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Potential for Sustainable Production of Natural Colorants in the Tropical Forest: A Biorefinery Case of Annatto Seeds

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Abstract: Tropical forests are a source of several high-value products that provide livelihood to small communities in different regions. Exotic fruits such as annatto are feedstock sources containing bioactive compounds with important applications in the food industry. Nevertheless, the integral use of annatto for community improvement and the crop's contribution to carbon sequestration in tropical forests have not been analyzed. This paper aims to demonstrate the economic and environmental performance of small-scale alternatives to obtain natural colorants using annatto seed. The extraction of natural colorants (bixin and norbixin) was analyzed using ethanol and NaOH as solvents. The experimental results were used to simulate two scenarios. Scenario one involved bixin production, and scenario two comprised bixin and norbixin production. The economic and environmental assessments were performed considering the life cycle assessment (LCA) methodology based on a Colombian context. The best extraction yield was 72.65 mg g^{-1} for bixin and 193.82 mg g^{-1} for norbixin. From a simulation perspective, scenario two showed the best economic performance since a payback period of 3.1 years was obtained. The LCA showed a high CO_2 sequestration potential (6.5 kg CO_2 eq kg^{-1} seed) of the annatto crop. Moreover, the solvents used during the colorant extraction proved to be the most environmentally representative. Nevertheless, the CO_2 sequestration of the crop continues to exceed the emissions generated by the process. This work demonstrates that the annatto is an alternative for small communities to reach equilibrium between the economic and environmental of the tropical forest.

Keywords: tropical forests; small communities; annatto; natural colorants; CO_2 capture

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1. Introduction

Biodiversity represents a very important role in maintaining and improving the quality of life for human beings in the world. It represents a large part of the renewable natural capital upon which livelihoods and development are based [1]. However, the constant exploitation of resources by humans has caused serious decreases in the abundance and distribution of species, and ecological functions within ecosystems [2]. Therefore, finding alternatives to reduce or control biodiversity losses is essential.

Biodiversity integration into sustainable processes has become a global challenge that has been reinforced by various strategies. The Strategic Plan for Biodiversity 2011–2020 from the Convention on Biological Diversity (CBD) has been successfully implemented in countries such as Costa Rica [3]. However, several barriers are evident, such as short-term economic gains in the primary production sector, fragmented decision-making, and limited communication with stakeholders [4]. Colombia has been categorized as a megadiverse country with approximately 10% of the planet's biodiversity. Among the sectors with the greatest participation is the biogeographic Chocó [5]. The biogeographic Chocó is a neotropical (humid) biogeographic region located in the eastern region of Panama, passing through the Pacific coast of Colombia and Ecuador. The department of Chocó hosts almost 3% of plant diversity, including endemic species, tropical forests, and exotic crops [6]. Furthermore, tropical forests have been shown to be essential ecosystems for carbon balance and climate change mitigation [7]. Indeed, Colombia's bioeconomy strategy for 2020–2030 prioritizes the biogeographic Chocó as one of the main regions for developing high-value-added products and services from the plant species that are characteristic of the region [8]. The challenge consists of balancing the environmental and economic sectors and associating with small communities within the sectors to obtain products of native origin but a low level of development in terms of valorization [9]. The annatto crop is an exotic crop found in tropical forests such as the Chocó region.

The extract of annatto (*Bixa orellana*) colorant is characterized as a biodegradable and non-toxic colorant for humans [10]. This characteristic makes annatto a possible substitute for synthetic colorants [11]. According to the United States Agency for International Development (USAID), most of the annatto production is identified in Latin America, with a production of more than 85% [12]. In Colombia, the department of Chocó represents approximately 80% of national production. The industrial application of annatto focuses on food additives, using seed colorants mainly constituted by carotenoids [13]. According to Natividad et al., the annatto seeds showed between four and nine times more total carotenoids (931 μg of carotenoid per gram of seed) than carrots, tomatoes, and corn, mostly represented by bixin [14]. In annatto seeds, more than 80% of the total colorant corresponds to bixin, a liposoluble orange-yellow apocarotenoid of the external seed layer. On the other hand, norbixin is a dark brown water-soluble carotenoid present inside the seed. Bixin is the most commercialized compound worldwide; however, norbixin has received considerable attention at the research and industrial level due to its solvating capacity [15].

Several methods can extract annatto colorant. The traditional method is the immersion of the seed in water. It can also be obtained from alkali compounds, mainly NaOH or KOH, achieving extraction yields of 7.6% [16]. However, this method does not allow for obtaining bixin and norbixin separately, and it is extensive compared to others. The use of solvents such as acetone, ethyl acetate, methanol, and ethanol has also been proposed since it is a faster, less expensive, and safer alternative process, achieving yields of 6.5–6.7%. However, additional costs may be incurred for solvent recovery [17]. Some techniques, such as microwave-assisted extraction, have also been studied to develop clean extraction technologies with environmental benefits ("green technologies"). This extraction method is simple, fast, and inexpensive, but can lead to the thermal degradation of carotenoids. Other methodologies, such as supercritical fluid extraction, are effective techniques; however, they require high operating costs [18].

Currently, the extraction of the colorants from the ecosystems of the biogeographic Chocó region is carried out based on traditional (ancestral) practices, without any technological development. However, at the research level, the technical extraction of colorants has been evaluated with different methods, solvents, and operating conditions. Nevertheless, these studies have not focused on dynamizing the value chain of annatto derivatives as a strategy for improving the region and small communities within the context of sustainability. On the other hand, several efforts have been made to know the contribution of tropical forests to carbon sequestration; however, these studies are unknown in areas such as the biogeographic region of Chocó. Therefore, this paper aims to demonstrate the sustainability of the economic and environmental dimensions (the social dimension does not merit additional studies since these crops become part of their own culture, i.e., they are ancestral) of small-scale production chains in the context of tropical forests, using the processing of annatto seeds in the biogeographic region of Chocó in the ecosystems of the Pacific coast of Colombia as a base case. Two scenarios were evaluated under a biorefinery scheme. Scenario one involved bixin production, and scenario two involved bixin and norbixin production. In addition, the environmental impact of the annatto crop was determined, and its contribution to carbon sequestration in tropical forests and feedstock processing was evaluated using life cycle assessment (LCA).

2. Materials and Methods

2.1. Raw Material

The annatto (*Bixa orellana L*) seeds were obtained from local communities in Unión Panamericana, Chocó—Colombia (5°14′38.4′′ N 76°39′33.3′′ W). Constant interaction and visits to the field were maintained with the communities and annatto growers to learn and understand their culture and habits concerning the economic activities characteristic of the region. The seeds were dried at 30°C until a moisture content close to 10% was obtained. After drying, the particle size of a portion of raw material was reduced in a blade mill and sieved until it was 0.4 mm. The rest was left as natural seed (5 mm). The samples were stored in hermetically sealed plastic bags for further use.

2.2. Raw Material Characterization

The physicochemical characterization of the seeds was categorized as proximate, lignocellulosic, and solid analysis. Furthermore, the total phenolic content, reducing sugars and antioxidant capacity, were analyzed from the extract content. All the characterizations were performed in triplicate, based on international standard methods.

2.2.1. Chemical Analysis

Moisture content was determined using a moisture balance at 105 °C. The mass difference determined the extract content after Soxhlet extraction, with water and ethanol as solvents based on the NREL/TP-510-42619 [19]. The chlorination method was used to estimate the holocellulose content [20]. The cellulose content was calculated using 17.5% NaOH, according to Machrafi (2012). The insoluble lignin was estimated as Klason lignin based on the NREL/TP-510-42618 [21]. The protein was calculated with the Kjeldahl method, using a factor conversion of 6.25 based on the NREL/TP-510-4262 [22]. Total pectin was extracted with concentrated sulfuric acid and continuous gentle stirring [23]. For protein quantification, the photometric galacturonic acid measurement was implemented via the carbazole method at 240 nm [23]. For holocellulose, cellulose, lignin, and protein determination, the sample must be on an extract-free basis.

2.2.2. Proximate Analysis

Volatile matter content was determined by mass difference using a muffle with a temperature of 950 °C for 7 min according to the ASTM D7582-15 [24]. The ash content was determined after slow heating at 500 °C, based on the NREL/TP-510-42622 [25]. The moisture content was quantified by mass difference using a preheated oven at 104–105 °C for 16 h. Finally, the fixed carbon content was determined by the difference between volatile matter, ash, and moisture (ASTM E870-82).

2.2.3. Solid Analysis

The total solids content was determined after sample heating at 105 °C for 6 h, according to the ASTM E1756-08 [26]. Then, the volatile solids content was determined, followed by the total solids content. Finally, the sample was calcined at 550 °C in a muffle for one hour according to the ASTM E1756-08.

2.2.4. Total Phenolic Content, Antioxidant Capacity, and Reducing Sugars

The total polyphenol content (TPC) of the Soxhlet extract was measured by the Singleton Folin–Ciocalteu colorimetric method with some modifications [26]. The antioxidant capacity was determined by inhibiting the radical DPPH (α, α-Diphenyl-β-picrylhydracil), using the method described by Marinova et al. [27]. The TPC and antioxidant capacity were carried out in duplicate. The reducing sugars were determined using the dinitro salicylic acid (DNS) methodology [28].

2.3. Bixin and Norbixin Extraction

The colorant extraction was performed using two particle sizes of annatto seeds: ground seed at 0.4 mm and natural seed (without milling). The operating conditions were selected based on the best extraction yields reported by Piedrahita et al. [29]. For bixin extraction, annatto seeds were mixed with ethanol (70% vol.) in a solid-to-liquid ratio of 1:25 at 70 °C. Thereafter, the exhausted seeds were used for norbixin extraction using NaOH 0.5 N at the same conditions as those in the bixin extraction. Both colorants were extracted at the continuous stirring of 200 rpm over 40 min. Moreover, ten extraction sequences were required for each colorant to ensure the near-complete extraction or colorant depletion, as reported elsewhere [30]. All extractions were performed in triplicate, and the extracts were stored in the dark at −20 °C.

The sample preparation and chromatographic quantification were carried out based on the methodology reported by Noppe et al. [31]. Stock standard solutions of 200 mg L^{-1} were prepared in ethanol. The samples and the calibration curves were dissolved in 0.1% acetic acid in acetonitrile/water (80:20 vol.) and were filtered on a 0.22 µm nylon [32]. The chromatographic separation was performed with an HPLC system (Shimadzu LC-2010AHT) equipped with a Kromasil C18 column (150 mm × 4.6 mm × 5 µm). The mobile phase consisted of a mixture of (A) acetonitrile and (B) aqueous formic acid (0.1% vol.) at 0.3 mL min^{-1}. The linear gradient method was: 80% A and 20% B initially, increasing to 95% A and 5% B over 10 min, increasing to 100% A over 5 min and holding it over 5 min, and finally, returning to the initial conditions of 80% A and 20% B and holding it for 10 min. The total run time was 30 min at 20 °C.

2.4. Process Simulation

Two scenarios were proposed based on the type of products from annatto. The production of annatto seeds in Chocó was estimated to be 5278 tons in 2019 [33]. Scenario 1 involves the bixin production (base case in Colombia), and scenario 2 involves the bixin and norbixin production. All scenarios were simulated in the Aspen Plus v9.0 software (Aspen Technology, Inc., Bedford, MA, USA). The activity coefficients of the liquid and vapor phases were calculated using the non-random two-liquid (NRTL) thermodynamic model and the Hayden–O'Connell equation of state.

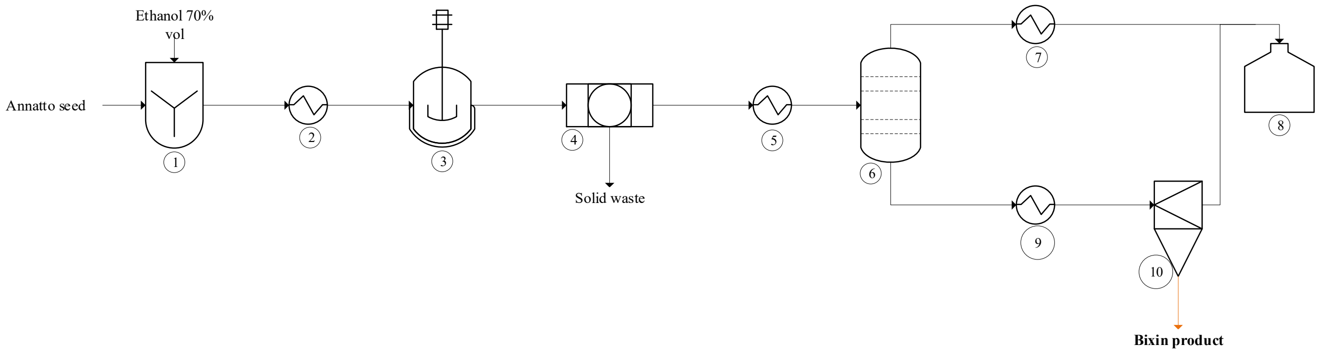
2.4.1. Process Description

Scenario 1

The raw material was dried and milled (depending on the experimental results). The bixin extraction was carried out using ethanol with a feed rate of 25 L per kilogram of seed at a temperature of 70 °C. Then, the bixin-rich extract was separated by filtration, and the remaining solid stream was disposed of as residue. Distillation technologies at 85 °C were implemented to separate bixin from the solvent [34]. Finally, solid bixin was produced with a crystallization stage. A representative process flowsheet is shown in Figure 1.

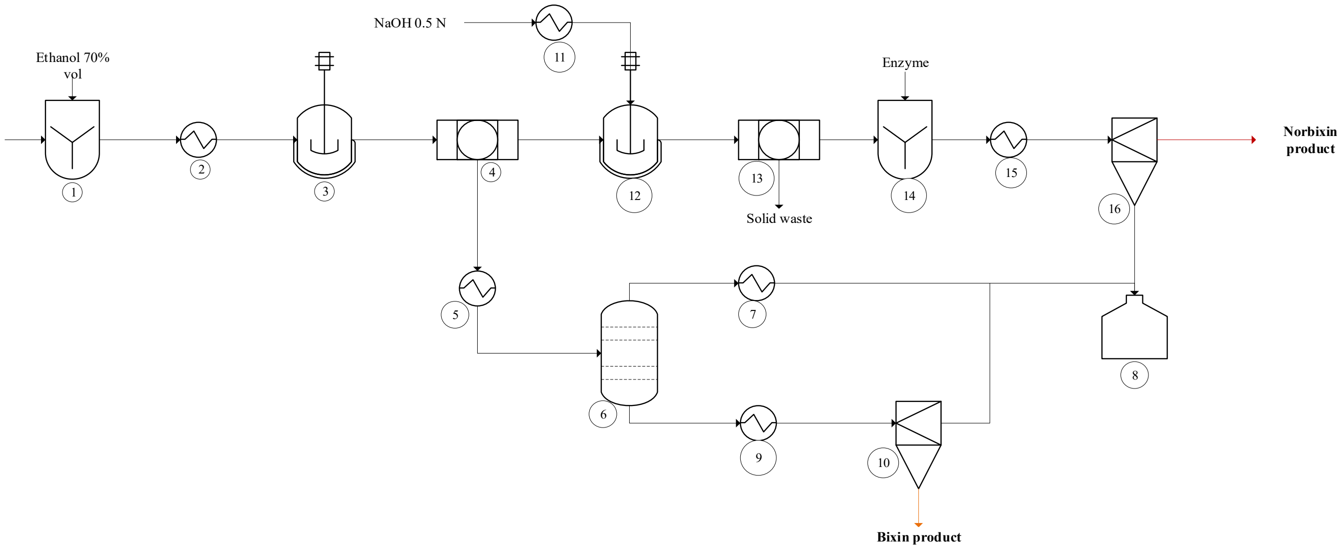
Scenario 2

For this scenario, the same operating conditions of scenario 1 for bixin extraction were considered. The solid phase (after bixin extraction) was mixed with NaOH 0.5 N in a solid:liquid ratio of 1:25 at 70 °C. Thereafter, the solution was filtered, the remaining solid stream was disposed of as residue, and the liquid phase (rich in norbixin) was mixed with Celluclast enzyme in a ratio of 0.2 g of enzymes per kilogram of liquor. According to Ruiz et al. [35], norbixin purification is performed by adding enzymes to precipitate the colorant. The remaining liquid after purification was disposed of as a residue, as shown in Figure 2.



Item	Equipment	Item	Equipment
1	Mixer	6	Distillation tower
2	Heat exchanger	7	Heat exchanger
3	Reactor for bixin extraction	8	Storage tank
4	Filter	9	Heat exchanger
5	Heat exchanger	10	Crystallizer

Figure 1. Schematic flowsheet of scenario 1.



Ítem	Equipment	Ítem	Equipment
1	Mixer	9	Heat exchanger
2	Heat exchanger	10	Crystallizer
3	Reactor for bixin extraction	11	Heat exchanger
4	Filter	12	Reactor for norbixin extraction
5	Heat exchanger	13	Filter
6	Distillation tower	14	Mixer
7	Heat exchanger	15	Heat exchanger
8	Storage tank	16	Crystallizer

Figure 2. Schematic flowsheet of scenario 2.

2.5. Technical and Economic Assessment

The economic analysis was performed using the commercial package of Aspen Process Economic Analyzer v9.0 (Aspen Technology, Inc., EE. UU.). The equipment sizing and capital cost were estimated based on the mass and energy balances of the simulations. The production cost was the sum of raw material, reagents, utilities (low and medium-pressure steam, cooling water, and electricity), maintenance, and operational and administrative costs. Likewise, the economic assessment was estimated in US dollars considering the linear depreciation method and a project lifetime of 20 years, using Colombian economic indexes of the Chocó region, such as an annual interest rate of 25%. Reagents and utility costs, as well as product prices, were taken from the literature, as shown in Table 1. The economic assessment also involves the production cost of the raw material based on the economic data of the annatto crop, such as chemical supplies (i.e., fertilizers and irrigation water), machinery that involves the use of fuels, and manual equipment, labor, and transportation. This economic analysis of the agronomic stage was performed until the first harvest of the annatto.

Table 1. Utilities and reagents cost, and product prices.

Item	Units	Value	Reference
Low-pressure steam	USD ton^{-1}	1.57	[36]
Medium-pressure steam	USD ton^{-1}	8.18	[36]
High-pressure steam	USD ton^{-1}	9.86	[36]
Electricity	kWh	0.1	[36]
Water	USD m^{-3}	0.74	[36]
NaOH	USD kg^{-1}	0.6	[37]
Celluclast	USD kg^{-1}	3.7	[38]
Ethanol	USD gallon^{-1}	2.05	[39]
Bixin	USD kg^{-1}	15.75	[40]
Norbixin	USD kg^{-1}	18.00	[41]

2.6. Environmental Assessment

The environmental assessment was performed according to the environmental life cycle assessment (LCA) approach and following the methodology proposed by ISO 14040:2006 [42], which includes four steps: (i) the definition of the objective and scope; (ii) inventory analysis; (iii) impact assessment; and (iv) the interpretation of the results. SimaPro v9.1 software (PRé Sustainability, The Netherlands) was used for the environmental assessment. In addition, the LCA was carried out quantitatively, by estimating representative indicators using the ReCiPe Midpoint method (hierarchical version H), the Ecoinvent database, and primary information provided by the small communities of the department of Chocó.

Carbon dioxide (CO_2) sequestration with the annatto crop was determined for a three-year plantation, considering the aerial biomass, belowground biomass, and soil organic carbon to determine its environmental contribution to the tropical forests of the region. To establish carbon sequestration, the aerial and belowground biomasses were determined using the indirect method through allometric equations applicable to the biomass of tropical forests in Colombia. Based on the forest characteristics of the biogeographic region of Chocó (tropical rainforest) and an average stem diameter of 10 cm (considering a three-year plantation), the allometric equation listed in Table 2 was used. For aerial biomass, the allometric equation proposed by Alvarez et al. was used [43]. The allometric equation proposed by Cairns et al. [44] was used for belowground biomass. Soil organic carbon was calculated according to the methodology of the IPCC 2006 and from soil survey data supplied by local growers. On the other hand, most studies on carbon storage in tropical forest biomass assume that the biomass of living trees contains approximately 50% carbon [45]; therefore, it is suggested to use the factor of 0.5 to transform biomass into carbon.

Table 2. Allometric equations for calculating carbon sequestration.

Item	Equation	Variables	Ref
Aerial biomass	$\ln(BA) = a + b \times \ln(D) + c \times (\ln(D))^2 + d \times (\ln(D))^3 + B1 \times \ln(\rho)$	BA = Aerial biomass D = Tree diameter ρ = Density a, b, c, d = Constants	[43]
Belowground biomass	$Br = e^{(-1.0587+0.8836\times Ln(BA))}$	BA = Aerial biomass Br = Biomass below ground	[44]
Soil organic carbon	$COS = \frac{SOC_{final} - SOC_{inicial}}{years}$ $COS_{final\ o\ inicial} = CO \times DA \times PM$	COS = Soil organic carbon CO = Organic carbon content DA = Density apparent PM = Depth of the sample	[45]

2.6.1. Goal and Scope Definition

The objective of the LCA is to determine the environmental impact of the previously mentioned scenarios. The specific objectives are: (i) to compare the previously mentioned scenarios and identify the process steps with the greatest contribution to environmental impact, (ii) to determine the environmental impact of the annatto crop and compare its impact when considering carbon sequestration for a three-year crop, and (iii) to analyze the environmental contribution of annatto crop to the tropical forest characteristic of the region. The LCA follows a cradle-to-gate approach in an attributional analysis, which considers the following stages: (i) crop/agronomic (i.e., nursery, site preparation, vegetative growth, fertilization, and harvesting, among others), (ii) agrochemical transport, and (iii) the processing of the raw material to produce the natural colorants. For the agronomic stage (annatto crop), the mass yield of 1 hectare (ha) of the crop is considered for a limit of three years, equivalent to obtaining the first harvest. Since the objective of the biorefinery is to produce natural colorants, a mass-based functional unit is the most suitable option to provide more useful information (i.e., environmental impact category values per unit mass) [46]. Bixin is the main colorant in annatto and is produced in both scenarios; therefore, the production of 1 kg of bixin was selected as the functional unit.

2.6.2. System Boundary

The system boundary defines which process will be included or excluded from the system and is divided into systems and subsystems. The system boundaries are presented in Figure 3. System 1 corresponds to the seed germination, and the subsystem of the germination is considered. System 2 involves the production of annatto plants (seedling production), and the subsystems are: (i) the soil preparation, and (ii) the plant growth for its subsequent harvest. System 3 corresponds to the two process scenarios mentioned above.

2.6.3. Inventory Data Selection and Description

The inventory is the LCA phase that involves compiling and quantifying inputs and outputs for a given product system throughout its life cycle. Data collection was obtained from primary sources. Several field visits were made to interact with small communities and annatto-growing families in the department of Chocó. In addition, to validate certain information, a search was made in secondary sources, mainly in technical manuals on the annatto crop. The inputs, reagents, and fertilizers required in the agronomic stage for 1 hectare of the crop are presented in Table 3.

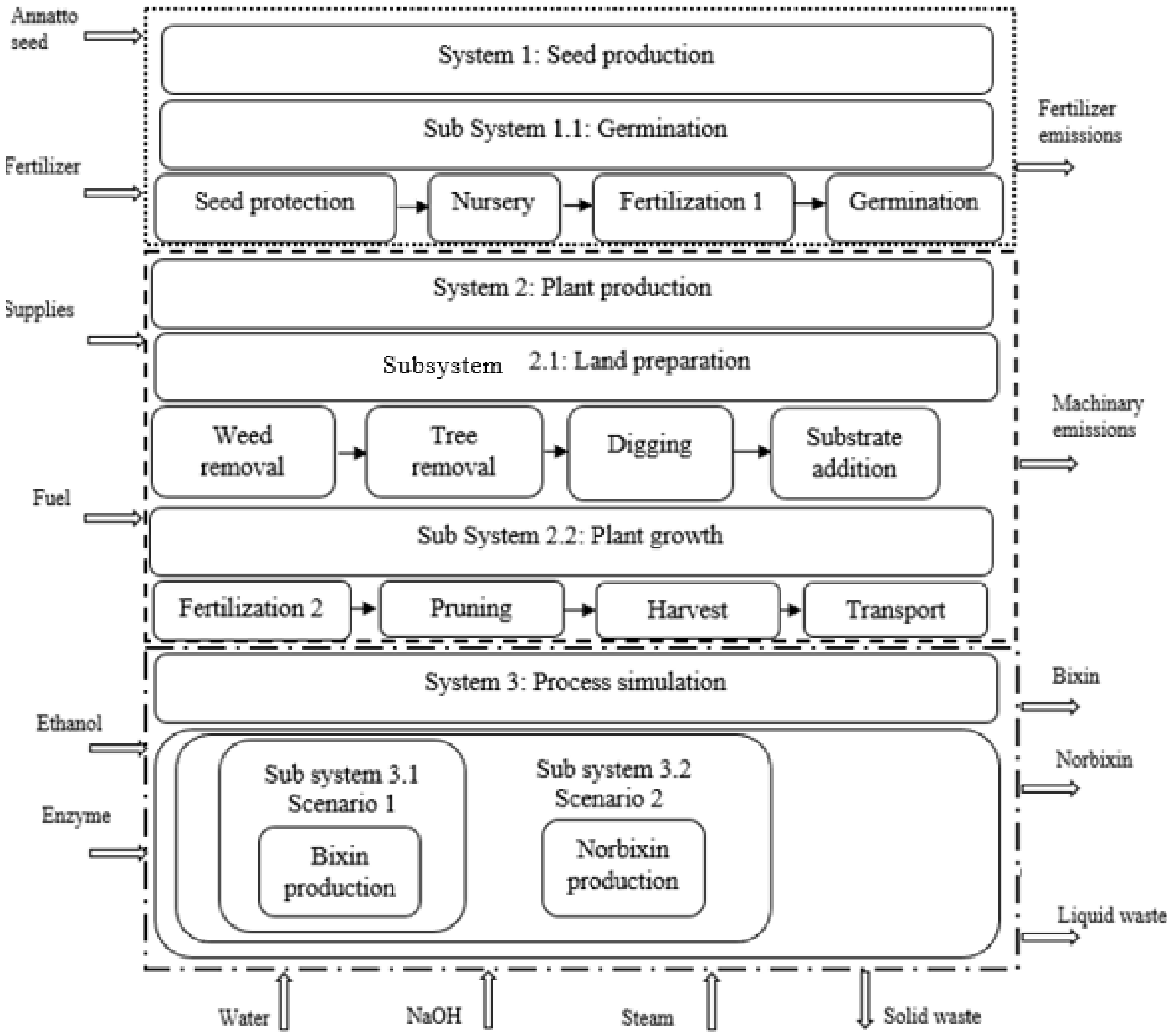


Figure 3. System boundary of the LCA.

System 1

The initial yield of the annatto crop is 625 trees ha^{-1}. The germination ratio of annatto seeds in the nursery stage is 98%. Therefore, excess seeds are required to favor the initial crop yield. The seeds are manually removed from the capsules and are covered with ash in a mass ratio of 1:1, to provide protection and minerals. Generally, the nursery is in the same crop sector; thus, no vehicle is required for seedling transport. The seeds are placed in polyethylene bags with the substrate, then carried in the seedbed (sprouter). The substrate is a mixture of sand and compost in a 2:1 mass ratio, and it must be sterilized with 2 L of hot water (>90 °C). The seed lifespan in the sprouter is around 2.5 months. Over this period, nutrients must be provided to the seedlings for proper growth. Foliar fertilizers are generally used. In this case, the addition of SuperMagro fertilizer, composed mainly of cattle manure and molasses, is applied. Approximately 1 L of fertilizer is dissolved in 19 L of water, and 0.5 L is dispersed in 1 m^2. This fertilization should be performed at least every two weeks over the lifespan.

Table 3. Inputs, reagents, and fertilizers required in the agronomic stage for 1 hectare of annatto crop.

					Input Compounds			Machinery/Supplies Inputs				
Stage	**Subsystem**	**Activity**	**Name**	**Time (Month)**	**Name**	**Value**	**Unit**	**Name**	**Value**	**Unit**	**Fuel Consumption L h^{-1}**	**Type of Machinery**
1	Seed protection	Seed protection	Ash	0.2	Ash	637.5	kg	-	-	-	-	Manual
2	Nursery	Nursery construction	Wood	1	-	-	-	Wood supports 1	53.3	kg ha^{-1}	-	Manual
			Sunshade greenhouse		-	-	-	Polyethylene	4.2	kg ha^{-1}	-	Manual
3	Germinator	Bed construction	Wood bed	3	-	-	-	Wood bed	144	kg ha^{-1}	-	Manual
			Wood supports		-	-	-	Wood supports 2	12.96	kg ha^{-1}	-	Manual
		Substrate	Sand		Sand	4060.26	kg	-	-	-	-	Manual
			Compost		Compost	523.9	kg	-	-	-	-	Manual
		Substrate sterilization	Water		Water	3056.11	kg	-	-	-	-	Manual
		Bags	Bags		-	-	-	Polyethylene	19.76	kg ha^{-1}	-	Manual
		First fertilization **	Super Magro		Super Magro	17.42	L	-	-	-	-	
					Cattle manure	5.807	kg	-	-	-	-	
					Molasses	5.807	kg	-	-	-	-	
					Water	330.97	kg	-	-	-	-	Manual
			Aspersion		-	-	-	-	-	-	-	Manual
		Irrigation	-		-	-	-		-	-	-	-

Table 3. *Cont.*

					Input Compounds			Machinery/Supplies Inputs				
Stage	**Subsystem**	**Activity**	**Name**	**Time (Month)**	**Name**	**Value**	**Unit**	**Name**	**Value**	**Unit**	**Fuel Consumption L h^{-1}**	**Type of Machinery**
4	Ground preparation	Weed removal	Machete	1	-	-	-	-	-	-	-	Manual
		Tree removal	Hatchet		-	-	-	-	-	-	-	Manual
		Herbicides	-		-	-	-	-	-	-	-	Manual
		Digging	Palin		-	-	-	-	-	-	-	Manual
		Substrate addition	Substrate 1		Sand	416.67	kg	-	-	-	-	Manual
					Compost	208.33	kg	-	-	-	-	Manual
		Irrigation	-		-	-	-	-	-	-	-	-
5	Plant growth	Second fertilization **	SuperMagro	18	SuperMagro	18.75	L	-	-	-	-	Manual
					Cattle manure	6.25	kg	-	-	-	-	
					Molasses	6.25	kg	-	-	-	-	
					Water	356.25	kg	-	-	-	-	Manual
			Aspersion		-	-	-	-	-	-	-	Manual
		Third fertilization **	SuperMagro		SuperMagro	937.5	L	-	-	-	-	Manual
					Cattle manure	312.5	kg	-	-	-	-	
					Molasses	312.5	kg	-	-	-	-	
					Water	17,812.5		-	-	-	-	
			Aspersion		-	-	-	-	-	-	-	Manual
		Pruning	Pruning shears		-	-	-	-	-	-	-	Manual
6	Production and harvest	Harvest	Pruning shears	0.5	-	-	-	-	-	-	-	Manual
		Gather	Bags		-	-	-	Fique *	34.28	kg ha^{-1}	-	Manual
		Transport	Transport		-	-	-	Transport	-	-	1.84	Truck

* Fique is a biodegradable fiber used for the manufacture of sacks. ** Three fertilizations are carried out at the crop stage.

System 2

Subsystem 2.1: The land requires adequate weed control. This process is carried out manually by a hatchet or machete and does not require pre-emergent herbicides. Due to the climatic conditions of the region, irrigation is not carried out. The land layout is made in a square shape with a spacing of 4 m per tree. The drowning is also done manually with a shovel, and 1 kg of the germination substrate is added per hole.

Subsystem 2.2: After sowing, fertilizers are added to the seedlings to ensure their growth. There are no specific studies on annatto crop fertilization. Instead, the expertise obtained from the Chocó growers is considered. They suggest that SuperMagro should be added during the first 2–3 months of the crop. For this fertilization, 20 L of SuperMagro is used to fertilize 100 trees; after three months, 20 L of SuperMagro is used to fertilize 10 trees. Likewise, after six months of sowing, manual pruning is carried out using pruning shears. Finally, after 1.5 years of sowing, the first harvest is performed manually with pruning shears. The capsules are packed in jute bags and transported to the collection center in a truck of 2.5 tons. This work assumes that the average distance from the annatto crop to the storage facility is 7 km. Furthermore, the transport distances of the substrates and biofertilizers range from 10 km.

System 3

An average distance from the collection center to the processing facility of 8 km is considered for this system. It is carried out from the data provided and the results of Section 2.6. All the input and output streams (feedstock, reagents, utilities, waste, and products) are considered.

Subsystem 3.1: In the process stage, the seeds are dried (using electricity as an energy source) and then mixed with ethanol. The stream is conditioned by increasing its temperature with a heat exchanger that uses steam for heating. When extraction occurs, the stream passes through a filter where the seeds (solid waste stream) are separated from the bixin-rich liquid (product). Then, the liquid stream is heated and carried into a distillation tower to separate the ethanol from the bixin. A liquid waste stream comes from the top of the tower (steam is condensed using a heat exchanger), composed mainly of ethanol. Finally, a heat exchanger is used to cool the bottom stream (using cooling water) to be crystallized for further sale.

Subsystem 3.2: The above-mentioned in subsystem 3.1 is considered. Furthermore, the solid stream obtained after bixin extraction is mixed with 0.5 N NaOH to produce norbixin. After extraction, the stream is filtered to separate the norbixin-rich liquid. Then, the liquor is mixed with enzymes to purify and precipitate norbixin.

3. Results and Discussion

3.1. Raw Material Characterization

The results of the physicochemical characterization of the annatto seeds are presented in Table 4. The extractives represent the highest content of the lignocellulosic analysis, being consistent with the literature reports [47]. According to Meñaca et al., annatto seeds contain high amounts of extracts, mostly represented by carotenoids such as bixin, β-bixin, methyl bixin, norbixin, and orelin, among others [47]. Moreover, the seeds present lower cellulose, hemicellulose, and lignin content than others, such as mandarin seed (53.26% cellulose, 12.36% hemicellulose, and 19.35% lignin) [48]. Regarding the proximate analysis, the seeds contain higher ashes than orange and mandarin seeds [48]. Annatto seeds are not suitable as fuel since ash content above 5% negatively affects the heat generation through combustion [48]. The volatile matter content is similar and comparable to the reports for different hulls, seeds, or agricultural residues [49]. A high volatile matter content indicates that biomass ignition begins at a relatively low temperature (high combustion reactivity), being consistent with the combustibility index (the ratio between the volatile matter and the fixed carbon). Moreover, a higher fixed carbon content indicates that the raw material has a higher heating value. For the annatto seeds, the fixed carbon is the lowest content;

therefore, a low calorific value (13.27 MJ kg^{-1}) is expected compared to *Eucalyptus grandis* (19.35 MJ kg^{-1}) and hazelnut shell (19.30 MJ kg^{-1}).

The physicochemical characterization data reported in the literature are very limited, focusing on the content of extracts, protein, lipids, ash, and fiber. Table 4 also shows some results regarding the annatto characterization reported in the literature. Kumar et al. report similar cellulose, ash, and lignin values, but higher content for hemicellulose and protein [50]. Stringheta et al. report a cellulose range of 40-45 %, similar values for protein (12.55%) and ash (5.82%), and higher values for lipids (7.20%) [51]. The variation in chemical composition may be due to the variation in soil and climatic conditions of the country where it is grown and to the determination method [52].

Table 4. Physicochemical characterization of the annatto seeds.

Analysis	Component	Mass Composition (%)		
		This Work	**Kumar et al. [50]**	**Valerio M et al. [53]**
Chemical [a]	Initial moisture	40 ± 0.25	-	-
	Cellulose	18.81 ± 1.4	14.40	-
	Hemicellulose	11.34 ± 1.26	34.00	-
	Lignin	13.92 ± 1.6	10.86	-
	Extractives	28.46 ± 0.94	-	-
	Fats	2.76 ± 2.12	-	2.23
	Protein	8.71 ± 1.41	14.35	11.50
	Pectin	16.00 ± 2.56	-	-
Proximate analysis	Ash	5.39 ± 1.63	5.23	-
	Fixed carbon	8.63 ± 1.5	-	-
	Volatile matter	72.78 ± 2.16	-	-
	Moisture	13.2 ± 1.5	-	-
	HHV (MJ/kg)	16.14	-	10.98
Solid content [b]	Total solid	87.29 ± 2.67	-	-
	Volatile solid	82.26 ± 2.51	-	-
Extract analysis	TPC (mg Gallic Acid/g sample)	5.45 ± 3.12	-	-
	AC IC50 (ug/mL sample)	111.04 ± 2.58	-	-
	RS g Glucose/L sample	1.27 ± 3.14	-	-

[a] dry basis, [b] wet basis, HPC higher calorific power, TPC total phenolic content, AC antioxidant capacity.

Concerning the bioactive analysis, the total phenolic content (TPC) was 5.45 mg of gallic acid per gram of extract. The polyphenols concentration in annatto seeds is highly variable because it depends on the raw material species and the operating conditions (mostly the solvent type). Mota et al. [54] concluded that the TPC in natural seeds is higher than in ground seeds, reporting values of 4.47 mg of gallic acid per gram for natural seeds, using KOH as solvent, whereas Chisté et al. reported TPC values of 1.5 mg of gallic acid/g, using aqueous methanol [55]. These differences are mainly due to the solvent polarity. On the other hand, the antioxidant capacity (AC) is the ability of phenolic compounds, among other substances, to capture free radicals or donate hydrogen atoms [47]. In this study, the ability of the extracts to interact with the DPPH and reduce it into DPPH-H was evaluated. To analyze the AC, a low value in the inhibitory concentration (IC) represents a lower amount of extract necessary to inhibit 50% (IC50) of the free radicals [55]. The antioxidant capacity of the annatto seed was lower compared with some supercritical extractions, when using methanol as a solvent. Some authors reported that by increasing the system pressure, there was an increase in the inhibitory capacity (23.55 μg mL^{-1}) [47]. Therefore, the annatto extracts represent ideal characteristics in the food and pharmaceutical industry.

3.2. Extraction and Quantification of Bixin and Norbixin

Figure 4 shows the results obtained for the bixin extraction sequences from ground seeds (Figure 4a) and natural seeds (Figure 4b). It is observed that the solvent is not only selective to bixin. Quantities of norbixin are also extracted. Concerning the ground seed, the bixin yield shows an increasing trend when sequential extractions are carried out. The maximum concentration is reached in the third extraction (414.94 mg L^{-1}). This yield increase was expected due to the superficial seed destruction and the consequent easy interaction of the solvent with the solute. After the third extraction, it is observed that the bixin yield decreases due to colorant exhaustion. Furthermore, it is observed that the norbixin yield decreases over sequential extractions due to the amount of this colorant in the first seed layers. The maximum concentration of norbixin was 108.36 mg L^{-1} in the first extraction. The total yield of bixin and norbixin extraction for the ground seed was 29.45% and 4.69%, respectively.

Regarding the natural seed (Figure 4b), an increase in the total yield (65%) of norbixin extraction is observed, with a maximum concentration of 130.23 mg L^{-1} (first extraction). In contrast to the ground seeds, the bixin yield tends to decrease from the first extraction. The total yield and the maximum bixin concentration were, respectively, 72.65 mg per gram of seed and 760.8 mg L^{-1}. This difference is possibly due to the location of bixin in the seed. The seed is covered with a peel where the colorant is located. Therefore, the solvent–peel interaction will be favored with the natural seed, whereas with the ground seed, the seed peel and seed are mixed, affecting the mass transfer of the colorant within the solvent. Compared to the ground seeds, higher bixin extraction yields were achieved.

There are several methods to extract the colorant from an annatto seed. The bixin content can vary between 1–4%, depending on the quality and origin of the seeds (crop conditions, weather, and annatto species, among others) [56]. The results obtained in this work agree with the data reported by other studies, showing that the bixin content varies from 0.26 to 311 mg per gram of seed [55]. Carderelli et al. reported a yield of 4.16 mg g^{-1} for four extraction sequences using ethanol as solvent [30]. Likewise, the average sequence extraction was 0.5 mg g^{-1}, slightly less than the results of Alcázar et al. (0.6 mg g^{-1}) [15].

Figure 4 shows the extraction results obtained for the norbixin sequences from ground seeds (Figure 4c) and natural seeds (Figure 4d). As a general result, the norbixin yield decreases when sequential extractions are performed. For the ground seed, the maximum concentration is reached in the first extraction (271.07 mg L^{-1}). A noteworthy difference is observed for the natural seed since a maximum concentration of 4529.32 mg L^{-1} is reached in the first extraction. This difference is possibly due to the location of norbixin in the seed. Although the seed structure is destroyed (seed–peel separation) in the bixin extraction, the peel remains almost intact, and the norbixin extraction is favored because there is greater interaction with the solvent. Therefore, a decrease in the norbixin yield is also observed in the natural seed.

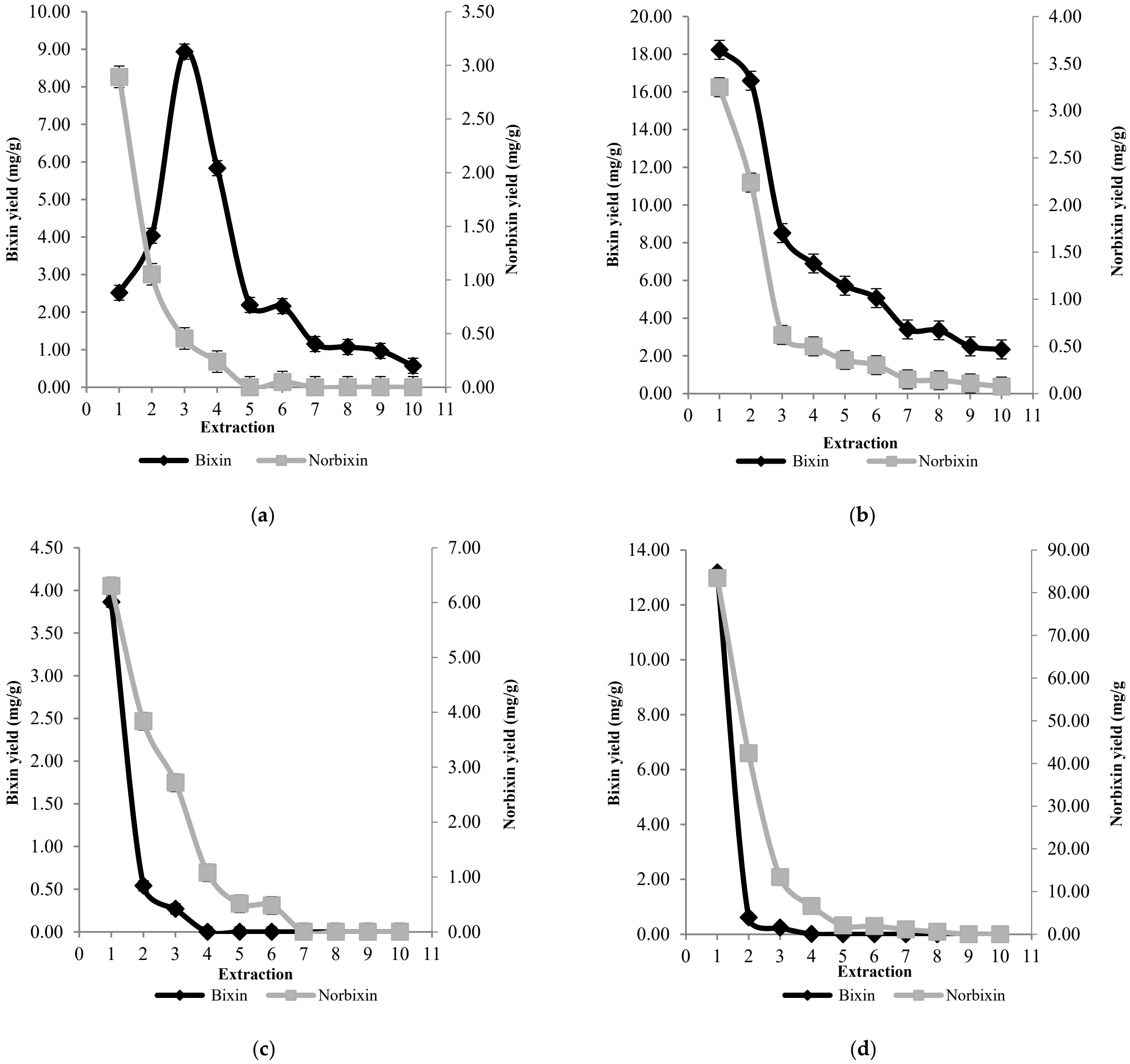


Figure 4. Colorant extraction yields for bixin using (**a**) ground seed and (**b**) natural seed, and norbixin from (**c**) ground seed and (**d**) natural seed.

3.3. *Process Simulation*

3.3.1. Technical and Economic Assessment

Based on the experimental results, it was observed that the maximum extraction yields of bixin and norbixin were in the natural seed extractions. Therefore, for simulation purposes, the milling stage was not considered. Tables 5 and 6 indicate the mass balances and energy requirements for the proposed scenarios, respectively. For scenario one, a bixin production yield of 0.05 kg per kg of annatto is obtained; in addition, concerning the extraction stage, a decrease of 27.19% is observed due to the separation and purification stages. For scenario two, a norbixin production yield of 0.17 kg per kg seed is obtained (with losses of 10.78% throughout the downstream process).

Table 5. Mass balances of the simulated scenarios based on the feedstock flow rate.

Scenario	Process	Input	Input Rate *	Output	Output Rate *
Scenario 1	Bixin extraction	Annatto seed	1.00	-	-
		Ethanol (70%v/v)	11.23	-	-
		Water	4.81	-	-
	Bixin filtration	-	-	Solid waste	0.59
	Bixin purification	-	-	Liquid waste	16.04
		-	-	Bixin	0.05
Scenario 2	Bixin extraction	Annatto seed	1.00	-	-
		Ethanol (70%v/v)	11.23	-	-
		Water	4.81	-	-
	Bixin purification			Liquid waste	16.04
				Bixin	0.05
	Norbixin extraction	Water	14.51	-	-
		NaOH	0.29	-	-
	Norbixin purification	Enzyme	0.003	Solid waste	0.45
	Norbixin separation	-	-	Liquid waste	14.78
		-	-	Norbixin	0.17

* Input and output rate in kilogram per kilogram of raw material (602.51 kg h^{-1}).

Table 6. Energy consumption of the biorefinery scenarios.

Scenario	Process	Energy Demand (MJ kg^{-1})	
		Cooling Water	Steam
Scenario 1	Colorant extraction	-	130.07
	Colorant purification	333.79	475.29
	Total	333.79	605.36
Scenario 2	Colorant extraction	-	380.12
	Colorant purification	364.44	644.46
	Total	364.44	1024.58

Based on the results reported in Table 5, two mass indexes for biorefineries were calculated: (i) the process mass intensity index (PMI), involving all input streams and the desired product, and (ii) the mass loss index (MLI), relating all waste streams in the process (including liquid wastes, solids, and gaseous emissions). As a result, PMI values of 321.66 and 141.03 kg of raw material per kg of product and MLI values of 319.48 and 138.98 of waste steam per kg of product are obtained for scenarios one and two, respectively. In addition, for scenario two, it is observed that less kg of raw material is required compared to scenario one, due to the addition of a second product involving less waste generation. Regarding the energy demand, Table 6 shows the energy requirement of the biorefinery scenarios. The bixin purification stage for scenario one, which involves ethanol separation and bixin crystallization, demands 475.29 MJ kg^{-1}, representing 78.51% of the overall energy. For scenario two, the norbixin extraction stage represents the second highest energy expenditure in steam (29.23% of the overall energy). Considering the energy consumption in cooling water, scenario one demands 333.79 MJ kg^{-1} in the bixin purification stage. Therefore, the norbixin purification for scenario two does not represent a considerable increase compared to scenario one (9.18%).

3.3.2. Economic Assessment

The analysis of the raw material cost was estimated based on the supplies (i.e., substrate, fertilizers, and chemicals), labor, equipment acquisition, and materials for the nursery construction, as shown in Table 2. Figure 5 shows the cost distribution to produce annatto seeds, composed of the nursery, land preparation, and start-up of the crop. It can be observed that the equipment investment represents the lowest value (10.39%), explained by the non-technified crop and highlighted by the purchase being considered a one-time investment. Therefore, it may be used for future crops, and the cost will be related to its maintenance. In contrast, labor represents a significant share of the investment (45.74%), since labor is constantly required in each stage of the agronomic process. The labor payment in Chocó is around USD 7.5 per 8 h of work, becoming an important aspect of the final cost of the raw material. For example, eight workers are needed for the harvesting and collection stage, ten for land preparation, and five for fertilization. Therefore, the time and capacity of laboring must be optimized. Considering all these expenses, the raw material cost is 2.9 USD/kg^{-1}.

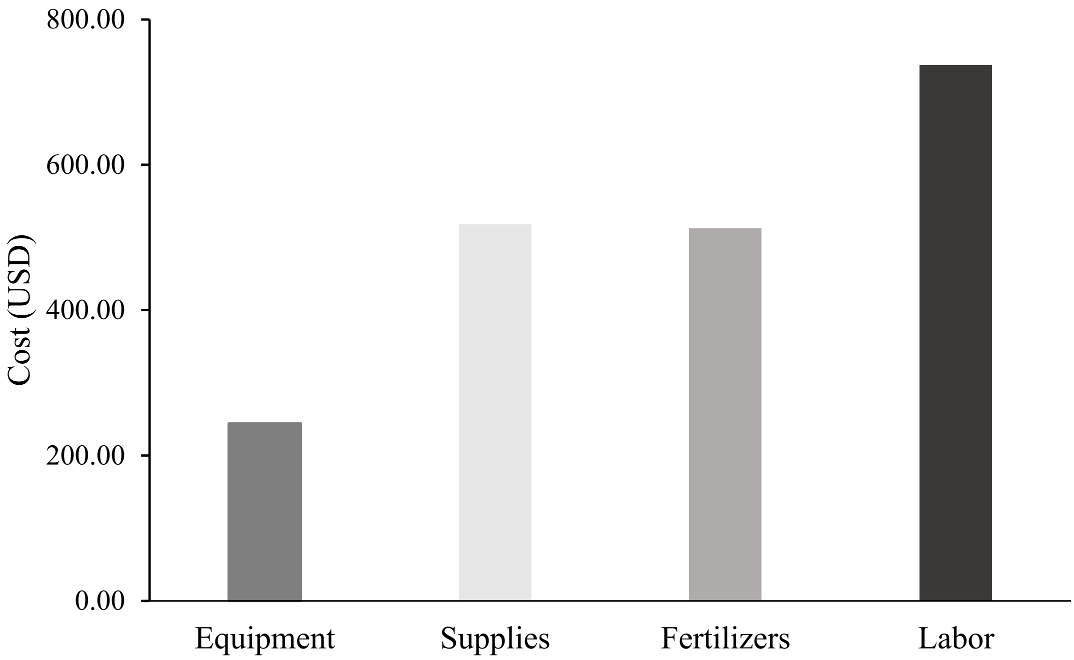


Figure 5. Cost distribution to produce annatto seeds in the first harvest.

For the economic assessment, a plant capacity baseline of 602.51 kg h^{-1} was selected according to the Chocó productivity, and the effect of different capacities on economic profitability was evaluated. The concept of the minimum processing scale for economic feasibility (MPSEF) was included in this analysis, representing the point where the net present value (NPV) is equal to zero throughout the total useful life of the project. Table 7 illustrates the profit margin of the proposed scenarios. It can be observed that the operating expenditure (OpEx) represents the highest distribution and lowest depreciation for all the scenarios. For the first scenario, the raw material cost represents 76.08% of the OpEx, followed by utilities (18.65%) and maintenance (0.92%). For the second scenario, an increase in the OpEx is observed due to the NaOH and enzyme costs used in the extraction and purification stages. However, the different reaction and separation technologies used in the process schemes are simple and do not require a high capital investment compared to OpEx.

Table 7. Profit margins of the proposed scenarios at a flowrate of 602.51 kg h^{-1} of raw material.

Scheme Type	CapEx (M-USD kg^{-1})	OpEx (M-USD/year)	Product	Sale Cost (USD kg^{-1})	Production Cost (USD kg^{-1})	Revenue (USD kg^{-1})
Scenario 1	0.74	4.99	Bixin	15.75	20.17	−4.42
Scenario 2	0.83	13.48	Bixin	15.75	15.52	0.23
			Norbixin	18	17.36	0.64
			Total	33.75	32.88	0.87
Base case (study region) *	-		Annatto seed	3.40	2.9	0.50

* The characteristic distribution of the region is the untreated annatto seed.

Comparing the profit margin of each scenario, negative values were observed for scenario one. These values are explained by the production cost being higher than the income. On the other hand, it is observed for scenario two that norbixin production increases the gross revenue from bixin production by 52% because it presents a higher yield and sale price. However, this decrease ignores the initial investment cost because the revenues are calculated as the difference between the profits and OpEx. The profits are the gains generated by the sale of products, and the OpEx includes the costs of raw materials, utilities, maintenance, and labor, among others. Therefore, it is not a decision criteria parameter to know if the process is profitable or not for the 20-year useful life of the project. In addition, when comparing scenario two with the practices carried out in the region (the sale of untreated seed), it can be observed that the income increases by 57.2%, which shows that the extraction of natural colorants in the annatto seed could provide a better economic livelihood for the region.

Due to the negative values of the profit margin from scenario one, an NPV sensitivity analysis was performed. The NPV analysis allows for calculating the economic profits through cash flow balances. Figure 6 illustrates the influence of the raw material flowrate on the NPV. The NPV collects the cost for the land acquisition, materials, and equipment to process the start-up, shown on negative year expenditures. However, the increase in raw material flowrates would decrease some process expenses. Therefore, production costs would be lower than market sale prices, offering a surplus in revenues to improve the economic profitability, as reflected in the positive values of the NPV analysis [57]. The economic allocation of market products and the simplified techniques used in the process would demonstrate this economic profitability at an industrial scale.

Figure 7 shows the sensitivity analysis of the NPV at different processing scales of the scenarios. This analysis shows, that for scenario one, the process does not demonstrate economic profitability in the baseline, mainly due to the cost of raw materials (ethanol) and the low bixin extraction yield. Consequently, the MPSEF of the process is reached with a scale-up of 7.1 times the base case (4277.45 kg h^{-1}). In contrast, for scenario two, the NPV tends to be positive, with a payback period of 3.2 years after the start-up. In this sense, this trend shows that the production of norbixin followed by the previous bixin has a favorable effect on the process, since it presents higher extraction yields than the bixin extraction in scenario one. Furthermore, the MPSEF of the process is reached at lower processing scales, 0.68 times the base case (409.7 kg h^{-1}).

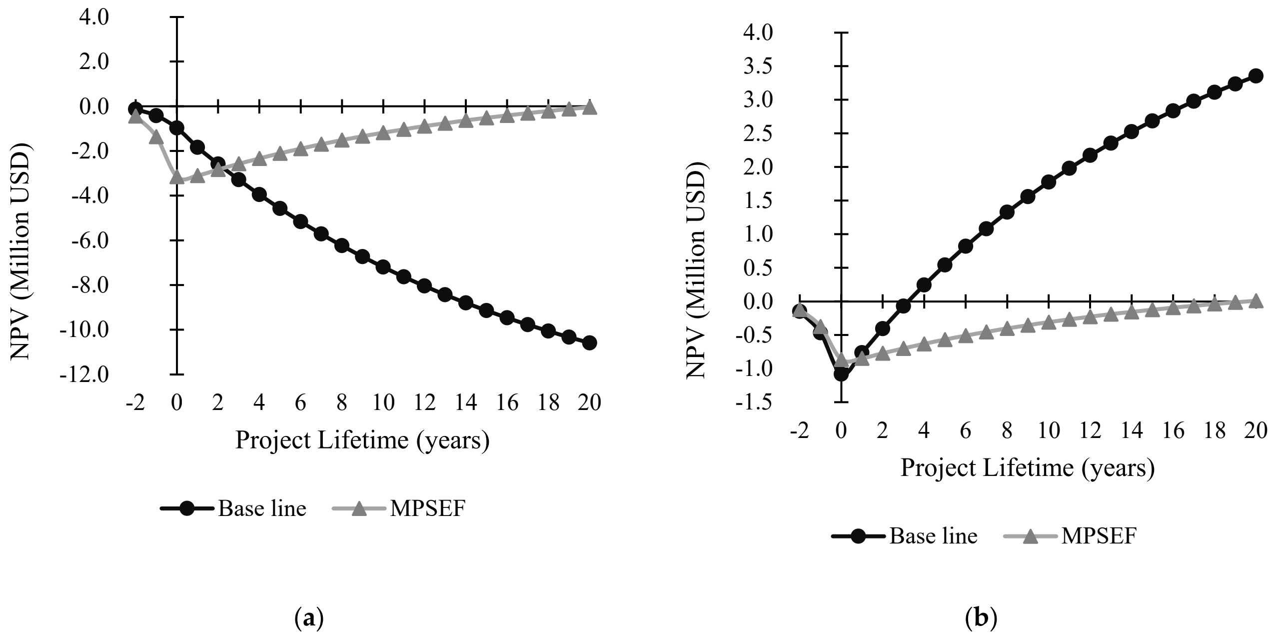


Figure 6. Sensitivity analysis of the raw material flowrates over the NPV for (**a**) scenario 1 and (**b**) scenario 2. The baseline represents a flowsheet of 602.51 kg h^{-1} based on the Chocó productivity, MPSEF is the minimum processing scale for economic feasibility.

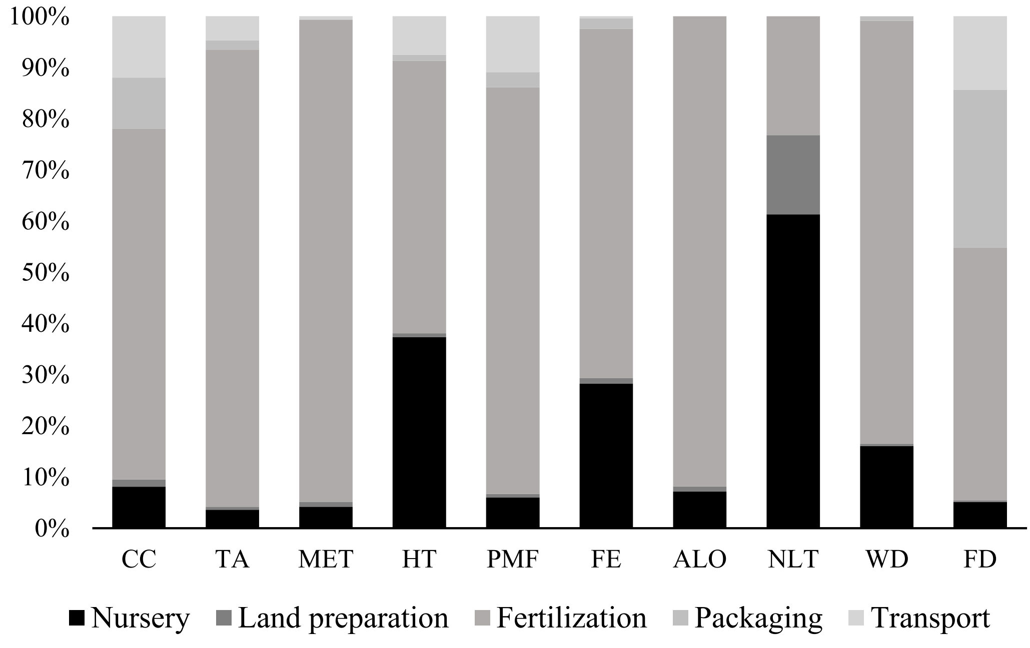


Figure 7. Contribution of the main systems to the total environmental impact of annatto crop. Graphics have the same color and plot for each contributor item.

The base case (scenario one) is related to a technified valorization of annatto in Chocó, which represents about 90% of the national production. Therefore, there would not be enough national production to satisfy the demand for the process. However, the economic pre-feasibility of the process is favored by the production of norbixin (as shown in scenario two). The production of natural colorants such as bixin and norbixin from annatto seeds is shown to have pre-feasibility in the economic dimension of sustainability. It is possible to link and associate small communities in the sector with the obtainment of high-value-added products of native origin while maintaining a low level of development. In addition, it can be a promising alternative to promote and protect these crops as substitutes for the illicit crops characteristic of the region.

3.3.3. Environmental Assessment

Environmental Assessment of the Annatto Crop in Tropical Rainforests (Bio Productive-Chocó Region)

The most representative categories that had the greatest impact on the annatto crop were climate change (CC), terrestrial acidification (TA), marine eutrophication (MET), human toxicity (HT), particulate matter formation (PMF), freshwater ecotoxicity (FET), agricultural land occupation (ALO), natural land transformation (NLT), water depletion (WD), and fossil depletion (FD). For the annatto crop stage, the impacts of fertilizers (organic fertilizers such as super lean manure) and the activities carried out in the nursery stage (seed disinfection, transplanting to bags, and the use of organic fertilizer) were grouped together. In the categories analyzed, organic fertilizers (manure, molasses, and SuperMagro) contributed the most, followed by the nursery. Figure 7 presents the relative contribution of the different stages of the annatto crop to the main impact categories. CC is the most representative category for comparing the results. This is because kg CO_2 eq can represent the carbon footprint of the system analyzed. The production of 1 kg of annatto generates 0.48 kg CO_2 eq in one crop cycle. The use of organic fertilizers represents 68% of this category. Several authors have reported that using chemical fertilizers for crops is the main contributor to the environmental impact [58], reporting similar contributions. For example, Ortiz M et al. mentioned that using fertilizers in orange crops represented about 50% of the environmental impact [59]. However, it has been shown that using organic fertilizers presents a greenhouse gas reduction rate comparable to chemical fertilizers. Kitamura et al. reported a reduction of 25% by using manure and slurry as fertilizers [60]. Similar results have been reported by Prateep et al. [61]. On the other hand, the categories most impacted by the addition of fertilizers were TA, MET, PMF, and ALO.

Using molasses (compost) in adequate doses is a source of energy for the microorganisms involved in compost fermentation [62]. However, water oxygen depletion and middle-term impact on the ecosystem could exist at high molasse dosages [61]. Furthermore, compost (used as organic fertilizer) and its leachate production generate air emissions (i.e., CH_4, N_2O, and NH_3), affecting the impact categories [63]. Due to the limited information on the environmental impact of the predominant crops in Chocó (e.g., banana, rice, cocoa, and illicit crops), it is not easy to compare. However, Table 8 presents the results of the environmental impact in terms of the climate change of crops from different regions, without considering CO_2 sequestration. These studies present differences in the approach and methodology used to define the environmental impact; however, they can indicate the environmental performance of these crops in the study region. The annatto crop represents lower environmental contributions to climate change than other crops characteristic of the region. These results corroborate the fieldwork performed. Furthermore, it was observed in the fieldwork that annatto is a non-technified crop that coexists with the biogeographic environment of Chocó in a natural way, where agricultural practices (the addition of agrochemicals) are carried out using organic compounds and in low quantities. In addition, it can be observed that the annatto crop represents a promising alternative in environmental terms for a substitution or transition from the illicit crops that are characteristic of Chocó to crops with low environmental impact and a high potential to generate high-value-added products [64].

Table 8. Comparison of the potential impact of different crops in the climate change category.

Crop	Climate Change (kg CO_2 eq/kg Product/Year)	Reference
Annatto	0.48	This work
Banana	1.3	[65]
Cocoa	2.6	[66]
Rice	1.46	[67]
Illicit crops (coca)	590	[68]

Environmental Assessment of the Annatto Crop in Tropical Forests Considering Carbon Sequestration

The climate change category is defined as the possible increase in temperature of the earth's atmosphere due to GHG emissions that derive mainly from anthropogenic activities. This impact category has been one of the most widely used to estimate the possible risk associated with human activities in developing their society [69,70]. In this sense, this category is of vital importance to be analyzed in the LCA because it allows for identifying the bottlenecks of the analyzed system, and the possible effects of absorption and reduction in emissions given the uptake, via photosynthesis, of the annatto crops and their contribution to the tropical forests of the biogeographic region of Chocó. Figure 8 presents the contribution to climate change of the annatto crop and the contribution considering the scenario where the biomass of the crop captures CO_2.

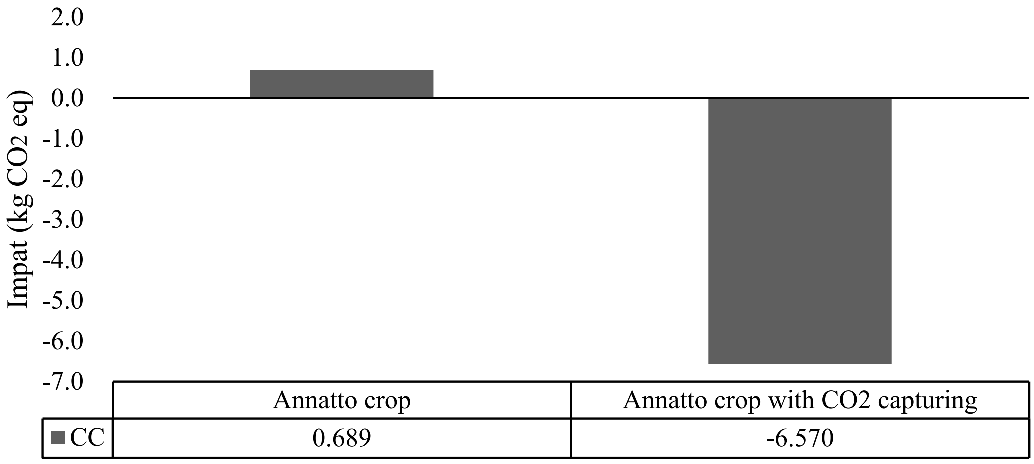


Figure 8. Contribution to climate change of annatto crop with and without CO_2 capture.

Considering the CO_2 capture of the annatto crop (three-year plantation), the contribution to climate change is −6.57 kg of CO_2 eq per kg of annatto seed. The negative values show that CO_2 capture exceeds the emissions generated by the different activities carried out in the crop because of a low level of agricultural practices. In this sense, it is evident that the annatto crop does not harm the environment, because the addition of agrochemicals and the use of fuel-consuming tools is very low. In addition, considering that the previously mentioned agricultural practices are only carried out during the first years of the crop, and that annatto plantations are characterized by being ancestral, it was determined that, for plantations older than 20 years, there is a contribution to climate change of approximately −16.45 kg of CO_2 eq per kg of annatto seed. This difference is mainly due to the size of the tree. Furthermore, the different tropical forests are fundamental in the carbon cycle and help to significantly mitigate GHG emissions produced by anthropogenic activities. In fact, Canadell J et al. mentioned that the CO_2 storage of tropical forests, in the context of global warming, can mitigate about 30% of annual CO_2 emissions [71]. Barragán J et al., [72] stated that the tropical rainforests of the Chocó biogeographic region are one of the main contributors to CO_2 sequestration in Colombia (−135.29 kg CO_2 eq). Other authors have obtained similar results, who stated that these forests have CO_2 sequestration of −219.86 kg

CO_2 eq [73]. Since the annatto crop coexists in the tropical forest environment, it can be said that it contributes directly to the fact that it is one of the most representative regions in environmental terms. In this sense, it is essential to join efforts to maintain and restore ecological connectivity as a key attribute in the functionality of small communities.

Environmental Assessment of the Biorefinery Schemes

Figure 9 represents the contribution of the main systems to the environmental impact of the two proposed scenarios. The most representative categories with the greatest impact on the biorefinery schemes were climate change (CC), human toxicity (HT), photochemical oxidant formation (POF), particulate matter formation (PMF), terrestrial ecotoxicity (TET), freshwater ecotoxicity (FET), agricultural land occupation (ALO), urban land occupation (ULO), water depletion (WD), and fossil depletion (FD). The impacts of each stage of the process were grouped. For scenario one, bixin extraction (due to ethanol and electricity) represents the main contributor in the impact categories, followed by the annatto crop. For scenario two, bixin extraction and the annatto crop remain the main contributors in the impact categories evaluated, followed by norbixin extraction (using NaOH). The bixin and norbixin purification stages do not represent considerable impacts because they only use steam and energy and come from renewable sources (hydroelectric plant).

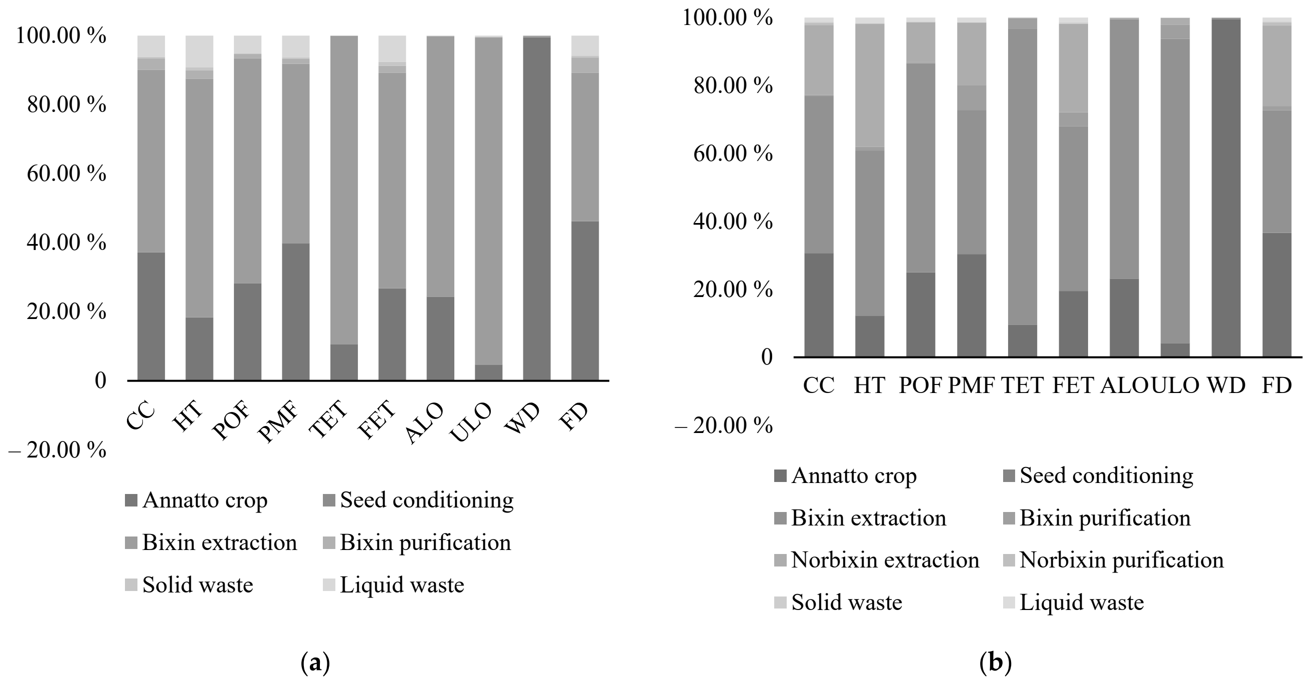


Figure 9. Contribution of the main systems to the total environmental impact of scenarios. Graphics have the same color and plot for each contributor item. (**a**) Scenario 1 and (**b**) Scenario 2.

Considering the CC category, 1 kg of bixin production represented 5.57 kg CO_2 eq for scenario one and 6.54 kg CO_2 eq for scenario two (see Table 9). Scenario two has a higher environmental impact due to the use of additional compounds and energy for the extraction and purification of norbixin. The extraction of bixin using ethanol as a solvent represented 52% in the CC category because, for the analysis, the entire processing part was considered until it was obtained, so there were direct GHG emissions to the atmosphere due to fermentation and transport [74]. In addition, large crop areas were required, which is reflected in the ALO category (representing 75%). On the other hand, chemical contamination caused by alkali substances in the extraction of norbixin, such as NaOH, compromised terrestrial and aquatic ecosystems; in addition, its mismanagement could cause health repercussions [75] (representing 27% in the HT category). In this sense, it is evident that the use of solvents to extract colorant is the main environmental contributor. Similar results have been reported by Kyriakopoulou K et al., where they performed an environmental comparison of different carrot and microalgae carotenoid extraction methods and mentioned that the use of solvents such as hexane and ethanol represented the major environmental contributor, and that methods such as ultrasound-assisted extraction could reduce these impacts [76]. Similar results have been reported in other studies [77]. Moreover, considering the CO_2 capture by the crop to evaluate the biorefinery schemes in the context of climate change, scenario one had a contribution of -14.73 kg CO_2 eq, and scenario two a contribution of -15.23 kg CO_2 eq per kg of bixin.

Table 9. Total contribution of the main systems to the environmental impact of scenarios.

Impact Category	Unit	Scenario 1	Scenario 2
CC	kg CO_2 eq	5.57	6.54
HT	kg 1,4-DB eq	1.168	1.724
POF	kg NMVOC	0.034	0.038
PMF	kg PM_{10} eq	0.021	0.025
TET	kg 1,4-DB eq	0.061	0.064
FET	kg 1,4-DB eq	0.036	0.047
ALO	m^2a	4.014	4.563
ULO	m^2a	0.441	0.471
WD	m^3	3.164	3.012
FD	kg oil eq	1.366	1.681

4. Conclusions

The extraction of bixin and norbixin was favored for natural seeds obtaining yields of 72.65 mg g^{-1} and 193.82 mg g^{-1}, respectively. On the other hand, the production of norbixin followed by bixin (scenario two) favored the economic feasibility in the context of the biogeographic region of Chocó. The annatto valorization did not have a negative impact in environmental terms. Indeed, when carbon sequestration by the crop was considered, it became evident that it contributed to CO_2 mitigation (-6.5 kg CO_2 eq per kg of seed). It was also determined that the cultivation and processing of annatto represented, in environmental and economic terms, an interesting alternative for substituting illicit crops and a high potential for generating value-added products. On the other hand, considering the biorefineries proposed for the valorization of annatto seeds, the solvents used in the colorant extraction process were the most representative of environmental impact. Therefore, it is possible to establish that the main ecological structure of the biogeographic region of Chocó includes sites that, due to the presence of cultural elements such as the cultivation of annatto, allow for the promotion of territorial rootedness and the conservation of traditional practices for the use of natural resources that are environmentally and economically sustainable. However, the results and expectations have limitations. First, the annatto crop at small-scale levels limits local farmers to control their productivity. Any good business in the tropical forest has limitations on scales to avoid extensive cropping and deforestation. Second, and consequently, future research is required to establish these limitations as well as adequate logistic and commercialization models preserving sustainability.

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