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The Bioeconomy of Microalgal Carotenoid-Rich Oleoresins Produced in Agroindustrial Biorefineries

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Abstract

The techno-economic evaluation of the obtaining process of a natural mixed carotenoid-rich oleoresin from microalgae dried biomass is presented in this paper. The process is based on solvent extraction on industrial scale, and the oleoresin obtained is suspended in soybean oil at a concentration of 20%. The oleoresin is compound of a mix of *trans* and *cis* isomers of carotenoids, having as the major carotenoid the all-*trans*-*β*-carotene, in amounts close to 37%. The experimental data were used to estimate the costs of an industrial plant that has the potential to generate 569,016 tons of microalgal biomass per year, in which 107,902.5 kilograms per year are represented by total carotenoids. Based on the determination of the cost analysis, it was demonstrated that the natural mixed carotenoid-rich oleoresin in soybean oil has a production cost estimated as USD 146.9 per kilogram.

Keywords: Cost analysis; Microalgae; Oleoresin; Wastewater

Introduction

Microalgae-based systems for the production of chemicals are an emergent area, representing, therefore, great promise for industrial application. Several processes have primarily demonstrated capabilities forthe food and feed industries, pigments and additives production and the cosmetics industries [1]. These microorganisms have a metabolic versatility that enables the biomass production based on organic sources without commercial value, such as industrial wastes [2].

Such possibilities become attractive for bioprospecting and exploitation as commercial sources in a wide range natural pigments primarily when their feedstock comes from biorefinery systems [3-9]. The biorefinery approach consists of a sustainable processing of biomass into a wide range of valuable bioproducts in an integrated process. The use of these strategies may provide an inexpensive alternative to the conventional technological routes of natural pigments production, e.g.carotenoids [10].

Commercially, carotenoids are used as food colorants and nutritional supplements, with an estimated global market of USD 935 million in 2005 [11]. The growing worldwide market value of carotenoids is projected to reach over USD 1 billion by the end of the decade [12]. This market was predicted to achieve USD 1.2 billion by 2009, and is expected to approach USD 919 million by 2015. Increased competition is the reason for a lower market value than previously predicted [13].

The process of synthesis and purification of carotenoids requires the use of techniques that would make production on a commercial scale very difficult and extremely expensive [14]. This process is highly complicated and involves different organic solvents and multiple steps for the purification [15]. Consequently, the purified form of carotenoids is not easily scaled up to an efficient commercial scale, wherein disposal considerations of various solvents play an important role in the overall feasibility of the process [16]. It is necessary, therefore, to find a more affordable way for commercializing this product, for instance fractions of different carotenoids in oily form in the same extract.

The oily extracts of pigments from different sources have a rather varied carotenoid composition, and according to Rios et al. [17] they are able to provide different tonalities from yellow to red, which are sufficiently concentrated to enable their large-scale commercial use (low doses are sufficient to achieve the desired color in a large amount of foodstuff). When carotenoids are extracted from natural sources and the solvent is evaporated, the residue is called oleoresin. According to these authors, the oleoresins are commercially available as food grade, and the natural carotenoids may also be manufactured as oil suspensions. Palm oil carotenes and carotenoids from *Dunaliella salina* and *Blakeslea trispora* are traded as 20-30% suspensions in vegetable oil. In the oil suspensions, the esters maybe hydrolyzed, and the free carotenoids may be suspended in vegetable oil to give a less viscous product than the isolated oleoresin [18].

A key issue on the viable production of the natural carotenoids is the general absence of low-cost processing technology. The biotechnological production of carotenoids originated of microalgae can circumvent the majority of these limitations, since the biomass carotenoid-rich production can be supported in agroindustrial wastes. These bioresources have low chemical risks, are potentially available on a large scale, and can generate feedstocks at a competitive cost [6,19]. Moreover, the utilization of these substrates might reduce the environmental and energetic problems related to their disposal [20].

In this regard, the aim of this study was to perform a techno-economic evaluation of a natural mixed carotenoid-rich oleoresin extracted from microalgae biomass produced in an agroindustrial biorefinery. The study focused on the carotenoid-rich biomass production, in the determination of the cost analysis and in the evaluation of the applicability of the process.

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Material and Methods

Standards

The standards of all-*trans*-violaxanthin, all-*trans*-lutein, all-*trans*-zeaxanthin, all-*trans*-zeinoxanthin, all-*trans*-lutein, all-*trans*-α-carotene, all-*trans*-β-carotene were donated by DMS Nutritional Products (BASEL, Switzerland) with purities ranging from 95.0% to 99.9%, as determined by HPLC-PDA. Methanol (MeOH), methyl tert-butyl ether (MTBE), hexane and potassium hydroxide (KOH) were obtained from Sigma Aldrich (St. Louis, MO, USA).

Microorganisms and culture media

Axenic cultures of *Phormidium autumnale* were originally isolated from the Cuatro Cienegas desert (26°59′N, 102°03′W-Mexico). Stock cultures were propagated and maintained in solidified agar-agar (20 g/L), containing synthetic BG11 medium [21]. The incubation conditions used were 25°C, a photon flux density of 15 μmol/m^2/s and a photoperiod of 12/12 hour light/dark.

Microalgal biomass production in a biorefinery

The biomass production was made in heterotrophic conditions, using the slaughterhouse wastewater as the culture medium. The cultivations were performed in a bubble column bioreactor (height/diameter (h/D) ratio equal to 1.3 and a nominal working volume of 5 L). The dispersion system of the reactor consisted of a 1.5 cm diameter air diffuser located inside the bioreactor.

The average composition of the wastewater, during one year of sampling, has the following composition (mg/L): pH of 5.9 ± 0.05, chemical oxygen demand of 4100 ± 874, total nitrogen of 128.5 ± 12.1, total phosphorus of 2.84 ± 0.2, total solids of 3.8 ± 2.7, suspended solids of 1.9 ± 0.8, volatile solids of 2.9 ± 1.4 and fixed solids of 0.9 ± 0.3. The operational conditions of the continuous process were previously optimized in order to define a pH adjusted to 7.6, temperature of 20°C, volumetric airflow rate per volume unit of 1 VVM (volume of air per volume of wastewater per minute), absence of light, and a dilution rate of 0.6/d.The wet biomass was separated from the wastewater by centrifugation and then dried on a tray dryer at 60°C. The cultivations were performed twice, and in duplicate.

Carotenoid extraction and carotenoid-rich oleoresin production

The carotenoids were extracted from the dried biomass based on an extraction phase composed by hexane/potassium hydroxide/methanol, which simultaneously affects an alkaline treatment to saponify susceptible lipids and extract the intended carotenoids [22-23]. The carotenoids extract was solubilized in soybean oil, at a concentration of 20%, and stabilized with antioxidant tert-butylhydroquinone (TBHQ) at a concentration of 0.02% (v/v). The final product obtained was a natural mixed carotenoid-rich oleoresin in soybean oil.

Sampling and analytical methods

Samples were collected aseptically in a laminar flow hood; the cell biomasswas monitored every 24 hours during the growth phase of microorganism; the analyses were performed in triplicate and the data refer to the average of six repetitions.

The cell biomass was gravimetrically evaluated by filtering anestablished volume of culture medium through a 0.45μm membrane filter (Millex FG®, Billerica-MA, USA), drying at 60°C for 24h.

The dissolved oxygen concentration in the wastewater was determined by a polarographic oxygen sensor (Mettler-Toledo, Zurich, Switzerland).

The carotenoid extract was analyzed by a high performance liquid chromatography HPLC-PDA-MS/MS (Shimadzu, Kyoto, Japan) equipped with quaternary pumps (model LC-20AD), online degasser, and injection valve with a 20 μL loop (Rheodyne, Rohnert Park, CA, USA). The equipment was connected in series to a PDA detector (model SPD-M20A) and a mass spectrometer with an ion-trap analyzer and atmospheric pressure chemical ionization (APCI) source (model Esquire 4000, Bruker Daltonics, Bremen, Germany). The carotenoid separation was performed on a C_{30}YMC column (5 μm, 250 × 4.6 mm) (Waters, Wilmington, DE, USA). HPLC-PDA-MS/MS parameters were set as previously described by De Rosso and Mercadante [24]. The mobile phase consisted in a mixture of methanol and MTBE. A linear gradient was applied from 95:5 to 70:30 in 30 min, to 50:50 in 20 min. The flow rate was 0.9 mL.min^{-1}. The identification was performed according to the following combined information: elution order on C_{30} HPLC column, co-chromatography with authentic standards, UV-visible spectrum (λ max, spectral fine structure, peak *cis*intensity), and mass spectra characteristics (protonated molecule ($[M+H]^+$) and MS/MS fragments), compared with data available in the literature [1,24-27].The carotenoids were also quantified by HPLC-PDA, using five-point analytical curves of all-*trans*-violaxanthin, all-*trans*-zeaxanthin, all-*trans*-zeinoxanthin, all-*trans*-lutein, all-*trans*-β-carotene and all-*trans*-α-carotene. All other xanthophyll and carotene contents were estimated using the curve of all-*trans*-lutein and all-*trans*-β-carotene, respectively. The *cis*-isomers were estimated by using the curve of the corresponding all-*trans*-carotenoid. The total carotenoid content was calculated as the sum of the contents of each individual carotenoid separated on the C_{30} column.

Scale-up of the microalgal biorefinery

The theoretical scale-up of the process was performed using the criteria of a constant oxygen transfer rate through the constant volumetric mass transfer coefficient (KL_a) method [28]. The estimation of large-scale process was based on an industrial plant operating at a wastewater flow rate of 10,000 m^3/day, working 24 h/day and 336 days/year, and performing one extraction of carotenoids per day.

Cost analysis methodology

The production cost of the mixed carotenoids-rich oleoresin was initially based on the description of the flowchart of the process (Figure 1). A list of the equipment used, its size, and the consumables of the process has also been given.The methodology applied to determine the total capital investment was established based on an estimation of the total capital investment (TCI), which is the somatory of the fixed capital investment (FCI) and the working capital (WC) [29].

The fixed costs were estimated through the factors of the major equipment costs (MEC) and all the required additional costs necessary to build the plant (e.g. installation of the equipment). These additional costs are related to the MEC through certain factors taken from the literature (Lang factors).

The different items were estimated as a percentage of the MEC, multiplying the corresponding Lang factors according to the nature of the item. The estimated cost for each piece of equipment was obtained from a website (www.matche.com) that estimates engineering prices in 2014 FOB in USD [30].

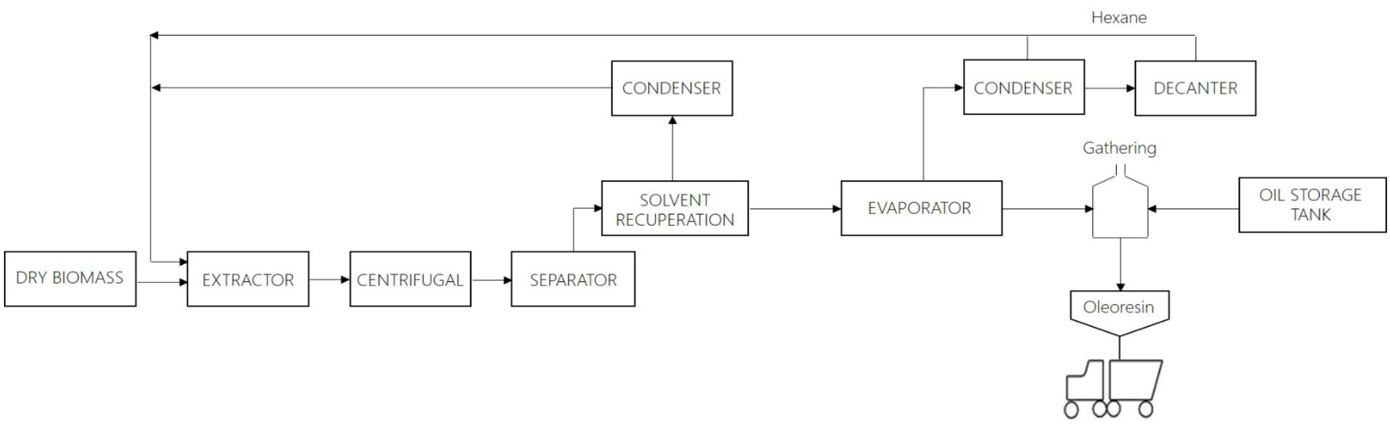

Figure 1: Flowchart diagram of the mixed carotenoid-rich oleoresin in soybean oil extraction process

The total of the operating capital represents the costs that are directly dependent on the production rate. It consists of the cost of raw materials (CRM) as well the cost of the solvent lost during the process, known asthe cost of utilities (CUT), which represents the demand for water that is required for the evaporator and condenser, electricity, waste treatment, and the cost of operational labor (COL).Within operating capital, the direct production costs included raw materials, utilities, labor costs and others (supervision, payroll charges, maintenance, operating supplies, general plant overheads, tax, and contingency). A percentage method was employed to calculate the different items [31]. The amount of the required raw materials was calculated from mass balances, whereas the consumption of utilities was estimated from the power consumption of the process that considered a value of 2% of the plant's capital for an overall utility cost [32,33]. The costs of raw materials were obtained through the selling prices of the market.

The direct labor costs were calculated estimating five workers, during three shifts a day, working 8 h/day and earning USD 8.50 per hour. This value was multiplied by two to include labor charges and then the costs were totaled.

Results and Discussion

Carotenoid-rich biomass production

The microalgae biomass is the primary bioproduct of a microalgal biorefinery and, considering that, the carotenoids are intracellular products, and the biomass productivity is a key performance indicator of the pigments production by micro-algae (Table 2). Thus, the microalgal biomass productivity in cultivation on wastewater was 0.63 kg/m^3/d, which enables the prediction of an annual production of 569,016 tons on an industrial scale (F=10.000 m^3/d). This biomass has a total carotenoids concentration of 183.03 mg/kg with the possibility of an annual production of 107,902.5 kg/year.

Qualitatively, Figure 2 shows the carotenoids profile of the microalgal biomass. Twenty carotenoids were found, the majority of which werethe isomers of β-carotene (42.7%), followed by the isomers of echinenone (19.4%), isomers of zeaxanthin (14.6%) and the isomers of lutein (13.1%). Others minority carotenoids comprised by 10.1% of the total.

Moreover, *Phormidium autumnale* biosynthesized some unique types of ketocarotenoids and glycosylated carotenoids (Figure 3), wherein all-*trans*-canthaxanthin and all-*trans*-myxoxanthophyll are exclusively present in the microalgal, besides the already reported isomers of echinenone. The potential bioactivity of these carotenoids should be considered in addition to the coloring capacity.

Determination of the cost analysis

The cost estimate of a natural mixed carotenoids-rich oleoresin production facility was determined using the description of the process proposed. The equipment that was utilized, including its size and type,is described in Table 1. The most costly of the MEC was the evaporator, followed by the extractor. The total amount of the MEC totals USD 25,796,500.00.

The installation expenses are shown, including the deployment, instrumentation, piping, and other elements necessary, resulting in a total fixed capital investment of USD 70,424,445.00. Considering a lifetime of 10 years, the annual fixed capital, required to keep the facility in operation, was estimated as USD 7,977,116.23 per year (Table 3).

Within the total operating capital, the direct production costs such as raw materials, utilities and labor were the main entries. Table 3 shows that the total of the raw materials was summarized as USD 19,218,814.90, wherein the consumption of hexane utilized for extraction was the major cost. The utilities expenses, based on water demand, power consumption and wastes treatment, were estimated as USD 31,683,528.60. In addition, other costs (labor, supervision, payroll charges, maintenance, operating supplies, overheads, taxes and contingencies) reached USD 14,826,276.16. In this sense, the total operating capital was estimated as USD 73,705,735.90 per year.

Regarding the analysis of the major costs of the process, the MEC showed that the evaporator represents an amount close to 54% of the total facility, followed by the extractor with 35%. The fixed capital investment, over 10 years of depreciation, contributed to approximately 10.8% to the cost of the process. The remaining 89.2% of the production cost originated the total operating capital. Depreciation charges contributed an approx. 9.2% to the annual production cost, and raw materials and utilities 26% and 43%, respectively, to the production cost.

Based on the determination of cost analysis, the calculation basis of the industry in analysis (1,693,500 kg per day of biomass), and considering the biomass microalgae formation, it is possible to predict a cost of USD 0.03 cents per kilogram of the dried biomass [34]. The natural mixed carotenoid-rich oleoresin in soybean oil cost production was estimated as USD 146.9 per kilogram.

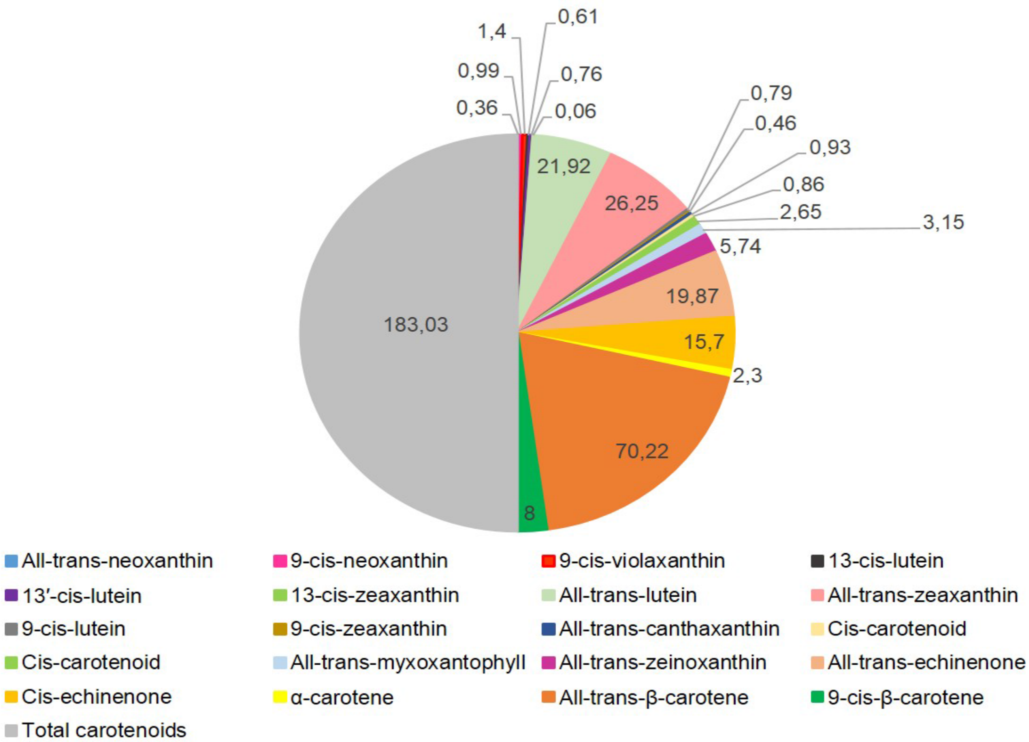

Figure 2: Identification of the carotenoids obtained by HPLC–PDA–MS/MS and their contents in μg/g

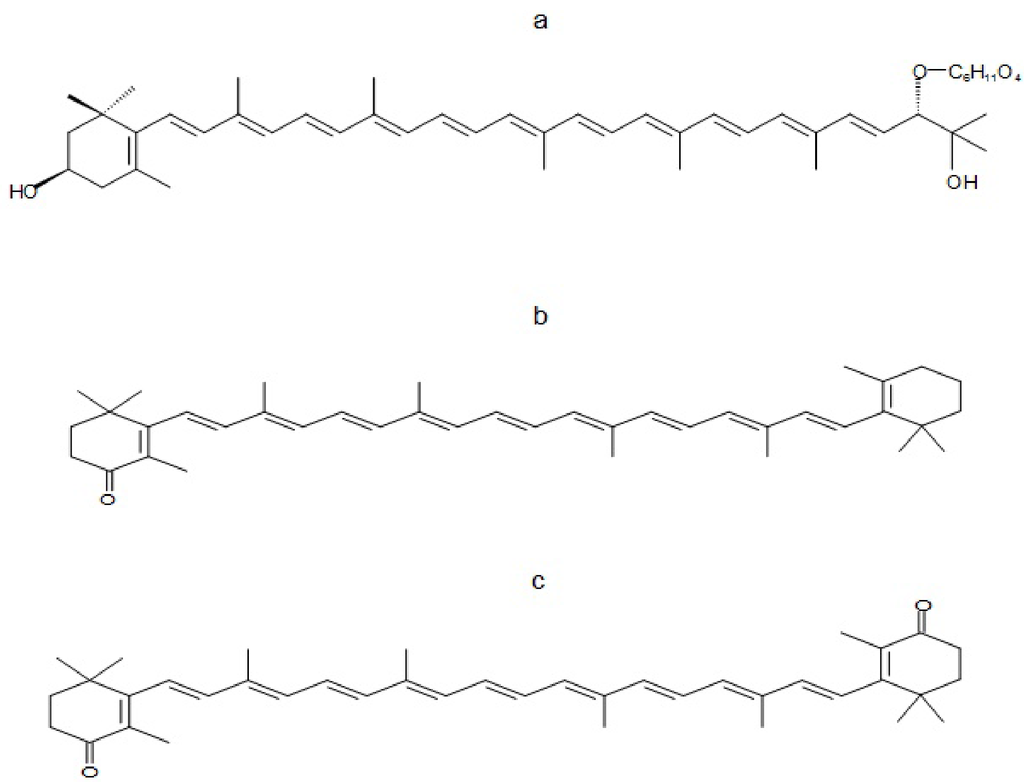

Figure 3: Ketocarotenoids and glycosylated carotenoids present in *Phormidium autumnale*. (a) all-*trans*-myxoxantophyll (b) all-*trans*-echinenone (c) all-*trans*-canthaxanthin.

Items	Units	Cost USD
Extractor (7,790.1 m³)	1	9,108,600.00
Centrifuge (13.5 m)	1	1,368,500.00
Separator (13.5 m)	1	190,000.00
Evaporator (6,397.6 m²)	1	13,946,800.00
Condenser (887.1 m²)	1	850,500.00
Storage tank hexane (6,774 m³)	1	85,000.00
Storage tank methanol (25.4 m³)	1	43,200.00
Storage tank oil (1.6 m³)	1	33,700.00
Storage tank water (11,290 m³)	1	90,400.00
Centrifugal pump (416.6 m³/h)	2	79,800.00
Total MEC (USD)		**25,796,500.00**

Table 1: Major equipment costs used in the extraction process of the oleresin

Parameter	Value
Biomass volumetric productivity (g/m³/d)	630
Biomass production (ton/year)	569,016
Total carotenoids concentration ($\mu g_{carotenoids}/g_{biomass}$)	183.03
Total carotenoids production (kg/year)	107,902.5

Table 2: Kinetic parameters and mass balance for microalgal biomass and carotenoids production in a microalgal biorefinery

Fixed capital investment		
Items	Factor	Cost (USD)
1. MEC	1.0	25,796,500.00
2. Instalattions	0.2	5,159,300.00
3. Instrumentation and control	0.4	10,318,600.00
4. Piping	0.4	10,318,600.00
5. Eletrical	0.09	2,321,685.00
6. Buildings	0.11	2,837,615.00
7. Services	0.14	3,611,510.00
8. Land	0.06	1,547,790.00
9. Engineering and supervision	0.13	3,353,545.00
10. Contractor's fee (0.05 Σ items 1-8)	0.05	3,095,580.00
11. Contingency	0.08	2,063,720.00
***A* (USD)**		**70,424,445.00**
Depreciation (Σ items 1-7, 9-11)/10 years		6,825,103.50
Property tax (0.01 depreciation)	0.01	68,251.03
Purchase tax (0.16 items 1-10/10)	0.16	1,083,761.70
***B* (USD)**		**7,977,116.23**
Total operating capital		
Raw materials	Total quantity	Cost (USD)
12. Hexane (USD 0.41/kg)[a]	29,466.9 m³	12,081,429.00
13. Methanol (USD 0.42/kg)[a]	25.4 m³	3,584,448.00
14. KOH (USD 0.40/kg)[b]	2,133,801.6 kg	853,520.60
15. Antioxidant (USD 28.66/kg)[c]	113,803.2 kg	3,261,599.70
16. Soybean oil (USD 0.54/kg)[d]	539.5 m³	291,338.20
Total, *C* (USD)		**19,218,814.90**
Utilities		
17. Power consumption (0.02 FCI)[e]	kWh	1,408,488.90
18. Water (USD 0.0003/kg)[f]	3.8×10^9 m³	1,138,032.00
19. Wastewater treatment (USD 2.99/m³)[g]	11,315.4 m³	11,367,903.50
20. Solid-waste disposal (USD 31.81/ton)[h]	558,601.2 tons	17,769,104.20
Total, *D* (USD)		**31,683,528.60**
Others		
21. Labor (USD 8.50/h, 3 shifts)	5 workers	685,440.00
22. Supervision (0.2 labor)		137,088.00
23. Payroll charges (0.25 labor + supervision)		205,632.00
24. Maintenance (0.04 MEC)		1,031,860.00

25. Operating supplies (0.004 *C*)		768,752.60
26. General plant overheads		1,019,913.40
(0.55 labor + supervision + maintenance)		
27. Tax (0.16 items 12-20, 24 and 25)		8,432,472.98
28. Contingency (0.05 items 12-20)		2,545,117.18
Total, *E* (USD)		**14,826,276.16**
Total production cost, *F* (*B* + *C*+ *D* + *E*) (USD)		**73,705,735.90**

[a](Koutinas et al., 2014); [b](Tabernero et al., 2012); [c](Almeida-Doria and Regitano-D'arce, 2000); [d](Glisic and Orlović, 2014); [e](Anderson, 2009); [f](Qureshi et al., 2013); [g](Buyukkamaci and Koken, 2010); [h](Meyers, 2004).

Table 3: Economic parameters used in the process

Comparatively, the products commercially sold today, characterized as natural mixed carotenoid, are Betatene®, Betanat®, Caromin® and Tocomin®. All of these products are a mixed suspension of natural carotenoids (preferentially *trans* and *cis* isomers of carotenes and xanthophylls) in vegetable oil, marketed in different concentrations. These products are isolated from different matrices as the alga *Dunaliella salina* (Betatene®), fungal *Blakeslea trispora* (Betanat®), palm fruit *Elaeis guineenses* (Caromin®), and crude palm oil (Tocomin®). Two of these products described here in above are more specifically similar with the product developed and presented in this study (Betatene® and Caromin®). The selling prices of these products are USD 12,774 and USD 12,642 per kilogram [35], respectively.Therefore, the new technological route presented in this paper could represent substantial savings per kilogram of natural mixed carotenoids-rich oleoresin produced, potentiating the economic viability of the process.

Applicability of the process

The major criteria for judging the feasibility of the process are the preliminary design and economic potential estimation, which are to be attained, and knowing the price of the final product is necessary for covering the costs involved [36]. The feasibility of the process was determined based on the techno-economic analysis a in a global scenario of the mixed carotenoid-rich oleoresin production, conducted based on a relationship of a benefit-cost ratio. In the present study, the main feasibility indicators were related, such as the economic equilibrium, profitability, rentability, and period of return on investment (Table 4).

Taking into account that the commercial products sold in the market as natural mixed carotenoids reach USD 12,800 per kilogram, the production cost of mixed carotenoid-rich oleoresin demonstrated in this study (USD 146.9 per kilogram) is shown to be extremely low. This occurs because the sources that are commercially available today are extracted of matrices that are highly expensive and difficult to obtain, handle, and extract. However, the oleoresin extracted in our process, is a product supported in a feedstock of negligible costs.

In addition, if our natural mixed carotenoid-rich oleoresin was sold at a value of USD 12,000/kg, the annual revenue would be more than USD 6 billion/year and with a profit margin of 98%. On the other hand, taking into consideration that the feedstock utilized has a negligible cost (USD 0.03 cents/kg of the dried biomass), the oleoresin may be quietly sold at a price of USD 500/kg, and yet, have a net profit estimated as USD 177,164,564.00 with a profit margin of 70.6%. The profitability of the process reports that, each year, the industry recovers approximately 251.5% of the amount invested, and when the revenue reaches the value of USD 89,144,867.10 the payment of the total costs is made. The time of return on investment was estimated as 0.39 years, which means when this period of operation is achieved the industry recovers the invested capital. These values are highly attractive, since the most companies use a value of 12% as minimum acceptable rate of return [37]. This rate is usually determined by evaluating existing opportunities in operations expansion, rate of return for investments, and other factors deemed relevant by management. However, companies operating in industries with more volatile markets might use a slightly higher rate in order to offset risk and attract investors [38]. In this sense, the feasibility of the process demonstrated that the natural mixed carotenoid-rich oleoresin obtained from the microalgae biomass of low production cost has a wide economic margin to explore industrial and commercially.

Parameter	Value
Economic equilibrium (USD)	89,144,867.10
Profitability (% per year)	70.62
Rentability (% per year)	251.50
Period of return on investment (year)	0.39

Table 4: Economical feasibility indicators of process.

Conclusion

The techno-economic modeling of the process demonstrated that the production cost of natural mixed carotenoid-rich oleoresin in soybean oil was USD 146.9/kg.

The feasibility analysis for the industrial applicability of the technology proposed showed that if the commercial value of mixed carotenoid-rich oleoresin is estimated as USD 500/kg, it is possible to obtain a 70.6% profit margin.

Accordingly, the oleoresin production in biorefinery systems can contribute to the technology consolidation of waste-pigment-utilization.

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