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Simultaneous extraction of sunflower oil and active compounds from olive leaves using pressurized propane

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ABSTRACT

Sunflower is grown in different parts of the world and oil from the grain has many uses, including cosmetics and food. Olive leaves are rich in active compounds with potential for industrial use. The simultaneous extraction of raw materials is an economical and sustainable way of using the same extraction process to obtain products with high added value. The aim of this work was to promote the incorporation of bioactive compounds from olive leaves in sunflower oil by two extraction techniques: pressurized propane (PRO) and Soxhlet (SOX) and to evaluate the increase in oxidative stability and antioxidant activity of oils. The techniques used were useful in producing sunflower oil incorporating olive leaf extract (SFO + OLE); 4.3% 1-octacosanol and 5.8% 1-triacontanol were incorporated, and β-sitosterol increased by at least 90%. Also, SFO + OLE showed an increase in the induction time of 2.7 and 3.7 h compared to SFO for the PRO and SOX methods, respectively. The profile of fatty acids was maintained, with the majority in all samples being oleic and linoleic acids. Consequently, with this procedure is possible to produce SFO + OLE with better antioxidant activity and better nutritional characteristics using PRO and SOX. The scaled-up of the simultaneous extraction process via pressurized propane is economically viable according to the process simulation and economic evaluation.

Practical Applications: This research has scientific relevance. It shows that sunflower oil is improved by incorporating active compounds from olive leaves, both by the conventional method of extraction and by the technique with pressurized propane. The use of clean technology to increase the nutraceutical content of products is necessary for the agri-food industry. Extraction with pressurized propane has become one of the most promising forms of technology for obtaining lipids and, at the same time, incorporates active compounds that provide a highly competitive potential to the final product. This research shows that olive leaves and sunflower seeds can be combined in the extractor in a structured bed, increasing the active compounds and essential fatty acids in the final product.

1. Introduction

The sunflower (Helianthus annuus L.) is an agricultural crop of great importance in the international market. It is one of the four most crucial oilseed crops globally (along with palm, soy, and rape). Around 90% of the sunflower grain oil (SFO) produced is for human consumption and almost 10% is used for biodiesel production and industrial applications (Martínez-Force et al., 2015). A projection of this scenario predicts that the production of sunflower grains will grow by at least 20% by 2050 (Domínguez Brando and Sarquis, 2012). Sunflower grains contain approximately 55% oil, which can vary according to several environmental factors such as the soil, climate, variety, and other cultivation factors (Sagiroglu and Arabaci, 2005).

About 90% of the fatty acids (FAs) present in SFO are unsaturated, and oleic and linoleic acids represent most of the FAs (Nimet et al., 2011; Pérez-Vich et al., 1998). The high concentration of α-tocopherol compared to other vegetable oils is advantageous (Martínez-Force et al., 2015; Ribeiro et al., 2015). Among its health benefits, SFO also induces a decrease in plasma lipoprotein total cholesterol, i.e., its ingestion avoids

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one of the main factors that cause arteriosclerosis.

During the processing of SFO and other oils of plant origin, lipid oxidation is considered one of the most critical factors affecting its quality attributes, polyunsaturated fatty acids (PUFAs) being the saturated fatty acids (FAs) most sensitive to factors such as temperature and oxygenation. Such deterioration reduces the oil's service life (da Silva and Jorge, 2012; Yang et al., 2016). This process is harmful to humans due to undesirable changes in oil characteristics (Malheiro et al., 2013). Synthetic antioxidants such as butylhydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ), and propyl gallate (PG) are used to prolong the shelf life of the product (Durán and Padilla, 1993). However, research shows that these compounds are toxic and carcinogenic when consumed frequently (Botterweck et al., 2000; Lanigan and Yamarik, 2002; Saito et al., 2003).

Due to its composition, the sunflower oil presents nutraceutical and therapeutic potential that are emphasized with the increase of other bioactive compounds and improvement in its antioxidant activity (Martínez-Force et al., 2015; Muhammad Anjum et al., 2012). Then, active plant compounds can be used as natural antioxidants in vegetable oils, promoting a reduction in the use of toxic compounds for health reasons (Chiou et al., 2009; Jaski et al., 2019a,b; Jimenez et al., 2011). For example, the use of plant phytosterols in the development of functional foods containing physiologically active components is reported in the literature (Herrero et al., 2011; Xavier and Mercadante, 2019). Consequently, it is important to quest new plant sources that can add quality to the oils and minimize the use of synthetic substances.

Examples of active compounds important for incorporation into foods are phytosterols, which in plants are structural components that stabilize biological membranes and also serve as precursors in the synthesis of essential compounds (Moreau et al., 2002; 2018a). When consumed, phytosterols can lower LDL cholesterol. Consequently, there has been a significant increase in the emergence of functional foods enriched with phytosterols in recent years (Moreau et al., 2018a). Due to the various benefits that phytosterols bring to the body when ingested, foods enriched with phytosterols are increasingly developed and intended by the food industry (Scholz et al., 2015). Among the advantages of ingesting phytosterols, there is a lower risk of diseases such as esophageal cancer (Ramprasath and Awad, 2015; Shahzad et al., 2017). The active compound octacosanol is a primary aliphatic alcohol and the main component of wax extracted from plant leaves (Taylor et al., 2003) Octacosanol is one of the main constituents of policosanol, a mixture of long-chain aliphatic alcohols (20–36 carbons) formed in addition to octacosanol (C28), triacontanol (C30), hexacosanol (C26), and heptacosanol (C27) (Fernández-Arche et al., 2009). Policosanol shows efficiency in reducing LDL and increasing HDL (Gong et al., 2018).

Also, Olive leaf extract (OLE) has been studied for its high capacity to promote the enrichment of vegetable oils, increasing their shelf life (Jaski et al., 2019a,b; Jimenez et al., 2011). However, there is no evidence from studies that assessed olive leaf active compounds being incorporated into SFO to promote more significant antioxidant activity and resistance to oxidation. Besides that, there are techniques to be explored for this incorporation, such as the use of pressurized propane. These technologies are exploited to improve agro-industrial products (Gullón et al., 2018; Herrero et al., 2011; Souilem et al., 2017).

Conventional extraction techniques, such as the Soxhlet method, and alternative techniques, such as extraction with pressurized fluids, are commonly used to obtain vegetable oils and plant extracts. Conventional extraction generally uses toxic organic solvents (such as methanol, hexane, and chloroform) that leave residues which can damage the health of the consumer, in addition to causing environmental concerns during their disposal. Pressurized fluid extraction techniques are considered more efficient and less aggressive to the environment and are gradually replacing conventional methods (Knez et al., 2019). In this way, ecologically cleaner extractions often use supercritical and subcritical conditions. The high selectivity, absence of light and oxygen, and use of lower temperatures, which avoids the degradation of thermolabile compounds, are the advantages of these techniques that result in high-quality extracts. Also, in these extraction methods, solvents are easily removed from the extract, and the extraction process is faster than the conventional one (Correa et al., 2016; Cuco et al., 2019a, b; C. M. da Silva et al., 2018; R. P. F. F. da Silva et al., 2016; Knez et al., 2019).

Extractions under pressurized conditions are also associated with a reduction in energy consumption and increased solvent efficiency when elimination of post-processing steps are achieved. The high solubility of propane in vegetable oils makes it widely used for obtaining oils. The results obtained are effective, using a small amount of solvent in a shorter time than carbon dioxide (CO2), which is the solvent most commonly used among pressurized fluids (Ahangari and Sargolzaei, 2012; Knez et al., 2019; Pederssetti et al., 2011; Zanqui et al., 2014). N-propane is a gas that does not leave any residue in food, and this is one of its advantages for use in the extraction of natural products. As a gas, propane evaporates quickly and does not remain in the oil obtained after extraction. Propane is a safer gas for obtaining residue-free oils, and for this reason, it is being widely studied as an alternative green solvent for obtaining natural and edible products. (Chemat et al., 2019; Knez Hrnčič et al., 2018; Oliveira et al., 2021). Due to the potential for exploiting OLE, the high quality of SFO, and the scarcity of studies in the literature on the use of OLE to incorporate bioactive compounds during the extraction process, obtaining a high value-added product is the intention of this study. Such combination of two high-quality vegetable matrices in the same extraction process aims to incorporate active OLE compounds in SFO, consequently an increase its oxidative stability and antioxidant capacity is also expected. Therefore, the open literature reports several processes for extracting bioactive compounds from olive leaves using a variety of solvents. The extraction using edible vegetable oils as co-solvent in a pressurized medium with n-propane, solvent, is innovative.

In this context, this work aimed to obtain sunflower oil with improved levels of active compounds, nutritional properties, and oxidative stability, using a combination of sunflower grains and olive leaves in the extraction process. The oil and extract yield, fatty acids profile, content of active compounds, antioxidant activity, and oxidative stability were determined and statistically analyzed. Next, results and insights of the experimental analyses are used to simulate a scaled-up extraction process aiming to assess the economic perspective of the investigated scenario.

2. Materials & methods

2.1. Materials

Peeled sunflower grains and dehydrated olive leaves were the two vegetable matrices used in the extractions. Sunflower grains were purchased at a store specializing in natural products in Maringá, Paraná, Brazil (November 2019 crop). Olive leaves of three varieties (Arbequina, Koroneiki, and Arbosana), cultivated commercially at high altitude in the Brazilian state of São Paulo (22° 00′ 48.6″ S, 46° 37′ 59.4″ W) were pruned for use in this research. Mid-December was the time chosen for collecting the leaves, before harvesting the fruits, which takes place between February and March in this region of Brazil. This moment was chosen due to previous studies carried out by the local research group and reports in the literature, which showed that there is greater production of active compounds before harvesting the fruits (Alowaiesh et al., 2018). *N*-propane gas (Messer, purity 99.5%) and *n*-hexane liquid (Synth, purity 99,9%) were used in the extractions. Helium (White Martins, purity >99%), BF_3 (Sigma-Aldrich, > 99% purity), Methanol (Panreac, purity 99,9%), Heptane (Anidrol, > 99% purity), *N*,*O*-bis(trimethylsilyl) trifluoroacetamide trimethylchlorosilane (BSTFA/TMCS, Sigma-Aldrich, > 99% purity) and 5α-cholestane standard (Sigma-Aldrich, > 99% purity) were used for the analysis of active compounds and fatty acids.

The chemicals, DPPH (1,1-diphenyl-2-picrylhydrazine) (Sigma-

Aldrich, purity ≥90%), ABTS$^{\bullet+}$[2,2-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid)] (Sigma-Aldrich, ≥ 98% purity), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Sigma-Aldrich, 97% purity), methanol (Panreac, 99.9% purity), ethanol (Anidrol, 99.5% purity) and potassium persulfate (0.140 mol L^{-1}) (Anidrol, > 99% purity) were used for the antioxidant analyses.

2.2. Methods

2.2.1. Samples preparation

Grinding of the sunflower grains was carried out in a manual household mill (size <2.0 mm). The olive leaves were dried in a forced ventilation oven (model 400/4[a], Ethik Technology, Vargem Grande Paulista, SP, Brazil) at 35 °C for 36 h to reduce the moisture content from 60% to approximately 5%. The dried leaves were ground with a rotary knife cutter (model SL-30, Solab, Piracicaba, SP, Brazil) with granulometry ≤2.0 mm and homogenized. Then the samples were destined for intensification of the extraction process was carried out by clean extraction, via pressurized propane, and classic extraction, via the Soxhlet method.

2.2.2. Extraction with compressed propane

Extractions with propane were performed, in duplicate, in the same experimental apparatus described by Trentini et al. (2017). The extraction of SFO was performed from ~ 20.0 g of sunflower grains added to the extraction vessel (53.4 cm^3). Olive leaves (60 g) were also added to the extraction container (165.2 cm^3) to obtain the extract (OLE). In extractions on a structured bed, to obtain SFO + OLE, the olive leaves were added first and then the sunflower grains, in a 1 : 3 (w/w) ratio, totaling ~ 60 g of sample in the extractor (165.2 cm^3).

When pressurized, propane first interacts with the grains, extracting the oil, which in turn acts as a co-solvent in this process, extracting the compounds of interest from the olive leaves. This strategy has shown efficiency in recent studies (Cuco et al., 2019a,b; Zanqui et al., 2021).

The experiments were conducted at temperature and pressure of 60 °C and 12 MPa, respectively, which favors a higher yield of SFO, as shown in previous studies (Nimet et al., 2011). The time taken for extraction was 60 min at a constant flow rate of 1.5 mL min^{-1}, as described by Trentini et al. (2017).

Propane was pumped and pressurized using a syringe pump (Teledyne ISCO 500D) in the extraction vessel loaded with the samples. The entire extraction procedure was performed in the absence of light. An amber glass bottle was used to collect the extracted oil, and the oil mass was determined at 5 min intervals. Likewise, the mass percentage yield of OLE was determined. The ratio of extracted oil mass to grain mass was used to determine the percentage yield by mass (Eq (1)).

$$Y\ (\%) = \left(\frac{M}{Gr}\right).100 \tag{1}$$

where Y is the percentage mass yield, M is the mass of oil accumulated in the extraction in grams and Gr is the mass of grains or leaves used in the extraction in grams.

2.2.3. Kinetic modeling

The mathematical model proposed by Sovová et al. (1994) was used to describe the kinetic data for the pressurized propane extraction of SFO and SFO + OLE. The model addresses two distinct structures in the matrices: one related to the cells that had their walls broken during the milling process, the oil contained in these being considered easy to access by the solvent, and the other to intact cells (i.e., difficult-to-access oil). The mass transfer of easily accessible oil extraction occurs by convection. In contrast, due to the difficult of interacting to the oil from the intact cells, the mass transfer occurs by intraparticle diffusion. With these characteristics, the extraction curves present three distinct stages, based on the different mechanisms of mass transfer. In the first period, with a constant extraction rate, the easily accessible solute is quickly extracted due to convective mass transfer; in the second period, of falling extraction rate, the mass transfer of the difficult-to-access oil begins by a diffusion mechanism due to the reduction of oil from broken cells, and both mechanisms co-occur; in the last period, diffusion-controlled extraction, only the solute contained in the intact cells is available for extraction, so the mass transfer rate is low and limited by intraparticle diffusion (Fornari and Stateva, 2015).

The model equations for the accumulated mass of extracted oil (m) as a function of time (t) in the three different periods are:

$$m(t) = \begin{cases} \dot{m}_F Y_S t\,[1 - \exp(-Z)] & t < t_{CER} \\ \dot{m}_F Y_S \left[t - t_{CER} \exp\left(\dfrac{ZY_S}{WX_0} \ln\left\{ \dfrac{1}{1-r}\left(\exp\left(\dfrac{W\dot{m}_F}{m_s} \right)(t - t_{CER}) - r \right) \right\} - Z \right) \right] & t_{CER} \le t \le t_{FER} \\ m_s \left[X_0 - \dfrac{Y_s}{W} \ln\left\{ 1 + \left(\exp\left(\dfrac{WX_0}{Y_S} \right) - 1 \right) \exp\left(\dfrac{W\dot{m}_F}{m_s} \right)(t_{CER} - t) r \right\} \right] & t > t_{FER} \end{cases} \tag{2}$$

where t_{CER} is the time (min) at which extraction of the oil from the inside of intact particles starts (min), and t_{FER} is the time (min) at which extraction of easily accessible oil ends. t_{CER} and t_{FER} are given by:

$$t_{CER} = \frac{(1-r)\, m_s\, X_0}{Y_S\, Z\, \dot{m}_F} \tag{3}$$

$$t_{FER} = t_{CER} + \frac{m_s}{W\, \dot{m}_F} \ln\left[r + (1-r) \exp\left(\frac{WX_0}{Y_S} \right) \right] \tag{4}$$

Z, W, and r (hardly accessible oil fraction) are dimensionless model parameters. Z and W are defined as:

$$Z = \frac{k_F a\, m_s\, \rho_F}{\dot{m}_F (1-\varepsilon) \rho_S} \tag{5}$$

$$W = \frac{m_s k_S a}{\dot{m}_F (1-\varepsilon)} \tag{6}$$

where $\dot{m}_F$ is the CO_2 mass flow rate (g min^{-1}); Y_S is the apparent oil solubility in supercritical CO_2 solvent (g_{oil} kg_{fluid}^{-1}), determined as the slope of the linear section of the extraction curves; X_0 is the initial concentration of the extractable solutein the solid matrix (g_{oil} g_{solid}^{-1}), calculated as the ratio of the initial oil mass in the raw material and the oil-free seed feed; m_S is the mass of oil-free seed feed; ε is the bed porosity given as: $\varepsilon = 1 - \rho_{bed}/\rho_S$, where ρ_S is solid density (g cm^{-3}) and ρ_{bed} is bed density (calculated as the ratio of the oil-free solid feed and bed volume, g cm^{-3}); and $k_F a$ and $k_S a$ are, respectively, the volumetric mass transfer coefficients in the solvent and solid phases (min^{-1}), obtained from Z and W model parameters.

Agreement between the experimental and model values was evaluated by the average absolute relative deviation (*AARD*), calculated from Eq. (6).

$$AARD(\%) = \frac{100}{N}\sum_{j=1}^{N}\left|\frac{m_{oil_j}^{Exp} - m_{oil_j}^{Calc}}{m_{oil_j}^{Exp}}\right| \quad (7)$$

where N is the number of experimental data points on the kinetic curve, $m_{oil,j}^{Exp}$ is the experimental mass of oil, and $m_{oil,j}^{Calc}$ is the mass of the oil calculated by the Sovová model, both at point j.

2.2.4. Soxhlet extraction

The classic extraction was performed in Soxhlet equipment (Marconi MA 491, Brazil) in duplicate. The extraction of SFO was carried out with 10 g of ground sunflower seeds, without peel, put into filter paper envelopes. Olive leaves already dried and ground, in the same way, were weighed (10 g) and added to filter paper envelopes to obtain the extract (OLE). In the simultaneous extraction, the grains were in the basal part and the leaves in the upper part of the filter paper envelope, in the proportion 1:3 (w/w), totaling 10 g in all. The solvent used was *n*-hexane to a normal boiling point for 8 h. Removal of solvent from the samples was carried out in an air circulation oven (model 400/4[a], Ethik Technology, Vargem Grande Paulista, SP, Brazil) at a temperature of 70 °C. Standard rules were used as the basis for this extraction process (AOAC, 2004).

2.2.5. Active compounds and fatty acids

The active compounds (phytosterols and tocopherols) and FAs present in the oils and extracts were analyzed on a gas chromatograph coupled to a mass spectrometer (Shimadzu, GC-MS QP2010 SE) equipped with an automatic injector (AOC-20i). Helium was used as the carrier gas at a flow rate of 1.0 mL min^{-1} with a split ratio of 1 : 40 and an injection volume of 2 μL. The injection temperature and the GC-MS interface temperature were maintained at 250 and 280 °C, respectively, for analysis of FAs and active compounds. The temperature of the ionic source was 260 °C for both analyses. Mass spectra were recorded at 70 eV with a range of m/z 50 to 550. Compound identification was performed from the library databases NIST14.lb and NIST14.lbs.

The FA composition was determined after saponification of the samples and derivatization with BF_3 (14% in methanol)for methylation of the FAs following the procedure described by Gonzalez et al. (2013). Heptane was used for sample dilution. A ZB-Wax™ capillary column (Zebron, 30 m × 0.25 mm × 0.25 μm) was used, and the oven temperature was set at 80 °C and heated till 180 °C at a rate of 10 °C min^{-1}, followed by further heating till 240 °C at a rate of 4 °C min^{-1}. This temperature was maintained for 2 min. The total area of FAs that flowed into the capillary column was used to determine the relative percentage of each FA.

For phytosterol and tocopherol analysis, the samples were derivatized with *N,O*-bis(trimethylsilyl) trifluoroacetamide trimethylchlorosilane (BSTFA/TMCS) for 30 min at 60 °C. The 5α-cholestane standard was added (80 μL) to the derivatized samples at a concentration of 5 mg mL^{-1}. Next, the samples were diluted with heptane, thereby obtaining a solution with a concentration of 40 mg mL^{-1}. Identification and quantification of the phytosterols and tocopherols were carried out by adopting a broad-based methodology (Du and Ahn, 2002) using an SH-Rtx-5MS™ capillary column (Shimadzu, 30 m × 0.25 mm × 0.25 μm). Santos et al. (2015) reported the heating ramp of the column used during the analysis, which was also used by Trentini et al. (2017), Cuco et al., 2019a,b and Stevanato and da Silva (2019) for analysis of other vegetable oils.

2.2.6. Antioxidant activity

Analysis of antioxidant activity was performed using the DPPH (1,1-diphenyl-2-picrylhydrazine) (Boroski et al., 2015; Gyamfi et al., 1999) and inhibition of $ABTS^{\bullet+}$ radical [2,2-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid)] (Boroski et al., 2015) free radical scavenging methods using duplicates of each sample, totaling four repetitions of each oil/extract.

2.2.6.1. DPPH method.

Ethanol was used to dilute the samples of SFO, SFO + OLE, and OLE to an initial concentration of 2000 μg mL^{-1}. Samples (250, 500, 750, 1000, 1500, and 2000 μL of the solution) were transferred to test tubes containing 2.0 mL of DPPH methanolic solution. After keeping them for 30 min in the dark, absorbance at 517 nm was measured by spectrophotometer (Hach/DR 2800). The reference used was methanol. DPPH antioxidant activity was calculated by Equation (8):

$$A_{DPPH}(\%) = \left(\frac{A_{DPPH} - (A - A_B)}{A_{DPPH}}\right).100 \quad (8)$$

where A_{DPPH} is the absorbance of the DPPH solution; and A and AB are the absorbance of the samples and blank, respectively.

The sample concentration capable of reducing 50% of DPPH (EC_{50}) was calculated from the linear equation, via percentage antioxidant activity versus sample concentration (μg mL^{-1}).

2.2.6.2. $ABTS^{\bullet+}$ method.

The reference antioxidant used for the calibration curve was the Trolox standard (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). The $ABTS^{\bullet+}$ reagent was dissolved in water at a concentration of 0.007 mol L^{-1} and then reacted with a solution of potassium persulfate (0.140 mol L^{-1}), forming the ABTS radical. The solution was diluted in ethanol and calibrated on a spectrophotometer (Hach/DR 2800) until the absorbance reached 0.70 ± 0.05 at a wavelength of 734 nm.

Samples (30 mL) of SFO and OLE at concentrations of 250, 1200, and 2000 mg mL^{-1} were prepared and pipetted in test tubes. Then, 3 mL of $ABTS^{\bullet+}$ solution was added to the test tubes and homogenized. After 6 min in the dark, absorbance at 734 nm was measured on a spectrophotometer (Hach/DR 2800). The results were calculated from the Trolox calibration curve equation and expressed in mmol of Trolox per g of oil or extract.

2.2.7. Oxidative stability

The oxidative stability of the oils was determined using an 873 Biodiesel Rancimat Oxidation Stability Analyzer (Metrohm). The reaction vessel containing 3.0 g of oil was connected to the heating block of the equipment, where the sample was exposed to the airflow of 20 L h^{-1} at a constant temperature of 120 °C (Damanik and Murkovic, 2018; Farhoosh and Hoseini-Yazdi, 2014). Secondary oxidation products were loaded into the measuring vessel containing 50 mL of ultrapure water and recorded in the order of increasing conductivity. The induction time (IT) was determined automatically from the second derivative of the conductivity curve using StabNet Software (version 1.1) (Metrohm, 2019).

2.2.8. Statistical analysis

The Tukey test was used to test the contrasts between the treatment means (extraction methods and plant matrices) and to verify whether there was a difference between the treatments (SFO, SFO + OLE, and OLE) concerning oxidative stability, antioxidant activity, FAs, and IT, using SISVAR software version 5.7 (Ferreira, 2014).

Mass percentage yield results were submitted to t-student test at 5% significance using Statistica software, version 8.0 for each group of samples in each analysis. Values followed by different letters indicate significant differences.

The multivariate method of principal component analysis (PCA) was used to reduce the number of variables and to evaluate the contribution of the FAs and active compounds in the SFO, SFO + OLE, and OLE, using the computational package FactoMineR (R Studio Inc., Boston, MA, USA) (Luo et al., 2009).

2.2.9. Process modelling and simulation

In view of the possibility of scaling up this extraction process, its requirements were modeled and simulated with the process simulator

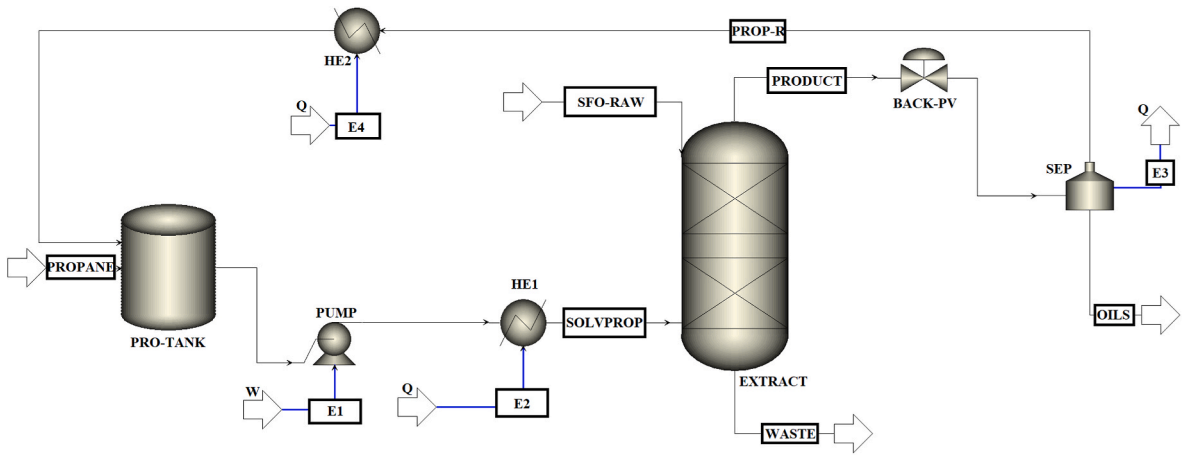

Fig. 1. Flowsheet of the continuous SFO extraction process using propane designed in Aspen Plus® V12.0.

Aspen Plus® V12.0 for the purpose of evaluating assessing its technical and economic feasibility. The chemical components present in the characterization analysis were considered and the process simulation was designed at the same experimental conditions. According to the simulator methods assistant, NRTL-RK was selected as the equilibrium calculation data and property method, with the Redlich-Kwong equation of state for fugacity coefficient (vapor phase) and the NRTL model for activity coefficient (liquid phase), based on the type of interactions existing in the system's compounds and operating conditions. The plant location for the scaled-up designed process is based on the availability of sunflower grains and olive leaves, being selected São Paulo in the southeast region of Brazil, and the process flowsheet is presented in Fig. 1. At the beginning of the process, a spare tank (TANK) is used to mix the make-up propane with process recycling in order to maintain the process solvent regeneration, which is posteriorly pressurized (PUMP) and heated and (HE1) for achieving the required process conditions. Heat losses of 5% were assumed for heat exchangers and isentropic efficiency of 80% was considered for pumping. Next, the solvent stream (SOLVPROP) is fed into the solid-liquid extractor (EXTRACT), which operates with a heating jacket to keep the isothermal process. At the exit of the extractor, the extract stream containing the products (PRODUCT) goes to a backpressure valve (BACK-PV) to return the pressure at the set point value. In sequence, the products and solvent are separated using a flash tank separator (SEP) operating at room temperature and 1 bar, where propane is recovered in the recycle stream (PROP-R) while the liquid phase containing all oils is removed at the bottom. The scaled-up scenario was designed for processing 2.4 tonnes of a mix containing sunflower grains and olive leaves per day considering 330 working days with 8 hours of work per day (i.e., 2,640 hours, and 1 month for industry maintenance), reaching an annual production of 256.6 tonnes of extracted oil.

2.2.10. Economic assessment methodology

The economic feasibility of the simulated scenario was performed considering a continuous process, and the industrial SFO extraction process using propane was assessed with evaluation of Aspen Process Economic Analyzer (APEA) supplement, which is supplement coupled to Aspen Plus® that provides operating and capital expenses (Al-Malah, 2016). The capital expenditure (CAPEX) refers to direct costs required for acquisition and installation of all process equipment with and associated components that are required for their implementation and associated facilities, also considering indirect costs of implementation (e.g., fees and remaining project expenses) (Eq. (9)). Additionally, operating expenses (OPEX) include the essential expenses for the continuous daily operation of the process and involve all general production costs (e.g., costs of utilities, maintenance, raw materials, labor and management). Complementarily, fixed costs of insurance, local charges and plant overhead, general administrative, distribution, research and development are also are also covered by OPEX (Eq. (10)) (Turton et al., 2018).

$$CAPEX\ [US\$] = C_{direct}\ [US\$] + C_{indicrect}\ [US\$] \tag{9}$$

$$OPEX\left[\frac{US\$}{yr}\right] = C_{operating}\left[\frac{US\$}{yr}\right] + C_{charges}\left[\frac{US\$}{yr}\right] + C_{general}\left[\frac{US\$}{yr}\right] \tag{10}$$

Table 1
Solvent stream and utilities prices.

Stream/Utility	Cost unity	Value	Reference
Propane	US$/kg	20	Messer Gases (2021)
Cooling Water	US$/kg	0.0004	Aspen Process Utility
Low pressure Steam	US$/kg	0.0179	Aspen Process Utility
Electricity	US$/kW	0.0775	Aspen Process Utility

For calculating part of operating costs, Table 1 presents the prices adopted based on the Aspen Process Utility costs databank and market references aligned to a similar work developed in the Brazilian scenario (Barros et al., 2022).

After evaluating capital and operating expenditures, it is necessary to correlate them by converting *CAPEX* to the equivalent annualized capital cost (*EACC*), and the capital expenses are dispersed out over the plant life time to a yearly cost according to Eq. (11). For such evaluation, the same conditions of Lima et al. (2021) (Magdeldin and Järvinen, 2020) were considered to annualize the capital expenditure in the economic analysis (i.e., the capital investment is applied on a plant life span along 10 years under an interest rate of 10%). Then, both expenditures are summed and the total annualized cost (*TAC*) is obtained according to Eq. (12).

$$EACC\left[\frac{US\$}{yr}\right] = CAPEX\ [US\$]\ .\ \frac{i\ (1+i)^n}{(1+i)^n - 1} \tag{11}$$

$$TAC\left[\frac{US\$}{yr}\right] = EACC\left[\frac{US\$}{yr}\right] + OPEX\left[\frac{US\$}{yr}\right] \tag{12}$$

where n represents the time horizon of the designed process (years) and i is the effective rate of return adopted for the investment.

Hereafter, the requisites of utilities obtained in the simulation results (i.e., electricity and thermal fluids) covered by the methodology of (Peters et al., 2003), previously mentioned, are taken into account to evaluate the remaining components of OPEX. For such costs, an important portion of the plant overhead costs and the manufacturing costs, is determined as a function of the total repair and maintenance

expenses, the supervision and direct administrative costs, and operating labor costs. Additionally, the general expenses are considered at around 15% of the sum of the EACC and remaining manufacturing costs (i.e., the total production cost). A straight-line depreciation was assumed considering 10% per year over the period of operation, which indicates a low residual value of the equipment at the end of the project's useful life.

Essentially, the formulated oil is priced higher than conventional seeds oils already produced on a large scale due to its health benefits and incorporation of bioactive compounds. The average price of the seeds oil in its fresh form is considered as US$ 66.83 per liter, which is obtained from online stores (Amazon, 2021; Americanas, 2021; Ebay, 2021; Sulu, 2021). It is necessary to highlight that instead of comparison purposes with other extraction techniques, the focus of this work is to investigate the possibility of establishing a pressurized propane extraction plant to get high-quality SFO + OLE close to the market requisites. Finally, the economic indicators selected to evaluate the economic performance of the designed scenario are the Net Present Value (*NPV*), calculated based on the difference between the annual gross profits obtained selling the product along and the equivalent annual costs (*EACC*) a whole year, and the payback period, representing the time to recuperate the capital investment (Turton et al., 2018).

3. Results and discussion

3.1. Mass percentage yield

Table 2 shows the mass percentage yields of the extractions using the different plant matrices (SFO, SFO + OLE, and OLE) and the two extraction methods, (SOX and PRO).

All the average yields of the matrices extracted alone (sunflower grains and olive leaves) or together (sunflower grains + olive leaves) were higher by the conventional method (SOX) when compared to the pressurized propane (PRO) extraction method: 46.8% higher for SFO, 3.1% for OLE, and 50% for SFO + OLE. Extraction via the Soxhlet method is known to promote the total extraction of lipids and nonpolar compounds from samples of plant origin by exhaustion, so this result was expected. Moreover, the coefficient of variation (CV) of less than 10% in all cases shows the consistency of the data obtained. The CV represents the variation of the data obtained in relation to the mean; is the sample standard deviation, an independent measure of variability and the smaller its value, the smaller the sample variation (Nicholas and Aaron, 2011).

Among the advantages of using pressurized propane as a solvent, the extraction takes place in a reduced time (1 h) and at a temperature below that used for SOX (60 °C), making it possible to minimize the degradation of thermolabile components present in the oil. According to (Nimet et al., 2011), who extracted oil from sunflower grains with supercritical carbon dioxide, it is possible to obtain up to 85% yield compared to the Soxhlet method.

SFO + OLE showed lower yields than SFO in the extraction using pressurized propane as a solvent. The same effect was reported by Cuco et al., 2019a,b, who used pumpkin peel in conjunction with pumpkin seeds and also used propane as a solvent. The low solubility of olive leaf components in propane and the shredded leaves within the extraction vessel (that act as physical impediments to the process) are factors that prejudiced the extraction yield. Besides that, the oil content in olive leaves is inherently low (Jaski et al., 2019a,b). Despite the low extraction yield using only olive leaves (1%), the composition's high quality is covered in the following sections.

Table 2
Mass percentage yield of sunflower oils (SFO), sunflower oil extracted with olive leaves (SFO + OLE), and olive leaves extract (OLE), obtained by conventional method by Soxhlet equipment (SOX) and pressurized propane (PRO).

Sample	Yield (%)		
	SOX	PRO	CV (%)
SFO	46.8^{a}±0.6	39.6^{b} ± 0.2	1.1
SFO + OLE	50.0^{a}±0.2	32.4^{b} ± 0.4	0.8
OLE	3.1^{a}±0.1	0.8^{b} ± 0.3	9.8

Mean ± Standard deviation; Different letters in the same line indicate significant difference ($p < 0.05$).

The Sovová kinetic model was used to evaluate the kinetic extraction curves. The following characteristics were used for the calculations: initial oil concentration in the inert solid = 0.67 g and bed porosity = 0.75 for sunflower grain samples; and initial oil concentration in the inert solid = 0.22 g and bed porosity = 0.75 for samples of sunflower grains + olive leaves.

Table 3 indicates the results of the adjustable parameters calculated for the Sovová model and the solubility of the oils in the solvent (propane). The dynamic method was used to calculate these parameters from the linear part of the extraction curves.

The model presented a good correlation coefficient for SFO (0.9926) and SFO + OLE (0.9988), meaning that there is a good correlation between the model variables, both for SFO and SFO + OLE.

The addition of olive leaves to the extraction bed provided an increase in the parameter r due to the more significant amount of oil mass available for extraction (oil mass of the grains + oil mass of the leaves).

The solubility values (S) calculated for the extraction with olive leaves are lower than those for the extraction performed with sunflower grains. In other words, the model indicates that the use of olive leaves in the extraction bed decreases the solvent's potential to solubilize the oil. This evidence corroborates the data from the kinetic oil extraction curves (Fig. 2) and mass percentage yield values (Table 2).

The values of the mass transfer coefficients in the solid phase (kSa) were lower than those of kFa (solvent phase) in the extraction pairs (with and without olive leaves), indicating that the reason for the highest extraction yield was the easily accessible oil. This indicates a certain barrier in the diffusion process and the consequent solubilization of oil that is difficult to access, which has been observed in other studies (Zanqui et al., 2020).

The tFER parameter indicates when the easily accessible oil extraction ends, generating a period of decreasing extraction rate. This value was lower in the sample extracted in a structured bed with olive leaves, corroborating the results that indicated a lower yield for these extractions, possibly justified by the mechanical impediment that the leaves generated in the extraction bed. This parameter also justifies not prolonging the extraction time since the difficult-to-access oil does not usually contribute significantly to the extraction yield (Sovová et al., 1994).

3.2. Fatty acids

Table 4 shows the FA composition in all plant matrices extracted by conventional (SOX) and pressurized (PRO) methods.

The lipid profile of SFO extracted with olive leaves remains the same. All the FAs present in pure SFO appear in the chromatographic profile of SFO + OLE. The primary purpose is to evaluate the acquisition of SFO + OLE. OLE also features FAs in its composition, with emphasis on saturated fatty acids (SFAs) and PUFAs (Ghanem et al., 2019), and the SOX method extracts a more significant amount of linolenic acid (25.7 ± 0.9%) while PRO extracts more oleic acid (24.0 ± 0.2%). SFO stands out for its high content of oleic and linoleic acids for the two extraction methods (PRO and SOX), an advantageous characteristic of the grains of this species, which can vary according to the agronomic characteristics of the species, varying conditions in plant cultivation, growing regions, material collection time, and other factors (Figueiredo et al., 2008; Grulova et al., 2015; Martínez-Force et al., 2015; Nimet et al., 2011; Onemli, 2012).

It is important to know the fatty acid profile of the oils, because n-6 FAs, as well as n-9 are considered essential because the human body does not produce them, and they provide health benefits such as the

Table 3
Adjustable parameters for the Sovová model.

Samples	Z	W	*r*	*S* (g oil/g solvent)	q_0	t_{CER} (min)	t_{FER} (min)	K_{Fa} (min)	K_{Sa} (min)
SFO	2.951	2.000	0.616	0.280	0.670	5.182	37.176	0.078	0.030
SFO + OLE	2.799	1.226	0.761	0.184	0.221	0.418	20.017	0.254	0.008

Z: dimensionless parameter of Sovová model, W: dimensionless parameter of Sovová model, *S*: solubility, *r*: easily accessible oil mass, q_0: initial oil concentration without solid inert, t_{CER}: time at which the extraction of the oil from the inside of particles starts, t_{FER}: time at which the extraction of easily accessible solute ends, K_{Fa}: solvent-phase mass transfer coefficient, K_{Sa}: solid-phase mass transfer coefficient.

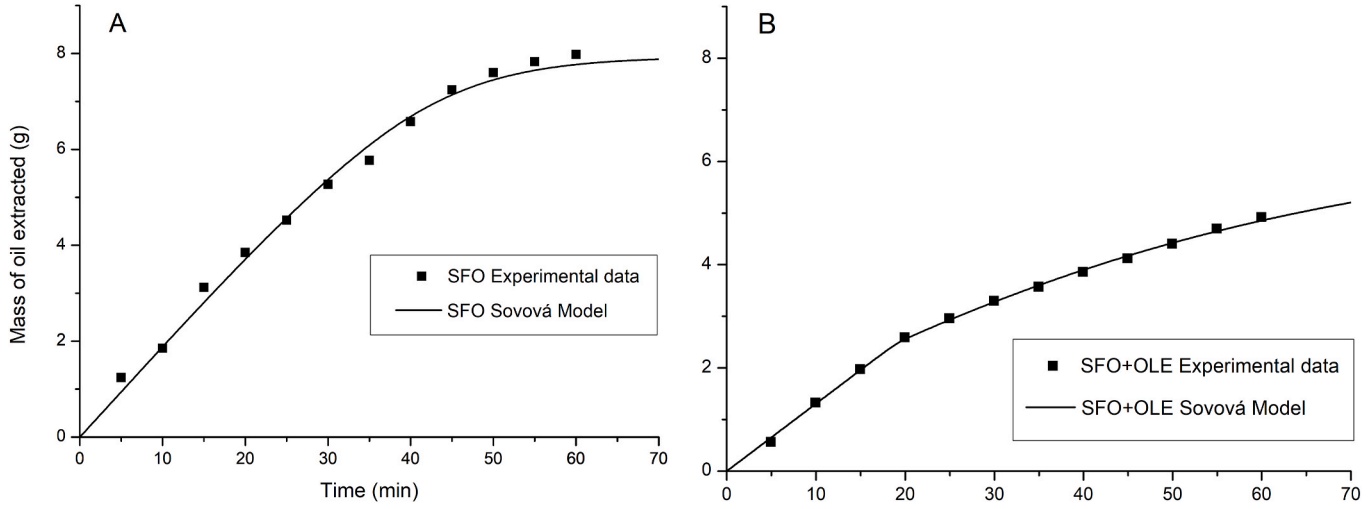

Fig. 2. Kinetic extraction curves and mathematical modeling of Sovová for sunflower oil (SFO) (A) and sunflower oil extracted with olive leaves (SFO + OLE) (B) by extraction with pressurized propane.

prevention of inflammatory diseases, cancer, cardiovascular disease, and other chronic diseases (Layé et al., 2018; Saini and Keum, 2018). The incorporation of OLE did not alter the lipid profile of oils. The process of extracting raw materials together (leaves and seeds) can be used to obtain high-quality oils, which is beneficial for consumers' health.

The first two dimensions summarize 95.56% of the chemical composition related to the FAs of SFO, SFO + OLE, and OLE (Fig. 3). The major contribution in the first principal component (PC1) is made by SFAs (palmitic, myristic, arachidic, and eicosanoic acids) and linoleic acid (a PUFA), in equidistant values (about 7%). In the second principal component, the most considerable contributions are made by the SFAs stearic acid (49.9%) and behenic acid (15.31%).

PC1 separates the oils extracted from the olive leaves on the negative axis, regardless of the method (conventional and pressurized propane), and groups the SFOs (with and without the addition of olive leaves) on the positive axis. PC2 separates the treatments by the extraction method, in the positive region by the conventional method, and in the negative region using pressurized propane as a solvent.

In Fig. 3B, on the right side of the graph, there is a higher concentration of SFAs (palmitic, myristic, arachidic, and eicosanoic acids), referring to OLE.

According to PC1 and PC2, the most significant average contribution to the final FA composition of SFO, SFO + OLE, and OLE comes from olive leaves (33%) regardless of the extraction method refers to SFA. In the experimental conditions analyzed, the olive leaves had a more significant contribution to the FA content of SFO + OLE, mainly the SFAs palmitic, myristic, arachidic, and eicosanoic acids, and linoleic acid (a PUFA).

3.3. Active compounds

To analyze the active compounds, the composition of SFO, SFO + OLE, and OLE was evaluated. The purpose of this analysis was to identify the main active compounds present in each of the matrices (isolated or together) and to quantify the increment promoted by the addition of olive leaves in SFO + OLE.

Table 5 presents the results of the quantification of active compounds for oils obtained by different methods.

In general, simultaneous extraction of olive leaves with sunflower grains provided the incorporation of active ingredients. This incorporation is evident when observing the amount of α-tocopherol in SFO and its increase in SFO + OLE, regardless of the extraction method. There was a 53% increase of this active compound using SOX and a 35% increase using SFO. The high content of α-tocopherol (about 66 mg 100 g^{-1}) in SFO is already known, and this addition of tocopherols to the oil further enhances its beneficial properties for use and consumption. In the literature, the data show that α-tocopherol is a potent antioxidant, neutralizing free radicals or reactive oxygen species (ROS) (Aggarwal et al., 2010). Also, α-tocopherol is studied to fight cancer and protect against bone, cardiovascular, eye, and neurological diseases (Aggarwal et al., 2010; Peh et al., 2016).

β-Sitosterol was the main phytosterol from olive leaves incorporated into SFO by both extraction methods. Extraction with pressurized propane promoted its incorporation in SFO + OLE (91%), while for extraction by the conventional method (SOX), 69% was incorporated when compared to SFO. Besides that, the active compounds present exclusively in the leaves of the olive tree (1-octacosanol and 1-triacontanol), which were not detected without SFO, now appear incorporated in SFO + OLE (Table 5).

The phytosterols observed in the extract and in the oil obtained are compounds of high added value that could be used in foods in the future (Moreau et al., 2018b). The extraction of these compounds with propane and with sunflower oil as co-solvent had not been studied in the literature. The present work showed that the extraction process is directly related to the incorporation of active compounds in sunflower oil.

Table 4
Composition of fatty acids in relative percentage present in sunflower oil (SFO), sunflower oil extracted from olive leaves (SFO + OLE) and olive leaf extract (OLE), obtained by conventional method by Soxhlet equipment (SOX) and pressurized propane (PRO).

	SOX			PRO		
Component (%)	SFO	SFO + OLE	OLE	SFO	SFO + OLE	OLE
Lauric acid (C12:0)	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	0.6 ± 0.1
Myristic acid (C14:0)	0.1 ± 0.1	0.1 ± 0.1	2.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	2.3 ± 0.2
Palmitic acid (C16:0)	6.1 ± 0.1	6.5 ± 0.1	23.2 ± 0.6	6.9 ± 0.1	7.2 ± 0.1	26.4 ± 0.1
Stearic acid (C18:0)	4.3 ± 0.1	4.3 ± 0.2	4.9 ± 0.3	5.1 ± 0.1	5.3 ± 0.1	6.0 ± 0.1
Linolenic acid (C18:3 3)	0.1 ± 0.1	0.4 ± 0.1	25.7 ± 0.9	0.1 ± 0.1	0.5 ± 0.1	15.5 ± 0.4
Arachidic acid (C20:0)	0.4 ± 0.1	0.4 ± 0.1	3.9 ± 0.2	0.4 ± 0.1	0.5 ± 0.1	4.5 ± 0.2
Palmitoleic acid (C16:l)	0.1 ± 0.1	0.1 ± 0.1	0.4 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.6 ± 0.1
Oleic acid (C18:l 9)	48.0 ± 0.1	47.5 ± 0.2	14.2 ± 0.2	45.6 ± 0.1	45.3 ± 0.1	24.0 ± 0.2
Linoleic acid (C18:2 6)	38.3 ± 0.1	39.0 ± 0.7	8.8 ± 0.6	38.4 ± 0.1	37.7 ± 0.1	10.6 ± 0.4
Lignoceric acid (C4:0)	0.4 ± 0.1	0.4 ± 0.1	N.D.	0.5 ± 0.1	0.5 ± 0.1	N.D.
Behenic acid (C22:0)	1.2 ± 0.1	1.2 ± 0.1	6.9 ± 0.6	1.5 ± 0.1	1.6 ± 0.1	3.2 ± 0.4
SFA	12.8 ± 0.2	13.4 ± 0.3	47.1 ± 0.1	14.8 ± 0.1	15.4 ± 0.1	45.9 ± 0.7
MUFA	49.1 ± 0.1	48.6 ± 0.6	16.3 ± 0.3	46.7 ± 0.2	46.4 ± 0.1	26.3 ± 0.7
PUFA	38.3 ± 0.1	38.5 ± 0.7	34.6 ± 0.4	38.5 ± 0.1	38.2 ± 0.1	26.1 ± 0.2

SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids. N.D.: not detected.

Ultrasonic extraction of phytosterols from olive leaves (Orozco-Solano et al., 2010) showed that the compounds found in this work (β-sitosterol, campesterol, and octacosanol) were also observed, and the major compound found was also β-sitosterol. Smaller fractions of olive oil produced from the residue containing bagasse, leaf, bark, and olive seeds contain octacosanol and triacontanol (which was also incorporated into SFO + OLE) (Fernández-Arche et al., 2009), both of which are indicated to inhibit cholesterol (Singh et al., 2006). Other studies have shown their cholesterol-lowering properties, as well as their anti-aggregating effect (Taylor et al., 2003).

The practical contribution of OLE compounds to SFO is evidenced by the PCA in the next section.

Fig. 4 shows Principal component analysis (PCA) relating the first and second principal components (Dm1 and Dm2) to the composition of active compounds (A) with the matrix and methods extraction.

The first two dimensions summarize 99.4% of the chemical composition relative to the active compounds of SFO, OLE, and SFO + OLE, extracted by different methods (conventional and pressurized propane). PC1 separates OLE on the positive axis, regardless of the extraction method, and on the negative axis, it groups OLE and SFO + OLE.

In PC1, the contribution of active compounds is very similar, both for total tocopherols (represented mainly by α-tocopherol) and for total phytosterols, with emphasis on 1-octacosanol (17.44%), β-sitosterol (16.98%), and campesterol (16.94%).

Fig. 4B shows that OLE extraction with pressurized propane (6) favored the extraction of phytosterols (1-triacontanol and stigmasterol), while OLE extraction by the conventional method (3) favored mainly the extraction of tocopherols (α-tocopherol).

According to the PCA, the extraction method has a low influence on the composition of SFO + OLE, as observed in the negative region of PC1, where all SFO and SFO + OLE are grouped. The active compound 1-octacosanol was not found in SFO but was found in SFO + OLE and OLE. Adding to its effective contribution as one of the main components, according to the PCA, it can be inferred that there was an enrichment of this phytosterol in SFO + OLE for both extraction methods. The same can be observed for the compound 1-triacontanol that was not detected in SFO but was detected in SFO + OLE and OLE in the extraction with pressurized propane. In this way, it is possible to observe the olive leaves' contribution to the formulation of an SFO enriched with phytosterols.

From this analysis, it can be concluded that the olive leaves acted as a source of phytosterols (1-octacosanol, 1-triacontanol) and tocopherol for the production of enriched SFO.

Considering the phytosterols found exclusively in olive leaves, the joint extraction of sunflower grains and olive leaves promoted the translocation of 4.6% of the 1-octacosanol and 5.8% of the 1-triacontanol in the leaves to SFO + OLE using propane as a solvent.

From this analysis, it can be inferred that there was an enrichment of these phytosterols in SFO + OLE, for both extraction methods, with more significant values obtained for extraction with pressurized propane. In this way, it is possible to observe the olive leaves' contribution to the formulation of an SFO enriched with phytosterols. This condition was viable due to simultaneous extraction of the two plant matrices, regardless of the extraction method (conventional or propane).

3.4. Antioxidant activity and oxidative stability

Table 6 shows the results for the antioxidant activity (DPPH and $ABTS^{\bullet+}$) and oxidative stability of SFO and SFO + OLE.

The statistical analysis ($p < 0.05$) shows that there was no significant interaction between the extraction method and the type of plant matrix (sunflower grains and sunflower grains + olive leaves) in the mean antioxidant activity measured by the DPPH method. However, for the ABTS method of assessing antioxidant activity, there was a difference in the average antioxidant activity (ABTS) between the extraction matrices as a function of the extraction method. For both propane extraction and the conventional method, SFO + OLE showed a higher antioxidant activity (ABTS) when compared to SFO. Olive leaves have a natural antioxidant potential to replace the synthetic antioxidants commonly used in vegetable oils.

Generally, the antioxidant content in SFO and other plant-based oils is not high. Sunflower seeds have an IC50 of around 1022 μg (Zilic et al., 2010), values similar to those found in this study (Table 6). Pumpkin seed oil has an IC50 of ~926 μg and when extracted together with the pumpkin peel results in an IC50 of ~750 μg (Cuco et al., 2019a,b), with a lower IC50 value indicating an increase in antioxidant potential, similar to the increase found in the present study with the use of propane as solvent (Table 6). Therefore, a significant increase in the antioxidant content in vegetable oils, even by small amounts, is of great interest for improving the quality of the oil, especially without the use of synthetic compounds.

Synthetic antioxidants are often used in commercial vegetable oils to increase their durability, favoring their commercialization for a longer time. The relative effectiveness of antioxidants depends on their solubility in oil, and lipophilic antioxidants more effectively protect oils against oxidation (Abdalla and Roozen, 1999; Frankel et al., 1982). Therefore, the joint extraction of vegetable matrices (sunflower grains and olive leaves) can favor the acquisition of soluble antioxidants in the same extraction solvents as vegetable oils (propane or hexane). This discovery explains the significant increase in the protection of the incorporated oil (SFO + OLE) with the compounds of the olive leaves.

Table 6 shows the comparison between the IT in oils extracted from grains (SFO) and from seeds + leaves (SFO + OLE). SFO extracted with propane showed an IT of approximately 1.8 h, similar to that found in previous experiments by other researchers (Nimet et al., 2011), and the conventionally extracted oil had an IT of 1.2 h. The statistical analysis showed no difference in the mean IT for the two oils ($p < 0.05$)

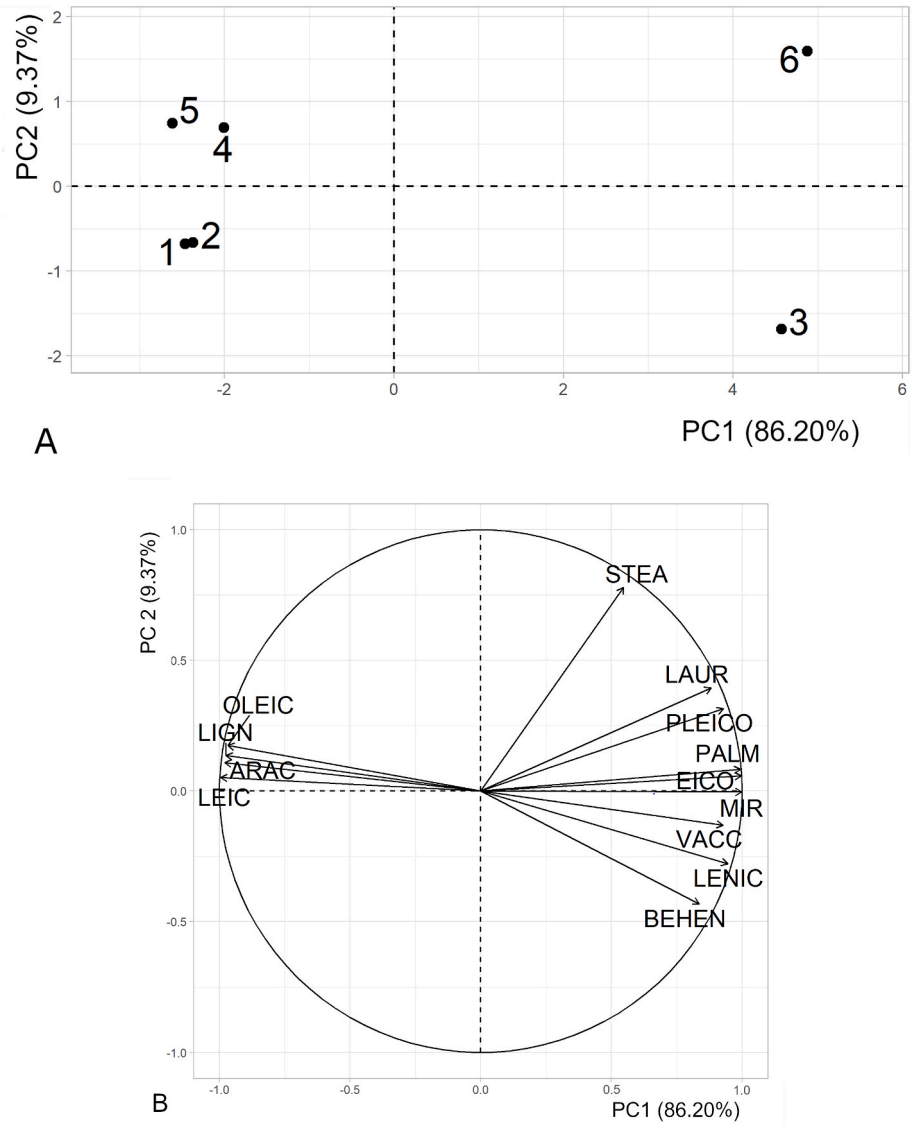

Fig. 3. Principal component analysis (PCA) relating the first and second principal components (PC1 and PC2) to the fatty acids composition (A) with the extraction matrix (B) of the SFO, SFO + OLE, and OLE. Mi: myristic acid; Pl: palmitic acid; Pleic: palmitoleic acid; Est: stearic acid; Ol: oleic acid; Leic: linoleic acid; Arac: arachidic acid; Lenic: linolenic acid; Eico: ecosenoic acid; Beh: behenic acid; Vacc: vaccenic acid; Lign: lignoceric. Codes 1 to 3: Soxhlet extraction method; Codes 4 to 6: Pressurized propane extraction method.

Table 5
Quantification of the active compounds (in mg.100g-1) present in sunflower oil (SFO), sunflower oil extracted from olive leaves (SFO + OLE), and olive leaf extract (OLE), obtained by conventional method by Soxhlet equipment (SOX) and pressurized propane (PRO).

Sample	Active compounds (mg per 100 g oil)											
	α-Tocopherol		1-Octacosanol		Campesterol		Stigmasterol		Triacontanol		β-Sitosterol	
	SOX	PRO	SOX	PRO	SOX	PRO	SOX	PRO	SOX	PRO	SOX	PRO
SFO	65.9 ± 0.3	66.3 ± 3.8	ND	ND	16.8 ± 1.1	12.7 ± 0.4	15.5 ± 0.9	15.4 ± 0.7	ND	ND	81.9 ± 1.9	66.4 ± 2.5
SFO + OLE	100.9 ± 2.6	89.8 ± 2.7	11.5 ± 2.8	15.7 ± 1.1	12.8 ± 1.3	12.4 ± 0.1	16.1 ± 1.7	16.3 ± 0.5	ND	8.5 ± 0.3	138.5 ± 0.1	127.0 ± 0.6
OLE	894.0 ± 0.3	631.4 ± 8.4	286.8 ± 1.0	287.0 ± 4.7	ND	ND	36.0 ± 1.8	53.6 ± 0.9	97.6 ± 0.3	147.3 ± 5.9	1158.6 ± 3.7	960.5 ± 22.7

ND: not detected.

according to the extraction method. Regarding matrices, SFO + OLE was more resistant to oxidation than pure SFO.

SFO + OLE showed an increase in IT of about 2.7 and 3.7 h compared to SFO for the PRO and SOX methods, respectively. There was an increase in IT of at least 150%. This increase is more significant than the 2.5 h reported by Farahmandfar et al. (2018), who used natural antioxidants from lemon essential oil in SFO after oil extraction. The increase in IT is similar to that found for the synthetic antioxidant TBHQ

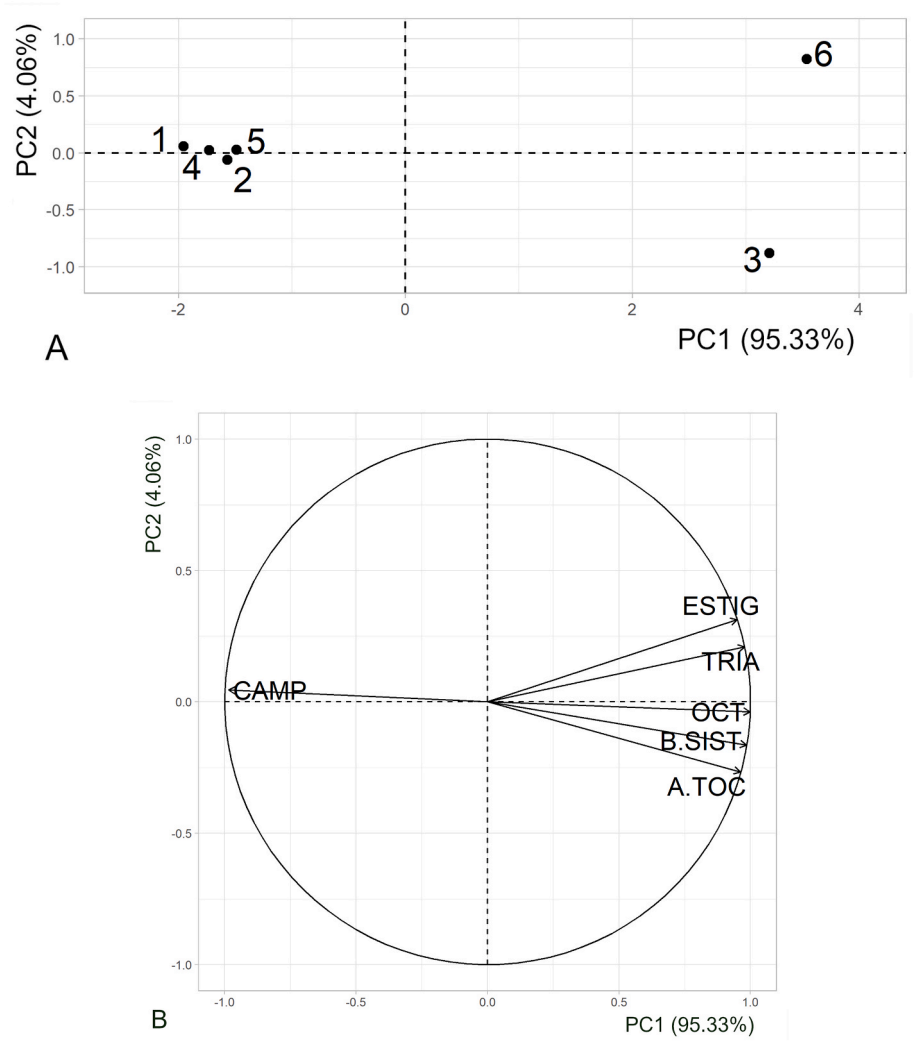

Fig. 4. Principal component analysis (PCA) relating the first and second principal components (PC1 and PC2) to the composition of active compounds (A) with the matrix and methods extraction (B) of the SFO, SFO + OLE, and OLE. OCT: 1- octacosanol; ATOC: α-tocopherol; CAMP: campesterol; STIG: stigmasterol; TRIA: 1-triacontanol; BSIS: β-sitosterol; Codes 1 to 3: Soxhlet extraction method; Codes 4 to 6: Pressurized propane extraction method.

Table 6
Antioxidant activity determined by the DPPH and ABTS • + methods and oxidative stability determined by Rancimat of sunflower oils (SFO) and sunflower oil extracted from olive leaves (SFO + OLE); oils obtained by conventional method by Soxhlet equipment (SOX) and pressurized propane (PRO).

	SOX			PRO		
	ABTS (μmol de Trolox g $^{-1}$oil)	IC_{50} (μg ml^{-1} oil)	OXS (h)	ABTS (μmol de Trolox g $^{-1}$oil)	IC_{50} (μg ml^{-1} oil)	OXS (h)
SFO	1.7 ± 0.6 ^{Ba}	1060.9 ± 127.5 ^{Aa}	1.2 ± 0.2 ^{Ba}	4.2 ± 0.7 ^{Ba}	1003.5 ± 39.5 ^{Aa}	1.8 ± 0.2 ^{Ba}
SFO + OLE	13.6 ± 4.1 ^{Aa}	1002.3 ± 71.7 ^{Aa}	4.9 ± 0.1 ^{Aa}	16.9 ± 1.4 ^{Aa}	882.8 ± 3.4 ^{Aa}	4.4 ± 0.2 ^{Aa}

Different capital letters in the columns indicate differences between the different plant matrices concerning the method. Different lowercase letters in the lines indicate the difference between the different methods about the same plant matrix. Both analyzes by the Tukey test at 5% significance.

(3.1 h) used in SFO (Upadhyay and Mishra, 2015). This technology may be feasible for replacing the synthetic antioxidants commonly added to SFO, taking advantage of a residue from olive growing.

These data demonstrate the high potential of using this process to obtain oils with better oxidative stability without synthetic antioxidants after extraction. The addition of OLE to the extraction process provided the oils with a longer IT than in other studies. The increased resistance of vegetable oils to degradation is essential for the food industry. Increasing the shelf life of vegetable oils without synthetic antioxidants is what industries need to add value to the final product and not harm consumers' health.

3.5. Scaled-up process performance and economic perspectives

Tables 7 and 8 presents the mass and energy balances, respectively, of the process simulation performed in Aspen Plus, indicating compositions and operating conditions of the main streams of the process, and the energy flows of equipment. From the perspective of energy consumption of the process, it is notable that heat exchangers for adequacy of solvent temperature and flashing the products for separation are the main responsible operations for thermal energy requirements, while pressurizing the solvent covers almost the total electricity requirement. Moreover, because of the process demand in each process unit, the equipment data sizing obtained by APEA supplement for the industrial

Table 7
Mass balance and conditions of the process streams.

Stream	PROPANE	SOLVPROP	SFO-RAW	PRODUCT	WASTE	PROP-R	OILS
Temperature (°C)	25	60	60	60	60	40	25
Pressure (bar)	10	120	120	120	120	10	10
Mass flow (kg/hr)	36.5	365.4	300.0	568.2		328.9	97.2
Mass frac propane	1.0000	1.0000	–	0.6785	–	1.0000	–
Mass frac solids	–	–	0.6796	–	0.9999	–	–
Mass frac lauric acid	–	–	0.0003	0.0003	7.1475E-10	–	0.0010
Mass frac myristic acid	–	–	0.0003	0.0003	7.3153E-11	–	0.0010
Mass frac palmitic acid	–	–	0.0233	0.0233	1.5963E-09	–	0.0728
Mass frac stearic acid	–	–	0.0171	0.0171	1.8389E-10	–	0.0536
Mass frac linolenic acid	–	–	0.0016	0.0016	7.3153E-11	–	0.0051
Mass frac arachidic acid	–	–	0.0016	0.0016	3.1067E-12	–	0.0051
Mass frac palmitoleic acid	–	–	0.0003	0.0003	1.4827E-11	–	0.0010
Mass frac oleic acid	–	–	0.1468	0.1468	7.9836E-09	–	0.4580
Mass frac linoleic acid	–	–	0.1221	0.1221	8.1228E-09	–	0.3812
Mass frac lignoceric acid	–	–	0.0016	0.0016	4.5938E-10	–	0.0051
Mass frac behenic acid	–	–	0.0052	0.0052	3.8297E-12	–	0.0162

Table 8
Energy balance of each process unit.

Unit	Electric energy (kWh)	Thermal energy (kW)
Pump	182.650	0.00
Heater (H1)	0.212	3,656
Heater (H2)	0.611	1,037
Flash	0.000	430
Total	183.473	5,123

Table 9
Equipment data sizing of the designed process.

Name	Description	Sizing	Cost (US$)
PRO-TANK	Mixer tank	Volume = 500 L	677,300
PUMP	Pressurization pump	Flow capacity = 138 L/min	347,700
HE1	Heat exchanger	Area = 95 m^2	485,430
EXTRACT	Isothermal extractor	Volume = 515 L	2,526,900
BACK-PV	Backpressure valve	–	10,120
HE2	Heat exchanger	Area = 21 m^2	231,600
SEP	Phase Separator	Volume = 100 L	336,400
	Total		**4,615,450**

scenario are listed in Table 9 following the nomenclature of Fig. 1, added by unit costs. The extractor plays a key role in the process and equipment highlights, with a volume of 515 L designed with an internal height and diameter of 4.88 and 0.46 m, respectively, which is a feasible proportion for reservoirs in extractive processes.

Based on the size of equipment previously described, the capital expenditures were predicted for acquisition and installing the industrial-scale plant, and are showed in upper section of Table 10 also considering indirect expenses demanded to build it. In view of a representative and complete economic estimation, the charges and property insurance taxes evaluated are around at 4% and 1%, respectively, referencing total CAPEX (Table 10).

Following the economic assessment with all costs, an industry with the potential of processing 792 tonnes per year of grains/leaves reaches a processing cost estimated at around 6.93 US$ per kg of raw material. Consequently, in terms of investment distribution, costs converted directly from the mass balances indicate that the final oil may be obtained at a cost of 31.5 US$ per tonne of oil. Overall, the associated costs in terms of their annual fractions represent 4.1% and 95.9% for the CAPEX and OPEX, respectively, and, depending on the applications and the commercialization market purity requirements, profitable scenarios are expected.

Under the economic assessment, Fig. 5 presents a cumulative cash flow diagram if the planning/construction period of the industrial plant is executed in 2 years. Along this period, the required initial investments

Table 10
Capital (CAPEX) and Operating (OPEX) expenses of the designed scenario.

Capital expenditure description		Cost (US$)
Direct cost	Purchased equipment and accessories (85% of CAPEX)	4,615,450
Indirect costs	Engineering and supervision (5% of CAPEX)	271,497
	Legal expenses (1% of CAPEX)	54,299
	Construction expenses and contractor's fee (4% of CAPEX)	217,198
	Contingency (5% of CAPEX)	271,497
Total CAPEX		5,429,941
Operating expenditure description		**Cost (US $/year)**
Manufacturing costs	**Production costs**	11,213,122
	Raw material (sunflower grain and olive leaf)	7,926,577
	Operating labor (OL)	343,600
	Utilities (Predicted by Aspen Plus)	825,454
	Make-up Propane	1,929,312
	Maintenance and repair (MR; 2% of CAPEX)	108,599
	Operating supplies (10% of MR)	10,860
	Laboratory charges (10% of OL)	34,360
	Direct Supervision and clerical labor (DSC, 10% of OL)	34,360
	Fixed charges	786,274
	Local taxes (4% of CAPEX)	217,198
	Insurance (1% of CAPEX)	54,299
	Interest (5% of CAPEX)	271,497
	Plant overhead costs (50% of OL, MR and DSC)	243,279
General costs	**15% (EACC + OPEX)**	1,889,640
	Administrative costs (5% of OPEX)	629,880
	Distribution and marketing (5% of OPEX)	629,880
	Research and development (5% of OPEX)	629,880
Total OPEX		13,889,036

before starting up the industry are the capital expenses to build the plant and the floating capital to start its operation (both values indicated in Table 10). The final NPV is calculated as positive (1.05×10^7 US$ after 10 years) and the required period to recuperate the initial investment (payback) is 6.2 years, i.e., 8.42 years if the construction period is considered. Consequently, even under different design and operating conditions the NPV and payback period values are in accordance with the results found in similar works (Kayathi et al., 2020, 2021). Overall, the studied extractive process may be considered economically feasible since the operating conditions and process performance are maintained.

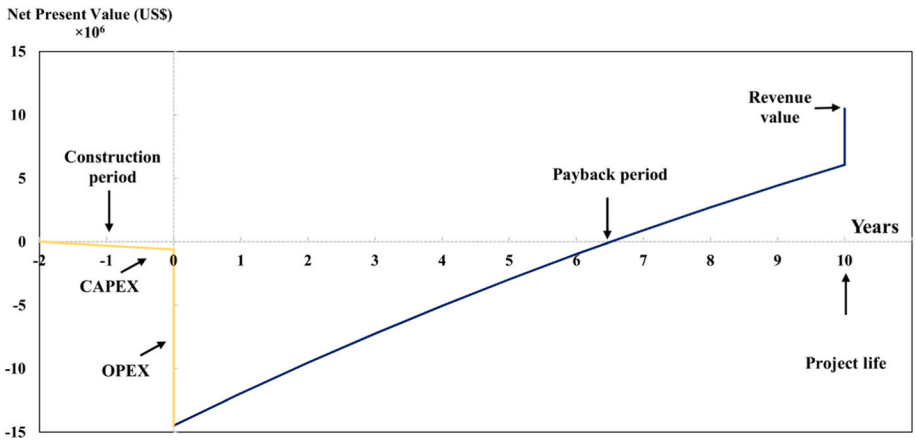

Fig. 5. Cash flow diagram of the scaled-up extractive plant of SFO + OLE.

4. Conclusion

The use of olive leaves simultaneously with sunflower grains in a structured bed for extraction with pressurized propane and for conventional Soxhlet extraction with hexane proved effective for incorporating active compounds, with emphasis on 1-octacosanol and β-sitosterol. There was a 53% increase in α-tocopherol in sunflower oil incorporated with olive leaf extract by the conventional extraction technique and a 35% increase in pressurized propane extraction. Also, there was a significant of increase greater than 3 hours in the IT for oils obtained from olive leaves, which implies a product with more significant antioxidant potential. The economic evaluation elucidated the economic potential of establishing a unit for simultaneous extraction of sunflower seeds and olive leaves with pressurized propane. The process is considered economically feasible with a positive NPV and a payback period of 6.2 years. Thus, this study concludes that it is possible to use an innovative and cleaner process without toxic solvents and a conventional process for the aggregation of chemically active compounds in sunflower oil by the combined use of vegetable matrices. This process has advantages due to the small amounts of solvent required, short extraction time, elimination of post-processing steps, and a high potential for promoting the products' healthiness.

CRediT authorship contribution statement

Jonas Marcelo Jaski: Writing – original draft, Writing – review & editing, Investigation, Conceptualization, Formal analysis, Data curation. **Karen Keli Barbosa Abrantes:** Writing – original draft, Formal analysis, Data curation. **Ana Beatriz Zanqui:** Writing – original draft, Writing – review & editing, Conceptualization. **Natalia Stevanato:** Formal analysis, Data curation, Writing – original draft. **Camila da Silva:** Formal analysis, Data curation, Supervision. **Carlos Eduardo Barão:** Supervision, Conceptualization, Investigation. **Lucas Bonfim-Rocha:** Writing – original draft, Formal analysis, Data curation. **Lúcio Cardozo-Filho:** Supervision, Conceptualization, Investigation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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