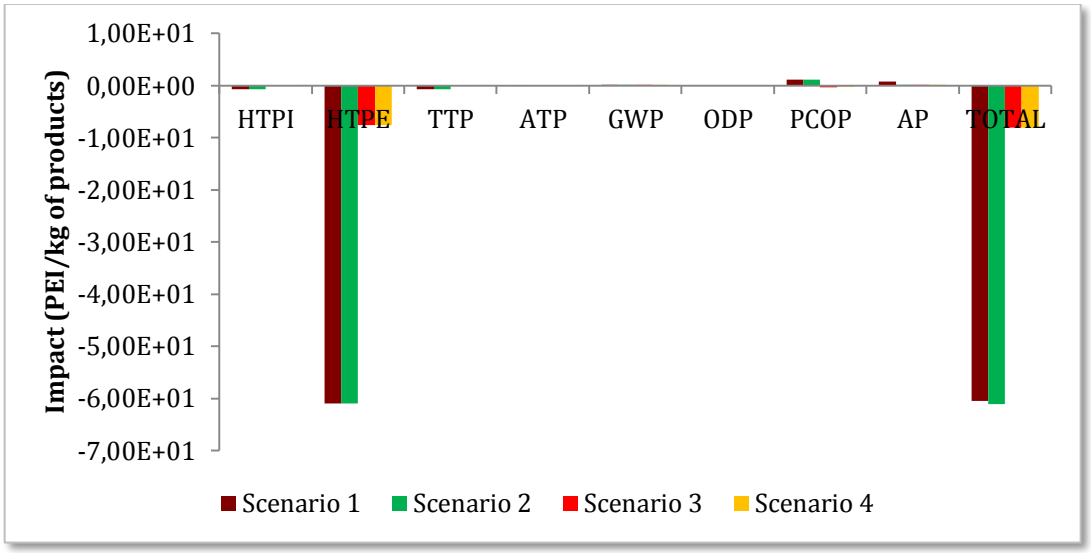


Figure 8.4. Generated PEI for naranjilla waste biorefinery

From these results, a biorefinery based on naranjilla waste is more environmentally friendly than SBP due to the lower level of generated PEI. However, both biorefineries can reduce the generated PEI, which in turn, becomes the biorefineries based on non-oil feedstocks in environmentally friendly biorefineries.

8.2 Potential Environmental Impact (PEI) for biorefineries based on oil feedstocks (avocado and SCG)

8.2.1 PEI for Avocado biorefinery

Figures 8.5 and 8.6 depict the leaving and generated PEI for avocado biorefinery respectively. For leaving PEI, avocado biorefinery presents lower values than those found for SBP and naranjilla waste biorefineries however; AP (acidification potential) appears with high values that corresponds to the consumption of external energy to cover the energy requirements (Moncada et al., 2014b). Both, heat and mass integrations give a reduction of leaving PEI but mass integration has the most significant effect. Therefore, scenarios 3 and 4 were the most environmentally friendly because total leaving PEI was lowest. For generated PEI, avocado biorefinery suggests that scenario with heat integration (scenario 2) has the most significant effect because the reduction of utilities decreases which, in turn, reduces the gases produced from energy generation.

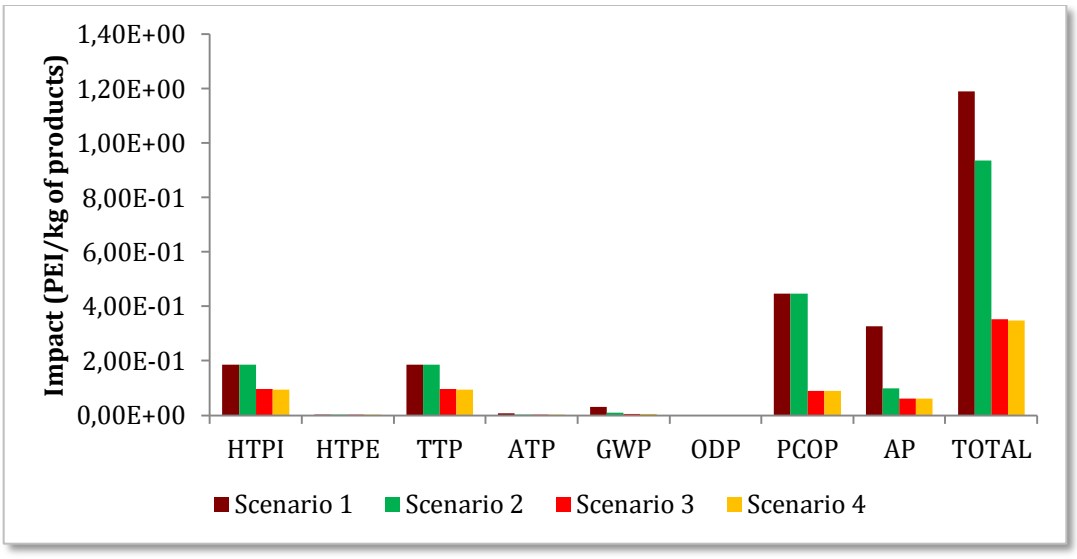


Figure 8.5. Leaving PEI for avocado biorefinery

The total leaving PEI has a similar behavior that those found for SBP and naranjilla waste. Scenario 1 continues having the highest leaving PEI and although heat integration has a good reduction of leaving PEI regard to scenario 1 (without heat integration), mass integration follows being the most important contribution on the reduction of leaving PEI (such scenarios 3 and 4).

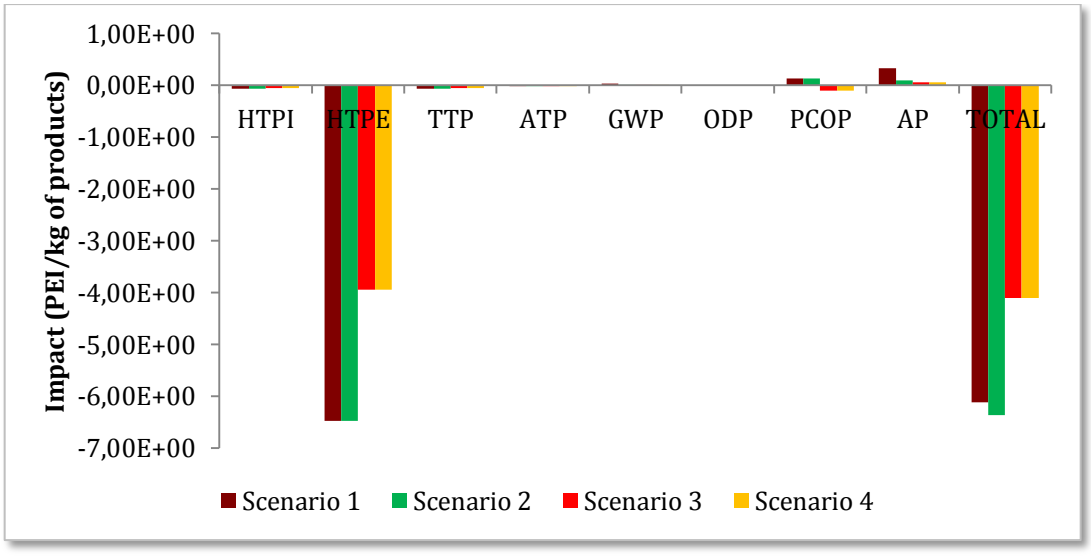


Figure 8.6. Generated PEI for avocado biorefinery

8.2.2 PEI for SCG biorefinery

Figures 8.7 and 8.8 depict the leaving and generated PEI for SCG biorefinery respectively. Leaving PEI for SCG biorefinery presents a similar behavior that of avocado biorefinery because both have the same plants and products. However, SCG biorefinery presents values of total leaving PEI higher than for avocado biorefinery because SCG has higher content of holocellulose (20.82% for SCG and 10.97% in average for peel and seed of avocado) and therefore, it leads to a higher consumption of reagents that affect the PEI of the SCG biorefinery.

AP environmental category also appears for SCG biorefinery such as for avocado biorefinery. However, PCOP has the most significant contribution to total leaving PEI in comparison with HTPI and HTPE categories. This suggest that CO_2 emissions from SCG biorefinery has an important contribution on leaving PEI and therefore, these emissions should be reduced if outlet streams with low PEI are desired.

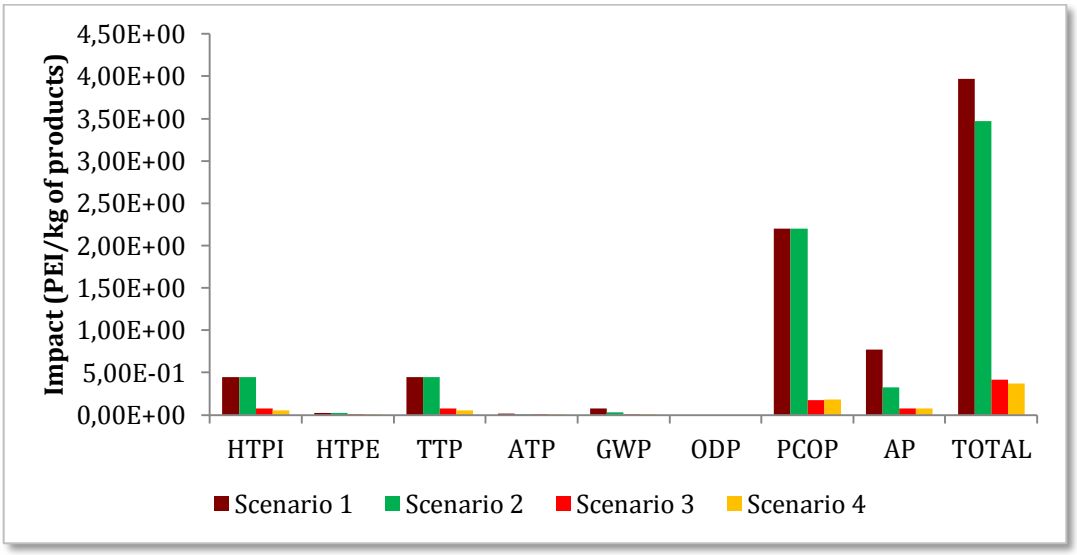


Figure 8.7. Leaving PEI for SCG biorefinery

Generated PEI of SCG biorefinery suggests that scenario 2 is the most attractive scenario because of its low level of generated PEI (negative bars). Therefore, these results indicate that heat integration has a significant effect on the environmental performance of SCG biorefinery. Scenarios 3 (with mass integration) and 4 (with cogeneration system) are less environmentally friendly because the mass integration allows reducing the impact of inlet streams and a cogeneration system produces gases that affect the PEI in a negative manner, similar to what happens for biorefineries based on non-oil feedstocks.

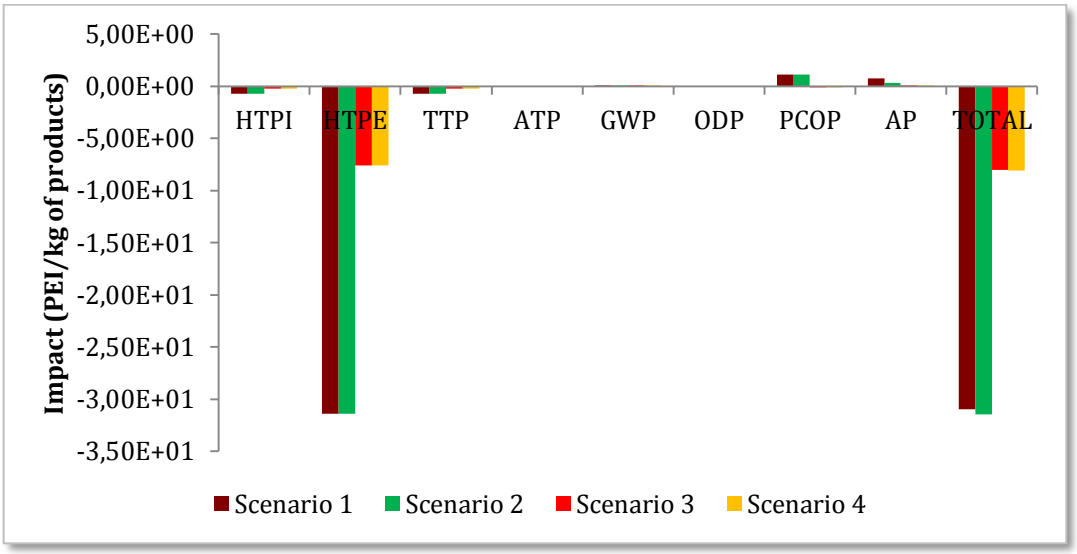


Figure 8.8. Generated PEI for SCG biorefinery

For all biorefineries (based on SBP, naranjilla waste, avocado and SCG), the generated PEI was reduced (negative bars). This reduction means that outlet streams are less contaminated than inlet streams. This suggests that the chemicals contained in inlet streams are more pollutants than outlet streams due to its characteristics and amounts used. Despite that all scenarios for all biorefineries are environmentally friendly; scenario 2 (with heat integration) is more efficient in reducing the generated PEI. The latter indicates that a plant for supply the energy requirements of the biorefineries have in an important effect over PEI.

8.3 Greenhouse Gases (GHG) assessments of the biorefineries

The quantification of GHG emissions for all biorefineries was based on the procedure described in **Appendix B3**. The emission factors used for all chemicals involved in the streams for all biorefineries are also showed in **Appendix B3**.

8.3.1 GHG emissions for biorefineries based on non-oil feedstocks (SBP and naranjilla waste)

Figure 8.9 depicts the GHG emissions for both, SBP and naranjilla waste biorefineries. According to these results, the balance of GHG emissions on the biorefineries gives negative GHG emissions (negative bars). This means that outlet streams of the biorefineries have less impact on GHG emissions than the inlet streams. This result is in agreement with the negative generated PEI values that were calculated for these biorefineries. Similar results have been reported by (Haro et al., 2015) where GHG balance gives negative emissions for a biorefinery with multi-production found GHG emissions of -75 g CO_2-eq/MJ of product. For both, SBP and naranjilla waste biorefineries, scenario 1 was the most environmentally friendly because the low values of GHG emissions. This is because in scenario 1 (without heat integration), the energy consumption is highest (that means high production of gases from energy generation) and therefore, the GHG emissions for inlet streams are more pollutant than the outlet streams. Ethanol was the product with the highest reduction of GHG emissions followed by xylitol and phenolic compounds. This is also associated with the energy consumption therefore; ethanol plant gives the highest reduction of GHG emissions on the biorefineries.

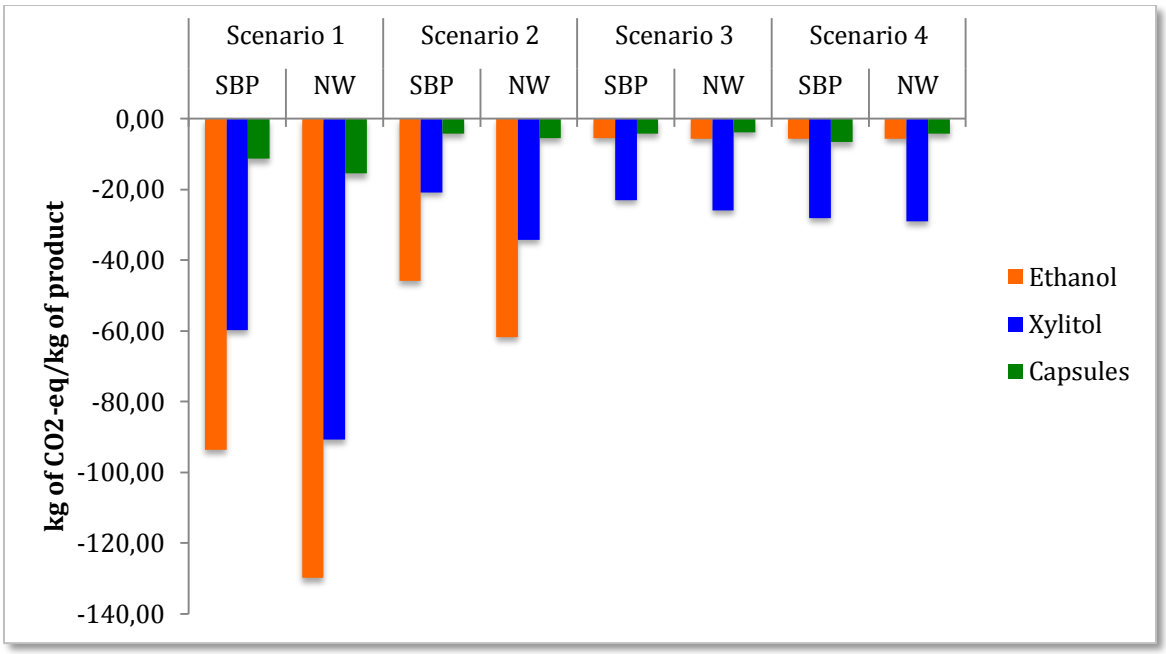


Figure 8.9. GHG for SBP and naranjilla waste (NW) biorefineries

8.3.2 GHG emissions for biorefineries based on oil feedstocks (avocado and SCG)

Figure 8.10 depicts the GHG emissions for both, avocado and SCG biorefineries. Similar to SBP and naranjilla waste biorefineries, scenario 1 was the most environmentally friendly for both, avocado and SCG biorefineries, because the low values of GHG emissions. For these biorefineries, ethanol also represents the product with highest contribution to the reduction of GHG emissions on the biorefineries. Oil plant of these biorefineries also has a significant contribution on the reduction of GHG emissions because it represents additional energy consumption in comparison with SBP and naranjilla waste biorefineries.

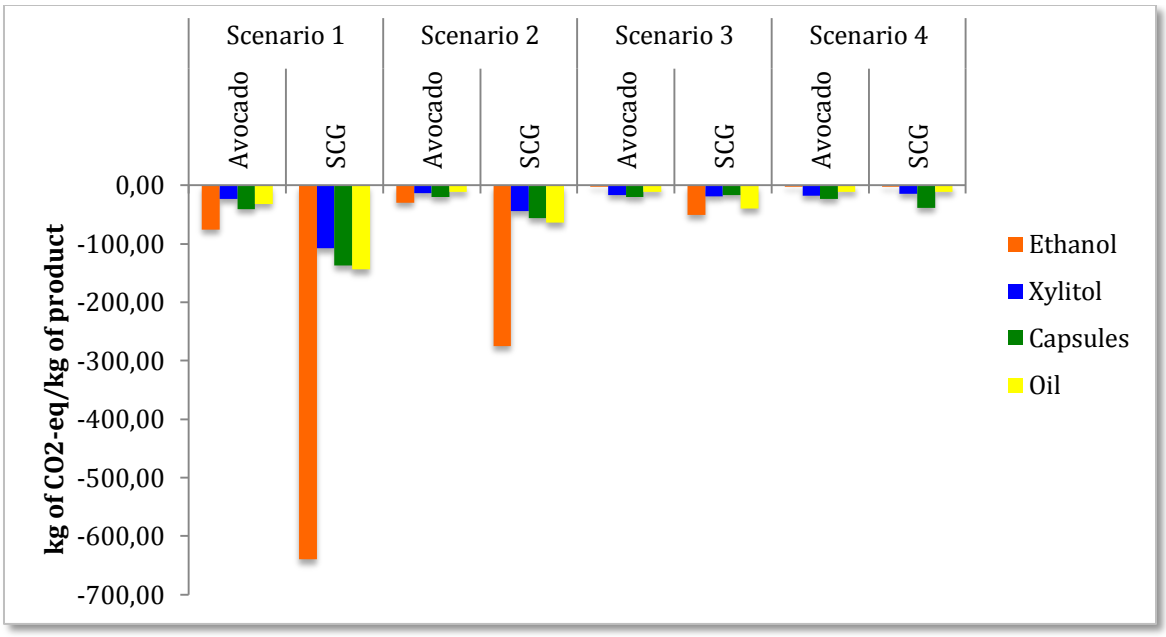


Figure 8.10. GHG for avocado and SCG biorefineries

For all scenarios, SCG biorefinery has the highest reductions on GHG emissions. For scenarios 1 and 2 in SCG biorefinery ethanol was the product with the highest contribution on GHG emissions followed by oil, phenolic compounds and xylitol while for avocado biorefinery the contributions are highest for ethanol, phenolic compounds, oil and xylitol.

Conclusions

The environmental assessment demonstrated that all of the evaluated biorefineries are environmentally friendly because the reduction of both generated PEI and GHG emissions. Mass integration is very important for reducing the leaving PEI of the biorefineries while heat integration becomes the most important contribution for reducing generated PEI.

The quantification of GHG emissions confirmed the results obtained for generated PEI that suggests that all biorefineries are environmentally friendly for any scenario. However, those scenarios that include mass and heat integrations are the most environmentally friendly. Ethanol was the most important product that contributes to the

reduction of both, PEI and GHG emissions because of the energy consumption. The types of pollutants contained in the outlet streams have an important effect on the leaving PEI. For instance, outlet streams containing acids and ethanol affect directly those environmental categories that are related to human toxicity potential by ingestion and dermal exposure (HTPI and HTPE). The most affected environmental category was PCOP because the CO_2 produced in xylitol and ethanol plants as well as for that CO_2 loosed in the phenolic compounds plant.

9. Conclusions and suggestions

Conclusions

This research allowed a better understanding of the design of biorefineries based on fruits and its wastes to obtain value added products. It is clear that Colombia has an attractive potential to develop biorefineries based on fruits and its wastes. Both, fresh fruit and derived streams from fruits processing (mainly peel and seed) are an important source for producing valuable compounds to be used as functional food or nutraceuticals with applications in chemical, food and pharmaceutical industries.

The chemical characterization of the raw materials explored in this thesis (Spent blackberry pulp (SBP), avocado, naranjilla waste and spent coffee grounds (SCG)) showed important contents of valuable compounds (such as phenolic compounds) and holocellulose (cellulose and hemicellulose) that allow obtaining interesting products. This thesis demonstrates that important products can be obtained from the selected feedstocks. Among these are: phenolic compounds, oil and holocellulose as a source of sugars (C_5 and C_6), which in turn, can become a raw material for producing other valuable products. Yet additionally, the lignin content can be considered, either alone or together with holocellulose, for producing bioenergy using thermochemical processes.

The experimental work that was carried out in this research helps to understand the potential of the selected raw materials as feedstocks for biorefineries. Variables such as yields, concentrations and compositions obtained from experiments at the bench confirmed the technical feasibility for obtaining different value added products.

Techno-economic analysis showed that biorefineries based on the selected raw materials have promising future. For the two types of biorefineries, based on non-oil (SBP and naranjilla waste) and oil (avocado and SCG) feedstocks presented phenolic compounds as the most promising product because of its high value in the market. Ethanol that is a product based on cellulose content, was not feasible in a stand-alone way because of the high associated energy consumption. This fact is common for

bioethanol production from lignocellulosic biomass in which the enzyme is a challenge for obtaining feasible processes. For xylitol, a similar result to that for ethanol was obtained. Thus, pretreatment and saccharification of raw materials is a challenge for products based on lignocellulosic biomass. However both, ethanol and xylitol are feasible products under biorefinery concept because the integration of these products in the biorefinery allows obtaining a sale-to-total-production cost ratio higher than 1.

Raw materials (including inputs for processing) and energy consumption were the most important economic factors that affected total production cost for all biorefineries. Enzyme has the most significant contribution among inputs for processing while energy consumption has the highest contribution in the case of ethanol plant.

However, this work demonstrates that different levels of integration can reduce significantly the total production cost. Energy consumption is the most important level of integration because more than 50% of the energy available in the biorefinery can be recovered. This fact decreases the external energy consumption of the plants associated to biorefineries. Mass integration also reduces total production cost and it is associated with water and ethanol consumption. A cogeneration system does not have a positive effect on total production cost for biorefineries based on non-oil feedstocks. This is because the capital cost associated to cogeneration system increases the total production cost. However, biorefineries based on oil feedstocks can accomplish a small reduction of total production cost using a cogeneration system. The latter is because the reduction of energy consumption (for steam and electricity produced) can overcome the capital cost associated to the cogeneration system.

From an environmental point of view, all biorefineries are environmentally friendly because the generated potential environmental impact (PEI) is negative. This fact indicates that streams leaving the system are less contaminated than the inlet streams. On the other hand, biorefineries based on non-oil feddstocks (SBP and naranjilla waste) can reduce significantly the environmental impact when mass integration is applied. The latter is because a reduction in the leaving mass can reduce the exit of possible pollutants. Similarly, for biorefineries based on oil feedstocks (avocado and SCG), the highest reduction of environmental impact is obtained when mass integration is applied. However, heat integration has a more important effect on the reduction of environmental impact than that observed for biorefineries based on non-oil feedstocks. The greenhouse

gases (GHG) emissions for all biorefineries were reduced, thus indicating that leaving streams have a smaller contribution to GHG emissions. This is in agreement with generated PEI that also was reduced for all scenarios and biorefineries.

Finally, the results obtained in this thesis can be a support for possible future analysis and decisions related to the future of fruits and the productive chains. The challenge to improve the fruits processing is not only to obtain value added products (representing in some cases just 1% to 2% of the raw material) but also to find technologies and adequate logistics to obtain integrally these products and reducing the wastes in a sustainable way. Besides, this research contributes with an important background on the design of biorefineries for the Colombian context. The last allows understanding all the potential that fruits and its wastes have for producing value added products and enlarge the products derived form fruits. At the same time, this research becomes in a base and strongly knowledge for future works and projects aimed to enhance the productive chain of fruits in Colombia.

Suggestions

The following suggestions can be taken into account for future works related with design of biorefineries based on fruits and its wastes.

Pretreatment process for raw materials (lignocellulosic biomass) must be addressed to reduce the inputs consumption, especially enzyme. Process such as simultaneous saccharification and fermentation (SSF) can enhance not only the yield of sugars obtained but also the efficiency of enzyme consumption.

It is important to explore the use of glucose obtained from the cellulose fraction of raw materials for other products instead of ethanol. This direction can allow producing biomolecules, such as organic acids, biomaterials and others, that have higher values in the market and thus can positively impact the total production cost of a biorefinery based on both, non-oil and oil feedstocks.

Lignin fraction of raw materials can be destined to obtaining other derived products such as activated carbon, binders, carbon fibers, motor fuel, phenols, plastic materials, and

sorbents among others. However, there is a need to identify the type of lignin and the proper process for obtaining value added products.

10. Appendix A. Chemical composition of some selected fruits

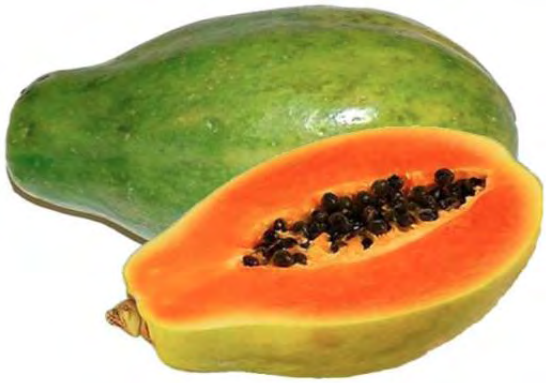
Fruits with major production and exotic fruits in Colombia

In chapter 1 was presented the 10 fruits with major production in Colombia and the chemical composition of the first four of them (Pineapple, citrus, banana and mango). Also, was presented the chemical composition of avocado an blackberry, which correspond the fifth and ninth position. Here, are presented the remaining four fruits (Papaya, tomato tree, guava and watermelon).

Tables A1 to A4 show the chemical composition of papaya, tree tomato, guava and watermelon respectively that correspond to the fruits with major production while Tables A5 to A8 show the chemical composition of some exotic fruits find in Amazonian region. The last fruits belong to the wide biodiversity of the Amazonian region. The potential of these Amazonian fruits has been proved therefore, there is necessary to consider additional applications for these fruits for take advantages from its chemical composition and its valuable compounds that cannot be found in other fruits in the same amounts.

The fruits considered here are attractive not only for its chemical composition but also for its production. This fact permits to take into account the possible use of these fruits for extracting valuable biological active compounds that could enhance the productive chain of these fruits.

Table A1. General chemical composition of Papaya (*Carica papaya*)

Papaya (*Carica papaya*)			
			
Component	Value	Unit	Reference
Water	88.06	g/100 g	
Energy	43	Kcal	
Protein	0.47	g/100 g	
Fat	0.26	g/100 g	
Carbohydrates	10.82	g/100 g	
Fiber	1.7	g/100 g	
Sugars	7.82	g/100 g	(USDA, 2012)
Calcium	20	mg/100 g	
Iron	0.25	mg/100 g	
Magnesium	21	mg/100 g	
Phosphorous	10	mg/100 g	
Potassium	182	mg/100 g	
Sodium	8	mg/100 g	

Zinc	0.08	mg/100 g
Vitamin C	60.9	mg/100 g
Thiamin	0.023	mg/100 g
Riboflavin	0.027	mg/100 g
Niacin	0.357	mg/100 g
Vitamin B-6	0.038	mg/100 g
Vitamin A	47	µg/100 g
Vitamin E	0.30	µg/100 g
Vitamin K	2.6	µg/100 g

Table A2. General chemical composition of Tomato (*Solanum betaceu*)

Tomato (*Solanum betaceu*)

Component	Value	Unit	Reference
Soluble solids	10.51	ºBrix	(Torres, 2012)
pH	3.5		
Moisture	87.72	g/100 g	
Protein	1.78	g/100 g	

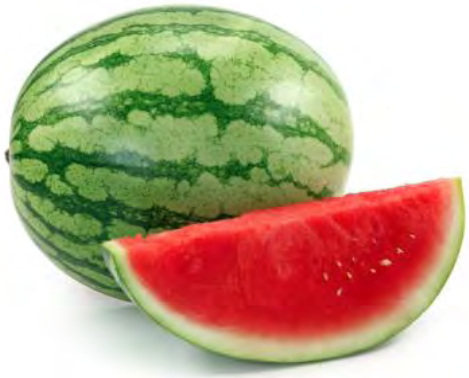
Fat	0.16	g/100 g	
Carbohydrates	5.36	g/100 g	
Fiber	8.2	g/100 g	
Ash	0.88	g/100 g	
Energy	30	Kcal	
Minerals			
PO4	331.32	mg/100 g	
Ca	21.25	mg/100 g	
Mg	21.18	mg/100 g	
K	17.03	mg/100 g	(Torres, 2012)
Fe	7.44	mg/100 g	
Zn	1.53	mg/100 g	
Mn	0.11	mg/100 g	
Bioactive compounds			
Ascorbic acid	23.32	mg/100 g	
Licopene	1.22	mg/100 g	
Polyphenols	1.39	mg/100 g	(Torres, 2012)
Tannins	0.4	mg/100 g	
Anthocyanins	0.29	mg/100 g	

Table A3. General chemical composition of Guava (*Psidium guajava*)

Guava (*Psidium guajava*)			
			
Component	Value	Unit	Reference
Water	80.80	g/100 g	
Energy	68	Kcal	
Protein	2.55	g/100 g	
Fat	0.95	g/100 g	
Carbohydrates	14.32	g/100 g	
Fiber	5.4	g/100 g	
Sugars	8.92	g/100 g	(USDA, 2012)
Calcium	18	mg/100 g	
Iron	0.26	mg/100 g	
Magnesium	22	mg/100 g	
Phosphorous	40	mg/100 g	
Potassium	417	mg/100 g	
Sodium	2	mg/100 g	

Zinc	0.23	mg/100 g
Ascorbic acid	228.3	mg/100 g
Thiamin	0.067	mg/100 g
Riboflavin	0.040	mg/100 g
Niacin	1.084	mg/100 g
Vitamin B-6	0.11	mg/100 g
Vitamin A	31	µg/100 g
Vitamin E	0.73	µg/100 g
Vitamin K	2.6	µg/100 g

Table A4. General chemical composition of Watermelon (*Citrullus lanatus*)

Watermelon (*Citrullus lanatus*)			
			
Component	Value	Unit	Reference
Water	91.45	g/100 g	(USDA, 2012)
Energy	30	Kcal	
Protein	0.61	g/100 g	

Fat	0.15	g/100 g
Carbohydrates	7.55	g/100 g
Fiber	0.4	g/100 g
Sugars	6.20	g/100 g
Calcium	7	mg/100 g
Iron	0.24	mg/100 g
Magnesium	10	mg/100 g
Phosphorous	11	mg/100 g
Potassium	112	mg/100 g
Sodium	1	mg/100 g
Zinc	0.10	mg/100 g
Ascorbic acid	8.1	mg/100 g
Thiamin	0.033	mg/100 g
Riboflavin	0.021	mg/100 g
Niacin	0.178	mg/100 g
Vitamin B-6	0.045	mg/100 g
Vitamin A	28	µg/100 g
Vitamin E	0.05	µg/100 g
Vitamin K	0.1	µg/100 g

Table A5. General chemical composition of Camu camu (*Myrciaria dubia*)

Camu camu (*Myrciaria dubia*)			
			
Component	Value	Unit	Reference
Energy	20.80	Kcal/100 g	(Sinchi, 2008)
Fat	0.00	g/100 g	
Carbohydrates	4.70	g/100 g	
Fiber	0.6	g/100 g	
Protein	0.5	g/100 g	
Calcium	27	mg/100 g	
Iron	0.5	mg/100 g	
Ascorbic acid	2994	mg/100 g	
Moisture	91.95	g/100 g	
Ash	0.53	g/100 g	
Phosphorous	28	mg/100 g	

Magnesium	46	mg/100 g	
Sodium	9.80	mg/100 g	(Vega, 2002)
Potassium	16.30	mg/100 g	
Copper	0.98	mg/100 g	
Zinc	2.90	mg/100 g	
Manganese	1.54	mg/100 g	
Ether extract	0.59	g/100 g	

Table A6. General chemical composition of Araza (*Eugenia spititata*)

Araza (*Eugenia spititata*)

Component	Value	Unit	Reference
Energy	34.86	Kcal/100 g	
Fat	1.08	g/100 g	(Sinchi, 2008)
Carbohydrates	5.53	g/100 g	
Fiber	0.92	g/100 g	

Protein	1.04	g/100 g	
Calcium	0.75	mg/100 g	
Sodium	4.80	mg/100 g	
Iron	0.14	mg/100 g	
Magnesium	2.35	mg/100 g	
Zinc	0.07	mg/100 g	
Copper	0.06	mg/100 g	
Potassium	37.42	mg/100 g	
Citric acid	2.19	%	
pH	2.88		
Soluble solids	3.4	ºBrix	(Soledad et al., 2006)
Sugars	0.542	% (DB)	
Ash	2.037	% (DB)	
Ether extract	12.32	% (DB)	

DB means Dry Basis

Table A7. General chemical composition of Copoazu (*Theobroma grandiflorum*)

Copoazu (*Theobroma grandiflorum*)


Component	Value	Unit	Reference
Energy	284.9	Kcal/100 g	
Fat	3.6	g/100 g	
Carbohydrates	52.3	g/100 g	
Fiber	16	g/100 g	
Proteins	10.9	g/100 g	
Ascorbic acid	9.2	mg/100 g	
Sodium	2.2	mg/100 g	(Sinchi, 2008)
Calcium	6.1	mg/100 g	
Iron	2.0	mg/100 g	
Magnesium	30	mg/100 g	
Zinc	0.5	mg/100 g	
Copper	0.3	mg/100 g	
Potassium	193.4	mg/100 g	
Manganese	0.8	mg/100 g	
pH	3.3		
Vitamin C	23.12	mg/100 g	(Mantilla et al., 2004)
Pectin	0.39	%	
Sugars	9.09	%	
Glycine	4.45	g/100 g	
Alanine	7.11	g/100 g	

Valin	6.06	g/100 g	
Leucine	6.82	g/100 g	(Hernandez, 2010)
Isoleucine	4.42	g/100 g	
Proline	4.56	g/100 g	
Phenylalanine	4.64	g/100 g	

Table A8. General chemical composition of Maraco (*Theobroma bicolor bonpl*)

Maraco (*Theobroma bicolor bonpl*)			
			
Component	Value	Unit	Reference
Energy	284.9	Kcal/100 g	(Sinchi, 2008)
Fat	3.19	g/100 g	
Carbohydrates	56’96	g/100 g	
Fiber	10.09	g/100 g	

Protein	10.96	g/100 g	
Moisture	87.90	%	
Oil	0.48	%	(Mantilla et al., 2004)
Ash	1.08	%	

11. Appendix B. Computational tools and methodologies used

Appendix B1. Information of the economic assessment using Aspen Process Economic Analyzer

Aspen Process Economic Analyzer gives to the user the capital cost, operating costs and the earnings based on the data extracted from the simulation (Mass and energy balances) as well as with data given by the user (Life of the project, tax rate, utilities cost and costs of other parameters such as operator, supervisor, potable water, etc.) (Moncada et al., 2013). The pathway followed by Aspen Process Economic Analyzer is showed in Figure B1:

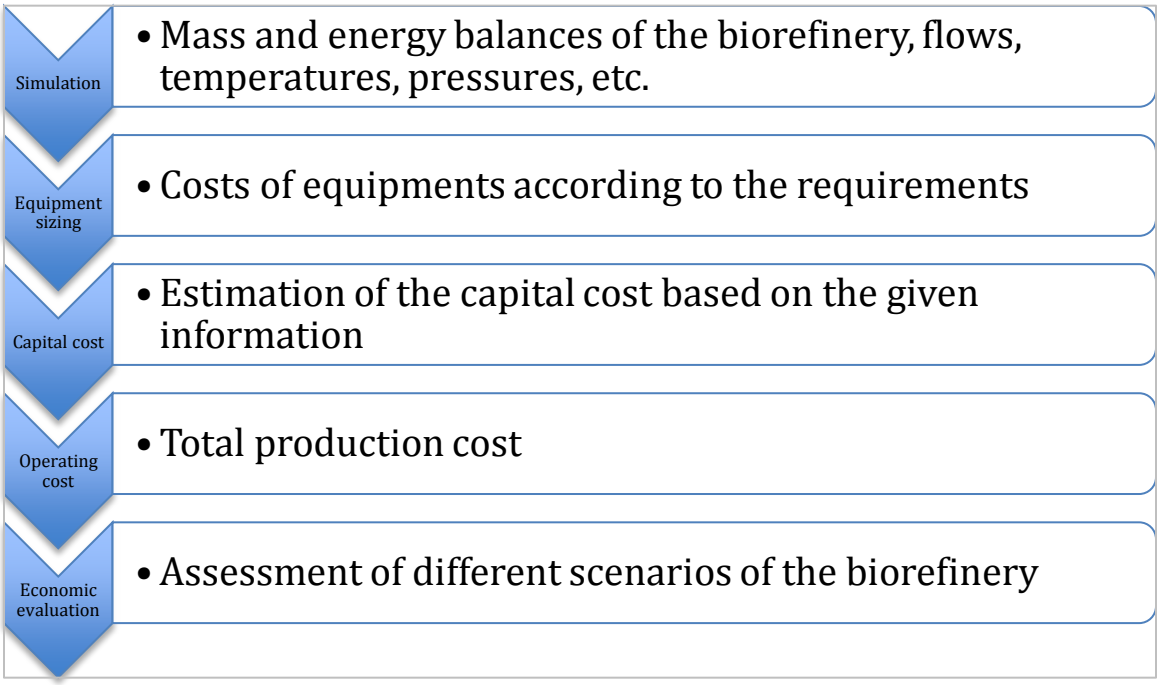


Figure B1. Pathway for economic analysis using Aspen Process Economic Analyzer

The main four steps made by Aspen Process Economic Analyzer correspond to sizing of units, capital cost, operating costs and economic evaluation. For Equipment sizing are used the data related with streams such as temperature, pressure, flows, physicochemical properties of the components involved in the streams as well as for the mixtures of them and operational conditions. As a result is obtained the sizing of the units such as pumps, compressors, vessels, heat exchangers, distillation towers, reactors and more. The interaction between all information for equipment sizing is showed in Figure B2.

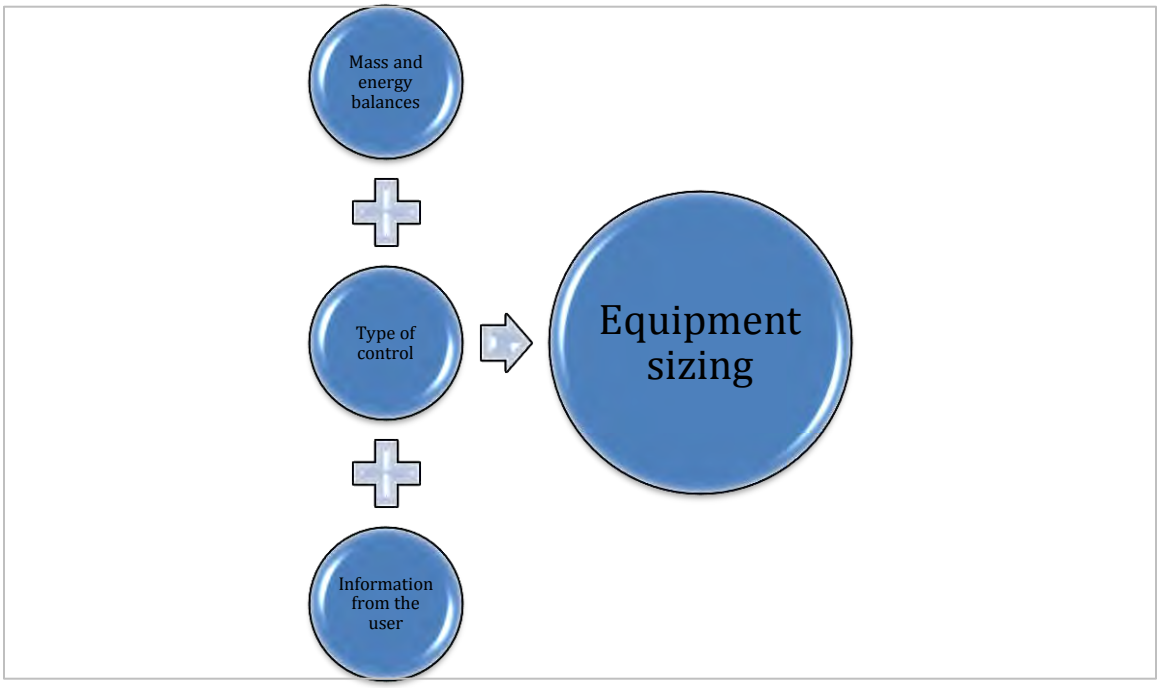


Figure B2. Flow of information for equipment sizing

For capital cost, the information of equipment sizing is extracted from the last step. Additionally, with the information from the user related with location of the biorefinery, cots index, tax rate, internal rate of return, plant startup, costs of reagents, costs of products, utilities and more then is determined the necessary investment for the biorefinery as well as the depreciation expense according to the depreciation method chosen. Figure B3 shows the information necessary for calculating capital cost.

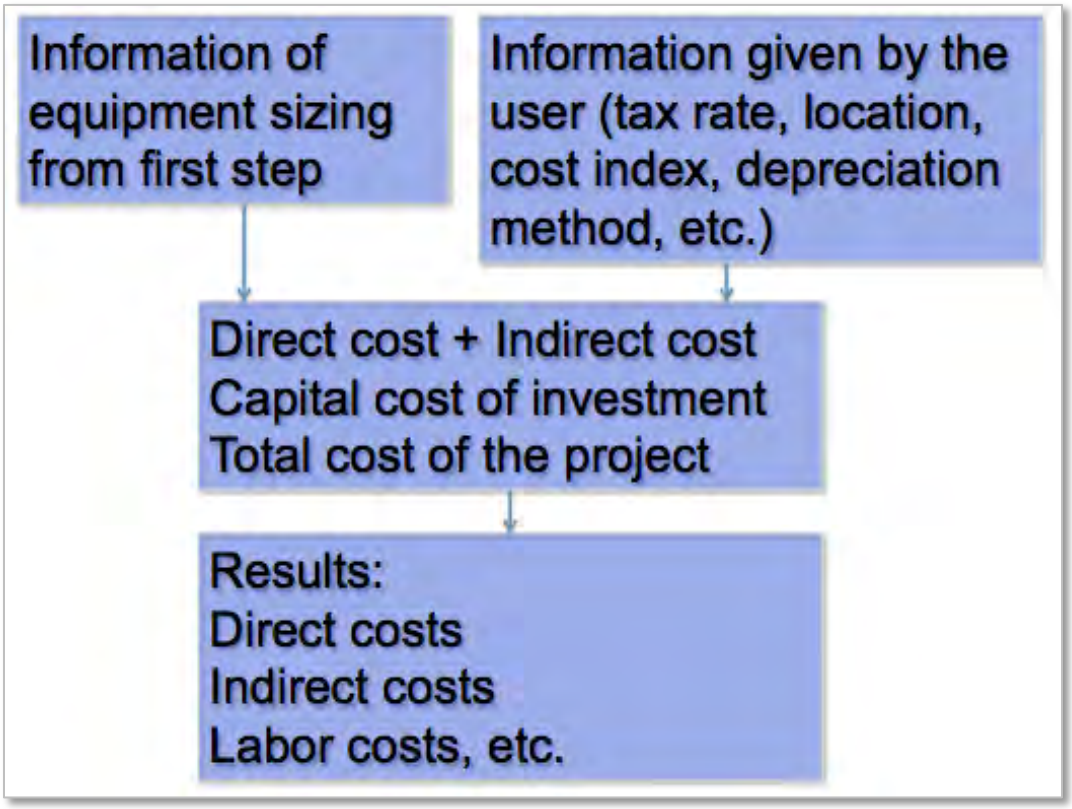


Figure B3. Information for capital cost evaluation

Operating costs are one of the most important in the economic evaluation because it reflects the costs of raw materials, reagents, utilities and other parameters per kilogram of product (Or cubic meter, cubic feet, molar and so on). For this step, Aspen Process Economic Analyzer takes the information of the previous steps such as flow of raw materials, amount of auxiliary services (utilities) among others parameters which are combined with the information given by the user such as mode of operation, period of work, type of utilities, type of plant, cost of raw materials and calculate the total cost of operation of the biorefinery. This cost can be transform to a cost per kilogram of product for comparison purposes. Figure B4 shows the information necessary for this step.

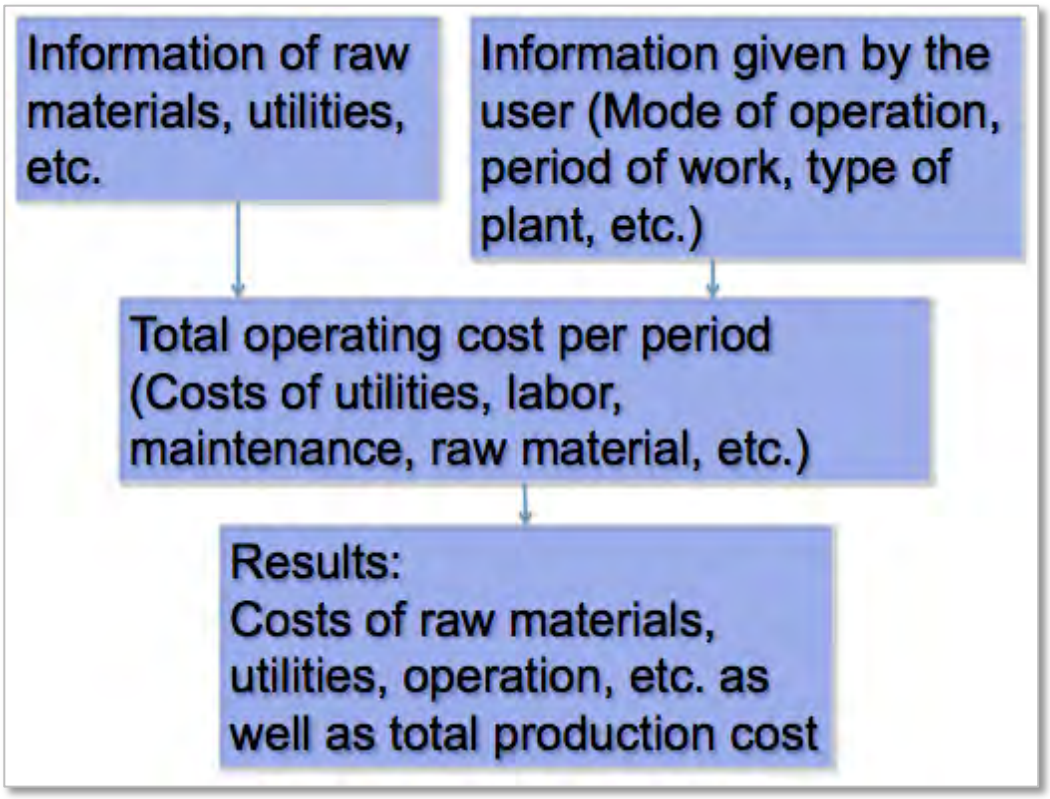


Figure B4. Information for operating cost evaluation

Finally, when the economic evaluation is made by Aspen Process Economic Analyzer, then there is possible to calculate the cash flow, Net Present Value (NPV) and other indicators that permit to evaluate the final earnings of the biorefinery. Figure B5 shows the flow of information for this step.

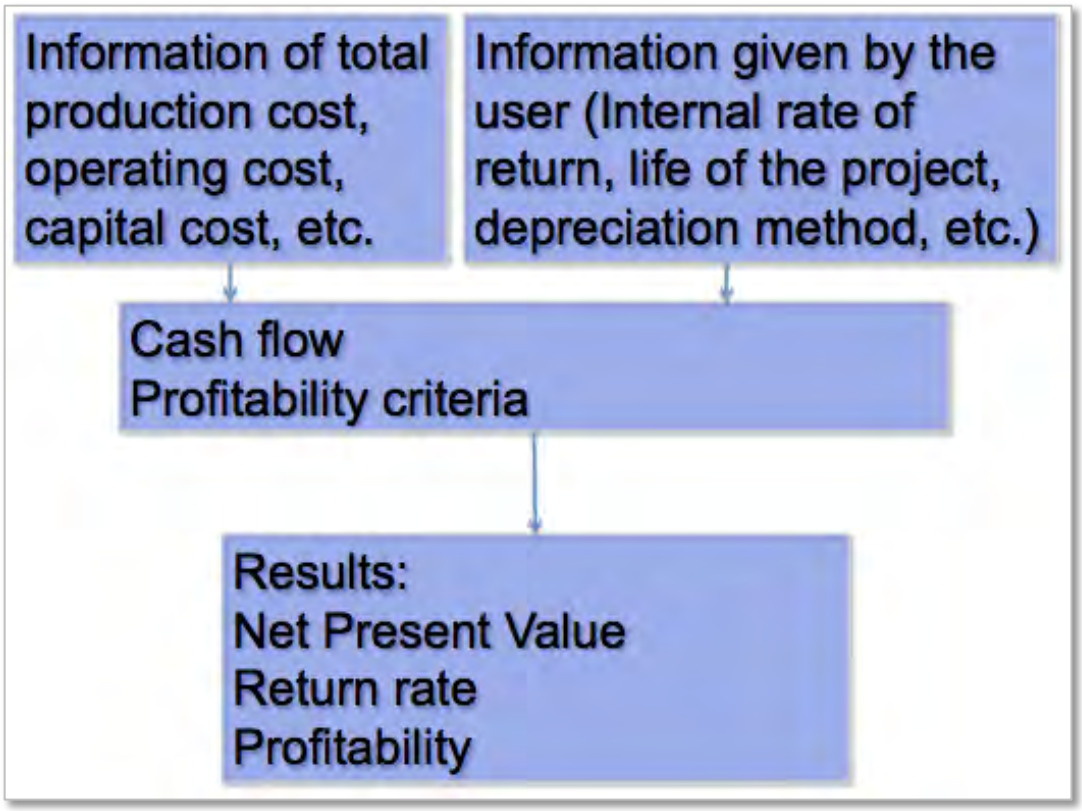


Figure B5. Flow of information for final economic analysis

Appendix B2. Pinch methodology for Heat Integration of processes

The Pinch methodology is widely used for heat integration of processes and in the case of biorefineries; this methodology is very useful because its permits to get a total heat integration of the biorefinery. This methodology is based on the thermodynamic of the streams involved in the process and by means of a simple representation of this information there is possible to obtain the minimum requirements of energy “Targets” for both, heating and cooling (Ng, 2010; Oliveira et al., 2015).

Pinch methodology also permits to obtain the Heat Exchanger Network (HEN) that allow supply all energy requirements of the process and guarantee the minimum energy consumption and therefore, the minimum cost of the HEN. Despite of the easy concept of this methodology there is necessary to carry out it with special software because the number of streams involved in the analysis. For this analysis, two softwares are available. The first one is HINT, which is free software and permits to obtain the heat integration in a simple way. The second one is Aspen Energy Analyzer that can obtain the heat integration after the simulation of the process using Aspen Plus (Oliveira et al., 2015; Shenoy and Shenoy, 2014).

Pinch methodology can be described in four steps such as it is shown in Figure B6. The first step is related with the Composite Curves (CC) of the process, followed by the selection of the utilities based on the Grand Composite Curve (GCC), then the construction of the Heat Exchanger Network (HEN) and finally, the possible use of a cogeneration system for supply the energy requirements.

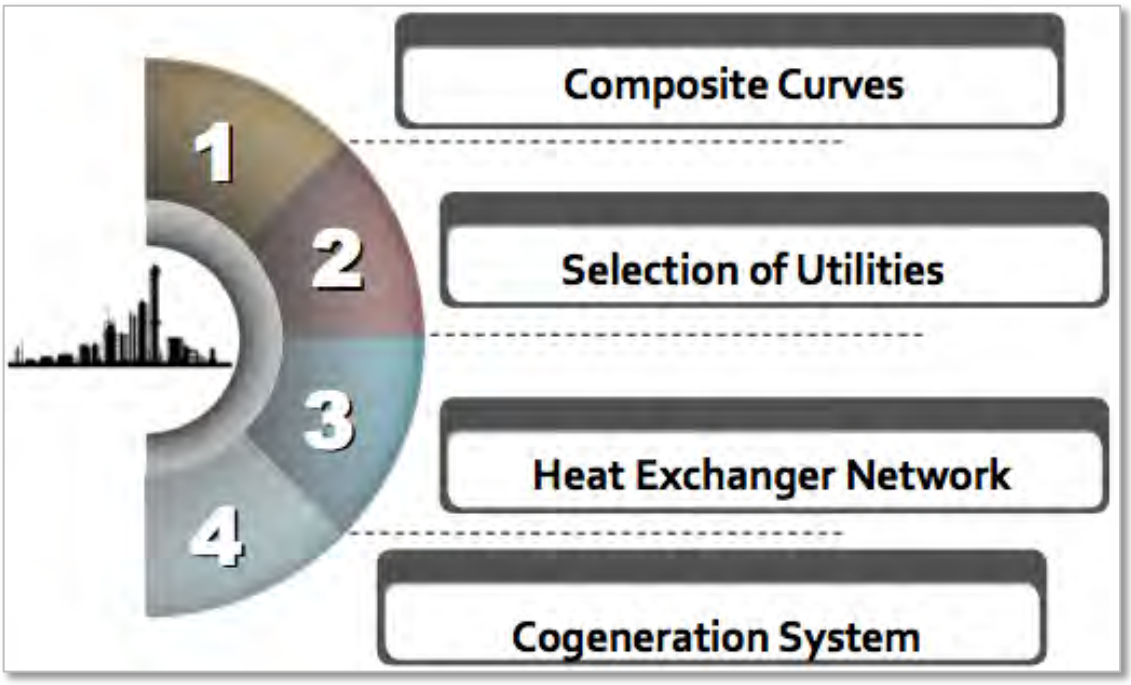


Figure B6. Steps for Pinch methodology for heat integration

The CC are made according to the temperature, enthalpy and heat capacities of the streams. Both, cool and heat streams are plotted in a diagram of temperature vs enthalpy. There, cool composite curve and heat composite curve are plotted based on a minimum difference of temperature (ΔT_{min}). So, there is possible to calculate the maximum heat recovery (The common region for cool and heat composite curves plotted in a temperature vs enthalpy diagram) and both, heating and cooling requirements that correspond to the targets as it is shown in Figure B7.

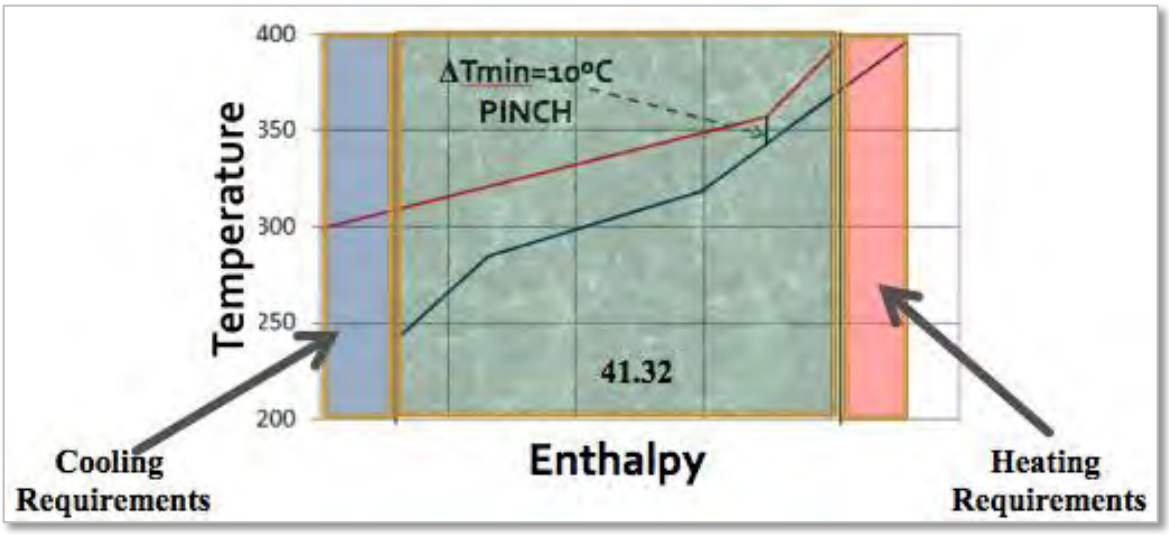


Figure B7. Composite curves for heat integration using Pinch methodology

Once the energy requirements of the process are known (Targets for heating and cooling) as well as maximum energy recovering, then the selection of the utilities is carried out based on the Grand Composite Curve (GCC). The GCC is made according to the ΔT_{min} selected (10 ºC commonly). According to the temperatures of the GCC and the pinch (Point in which the enthalpy is zero), there is possible to separate the zones for heating and cooling requirements. The zone above the pinch corresponds to the heating requirements and the zone below the pinch is for cooling requirements such it is shown in Figure B8. For almost all cases, the common option to cover the heating requirements is steam at high, medium or low pressure while for cooling requirements is cooling water however, it depends on the process because sometimes there is more convenient the use of oil to cover heat requirements or a refrigerant or steam at low pressure for cooling requirements.

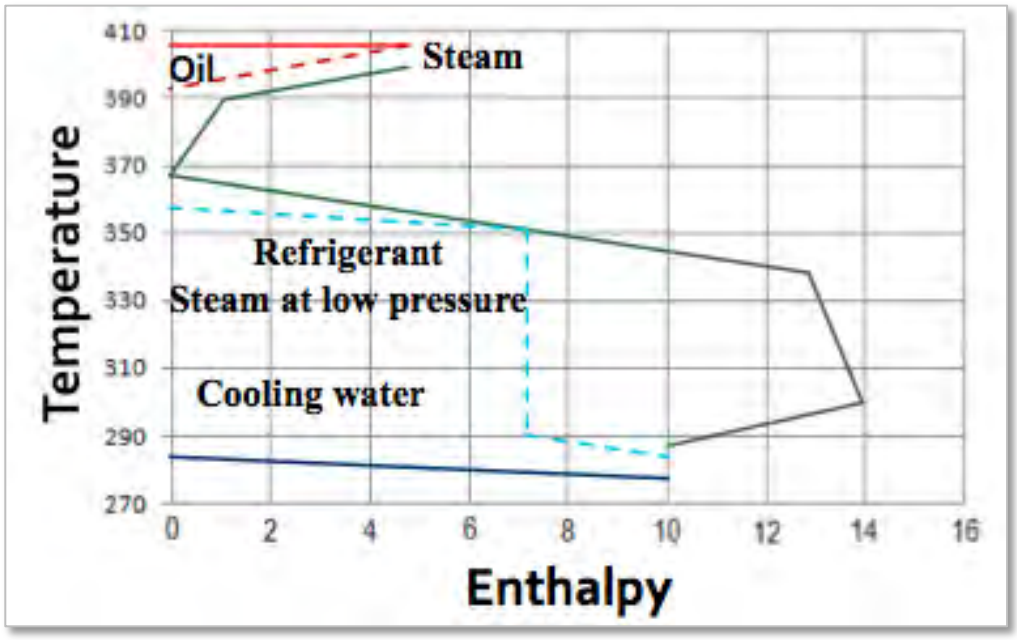


Figure B8. Gran Composite Curve for utilities selection

After define the utilities to be used, there is possible to construct the HEN by means of the possibilities of heat transfer above and below the pinch. The heat exchangers that use streams of the process (not utilities) correspond to heat recovery between streams of the process; the remaining heat exchangers are for the use of utilities (steam and cooling water) as it is shown in Figure B9.

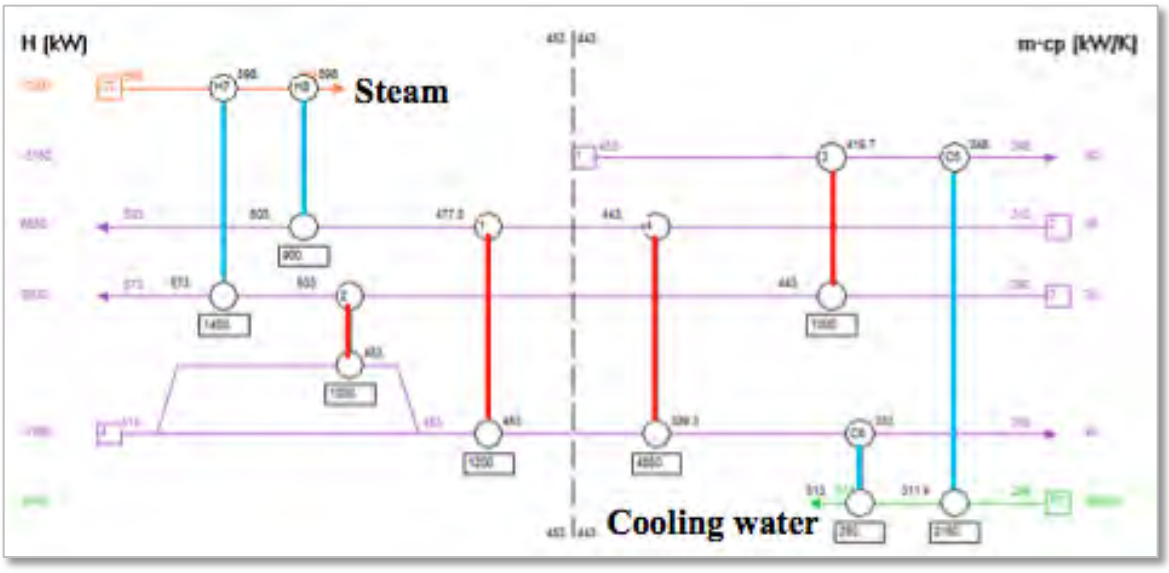


Figure B9. Heat Exchanger Network using Pinch methodology

Finally, the use a cogeneration system can be considered according to the requirements of the biorefinery and the availability of a fuel. Typically, one of the solid residues from biorefineries is lignin after hydrolysis processes and conversion of cellulose into sugars; this residue can be used for energy purposes under gasification schemes. Therefore, as a final level of integration, there is possible to consider the use of a cogeneration system for supply part or all energy requirements of the biorefinery (between all plants of the biorefinery) as it s shown in Figure B10.

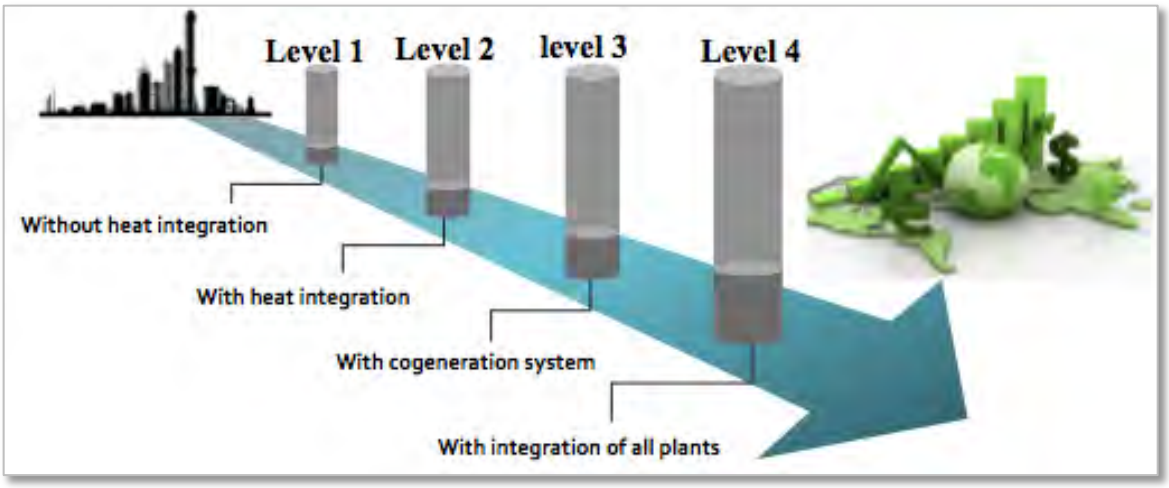


Figure B10. Levels of heat integration

Appendix B3. Potential Environmental Impact using WAR software and GHG emissions

For evaluating Potential Environmental Impact (PEI) of a biorefinery, the WAste Reduction Algorithm (WAR) was used. This software is free and it can be downloaded from the Environmental Protection Agency (EPA) of the Unite States of America. This method uses the direct sum of environmental data based on the information of mass and energy streams of the process and the compounds involved in these streams. WAR evaluates the eight impact categories as follow (Cabezas et al., 1999; Young and Cabezas, 1999):

- HTPI: Human Toxicity Potential Impact
- HTPE: Human Toxicity Potential by Either
- TTP: Terrestrial Toxicity Potential
- ATP: Aquatic Toxicity Potential
- GWP: Global Warming Potential
- ODP: Ozone Depletion Potential
- PCOP: Photo Chemical Oxidation Potential
- AP: Acidification Potential

The PEI is measured for the non-products that are considered, as pollutants therefore the PEI for these streams will be different to zero while for the products PEI takes zero value. The streams involved in an environmental evaluation of a biorefinery and its energy plant is showed in Figure B11. A balance over a biorefinery and its plant for energy generation involving mass and energy streams of the system is showed in Equation (B1).

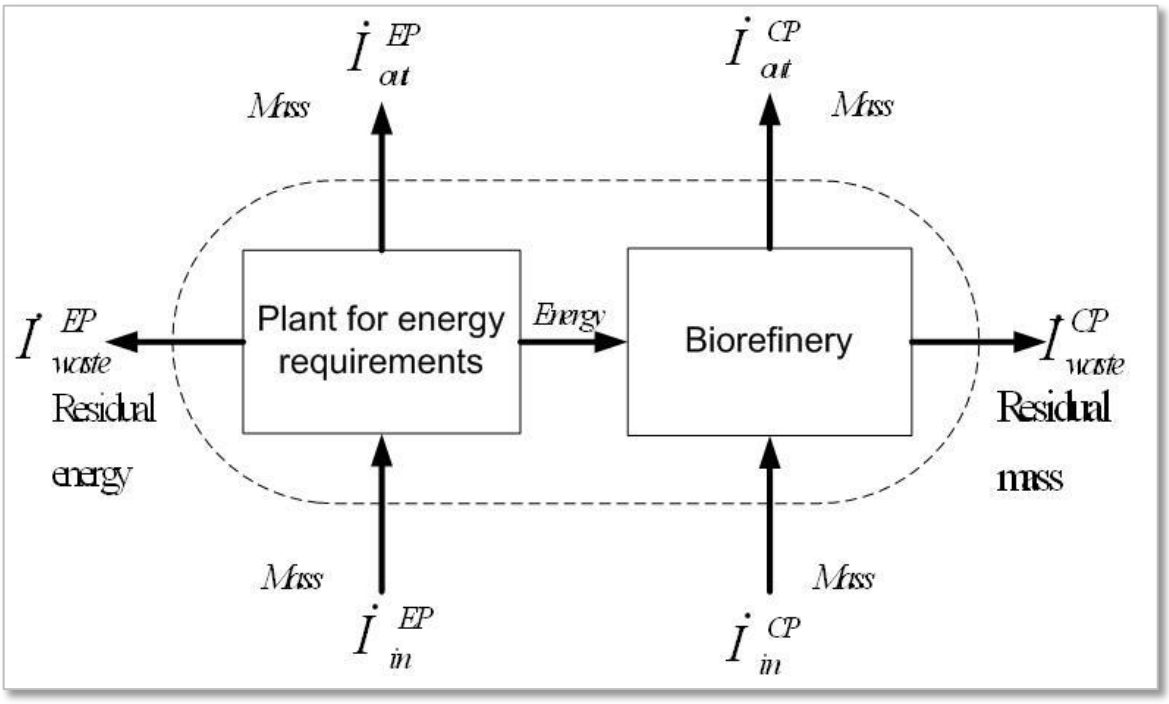


Figure B11. Streams involved in an environmental assessment of biorefineries

$$\frac{dI_{System}}{dt} = \dot{I}_{in}^{CP} + \dot{I}_{in}^{EP} - \dot{I}_{out}^{CP} - \dot{I}_{out}^{EP} - \dot{I}_{we}^{CP} - \dot{I}_{we}^{EP} + \dot{I}_{Gen}^{System} \qquad \text{Equation (B1)}$$

This equation involves all incoming and leaving streams of the biorefinery. But in stable state, the accumulation term does not exist therefore, this term becomes zero and the balance is so Equation (B2):

$$0 = \dot{I}_{in}^{CP} + \dot{I}_{in}^{EP} - \dot{I}_{out}^{CP} - \dot{I}_{out}^{EP} - \dot{I}_{we}^{CP} - \dot{I}_{we}^{EP} + \dot{I}_{Gen}^{System} \qquad \text{Equation (B2)}$$

If all incoming and leaving streams are grouped then, a general balance can be obtained such Equation (B3).

$$0 = \dot{I}_{In} - \dot{I}_{out} + \dot{I}_{Gen}$$ Equation (B3)

The PEI of leaving and incoming streams ($\dot{I}_{out}$ and $\dot{I}_{in}$) are calculated with Equation (B4). There, the sub indexes j, i and k refer to the streams, in or out and component respectively. $\dot{M}$ refers to the flows of mass and X refers to the compound. Ψ corresponds to the PEI of the compound associated to an impact category that can be found for each compound.

$$\dot{I}_i = \sum_j \dot{M}_j^{(i)} \sum_k X_{k,j} \psi_j$$ Equation (B4)

Two types of indices can be measured with WAR. The first one is related with the generated PEI and the second one is the leaving PEI. The generated PEI is calculated using the Equation (B5) which is the PEI generated divided by the sum of products.

$$\hat{I}_{Gen} = \frac{\dot{I}_{Gen}}{\sum_P \dot{P}_P}$$ Equation (B5)

The leaving PEI is calculated using the PEI for leaving streams by means of Equation (B6) and dividing it by the sum of all products of the biorefinery using Equation (B7).

$$\dot{I}_{Out} = \sum_{j} \dot{M}_{j}^{Out} \sum_{K} X_{K,j} \psi_{j}$$

Equation (B6)

$$\hat{I}_{Out} = \frac{\dot{I}_{Out}}{\sum_{P} \dot{P}_{P}}$$

Equation (B7)

At this point, there is necessary the evaluation of the PEI (ψ) of a compound for assess all impact categories. WAR includes base data of compounds with these values which is calculated from several measurements of environmental impact for the different categories. The parameter ψ also is named Environmental Chemical Impact (ECI) that can be calculated according to Equation (B8).

$$\psi_{K} = \sum_{l} \alpha_{l} \psi_{K,l}$$

Equation (B8)

In this equation k refers to the compound, l is the category, α is a relative factor associated to a category and ψ is the ECI of a compound. For the measurement of the eight impact categories evaluated by WAR, different parameters are taken into account such it is shown in Table B1.

Table B1. Environmental categories for measuring PEI

Impact Category	Measure	Characteristic
HTPI	$HTPI_i = \frac{1}{LD50_i}$	Inversely to LD_{50}
HTPE	$HTPE_i = \frac{1}{LTV_i}$	Inversely to LTV (Limit Threshold Values)
TTP	$TTP_i = \frac{1}{LD50_i}$	Inversely to LD_{50}
ATP	$ATP_i = \frac{1}{LC50_i}$	Inversely to LC_{50}
GWP	$GWP_i = \frac{Ton/year \text{ de gas i}}{Ton/year \text{ de } CO_2}$	Contribution of a gas related to CO_2
ODP	$ODP_i = \frac{\text{Contribucion de gas i}}{\text{Contribucion de CFC}}$	Equivalent emission of a gas related to CFC ($CFCl_3$)
PCOP	$PCOP_i = \frac{\text{Contribucion de gas i}}{\text{Contribucion de } C_2H_4}$	Equivalent emission of a substance related to Ethylene (C_2H_4)
AP	$AP_i = \frac{\alpha}{2}\frac{MW_{SO2}}{MW_x}$	Contribution of a substance x equal to an equivalent amount of SO_2

Finally, the total PEI of a process is the sum of all categories. WAR provides a platform to compare scenarios, therefore there is necessary use this computational tool for comparison purposes. If the generated PEI value is negative this means that there was a mitigation of the PEI due to the leaving streams are less pollutants than the incoming streams however, these streams can have an effect on the environment. On the other

hand, if the generated PEI is positive this means that the biorefinery delivers more pollutants in comparison with the incoming streams to the biorefinery.

For calculating GHG emissions, the following methodology was made (Mussatto et al., 2013). Figure B12 shows a system (biorefinery) for which, a GHG emissions balance will be made. This system takes into account all inlet and outlet streams of mass and energy and for each one of the compounds involved in each stream is calculated the activity which is done by equation (B9). After that, the emission factor (taken from literature) of each compound is used for obtaining the CO_2-eq per kg of product according to equation (B10).

$$A_i = \frac{\text{kg of compound i}}{\text{kg of product}}$$

Equation (B9)

$$\frac{\text{kg of compound i}}{\text{kg of product}} * \frac{\text{kg of } CO_{2\text{-}eq}}{\text{kg of compound i}} = \frac{\text{kg of } CO_{2\text{-}eq}}{\text{kg of product}}$$

Equation (B10)

The same calculation is made for each one of the compounds involved in each one of the streams for inlet and outlet streams. Finally, a balance of GHG emissions is made according to equation (B11).

$$Outlet - inlet = generation$$

Equation (B11)

Table B2 shows the emission factors used for all chemicals involved in the streams for all biorefineries.

Table B2. Emission factors for GHG calculation.

Chemical	Emission factor (kg of CO_2/kg of chemical)	Reference
Fruit waste [a]	0.12	(EPA, 2015)
Ethanol	0.13	(Lee and Pereira, 2011)
CO_2	1	(Biograce, 2015)
H_2SO_4	0.21	(Biograce, 2015)
NaOH	0.47	(Biograce, 2015)
Enzyme	2.26	(Kumar and Murthy, 2012)
Maltodextrin [b]	1.1	(Tsiropoulos et al., 2013)
Biomass	1.04	(2BSvs, 2011)
Nitrogen	0.43	(Biograce, 2015)
Oxygen	0.41	(Biograce, 2015)
Xylitol	0.5	(Shen, 2012)
Capsules [c]	3.5	(Cherubini et al., 2010)
Methane	0.023	(Biograce, 2015)
Hexane	3.63	(Biograce, 2015)
CO	1.57	(Biograce, 2015)
Hydrogen	1.63	(Winnipeg, 2014)
Wastewater treatment	0.00036	(2BSvs, 2011)
Electricity [d]	0.048	(2BSvs, 2011)
Steam [e]	66.33	(IPCC, 2014)
Process water	0.0003	(ISCC, 2011)
Lignin	2.19	(Boldrin and Astrup, 2015)
Na_2SO_4 [f]	0.3	(Winnipeg, 2014)
Avocado	0.44	(EPA, 2015)
Oil	9.79	(IPCC, 2014)

[a] This value corresponds to a general value for fruit wastes therefore, it is used for SBP, naranjilla waste and SCG.

[b] This value was taken for glucose taken into account that maltodextrin is a chain of 5 glucose molecules

[c] This value is referred to the phenols contained in lignocellulosic biomass

[d] This value is reported as kg of CO_2-eq/MJ for Colombia

[e] This value is reported as kg of CO_2-eq/MMBTU

[f] This value was taken for $MgSO_4$ as reference component

12. Appendix C. Calibration curves and volatiles profiles

Appendix C1. Total Phenolic Compounds measurements

Total phenolic content of the samples were measured using a modified colorimetric Folin-Ciocalteu method as was explained in chapter 3. Figure C1 depicts the standard calibration curve using Gallic Acid (GA) for Total Phenolic Compounds (TPC) measurements for spent pulp of blackberry. Concentrations between 0 and 500 mg GA/l were prepared and the absorbance was measured.

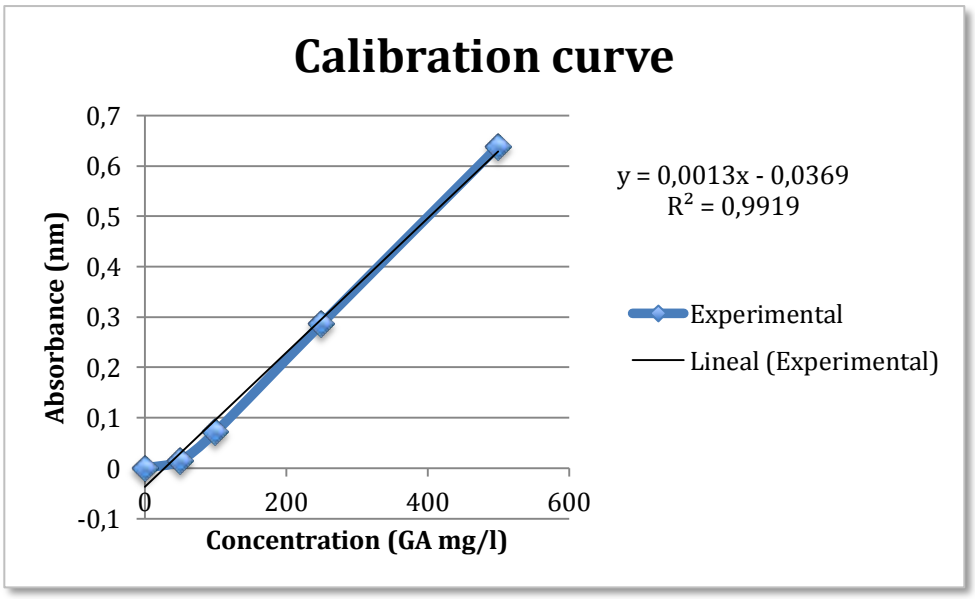


Figure C1. Calibration curve for TPC measurements for spent pulp of blackberry

Thus, the TPC were measured according to the absorbance measured for each sample and the corresponding concentration on the calibration curve.

Appendix C2. Trolox standard curve and IC_{50} values

The initial antioxidant activity (AA) of spent pulp of blackberry (SPB) was calculated according to procedure described in chapter 3. The IC_{50} values for four extractions (for initial AA of SPB) were calculated for several concentrations (Dilution Factors (DF)) of extracts. Tables C1, C2, C3 and C4 show the IC_{50} values calculated for several concentrations of extracts for extractions 1, 2, 3 and 4 respectively.

For all measurements, the IC_{50} was calculated using the equation (C1) for percentage of scavenging activity.

$$\%\ Scavenging\ Activity = \left(1 - \frac{Abs_{extract}}{Abs_{Reference}}\right) 100 \quad \text{(C1)}$$

The final values of AA were expressed as IC_{50} (the amount of antioxidant necessary to halve the initial DPPH concentration). Table C5 shows the IC_{50} values for all extractions and the final AA (IC_{50}) for SPB.

Table C1. IC_{50} values were calculated for several concentrations of extracts for extraction 1

Concentration (DF)	Reply	Absorbance	% Inhibition (%)	Average IC_{50} (ml sol DPPH/ml extract)	Average IC_{50} (mg SPB/ml DPPH)
	1	0.144	76.95		
10	2	0.386	38.21	26.98	4.46
	3	0.484	22.52		
	1	0.112	82.07		
30	2	0.390	37.57	28.40	4.32
	3	0.479	23.32		

	1	0.116	81.43		
50	2	0.383	38.69	28.33	4.45
	3	0.484	22.52		

Table C2. IC_{50} values were calculated for several concentrations of extracts for extraction 2

Concentration (DF)	Reply	Absorbance	% Inhibition (%)	Average IC_{50} (ml sol DPPH/ml extract)	Average IC_{50} (mg SPB/ml DPPH)
	1	0.068	89.11		
5	2	0.420	32.76	20.19	5.95
	3	0.502	19.64		
	1	0.061	90.23		
20	2	0.379	39.33	21.89	5.60
	3	0.489	21.72		
	1	0.037	94.08		
40	2	0.354	43.33	22.99	5.48
	3	0.495	20.76		

Table C3. IC_{50} values were calculated for several concentrations of extracts for extraction 3

Concentration (DF)	Reply	Absorbance	% Inhibition (%)	Average IC_{50} (ml sol DPPH/ml extract)	Average IC_{50} (mg SPB/ml DPPH)

	1	0.037	94.08		
1	2	0.387	38.05	7.92	15.18
	3	0.540	13.55		
	1	0.080	87.19		
5	2	0.396	36.61	6.85	17.92
	3	0.553	11.47		
	1	0.045	92.80		
20	2	0.383	38.69	7.91	15.95
	3	0.536	14.19		

Table C4. IC_{50} values were calculated for several concentrations of extracts for extraction 4

Concentration (DF)	Reply	Absorbance	% Inhibition (%)	Average IC_{50} (ml sol DPPH/ml extract)	Average IC_{50} (mg SPB/ml DPPH)
	1	0.183	73.97		
1	2	0.615	12.52	1.83	65.55
	3	0.632	10.10		
	1	0.193	82.22		
5	2	0.500	45.52	1.74	69.20
	3	0.591	19.77		
	1	0.152	78.28		
15*	2	0.193	72.93	3.81	33.12
	3	0.593	15.65		

* This sample was not taken

Table C5. Initial AA for SPB expressed as IC_{50} values

Extraction	Average IC_{50} (mg SPB/ml DPPH)	S. Dev.	% Error (%)	$1/IC_{50}$ (ml sln DPPH/mg SPB)	Initial IC_{50} (mg SPB/ml DPPH)
1	4.41	0.063	1.43	0.227	
2	5.68	0.19	3.51	0.176	
3	16.35	1.15	7.06	0.061	2.09
4	67.38	1.82	2.71	0.014	
Total				0.478	

Thus, the Initial IC_{50} for SPB is calculated as the inverse of $1/IC_{50}$ (0.478) found 2.09 mg SPB/ml DPPH solution. The Trolox standard curve was made for concentrations ranging from 80 to 550 μM. The IC_{50} of Trolox was calculated in the same way that for the extracts using the equation (C1) for percentage of scavenging activity. IC_{50} of Trolox was calculated for triplicate. The Trolox standard curves are showed in Figures C2, C3 and C4.

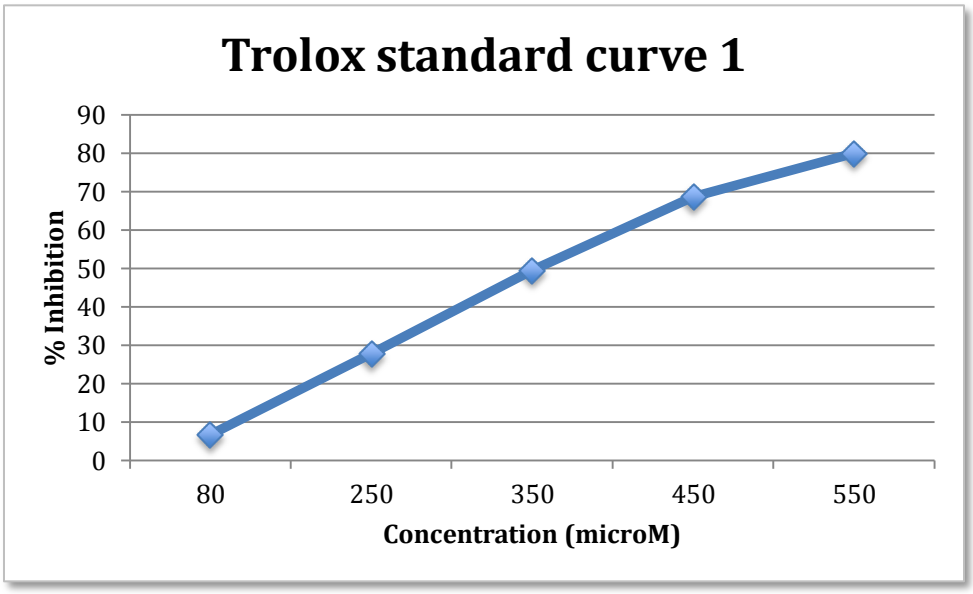


Figure C2. Trolox standard curve 1 for AA measurements

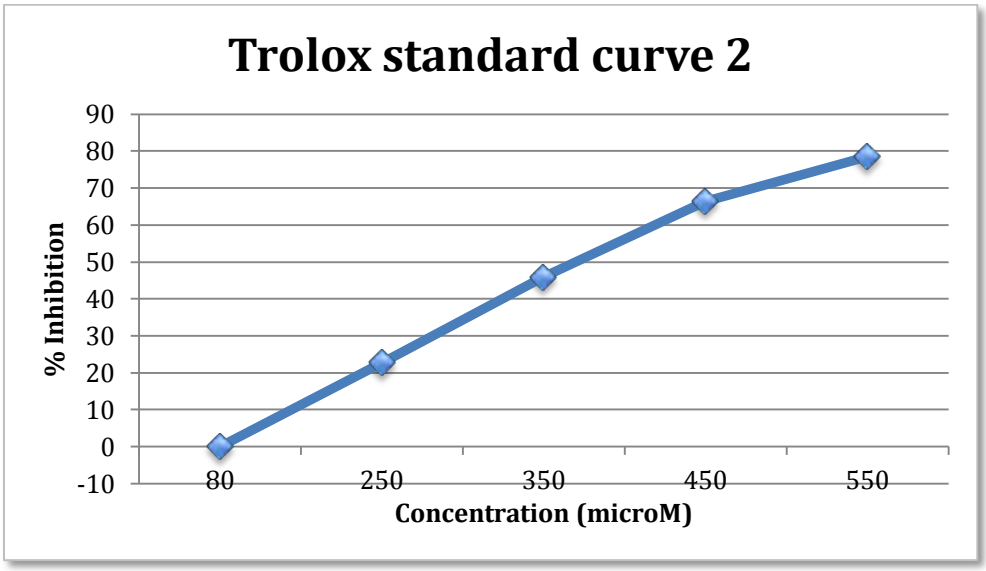


Figure C3. Trolox standard curve 2 for AA measurements

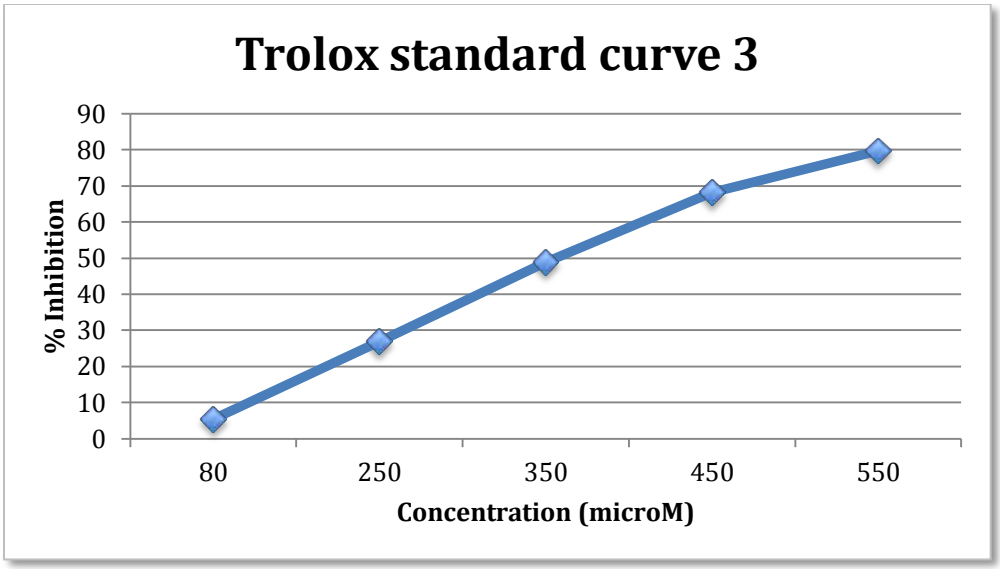


Figure C4. Trolox standard curve 3 for AA measurements

The IC_{50} values for Trolox from the three curves are 357.5, 377.8 and 361.2 μM TE (μmol/l) where TE is Trolox Equivalent (Molecular weight of Trolox is 250.29 g/mol). Thus, the average value for IC_{50} of Trolox is 365.5 μM (365.5 μmol/l). This measurement is used to give AA of the extracts in μmol TE/g of SPB. Taking into account the calculated AA in terms of IC_{50} was 2.09 mg SPB/ml DPPH solution then, the AA of SPB is terms of Trolox is:

$$IC_{50} = 2.09\ \frac{mg\ SPB}{ml\ DPPH\ Solution} = 2090\ \frac{mg\ SPB}{l\ DPPH\ Solution}$$

$$AA = \frac{IC_{50}\ Trolox}{IC_{50}\ Extract} = \frac{365.5\ \mu mol\ TE/l}{2090\ mg\ SPB/l} = 0.1748\ \frac{\mu mol\ TE}{mg\ SPB}$$

$$AA = 0.1748 \frac{\mu mol\ TE}{mg\ SPB} * \frac{1000\ mg}{1\ g\ of\ SPB} = 174.8\ \frac{\mu mol\ TE}{g\ of\ SPB}$$

Appendix C3. Volatiles profile for naranjilla waste, peel and seed of avocado and SCG

Volatile compounds present in naranjilla waste

Figures C5 and C6 depict the chromatogram obtained for naranjilla waste using the SPME method. Table C6 shows the concentration for each one of the compounds (volatiles) present in naranjilla waste.

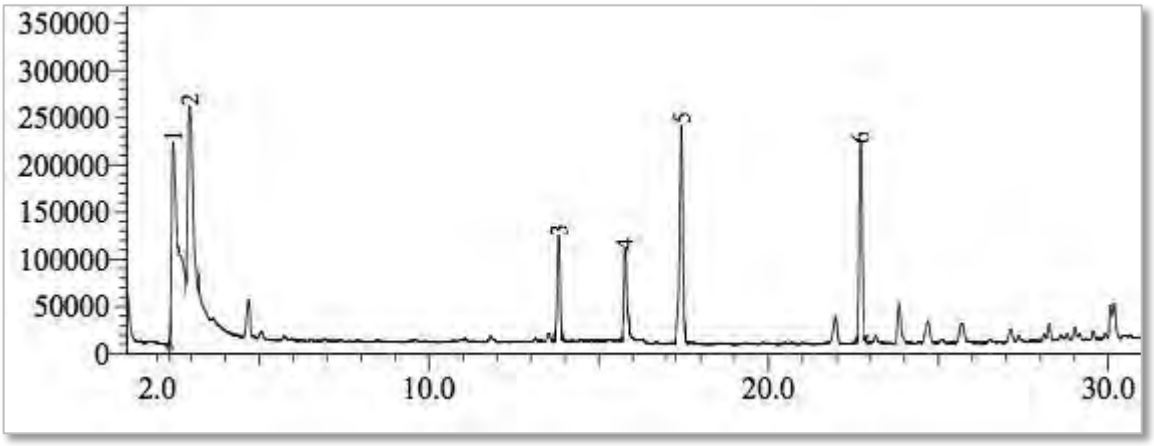


Figure C5. Chromatogram of volatiles profile for naranjilla waste until first 30 minutes

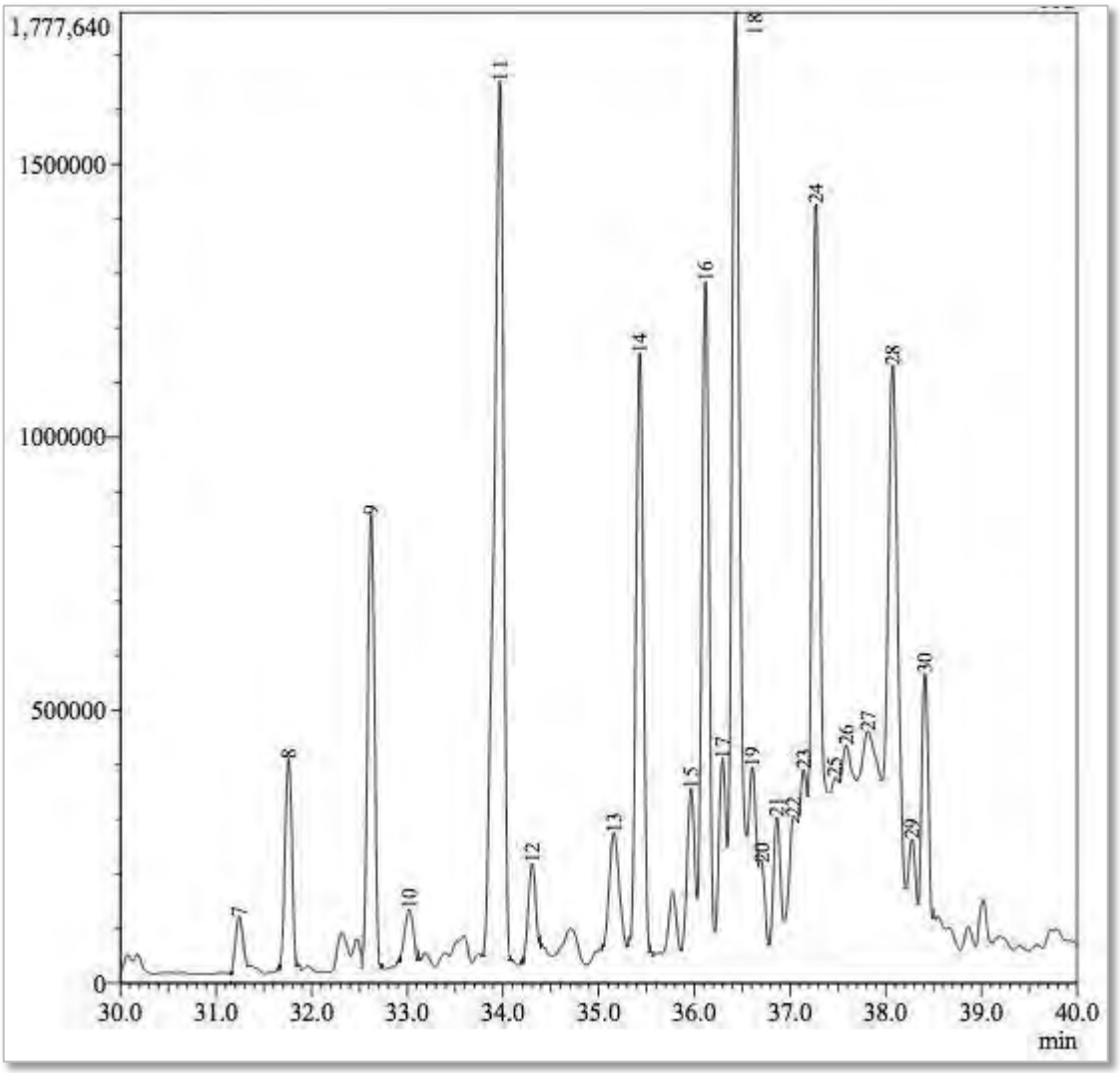


Figure C6. Chromatogram of volatiles profile for naranjilla waste after 30 minutes

Table C6. Concentration of volatiles present in naranjilla waste. NW means Naranjilla Waste

Peak	Compound	Concentration (µg/kg NW)
2	Acetic acid	64,333.77
3	Limonene	20,522.71

4	1-Octanol	19,373.14
6	3-Cyclohexene-1-methanol, .alpha.,.alpha.4-trimethyl-	46,532.88
7	Camphene	19,507.66
8	.alpha.-Cubebene	66,767.84
9	Copaene	147,156.47
10	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-	19,450.32
11	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]-	393,031.63
12	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [s-(E,E)]-	29,337.26
13	cis-.alpha.-Bisabolene	51,593.86
14	1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-, [1aR-(1a.alpha.,4a.beta.,7.alpha.,7a.beta.,7b.alpha.)]-.beta.-Vatirenene	208,682.31
15	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,7.beta.,8a.alpha.)]-	56,691.59
16	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [s-(E,E)]-	224,342.14
17	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-	63,963.24
18	1H-Cyclopropa[a]naphthalene, 1a,2,3,3a,4,5,6,7b-octahydro-1,1,3a,7-tetramethyl-, [1aR-(1a.alpha.,3a.alpha.,7b.alpha.)]-	352,408.86
19	Seychellene	67,304.55
20	(Z,Z)-.alpha.-Farnesene	42,742.04
22	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-	50,840.20
23	Diethyl Phthalate	60,238.14

24	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	315,612.86
25	Phthalic acid, di-(1-hexen-5-yl) ester	69,953.39
28	Diethyl Phthalate	278,086.33
30	1,5,9,11-Tridecatetraene, 12-methyl-, (E,E)-	75,039.13

Peaks 1, 5, 21, 26, 27 and 29 were not detected

Volatile compounds present in avocado seed

Figure C7 depicts the chromatogram obtained for avocado seed using the SPME method. Table C7 shows the concentration for each one of the compounds (volatiles) present in avocado seed.

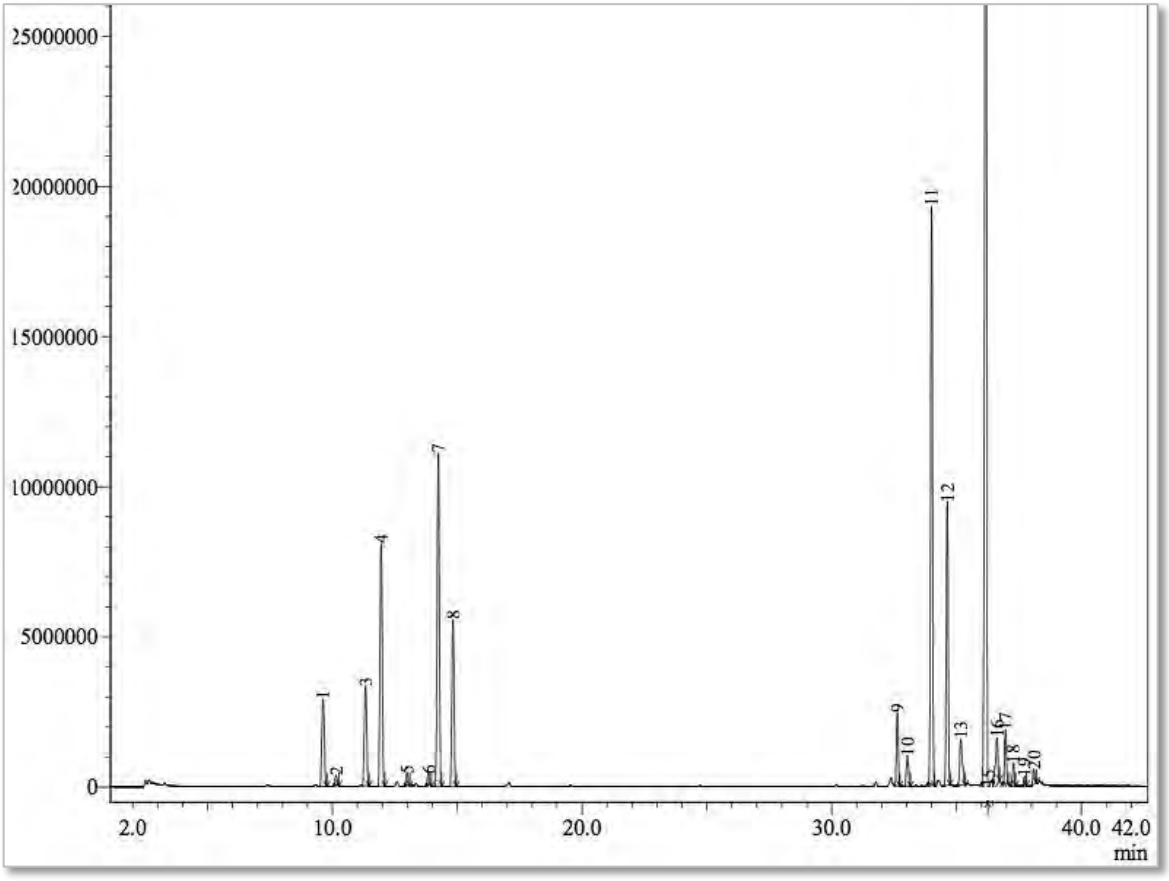


Figure C7. Chromatogram of volatiles profile for avocado seed

Table C7. Concentration of volatiles present in avocado seed

Peak	Compound	Concentration (µg/kg Avocado seed)
1	.alpha.-Pinene	4,656.54
2	Camphene	428.10
3	.beta.-Pinene	5,088.08
4	.beta.-Myrcene	11,701.91
5	1,3,6-Octatriene, 3,7-dimethyl-	475.15
6	Limonene	477.67
7	1,3,6-Octatriene, 3,7-dimethyl-, (E)-	15,606.17
8	1,3,6-Octatriene, 3,7-dimethyl-	7,628.34
9	Copaene	2,834.71
10	Aromadendrene	1,451.54
11	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]-	23,492.41
12	trans-.alpha.-Bergamotene	11,382.26
13	.alpha.-Caryophyllene	2,606.90
14	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [s-(E,E)]-	52,713.19
16	3,4-Dimethyl-2,5-diprop-2-enyl-2,5-dihydrothiophene 1,1-dioxide	2,749.60
17	Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-, (S)-	1,998.53
18	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	828.10

19	cis-.alpha.-Bisabolene	298.72
20	Diethyl Phthalate	597.33

Peak 15 was not detected

Volatile compounds present in avocado peel

Figures C8 and C9 depict the chromatogram obtained for avocado peel using the SPME method. Table C8 shows the concentration for each one of the compounds (volatiles) present in avocado peel.

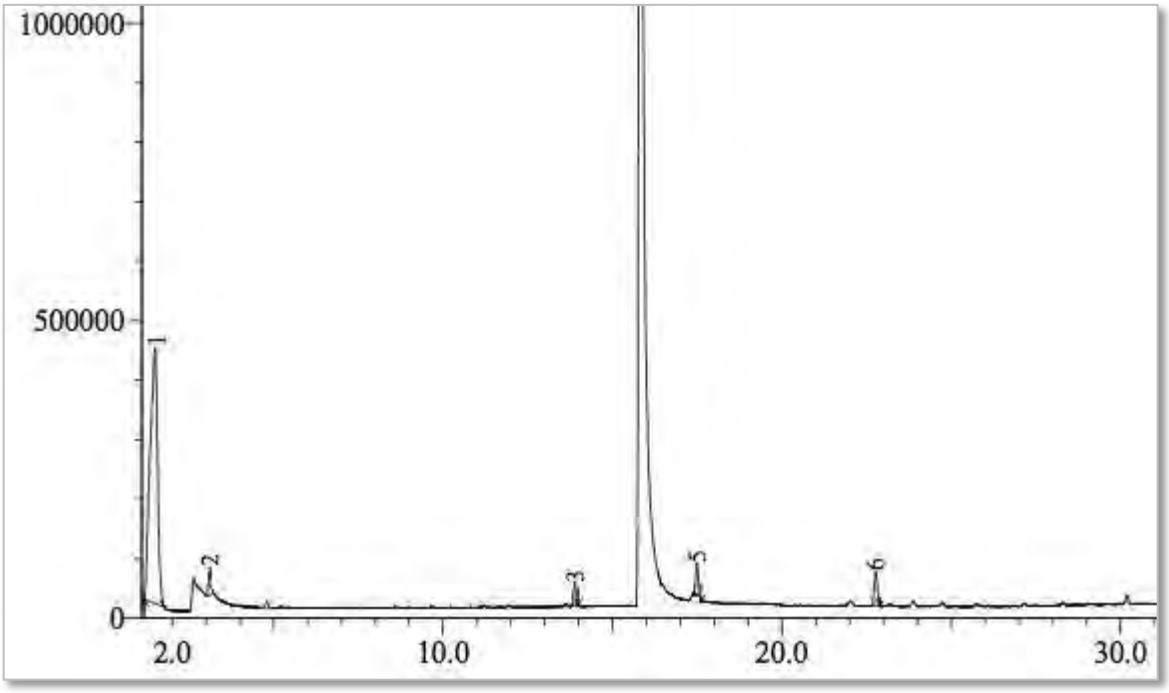


Figure C8. Chromatogram of volatiles profile for avocado peel until 30 minutes

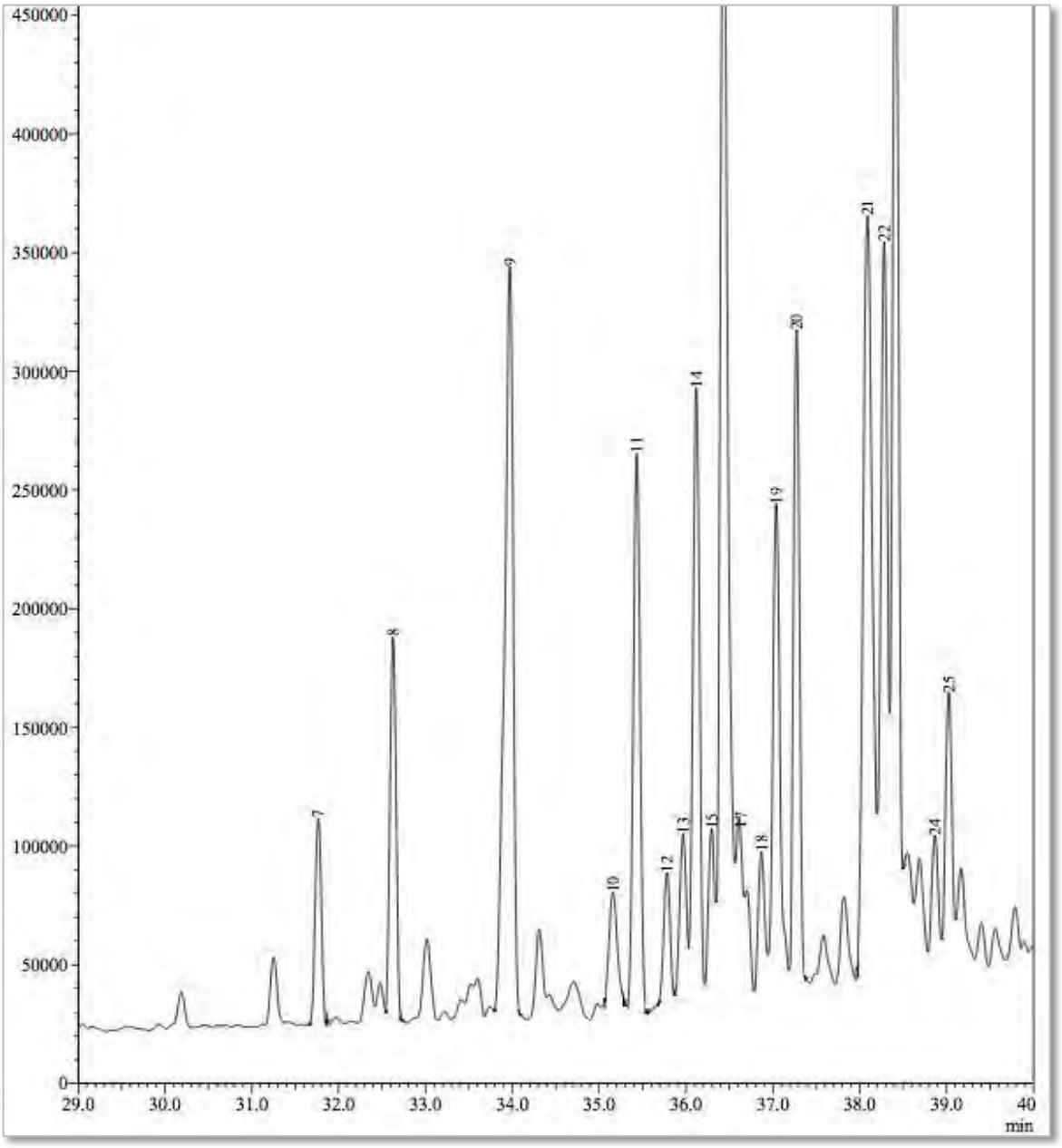


Figure C9. Chromatogram of volatiles profile for avocado peel after 30 minutes

Table C8. Concentration of volatiles present in avocado peel

Peak	Compound	Concentration (µg/kg Avocado peel)
2	Acetic acid	216.35
3	Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (S)-	164.33
4	1-Octanol	19,373.14
5	1,6-Octadien-3-ol, 3,7-dimethyl-	235.97
6	3-Cyclohexene-1-methanol, .alpha.,.alpha.4-trimethyl-	293.58
7	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [s-(E,E)]-	340.42
8	Copaene	626.62
9	Aromadendrene	1,749.15
10	1,3,7-Octatriene, 3,7-dimethyl-	234.16
11	Aromadendrene	1,022.11
13	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,7.beta.,8a.alpha.)]-	305.92
14	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [s-(E,E)]-	1,089.19
16	1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.alpha.,4a.beta.,7b.alpha.)]-	2,451.82
19	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [s-(E,E)]-	930.95
20	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [s-(E,E)]-	1,061.23
21	Phthalic acid, di-(1-hexen-5-yl) ester	1,762.62
23	Caryophyllene oxide	1,635.08

Peaks 1, 12, 15, 17, 18, and 22 were not detected

Volatile compounds present in SCG

Figure C10 depicts the chromatogram obtained for SCG using the SPME method. Table C9 shows the concentration for each one of the compounds (volatiles) present in SCG.

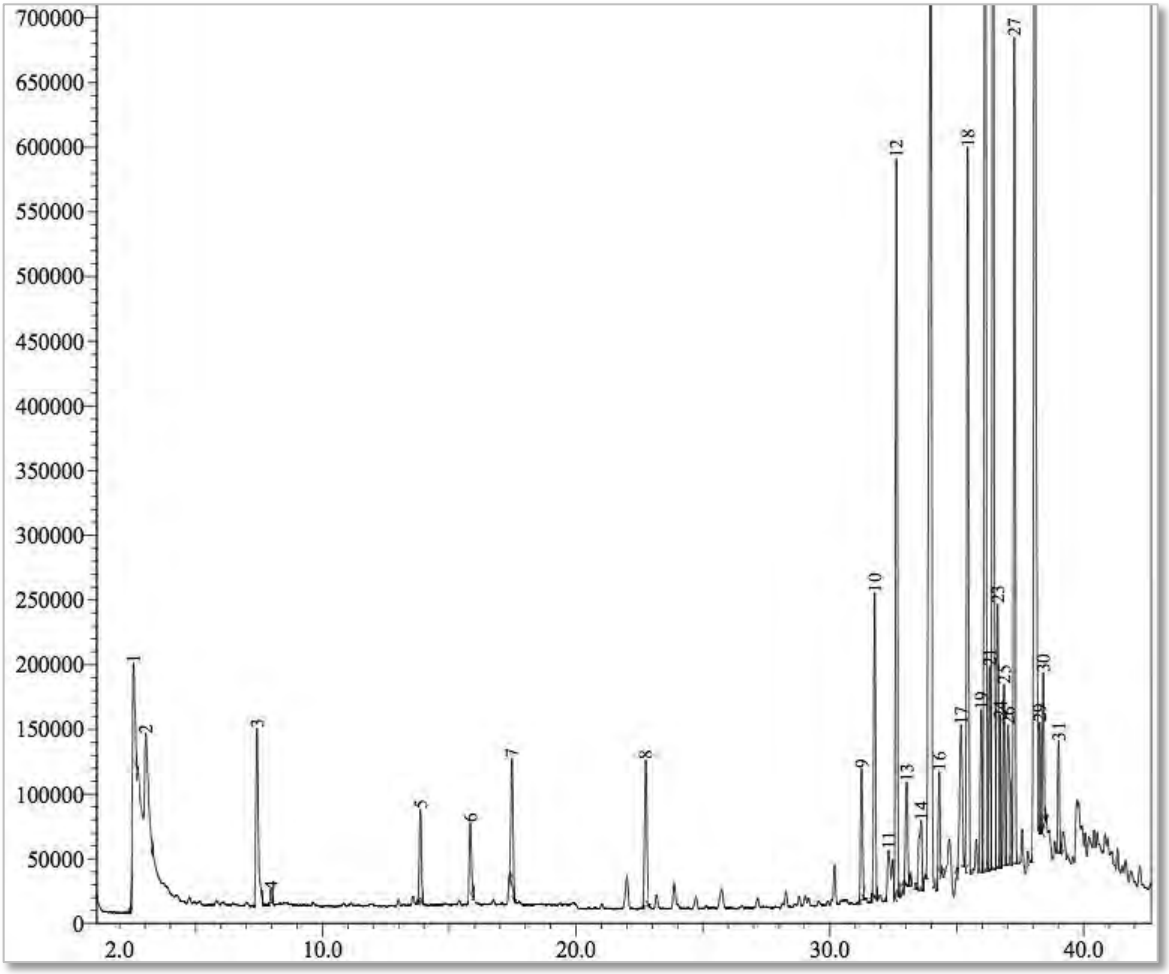


Figure C10. Chromatogram of volatiles profile for SCG

Table C9. Concentration of volatiles present in SCG

Peak	Compound	Concentration (µg/kg SCG)
3	2-Heptanone	46,556.46
4	(+-)-5-Methyl-2-hexanol	2,875.43
5	Limonene	20,307.51
6	1-Octanol	19,373.14
7	1,6-Octadien-3-ol, 3,7-dimethyl-	27,762.78
8	3-Cyclohexene-1-methanol, .alpha.,.alpha.4-trimethyl-	37,542.20
9	Bicyclo[4.1.0]heptane, 7-(1-methylethylidene)-	28,784.68
10	.alpha.-Cubebene	59,430.42
12	Copaene	144,570.32
13	.gamma.-Elemene	23,910.72
14	Decane, 2-methyl-	22,668.48
15	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]-	315,166.56
16	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [s-(E,E)]-	21,968.48
17	1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-	42,521.60
18	Aromadendrene	154,499.78
19	1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-, [1aR-(1a.alpha.,4a.beta.,7.alpha.,7a.beta.,7b.alpha.)]-	35,946.28
20	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [s-(E,E)]-	264,404.09
21	.gamma.-Elemene	41,339.18
22	1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-	252,511.49

	(1a.alpha.,4.alpha.,4a.beta.,7b.alpha.)]-	
23	Seychellene	67,548.82
24	.alpha.-Farnesene	37,079.47
27	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	158,034.62
28	Diethyl Phthalate	297,754.11
30	3-Tridecen-1-yne, (E)-	28,198.61
31	Decane, 2,3,5,8-tetramethyl-	20,602.10

Peaks 1, 2, 11, 25, 26 and 29 were not detected

Appendix C4. Calibration curve for reducing sugar

The reducing sugars were measured according to procedure described in chapter 3. Figure C11 depicts the calibration curve for reducing sugar measurements for acid hydrolysis. Concentrations from 1 to 5 g/l were evaluated. Figure C12 depicts the calibration curve for enzymatic hydrolysis with concentrations from 1 to 5 g/l.

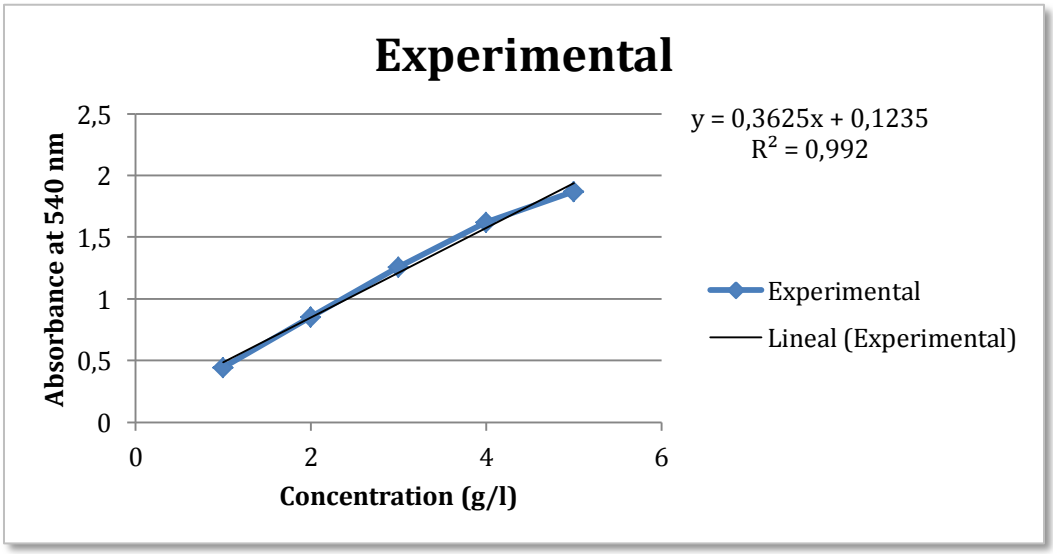


Figure C11. Calibration curve for sugar production from acid hydrolysis

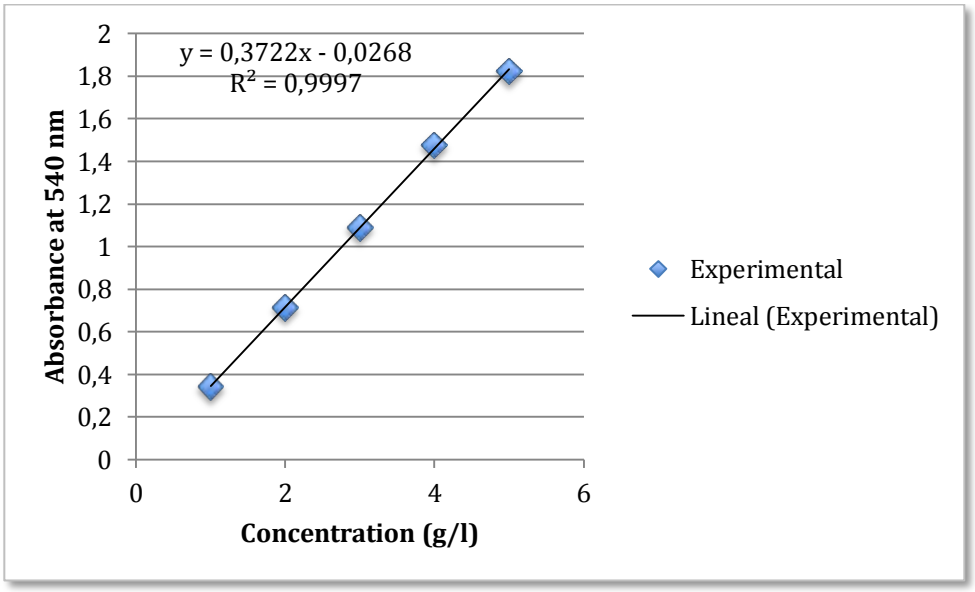


Figure C12. Calibration curve for sugar production from enzymatic hydrolysis

13. Appendix D. Simulation descriptions and results

Appendix D1. Processes, units and conditions for biorefineries based on non-oil feedstocks (SBP and naranjilla waste)

Table D1 shows the chemical characterization for both feedstocks (SBP and naranjilla waste) that was used for simulation purposes.

Table D1. Chemical characterization for SBP and naranjilla waste

Component	*SBP (%)	Naranjilla waste (%)
Moisture	89.25	86.62
Extractives	1.47	1.71
Cellulose	4.73	6.65
Hemicellulose	2.24	1.92
Lignin	2.18	2.17
Ash	0.13	0.93
Phenolic compounds	0.03	0.01
Total	**100**	**100**

*Composition of SBP was taken in dry basis (from chemical characterization) thus; the chemical composition was corrected using moisture of 89.25 (measured in the laboratory).

The description and considerations made for each one of the plants that compose the biorefineries based on non-oil feedstocks are presented below.

Extraction plant

For the extraction plant, an enhanced-fluidity liquid extraction process (EFLE) with CO_2 and ethanol was used (Cerón et al., 2012). The feedstock (SBP or naranjilla waste) is milled to obtain particle sizes smaller than 0.45 millimeters. This reduction allows obtaining a large surface area and increase the yield of extraction and at the same time, it permits to have a best handle of the solid material (Davila et al., 2014b). Then, the raw material is dried at 40 ºC to avoid the losses of antioxidant compounds that are degraded at high temperatures (Typically 60ºC) (Cerón et al., 2012). Besides, the sequencing concept discussed in chapter 2 suggests that these kinds of compounds should be extracted first. Once the raw material is pretreated (small dried particles) then, the extraction process begins. The feedstock (SBP or naranjilla waste) is loaded in the extraction chamber and it is mixed with CO_2 (solvent) and ethanol (co-solvent), The extraction chamber works at supercritical conditions, which are 300 bar of pressure and 40 ºC of temperature. To reach the supercritical conditions, the CO_2 (solvent) is turned to a liquid phase using a heat exchanger. Then the CO_2 is pressurized at 300 bar by a pump and finally, CO_2 enters to the chamber extraction. On the other hand, the ethanol (co-solvent) is pumped at 300 bar and added to the extraction chamber, thus the extraction begins. The yield of the extraction was 92% (experimental yield discussed in chapters 4 and 5) related to the initial content.

After the extraction of the antioxidant compounds from the solid matrix, the extract is passed to a collector vessel that operates at 50 bar and 25 ºC. In this vessel it is possible to separate the CO_2 from both, solid material and liquid extract. The CO_2 is recycled to the process however, part of the solvent is lost (around 10%) therefore it is necessary to add new solvent to the process. Then, the extract is separated from the solid material, which is used for microencapsulation.

Then, the extract is sent to a concentration process by means of evaporation. This allows separating part of the co-solvent (ethanol) for recycling to the process. However, because part of the ethanol is lost in the depressurization it is necessary to feed new ethanol to the process.

Finally, the extract is sent to a microencapsulation step by means of a spray drying using maltodextrin as wall material or carrier agent according to (Warner, 2015). The extract is filtered to remove possible solids that can block the nozzle of the spray drying and then mixed with maltodrextrine DE10. The carrier agent is prepared at 6% (w/w) and added in a proportion of 2:1 carrier agent to antioxidant compounds in the extract according to (Tonon et al., 2010). The mix is sent to a spray drying using air at 0.06 MPa of pressure and the microcapsules are collected after a cyclone. Table D2 shows the operational conditions of each unit in the extraction plant. Figure D1 depicts the process flow diagram for extraction plant.

Table D2. Operational conditions for plant extraction

Unit	Conditions	Purpose
Mill	1 Ton/h	Reduction of particle size to 0.45 mm (increase the superficial area)
Dryer	40 ºC, 1 bar	Decrease the moisture content in the raw material
Extractor	45 ºC, 250 bar	Extraction of phenolic compounds
Valve	45 ºC, 1 bar	Decrease the pressure
Collector	30 ºC, 1 bar	Separation of CO_2 from extract
Evaporator	40 ºC, 0.25 bar	Concentration of the extract and

		recover ethanol
Ethanol pump	45 ºC, 250 bar	Presurization of cosolvent
CO_2 heat exchanger	-40 ºC, 1 bar	Reach the liquid phase of CO_2
CO_2 pump	25 ºC, 250 bar	Presurization of solvent
Mixers	Temperatures y pressures of the last streams	Mix the recycled streams (ethanol and CO_2) and storage
Spray drying	Air pressure 0.06 MPa, drying air flow rate 73 m3/h, feed flow rate 17.85 l/h. air temperature 140ºC	Obtain microcapsules containing phenolic compounds

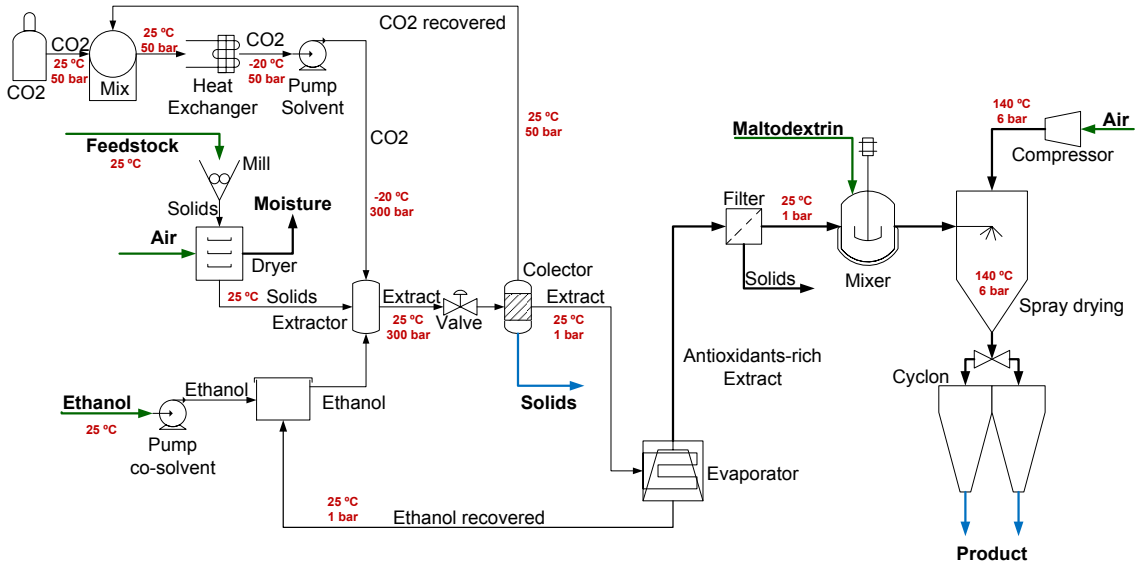


Figure D1. Process flow diagram for extraction plant

Sugar plant

The remaining solid material (Spent biomass) obtained after extraction plant is used for sugar production plant. In order to improve the cellulose accessibility, a diluted acid hydrolysis using sulfuric acid (2% v/v) at 121 ºC was carried out (Jin et al., 2011). The kinetic model used considered xylose production from hemicellulose as well as the degradation of xylose to furfural is showed in Table D3.

Table D3. Kinetic model and its parameters for acid hydrolysis

Kinetic model for acid hydrolysis taken from (Jin et al., 2011)
Reaction $Hemicellulose \rightarrow Xylose \rightarrow Furfural$
Kinetic model

$$\frac{dC_{Hemiellulose}}{dt} = -k_1 * C_{Hemicellulose}$$

$$\frac{dC_{Xylose}}{dt} = k_1 * C_{Hemicellulose} - k_2 * C_{Xylose}$$

$$\frac{dC_{Furfural}}{dt} = k_2 * C_{Xylose}$$

$$k_1 = k_{1,0} * C_{Acid}^{n} * exp\left(-\frac{Ea_1}{RT}\right)$$

$$k_2 = k_{2,0} * C_{Acid}^{n} * exp\left(-\frac{Ea_2}{RT}\right)$$

Parameters

$k_{1,0}$=1x10^{14} , $k_{2,0}$=9x10^{11}

R=8.314 J/mol*K

Ea_1=115100 J/mol, Ea_2=118000 J/mol

After acid hydrolysis, the liquid stream is separated from remaining solids and subjected to a neutralization adjusting the pH at 6.5 using NaOH. The precipitate (Na_2SO_4) is separated by centrifugation (Mussatto et al., 2013). The pH-adjusted hemicellulosic hydrolysate (rich in xylose) is then send to xylose plant.

The solid fraction (rich in cellulose and lignin) was subjected to an enzymatic hydrolysis at 50ºC previous neutralization. The kinetic model used correspond to that proposed by (Morales-Rodriguez et al., 2011). This model considers the decomposition of cellulose to cellobiose and glucose as well as cellobiose hydrolysis into glucose. This model also describes the enzyme adsorption. The kinetic model for enzymatic hydrolysis as well as its parameters is shown in Table D4.

Table D4. Kinetic model and its parameters for enzymatic hydrolysis

Kinetic model for enzymatic hydrolysis taken from (Morales-Rodriguez et al., 2011)

Kinetic model	
Cellulose to cellobiose	$r_1 = \frac{k_{1,r} C_{E1B} R_S C_S}{1+\frac{C_{G2}}{k_{11G2}}+\frac{C_G}{k_{11G}}+\frac{C_{xy}}{k_{11xy}}}$
Cellulose to glucose	$r_2 = \frac{k_{2,r}(C_{E1B}+C_{E2B}) R_S C_S}{1+\frac{C_{G2}}{k_{21G2}}+\frac{C_G}{k_{21G}}+\frac{C_{xy}}{k_{21xy}}}$
Cellobiose to glucose	$r_3 = \frac{k_{3,r} C_{E2F} C_{G2}}{K_{3M}\left(1+\frac{C_G}{k_{31G}}+\frac{C_{xy}}{k_{31xy}}\right)+C_{G2}}$
Enzyme adsorption	$C_{EiB3} = \frac{E_{imax} k_{iad} C_{EiF} C_S}{1+k_{iad} C_{EiF}}$
Enzyme	$C_{iET} = C_{EiF} + C_{EIB}$
Substrate reactivity	$R_S = \alpha C_S / S_0$
Temperature dependence	$K_{ir(T2)} = K_{ir(T1)*exp\left(-\frac{E_{ai}}{R(1/T1-1/T2)}\right)}$
Cellulose kinetic	$\frac{dC_S}{dt} = -r_1 - r_2$
Cellobiose kinetic	$\frac{dC_{G2}}{dt} = 1.056 * r_1 - r_3$
Glucose kinetic	$\frac{dC_G}{dt} = 1.111 * r_2 + 1.053 * r_3$

Parameters

$K_{1,r}$=22.3 g*mg^{-1}*h^{-1} , $K_{2,r}$=7.18 g*mg^{-1}*h^{-1} , $K_{3,r}$=285.5 g*mg^{-1}*h^{-1}

k_{11G2}=0.015 g*kg^{-1} , k_{11G}=0.1 g*kg^{-1}, k_{11xy}=0.1 g*kg^{-1}

k_{21G2}=132 g*kg^{-1} , k_{21G}=0.04 g*kg^{-1}, k_{21xy}=0.2 g*kg^{-1}

k_{31G}=3.9 g*kg^{-1} , k_{3M}=24.3 g*kg^{-1}, k_{31xy}=201 g*kg^{-1}

E_{1max}=0.06 g/g, k_{2ad}=0.1 g/g

R=8.314 J/mol*K

After enzymatic hydrolysis, the liquid phase (rich in glucose) was separated from the remaining solid (rich in lignin) by means of a filter. Table D5 shows the purpose, operational conditions and assumptions made for sugar plant. Figure D2 depicts the process flow diagram for sugar plant.

Table D5. Purposes, conditions and assumptions for the units used in the sugar plant.

Unit	Purpose	Conditions and unit specifications	Assumptions
Neutralizations	Neutralization	25 ºC, 1 bar	No
Acid hydrolysis	Improve the accessibility of the cellulose and xylose production	121 ºC, 1 bar, (2% v/v of H_2SO_4) Agitated tank enclosed	Low production of glucose, HMF and acids. Based on kinetic model of Table 7.2
Evaporators	Remove part of the water (60% for both xylose and glucose)	111 ºC, 1 bar for both xylose and glucose Standard tube vertical evaporator, one effect	No
Enzymatic hydrolysis	Glucose production	50 ºC, 1 bar, 7% (wt) biomass/enzyme Agitated tank enclosed	Based on kinetic model of Table 7.4

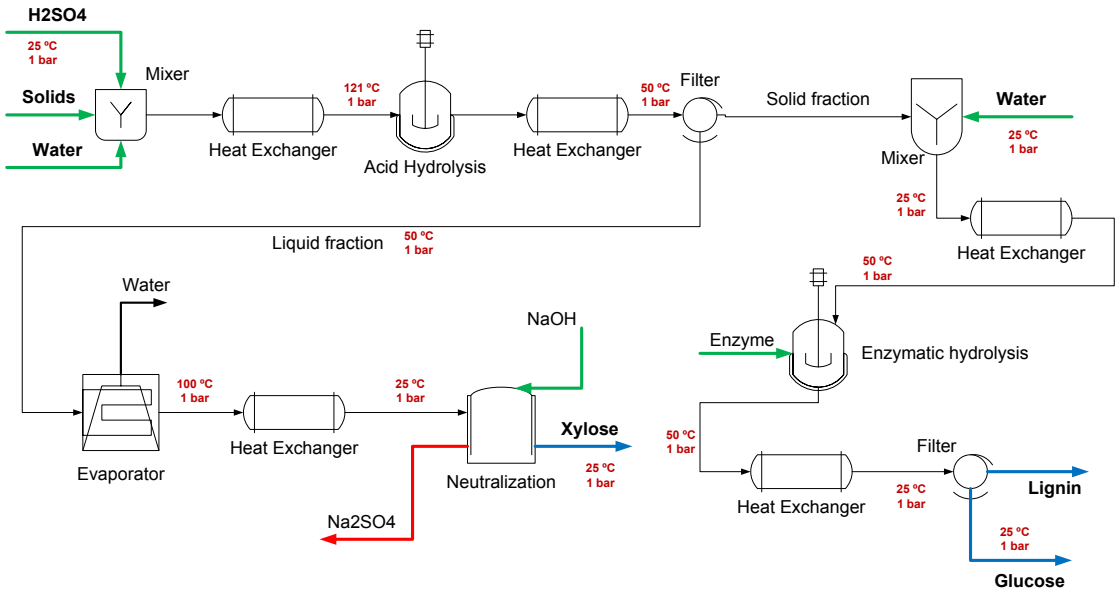


Figure D2. Process flow diagram for sugar plant

Xylitol plant

Xylose-rich hydrolysate from sugar plant is used to produce xylitol. The hydrolysate is concentrated up to 70 g/l by means of a flash evaporator at 121 ºC and 1 bar of pressure and is then cooled at 30ºC. After, the hydrolysate is fermented, using *Candida guilliermondii* yeast at 30 ºC and 200 rpm according to optimized conditions reported by (Mussatto and Roberto, 2008). The CO_2 that is generated by the fermentation is separated and the liquid stream is filtered to separate the cell biomass. Finally, the xylitol stream is concentrated using a flash unit (at 40 ºC) and the xylitol is crystallized out. After, ethanol (at 95.3%, w/w) is added in order to lower solubility of xylitol and enhance the process efficiency (Vyglazov, 2004). Table D6 shows the purpose, conditions and assumptions made for xylitol plant. Figure D3 depicts de process flow diagram for xylitol plant.

Table D6. Purposes, conditions and assumptions for the units used in the xylitol plant.

Unit	Purpose	Conditions and unit specifications	Assumptions
Evaporation	Remove part of the water (Concentration of xylose at 70 g/l)	121 ºC, 1 bar Standard tube vertical evaporator, one effect	No
Fermentation	Production of Xylitol	30 ºC. *Candida guilliermondii*	Based on experimental data (Mussatto and Roberto, 2008)
Crystallizer	Xylitol crystals formation	40 ºC, Ethanol at 95.3%	No

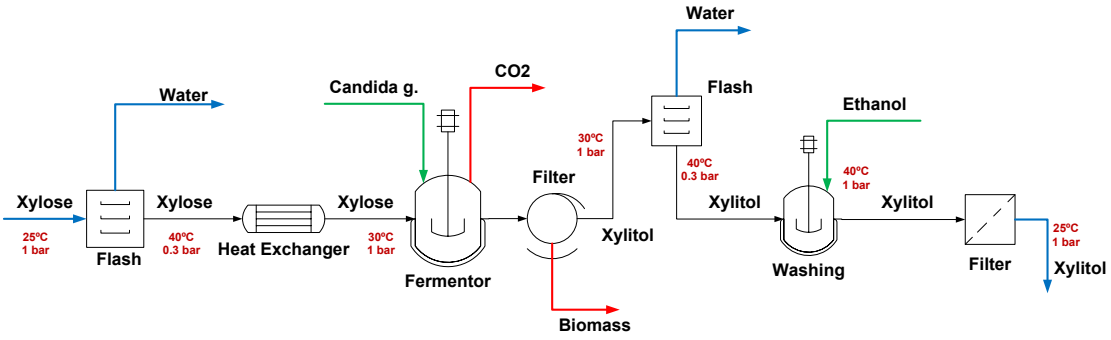


Figure D3. Process flow diagram for xylitol plant

Ethanol plant

Ethanol is produced from the glucose fraction that is obtained from the glucose plant. The glucose is fermented to ethanol using *Saccharomyces cerevisiae,* at 37 ºC according to the kinetic model proposed by (Rivera et al., 2006). After fermentation, a concentration step was considered using distillation and rectification towers, where the ethanol concentration is increased to 96%. Finally, the ethanol is dehydrated by means

of molecular sieves according to operational conditions and yield reported by (Quintero et al., 2007). Table D7 shows the kinetic model and its parameters. Figure D4 depicts the process flow diagram for ethanol plant.

Table D7. Kinetic model and its parameters for acid hydrolysis

Kinetic model for fermentation of glucose taken from (Rivera et al., 2006)	
Kinetic model	
Biomass	$r_x = \mu_{max}\frac{S}{K_S+S}\exp(-K_i S)\left(1-\frac{X}{X_{max}}\right)^m\left(1-\frac{P}{P_{max}}\right)^n X$
Product	$r_p = Y_{px}r_x + m_p X$
Substrate	$r_s = \frac{r_x}{Y_x} + m_x X$
Concentration of biomass	$\frac{dX}{dt} = r_x$
Concentration of substrate	$\frac{dS}{dt} = -r_S$
Concentration of Product	$\frac{dP}{dt} = r_p$
Parameters	
μ_{max} = 0.426 h^{-1}, X_{max} = 54.47 kg/m^3, P_{max} = 86.07 kg/m^3	
Y_x = 9.763 kg/kg, Y_{px} = 0.03831 kg/kg	
K_s = 4.1 kg/m^3, K_i = 0.002 m^3/kg,	
m_p = 0.1 kg/(kg*h), m_x = 0.2 kg/(kg*h), m = 1, n = 1.5	

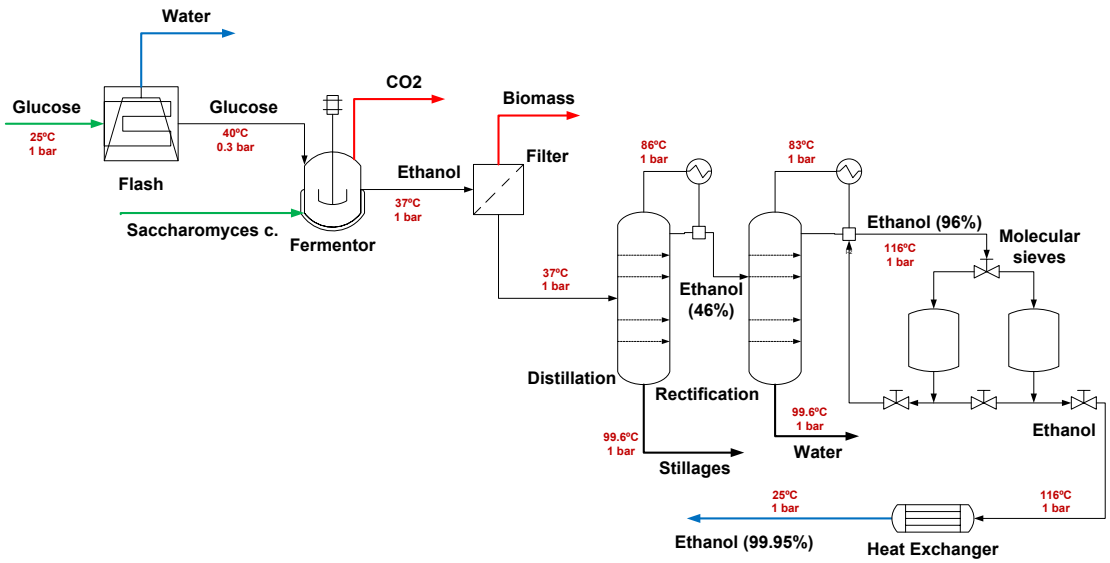


Figure D4. Process flow diagram for ethanol plant

Cogeneration plant

For this plant, the solid stream from the sugar plant (rich in lignin) was used as feedstock. For this purpose, a simple gas turbine gasification scheme was used. The feedstock (residual solid rich in lignin obtained from sugar plant) was dried and then gasified at 850ºC of temperature. A ratio of 0.4 air to fuel was used (Ruiz et al., 2013). Depending on the composition of the biomass, a mixture of gases consisting of CO, CO_2, H_2O, H_2, and CH_4, among other constituents, can be obtained. After gasification, the hot gases are used in a gas turbine to produce electricity and the exhausted gases are used for obtaining steam (at low pressure). Table D8 shows the purpose, conditions and assumptions for cogeneration plant. Figure D5 depicts de process flow diagram for cogeneration plant.

Table D8. Purposes, conditions and assumptions for the units used in the cogeneration plant.

Unit	Purpose	Conditions and unit specifications	Assumptions
Gasifier	Hot gases generation	850 ºC, 60 bar	No
Turbine	Electricity generation	1 bar, outlet temperature 143 ºC	70% of efficiency
Heat exchanger	Steam generation	3 bar	No

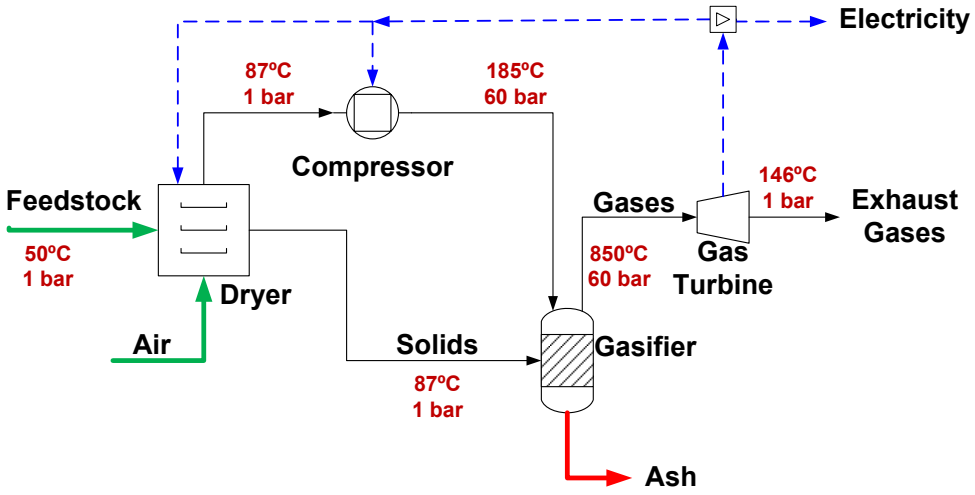


Figure D5. Process flow diagram for cogeneration plant

Appendix D2. Cost of raw material, inputs and services for economic assessments for all biorefineries

The cost of raw materials, inputs and services as well as operator, supervisor and other important cost associated to the biorefinery are showed in Table D9.

Table D9. Costs of raw materials and services to the Biorefinery

Item	Price	Unit
Feedstock [a]	21	(USD/Ton)
Water [b]	1.25	(USD/m^3)
Sulfuric acid [c]	0.094	(USD/kg)
Sodium hydroxide [c]	0.098	(USD/kg)
Enzyme [d]	3	(USD/kg)
Operator labor cost [b]	2.14	(USD/h)
Supervisor labor cost [b]	4.19	(USD/h)
Electricity cost [b]	0.1	(USD/KWh)
Fuel [e]	7.28	(USD/MMBTU)
Ethanol at 99.5% [f]	0.94	(USD/l)
CO_2 [g]	0.01	(USD/kg)

Maltodextrine [h]	0.2	(USD/kg)
Phenolics [i]	167.48	(USD/kg)
Xylitol [j]	2.95	(USD/kg)
Hexane [k]	0.3	(USD/kg)
Avocado fruit [l]	0.47	(USD/kg)
Avocado oil [m]	8.13	(USD/kg)
SCG oil [n]	0.61	(USD/kg)

[a] Calculated for transportation (of SBP or naranjilla waste and SCG) over a distance of 140 Km with a truck of three axles.

[b] Typical price in Colombia.

[c] Taken from ICIS Prices (ICIS, 2013)

[d] Prices based on Alibaba International Prices (ALIBABA, 2013a)

[e] Estimated cost of Gas for the years 2015 – 2035 (NME, 2013a)

[f] National price in Colombia (Fedebiocombustibles, 2013)

[g] Taken from international prices (Luckow, 2014)

[h] Taken from (ALIBABA, 2013b)

[i] Taken from (Warner, 2015)

[j] Taken from (Mussatto et al., 2013)

[k] Taken from (ICIS, 2013)

[l] Taken from (mayorista, 2015)

[m] Taken from (Peña et al., 2012)

[n] Taken from (Index, 2015)

Appendix D3. Share and costs for each plant and each biorefineries based on non-oil feedstocks

Figures D.6 and D.7 depict the share of cost for each plant for SBP and naranjilla waste biorefineries respectively. For all plants, the raw material cost (That include input costs) continues being the more important economic factor (as well for entire biorefinery) over total production cost.

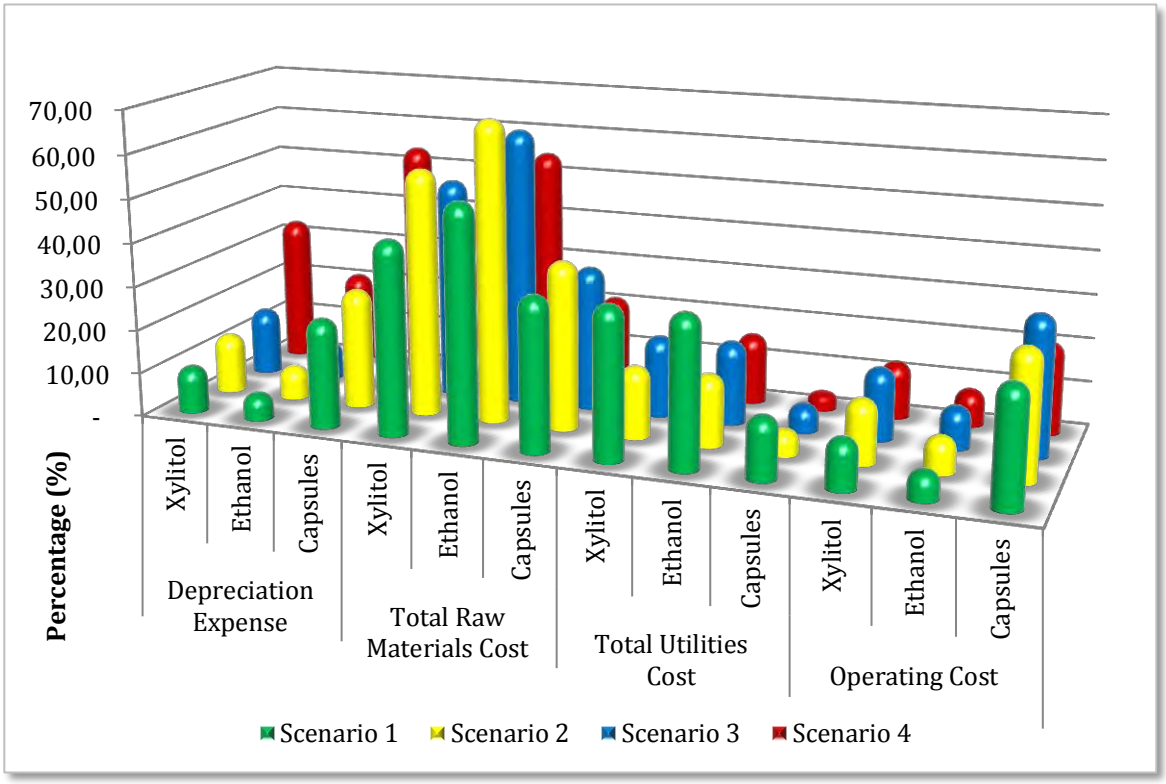


Figure D.6. Share of cost for SBP biorefinery for each scenario

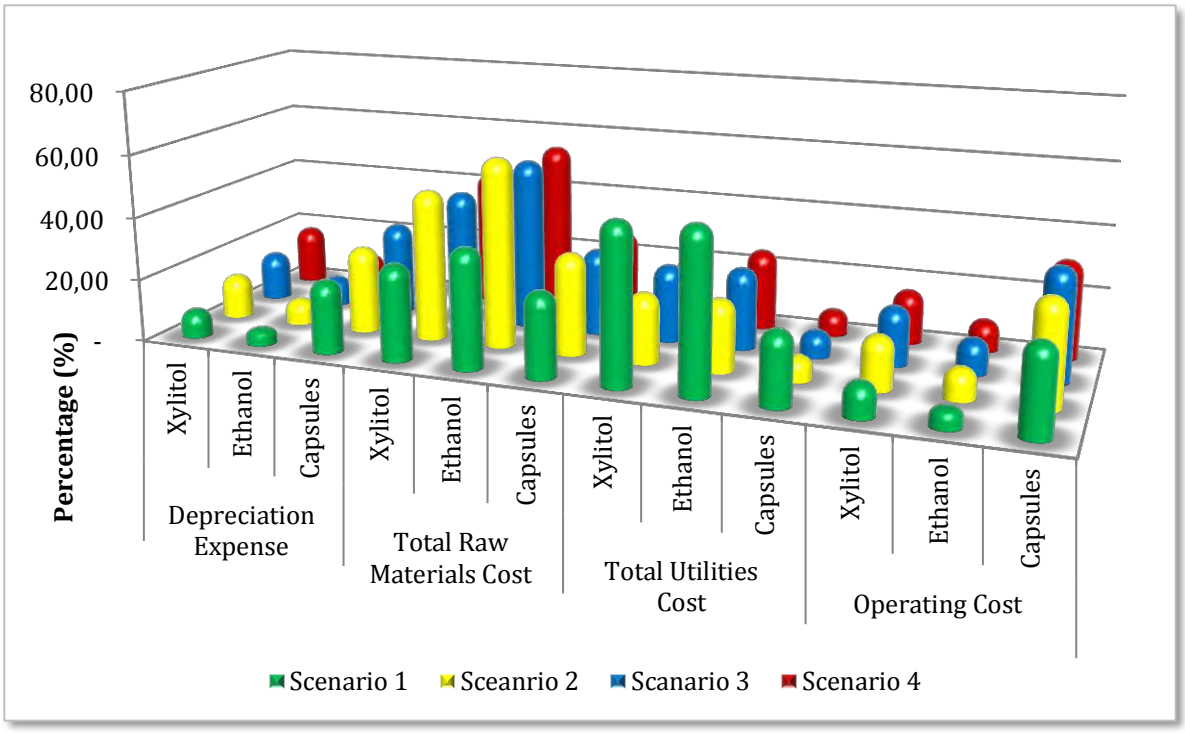


Figure D.7. Share of cost for naranjilla waste biorefinery for each scenario

Appendix D4. Composite curves for each biorefinery after heat integration

Figures D.8 and D.9 depict the composite curves after heat integration obtained applying the Pinch methodology according to procedure described in **Appendix B2**.

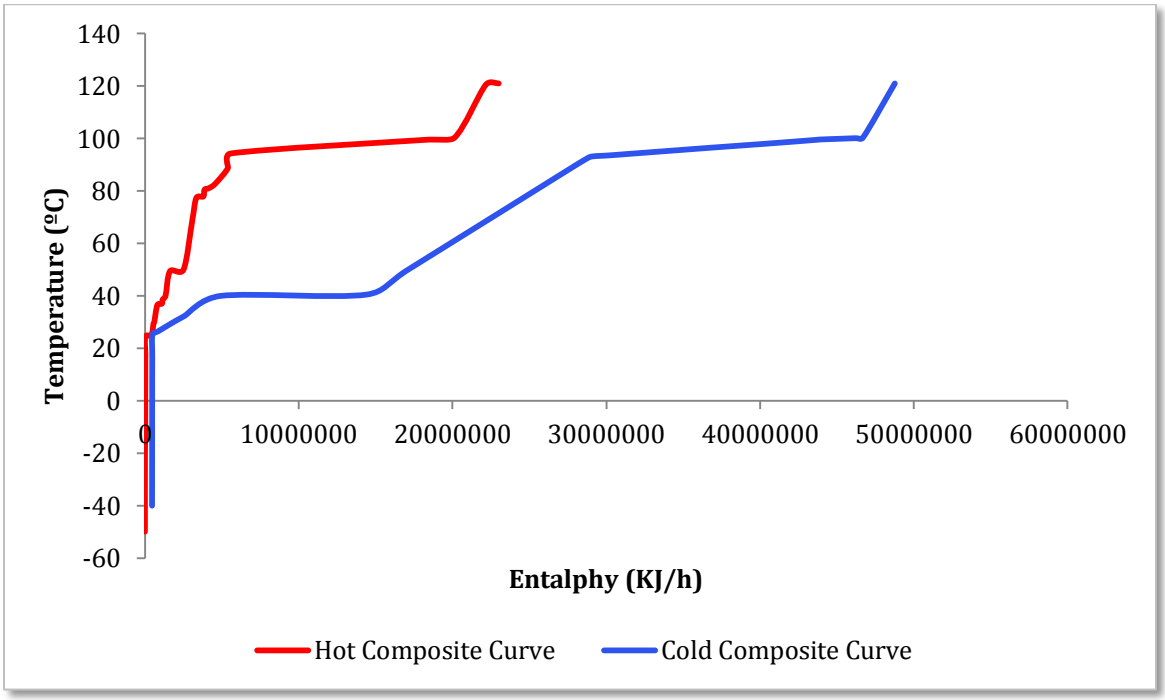


Figure D.8. Composite curves for SBP biorefinery with heat integration

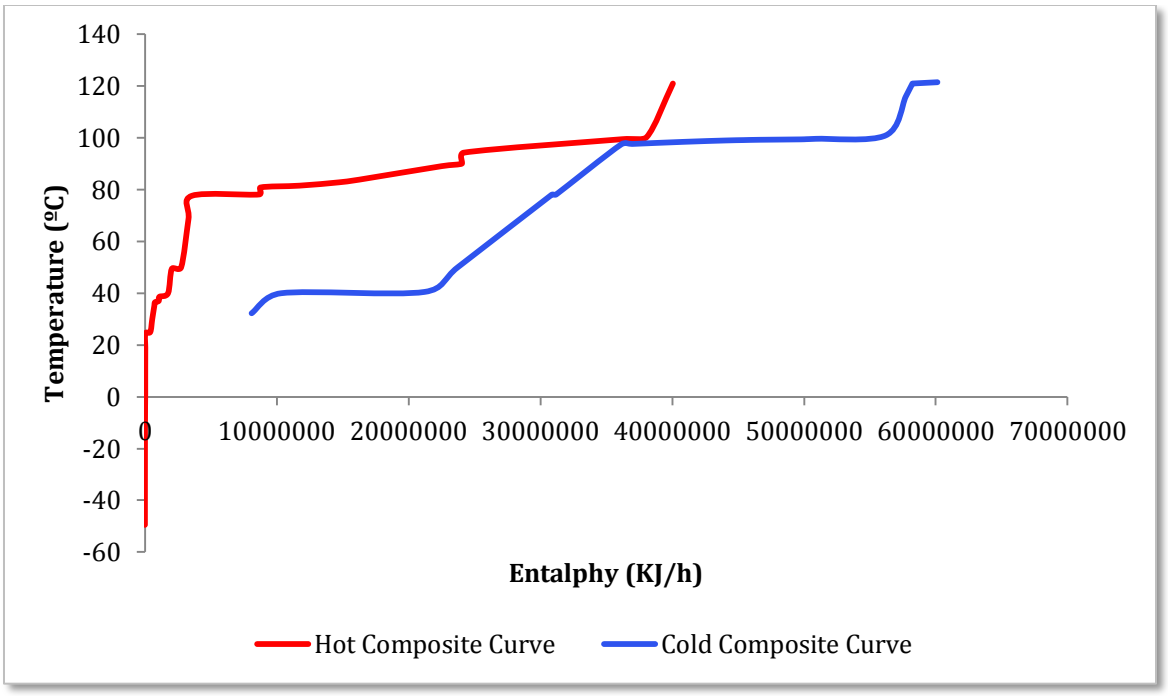


Figure D.9. Composite curves for naranjilla waste biorefinery with heat integration

Appendix D5. Processes, units and conditions for biorefineries based on oil feedstocks (Avocado and SCG)

The chemical characterization for both feedstocks is showed in Table D.10. For avocado, were taken the composition of the lignocellulosic biomass of peel and seed while for avocado pulp was taken its content of oil according to experimental results (chapter 4).

Table D.10. Chemical characterization for avocado and SCG

Component	***Avocado (%)**		****SCG (%)**
	Peel	Seed	
Moisture	76.32	81.37	60.00
Extractives	8.14	6.22	5.21
Cellulose	6.53	1.12	12.46
Hemicellulose	5.99	8.28	8.36
Lignin	1.03	0.31	7.2
Ash	0.25	0.15	0.8
Fat	***1.72	***2.54	5.96
Phenolic compounds	***0.02	***0.01	0.01
Total	**100**	**100**	**100**

* The composition was taken in dry basis (from chemical characterization) thus; the chemical composition was corrected using moisture of 76.32 and 82.47 for peel and seed respectively (measured in the laboratory).

** This chemical characterization was corrected according to moisture content in wet basis which is 60.01% for SCG (Rodriguez, 2011).

*** These values were taken from the literature (Arukwe et al., 2012).

Similarly to biorefineries based on SBP and naranjilla waste, the biorefineries based on avocado and SCG have the same plants (plant for the extraction of phenolic compounds and microencapsulation, a plant for sugars production, a plant for xylitol and a plant for ethanol production) and one additional plant which is aimed to recover the oil contained in avocado pulp and SCG. Also, the biorefineries were evaluated considering a plant for cogeneration. Below is the description and considerations made for the additional plant aimed to recover the oil from avocado pulp and SCG.

Oil plant

For oil extraction from SCG, this feedstock was introduced in an extractor using hexane as solvent. The extraction was carried out at the experimental conditions established in chapter 3 that allows the maximum yield (160 ºC and 1 bar). Solvent to SCG ratio of 4.2 (ml/g) was used. After, an evaporation of the solvent was carried out to recover and recycle part of the solvent used: Finally, the oil is cooled at 25ºC. Figure D.10 shows the oil extraction plant for SCG.

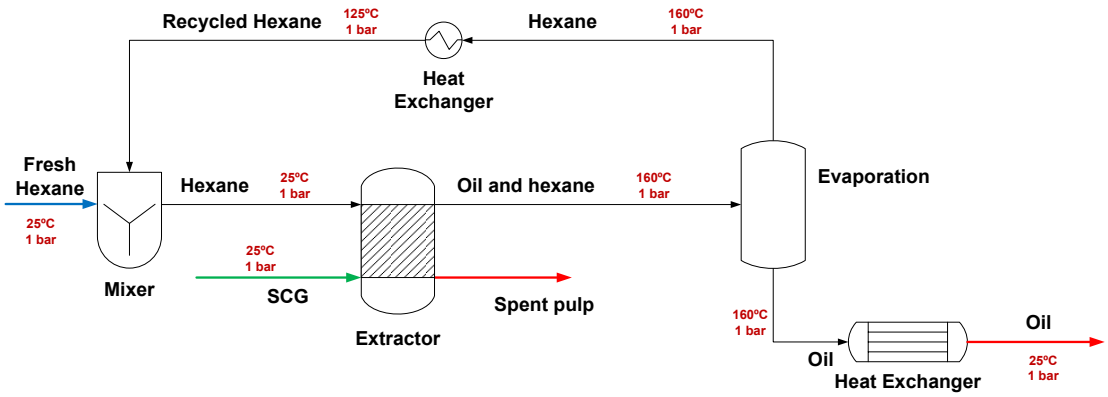


Figure D.10. Oil extraction plant for SCG

For avocado pulp, the extraction of oil was carried out at the experimental conditions established in chapter 3 (50ºC, 1 bar). The oil was separated from the pulp using a thermo-mechanical method and then, oil was centrifuged at 6000 rpm. Finally, the oil is cooled at 25 ºC. Figure D.11 shows the oil extraction plant for avocado pulp.

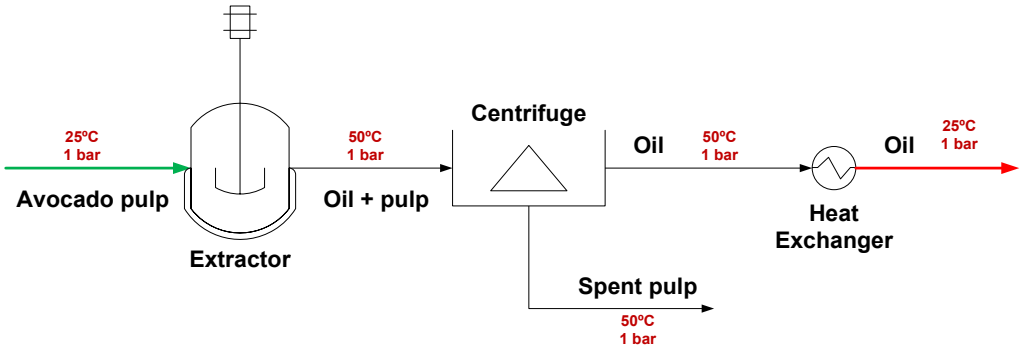


Figure D.11. Oil extraction plant for avocado pulp

Table D.11 shows the purpose, conditions and assumptions made for oil extraction plants for avocado pulp and SCG.

Table D.11. Purposes, conditions and assumptions for the units used in the oil extraction plants for avocado pulp and SCG.

Unit	Purpose	Conditions and unit specifications	Assumptions
Extraction plant for SCG			
Extractor	Recover oil from the solid matrix	160 ºC, 1 bar	No
Evaporation	Recovering hexane to be recycled	40 ºC, 335 mbar	No
Heat exchangers	Temperature conditioning	25 ºC for hexane and oil	No
Extraction plant for avocado pulp			

Extractor	Recover oil from the solid matrix	50 ºC, 1 bar	No
Centrifugation	Separate oil from spent pulp	50 ºC, 1 bar	No
Heat exchanger	Temperature conditioning	25 ºC, 1 bar	No

Appendix D6. Share and costs for each plant and each biorefineries based on oil feedstocks

Figures D12 depicts the share of costs for each plant for avocado and SCG biorefineries respectively. Raw material cost (that include inputs costs) represents the most important economic factor over total production cost followed by utilities. It is noted that oil plant is the higher consumer of raw materials; this is due to the consumption of avocado pulp that represents almost 80% of the entire fruit

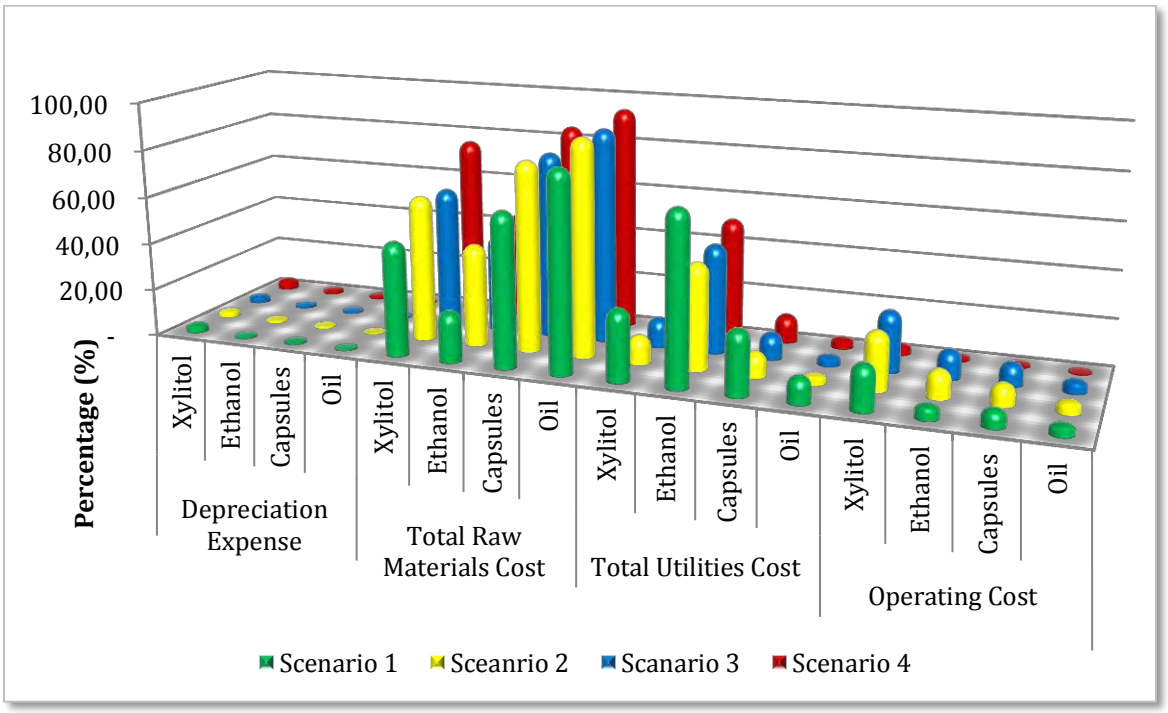


Figure D.12. Share of cost for each plant in avocado biorefinery for each scenario

Figure D.13 depicts the contribution of each economic factor over total production cots in each plant for SCG biorefinery. As was explained with Table 7.12, utilities have the highest contribution over total production cost; this is due to the content of holocellulose of SCG that is very high (20.82% of the SCG) in comparison with avocado (12.52% and 9.4% for peel and seed respectively). Even, holocellulose content of SCG is higher than SBP (6.97%) and naranjilla waste (8.57%). As a consequence of high content of holocellulose, more utilities are required for processing cellulose and hemicellulose in each plant for obtaining products.

Indeed, SCG was the feedstock with higher yields for xylitol and ethanol because the holocellulose content. Only, SBP had a huge yield of phenolic compounds in comparison of SCG because the content of phenolic compounds is higher in SBP. As it was expected, ethanol plant is the higher consumer of utilities such as it happened with SBP and naranjilla waste biorefineries.

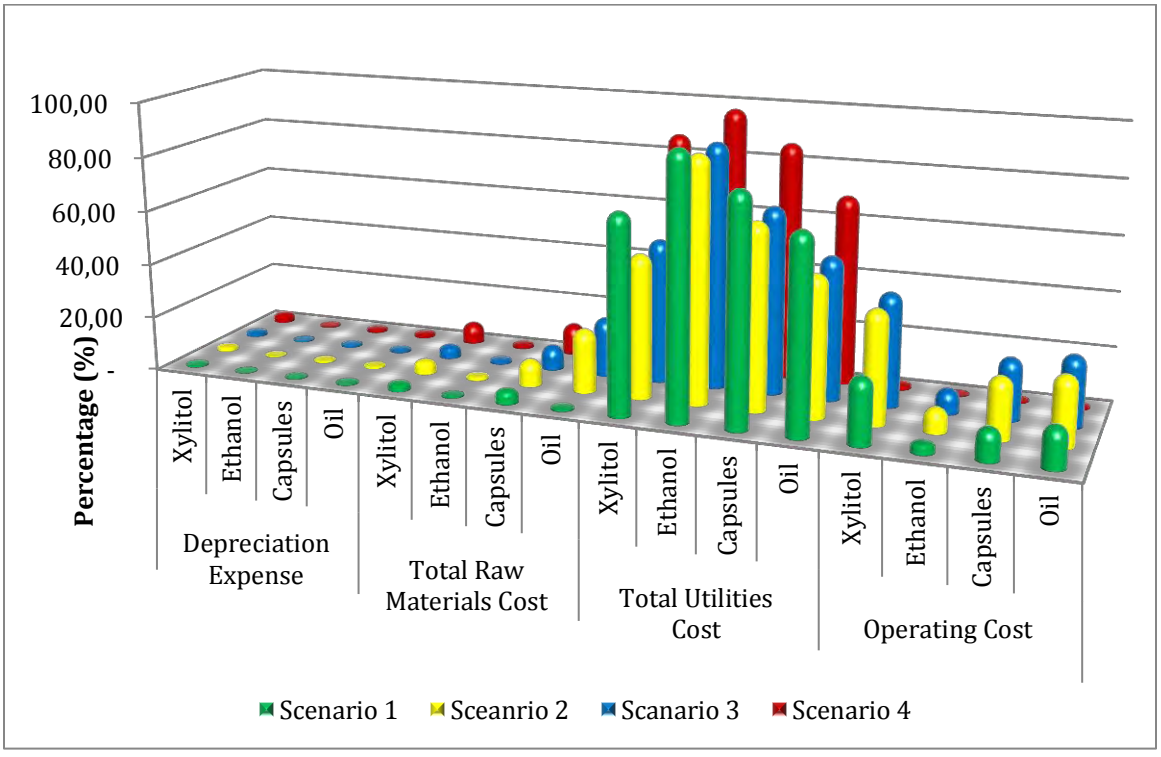


Figure D.13. Share of cost for each plant in SCG biorefinery for each scenario

Appendix D7. Composite curves for each biorefinery after heat integration

Figures D.14 and D.15 depict the composite curves after heat integration obtained applying the Pinch methodology according to procedure described in **Appendix B2**.

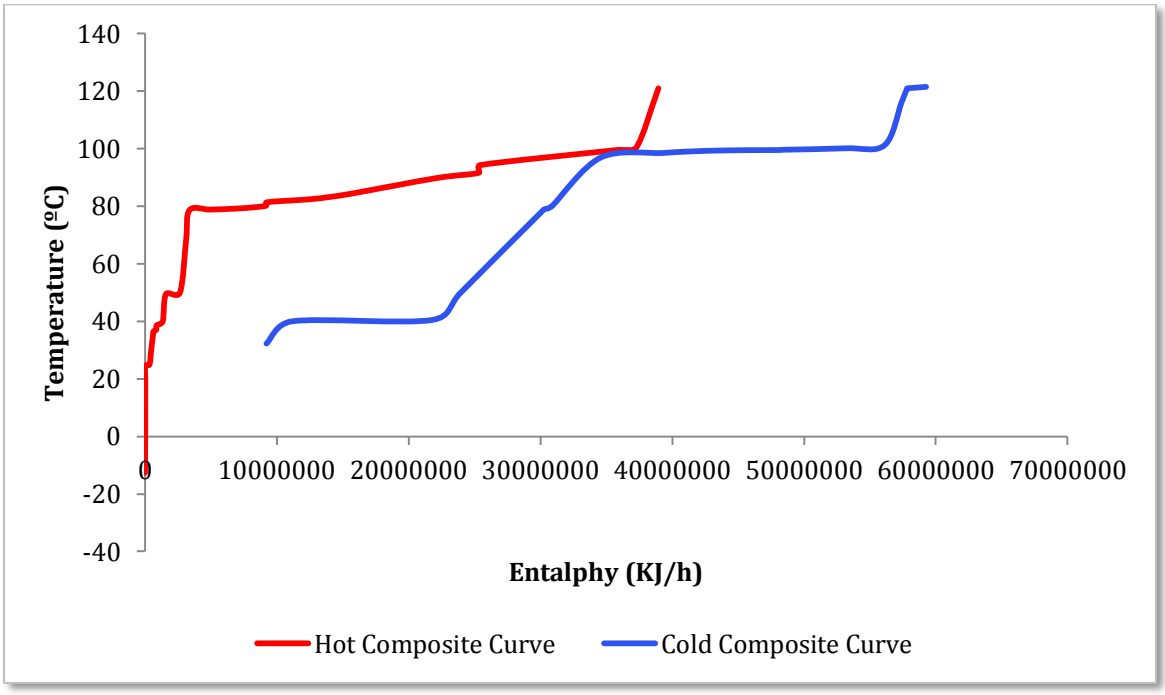


Figure D.14. Composite curves for Avocado biorefinery with heat integration

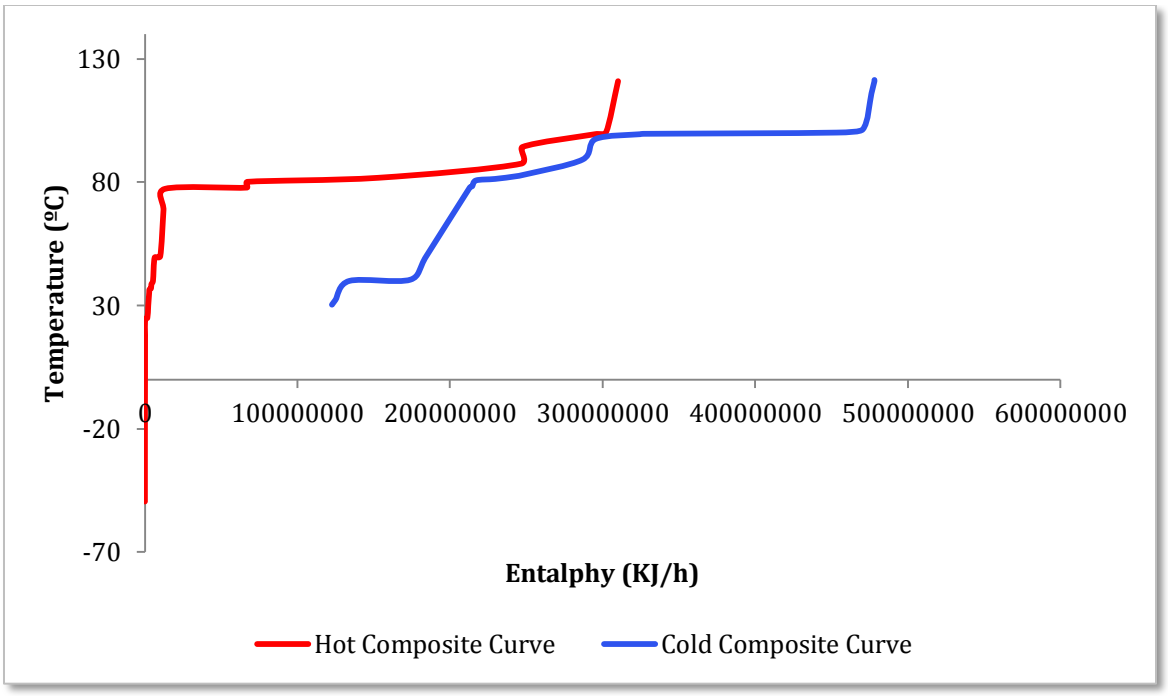


Figure D.15. Composite curves for SCG biorefinery with heat integration

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