SUPERCRITICAL TECHNOLOGY APPLIED TO THE PRODUCTION OF BIOACTIVE COMPOUNDS: COMPILATION OF RESEARCHES DONE AT LASEFI FROM 2009-2012

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Abstract. A review of the scientific investigations developed from 2009-2012 at LASEFI (LAboratory of Supercritical Technology: Extraction, Fractionation and Identification of Vegetal Extracts) – Department of Food Engineering (DEA) / School of Food Engineering (FEA), University of Campinas (UNICAMP) – is presented in this study. The current research projects cover the production of micro and nano encapsulated bioactive compounds using Supercritical Antisolvent (SAS) and Supercritical Fluid Extraction from Emulsions (SFEE) techniques. Obtaining anthocyanins, carotenoids, flavonoids, volatile oils and tocotrienols applying supercritical technology: process parameters and chemical characterization of the extracts is carried out. Hydrolysis of agroindustrial waste using sub/supercritical water + CO_2 for sugar production and second generation ethanol production is performed. Assembling of multipurpose equipment to perform supercritical fluids extraction, coupling processes to produce and encapsulate vegetal extracts of high added value and supercritical fluid extraction (SFE) of bioactive compounds in continuous mode are also done. These projects are performed in 5 SFE extraction equipments equipped with 0.005 L – 5 L extractors and 1 hydrolysis equipment equipped with 0.05 L reactor. In the past 3 years 30 articles and over 80 conference papers (full length and abstracts) have been published. A large number of botanic matrices have been selected to study because of their functional properties for application on the food and pharmaceutics industries.

Keywords: Supercritical fluid extraction, SFE, SAS, botanic matrices, bioactive compounds.

1. Introduction

Supercritical technology is investigated in several international research centers. This technology utilizes renewable solvents, as CO_2, and is identified as a green technology because supercritical processes avoid or minimize environmental damages. The characteristics associated to this technology comprise the use of solvents stated above or near the critical conditions of temperature and pressure. The solvent in supercritical region easily penetrates inside the solid matrix and solubilizes the solute even that strongly attached to the cellular wall. The obtained products possess high quality and they are free of toxic solvents. These characteristics are hardly obtained with conventional techniques.

The availability of expressive amounts of raw materials in regions near to the manufacture local contributes to the development of supercritical fluid processes and improves the economic feasibility for industrial applications. This fundamental point is satisfied in several Brazil regions. The country owns one of the largest biodiversities in the world, which allows the production of low cost raw materials.

In the latest 28 years, the LASEFI has been developing processes using supercritical technology. Some patents were registered and many scientific investigations were published in journals and conference proceedings. Therefore, the objective of this review is to present the knowledge acquired from the recent results obtained in the laboratory and to conduct a brief discussion about the advances occurred from 2009-2012.

Nomenclature	
SFE	Supercritical fluid extraction
PLE	Pressurized liquid extraction
LPSE	Low pressure solvent extraction
HPCDAE	High pressure carbon dioxide assisted-extraction
UAE	Ultrasound assisted extraction
ABE	Agitated bed extraction
GYIs	Global yield isotherms
OECs	Overall extraction curves
EY	Extraction yield
UV-vis	Spectrophotometry
GC-MS	Gas chromatography – mass spectrometry
GC-FID	Gas chromatography coupled to flame ionization detector
HPLC	High performance liquid chromatography
TLC	Thin layer chromatography
DSC	Differential scanning calorimetry
X_0	Total yield of the soluble matter in the solvent
TPC	Total phenolic content
TFC	Total flavonoid content
Aa	Antioxidant activity
COM	Cost of manufacturing
TMA	Total monomeric anthocyanin
DPPH	2,2-diphenyl-1-picrylhydrazyl
GAC	Gallic acid content
d_E	Extractor/reactor internal diameter
H_E	Extractor/reactor height
V_E	Extractor/reactor volume
V_S	Separator volume

2. Researches done at LASEFI from 2009-2012

The current scientific investigations carried out by the LASEFI's researchers group that enclose supercritical technology comprise: extraction of bioactive compounds from botanic matrices and evaluation of the process parameters; production of micro and nano encapsulated compounds using Supercritical Antisolvent (SAS) and Supercritical Fluid Extraction from Emulsions (SFEE) techniques; and hydrolysis of agroindustrial waste using sub/supercritical water + CO_2 for sugar production that can be eventually utilized for second generation ethanol production. Thus, the laboratory has seven equipments. Table 1 and Figure 1 present the characteristics of each one of the equipments belonging to LASEFI.

Table 1. Characteristics of the LASEFI's equipment of SFE, PLE, micronization and hydrolysis

Item	Equipment	Characteristics of the extractors/reactors			Solvent	Cosolvent	Separators
		d_E (cm)	H_E (cm)	V_E (L)			V_S (L)
1	SFE-2×1L (Extraction)	5.7 7.7	21.2 40.7	1 1	CO_2	No	NP
2	SFE-I (Extraction)	3.4	40.5	0.37	CO_2	Yes	NP
3	SFE-Spe-ed (Extraction)	5.4 2.0 2.0	12.3 6.3 1.6	0.28 0.02 0.005	CO_2	No	NP
4	SFE-Pilot (Extraction)	10.2	61.4	2*×5	CO_2	Yes	3*×1000
5	PLE-I (Extraction)	2.0	2.0	0.006	Ethanol/water	No	NP
6	ARADIME (Micronization)	7.0	16.8	0.65	CO_2	Yes	NP
7	HYDRO (Hydrolysis)	2.8	8.4	0.05	Water	Yes	NP

*: indicates the number of extractors/reactors or separators belonging to the equipment; NP: Not present.

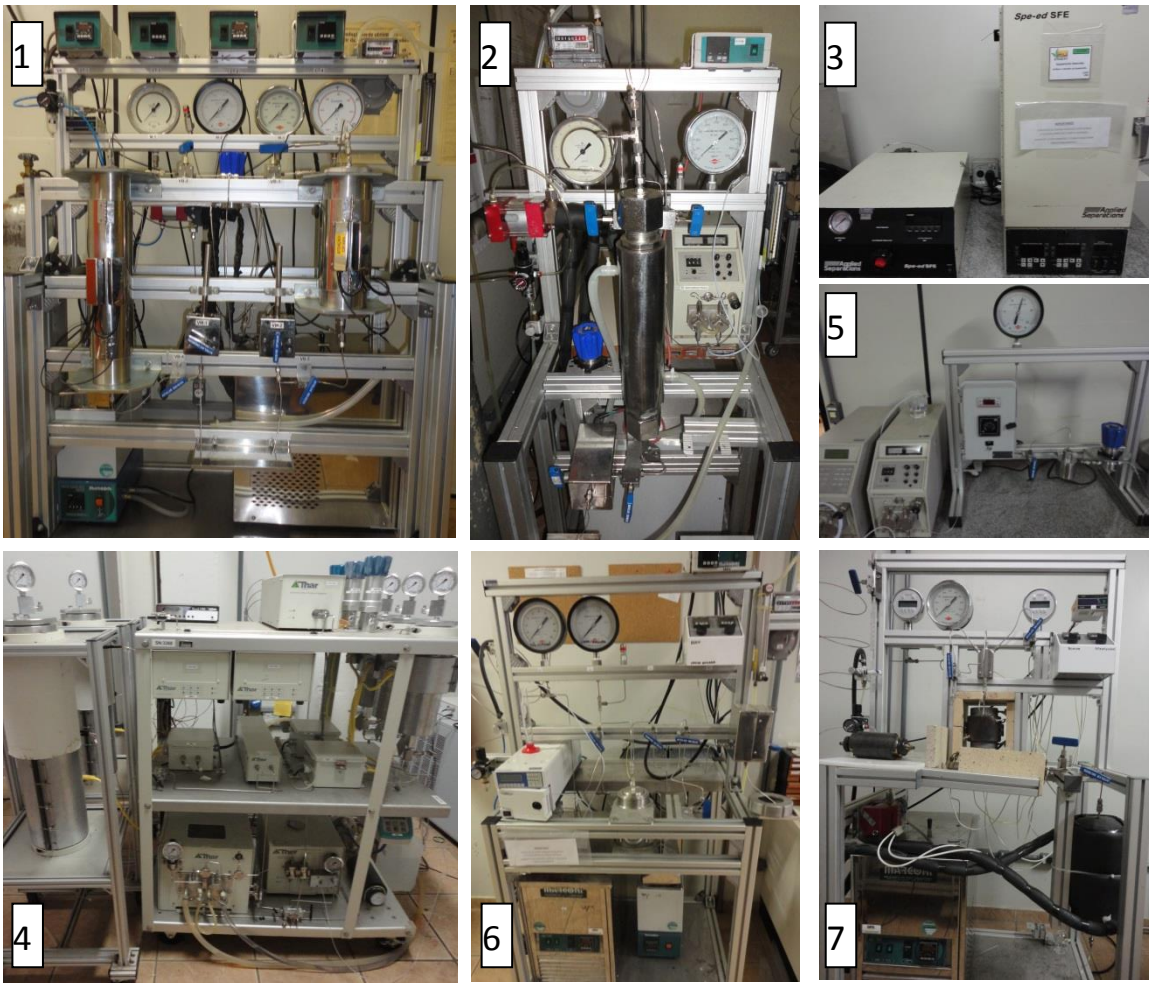


Figure 1. Equipment of supercritical technology belonging to LASEFI.

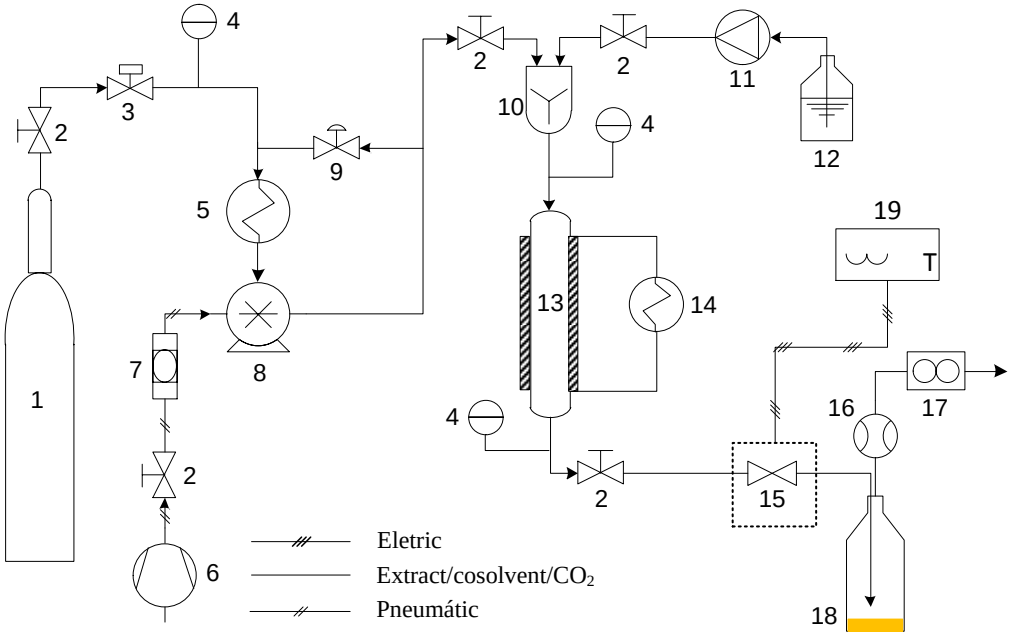
Item 3 (SFE-Spe-ed) is a commercial equipment of extraction (Applied Separations, model 7071, Allentown, USA) that can be used with extractors from 0.005 L to 0.29 L. Item 4 (SFE-Pilot) is the larger equipment owned by LASEFI. The respective equipment of extraction possesses two extractors of 5 L (Thar Technologies, model SFE-2X5LF-2-FMC, Pittsburgh, USA) disposed in parallel and three separators of 1 L disposed in series. The other equipment were assembled and validated by the LASEFI's researchers.

2.1 Extraction of bioactive compounds

Several botanic matrices are studied, which contain compounds for potential applications in the food, chemical and pharmaceutical industries. One of the compounds is the β-ecdysone, a saponin with therapeutic properties found in Brazilian ginseng (*Pfaffia glomerata*). Leal *et al.* [1] obtained Brazilian ginseng extracts using supercritical CO_2 in the SFE-I equipment. According to the results, the antioxidant activities of the extracts were different for each extraction conditions, where the largest antioxidant activity was obtained at 30 MPa and 303 K.

A patent by Meireles *et al.* [2], PI0900551-0A2, was deposited in the Brazilian National Institute of Industrial Property (INPI). The patent presents a supercritical fluid extraction process of active compounds from Brazilian ginseng roots. The referred process can occur in continuous or semi-continuous mode. The choice of one extraction mode depends of the quantity of extractors and control devices, as valves, which compose the operational plant.

Takeuchi *et al*. [3] extracted phenolic compounds from macela (*Achyrocline satureioides*) using mixtures of CO_2 plus ethanol in the SFE-I equipment (Figure 2). The addition of cosolvent provided another means of manipulating the solvent selectivity, improved the extraction yield and intensified the functional properties of the extracts. High and stable antioxidant activities were also obtained.



List of equipments	
1	CO_2 reservoir
5	Cooling bath
6	Air compressor
8	Air-driven CO_2 pump
11	Air driven cosolvent pump
12	Cosolvent reservoir
13	Extraction cell
14	Heating bath
18	Extract collecting vessel

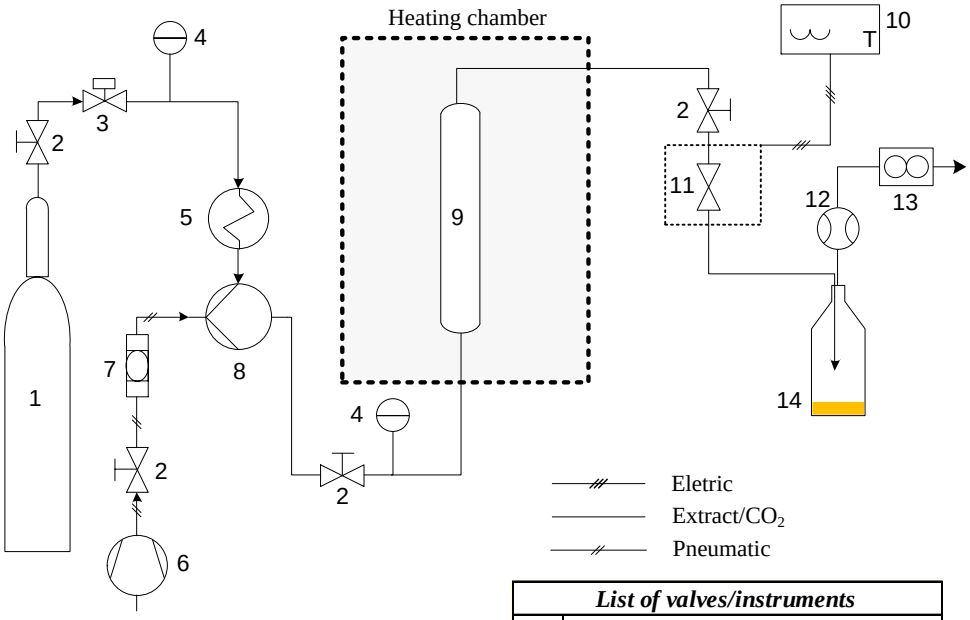
List of valves/instruments	
2	Blocking valve
3	Non-return valve
4	Pressure gauge
7	Air filter
9	Back Pressure Regulator
10	Mixer
15	Micrometering valve
16	Flowmeter
17	Flow totalizer

Figure 2. Flow diagram for the SFE-I experimental apparatus able to operate using cosolvent (Adapted from Veggi [4]).

Phenolic compounds were extracted from pomegranate (*Punica granatum*), since the compounds of this fruit present anti-inflammatory and antimicrobial properties. The supercritical fluid extraction was efficient, because the extract presented a high TPC (389 $mg.g^{-1}$). The appropriated operational condition was 30 MPa and 323 K [5].

The extraction of antioxidant compounds from jabuticaba (*Myrciaria cauliflora*) byproducts was investigated using SFE with co-solvent. The SFE-I equipment contains a co-solvent line, as can be seen in Figure 2, and it was used for performing the experimental assays. The larger extraction yield was achieved at 30 MPa and 333 K, resulting in 25 g extract/100 g raw material. However, the larger antioxidant activity was obtained at 20 MPa and 323 K. An increase in temperature resulted in a decrease in antioxidant compounds recovery, indicating the extraction of undesirable compounds. This is likely due to the fact that most of the antioxidant compounds are unstable and highly susceptible to thermal degradation [6].

Another patent developed at LASEFI was deposited by Meireles e Rosa [7] in the INPI. This patent of number PI0903275-4A2 mentions the extraction and purification process of artemisinin from solid mass of *Artemisia annua* using supercritical technology. The extraction process is divided in three stages. The first stage consists in the contact and dissolution of the solid particulate mass in supercritical CO_2 inside the extractor. The second stage aims to obtain the purified extract and consists in the contact between the CO_2 and the vegetal extract dissolved with a fixed polar phase in a fractionation column. The third stage, called elution, consists in the contact between the CO_2 and co-solvent mixture with a fixed polar phase in a fractionation column.



List of equipaments	
1	CO_2 reservoir
5	Cooling bath
6	Air compressor
8	Air-driven CO_2 pump
9	Extraction vessel
14	Extract collecting vessel

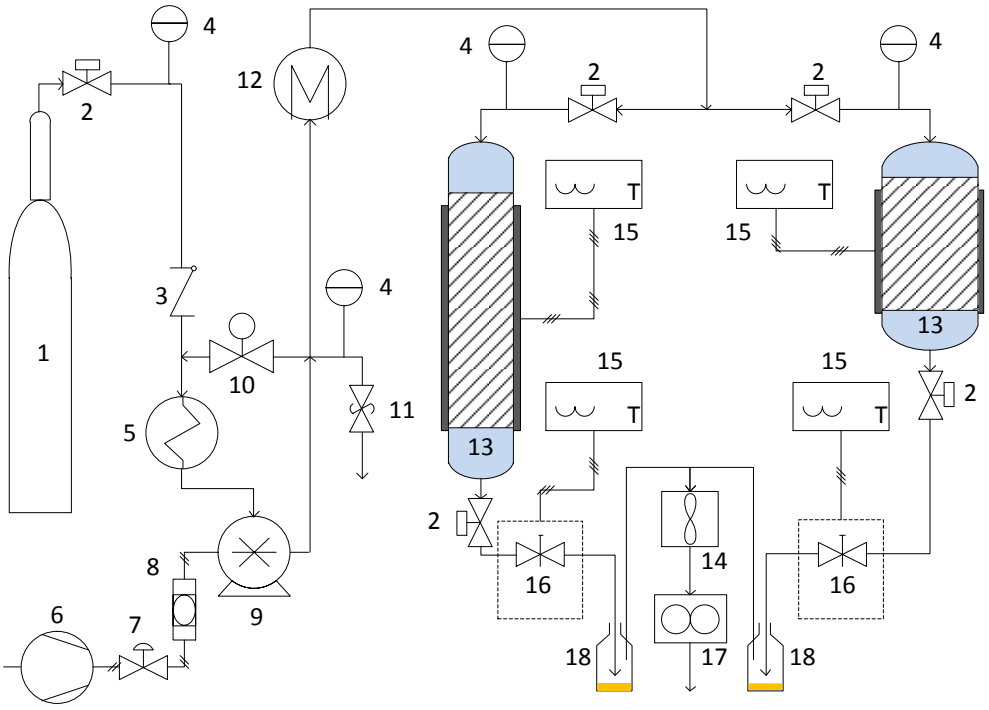
List of valves/instruments	
2	Blocking valve
3	Non-return valve
4	Pressure gauge
7	Air filter
10	Temperature controller
11	Micrometering valve
12	Flowmeter
13	Flow totalizer

Figure 3. Flow diagram for the SFE-Spe-ed experimental apparatus (Adapted from Vasconcellos [8]).

The Figure 3 shows a flow diagram of the SFE-Spe-ed equipment (Applied Separations, model 7071, Allentown, USA). The respective equipment is an efficient and simple apparatus used to obtain various target compounds. SFE of chamomile (*Chamomilla recutita* L.) extract was studied in SFE-Spe-ed equipment using a 0.29 L extractor. Yield of approximately 3.5 g extract/100 g raw material was obtained. Compounds such as En-in-dicycloether and cis-β-farnesene were identified in the extracts. Mathematical modeling and optimization were carried out focusing to attain the optimum condition that maximizes the extract amount. The effect of particle diameter on extraction yield was investigated and the results show that the evaluated response is a weak function of particle diameter for chamomile. Nevertheless, the process parameters as temperature and pressure presented influence on the evaluated response. The extraction yield is a strong function of these parameters and their increment allows an increment on the extracted mass chamomile bioactive compounds [9].

SFE of annatto (*Bixa orellana* L.) was performed in the SFE-Spe-ed equipment aiming to obtain an extract rich in tocotrienols and the defatted rich-bixin seeds. The major extraction yield (2.2 g extract/100 g raw material) was reached at 40 MPa and 333 K. In this study, cycles of CO_2 pressurization/depressurization were tested, although their influences on the bixin yield were negligible. The effects of pressure release in modifying the cell membrane due to rapid gas expansion did not provide an improvement in the process efficiency [10].

Other equipment belonging to LASEFI is the SFE-2×1L (Figure 4), which includes two extractors of 1 L disposed in parallel. The equipment was assembled to test and to validate an extraction process of bioactive compounds in continuous mode. The referred configuration allows obtaining vegetal extracts uninterruptedly, so the productivity can be improved and the costs of production can be reduced. In this sense, the kinetic curves need to be identical for both extractors with different geometries. Currently, experimental assays are being carried out for establishing criterions and process parameters to relate the extraction curves in the extractors. The purpose is to obtain equals mass transfer rates for both extractors. Thus, one of the criterions can be the maintenance of the S/F (mass of solvent/mass of raw material) ratio and the extraction time constants. The review of Zabot *et al.* [11] brings up more detailed information about the influence of the extractor geometries on the extraction curves profiles.



List of equipments	
1	CO_2 reservoir
5	Cooling bath
6	Air compressor
9	Air-driven CO_2 pump
12	Heating bath
13	Extraction cell

List of valves/instruments	
2	Blocking valve
3	Non-return valve
4	Pressure gauge
7	Control valve (air flow)
8	Air filter
10	Back pressure regulator
11	Safety valve
14	Flowmeter
15	Temperature controller
16	Micrometering valve
17	Flow totalizer
18	Extract collecting vessel

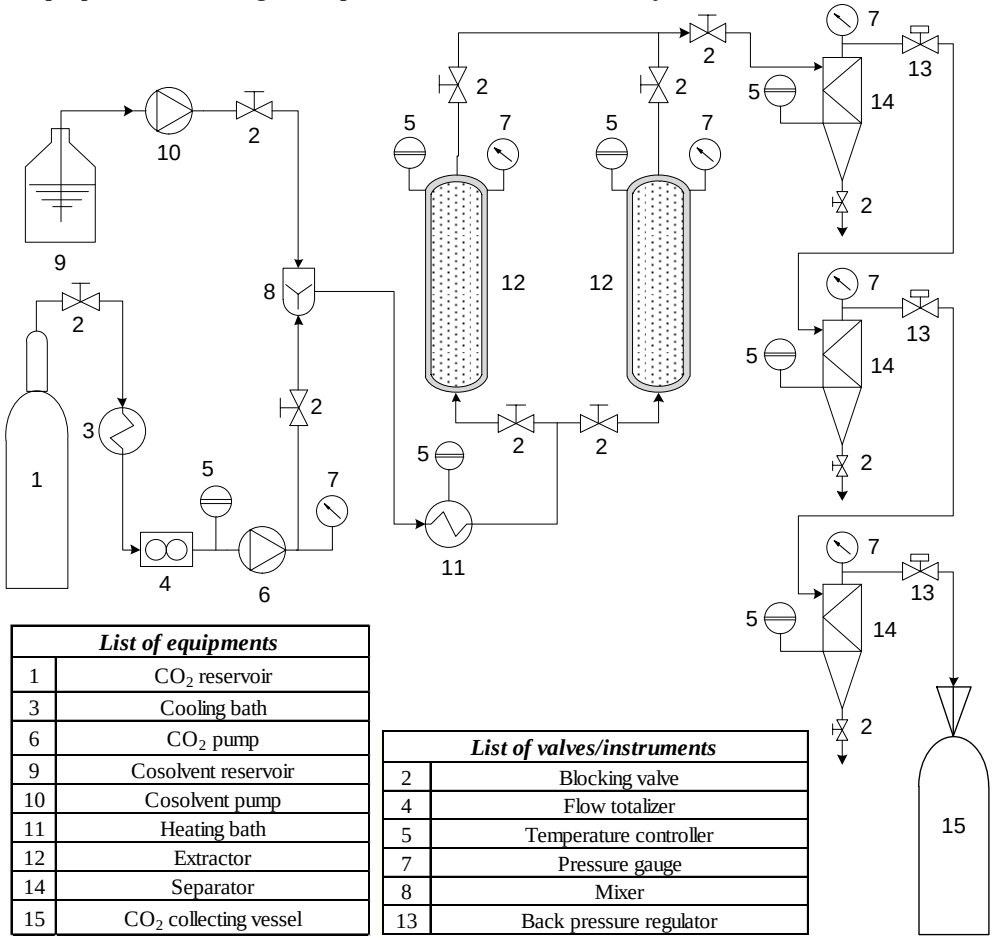
Figure 4. Flow diagram for the SFE-2×1L experimental apparatus.

For making feasible an industrial application of SFE, the knowledge obtained in laboratory scale should be transferred, initially, to pilot scale. In this way, criterions of scale up which can successfully satisfy the reproduction of the overall extraction curves (OEC) need to be defined. Prado *et al.* [12] utilized the available equipment in LASEFI and studied process involving the SFE scale up. The investigations met the obtainment of compounds from clove (*Eugenia caryophyllus*) and sugarcane residue. The SFE-Sp-ed equipment (extractor of 0.29 L) and the SFE-Pilot equipment (Figure 5) were used in the research. The adopted criterion consisted of maintaining the S/F ratio and the extraction time constants. Considering a 15 times increase in raw material mass for the SFE-Pilot, the solvent mass was also increased 15 times. Operationally, the mass solvent flow rate was 15 times higher and the extraction time was maintained. According to the kinetic profiles of the OEC, the simple adopted criterion of maintaining the S/F ratio and the extraction time constants satisfied the curves reproduction. Similar yields were reached in both SFE-Spe-ed (I) and SFE-Pilot (II) equipment. The yields of clove extracts were 15 g/100 g (I) and 14.5 g/100 g (II). The yields of sugarcane residue were 2.5 g/100 g (I) and 2.8 g/100 g (II).

Prado *et al.* [13] investigated the same criterion of scaling up mentioned above on SFE of grape (*Vitis vinifera*) seed extract. The results were satisfactory, because the yields were 11.9 g/100 g (I) and 11.2 g/100 g (II) using an S/F ratio of 8.4. The authors still tested the separation step in the SFE-Pilot equipment using three separators of 1 L disposed in series (Figure 5). The temperature was kept in 313 K and pressures of 10 MPa, 6 MPa and 3 MPa were used for the separators 1, 2 and 3, respectively. Most of extract was recovered in separator 1 (86%) and the rest of it was recovered in separator 2. There was no extract in separator 3, which indicates that using the operational conditions selected for separator 1 and 2 it was possible to precipitate all the extract. Similar results were obtained with clove [12].

Figure 6 shows a flow diagram of PLE-I equipment that uses pressurized liquid, generally water or ethanol, for extracting mainly polar compounds which possess chemical affinity with these solvents.

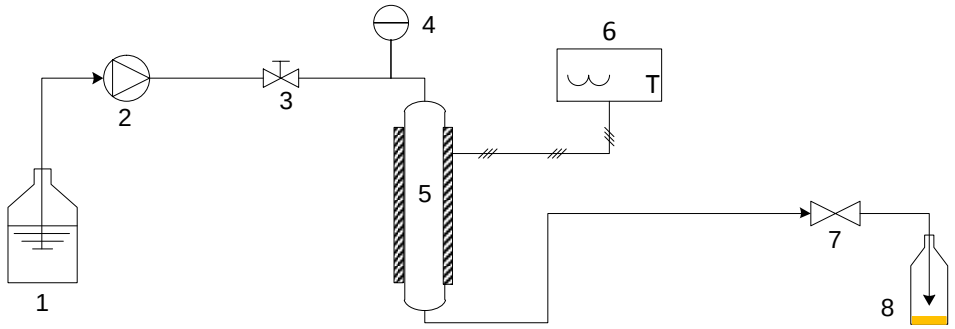
In summary, the scientific investigations developed for extracting bioactive compounds in LASEFI emphasize the obtainment of the largest extraction yields matching process parameters and performing mathematical modeling for optimization. Additionally, the chemical composition of the extracts and the functional properties of the target compounds, as antioxidant activity, are researched.



List of equipments	
1	CO_2 reservoir
3	Cooling bath
6	CO_2 pump
9	Cosolvent reservoir
10	Cosolvent pump
11	Heating bath
12	Extractor
14	Separator
15	CO_2 collecting vessel

List of valves/instruments	
2	Blocking valve
4	Flow totalizer
5	Temperature controller
7	Pressure gauge
8	Mixer
13	Back pressure regulator

Figure 5. Flow diagram for the SFE-Pilot experimental apparatus able to operate using cosolvent (Adapted from Prado [14]).



List of equipments	
1	Solvent reservoir
2	Liquid pump
5	Extractor
8	Extract collecting vessel

List of valves/instruments	
3	Blocking valve
4	Pressure gauge
6	Temperature controller
7	Back pressure regulator

Figure 6. Flow diagram for the PLE-I experimental apparatus.

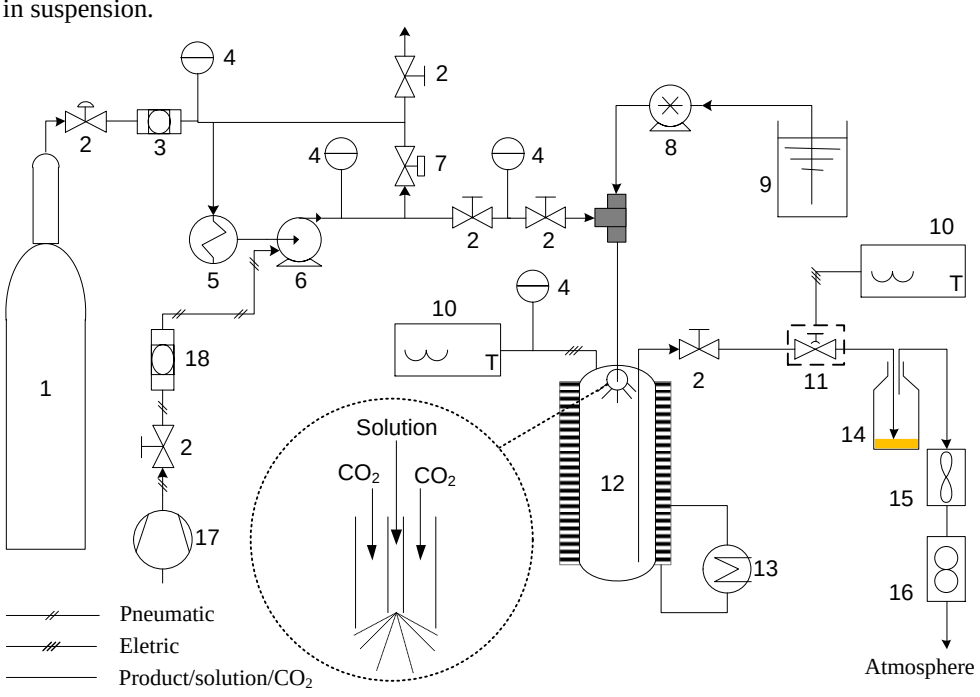
2.2 Production of micro and nano particles

Lately, novel techniques of producing micro and nano particles using supercritical technology are being developed to overcome the drawbacks found in conventional processes. In our research group, studies enclosing production of micrometric particles are being carried out since 2009. Santos [15] designed, assembled and tested the ARADIME equipment, as a flow diagram showed in Figure 7. The home-made multipurpose equipment is used for performing process with pressurized fluids which allows the production of particles of functional pigments using RESS (Rapid Expansion of Supercritical Solutions) and SAS (Supercritical fluid Anti-Solvent).

Santos *et al.* [16] demonstrated that SAS process can be successfully utilized to co-precipitate microparticles of polyethylene glycol (PEG) loaded with bixin-rich extract. Besides, the RESS process using ethanol as cosolvent can be effectively employed to encapsulate rutin and antocyanin-rich extract in PEG matrix.

Anthocyanins extracted from jabuticaba (*Myrciaria cauliflora*) skins were encapsulated using supercritical CO_2 and ethanol as cosolvent. Encapsulated particles by RESS at different pressures and temperatures retained the extracts' biological activity, keeping the stability of the extracts against the light and heat degradation. The best operational condition was 313 K and 20 MPa [17].

Sub-micrometric particles of carotenoids were produced using SFE of oil-in-water emulsion. Suspensions containing stabilized carotenoids with final particle size of 344–366 nm, encapsulation efficiency of 34–89% and degree of isomerization from carotenoid trans to cis forms in the range of 0.02–15% were obtained [18]. A novel process, known as OEPO (organic solvent extraction and on-line particle formation), was developed by Santos *et al.* [19]. The process consists of hyphenated Pressurized Liquid Extraction (PLE)-Supercritical Anti Solvent (SAS) precipitation, PLE-SAS co-precipitation and PLE-Supercritical Fluid Extraction of Emulsions (SFEE). This is a suitable and promising process to obtain, in only one step, different products as precipitated extract, co-precipitated extract or encapsulated extract in suspension.



List of equipments	
1	CO_2 reservoir
5	Cooling bath
6	Air-driven CO_2 pump
8	Solution pump
9	Solution reservoir
12	Precipitation vessel
13	Heating bath
14	Glass flask
17	Air compressor

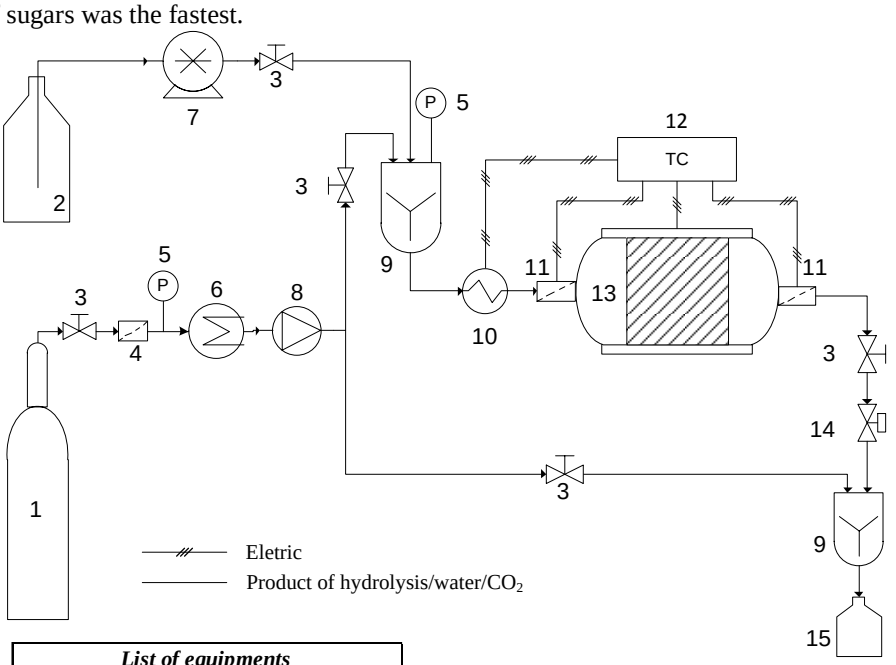
List of valves/instruments	
2	Blocking valve
3	CO_2 filter
4	Pressure gauge
7	Back pressure regulator
10	Temperature controller
11	Micrometering valve
15	Flowmeter
16	Flow totalizer
18	Air filter

Figure 7: Flow diagram for the ARADIME experimental apparatus (Adapted from Santos [15]).

2.3 Hydrolysis of agroindustrial waste using supercritical technology

Other research line, exploited in LASEFI since 2004, consists in the hydrolysis of agroindustrial waste using sub/supercritical water + CO_2 for sugar production that can be eventually utilized for second generation ethanol production. The HYDRO equipment (Figure 8), containing a 0.05 L reactor, was designed, assembled and tested by Prado *et al.* [20]. The system was designed to work with pressures up to 40 MPa and temperatures up to 673 K. The water is pumped from reservoir 2 and, whether there is addition of CO_2, the static mixer (instrument 9) serves to homogenate the solvents before their percolation inside the reactor containing the biomass. Generally, the biomass consists in lignocellulosic material, non-edible source of fermentable sugars profusely found in the nature. To validate the HYDRO equipment, hydrolyses of cellulose and sugarcane bagasse were performed. The glucose equivalent concentrations in the hydrolysate fractions were approximately 6 g/100 g of cellulose in reaction time of 60 min and 5.9 g/100 g sugarcane bagasse in reaction time of 40 min.

The reducing sugars obtained in the hydrolysate fractions can be used in fermentative process for producing renewable energetic sources. This mode of obtaining energy can futurely become the basis to the development of modern industrial economies. The ethanol is emerging as an efficient and economic feasible fuel. Considering it, Follegatti-Romero *et al.* [21] also performed experimental assays in the HYDRO equipment using cellulose with water in subcritical conditions of 457 K, 470 K and 482 K, in reaction time of 68 min and 20 MPa. The authors concluded that the obtained results were satisfactory and the total reducing sugars content recovered in 482 K was the highest. In this temperature, the recovery of sugars was the fastest.



List of equipments	
1	CO_2 reservoir
2	Water reservoir
6	Cooling bath
7	Liquid pump
8	CO_2 pump
10	Heating bath
13	Reactor
15	Product collecting vessel

List of valves/instruments	
3	Blocking valve
4	CO_2 filter
5	Pressure gauge
9	Mixer
11	Thermocouple
12	Temperature controlles
14	Micrometering valves

Figure 8. Flow diagram for the HYDRO experimental apparatus (Adapted from Prado *et al.* [20]).

3. Analytical procedures

The chemical characterization of the products is important to know their qualities. At LASEFI, usually the researchers perform thin layer chromatography (TLC) for qualitatively analysis of the extracts. An important method of quantitative analysis is accomplished in gas chromatography (GC) coupled to flame ionization detector (FID). HPLC (high-performance liquid chromatography) analysis are also done to identify and to quantify the target compounds obtained. Futurely, supercritical fluid chromatography (SFC) will be also done in the laboratory.

4. Economic evaluation of supercritical technology process

Processes which comprise supercritical technology commonly present technic feasibility. Nonetheless, detailed investigations about the economic feasibility need to be performed for the process to hold industrial application. Therefore, simulations of cost of manufacturing (COM) extracts from several botanic matrices are developed by the LASEFI's researchers. Cavalcanti *et al.* [6] simulated the COM of jabuticaba extract obtained by SFE in extractors of various sizes using a commercial simulator. According to the results, the cost of extract production was lower than US$ 10.00/kg in a 300 L extractor. Veggi *et al.* [22] compared the COM of jabuticaba extract obtained by PLE and conventional techniques in a 300 L extractor. The values of COM of the extracts were US$ 15.53/kg in PLE, US$ 410.21/kg in ultrasound assisted extraction and US$ 778.42/kg in Soxhlet extraction. The values of COM of extracts from Amazonian plants as buriti, pupunha and pressed palm fiber [23], annatto [10], pomegranate [5], grape [13] and sugarcane residue [14] were also simulated.

5. Summary of the current scientific investigations conducted at LASEFI

Table 2 shows a summary of the recent projects developed at LASEFI. The present objectives of the research group are to scale up processes and products for effectively installing an industrial plant of supercritical technology in Latin America.

Table 2. LASEFI's studies from 2009-2012

Raw materials studied	Extraction methods	Experimental determinations	Extracts' characterization	Theoretical studies	References
Annatto (*Bixa orellana* L.)	SFE	GYIs; OECs; X0	Bixin (UV-vis); Vitamin E (HPLC)	Mathematical modeling; COM	[10]
Pomegranate (*Punica granatum* L.)	SFE	GYIs	TPC (UV-vis); Chemical composition (GC-MS); Aa	Process simulation; scale up; COM	[5]
Jabuticaba (*Myrciaria cauliflora*)	SFE; PLE; LPSE; HPCDAE; UAE; ABE; UAE+ABE	GYIs; EY; OECs; Recovery of anthocyanins and phenolic compounds	TMA (UV-vis); TPC; Aa (DPPH method); Fractionated separation (TLC)	Process simulation; COM; scale up	[6; 19; 22; 24-26]
Brazilian ginseng (*Pfaffia glomerata*)	SFE; PLE; LPSE	OECs; EY; X0	Fractionated separation (TLC); Ecdysteroids (HPLC); Aa (DPPH method)	Process simulation; energetic analysis; COM;	[1; 27; 28]
Grape (*Vitis vinífera* L.)	SFE	OECs; scale up; Separation step of compounds	Fatty acid (GC); crystallization of the oil (DSC)	COM; scale up	[13]
Clove (*Eugenia caryophyllus*)	SFE	OECs; scale up; Separation step of compounds	Chemical composition (GC-FID)	Mathematical modeling; process simulation and optimization	[12; 29]
Macela (*Achyrocline satureioides*)	SFE; LPSE	GYIs; EY; scale up	Aa; TPC; TFC; GAC (UV-vis); chemical composition (GC-FID)	Mathematical modeling; COM	[3; 4; 30; 31]
Chamomile (*Chamomilla recutita* L.)	SFE	OECs	Chemical composition (GC-FID)	Mathematical modeling; process optimization	[9]
Vetiver (*Vetiveria zizanioides*)	SFE	Amount of extract in the light and heavy phases	Aa (DPPH method); TPC (UV-vis); chemical composition (GC-FID)	Simulation of the phases equilibrium	[32]
Ginger (*Zingiber officinale*)	SFE	GYIs; OECs; EY; scale up	Fractional separation (TLC); chemical composition (GC-FID)	Scale up	[14]

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