Food and Public Health 2014, 4(2): 36-48
DOI: 10.5923/j.fph.20140402.04

Supercritical Technology Applied to the Production of Bioactive Compounds: Research Studies Conducted at LASEFI from 2009 to 2013

Giovani L. Zabot, Moyses N. Moraes, M. Angela A. Meireles*

LASEFI/DEA/FEA (School of Food Engineering), UNICAMP (University of Campinas), RuaMonteiroLobato, 80, Campinas-SP, CEP:13083-862, Brazil

Abstract This paper presents a review of the scientific research studies conducted in the period from 2009 to 2013 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)/Brazil. The current research projects revolve around the use of supercritical fluids in several areas. One of these projects covers the production and encapsulation of micro- and nano-bioactive compounds using Supercritical Antisolvent (SAS) and Supercritical Fluid Extraction from Emulsions (SFEE) techniques. Another project is focused on obtaining anthocyanins, carotenoids, flavonoids, volatile oils and tocotrienols by applying supercritical technology. The experiments involve the determination of process parameters and the chemical characterization of the extracts. The hydrolysis of agroindustrial co-products using sub/supercritical water + CO_2 for sugar production and second generation ethanol production are also performed. Recently, a home-made multipurpose system containing two extractors of 1 L each with different shapes was assembled to evaluate the influence of the bed geometry on the kinetic extraction yields and on the chemical composition of the extracts. In yet another project, online processes that produce and encapsulate vegetal extracts of high added value are coupled with supercritical fluid extraction (SFE) of bioactive compounds in continuous mode. These projects are performed using SFE extraction systems equipped with 0.005 L – 5 L extractors, 1 hydrolysis systems containing a 0.05 L reactor and 1 micronization systems containing a 0.65 L reactor. In the past 4 years, 57 articles and over 100 conference papers (full length and abstracts) have been published. A large number of botanic matrices have been selected for study due to their functional properties and their potential applications in the food and pharmaceutical industries.

Keywords Supercritical fluid extraction, Botanic matrices, Bioactive compounds, Hydrolysis, Micronization

1. Introduction

Supercritical technology is studied in several international research centers, where some studies are mentioned [1-15]. This technology utilizes renewable solvents, such as CO_2, and is a green technology because supercritical processes avoid or minimize environmental damage. The main characteristic of this technology is the use of solvents above or near their critical conditions for temperature and pressure. A solvent in a supercritical state easily penetrates inside a solid matrix and solubilizes the solute even when it is strongly attached to the cellular wall.

The availability of substantial amounts of raw materials in regions near the place of manufacture contributes to the development of supercritical fluid processes and improves their economic feasibility for industrial applications. This fundamental point is satisfied in several regions of Brazil. This country has one of the highest levels of biodiversity worldwide, which allows the production of low-cost raw materials.

In the latest 29 years, LASEFI (Laboratory of Supercritical Technology: Extraction, Fractionation and Identification of Vegetal Extracts) has been developing processes using supercritical technology. Several patents have been registered, and many scientific research studies have been published in Journals and Conference Proceedings (44 full length and 65 abstracts). Therefore, the objective of this review is to present the knowledge acquired from the recent results obtained in the laboratory and to provide a brief discussion of the advances that occurred from 2009 to 2013.

* Corresponding author:
meireles@fea.unicamp.br (M. Angela A. Meireles)
Published online at http://journal.sapub.org/fph

Nomenclature

SFE	Supercritical fluid extraction
PLE	Pressurized liquid extraction
LPSE	Low pressure solvent extraction
HPCDAE	High pressure CO_2-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. Research Studies Conducted at LASEFI from 2009 to 2013

Some of the current scientific investigations focused on supercritical technology being carried out by LASEFI's research group are as follows: the extraction of bioactive compounds from botanic matrices and the evaluation of the process parameters, the production of micro- and nano-encapsulated compounds using Supercritical Antisolvent (SAS) and Supercritical Fluid Extraction from Emulsions (SFEE) techniques and the hydrolysis of agroindustrial co-products using sub/supercritical water + CO_2 for the production of sugars that can be eventually utilized for second-generation ethanol production. The laboratory has seven systems to perform these types of experiments. Table 1 and Figure 1 present the characteristics of each one of the systems belonging to LASEFI.

Item 1 (SFE-2×1L, Figure 1-1) is a home-made system containing two extractors of 1 L each with different geometries. It is a multipurpose unit that can be used for extracting bioactive compounds in continuous mode from several raw materials. Item 2 (SFE-I, Figure 1-2) is also a system that was assembled at LASEFI. It is used when the addition of co-solvents is required during extraction. Both Items 2 and 5 (PLE-I, Figure 1-5) can be used for PLE.

Item 3 (SFE-Spe-ed, Figure 1-3) is a commercial unit for extraction (Applied Separations, 7071, Allentown, USA) and can be used with extractors with volumes from 0.005 L to 0.29 L. Item 4 (SFE-2×5L, Figure 1-4) is the largest unit owned by LASEFI. The respective extraction system possesses two extractors of 5 L each (Thar Technologies, Pittsburgh, USA) arranged in parallel and three 1-liter separators operating in series.

Item 6 (ARADIME, Figure 1-6) is a system that enables the development of several types of processes using pressurized fluids such as sub/supercritical fluid extraction with or without co-solvent and the production of particles via RESS or SAS. The integration of unit operations is one of the recent research topics undertaken by the scientists at LASEFI. As a result of this research, the HYDRO system (Item 7, Figure 1-7) was integrated with upstream extraction units to achieve biomass hydrolysis.

2.1. Extracting Bioactive Compounds

Several botanic matrices that contain compounds with potential applications in the food, chemical and pharmaceutical industries have been studied. One of these compounds is β-ecdysone, a saponin with therapeutic properties found in Brazilian ginseng (*Pfaffia glomerata*). Leal *et al* [16] obtained Brazilian ginseng extracts using supercritical CO_2 in the SFE-I system. According to their results, the extract's antioxidant activity was a function of the extraction conditions, where the largest antioxidant activity was obtained at 30 MPa and 303 K.

Table 1. Characteristics of LASEFI's systems for SFE, PLE, micronization and hydrolysis

Item	System	Characteristics of the extractors/reactors			Solvent commonly used	Co-solvent	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-2×5L (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/CO_2	Yes	NP

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

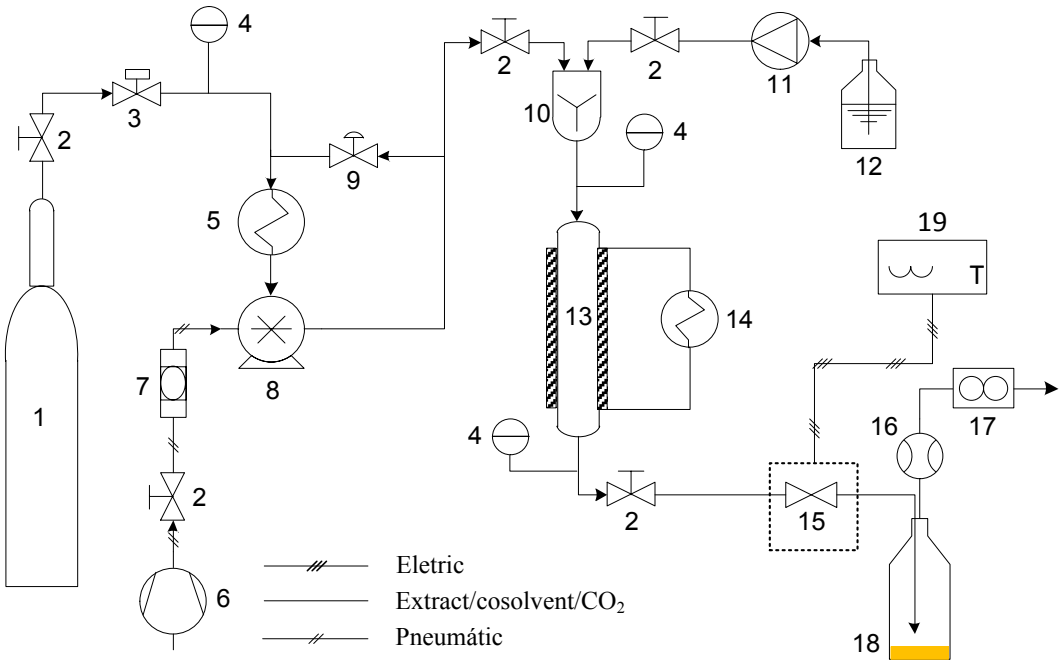
A patent by Meireles *et al* [17], PI0900551-0A2, was deposited with the Brazilian National Institute of Industrial Property (INPI). The patent presents a supercritical fluid extraction process for active compounds from Brazilian ginseng roots. The described process can be performed in continuous or semi-continuous mode. The choice of one extraction mode over the other depends on the number of extractors and on the control devices that make up the process operation plant.

Takeuchi *et al* [18] extracted phenolic compounds from macela (*Achyroclinesatureioides*) using mixtures of CO_2 plus ethanol using the SFE-I system (Item 2, Figure 1). The addition of a co-solvent 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 obtained.

Phenolic compounds were extracted from pomegranate (*Punicagranatum*). The compounds present in this fruit show anti-inflammatory and antimicrobial properties. The supercritical fluid extraction was efficient because the extract presented a high TPC (389 $mg.g^{-1}$). The appropriate operating conditions were 30 Pa and 323 K [19].

The extraction of antioxidant compounds from jabuticaba (*Myrciaria cauliflora*) byproducts was investigated using SFE with a co-solvent. The SFE-I system has a co-solvent line (as seen in Scheme 1) and was used for performing the experimental assays. A greater extraction yield was achieved at 30 MPa and 333 K, resulting in 25 g extract/100 g raw material. However, the highest antioxidant activity was obtained at 20 MPa and 323 K. An increase in temperature resulted in decreased recovery of the antioxidant compounds, indicating the extraction of undesirable compounds. This is likely because most of the antioxidant compounds are unstable and highly susceptible to thermal degradation [20].

Figure 1. Supercritical technology unitsbelonging to LASEFI for extraction: (1) SFE-2×1L; (2) SFE-I; (3) SFE-Spe-ed; (4) SFE-2×5L; for micronization: (5) ARADIME; and for hydrolysis: (6) HYDRO



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

Scheme 1. Flow diagram for the SFE-I experimental apparatus for extraction, including cosolvent feed system (adapted from Veggi [21])

Another patent (PI0903275-4A2) developed at LASEFI was deposited by Meireles and Rosa [22] with the INPI. This patent mentions the extraction and purification process of artemisinin from the solid mass of *Artemisia annua* using supercritical technology. The extraction process is divided into three stages. The first stage entails 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 requires contact between the CO_2 and the vegetal extract dissolved in a fixed polar phase in a fractionation column. The third stage, elution, involves contact between the CO_2 and co-solvent mixture with a fixed polar phase in a second fractionation column.

Scheme 2 shows the flow diagram of the SFE-Spe-ed system (Applied Separations, model 7071, Allentown, USA). This unit is an efficient and simple apparatus used to obtain various target compounds. SFE of chamomile (*Chamomillarecutita*L.) extract was studied in the SFE-Spe-ed system using a 0.29 L extractor. A 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 to determine the optimum conditions that maximize the extract amount. The effect of particle diameter on extraction yield was also investigated. The results show that, for chamomile, the extraction yield is a weak function of particle diameter. Nevertheless, the other process parameters, temperature and pressure, strongly influenced the extraction yield. Increasing either of these parameters led to enhanced extraction of bioactive compounds from chamomile [23].

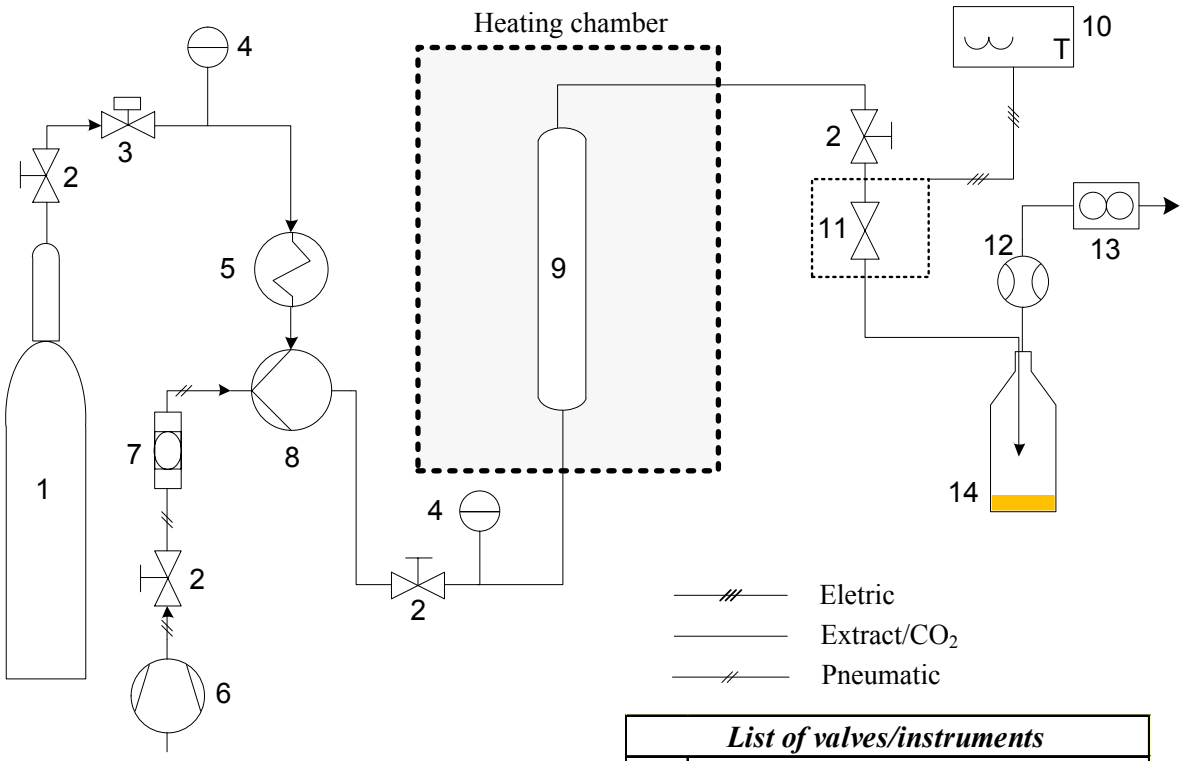
SFE of annatto (*Bixaorellana* L.) was performed in the SFE-Spe-ed apparatus to obtain an extract rich in tocotrienols and defatted bixin-rich seeds. The highest extraction yield (2.2 g extract/100 g raw material) was reached at 40 MPa and 333 K. In this study, cycles of CO_2 pressurization/depressurization were tested, but their influences on the bixin yield were negligible. The effects of pressure release on modifying the cell membrane due to rapid gas expansion did not provide an improvement in the process efficiency [24].

Another piece of equipment belonging to LASEFI is the SFE-2×1L system (Scheme 3), which includes two extractors of 1 L each operating in parallel. The system was assembled to test and validate an extraction process for bioactive compounds in continuous mode. This

configuration allows vegetal extracts to be obtained uninterruptedly, and thus, the productivity can be improved and the costs of production can be reduced. However, operating in this configuration requires the kinetic curves of both extractors to be identical, despite their different geometries. Currently, experimental assays are being carried out to establish the operation criteria and process parameters that will enable comparison of the extraction curves in these extractors. The purpose is to obtain equals mass transfer rates for both extractors. Thus, one of the criterion can be the maintenance of the S/F (mass of solvent/mass of raw material) ratio and the extraction time constants. The review by Zabot*et al* [26] provides detailed information about the influence of extractor geometries on the kinetic profiles of the extraction.

To assess the feasibility of SFE in industrial applications, knowledge obtained from laboratory scale experiments should be used to conduct a pilot run. Therefore, scale-up criteria that are able to reproduce the overall extraction curves (OEC) need to be defined.

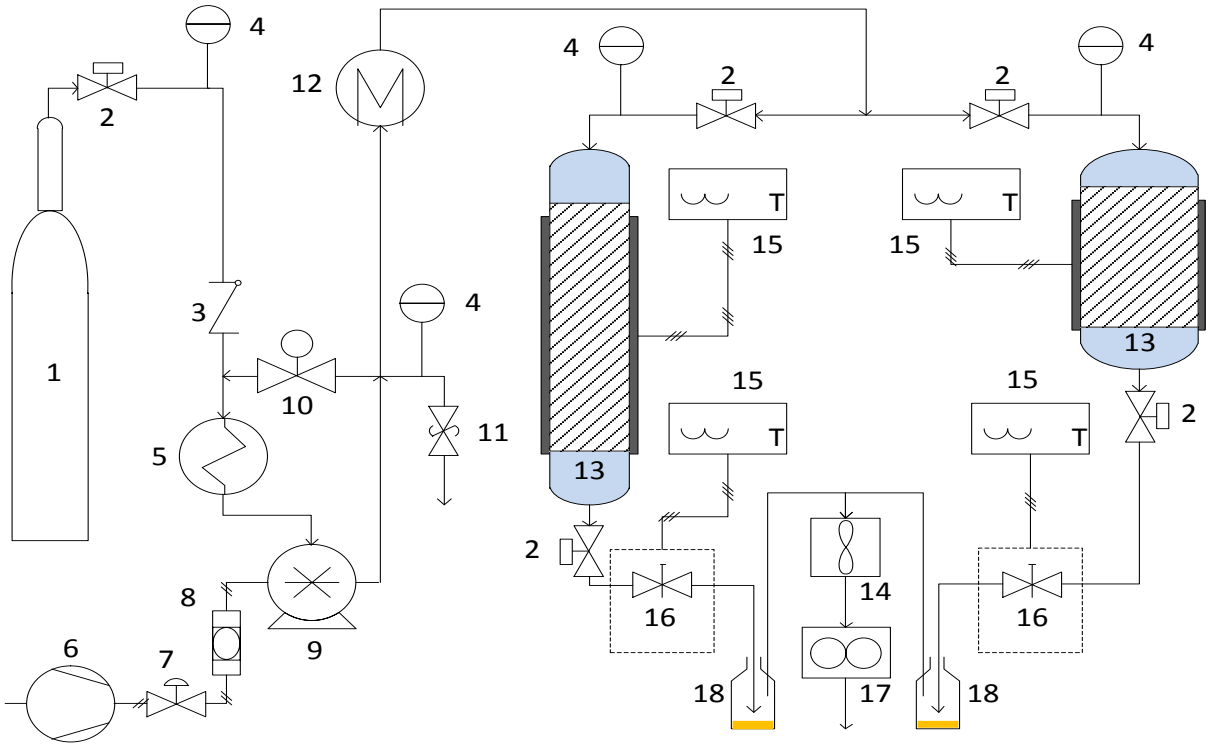
Prado *et al* [27] utilized the available equipment at LASEFI and studied processes involving SFE scale up. The investigations aimed to obtain extracts from clove (*Eugenia caryophyllus*) and sugarcane residue. The SFE-Spe-ed system (extractor of 0.29 L) and the SFE-2×5L system (Scheme 4) were used in this research. The proposed scale-up criteria consisted of maintaining the S/F ratio and the extraction time constants. Considering a 15-fold increase in the raw material mass for the SFE-2×5L, the solvent mass was also increased 15 times. Operationally, the solvent mass flow rate was 15 times higher and the extraction time was maintained. Adopting these simple criteria of maintaining the S/F ratio and the extraction time constants resulted in the appropriate replication of the kinetic profiles of the OEC in the scale-up experiment. Similar yields were reached in both the SFE-Spe-ed (I) and the SFE-2×5L (II) system. 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).



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

Scheme 2. Flow diagram for the SFE-Spe-ed experimental apparatus for extraction (adapted from Prado [25])



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

Scheme 3. Flow diagram for the SFE-2×1L experimental apparatus for extraction (adapted from Zabot*et al* [28])

Prado *et al* [29] investigated the same scale-up criteria mentioned above on SFE of grape (*Vitisvinifera*) 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 further tested the separation step on the SFE-2×5L system using three separators of 1 L operating in series (Scheme). The temperature was kept at 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 operating conditions selected for separators 1 and 2, it is possible to precipitate all the extract. Similar results were obtained for extraction from clove [27].

Figure 6 shows a flow diagram for the PLE-I system, which uses pressurized liquid, generally water or ethanol, for extracting mainly polar compounds that possess chemical affinity for these solvents. To summarize, the research studies conducted at LASEFI emphasize the extraction of bioactive compounds at the highest extraction yield by optimizing process parameters through experimental work coupled with mathematical modeling. In addition, these studies investigate the chemical composition of the extracts and the functional properties, such as antioxidant activity, of the target compounds.

2.2. Production of Micro- and Nano-particles

Currently, novel techniques to produce micro- and nano-particles using supercritical technology are being developed to overcome the drawbacks found in conventional processes. In our research group, studies aiming to produce micrometer-scale particles have been carried out since 2009. Santos [30] designed, assembled and tested the ARADIME apparatus, the flow diagram of which is shown in Scheme. This home-made multipurpose system is used to conduct

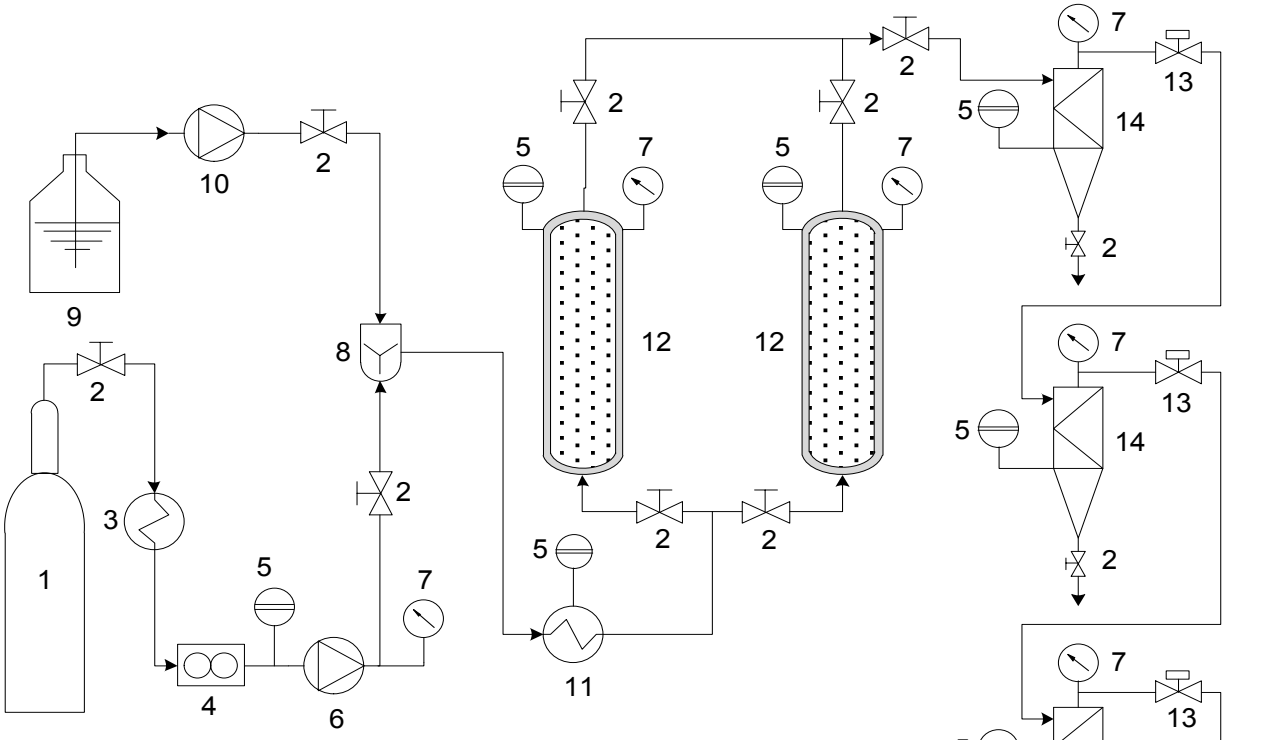
experiments with pressurized fluids that allow the production of particles of functional pigments using RESS (Rapid Expansion of Supercritical Solutions) and SAS (Supercritical fluid Anti-Solvent).

Santos *et al* [31] demonstrated that the SAS process could be successfully utilized to co-precipitate microparticles of polyethylene glycol (PEG) loaded with bixin-rich extract. Moreover, the RESS process using ethanol as co-solvent can be effectively employed to encapsulate rutin and anthocyanin-rich extract in the PEG matrix.

Anthocyanins extracted from jabuticaba (*Myrciaria cauliflora*) skins were encapsulated using supercritical CO_2 and ethanol as the co-solvent. Particles encapsulated by RESS at different pressures and temperatures retained the extracts' biological activity and preserved the extract's stability against light and heat degradation. The best operational conditions were 313 K and 20 MPa [32].

Carotenoid particles of sub-micron size were produced using SFE with an oil-in-water emulsion. Suspensions containing stabilized carotenoids with final particle size of 344–366 nm, an encapsulation efficiency of 34–89% and a degree of isomerization from trans- to cis-carotenoid forms in the range of 0.02–15% were obtained [33]. A novel process, known as OEPO (organic solvent extraction and online particle formation), was developed by Santos *et al* [34]. The process consists of the combination of 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.

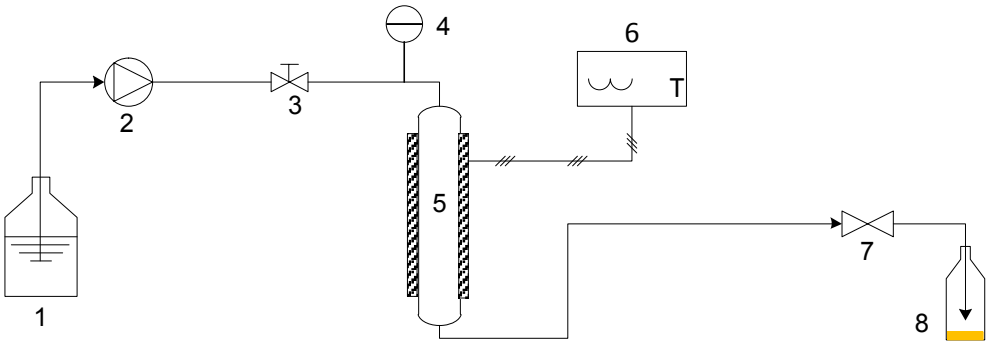
All of the recent works completed at LASEFI related to this emerging technology [31-37] belong to a broader project whose overall goal is the development of alternative sustainable systems for the formation of particles of sensitive bioactive substances from many vegetable matrices.



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

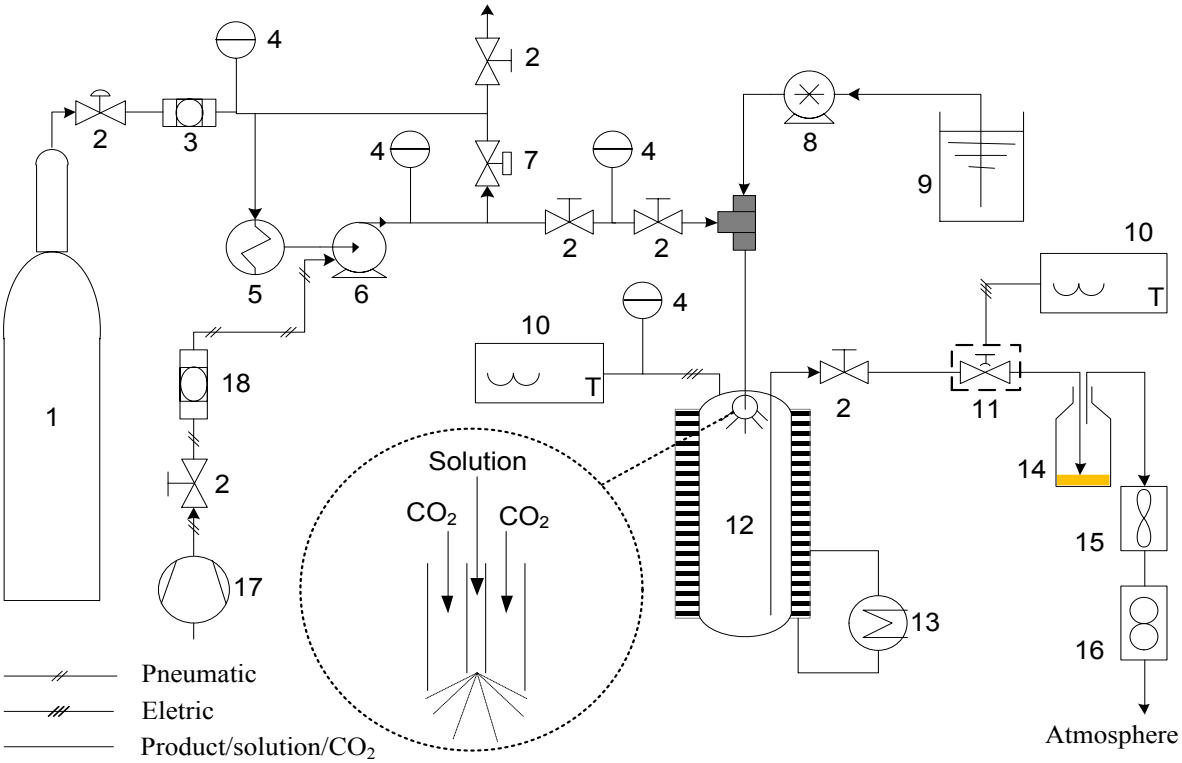
Scheme 4. Flow diagram for the SFE-2×5L experimental apparatus for extraction, including the co-solvent feed system (adapted from Prado [25])



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

Scheme 5. Flow diagram for the PLE-I experimental apparatusfor extraction (adapted from Rodrigues *et al* [38])



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

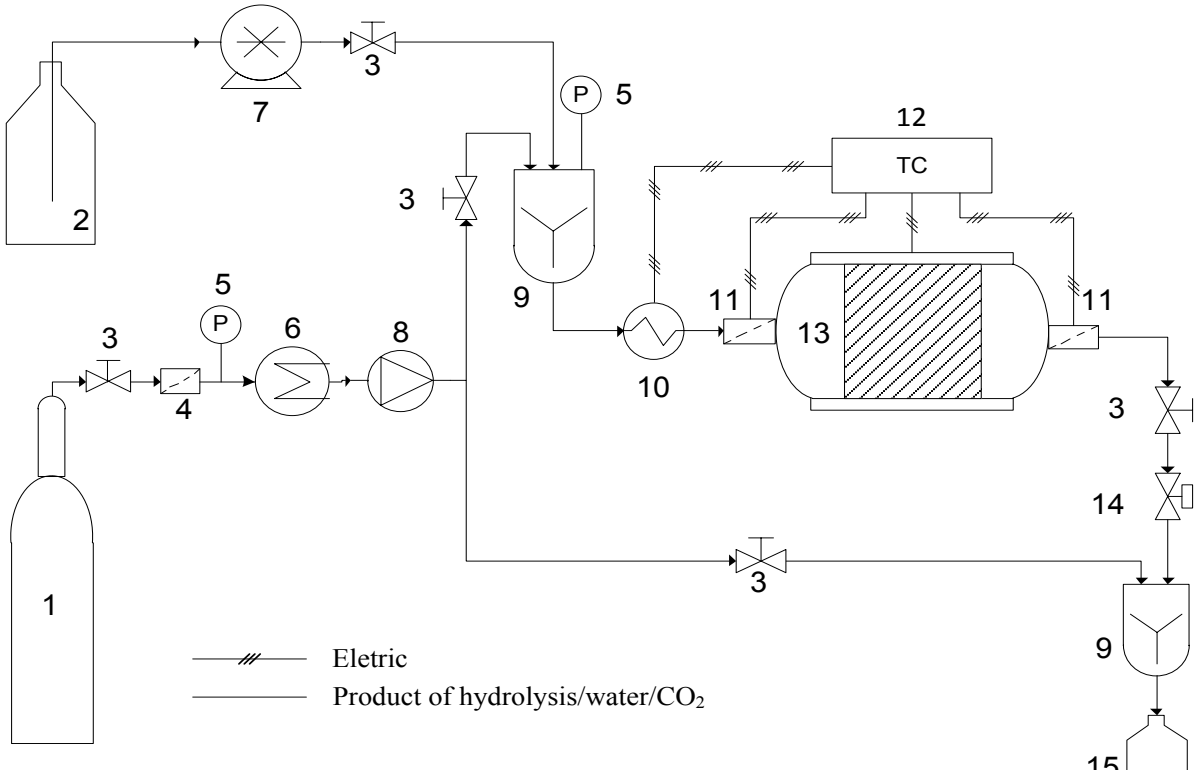
List of valves/instruments	
2	Blocking valve
3	CO_2 filter
4	Pressure gauge
7	Back pressure regulator
10	Temperature controller
11	Micrometering valve
15	Flowmeter
16	Flow totalizer
18	Air filter

Scheme 6. Flow diagram for the ARADIME experimental apparatus for micronization (adapted from Santos [30])

2.3. Hydrolysis of Agroindustrial Co-products Using Supercritical Technology

Another research line, pursued at LASEFI since 2004, relates to the hydrolysis of agroindustrial waste using sub/supercritical water + CO_2 for the production of sugars that can be eventually utilized for second-generation ethanol production. The HYDRO system (Scheme 7), containing a 0.05 L reactor, was designed, assembled and tested by Prado *et al* [39]. The system was designed to operate at pressures up to 40 MPa and temperatures up to 673 K. Water is pumped from reservoir 2, and if addition of CO_2 is enabled, the static mixer (instrument 9) serves to homogenate the solvents before their percolation inside the reactor containing the biomass. Generally, the biomass consists of lignocellulosic material, a non-edible source of fermentable sugars widely available in the nature. To validate the HYDRO system, hydrolyses of cellulose and sugarcane bagasse were performed. The glucose equivalent concentrations in the hydrolysate fractions were approximately 6 g/100 g of cellulose for a reaction time of 60 min and 5.9 g/100 g sugarcane bagasse for a reaction time of 40 min.

The reducing sugars obtained in the hydrolysate fractions can be used in fermentative processes for the production of renewable energy sources. This method of obtaining energy can become the basis for the development of modern industrial economies in the future. Ethanol is emerging as an efficient and economically feasible fuel. With the goal of obtaining sugar precursors for ethanol production, Follegatti-Romero *et al* [40] performed experimental assays with the HYDRO system using cellulose with water under subcritical conditions of 457 K, 470 K and 482 K with a reaction time of 68 min and at a pressure of 20 MPa. The authors concluded that the results obtained were satisfactory and that the total reducing sugar content recovered at 482 K was the highest. At this temperature, the recovery of sugars was also 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

Scheme 7. Flow diagram for the HYDRO experimental apparatus for hydrolysis (adapted from Prado *et al* [39])

3. Analytical Procedures

Chemical characterization of the products is important to assess their qualities. At LASEFI, researchers usually perform thin layer chromatography (TLC) for qualitative analysis of the extracts. An important method of quantitative analysis is gas chromatography (GC) coupled to a flame ionization detector (FID). HPLC (high performance liquid chromatography) analyses are also performed to identify and quantify the target compounds obtained. Additionally, supercritical fluid chromatography (SFC) has been recently implemented in the laboratory. The system will be used to develop methodologies for analyzing a wide range of components (phenolic acids, catechins, flavonoids, alkaloids, carotenoids, sterols, etc.) present in different natural sources.

4. Economic Evaluation of Processes Employing Supercritical Technology

Processes that make use of supercritical technology are usually technically feasible. Nonetheless, detailed investigations about their economic feasibility need to be performed if these processes are to be scaled up for industrial applications. Therefore, simulations of the cost of manufacturing (COM) extracts from several botanic matrices are being developed by researchers at LASEFI. Cavalcanti*et al* [20] simulated the COM of jabuticaba extract obtained by SFE in extractors of various sizes using a commercial simulator. According to their results, the cost of extract production was lower than US$ 10.00/kg in a 300 L extractor. Veggi*et al* [41] compared the COM of jabuticaba extract obtained by PLE and by 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 [42], annatto [24], pomegranate [19], grape [29] and sugarcane residue [27] were also simulated.

Table 2. LASEFI's research studies from 2009 to 2013

Raw materials studied	Extraction methods	Experimental determinations	Extracts' characterization	Theoretical studies	References
Annatto (*Bixaorellana* L.)	SFE	GYIs; OECs; X0	Bixin (UV-vis); Vitamin E (HPLC)	Mathematical modeling; COM	[24]
Pomegranate (*Punicagranatum* L.)	SFE	GYIs	TPC (UV-vis); Chemical composition (GC-MS); Aa	Process simulation; scale up; COM	[19]
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	[20],[34], [41],[45-47]
Brazilian ginseng (*Pfaffia glomerata*)	SFE; PLE; LPSE	OECs; EY; X0	Fractionated separation (TLC); Ecdysteroids (HPLC); Aa (DPPH method)	Process simulation; energetic analysis; COM;	[16],[48], [49]
Grape (*Vitisvinifera*L.)	SFE	OECs; scale up; Separation step of compounds	Fatty acid (GC); crystallization of the oil (DSC)	COM; scale up	[29],[50]
Clove (*Eugenia caryophyllus*)	SFE	OECs; scale up; Separation step of compounds	Chemical composition (GC-FID)	Mathematical modeling; simulation and optimization	[27],[51]
Macela (*Achyroclinesatureioides*)	SFE; LPSE	GYIs; EY; scale up	Aa; TPC; TFC; GAC (UV-vis); chemical composition (GC-FID)	Mathematical modeling; COM	[18],[21], [52],[53]
Chamomile (*Chamomillarecutita* L.)	SFE	OECs	Chemical composition (GC-FID)	Mathematical modeling; process optimization	[23]
Vetiver (*Vetiveriazizanioides*)	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	[54]
Ginger (*Zingiberofficinale*)	SFE	GYIs; OECs; EY; scale up	Fractional separation (TLC); chemical composition (GC-FID)	Scale up	[27]
Jambul (*Syzygiumcumini*)	UAE; ABE	EY; Anthocyanin extraction	TPC; TMA (UV-vis); TLC; Electrospray Ionization Tandem Mass Spectrometry	-	[55]
Jatoba (*Hymenaeacourbaril* L.)	UAE; ABE	OECs; EY	TPC (UV-vis); Aa (DPPH method); TLC; Polyphenols (HPLC)	Scale up	[56]

5. Summary of the Current Scientific Investigations Conducted at LASEFI

Table 2 shows a summary of the recent projects developed at LASEFI coveringthe application of supercritical fluids to several experimental processes. The large biodiversity of bioresources in Brazil has favored the use of different raw materials, as annatto (*Bixaorellana* L.), which contains tocotrienols and bixin [24] to be used in the treatment of cardiovascular diseases [43] and to be applied as colorant in food processing [44], respectively.

6. Concluding Remarks

Some progress has been made compared to the recent research results. For instance, one ongoing projectaims to develop an integrated analysis system by gathering the sample preparing with the chromatographic analyses. The sample preparing step is done by pressurized liquid extraction (PLE) assisted by ultrasound or supercritical fluid extraction (SFE) online coupled to a purification step using solid phase extraction (SPE). This integrated system will be applied to develop methodologies for determining a wide range of bioactive compounds.

Likewise, producing high-added vegetal extracts using green processes is a continuous effort done by the research group. Emerging applications of supercritical fluids, such as micronization and the encapsulation of nanoparticles, are being studied to produce several phytochemicals useful in the food and pharmaceutical industries. The processes include the encapsulation of nanoparticles containing target components from annatto, jabuticaba waste and Brazilian ginseng. Moreover, the use of environmentally friendly pressurized fluid technologies to obtain products from biomass has shown encouraging results. Another ongoing project is focused on the attainment of multiple products through a combination of different processes and sequenced with the use of multiple fluids. The goal is to use the knowledge and available tools on energy integration and life cycle analysis seeking an integral use of all parts of the Brazilian ginseng (root and aerial parts), expecting a Brazilian ginseng biorefinery based on supercritical technology.

Process intensification, process integration, extraction in continuous mode and hydrolysis of agroindustrial residues are also ongoing projects performed at LASEFI. In summary, the present objectives of the research group revolve around the scale up of the laboratory processes for the production of various extracts to effectively start up an industrial plant employing supercritical technology in Latin America.

ACKNOWLEDGMENTS

Giovani L. Zabot thanks FAPESP (2011/23665-2) and Moyses N. Moraes thanks CAPES for granting their Ph.D. assistantships. M. A. A. Meireles acknowledges a productivity grant (301301/2010-7) from CNPq. The authors acknowledge the financial support from CAPES, CNPq and FAPESP.

REFERENCES

[1] M. N. Moraes, G. L. Zabot, J. M. Prado, M. A. A. Meireles, "Obtaining Antioxidants from Botanic Matrices Applying Novel Extraction Techniques", Food and Public Health, vol.3, no.4, pp.195-214, 2013.

[2] G. A. Núñez, C. A. Gelmi, J. M. del Valle, "Simulation of a supercritical carbon dioxide extraction plant with three extraction vessels", Computers and Chemical Engineering, vol.35, no.12, pp.2687-2695, 2011.

[3] D. A. Oliveira, A. A. Salvador, A. Smânia, E. F. A. Smânia, M. Maraschin, S. R. S. Ferreira, "Antimicrobial activity and composition profile of grape (*Vitis vinifera*) pomace extracts obtained by supercritical fluids", Journal of Biotechnology, vol.164, no.3, pp.423-432, 2013.

[4] G. Brunner, N. T. Machado, "Process design methodology for fractionation of fatty acids from palm fatty acid distillates in countercurrent packed columns with supercritical CO_2", Journal of Supercritical Fluids, vol.66, pp.96-110, 2012.

[5] E. Kiran, "Foaming strategies for bioabsorbable polymers in supercritical fluid mixtures. Part I. Miscibility and foaming of poly(l-lactic acid) in carbon dioxide + acetone binary fluid mixtures", Journal of Supercritical Fluids, vol.54, no.3, pp.308-319, 2010.

[6] J. P. S. Queiroz, M. D. Bermejo, M. J. Cocero, "Kinetic model for isopropanol oxidation in supercritical water in hydrothermal flame regime and analysis", Journal of Supercritical Fluids, vol.76, pp.41-47, 2013.

[7] S. Machmudah, Wahyudiono, N. Takada, H. Kanda, K. Sasaki, M. Goto, "Fabrication of gold and silver nanoparticles with pulsed laser ablation under pressurized CO_2", Advances in Natural Sciences: Nanoscience and Nanotechnology, vol.4, no.4, 2013.

[8] D. Villanueva Bermejo, E. Ibáñez, R. P. Stateva, T. Fornari, "Solubility of CO_2 in ethyl lactate and modeling of the phase behavior of the CO_2 + ethyl lactate mixture", Journal of Chemical and Engineering Data, vol.58, no.2, pp.301-306, 2013.

[9] V. Marulanda, G. Bolaños, "Supercritical water oxidation of a heavily PCB-contaminated mineral transformer oil: Laboratory-scale data and economic assessment", Journal of Supercritical Fluids, vol.54, no.2, pp.258-265, 2010.

[10] H. Mhemdi, E. Rodier, N. Kechaou, J. Fages, "A supercritical tuneable process for the selective extraction of fats and essential oil from coriander seeds", Journal of Food Engineering, vol.105, no.4, pp.609-616, 2011.

[11] H. Machida, M. Takesue, R. L. Smith, "Green chemical processes with supercritical fluids: Properties, materials, separations and energy", Journal of Supercritical Fluids, vol.60, pp.2-15, 2011.

[12] J. W. King, "Supercritical fluid-based extraction/processing: Then and now", International News on Fats, Oils and Related

Materials, vol.23, no.2, pp.124-128, 2012.

[13] D. Ciftci, M. D. A. Saldaña, "Enzymatic synthesis of phenolic lipids using flaxseed oil and ferulic acid in supercritical carbon dioxide media", Journal of Supercritical Fluids, vol.72, pp.255-262, 2012.

[14] K. Gairola, I. Smirnova, "Hydrothermal pentose to furfural conversion and simultaneous extraction with SC-CO_2 - Kinetics and application to biomass hydrolysates", Bioresource Technology, vol.123, pp.592-598, 2012.

[15] S. Kazemi, V. Belandria, N. Janssen, D. Richon, C. J. Peters, M. C. Kroon, "Solubilities of ferrocene and acetylferrocene in supercritical carbon dioxide", Journal of Supercritical Fluids, vol.72, pp.320-325, 2012.

[16] P. F. Leal, M. B. Kfouri, F. C. Alexandre, F. H. R. Fagundes, J. M. Prado, M. H. Toyama, M. A. A. Meireles, "Brazilian ginseng extraction via LPSE and SFE: global yields, extraction kinetics, chemical composition and antioxidant activity", J. Supercrit. Fluids, vol.54, no.1, pp.38-45, 2010.

[17] M. A. A. Meireles, P. F. Leal, F. C. Alexandre, M. B. Kfouri, "Extraction process of active substances from brazilian ginseng", PI0900551-0A2, Brazil, 2009.

[18] T. M. Takeuchi, M. L. Rubano, M. A. A. Meireles, "Characterization and functional properties of macela (*Achyrocline satureioides*) extracts obtained by supercritical fluid extraction using mixtures of CO_2 plus ethanol", Food Bioproc. Technol., vol.3, no.6, pp.804-812, 2010.

[19] R. N. Cavalcanti, H. J. J. Navarro-Díaz, D. T. Santos, M. A. Rostagno, M. A. A. Meireles, "Supercritical carbon dioxide extraction of polyphenols from pomegranate (*Punica granatum* L.) leaves: chemical composition, economic evaluation and chemometric approach", J. Food Research, vol.1, no.3, pp.282-294, 2012.

[20] R. N. Cavalcanti, P. C. Veggi, M. A. A. Meireles, "Supercritical fluid extraction with a modifier of antioxidant compounds from jabuticaba (*Myrciaria cauliflora*) byproducts: economic viability", Procedia Food Sci., vol.1, pp.1672-1678, 2011.

[21] P. C. Veggi, "Obtaining vegetable extracts by different extraction methods: experimental study and process simulation", Master Thesis, University of Campinas/ Brazil/ Department of Food Engineer, Campinas-SP/Brazil, 2009.

[22] M. A. A. Meireles, P. T. V. Rosa, "Extraction and purification process of artemisin from solid mass of *Artemisia annua* using carbon dioxide", PI0903275-4A2, Brazil, 2009.

[23] E. Rahimi, J. M. Prado, G. Zahedi, M. A. A. Meireles, "Chamomile extraction with supercritical carbon dioxide: mathematical modeling and optimization", J. Supercrit. Fluids, vol.56, no.1, pp.80-88, 2011.

[24] C. L. C. Albuquerque, M. A. A. Meireles, "Defatting of annatto seeds using supercritical carbon dioxide as a pretreatment for the production of bixin: experimental, modeling and economic evaluation of the process", J. Supercrit. Fluids, vol.66, pp.86-95, 2012.

[25] J. M. Prado, "Scale-up study of supercritical fluid extraction process in fixed bed", Doctoral in Food Engineering, Unicamp/Food Engineering, Campinas-Brazil, 2010.

[26] G. L. Zabot, M. N. Moraes, M. A. A. Meireles, "Supercritical fluid extraction of bioactive compounds from botanic matrices: experimental data, process parameters and economical evaluation", Recent Patents Eng., vol.6, no.3, pp.182-206, 2012.

[27] J. M. Prado, G. H. C. Prado, M. A. A. Meireles, "Scale-up study of supercritical fluid extraction process for clove and sugarcane residue", J. Supercrit. Fluids, vol.56, no.3, pp.231-237, 2011.

[28] G. L. Zabot, M. N. Moraes, A. J. Petenate, M. A. A. Meireles, "Influence of the bed geometry on the kinetics of the extraction of clove bud oil with supercritical CO_2", Journal of Supercritical Fluids, 2013, *In press*.

[29] J. M. Prado, I. Dalmolin, N. D. D. Carareto, R. C. Basso, A. J. A. Meirelles, J. V. Oliveira, E. A. C. Batista, M. A. A. Meireles, "Supercritical fluid extraction of grape seed: process scale-up, extract chemical composition and economic evaluation", J. Food Eng., vol.109, no.2, pp.249-257, 2012.

[30] D. T. Santos, "Extraction, micronization and stabilization of functional pigments: construction of multipurpose unit for pressurized fluid process development", Doctoral Thesis, University of Campinas/Brazil, Department of Food Engineering, Campinas-SP/Brazil, 2011.

[31] D. T. Santos, M. A. A. Meireles, "Micronization and encapsulation of functional pigments using supercritical carbon dioxide", J. Food Process Eng., vol.36, no.1, pp.36-49, 2011.

[32] D. T. Santos, J. Q. Albarelli, M. M. Beppu, M. A. A. Meireles, "Stabilization of anthocyanin extract from jabuticaba skins by encapsulation using supercritical CO_2 as solvent", Food Research Int., vol.50, no.0, pp.617-624, 2012.

[33] D. T. Santos, A. Martin, M. A. A. Meireles, M. J. Cocero, "Production of stabilized sub-micrometric particles of carotenoids using supercritical fluid extraction of emulsions", J. Supercrit. Fluids, vol.61, pp.167-174, 2012.

[34] D. T. Santos, D. F. Barbosa, K. Broccolo, M. T. M. S. Gomes, R. Vardanega, M. A. A. Meireles, "Pressurized organic solvent extraction with on-line particle formation by supercritical anti solvent processes", Food Public Health, vol.2, no.6, pp.231-240, 2012.

[35] M. T. M. S. Gomes, D. T. Santos, M. A. A. Meireles, "Trends in Particle Formation of Bioactive Compounds Using Supercritical Fluids and Nanoemulsions", Food Public Health, vol.2, pp.142-152, 2012.

[36] D. T. Santos, D. F. Barbosa, R. Vardanega, J. Q. Albarelli, M. A. A. Meireles, "Experimental and simulation study on formulation of clove essential oil products using alternative surfactant", J. Coll. Sci. Biotech., vol.2, pp.112-122, 2013.

[37] D. T. Santos, D. F. Barbosa, R. Vardanega, M. T. M. S. Gomes, M. A. A. Meireles, "Novel method to produce emulsions containing essential oils from saponin-rich pressurized aqueous plant extracts", J. Coll. Sci. Biotech., vol.2, pp.93-99, 2013.

[38] L. M. Rodrigues, S. C. Alcázar-Alay, A. J. Petenate, M. A. A. Meireles, "Bixin extraction from defatted annatto seeds", Comptes Rendus Chimie, 2014, *In press*.

[39] J. M. Prado, L. A. Follegatti-Romero, F. P. Cardenas-Toro, T. Forster-Carneiro, M. A. Rostagno, M. A. A. Meireles, 2012, Cellulose and sugarcane bagasse hydrolysis using subcritical

water, Proc. 10th International Symposium on Supercritical Fluids, San Francisco, CA-USA.

[40] L. A. Follegatti–Romero, J. M. Prado, T. F. Carneiro, F. P. Cardenas–Toro, M. A. Rostagno, M. A. A. Meireles, 2012, Semi–batch equipment for the hydrolysis of lignocellulosic raw materials, Proc. 10th International Symposium on Supercritical Fluids, San Francisco, CA-USA.

[41] P. C. Veggi, D. T. Santos, M. A. A. Meireles, "Anthocyanin extraction from Jabuticaba (*Myrciaria cauliflora*) skins by different techniques: economic evaluation", Procedia Food Sci., vol.1, pp.1725-1731, 2011.

[42] J. M. Prado, A. R. Assis, M. R. Marostica, M. A. A. Meireles, "Manufacturing cost of supercritical-extracted oils and carotenoids from amazonian plants", J. Food Proc. Eng., vol.33, no.2, pp.348-369, 2010.

[43] B. Tan, "Appropriate spectrum vitamin E and new perspectives on desmethyl tocopherols and tocotrienols", Journal of American Nutraceutical Association, vol.8, pp.35-42, 2005.

[44] V. Akshatha, P. Giridhar, G. A. Ravishankar, "Morphological diversity in *Bixa orellana* L. and variations in annatto pigment yield", Journal of Horticultural Science and Biotechnology, vol.86, pp.319-324, 2011.

[45] D. T. Santos, M. A. A. Meireles, "Optimization of bioactive compounds extraction from jabuticaba (*Myrciaria cauliflora*) skins assisted by high pressure CO_2", Innov. Food Sci. Emerg. Technol., vol.12, no.3, pp.398-406, 2011.

[46] D. T. Santos, P. C. Veggi, M. A. A. Meireles, "Extraction of antioxidant compounds from jabuticaba (*Myrciaria cauliflora*) skins: yield, composition and economical evaluation", J. Food Eng., vol.101, no.1, pp.23-31, 2010.

[47] D. T. Santos, C. L. C. Albuquerque, M. A. A. Meireles, "Antioxidant dye and pigment extraction using a homemade pressurized solvent extraction system", Procedia Food Sci., vol.1, pp.1581-1588, 2011.

[48] D. T. Santos, J. Q. Albarelli, M. A. A. Meireles, "Simulation of an integrated sustainable production of extract from Brazilian ginseng roots with a cogeneration plant", Chem. Eng. Transactions, vol.29, pp.91-96, 2012.

[49] R. Vardanega, D. T. Santos, I. C. N. Debien, M. A. A. Meireles, 2012, Pressurized liquid extraction of bioactive compounds from brazilian ginseng roots (*Pfaffia glomerata*), Proc. 16th world congress of food science and technology, Foz do Iguaçu-PR/Brazil.

[50] A. M. Farías-Campomanes, M. A. Rostagno, M. A. A. Meireles, "Production of polyphenol extracts from grape bagasse using supercritical fluids: yield, extract composition and economic evaluation", J. Supercrit. Fluids, vol.77, pp.70-78, 2013.

[51] T. Hatami, M. A. A. Meireles, G. Zahedi, "Mathematical modeling and genetic algorithm optimization of clove oil extraction with supercritical carbon dioxide", J. Supercrit. Fluids, vol.51, no.3, pp.331-338, 2010.

[52] T. Hatami, R. N. Cavalcanti, T. M. Takeuchi, M. A. A. Meireles, "Supercritical fluid extraction of bioactive compounds from macela (*Achyrocline satureioides*) flowers: kinetic, experiments and modeling", J. Supercrit. Fluids, vol.65, pp.71-77, 2012.

[53] A. R. Assis, "Assembly, test and validation of a supercritical extraction unit with recycle and continuous operation", Doctoral Thesis, University of Campinas/Brazil, Department of Food Engineering, Campinas-SP/Brazil, 2010.

[54] T. M. Takeuchi, "Supercritical extraction of macela, clove and vetiver: technological and economical aspects", Doctoral Thesis, University of Campinas/Brazil, Department of Food Engineering, Campinas-SP/Brazil, 2009.

[55] D. T. Santos, R. N. Cavalcanti, M. A. Rostagno, C. L. Queiroga, M. N. Eberlin, M. A. A. Meireles, "Extraction of polyphenols and anthocyanins from the jambul (*Syzygium cumini*) fruit peels", Food Public Health, vol.3, no.1, pp.12-20, 2013.

[56] P. C. Veggi, D. T. Santos, A. S. Fabiano-Tixier, C. Bourvellec, M. A. A. Meireles, F. Chemat, "Ultrasound-assisted extraction of polyphenols from jatoba (H*ymenaea courbaril* L. var stilbocarpa) bark", Food Public Health, vol.3, no.3, pp.119-129, 2013.