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FRUIT AGROINDUSTRIAL WASTES FOR PREPARING BEVERAGES FOR MEDICINAL PURPOSES BY SUPERCRITICAL FLUID EXTRACTION TECHNOLOGY: ANDES BERRY (*RUBUS GLAUCUS BENTH*) CASE

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5.1 Overview

Tropical countries have a wide variety of fruits that contain valuable compounds, which could be obtained not only from the pulp but also from the peel and seed. Fruit processing generates a significant amounts of residues that correspond to the nonedible parts that are disposed in an inadequate manner but its valuable compounds have different potential applications. Since a lot of these valuable compounds contained in fruits could be used for pharmaceutical, cosmetic, and chemical applications; the valuable biologically active compounds such as antioxidants, vitamins, phenolic compounds, and anthocyanins are the most attractive compounds to be extracted, and thus, could be used for preparing beverages with high functional quality to be used as health promoters. However, the applications of these compounds are constrained for the extractions and concentrations yields because the technologies are not well established yet. Because for conserving the chemical properties of the valuable compounds it is an important factor in the extraction process, it is necessary to improve such processes to increase the yields of the extraction and to

Production and Management of Beverages. https://doi.org/10.1016/B978-0-12-815260-7.00005-5

obtain solvent-free extracts as well as to enhance the concentration and stabilization of valuable compounds for allowing medicinal applications for human health and well-being.

In this sense, Andes berry (*Rubus glaucus benth*) has antioxidant properties due to its phenolic compounds such as cafeic, chlorogenic acids, and quercitin. This fruit also has significant contents of ascorbic acid and it is an excellent source of anthocyanins, which have applications in the field of cancer. Owing to this fact Andes berry is a good source of valuable compounds (phenolic compounds, anthocyanins among others), which can be obtained as extracts and can be used for beverage preparation aimed for medicinal applications. In this sense, this chapter describes the use of Andes berry waste for obtaining extracts rich in phenolic compounds and anthocyanins using supercritical CO_2 extraction. The functional quality of the extracts is assessed according to the contents of total phenolic compounds (TPCs), anthocyanins content, and the total antioxidant activities as the main characteristics for beverages preparation. Pressure and cosolvent (ethanol) to solid matrix ratio are evaluated for assessing the extraction yield as well as functional quality. Thus, potential applications of fruit agroindustrial wastes from Andes berry processing are evaluated for obtaining extracts that can be aimed to prepare beverage for medicinal purposes.

5.2 Fruits as Source of Valuable Compounds

Because of its geographic location and its wide range of climates, Colombia is the third-ranked country in the world in terms of biodiversity, housing approximately the 10% of the world species, and the fourth-ranked country in terms of hydrographic resources (Mongabay, 2010). According to Procolombia (2016) 10.9% of the country was devoted to fruit production in Colombia in 2013, which corresponded to a production of 9.5 million tons of fruits. In the country, approximately 48 fruit species can be obtained, including both perennial and nonperennial fruits (Alarcón García et al., 2015). Among the perennial fruits, the top five species cultivated in Colombia are orange (*Citrus sinensis*), mango (*Mangifera indica*), avocado (*Persea americana*), guava (*Psidium* spp.), and tangerine (*Citrus* spp.), comprising 44.8% of the total cultivated area devoted to fruit production, without considering domestic plantain and banana production (Alarcón García et al., 2015). Similarly, the most important nonperennial fruits (without considering plantain and banana) are pineapple (*Ananas comosus*), blackberry (*Rubus ulmifolius*), tamarillo fruit (*Solanum betaceum*), and lulo (*Solanum quitoense*), comprising 23.48% of the total cultivated area (Alarcón García et al., 2015). Table 5.1 summarizes the crop production per year of some fruits in Colombia and its respective agroindustrial wastes.

Table 5.1 Some Fruits and Agroindustrial Wastes Produced in Colombia

Agroindustrial Waste	Crop Production (tons/year)	Agroindustrial Wastes (tons/year)	References
Plantain peel	2.724.888	416.908	de Fonseca (2009), Motato et al. (2006), Castellanos and Lucas (2011), and Duque et al. (2015)
Rejection bananas	2.034.340	364.420	Motato et al. (2006)
Banana stem		226.037	Motato et al. (2006) and Duque et al. (2015)
Coffee pulp	514.128	216.962	Rodríguez (2009) and Pandey et al. (2000)
Coffee husks		71.493	Rodríguez (2009) and Pandey et al. (2000)
Guava peel and seed	440.102	59.854	Torres (2010), Yepes et al. (2008), and González (2010)
Pineapple peel	397.824	149.184	Duque et al. (2015), Yepes et al. (2008), Rodríguez and Hanssen (2007), and Ministerio de Agricultura y Desarrollo Rural and Corporación Colombia Internacional (2002)
Pineapple leaf		79.565	Yepes et al. (2008), Ministerio de Agricultura y Desarrollo Rural and Corporación Colombia Internacional (2002) and García and Torres (2003)
Orange peels, seeds, and bagasse	322.989	161.495	de Fonseca (2009), Yepes et al. (2008), Rodríguez and Hanssen (2007), López et al. (2011), and Espinal et al. (2005)
Papaya peel and seed	262.914	93.710	Yepes et al. (2008)
Peel, seeds, and whole mango	243.375	65.711	Duque et al. (2015), Yepes et al. (2008), Ospina et al. (2012), and Mejía et al. (2007)
Tamarillo peel	130.211	11.719	Yepes et al. (2008), Cerón et al. (2010), Revelo (2011), and Sánchez and Murillo (2010)
Mandarin wastes, seeds, and peel	123.641	68.003	Espinal et al. (2005) and Navarrete et al. (2010)
Coconut fiber	93.206	27.962	Bonilla-Lavado et al. (2006)
Blackberry seeds	86.176	56.014	Sánchez and Murillo (2010), Franco and Giraldo (1998), and Díaz (2011)
Passion fruit peel	81.089	42.166	Yepes et al. (2008), Durán and Méndez (2008), and Ministerio de Agricultura y Desarrollo Rural and Corporación Colombia Internacional (2003)
Cocoa pulp	39.534	13.178	Cuéllar (2010) and Villegas et al. (2007)
Cocoa seed hulls		3.953	Villegas et al. (2007) and Serra Bonvehí and Ventura Coll (1999)

Continued

Table 5.1 Some Fruits and Agroindustrial Wastes Produced in Colombia—cont'd

Agroindustrial Waste	Crop Production (tons/year)	Agroindustrial Wastes (tons/year)	References
Soursop peel and seed	13.029	2.840	Márquez (2009)
Goldenberry calyx	8.211	821	Yepes et al. (2008), Cerón et al. (2010), Hernández and López (2007), Ministerio de Agricultura y Desarrollo Rural and Corporación Colombia Internacional (2001), Cedeño and Montenegro (2004), and Fischer et al. (2005)

In Colombia, cutting and transformation processes of the different crops lead to the generation of several million tons of wastes per year (Duque et al., 2015; Escalante et al., 2007), as illustrated in the table. These agricultural crop residues can be classified as field and processing residues (Escalante et al., 2007; Hadar and Faraco, 2013). According to the Ministerio de Minas y Energía (Escalante et al., 2007), 75% of the total biomass produced in Colombia corresponds to crop residues, while processing residues represent the remaining 25%. In the case of fruit residues, its pharmacological and nutritional properties have attracted the attention of food, cosmetic, and pharmaceutical industries for obtaining valuable products (Gupta et al., 2015).

5.2.1 Valuable Compounds of Tropical Fruits and Its Residues

Fruits contain substances such as vitamins and secondary metabolites (polyphenols, carotenoides, sterols, flavors, dyes, essential oils, flavonoids, alkaloids, tannins, coumarins, lactones, terpenes, and saponins, among others) (Ramful et al., 2010; Genovese et al., 2008; Contreras-Calderón et al., 2011), with a biological activity which can protect the human body against cellular oxidation reactions. Besides, fruit wastes, which usually represent an environmental problem due to the current disposal methods (dumping, burning, or land filling), are an attractive sources of several phytonutrients.

5.2.1.1 Antioxidant Compounds

The oxidative stress contributes to a wide variety of deceases such as cancer, neurological disorders, atherosclerosis, hypertension, ischemia, diabetes, acute respiratory distress syndrome, idiopathic pulmonary fibrosis, chronic obstructive pulmonary decease, heart failure, chronic inflammation, hemorrhagic shock, kidney damage, rheumatoid arthritis, aging, and asthma, among others. In order to counteract the harmful effects of the oxidative stress, antioxidants can be either naturally produced in the body (endogenous antioxidants) or supplied externally through the diet (exogenous antioxidants) (Dávila et al., 2014). Under specific low concentrations, the antioxidants can inhibit or delay the oxidative process or even inactivate the ROS at cellular and molecular levels by interrupting the radical chain reaction (Flora, 2009). Plants contain different types of phytochemicals with antioxidant properties. Some of the most important exogenous antioxidants and its benefit effects over the human health are explained below.

Vitamin C is also called ascorbic acid and cannot be synthetized by the human body. It is a water-soluble vitamin essential for the biosynthesis of neurotransmitters, collagen, and carnitine (Dávila et al., 2014). In the cells the ascorbic acid reacts with glutathione for maintaining its reduced form (Lobo et al., 2010). Health benefits associated with vitamin C are its antioxidant, antiatherogenic, anticarcinogenic, and immunomodulator properties. Several studies have demonstrated the positive effects of vitamin C in reducing the incidence of stomach cancer and the prevention of lung and colorectal cancers (Dávila et al., 2014).

Vitamin E is a fat-soluble vitamin and a chiral metabolite with eight stereoisomers:α-,β-,γ-,andδ-tocopherolandα-,β-,γ-,andδ-tocotrienol, α-tocopherol being the most bioactive for humans. It is also considered to be the major membrane bound antioxidant used by the cell (Dávila et al., 2014). The main function of vitamin E is to protect against lipid peroxidation by reacting with lipid radicals produced in the peroxidation chain reaction (Dávila et al., 2014). During this antioxidant reaction, α-tocopherol radical is obtained and can be reduced to its original form (α-tocopherol) by vitamin C.

Flavonoids are metabolites with variable phenolic structures that can be found in different sources such as fruit, vegetables, grains, bark, roots, stems, flowers, tea, and wine. The antioxidant effect of flavonoids lays over its ability of scavenging the oxygen-derived free radicals as well as its antiinflammatory, antiallergic, antiviral, and anticarcinogenic properties (Dávila et al., 2014).

Carotenoids have several biological activities to help promote health and can be synthetized by plants and microorganisms. Studies have demonstrated that these pigments are involved in the scavenging of two of the ROS: molecular oxygen (O_2) and peroxyl radicals

(Dávila et al., 2014). Beneficial effects of carotenoids on health have been related to the decreasing the risk of certain cancers, cardiovascular diseases, and disorders related to the eyes (Dávila et al., 2014).

Antioxidants are always present in fruits as very complex mixtures and its activity is attributed to a group of compounds rather than one single compound. Therefore, knowing the composition and characterization (antioxidant capacity and total polyphenolic compounds) is important to generate agroindustrial uses. The overall nutritional value depends on the specific plant genotype and interactions of cultivation conditions; Table 5.2 summarizes the polyphenolic concentration and antioxidant activity contents of different tropical fruits.

Table 5.2 Polyphenolic Compounds and Antioxidant Capacity Contents of Tropical Fruits

Fruit	Polyphenolic compounds (mg GAE 100 g^{-1} fw)	Antioxidant capacity (μmol Trolox g^{-1} sample FW)	Country	References
Tamarillo pulp	78 ± 2	2.3 ± 0.1	Ecuador	Vasco et al. (2009)
Tamarillo peel	387 ± 8	22 ± 4		Vasco et al. (2009))
Tamarillo seed	94 ± 1	3.8 ± 0.6		Vasco et al. (2009)
Naranjilla pulp	58.3 ± 2.39	12.2 ± 0.85	Colombia	Acosta et al. (2009)
Naranjilla peel	83.6 ± 0.64	21.1 ± 0.23		Acosta et al. (2009)
Naranjilla pulp	48 ± 3	NR	Costa Rica	Acosta et al. (2009)
Naranjilla peel	91 ± 17	14.02	Ecuador	Acosta et al. (2009)
Goldenberry fruit	40.45 ± 0.93	2.11 ± 0.09	Colombia	Restrepo Duque (2008)
Goldenberry fruit	39.15 ± 5.43	1.93 ± 0.30	Colombia	Botero Echeverri (2008)
Goldenberry peel	154 ± 3	NR	Peru	Repo-de-Carrasco and Encina Zelada (2008)
Araza frozen pulp	64 ± 3	3.7 ± 0.1	Brazil	Genovese et al. (2008)
Araza fruit	129 ± 9	4.1 ± 0.2	Brazil	Genovese et al. (2008)
Araza fruit	111 ± 3.64	20.2 ± 2.44	Colombia	Contreras-Calderón et al. (2011)
Araza seed	1624 ± 44.9	440 ± 7.77	Colombia	Contreras-Calderón et al. (2011)
Cupuazu fruit	4.03 ± 0.57	9.59 ± 0.25	Colombia	Contreras-Calderón et al., (2011)
Cupuazu peel	497 ± 17.8	145 ± 0.09	Colombia	Contreras-Calderón et al. (2011)

Table 5.2 Polyphenolic Compounds and Antioxidant Capacity Contents of Tropical Fruits—cont'd

Fruit	Polyphenolic compounds (mg GAE 100 g^{-1} fw)	Antioxidant capacity (μmol Trolox g^{-1} sample FW)	Country	References
Cupuazu peel	252 ± 28.7	65.3 ± 1.00	Colombia	Con treras-Calderón et al. (2011)
Algarrobo peel	1712 ± 42.5	428 ± 9.38	Colombia	Contreras-Calderón et al. (2011)
Coastal sapote peel	1488 ± 20.1	377 ± 8.06	Colombia	Contreras-Calderón et al. (2011)
Buriti	378.07 ± 3.12	NR		Koolen et al. (2013)
Gabiroba fruit	851.03 ± 40.79	8027.52 ± 378.63	Brazil	Malta et al. (2013)
Açaí	454 ± 44.6	15.1 ± 4.1	Brazil	Rufino et al. (2010)
Acerola	1063 ± 53.1	96.6 ± 6.1	Brazil	Rufino et al. (2010)
Camu camu	1320 ± 102	167 ± 11	Peru	Chirinos et al. (2010)
Borojo fruit	41.8 ± 1.54	6.29 ± 0.86	Colombia	Contreras-Calderón et al. (2011)

GAE, Gallic acid equivalent.

5.2.2 Potential of Fruits for Preparing Beverages

Currently the world trade of fresh fruits is earning approximately 20 billions dollars every year (Almeida et al., 2011). In addition, production of functional products has been promoted by the demand in the market by sustained product rich in natural compounds. The United States is the largest importer of fresh tropical fruits followed by the European Union, Japan, and China. In the case of functional beverages, its market represents the largest and fastest growing segment of the functional foods sector, with an annual growing rate of almost 20% in the United States (Shahidi and Alasalvar, 2016). Besides, production and consumption of fruit juices, beverages and hot drinks have gained much importance since they are inversely associated with morbidity and mortality from degenerative diseases.

Currently several researchers have dedicated their efforts to study the nutritional value of different fruit beverages rich in antioxidant compounds. For instance, Carlsen et al. (2010) studied 283 beverages, including fruit-based products (see Table 5.3). According to this study, red wine presented the highest antioxidant contents. Some fruits currently used in commercial beverages, due to the attractive antioxidant

Table 5.3 Antioxidant Capacity of Different Fruit Beverages

Fruit Beverage	Antioxidant Content (nmol/100 g)
Apple juice	0.27
Cranberry juice	0.92
Grape juice	1.20
Orange juice	0.64
Pomegranate juice	2.10
Tomato juice	0.48
Red wine	2.50

Data from Carlsen MH, et al. The total antioxidant content of more than 3100 foods, beverages, spices, herbs and supplements used worldwide. Nutr. J. 9:3–3, 2010.

content, include cranberry, pomegranate, black currant, acai, guarana, mango, bilberries, grapes, cherries, kiwi, strawberries, feijoa, peach, watermelon, sapodilla, orange, and plums (Corbo et al., 2014).

Different studies have established that the consumption of fruits and vegetables are beneficial to human health such as reducing cardiovascular and coronary heart diseases (Franzini et al., 2012; Wang et al., 2011; Egan et al., 2001), diabetes (McCune and Johns, 2002), inflammatory processes (Wu et al., 2006; Gene et al., 1992), neurodegenerative diseases (ArunaDevi et al., 2010; Kris-Etherton et al., 2002), and cancer (Kris-Etherton et al., 2002; Collins, 2005; de Mesquita et al., 2009; Forster et al., 2013; Habib et al., 2013; Tsai et al., 2013; Zhang et al., 2013), among others. The constituents responsible for these protective effects are bioactive compounds known as antioxidants. An antioxidant is a metabolite capable of preventing, reducing, or delaying the effect of the oxidative stress of target biomolecules. The oxidative stress is considered a consequence of an insufficiency of the antioxidant defense system and/or the overproduction of free radicals (Dávila et al., 2014). A free radical is defined as molecule with one or more unpaired electron in its outer shell. The free radicals can be derived from the essential metabolic processes in the human body as well as from external exposure (Lobo et al., 2010). The most studied free radicals are those derived from molecular oxygen and nitrogen (ROS and RNS, respectively). The ROS includes oxygen radicals [e.g., superoxide anion radicals (O_2^-) and hydroxyl radicals (OH^-)] and some nonradicals which are oxidizing agents and/or can be converted to free radicals (e.g., H_2O_2 and O_2) (Dávila et al., 2014). The ROS and RNS are generally the result of radiation by UV light, metal-catalyzed reactions,

Table 5.4 Physicochemical Composition of Andes Berry (Dávila et al., 2014)

Feature	Value	Feature	Value
Physicochemical characterization		**Total phenolic, vitamin C, and anthocyanin content (mg/100 g FW)**	
Ash (%)	0.29	Total phenolics	499.0
Moisture (%)	90.88	Antioxidant capacity	71.0
pH	3.42	Vitamin C	21.0
TSS (°Brix)	7.60	Anthocyanins	125.6
TA (% citric acid)	1.26		
Carbohydrates (%)	5.04		
Total soluble sugars (g/100 g FW)		**Flavonoids content (mg/100 g FW)**	
Glucose	2.44	Quercetin	19.0
Fructose	1.99	Cyanidin	142.0
Total	4.35	Epicatechin	52.0
		Total	213.0

atmospheric pollutants, neutrophils, and macrophages during inflammation and mitochondria-catalyzed electron transportation reactions, among other causes (Dávila et al., 2014).

5.2.2.1 Andes Berry (Rubus glaucus Benth)

Andes berry is a perennial climbing shrub from the *Rosaceae* family. The fruit is a berry ellipsoid with a size between 15 and 25 mm and with a weight of 3–5 g approximately. This fruit is rich in vitamin C, calcium, and phosphorus and used commonly for juices, nectars, jams, jellies, ice cream, pastries, and confectionery. Two annual productions are there. Several studies have demonstrated the presence of antioxidants in Andes berry (Cerón et al., 2012; Hassimotto et al., 2008; Dávila et al., 2017). The chemical composition of the blackberry fruit is summarized in Table 5.4.

5.3 Industrial Production of Beverages

5.3.1 State of the Art about Beverage Production

Scientific studies about fruit-based beverages have increased in the last years, as illustrated in Table 5.5, due to the increasing interest in healthy food consumption around the world. There is a wide range

Table 5.5 Active Compounds Present in Fruit-Based Beverages

Fruit-Based Beverage	Active Compounds
Fortified-strawberry beverage	Polyphenols
Grape-based fermented beverage	Polyphenols
Fortified blackcurrant juice	Polyphenols
Fortified fruit juice	Antioxidants
Pomegranate fermented juice	Phenolic compounds
Apple-based beverage	Secoiridoid glycosides

Data from Corbo MR et al.: Functional beverages: the emerging side of functional foods. Compr. Rev. Food Sci. Food Saf.:13(6): 1192–1206, 2014.

of functional beverage in the market such as beverages based on dairy as well as beverages with probiotics and minerals/ω-3, vegetable and fruit beverages, and sports and energy drinks (Corbo et al., 2014). The most typical beverages based on dairy drinks are fresh milk, fermented milk, and yogurt. In the case of fruits, the most used are cranberry, blueberry, black currant, acai, acerola, guarana, mango, bilberries, grapes, cherries, kiwi, strawberries, feijoa, peach, watermelon, sapodilla, orange, and plums. On the other hand, sport drinks are flavored beverages to be consumed before or during exercise. This chapter is focused on the production of fruit-based beverages with antioxidant capacity.

There are different processes to obtain functional beverages from fruits and can be divided into five groups: (1) exploration of functionality of microorganism, (2) production of new functional beverages, (3) use of prebiotics and synbiotics, (4) use of natural ingredients for beverages, and (5) use as functional ingredients, by-products based on fruits (Corbo et al., 2014). This chapter is focused on the last two methods, in which fruits and its by-products are used as functional ingredients for producing beverages.

5.3.2 Supercritical CO_2 Extraction of Valuable Compounds

Different extraction technologies have been evaluated for performing the extraction of antioxidants from plants. Although, in general four main steps are involved in the antioxidant production process: pretreatment, extraction, concentration, and stabilization. In the pretreatment stage, the feedstock is dried allowing a rapid cell wall

breakdown during the subsequent milling and homogenization processes and minimizing the enzymatic degradation of the fruits and the antioxidant compounds (Dávila et al., 2014). The most used technologies in the pretreatment stage for the extraction of antioxidants are air drying, freeze drying or lyophilization, and vacuum drying (Dávila et al., 2014).

In the second stage, the extraction of antioxidant-rich extracts is carried out. This process have been performed through different extraction technologies such as conventional solvent extraction (CSE), supercritical fluids (SFE), and ultrasonic- and microwave-assisted extraction (MAE) (Dávila et al., 2014).

In the concentration stage the most used technologies are the vacuum distillation (Chumsri et al., 2008), ultrafiltration, and nanofiltration (Dávila et al., 2014). In the concentration process, low oxygen presence, low temperatures and low residence time are required in order to preserve the high quality of the extract.

Since the antioxidants extracted from fruits are sensitive to many environmental conditions such as pH, light exposure, temperature, and water activity (Dávila et al., 2014), the fourth stage involves the stabilization of the concentrated extracts. The microencapsulation is the most used technology for performing the stabilization of the antioxidants leading to preservation their effectiveness (Dávila et al., 2014).

5.3.2.1 Supercritical Extraction of Antioxidants

In the supercritical extraction process, a solvent at supercritical conditions is used to separate the extractant from the matrix, which is usually solid but that can be liquid or even viscous. There is a possibility of modifying the selectivity of the process when the density of the fluid is changed, which is the main advantage of the supercritical extraction process (Dávila et al., 2014). A general flow diagram of a supercritical extraction process is illustrated in Fig. 5.1.

In the supercritical extraction process, the carbon dioxide is first subcooled in order to guarantee the liquid phase before entering into the pump to avoid cavitation. Then the pressurized CO_2 is heated above its critical temperature, while the extractor is maintained at the operating temperature through electricity or hot water. The CO_2, at supercritical conditions, flows through the feedstock into the extractor extracting its soluble compounds. Then the solvent together with the extract leaves the extractor, which is at the operating pressure, and goes to the separator (at atmospheric pressure). In the separation vessel due to the depressurization the extract is precipitated.

The supercritical fluid extraction using CO_2 as solvent has been widely used for extracting antioxidants from tropical fruits such as blackberry (Cerón et al., 2012), citrus (Moncada et al., 2013), zapote

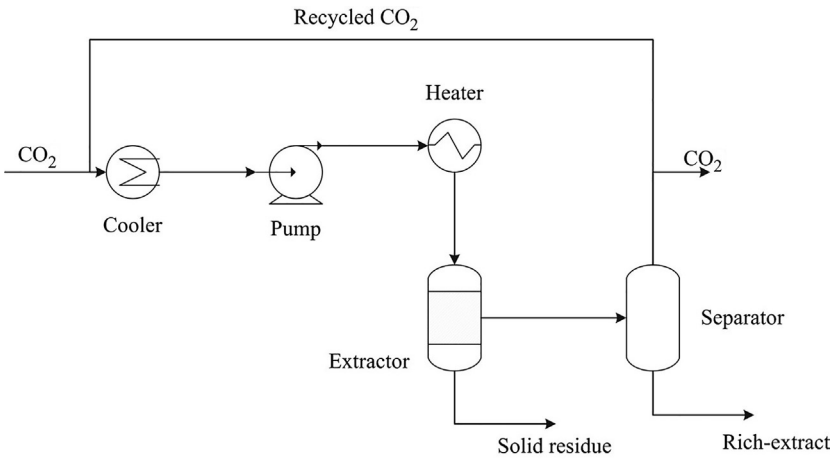

Fig. 5.1 Simplified flow diagram of the supercritical fluid extraction with CO_2 (Dávila et al., 2014).

(Cerón et al., 2014), tamarillo (Cerón, 2013), goldenberry (Cerón, 2013), and Andes berry (Dávila et al., 2014; Dávila et al., 2017).

5.4 Supercritical CO_2 Extraction for Phenolic Compound-Rich Extracts

5.4.1 Supercritical CO_2 Extraction of Valuable Compounds From Andes Berry Residues

The extraction of supercritical fluid has been considered a green method due to a substitute with advantageous results with respect to a traditional extraction. The low viscosity and the higher diffusion coefficient allow reaching a great thermal compressibility. This process involves a simple separation, changing the temperature or pressure, which generates a dissolution capacity similar to a liquid, which has an effect of a solvent with high power (Sanchez et al., 2017). This kind of process combines advantages of distillation and extraction with liquids since it works at low temperatures, in this case a process at low temperatures and pressures carried out with CO_2 will be analyzed (Sanchez et al., 2017; Brunner, 2005).

The CO_2 improves the condition of the extraction since the critical point is reached at low temperatures and pressures (Sahena et al., 2009; Machado et al., 2015). The CO_2 is an ideal solvent due to the low viscosity and high density that allows a better mass transfer in supercritical conditions. This solvent is nontoxic and nonflammable; besides, the obtained extracts have a higher purity in comparison

with other extraction methods such as solvent extraction techniques. Because CO_2 is a nonpolar molecule, it is necessary to use a cosolvent to increase the solubility of polar components to obtain high yields of anthocyanins. In this case the water-ethanol mixture helps in the production of upper extracts with a great ranking of purity, improving the polarity of the supercritical CO_2 which allows to dissolve polar compounds (Domínguez and Parzanese, 2005; Zulkafli et al., 2014a).

This technique uses supercritical conditions to modify the solubility between target compounds and the solvent by the fluid density. CO_2 is accentuated as the first component subcooled in all the process to make it sure that it stay in liquid phase and avoid a cavitation. Thus, CO_2 must be pressurized to a temperature near or above the critical temperature, thus, supercritical conditions should be maintained in order to allow a flow through the soluble components contained in the vegetable matrix (Brunner, 2005; Sahena et al., 2009). Then, the solvent comes out with the extract at a low pressure through the separator at an atmospheric pressure. Thus, the precipitated extract is obtained in a separator vessel and at the same time, CO_2 is cooled to −25°C to have a liquid phase. Theoretically, the pumps maintain an approximate pressure of 200 bar looking for the supercritical conditions to enter the extraction; the cosolvent is added to increase the yield in the extraction, thus improving the anthocyanins content in the final extracts (Cerón et al., 2012).

The company Frugy S.A gave samples of Andes berry residues (Manizales, Colombia). These samples were dried by a conventional method (Chemical Industries FIQ LTDA, Colombia y Memmert, 854 Schwabach, Germany) to get a regular weight between every sample at a regular temperature of 35°C that avoid the degradation of antioxidants and phenolic compounds. Then, the sample was processed by a hammer mill (Raymond No. 82, China) and sifted by a series of Tyler-type sifts (ASTM E-11) obtaining an average of 756 μm diameter particle. The reagents used were CO_2 at 99.9% (Linde Colombia S.A.), ethanol at 99.8% (Merck, Germany), which was acidified with distilled water and citric acid to have a pH of 2.0 obtaining a mixture of water-ethanol at 57%v/v, chloride acid at 37% (Alquera), sodium acetate, and potassium chloride (Bioquigen) (Garzón, 2008).

The supercritical fluid extraction equipment is located at the high pressures in the laboratory of the university Jorge Tadeo Lozano, Bogota Colombia. This system has a pressurization pump (piston bomb-Milroyal B Pump), a tank extractor (1.3 L), and a separator vessel (1 L) with a capacity to operate at a pressure of 4500 psi to obtain all the extracts. Besides, this system has an automatic control (SuperView 2.9.9) where it is possible to verify the temperatures and pressures of the extractor and separator vessel (Acosta, 2017; Santacruz, 2016).

An amount of 30 g of samples were used and placed in the 550 mL extractor while the cosolvent (acidified ethanol) were added in the extraction chamber and both the pressure and the temperature were fixed at the desired supercritical conditions. In parallel, the refrigerant (propilenglicol) was recirculated using a chiller at a temperature of approximately −20°C to allow CO_2 (99.9% of purity) to remain in a liquid state (Acosta, 2017). There is necessary for the system, 1 hour to stabilize thermally the system and starting the pressurizing to the desired pressure. The extractions have been carried out in static mode, which means the batches of approximately 15 min was followed for depressurization and then pressurized again to the desired pressure. Once the operating conditions have been reached, the extraction time is calculated, waiting 15 min to start another discharge, which is necessary to renovate the CO_2, thus, improving the sample-mass transfer (Santacruz, 2016).

After, the equipment is depressurized conducting the CO_2 and the sample toward the tank separation. As the CO_2 passes through the valve, it expands and losses all its proprieties as solvent, therefore, the extract (rich in anthocyanins) tends to precipitate, facilitating the recovery process (Acosta, 2017; Santacruz, 2016).

After the depressurization of CO_2, the solute compounds (anthocyanins) are obtained free of solvent and because of the photosensitive character of the extracts, the samples were collected and protected from light and stored at −10°C to further analyze the physicochemical properties (del P Garcia-Mendoza et al., 2017; De Cássia Rodrigues Batista et al., 2015) and to avoid any type of degradation. The process flow diagram of the supercritical extraction is depicted in Fig. 5.2.

5.4.2 Anthocyanins Analysis (Measurement and Analysis of the Anthocyanins Content in the Obtained Extracts)

For the determination of anthocyanins, the colorimetric method was used. This method is defined by Giusti and Wrolstad (2001). Two types of buffer were required for the extraction solutions: sodium acetate with a pH of 4.5 and potassium chloride with a pH of 1.0 (Giusti and Wrolstad, 2001). The anthocyanins concentration is expressed in mg/L of extract determined as cyanidin 3-glucoside because this type of anthocyanin is predominant in this fruit. The calculation of anthocyanins concentrations have been carried out by Acosta (2017) where the absorbance method was used with a wavelength between 300 and 700 nm for each of the buffers. The concentration is expressed according to Eq. (5.1) where Abs is the absorbance calculated in Eq. (5.2),

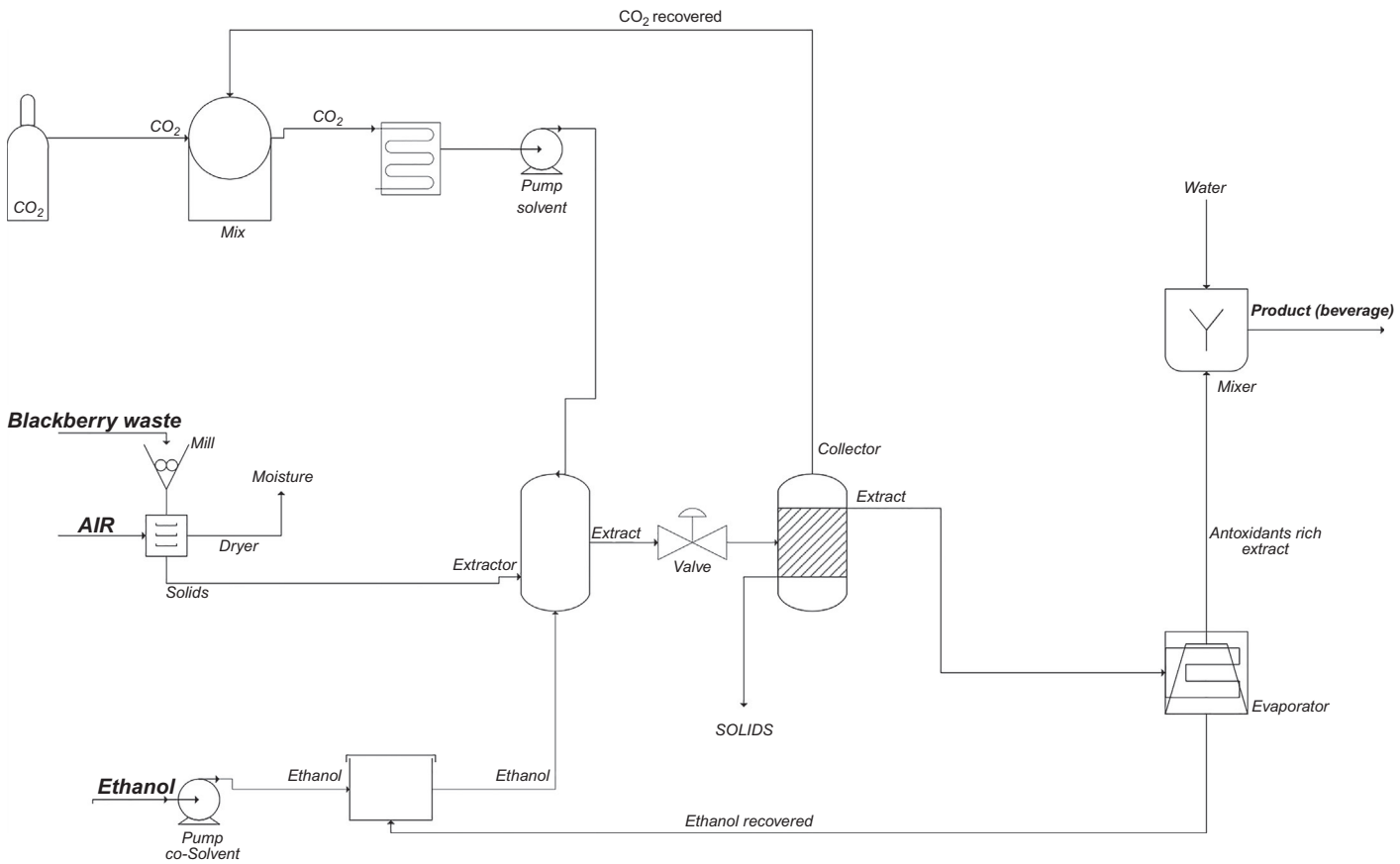

Fig. 5.2 Process flow diagram for the supercritical extraction.

MW is the molecular weight (449.2 g/mol), and ε is the molar extinction coefficient (26,900 $L \times mol^{-1} \times cm^{-1}$) (Giusti and Wrolstad, 2001). The anthocyanins concentration for all the samples is presented in Table 5.6.

$$[\text{Antocianinas}] = \frac{\text{Abs} \times \text{MW} \times \text{DF} \times 1000}{\mu \times 1} \quad 5.1$$

$$\text{Abs} = \left(A_{515\,nm} - A_{700\,nm}\right)_{pH1} - \left(A_{515\,nm} - A_{700\,nm}\right)_{pH4.5} \quad 5.2$$

The maximum concentration of anthocyanins (20.74 mg/L) corresponds to a temperature of 40°C and a pressure of 200 bar. This value is in agreement with other authors, who have found the value of 68.42 mg/L (Acosta, 2017). Other authors have found that low pressures can enhance the extraction yield of anthocyanins for it generates low CO_2 flows, which in turn can increase the mass transfer from vegetable matrix to the solvent (Porto and Natolino, 2017).

The relation between the temperature and the cosolvent/sample established the postulation that at high temperature properties such as pigmentation is lost due to loss of the glycosylated sugar, which is in the third position in the molecule and produces colorless chaconnes, therefore, the yield of the extraction should be carried out at low temperatures (Gorriti Gutierrez et al., 2009; Porto and Natolino, 2017; Colchao et al., 2011).

Table 5.6 Experimental Results of Anthocyanins Concentration

Factor			Results
Pressure	Temperature	Cosolvent/Sample	Anthocyanins (mg/L)
1	−1	−1	12.847
1	1	−1	7.586
1	−1	1	12.919
1	1	1	6.278
−1	−1	−1	20.740
−1	1	−1	15.329
−1	−1	1	16.810
−1	1	1	15.580
0	0	0	12.874
0	0	0	15.607

5.4.3 Total Phenolic Compound Analysis (Measurement and Analysis of the Total Phenolic Compounds in the Obtained Extracts)

The TPCs turn out to be of a great importance in the secondary metabolites of vegetables where they have multiple functions. These compounds are considered antinutritive substances with the effect of digestibility of proteins (Cueva et al., 2010; García et al., 2015). The amount of polyphenols varies according to the species, sun exposure, culture conditions, degree of maturity, storage that makes them present with different rings of benzenes or hydroxyl groups in the structure (García et al., 2015).

These molecules can be combined with sugars such as galactose, xylose, or galacturonic acids. These type of compounds can be responsible for sensory properties.

The method used in this process was Folin-Ciocalteu that standout for the capacity to react with any type of phenol. The phenolic compounds react with the Folin reagent which is formed by sodium tungstate and sodium molybdate in acid medium. Thus, this reaction produces a yellow complex that can be reduced with the phenolic compounds and identified with a spectrophotometric method at a wavelength of 765 nm (Cueva et al., 2010).

Thus, the method proposed by Singleton and Rossi Jr. (1965) was used, with some modifications suggested by Cicco et al. (2009), where it was necessary multiple dilutions were made (1:100, 1:50, 1:20, 1:10) due to the concentration of phenols in the samples and was not allowed to the ranges of the spectrophotometer as neither perform according to the Lambert and Beer laws that has to be carried out when using a spectrophotometric method.

For the analysis of TPCs, the samples obtained from supercritical fluid extraction method were protected from light and stored at −20°C. Then, each sample was taken to a centrifuge for a minimal time of 15 min and 4000 rpm for the particles to settle in suspension. Every reaction was carried out in Eppendorf tubes of 2 mL each one performing the following steps:

The standard solution to this method was gallic acid, which has to be diluted to values of 0–500 mg GA/L which is shown in calibration curve (Fig. 5.3). It is used to realize the total concentration of phenols in the samples analyzed. Then, 10 μL of the diluted sample was taken and added to 1600 μL of distilled water finally completing with 100 μL of the Folin reagent (1 N). These samples were shaken in a vortex and kept at room temperature for approximately 8 min. After that, 200 μL of sodium carbonate (20% W/V) was shaken and placed in the dark for 60 min. At the end of this time, the absorbance reading was performed on a UV/VIS spectrophotometer (Thermo Fisher Scientific,

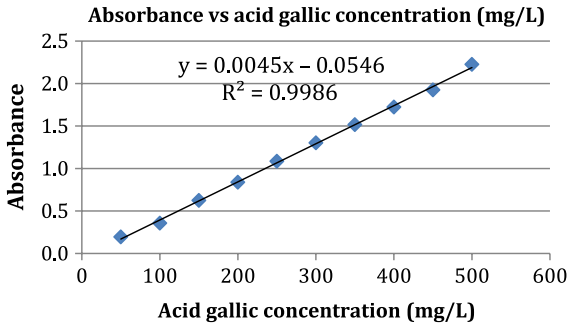

Fig. 5.3 Calibration curve for total phenolic compound measurements.

United States) at a wavelength of 765 nm. Phenolic compound content is expressed as milligrams of gallic acid equivalent (GAE) per dry mass unit (mg GAE/g). To know the phenolic content, it was determined in units of milligrams of equivalent gallic acid for each unit of dry mass (mg AGE/g).

The contents of TPCs are illustrated in Table 5.7. The higher concentration of TPC was obtained at 4200 psi and cosolvent to solid ratio of 180 mL. According to the results, at higher temperatures the TPC have to be solubilized because high temperatures increase the volatility of the solutes (TPC) present in the vegetable matrix, which in turn, increases the solubility of the solvent. The high pressures (2400 and 4200 psi) used allow to increase the diffusivity in the solvent medium and thus, improving the mass transfer since the cell walls and the

Table 5.7 Phenolic Compounds Results

Cosolvent: Solid Relation (mL)	Pressure (Psia)	Temperature (°C)	Phenolic Content (mg A.G.E/g de pulp)
60	4200	40	11.19
60	4200	60	19.75
180	4200	40	21.93
180	4200	60	24.16
60	2900	40	4.15
60	2900	60	4.93
180	2900	40	4.49
120	3550	50	12.44
180	2900	60	7.33
120	3550	50	13.45

molecules bond become weak due to the desorption process of the solvent next to the solute tend to magnify.

The yield of the extraction depends strongly on the combination of variables such as temperature, pressure, and cosolvent charge, to obtain the best results for solubility and mass transfer (Espinosa-Pardo et al., 2017). Thus, from this research, it is recommended that intermediate pressures as well as the use of a cosolvent for is necessary to obtain higher yields. In spite of the fact that high pressures can improve the mass transfer due to best penetration in the plant matrix and their pores, for generating a better drag of the solutes from the inside of the matrix, it is necessary to have optimal conditions of all variables that generate maximum solubilization of solutes, and thus, attain higher yields (del P Garcia-Mendoza et al., 2017; Mustafa and Turner, 2011).

5.4.4 Total Antioxidant Activity Analysis (Measurement and Analysis of the Total Antioxidant Activity in the Obtained Extracts)

The DPPH technique (2,2-diphenyl-1-picrylhydracil-Sigma Aldrich) proposed by Brand-Williams et al. (1995) was used in this research. Diluted samples in ethanol (1:10, 1:100, 1:1000) were shaken on a vortex using a 60-μM DPPH and taken to an ultrasound bath for 5 min at 40 kHz (Branson 3800, United States). It is important to keep the samples in the dark to protect the samples from sunlight. Afterwards, 75 μL of diluted samples were taken and mixed with 1425 μL of the solution of DPPH previously prepared (saved and kept in the dark for 60 min), which allowed a reading at 515 nm in Evolution 300 UV/VIS spectrophotometer (Thermo Fisher Scientific, United States). To know the inhibition percent Eq. (5.3) was used where the Abs control is the absorbance of the DPPH solution (60 μM) and Abs sample is the absorbance of the sample plus the DPPH solution (60 μM).

$$\%\text{Inhibition} = \frac{\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}}}{\text{Abs}_{\text{Control}}} \times 100 \qquad 5.3$$

The berry has been identified by its antioxidant capacity, the ranges of antioxidant capacity of the extracts varied from 23.83 to 112.89 μmol of Trolox/g pulp that is derived using high pressure (4200 psia), high temperature (60°C), and 180 mL of cosolvent (ethanol). These conditions improve significantly the extraction yield of phenolic compounds using supercritical fluid extraction. Other authors have evaluated the antioxidant capacity of extracts of Andes berry using the DPPH method. Pasquel Reátegui et al. (2014) obtained 99.11 μmol of Trolox/g pulp. Because the final characteristics of the extracts obtained from the supercritical fluid extraction depend on the nature of

the raw material as well as the operational conditions (temperature, pressure, flows, cosolvent use, etc.), it is necessary to explore a wide range of the variables. Therefore, an optimal combination of these variables results in an economic process as well as in an improvement of the final characteristic of the extracts for human consumption.

Table 5.8 illustrates the antioxidant activities where the maximum value registered was 112.89 μmol of Trolox/g pulp that corresponds to the higher conditions of temperature and pressure. Therefore an increase in pressure is directly related to the solvation power of CO_2 and thus, high pressures are suggested to obtain extracts rich in phenolic compounds with high antioxidant capacities (Zulkafli et al., 2014b).

Fig. 5.4 depicts the relation between TPCs and antioxidant capacity of the extracts of Andes berry. According to these results, 81.66% of the antioxidant activity can be attributed to the phenolic compounds in the extracts. Similar results have been found for Andes berry extracts where 91% of the antioxidant activity is due to the phenolic compounds content (Cerón et al., 2012).

According to the results (Table 5.8), the antioxidant activity of the recovered extract increased as the temperature, pressure, and cosolvent ratio levels increase, similar to the behavior of the phenolic compounds content. The extracts with the highest phenolic compounds content also exhibited the maximum antioxidant activity, thus suggesting again that high pressures and temperatures should be used to obtain extracts with valuable characteristics. Besides, it became evident from the relation between TPCs and antioxidant capacity that the phenolic compounds including anthocyanins should be extracted

Table 5.8 Antioxidant Capacity Results

Cosolvent: Solid Relation (mL)	Pressure (Psia)	Temperature (°C)	Antioxidant Activity (μmol de Trolox/g Pulp)
60	4200	40	39.75
60	4200	60	56.17
180	4200	40	97.56
180	4200	60	112.89
60	2900	40	23.79
60	2900	60	28.62
180	2900	40	23.83
120	3550	50	31.79
180	2900	60	30.93
120	3550	50	36.88

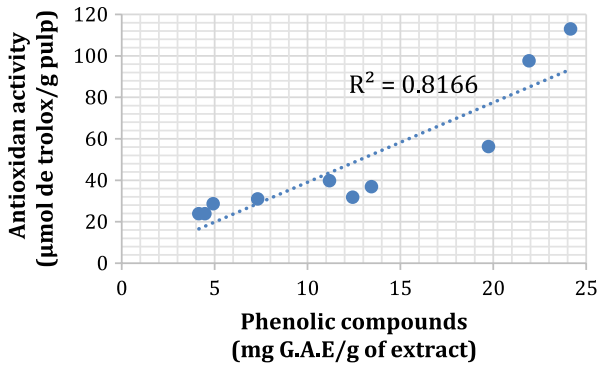

Fig. 5.4 Correlation between the phenolic compounds and the antioxidant activity.

because they are associated with the total antioxidant activity of the extracts as other authors have also confirmed a correlation between antioxidant activity and phenolic contents of 96.50% (Da Fonseca Machado et al., 2015).

5.5 Conclusions

Beverage preparation can be carried out by several techniques, however, supercritical fluids extraction is a promising technology to take advantages of agroindustrial wastes since it allows to obtain phenolic-rich extracts that also contains anthocyanins and important antioxidant activity that suggest applications in different fields such as pharmaceutical, food, and cosmetic industries. As it was shown here, extracts obtained from supercritical fluid extraction technique have valuable characteristics that can be used for beverage preparation and should be refined according to the level of concentration and final presentation. Thus, processes such as evaporation, microfiltration, and nanofiltration can be utilized for adding value to these extracts and thus obtaining beverages with valuable applications.

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Further Reading

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