Sterols and Fatty Acids Extraction Process from the Cactus
_Opuntia ficus-indica_ [(L.) Miller] by Means of Supercritical CO₂

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THESIS

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Hiermit übergebe ich euch alle Pflanzen auf der ganzen Erde, die Samen tragen, und alle Bäume mit samenhaltigen Früchten. Euch sollen sie zur Nahrung dienen.

See, I give you every seed-bearing plant all over the earth and every tree that has seed-bearing fruit on it to be your food.

Yo les doy todas las plantas que producen semilla sobre la tierra, y todos los árboles que dan frutos con semilla: ellos les servirán de alimento.

Genesis 1: 29
ABSTRACT

After 120 years of Supercritical Fluids (SCF) discovering, practical applications began to be developed. The SCF extraction (SCFE) of sterol fraction and fatty acids from the prickly pear seeds’ oil, considered sub-product from sweets processing, is compared to traditional extraction methods varying extraction time, modifier influence, temperature and pressure of supercritical CO$_2$ as main solvent. The main substances found were β-sitosterol (BS), Linoleic Acid (LA) and Palmitic Acid (PA). Low BS extraction velocities were found during the pure CO$_2$ extractions reaching the maximum yield after 55 min (0.09 mgBS/min), while by modified CO$_2$ at 35 min (0.16 mgBS/min). Best BS extraction conditions were found 35 °C and 175 bar while for LA and PA 55 °C and 125 bar offering the possibility for a further fractionation given the different affinities ruled by the difference in molar mass. SCFE confirmed a higher selectivity by adjusting the extraction conditions, saving time and aggressive solvent handling compared to traditional extraction.

ZUSAMMENFASSUNG

Nachdem vor 120 Jahren der überkritische Zustand von Fluiden entdeckt wurde, entwickelte sich danach der Einsatz in praktischen Anwendungsfällen. In der Arbeit wird die Extraktion von Sterolen und Fettsäuren aus dem Öl der Kaktusfeigensamen, die ein Abfall bei der Süßwarenherstellung darstellen, mit überkritischem Fluid und konventionellen Extraktionsmethoden miteinander verglichen. Dabei wird die Extraktionszeit, der Modifier, die Temperatur und der Druck des Lösungsmittels CO$_2$ variiert. Als Hauptkomponenten wurden β-Sitosterol (BS), Linolsäure (LA) und Palmitinsäure (PA) identifiziert. Bei der Extraktion mit reinem CO$_2$ wurden geringe Extraktionsgeschwindigkeiten ermittelt, wobei die maximale Ausbeute nach 55 min (0,09 mgBS/min) erreicht wurde – für CO$_2$ mit Modifier nach 35 min (0,16 mgBS/min). Die besten Extraktionsbedingungen sind für die Komponente BS 35 °C und 175 bar, für LA und PA lagen diese bei 55 °C und 125 bar. Daraus leitet sich die Möglichkeit einer weitergehenden Fraktionierung aufgrund unterschiedlicher Löslichkeiten durch die unterschiedlichen molaren Massen ab. Die Extraktion mit überkritischen Fluiden erlaubte verglichen mit konventionellen Extraktionsmethoden höhere Selektivitäten durch die Anpassung der Extraktionsparameter, geringere Extraktionszeiten und den Verzicht auf gefährliche Lösungsmittel.
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1 INTRODUCTION

Substances near or above their critical point, that means, those who follow the border line between the liquid and the gas phases until getting into an intermediate condition with advantageous characteristics of those both matter states, have become more and more important in production, transformation and remediation processes. One of the most exploited uses of this fast developing supercritical condition is the extraction, the separation of a solute from a matrix referring commonly cleaning metallic parts, removing pollutants from the soil and enrichment or depuration of certain living tissue.

Tons of material are wasted per year due to the lack of infrastructure for treatment, recovering and reuse, throwing ‘through the window’ valuable sub-products and getting a co-problem due to the prices for confinement places. Different governments invest in exchange of people to share ideas on using technology like the one of supercritical fluids on exploitable resources and find alternative paths to the traditional ones in the improvement of the processes.

Due to its orography and tropical proximity, Middle America possesses a high flora and fauna richness. Representatives of the Mexican tablelands are the cacti, with different advantages, adaptation and uses as native plant. Worldwide the economically most important cactus is the prickly pear cactus or ‘Nopal’ harvested all over the world because of the fruit, but with consumption of the tender cladodes (‘racquets’) only in Mexico due to its reduced number and size of spines. After the processing of the fruits we could consider a quasi holistic harvesting of the resource but the seeds are not considered in the majority of the sweets, candies or alcoholic beverages production. What to do with this sub-product?

Within Europe, Germany has been home of prominent researchers in scientific fields, recognized quality standards and innovative technology which offer affordable processes to take advantage of these materials. Placing the crushed seeds in a carbon dioxide chamber and reaching the critical pressure and temperature the CO$_2$ will present higher diffusion like a liquid and lower viscosity like a gas while the solvency increases together with the density.
Within the seeds tissues, the fats and lipids are the first energy sources for the new plants, other defense compounds: the sterols, are large polar molecules which are difficult to solve in pure CO$_2$, hence the solvent shall be modified by a co-solvent usually ethanol which adds polarity to the main solvent. Talking about fatty acids, in most of the cases includes linoleic acid and palmitic acid; the first one: unsaturated, ‘unstable’, digestible; the second one: saturated, more stable, easy to accumulate into the veins and arteries. The main sterolic fraction component in the plant’s oil is the $\beta$-sitosterol currently studied in the growing control of human prostate and colon cancer cells.

Another advantage of this kind of process compared to the traditional cold press or solvent extraction is the selectivity, adjusting the pressure and the temperature, the density of the fluid be adjusted to determine the solvency in a scale that it is possible to finely select the desired solutes in the extract. The solubility of the different compounds from the same matrix can be directed to the substance of our interest yielding an extract richer or more concentrated in certain solutes.

After the extraction, the product can be used in the pharmacological or nutrition industry. Besides this the supercritical CO$_2$ offers the advantage of a reduced solvent treatment in comparison with conventional methods without separation, cleaning, and/or confinement, besides to the low energy and time required for the process. The dried, deoiled, woody material post extraction can be used in briquettes and pellets production for thermal processes, finishing a holistic advantaging of the natural resource.
2 ANTECEDENTS

2.1 Supercritical Fluids

2.1.1 Historical development

Supercritical Fluids (SFC’s) have been used from 1822, when Cagniard de la Tour [34] while rocking the cannon autoclave back and forth realized that at certain temperature the CO₂ splash ceased. Also in 1860 [79] the English Chemist Thomas Andrews (1813-1885) researched the basis for gases compressibility, being for him especially interesting the aggregate state changes between fluids and gases. On the way of one of his experiments on carbon dioxide he discovered that the borderline between liquid and gas at 31 °C and high pressure disappears. Further systematic experiments brought that a gas with a temperature above certain point can not be liquefied by a simple pressure increase. Such point would be defined as the Critical Point (Figure 2.1) by Andrews in 1866.

In the same year [79] he postulated the thesis that the gases air, oxygen, nitrogen, carbon dioxide and hydrogen which were considered as permanent or incondensable, could be solidified or liquefied. The continuation of his experiments on substances at the Critical Point was performed by the English James B. Hannay (1855-1931). This reached to an idea about the meaning of a critical behavior and lead together with J. Hogarth for the first time to experiments on supercritical fluids solvent properties. Both scientists investigated about a higher concentration of inorganic salts in supercritical ethanol as it is in the pure vapor pressure. Only few years later, Johannes Diderik van der Waals (1837-1923) was in a position to explain theoretically the critical phenomenon.

In his brilliant dissertation in 1873 [79] “On the continuity of the gas and liquid state” published the after him well known formula in which he establishes the deviation of real gases from ideal behavior by the influence of intermolecular forces. Van der Waal’s theory had a basic meaning for further works on the field of Thermodynamics of same weight phases as for instance, the liquefaction of hydrogen and helium by James Dewar and Heike Kammerling Onnes.
However, supercritical fluids didn’t find any practical use until the forties, thus they became important after improving the high pressure techniques. From this time on, the investigations increased to the supercritical outstanding solvent and transport properties, from which both still agree today with the application area of these substances. Stahl, Drescher and Lürken –[132], [40] and [89] respectively– gave an exemplar overview of the further application area and development.

2.1.2 Supercritical fluids basics

The curves in a phase diagram represent coexistence between two of the phases. If we move upward along the gas-liquid coexistence curve which is a plot of vapor pressure vs. temperature, both temperature and pressure increase. The liquid becomes less dense because of thermal expansion and the gas become denser as the pressure rises. At the critical point the densities become identical, the distinction between the gas and the liquid disappears, and the curve comes to an end at the critical point. The substance is now described as a fluid.

![Phase diagram of carbon dioxide with the critical point at Tc 31.1 °C and Pc 73.8 bar](#)

Figure 2.1 Phase diagram of carbon dioxide with the critical point at $T_c$ 31.1 °C and $P_c$ 73.8 bar [65].
The critical point has pressure and temperature co-ordinates on the phase diagram, which are referred to as the critical temperature, \( T_c \), and the critical pressure, \( P_c \), and which have particular values for particular substances as exemplified in the table 2.1.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Critical Temperature, ( T_c ) (K)</th>
<th>Critical Pressure, ( P_c ) (bar)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon Dioxide</td>
<td>304</td>
<td>74</td>
</tr>
<tr>
<td>Water</td>
<td>674</td>
<td>221</td>
</tr>
<tr>
<td>Ethane</td>
<td>305</td>
<td>49</td>
</tr>
<tr>
<td>Ethene</td>
<td>282</td>
<td>50</td>
</tr>
<tr>
<td>Propane</td>
<td>370</td>
<td>43</td>
</tr>
<tr>
<td>Xenon</td>
<td>290</td>
<td>58</td>
</tr>
<tr>
<td>Ammonia</td>
<td>406</td>
<td>114</td>
</tr>
<tr>
<td>Nitrous oxide</td>
<td>310</td>
<td>72</td>
</tr>
<tr>
<td>Fluoroform</td>
<td>299</td>
<td>49</td>
</tr>
</tbody>
</table>

The liquid region to the left of the critical region has many of the characteristics of SCF’s and it is exploited in a similar way. For this reason some people prefer the term near-critical fluids and the adjective subcritical is also used.

Supercritical Fluids, [34] exhibit important characteristics, such as compressibility, homogeneity, and a continuous change from gas-like to liquid-like properties.

Clifford, [34] emphasizes that the use of SCF’s in extraction compared to liquid extraction:

- It is usually more rapid
- The solvent is easier to remove
- The pressure (as well as the temperature and the nature of the solvent) can be used to select, to some extent the compounds to be extracted.
- Carbon dioxide is available, to be used as a pure or modified solvent with its convenient critical temperature, its cheapness and non-toxicity.

---

1 Parameters from Reid et al. in Clifford [34].
Poliakoff [24] mentions Green Chemistry as one of the latest buzzwords in the drive to improve our environment, and SCF’s replace the toxic and volatile solvents of chemical manufacturing. “By definition it is the design, development and implementation of chemical products and processes to reduce or eliminate the use and generation of substances hazardous to human health and the environment”.

Carbon dioxide [34] has so far been the most widely used because of its convenient critical temperature, cheapness, chemical stability, non-flammability, stability in radioactive applications, and non-toxicity.

Perhaps one of the most successful application areas of supercritical fluids is supercritical fluid chromatography [77].

2.2 Supercritical Fluids Extraction

Substances in supercritical conditions have been explored as solvents because of different advantages: It’s usually more rapid (the extraction) because of the low viscosity and high diffusion coefficient [88] the solvent is easier to remove. Pressure (as well as temperature and the nature of the solvent) can be used to select, to some extent, the compounds to be extracted; carbon dioxide is available to be used as a pure or modified solvent [34].

Traditional solvent extraction or ‘TSE’ [102] requires multiple steps, consumes a large amount of organic solvent and most importantly, extracts products have solvent residue. On the other hand, SFE in most cases supercritical CO₂ (SC-CO₂) extraction provides an extraction environment with reduced potential for oxidation of carotenoids. Extraction with carbon dioxide [110] provided very low liquid yields but with the presence of relevant compounds not observed in the conventional extraction methods.

Extractions [77] are carried out either to isolate a desired compound of higher value or remove undesirable components from a raw material to obtain a product with improved properties. Removal of impurities from polymers, cleaning of electronic parts, binder removal from ceramics, separation of buckminsterfullerene and environmental remediation are among more specific application.
At the present, an application area of much activity is the environmental remediation and removal of toxic contaminants from soils and industrial waste using supercritical fluids. It is also possible to transform skins into leather in supercritical CO\(_2\) medium. The green solvent SC-CO\(_2\) was used also to extract the volatile part, rich in thermolabile compounds, from a Romanian mint hybrid. Hydrolization for the extraction of essential oil rich in sabinene hydrates favors isomerizations and hydrolysis because of the prolonged water contact time at high temperature. SC-CO\(_2\) avoids this thermal degradation and solvent pollution.

Comparison between hydrodistillation and SC-CO\(_2\) extraction reveals the superiority of the green solvent, SC-CO\(_2\), to obtain a good yield and a flavor similar to plant material and to keep the native composition present in the genuine plant. This study suggests a way to obtain z-sabinene hydrate in high yield. Kery et al. [76] support this idea saying that SCF extracts prove to be a true alternative to the conventional procedures. To obtain such extracts carbon dioxide is an extractant which is physiological acceptable and products obtained this way are free of solvents.

Givenç [55] describes the energy costs for recovery of ethanol for two column conventional distillation, conventional + azeotropic distillation with pentane and supercritical fluid extraction with carbon dioxide are 4730 kJ/L, 1800 kJ/L and 2500 kJ/L respectively. Hence the energy consumption of the supercritical fluid extraction process is more than that of distillation process. Experiments, explains Givenç, were carried out at 313 K and 120 atmospheres, for 60 minutes because the density of the SC-CO\(_2\) is near to the normal liquid density under these conditions and consequently it has a high solvent power. At 313 K extraction yield rose from 8.6 % to 11.2 % with increasing pressure from 80 to 160 atmospheres. At 330 K, the effect of pressure was not as obvious. The first batch extraction experiment was carried out for 60 minutes. After the CO\(_2\) was bled down to the atmospheric pressure, the system pressure was raised again to 120 atmospheres with fresh CO\(_2\). This operation was repeated at the end of 30 minutes. Thus the feed solution had contact with fresh SC-CO\(_2\) at three consecutive steps consecutive solvent feeding increased extraction yield. Extraction yield rose from 6.5 to 21.9 % at the end of 3 steps. This shows that consecutive extraction is superior to batch extraction.
The extraction selectivity [129] can be manipulated by using modifiers [34], moderators\(^2\), co-solvents or entrainers [25]. Modifiers may increase significantly artemisinin yield, it has to be taken into account that this measure strongly affects selectivity. The phospholipids can not significantly be extracted with SC-CO\(_2\). The polarity of the SCF [25] needs to be increased by adding ethanol as modifier. The extraction of phospholipids strongly depends on the composition of the SC-Fluid and the extraction pressure applied. During the determination of total fat content of fish products [47] one of the optimized parameters was the use of EtOH (5\% mol/mol) as modifier.

2.2.1. Extraction of oil, lipids and other organic compounds by SCF’s

Several experiments have been recently performed around the world due to the benefits offered by SCF’s. Tocopherols, tocotrienols [120], carotenoids, flavonoids [14] have been selectively extracted with very good results.

Henry et al. [67] report a better quality egg yolk constituents, including the consumer-desired flavor retained by SCFE, while the undesired components, as cholesterol and triglycerides are selectively removed from the natural egg yolk. For Mostert et al. [105] the use of SC-CO\(_2\) may have more selectivity creating paths of less resistance through the vegetal substrate while hexane has possibly higher oil yields but, at the same time less selectivity permeating the whole substrate.

Looking for sterols and tocopherols, Guclu-Ustundag and Temelli [61] found the potential of canola oil deodorizer as a source of sterols. Ashtiani [10] found 1,8-cineole and pinene as the main volatile oil constituents of two different *Eucalyptus* species leaves.

Conditions of 90 bar and a 1.32 kg/h CO\(_2\) mass flow at 40 °C brought the best results for Coelho et al. [36], while extracting volatile compounds from dried flowers and leaves of *Satureja fruticosa* Beguinot.

\(^2\) G. M. Schneider in Wenclawiak B. [147]
Oil extraction of vetiver and clove performed by Martinez et al. [101] studying the kinetics of the Supercritical fluids extraction showed the Sovová Model as appropriate to predict the extraction curves on large scale.

Comparing the extractions of black seed (*Nigella sativa*) oil rich in antioxidants by means of soxhlet extraction method and the supercritical extraction, Rao et al. [121] found superior those by supercritical extraction with low yield.

Fiori [49] reports also by modeling of the complete curves, transport parameters regulating the oil mass transfer inside the seed particles, that the internal transport coefficient and the effective diffusivity have been calculated.

Hard resins, lipids and waxes in a mixture obtained by solvent extractions of hops by SC-CO$_2$, mentioned as ‘liquid carbon dioxide’ by Marriot [100]. This mixture, affirms Marriot, can be used as foam suppresser in brewing processes.

*Pinus nigra* ssp. Laricio can be considered as a diterpene source according to the research of Duquesnoy et al. [41], after extracting volatile compounds from the pine’s needles.

Pourmortazavi et al. [118] confirmed the expected advantages of the supercritical fluids, while analyzing essential and volatile oils from plants, they mention the rapidness, selectivity and convenience performing the SFE’s with CO$_2$ pure or modified.

Brunner [26] differentiated the use of SC-CO$_2$ for the tocochromanol enrichment of the palm oil and *near critical* propane (below the critical temperature) was used for carotenoid enriched oil.

Wang [144] obtained oil samples from *Nitraria tangutorum* by SC-CO$_2$ extraction methods, reporting its coupling with mass spectrometry detection (MSD) as a high sensitive method for analyzing free fatty acids. Together SCFE and MSD, it is possible to perform high efficient oil extractions and extremely sensitive fatty acid profile determinations.
While extracting volatile compounds of *Zanthoxylum rhesta* DC and analyzing their composition Rout *et al.* [125] found the essential oil concentrated in pericarp and the fatty oil in the seeds. They compared subcritical CO₂ extraction and traditional extraction processes finding superior the results obtained by subcritical CO₂ requiring a pretreatment of the pericarp by methanol.

Setting different conditions it is possible to extract different flavor and fragrance constituents like the ones from *Hyssopus officinalis* L. performed by Kazazi *et al.* [75]. Concentrating natural tocopherols [45] supercritical methanol batch extraction experimental results show the process from the soybean refining process byproduct as an energy saving process, moreover the shorter time and smaller amounts of waste water. It leads to more fatty acids methyl esters and sterols than conventional pretreatment without damage to the tocopherols at 573 K, 20.1 MPa for 30-45 min.

Volatile oil extracts of different aerial parts of *Ridolfia segetum* (L.) Moris, have been analyzed by Marongiu *et al.* [96] reporting the extraction conditions as follows: 90 bar, 50 °C and a CO₂ flow of 1.0 kg·h⁻¹.

2.2.2 Alternative uses of SCF’s

Zhang *et al.* [152] extracting and micro-encapsulating procyanidins take advantage of the fact that grape seeds, as byproducts of grape juice and wine production, are a rich source of procyanidins. Even though, they are discarded as waste.

Trejo Rodriguez *et al.* [136] have used supercritical solvents to regenerate lubricating oils and, according to their publication, this is an environmentally friendly process because neither aggressive nor toxic substances are used.

The extraction of kerogen by means of dense phase fluids like supercritical CO₂ from underground oil shale formations is described by Looney *et al.* [84].

Supercritical ethane has been used by Avila-Chávez *et al.* [11] to study the extraction of hydrocarbons from crude oil tank bottom sludge.
Uses of fluids at supercritical temperatures and pressures can vary like the one of Hough [69], who implemented a method to break cross links in vulcanized rubber by swelling the rubber in the supercritical fluid (like CO\textsubscript{2}, N or a refrigerant) followed by a rapid decompression.

The extraction by means of supercritical pentane [154] followed by fractionation allows cutting deep into the bottoms of the vacuum residues of heavy oils while readily yielding enough of each fractionation to allow characterization and reactivity studies.

2.3 Importance of Prickly Pear Opuntia spp.

2.3.1Origin of Opuntia spp.

\textit{Opuntia ficus-indica} (L.) Miller is a native cactus to Mexico [123]. Wild plants have not been found and it has been proposed that it derived from \textit{O. amyclae} or \textit{O. megacantha}, two species distributed in north central Mexico tablelands. The most extreme degree of domestication in any given species is characterized by its dependence on man-made habitats to survive. All \textit{Opuntia} cultivars are located in protected environments, either plantations or home gardens, since their survival in areas exposed to herbivore vertebrates is unlikely.

The domestication process of Opuntia was directed towards producing plants with cladodes (Figure 2.2, B) lacking spines and with large sweet fruits, a process developed in the south of the meridional highlands of Mexico. There is archaic-botanical evidence indicating the use of \textit{Opuntia} spp. by several ethnic groups since about 8000 years ago, none of it can be directly associated to \textit{O. ficus-indica}.

The Spanish took this species to their homeland, given its morphological peculiarities, and because of its edible fruits, anti-scurvy properties and for being the host of the cochineal insect, from which the commercially important cochineal dye was obtained. Afterwards, it was introduced to other parts of the world, particularly to Mediterranean region.
Figure 2.2 Left (from Reyes [123]) *Opuntia ficus-indica*. A, habit; B, cladode; C, areole of cladode; D, flower; E areole of flower; F, longitudinal section of flower; G, fruit; H, dorsal view of seed; I, ventral view of seed. Bars = 1 m (A), 10 cm (B), 5 mm (C, E), 4 cm (D, F), 5 cm (G), 4 mm (H, I).

*Opuntia ficus indica* “tree” in Neapel, Italy. Right (personal archive): detail of the flowers, young fruits and cladodes.

About the reproductive biology, Reyes [123] mentions that among Cactaceae, *Opuntia* is the most diverse and widely distributed genus in the Americas.

Reyes [123] also mentions that the Nahuatl pre-Columbian name of *O. ficus-indica* was *Tenochtli* or *Tzapotlnochtli*. *Nochtli* is the Nahuatl word for cactus pear. The first name *-Tenochtli-* means divine cactus pear; the latter derives from the similarity between the cactus pear fruit with the spherical fleshy fruits generically denominated *Tzapotl* (*Manilkara zapota* and *Diospyros digyna*).

The name most extensively used outside México is Indian Fig [123], because of its supposed resemblance to Ficus fruits and its American origin; its scientific epithet derived from this name, as well as some common names in other languages (Indischer Feigenkaktus and Fico d’India). Other common names refer to the lack of spines (Smooth Prickly Pear) or shape (Higo de Pala).
Figure 2.3 Dr. Rigoberto Vazquez Alvarado in his nopal plantation, at the Faculty of Agronomy Sciences. Mina N. L. UANL, Mexico.

The Spanish name *Tuna* is a word of the Caribbean Taino language; the Arabs call it Christian Fig, and the Portuguese name it *Figuiera Moura*; its name is Mission Cactus in the United States, since Franciscan monks established missions in California during the 18th century, using it as fruit, decoration and building material. It is called *Sabra* in Israel, a name also given to some local inhabitants in the country; in Brazil it is called *Palma Forrageira*, because it is used almost exclusively as fodder. The richness of common names for *O. ficus-indica* (L.) Miller is a signal of its importance. People name only plants with economical, social or ecological value.

Reyes [123] offers a plant description as shrubby to arborescent, to 1.7(−3) m tall, with a lignified, well-defined primary stem. Stem dark-brown, green or gray, cylindrical, to 45 cm high, to 20 cm diameter. Cladodes usually elliptic, but also obovate, ovate, circular, oblong, ob lanceolate or rhombic, (27−) 32-44 (−63) cm long in 2-3 year old cladodes, (14−) 18- 25(−31) cm wide, (1−) 1.8-2.3 (−3) cm thick, with an area of (356−) 462-796 (−1182) cm², usually pale green (Munsell color 2.5GY 6/2), ranging from pale green (2.5GY 8/2) to dark green (7.5GY 7/4), with (6−) 8-11 (−19) spiral areole series, with a distance between series of (2.7−) 3-4 (−5) cm; young cladodes with prominent podaria, with leaves conical to 5-7 mm long, the areoles with 0-1 acicular spine and 0-2 setose spines. Areoles of developed cladodes (38−) 52-69 (−80) per face, with a density of (4−) 8-14 (−18) areoles per each 100 cm², elliptic, obovate, ob lanceolate or ovate, rarely circular or rhombic, (2−) 3-4 (−8) mm long, 2-3 (−5) mm wide.
Spines usually absent, but sometimes a few areoles in the cladode with one spine, usually acicular, depressed, white, (3−) 4-7 (−10) mm long. Glochids usually abundant in the proximal areoles of the cladode, sometimes absent.

Fruit usually turbinate, sometimes spherical, cylindrical or ellipsoidal, frequently bright yellow (Munsell color 2.5GY 8/6) ranging from pale yellow (2.5Y 7/10) to red purple (5RP 4/6), less frequently of a combination of yellow/red, (5-) 7-9 (-10) cm long, (4-) 5-6 (-7) cm wide, (45-) 86-146 (-223) g weight; non-umbilicate to deeply umbilicate, with the umbilicus (0-) 3-8 (-13) mm deep, (13-) 20-27 (-33) mm diameter; peel with (39-) 51-69 (-81) areoles, (1-) 2-4 (-10) mm tick; pulp same color as the peel, but very often of a contrasting color, fleshy and juicy, from slightly to very sweet (7.4-) 12.4-15.5 (-16.8) °Brix. The main diagnostic character in *O. ficus-indica* (L.) Miller is the partial or total absence of spines; however, other important features are longer areoles, a longer pericarpel, wider fruits, and a broader variability in fruit color.

Figure 2.4 Size for an *Opuntia ficus-indica* (L.) Miller plant. Dr. Rigoberto Vazquez Alvarado and the author in Vazquez’s nopal plantation, at the Faculty of Agronomy Sciences. Mina, N. L. UANL, Mexico.
2.3.2 Uses of *Opuntia ficus-indica* (L.) Miller

Mexicans have used historically prickly pear (*Opuntia* spp.) in a number of ways [140]:

1. **Fruit.** The cactus pear or fleshy fruits called tunas are in great demand on the local market, and are exported to the US, Canada, Japan, and some European countries. Duru and Turker [42] describe the fruit as a berry with a thick peel enclosing a delicately flavored very seedy pulp. 30% to 45% of the fruit is composed of a thick pericarp (peel) and the remainder is a juicy pulp comprising many hard seeds. Seeds comprise 5% to 10% of the pulp weight. The percentage of seeds changed from 2.19 to 5.59 per whole fruit and from 4.32 to 10.51 per pulp for different clones of *Opuntia* grown in Argentina and the United States. The number of seeds initially increased to 5.44 per g fruit, then decreased and remained more or less constant at 2.96 per g fruit during development.

![Figure 2.5 Market presentation of peeled prickly pear on ice (left) and detail of its size.](image)

2. **Vegetable.** The tender cladodes of certain species are cut up and eaten as a vegetable called ‘nopalito’. Because there is an abundant supply of nopal throughout the year, they are also used as an ingredient in many traditional dishes. According to Vigueras [140] *Opuntia ficus-indica* (L.) Miller var. Copena VI is the most sought after as vegetable, due to its excellent flavor and low acidity.

3. **Fodder.** Prickly pear is used to feed cows on cattle farms in the north of Mexico, especially during the dry seasons. The cladodes are first scorched to remove the spines and then chopped up before mixing them into fodder. It is also mentioned by Vigueras *et al.* [140] as a valuable addition to fodder for cattle, goats, horses and a variety of wild animals due to its high palatability and digestibility, its great abundance, and its high productivity and quick recovery after harvesting.
4. **Medicinal products.** The consumption of nopal and the acid fruits (xonostle) of certain species have been shown to reduce blood glucose and cholesterol levels. The plant parts are consumed cooked, in capsules and in pill form.

5. **Agro-industry.** The cladodes are canned and used in a variety of processed foods for the commercial market. The fruit is used to produce marmalade, juice, nectars, pigments, pectin [57] and fructose.

6. **Cosmetics.** Extracts from *Opuntia* cacti are used to make soaps, body creams, shampoos and cosmetic gels, that are said to reduce body fat.

7. **Pigments.** About 12 *Opuntia* species are used to rear the cochineal insect (*Dactylopius coccus* Costa) for dye production. Also Duru and Turker [42] mention the use of prickly pear looking for production of natural food colorant.

8. **Fencing.** Some of the more spiny *Opuntia* species are used to delimit terrains, family farms and cattle camps. This is an ancient and common activity in Mexico.

9. **Reforesting.** *Opuntia* species are also used to protect eroded lands and or as a first step in reforestation on remediated soils [54].

In addition to the uses listed above prickly pears are now being looked at as a potential source of anti-polluting agents to clean dirty water, as a source of oil, and as a mechanism to prevent soil erosion. Goycoolea and Cárdenas [57] highlight that the pectin content in *Opuntia robusta* is within the reported range for citrus peel (20% to 30% d. m. b.), an industrial source of pectin. At present, apple pomace and citrus peels are the main sources of commercially acceptable pectins. Other sources of pectins that have been considered are sugar beet and residues from the seed heads of sunflowers. The intake of nopal pectin includes anti-inflammatory activity for the treatment of enteritis and relief of the symptoms caused by benign prostate hypertrophy.

In cultivars of this genus [1] it is considered that the presence of a high amount of normal seed within the fruit is an obstacle for its consumption, since they are usually big and hard. Thus, during the production of sweets and beverages seeds are wasted [39]. Salvo et al. [128] mention that nowadays, agricultural and agro industrial wastes disposal represent a serious problem from various angles, mainly economical and environmental. *Opuntia ficus-indica* (L.) wastes and eventually surpluses may be considered among these materials, since in the last few years specialized plantation have been set up.
2.3.3 Systematic Data of *Opuntia ficus-indica*

Aguilar Estrada [1] reports an average of 228.5 seeds per fruit weighting 3.9 g (from 0.02 to 8.94 g). Seed hardness [123] was assessed using an Instron universal test machine. Seeds with hard testa, thick funiculate, hard, white that surrounds the seed and with curved embryo, lenticulate [1] [123] to widely ellipsoidal, (81-) 188-355 (480) per fruit, of which ca. 35-40% are abortive, the normal ones (3.2-) 4-4.5 (-5) mm long, (2.7-) 3.2-3.8 (-4.2) mm wide, (0.8-) 1.2-1.6 (-1.9) mm thick, (32-) 96-253 (-392) kgf hardness.

![Prickly pear seeds](image1.png)

Figure 2.6 Prickly pear seeds (left) with mucilaginous material and after blending (right).

While Coskuner and Tekin [39] mention that seeds of certain *Opuntia* species are referred to contain good quality edible oil, Salvo *et al.* [128] report that the seeds contained 9.14% oil, where the first sterolic component was β-sitosterol (BS) (61.42%). This sterol composition of *Opuntia* seed oil is compared with those of other oils. It is possible to note that it is similar to that of corn oil, mainly because of the percentage of total β-sitosterol. According to its chemical composition *Opuntia ficus-indica* (L.) Miller seed oil can be utilized in alimentary field.
2.4 Fats and Oils

According to Taiz and Zeiger [133] fats and oils are important storage forms of reduced carbon in many seeds including peanut and sunflower among others insoluble in water but soluble in organic solvents such as chloroform and methanol [142]. Hence they are easily separated from other biological materials extracting them into inorganic solvents. In higher plants and animals the predominant fatty acid residues are C\textsubscript{16} and C\textsubscript{18} species: Linoleic, Stearic and Palmitic acids.

From these compounds the germinating seeds obtain metabolic energy. Among the plant defense substances it is possible to identify surface protectants and secondary metabolites, the last ones are a large and diverse array of organic compounds that apparently don’t have a direct function in development and growth. Other names for these compounds are natural products or secondary products with a restricted distribution in the plant kingdom. The largest class of secondary products is constituted by the terpenes or terpenoids divided in five major subclasses: monoterpenes, sesquiterpenes, diterpenes, triterpenes and polyterpenes. The triterpenes include steroids and comprise structurally diverse compounds; several steroid alcohols (sterols) are important components of plant cell membranes decreasing the permeability of the membrane to small molecules by reducing the motion of the fatty acid chains.

From the extraction of vegetable material (e. g. hops, coffee, beans, wood, etc.) it is known that the rate of mass transfer is affected by the water content. The extraction rate is low for dry vegetable materials but increases with the content of swelling moisture. According to Beer & Peter\textsuperscript{3} in general, there exist an optimal content of swelling water. Clifford [34] makes stress on three factors during the extraction, namely: the solute must be sufficiently soluble in the supercritical fluid to be removed by the solution in the fluid flow. The solute must be transported sufficiently rapidly and the solute must be released by the matrix. However it can be slow and in some cases it appears, that part of the substance being extracted is locked into the structure of the matrix. A related problem is the presence of water since water is not very soluble in many fluids, such as carbon dioxide, and it can ‘mask’ the substances to be recovered. The rate of extraction may sometimes be equal to the rate of water removal. Reduction of water content of

\textsuperscript{3} In Penninger [112]
plant material from say, 80 % (as measured by mass loss at 100 °C) towards 10 % may be desirable, because provided valuable volatiles are not lost in the process. However, water may assist extraction by acting as a modifier, as is believed to be the case for coffee decaffeination.

Menta Hybrid was cultivated at Grid (County of Brasov), dried, vacuum, packed and stored in a cool, dry place. Supercritical fluid extraction from only 10-20 g of vegetal material was, upon the knowledge of Pop and Barth [117], not mentioned in the literature. Usual quantities are, for preparative scale, around hundreds of grams, and for analytical scale are around hundreds of milligrams. It is well known [8] that even at low temperatures solid matrices with high lipid contents are subject to oxidation reactions.
2.5 Prickly Pear Oil main Compounds

2.5.1 β-sitosterol (BS)

24-Ethylcholest-5-en-3β-ol; Beta-sitosterol; beta-Sitosterol, with ca. 10\% campesterol and sitosterol.

Table 2.2 Compound β-sitosterol properties (chemfinder.com).

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<td>—</td>
</tr>
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<td>—</td>
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<td>—</td>
</tr>
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<td>—</td>
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</tr>
<tr>
<td>Comments</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Figure 2.7 Chemical Structure of β-sitosterol.
Different authors, as Awad et al. [12], consider β-sitosterol is the most abundant phytosterol that can be found in legumes, oil seeds, and unrefined plant oils as in foods such as peanut butter, pistachios and sunflower seeds. It has been related with the inhibition of the growth of specific tumor cells in vitro, decreasing the size and the extent of tumor metastases in vivo, remark Awad et al., whose findings support the hypothesis that β-sitosterol is an effective apoptosis-promoting agent and its dietary inclusion may serve as prevention for breast cancer. The in folk medicine widely used root bark, Calotropis gigantean (Linn.) was studied by Habib et al. [64] to identify and analyze β-sitosterol between the main constituents. Nevertheless, although it is a recent work, the experiments are reported to be performed by hot extraction and the crude methanol fractionated with petroleum ether, chloroform and acetate.

Isolating important substances from Bergia suffruticosa, from the Elatinaceae family, because of its medicinal use, Anandjiwala et al. [6] found 0.034% wt/wt of β-sitosterol. After the extraction and separation by column chromatography, Aguirre-Hernández [1] identified β-sitosterol as one of the major constituents of Tilia americana var mexicana, and the hexane extracts produced depressant actions of the central nervous system at least in part, because of the presence of β-sitosterol and some fatty acids.

The possibility of lactose-β-sitosterol and β-sitosterol as potential therapeutic molecules in asthma is stressed by Yuk et al. [149] during their experiments with mice, contributing to the need to improve current therapeutic drugs. Root extract from Pluchea indica Less (ASTERACEAE), has been studied by Gomes et al. [58] where β-sitosterol was extracted and found to have a possible important role in neutralizing snake venom-induced actions, working along with antiserum.

Park et al. [111] also refer the use of β-sitosterol as apoptosis inductor in human leukemic U937 cells and this apoptosis is related to the selective activation of caspase-3 and induction of Bax/Bcl-2 ratio.

Stigmasterol and β-sitosterol are the most common phytosterols in traditional Chinese medicine. Huang et al. [72] found 2.89% of β-sitosterol under optimized extraction conditions, namely 25 mPa and 45 °C using ethanol as modifier.
Mothes et al. [106] had found sterols in important quantities in Sea Buckthorn. In certain samples up to 0.9 % contents were reached. This is comparable with the corn oil. Choi et al. [33] demonstrate that β-sitosterol, the main dietary phytosterol, induces apoptosis in HCT116 human colon cancer cells, which appears to account for its antiproliferating activity. It was shown that β-sitosterol induced a dose dependent apoptotic body fragmentation, which is an end stage apoptotic event. By regulation of the intracellular redox balance, Li et al. [83] indicate that β-sitosterol confers a radioprotective effect on thymocytes, carried out by scavenging of reactive oxygen species and maintenance of mitochondrial membrane stability.

Choi et al. mention as well that β-sitosterol induces apoptosis in HCT116 human colon cancer cells, as characterized by morphological features of programmed cell death and flow cytometric studies. These apoptotic mechanisms were mediated by caspase-3 and caspase-9 activation and PARP cleavage, and associated with an increase in Bax expression as well as with release of cytochrome c from the mitochondria into the cytosol.
2.5.2 Linoleic acid

Table 2.3 Compound Linoleic acid properties (chemfinder.com).

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula</td>
<td>C\textsubscript{18}H\textsubscript{32}O\textsubscript{2}</td>
</tr>
<tr>
<td>Molecular Weight</td>
<td>280.4496</td>
</tr>
<tr>
<td>CAS RN</td>
<td>60-33-3</td>
</tr>
<tr>
<td>Melting Point</td>
<td>-5 °C</td>
</tr>
<tr>
<td>ACX Number</td>
<td>X1009881-2</td>
</tr>
<tr>
<td>Boiling Point</td>
<td>229-230 16 mm Hg</td>
</tr>
<tr>
<td>Density</td>
<td>0.902</td>
</tr>
<tr>
<td>Vapor Density</td>
<td>—</td>
</tr>
<tr>
<td>Refractive Index</td>
<td>—</td>
</tr>
<tr>
<td>Vapor Pressure</td>
<td>—</td>
</tr>
<tr>
<td>Evaporation Rate</td>
<td>—</td>
</tr>
<tr>
<td>Water Solubility</td>
<td>Insoluble</td>
</tr>
<tr>
<td>Flash Point</td>
<td>—</td>
</tr>
<tr>
<td>EPA Code</td>
<td>—</td>
</tr>
<tr>
<td>DOT Number</td>
<td>—</td>
</tr>
<tr>
<td>RTECS</td>
<td>RF9990000</td>
</tr>
<tr>
<td>Comments</td>
<td>Colorless to straw colored liquid. AIR SENSITIVE.</td>
</tr>
</tbody>
</table>

Figure 2.8 Chemical Structure of Linoleic acid (9, 12 Octadecadienoic acid), with two cis double bonds at the 9\textsuperscript{th} and the 12\textsuperscript{th} carbon from the carboxyl group.

According to Vles [141] numerous studies have shown that a limitation of dietary saturated fatty acids and cholesterol associated with an increase in linoleic acid consumption lowers high blood cholesterol level (a risk factor in the development of atherosclerosis).

Besides the importance of dietary fatty acid composition in determining blood lipoprotein concentrations, it has been shown, that increased intake of dietary linoleic acid influences arterial thrombosis tendency in rats and improves blood platelet function in man. Moreover linoleic acid is known to regulate the endogenous prostaglandin biosynthesis.
In the review made by Harris [66] about omega 3 fatty acids, he mentions that the cardiovascular benefits of omega (n)-3 fatty acids become clearer through time. The relative importance of the n-6 FA linoleic acid (LA), the short-chain n-3 FA alpha linolenic acid (ALA), and the long-chain n-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) is becoming clearer. If consumption of the last mentioned are adequate (suggesting over 250 mg/d), then there appears to be less need for higher intakes of ALA or less LA.

2.5.3 Palmitic acid

Being among the saturated acids the most widely occurring, Palmitic acid [62] is present almost in every fat examined: fish oils, milk, vegetables, and special sources are palm oil, tallow fats from pig, sheep and cattle.

Table 2.4 Compound Palmitic acid properties (chemfinder.com).

<table>
<thead>
<tr>
<th>Formula</th>
<th>C_{16}H_{32}O_{2}</th>
<th>Molecular Weight</th>
<th>256.42 g/mol</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAS RN</td>
<td>57-10-3</td>
<td>Melting Point</td>
<td>63-64 °C</td>
</tr>
<tr>
<td>ACX Number</td>
<td>—</td>
<td>Boiling Point</td>
<td>351-352 °C</td>
</tr>
<tr>
<td>Density</td>
<td>0.853 g/cm³ at 62 °C</td>
<td>Vapor Density</td>
<td>—</td>
</tr>
<tr>
<td>Refractive Index</td>
<td>—</td>
<td>Vapor Pressure</td>
<td>—</td>
</tr>
<tr>
<td>Evaporation Rate</td>
<td>—</td>
<td>Water Solubility</td>
<td>Insoluble</td>
</tr>
<tr>
<td>Flash Point</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>DOT Number</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Comments</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Figure 2.9 Structure of Palmitic acid (Hexadecanoic acid).
2.6 Extraction Processes

2.6.1 Extraction Parameters

Supercritical extraction kinetics can be conditioned varying the pressure, temperature, extraction time, solvent/solute rate, solvent flow, substrate moisture and/or addition of a co-solvent or modifier. Examples are experiments like those performed by del Valle et al. [137] who extracted oleoresins from pelletized jalapeño pepper flakes at 40 °C and 120 or 320 bar with a particle size of 0.28-3.19 mm and a superficial solvent velocity of 0.14–2.26 mm s⁻¹. For Li et al. [83] the sterols extraction by pressurized fluids from marine sediments samples was superior to that by soxhlet, reporting simple, fast, efficient and amenable to automation. Kiriamiti et al. [78] worked with sunflower seed extracting oil at 260 bar finding detrimental to rise the temperature up to 60 °C. They ground the seeds working the particle size 0.6 mm and 1.25 mm. Kiriamiti et al. mention for vegetable and essential oil extraction, the simplest models like Shrinking Core Model and Desorption-Dissolution-Diffusion Model and found the Sovová model as the most adequate. Sovová model, as Kiriamiti et al. explain, assumes that the seeds contain oil divided into accessible and inaccesible forms described as bound and unbound oil and the limit corresponds to a value $x_k$ expressed in oil free solid bases.

Coelho et al. [37] also report their results by the adoption of Sovová’s model to interpret the mass transfer phenomena in the extraction of volatiles from Brazilian rosemary (Rosmarinus officinalis L., Labiate). Coelho and his team performed extractions from 310.15 to 320.15 K and a pressure range from 100 to 160 bar maintaining a CO₂ flow rate of 12.2 L/h showing an endless curve still after 400 min.

Working with oleoresin extraction from Calendula officinalis known for its potential against skin problems, Campos et al. [27] also report a good interaction between the experimental results and Sovová model, Logistic model, Desorption model mentioning also heat transfer analogy models like the Single Simple Plate (SSP) and the Diffusion model. Experimental and correlated data found the lowest deviation with the Logistic model, explained by Luanda as an adaptation of Sovová’s model. Their experiments offer an overview of the temperature, pressure and solvent flow effects working with 293 to 313 K, 12 to 20 MPa and 1.3 to 5.0X10⁻⁵ kg CO₂ respectively.
Sovová [130] makes adjustments to the former self-proposed model\(^4\), where the concept of broken and intact cells was considered. The broken cells or damaged by a mechanical pretreatment, perform a rapid mass diffusion in the first section of the extraction curve, followed by a slower extraction dominated by internal diffusion within the substrate.

About the solvent flow, Sovová emphasizes the risk of axial dispersion in a real extractor the higher the ratio length-to-diameter is, stressing a plug flow as an ideal flow pattern. This ratio can affect the extraction scale up due to a channeling effect of the solvent especially when the extracting bed consists of \(<0.4\text{mm}\) particles, and it is mentioned in one of the examples the addition of diatomaceous earth serving as a distributor for the fluids.

Sovová mentioned an example with solvent flow rates varying from 0.31-0.62 g·s\(^{-1}\). In the Sovová’s adjusted model, the mass transfer resistance is neglected in the damaged wall cells, since it is much lower than the resistance in the cells without damage. This new model also considers the estimated time when the easy extractable oil ceases and the ‘hard to extract’ oil begins to be solved by the internal diffusion.

The advancements on how the extraction process became automatic and intensive are very tightly related to the development of the use of models to depict the processes. The building of the processes occurs based on a thermodynamic background and experimental data.

2.6.2 Extraction Mechanisms

Freese [52] defines the soluble, solid or liquid separation of the constituents from a crude mineral as solid extraction while by authors like Perry and Green [115] the concept of *leaching* is used, when the soluble fraction in form of a solution is removed from a solid phase. This separation process can work by solution or ‘dissolution’ when a chemical reaction is involved.

1. Solvent transport in free solution (convection)
2. Solvent transport through the border film (film diffusion)
3. Solvent transport in capillaries (pore diffusion)
4. Solution process
5. Extract solution transport in capillaries (pore diffusion)
6. Extract solution transport through the border film (pore diffusion)
7. Extract solution transport (convection)
8. Extract solution transport in solid material (Diffusion)

Figure 2.10 Mass transport steps [68].

For soil remediation, Freese [52] mostly used organic solvents, which are removed from the solid matrix, which at the end are separated again. The removable substances can be present in different forms:

- as a film on the solid particles surface,
- enclosed in the solid particles pores,
- as free particles between the material.

The union between the solute and the matrix can be due to chemisorption or by physical boundary forces. Material transfer mechanisms in porous systems are based on four steps.
1st Step: Desorption

The desorption of the solute from the particle surface will be analog to the equation of a 1st Order reaction.

\[
\frac{\partial c_s}{\partial t} = k_1 \cdot c_s \left( \frac{c_p}{c_{p_{\text{max}}}} - 1 \right)
\]  

(2.1)

\[
\frac{\partial c_s}{\partial t} = -k_1 \cdot c_s \quad \text{when } c_p << c_{p_{\text{max}}}
\]

(2.2)

\[
\frac{\partial c_s}{\partial t} = 0 \quad \text{when } c_p = c_{p_{\text{max}}}
\]

(2.3)

\(c_s\) mass load of solid material  
\(t\) time  
\(k_1\) velocity coefficient  
\(c_p\) concentration in the porous phase  
\(c_{p_{\text{max}}}\) solubility in the pores’ fluid

The velocity coefficient is the adjustment parameter. When the oil content in the pore stage is small compared to the solubility, there is a first order kinetic, and respectively reaching the maximum oil content it exist an equal phase weight. For the velocity coefficient, Nowak [108] assumed that the pollution exists in form of droplets with diameter with units \(d_*\) and with density \(\rho_*\) and a general approach for the mass transfer at the relation:

\[
k_1 = \frac{6 \beta}{d_*}
\]

(2.4)

\[
k_1 = \frac{6 \text{Sh} D_{\text{AB}}}{d_*^2}
\]

(2.5)

\[
k_1 = \text{const.} D_{\text{AB}} \frac{1}{d_*^2}
\]

(2.6)

\[
k_1 \approx D_{\text{AB}} = f(T, p, ...)
\]

(2.7)

\[
k_1 \approx 1/d_* = f(\text{Substrate}...)
\]

(2.8)
where

\[
\begin{align*}
\beta & \quad \text{mass transfer coefficient} \\
Sh & \quad \text{Sherwood number} \\
D_{AB} & \quad \text{molecular diffusion coefficient}
\end{align*}
\]

Therefore, \( k_1 \) depends on two factors:

- On one hand, on the extractions parameters (2.7)
- On the other hand, on the properties of the substrate (2.8).

2nd Step: Diffusion

For the oil transport in the agglomerated material, the next considerations are assumed:

- The agglomerates have the form of a sphere with radius \( r \).
- The hollow space inside the agglomerates is completely full with solvent (in this case: supercritical fluid).
- Inside the agglomerate reigns an equilibrium between solid and fluid loads.
- The mass transfer within the agglomerates takes place exclusively by diffusion. There the diffusion can not be messed directly and in all the pore volume, it will be calculated through the efficient diffusion coefficient \( D_{eff} \).

All the mass transfer activities within the solid particles and agglomerates will be comprised by the principle of linear driving forces and described mathematically by the 2nd Fick’s law.

\[
\frac{\partial c_p}{\partial t} = D_{eff} \left( \frac{\partial^2 c_p}{\partial r^2} + \frac{2}{r} \frac{\partial c_p}{\partial r} \right), \quad \text{for} \quad 0 < r < R \quad (2.9)
\]

With the boundary condition

\[
\frac{\partial c_p}{\partial t}_{r=0} \equiv 0. \quad (2.10)
\]
The effective diffusion coefficient $D_{\text{eff}}$ is calculated from the diffusion coefficient $D_{AB}$

$$D_{\text{eff}} = \frac{e_p}{\tau} D_{AB} \tag{2.11}$$

$r$ radial coordinate in the agglomerates

$D_{\text{eff}}$ effective diffusion coefficient

$\tau$ tortuosity factor

$e_p$ particle porosity

3rd Step: Mass Transfer

In the present system the supercritical fluid streams must run out by a convective mass transfer. The mass flow concentration is defined as follows

$$m = \beta_f A_\perp (c_f|_{rad_p/2} - c_f) \tag{2.12}$$

$m$ oil mass flow from the agglomerates in the streaming fluid

$\beta_f$ mass transfer coefficient in the fluid side

$A_\perp$ mass exchange area

$c_f|_{rad_p/2}$ concentration in the pore fluid directly at the agglomerate surface

$c_f$ concentration in streaming fluid

With the help of dimensionless numbers like the Sherwood’s number $Sh$, Schmidt’s number $Sc$ and Reynolds number $Re$, the coefficient of mass transfer can be empirically calculated by equations of the form:

$$Sh = A + BRe^m \cdot Sc^n \tag{2.13}$$
Nowak [108] found by own experiments the best reproduction of his results with the next correlation from Dwivedi and Upadhyay

\[
\text{Sh} = \frac{1}{\varepsilon_{pb}} \left( 0.765 \text{Re}^{0.18} + 0.365 \text{Re}^{0.614} \right) \beta^{1/3}
\]  

(2.14)

in which the Sherwood number is defined as

\[
\text{Sh} = \beta_{pf} d_p / D_{AB}
\]

(2.15)

the Schmidt number as

\[
\text{Sc} = v / D_{AB}
\]

(2.16)

and the Reynolds number as

\[
\text{Re} = d_p u_z / v
\]

(2.17)

where

- \( \varepsilon_{pb} \) porosity in the packed bed
- \( d_p \) solid particle diameter
- \( u_z \) empty pipe velocity
- \( v \) kinematic viscosity in the packed bed

Porosity in the packed bed \( \varepsilon_{pb} \) and solid particle diameter \( d_p \) are two additional adjusting parameters.
4th Step: Convection

Considering convection, the axial dispersion especially in low Reynolds values, can not be left unattended. To avoid the relative unsure determination of a reliable axial dispersion coefficient, Nowak describes the streaming process with a model cell. There he divided the packed bed in a stirrer tank battery of ideal stirrer tanks. Through increasing number of stirrer tanks it approached the behavior of an ideal piston flow. He found the next relation for the stirrer tank number:

\[ N = -0.59 + 0.49 \text{Pe}_p \frac{L_{fb}}{\varepsilon_{fb}} \tag{2.18} \]

\( \text{Pe}_p \) Peclet number in the porous phase

The stages number \( N \) is the 4th adjust parameter.

The balance equation for the fluid phase reads

\[
\frac{\partial c_{f,n}}{\partial t} = \frac{u_z}{\Delta L_{fb} \varepsilon_{fb}} (c_{n,f-1} - c_{f,n}) + \frac{1 - \varepsilon_{fb}}{\varepsilon_{fb}} \frac{6 \beta}{d_p} [c_{p,f}^{R,n} - c_{f,n}] \tag{2.19}
\]

where

- \( c_{f,n} \) concentration in the packed bed in the \( n^{th} \) section
- \( r \) radius
- \( \rho_s \) substrate density
- \( \Delta L_{fb} \) packed bed cut longitude

A good correlation of the simulation of the packed bed extraction could be reached by supercritical water towards the measured results with this model. In the range of partial solubility, at the subcritical area of the water, the correlation between the experimental and calculated data were less good. Firus [51] in Freese [52] used this model on packed bed CO₂ extraction. Using this model in artificial contaminated soil samples, it reproduced well the performance of two temperatures, what means two desorption velocity coefficients.
Unfortunately the calculated contamination grades in real contaminated soil samples could not be verified by experimental values. Nevertheless, it was shown that only a linear extraction process can be expressed with the model since on a theoretical extension of the extraction time the fail of the extraction rate was too high. Considering the extraction yield as function of solvent amount and also versus the extraction time (residence time) in a discontinuous approach it is possible to divide the extraction process in three phases (Figure 2.11):

At the beginning the extraction curve increases almost linear, because the solvent load is almost constant in the elution phase (area I). Subsequently, in the area II the extract concentration in the solvent decays, this indicates the end of the elution process. In area III of the extraction curve the extract yield increases only slightly. In this area, the process will be born only by pure diffusion, which marks the velocity of this process step. The grinding degree (particle size) in the extraction substrate defines the time point of the transition from elution to the diffusion area and in the same way the decision of the extraction duration measure. In case of compact plant material, the particle size is the factor that determines the yield, if too long extraction times due to slow flow diffusion processes can not be accepted.

![Figure 2.11 Typical course of the natural substances extraction from plant substrates, where $A_{\text{max}}$ = maximal possible yield [68].](image-url)
3  HYPOTHESIS

It exist a difference between the extractions by supercritical CO\textsubscript{2} and the conventional extraction methods, namely: ultrasound assisted extraction and Soxhlet values found in publications, showing more advantages in amount—kind of solvents and quality of the oil yield, expressed in this case as higher $\beta$-sitosterol concentration.

Higher or lower yields of specific substances can be found varying the extraction parameters, as solvent, co-solvent, temperature, pressure and extraction time.

Sub products from the prickly pear sweets and beverages production processes can be used as a source of valuable enriched oils and selected fatty acids.
4 AIMS

To corroborate the advantages of extracting natural substances by gases in near or supercritical state compared to those of the conventional extraction methods.

Find out the extraction parameters with the best yield in quantity and quality of β-sitosterol in combination with linoleic acid by near and supercritical carbon dioxide extraction compared to traditional extraction methods, selecting at the same time those parameters with lower palmitic acid based on the mentioned properties for every substance.

Determine the viability of using cacti seeds as an oil and sterols supply for nutritional and pharmacological industries.
5 MATERIALS AND METHOD

About 5.7 kg of *Opuntia ficus-indica* [(L) Miller], were received from the UASLP in San Luis Potosi, Mexico. Once we got the permission form the "(Mexican) National Service of Seed Inspection and Certification" that has the role of control the exportation of biological material in this field, the material was obtained.

![Overview of the Retsch grind machine model ZM1 used to comminute the seeds as substrate for the experiments.](image1)

Figure 5.1 Overview of the *Retsch* grind machine model ZM1 used to comminute the seeds as substrate for the experiments.

The samples were comminuted, first fed by a dose machine *Retsch* model DR15/40 to the *Retsch* grind machine model ZM1 (Figure 5.1) with a 3 mm sieve (Figure 5.2).

![Detail of the Retsch grind machine model ZM1 with the seeds already comminuted through the 3 mm sieve.](image2)

Figure 5.2 Detail of the *Retsch* grind machine model ZM1 with the seeds already comminuted through the 3 mm sieve.
The ground seeds are sieved 15 min in a *Retsch* sieve machine model VE1000 with 0.85 mm of amplitude (Figure 5.3).

![Sieve machine model VE1000, set for 15 min sieving at 0.85 mm of amplitude.](image)

The <0.5 mm ground material (Figure 5.4) is stored, and a sample is weighted and dried for 15 h at 60° C to determine the humidity and real mass of substrate subject to extraction.

![Ground substrate <500 µm before the storage.](image)
5.1 Extraction Devices

5.1.1 Control Experiment: Ultrasonic Extraction

Ground nopal seed <500 µm fraction, are placed in filtering medium in amounts of 20 g. The full filtering media are submerged in bottles with 200 mL of solvent; in this case n-heptane in an Ultrasonic bath Bandelin SONOREX Super PK 255 H during 2 h (Figure 5.5).

Once the extraction time is over, the mixture solvent-solute is filtered in order to condition the sample for the GC analysis and a 2 mL sample is taken for this purpose.

Figure 5.5 Ultrasonic extraction process, where it is possible to see immersed in n-heptane the filtering medium with the substrate showing at the end of the extraction a lot of fine dust suspended.
5.1.2 Supercritical Fluids Extraction

5.1.2.1 Semi-continuous Flow Process Extraction

The extraction chamber in the semi-continuous flow extraction process, is a 25 cm long, 1 cm Ø column with porous stainless steel filters at both edges helping the distribution of the solvent pure or modified, retaining the substrate and allowing the free flow of the permeate out of the column.

![Semi-continuous Extraction Flow Diagram](image)

Figure 5.6 Detail of the Semi-continuous Extraction Device (PIR: Pressure measurement-Indication-Registration; TIC: Temperature measurement-Indication-Control).

The column is filled with 7.5 g of <500 µm fraction ground seeds as the extraction matrix which can be accompanied by stainless steel bearings when the substrate requires moisture or the solvent is modified by ethanol, to avoid the compaction (‘cake’ formation). The column is then submerged in a M12 LAUDA warm bath to control the process temperature at 35, 45 and 55 °C, the ethanol is added as co-solvent by an ABIMED GILSON pump at Q = 0.5 mL/min and the pressure is measured by a HAENNI pressure meter Type ED 513/373.211/155 and registered by an ALMEMO® 3290 pressure register.
The solvent flow rate is set on 2 L/min and the solvent extract mixture is expanded after the extraction chamber collecting the extract in a cyclone and measuring the flow speed and solvent consumption with a gas clock. Beginning on the fifth minute a two minutes sample was taken every 7 minutes, performing a 5 min running x 2 min sample pattern for 131 min. About 20 samples were taken from every experiment. Samples were collected in 4 mL previously weighed vials and 2 mL of chloroform were added for GC analysis. When the solvent was modified by ethanol, the samples were weighed after volatilization of the modifier and the procedure was continued as described before.

5.1.2.2 Batch Process Extraction

The batch supercritical extraction process consists of two different autoclaves: stationary and mobile. Both designs have the same peripheral devices to control, measure or conduct the flow, the difference lays at the reactor, which stays static for the stationary device (Figure 5.8), while it possesses an engine to move the embracing frame forcing the convection within the autoclave (Figure 5.9). The system comprehends a CO₂ tank followed by a CO₂ pump and the line whether modified or not can be connected to a modifier pump for the tests to be performed with a co-solvent (Figure 5.7).

Figure 5.7 Flow diagram of the Batch Process Extraction Device.
Stationary Device.

The stationary autoclave has a volume of 1 L and it is used with 3 stainless steel blocks for volume reduction getting at the end a volume of 0.515 L.

![Stationary Autoclave](image)

Figure 5.8 Overview of the stationary autoclave surrounded by the peripheral extraction devices.

Stationary autoclave is preheated and the substrate placed within it, fed with modifier and reaching the target pressure. The pressure is measured every hour and the data are registered.

Mobile device.

The mobile device consists of a shaker block with a set of 4 autoclaves; every autoclave has a volume of 0.2 L mounted in the stainless steel block. The material is placed into filtering system, closed and introduced into its respective autoclave. Every autoclave holds 15 g of substrate and the heating block has 4 autoclave holes. The block is preheated and the autoclave(s) is (are) placed within the block, connected to the CO\textsubscript{2} line, pressurized one at a time until the target pressure is reached. After pressurization, the block is unplugged from the CO\textsubscript{2} line and the shaking begins in order to get a better diffusion through forced convection within the system.
Once the autoclaves are fixed with a safety ring, the feeding line is released and the motor is switched on. The pressure is measured every hour and the data are registered. At the end of the extraction period, the autoclaves are depressurized and the CO₂ is expanded making it flow through a cyclone to catch the extract; every container takes 30 to 40 min for decompression and cleaning of pipes and cyclone. The co-solvent is vaporized by a Heidolph WB 2000-VV 2000 rotation device and the recovered oil amount is weighted. The analysis of the samples is performed with an Agilent Technologies 7890 A GC System described in table 5.1. Curves with 20 sampling points are exported to the software Origin Pro 7.5 to fit the extraction behavior with a model which is integrated by the same program getting the area under the extraction curve which represents the mass in grams of the analyzed substance.

Table 5.1 GC Analyzing parameters and Technical data [65].

<table>
<thead>
<tr>
<th>Analyze System</th>
<th>GC: HP 5890 II</th>
</tr>
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<tbody>
<tr>
<td>Detector</td>
<td>HP 5972A MSD</td>
</tr>
<tr>
<td>Separation column</td>
<td>HP5-MS 30m x 0.25mm x 0.25µm</td>
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</table>

<table>
<thead>
<tr>
<th>Injection parameter:</th>
<th>$T = 250°C$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume</td>
<td>1µl without split</td>
</tr>
<tr>
<td>Carrier gas</td>
<td>Helium 0.8 mL/min</td>
</tr>
</tbody>
</table>

| Oven program: | 35°C (1min), 10°C/min, 300°C (5min) |

<table>
<thead>
<tr>
<th>Detector Parameter:</th>
<th>$T$ Transfer conductor: 280°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent Delay</td>
<td>3min</td>
</tr>
<tr>
<td>Scan Modus</td>
<td>33-450 amu, SEV 2600eV</td>
</tr>
</tbody>
</table>
6 RESULTS

While the material was ordered from Mexico, some experiments were performed in order to test and calibrate the extraction devices additionally to compare some of the published values for peanut, walnut and maize oil.

Herewith are shown these preliminary experiments finishing the series with results of extractions performed on prickly pear seeds oil.

6.1 Peanut tests (*Arachis hypogaea*).

The results obtained from the extraction of peanut oil by supercritical CO\(_2\) were analyzed, where the material was submitted to a relative low pressure and high temperature, compared to those used during the extractions from prickly pear seeds as stated below.

Table 6.1 Summary of peanut oil extractions showing sort, modifier substances, ratio of substances in co-solvent, experiment duration, pressure and temperature averages, CO\(_2\) consumption, \(\beta\)-sitosterol and oil mass per mass of substrate.

<table>
<thead>
<tr>
<th>Sort</th>
<th>Modifier</th>
<th>Ratio</th>
<th>t (h)</th>
<th>P bar</th>
<th>T °C</th>
<th>(\text{kg}_{\text{CO}_2})</th>
<th>(\text{mg}<em>{\beta\text{-sitsterol}}/\text{g}</em>{\text{oil}})</th>
<th>Oil Load %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrasonic Bath</td>
<td>Ac-EtOH</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>40.0</td>
<td>1.060</td>
<td>23.2</td>
<td></td>
</tr>
<tr>
<td>SC- CO(_2)</td>
<td>Ac-EtOH</td>
<td>2:1</td>
<td>18:55</td>
<td>100.5</td>
<td>43.8</td>
<td>0.29</td>
<td>1.166</td>
<td>19.0</td>
</tr>
<tr>
<td>SC- CO(_2)</td>
<td>Isop-EtOH</td>
<td>3:1</td>
<td>17:22</td>
<td>102.2</td>
<td>50.3</td>
<td>0.27</td>
<td>1.369</td>
<td>21.1</td>
</tr>
<tr>
<td>Commercial Sample</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.616</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

The modifier rate was previously tested with some other edible oils giving the best results in 2:1 ratio of acetone to ethanol. The supercritical extractions were performed using different mixtures of acetone-ethanol with very good oil yields. However, the presence of acetone seems to damage the cyclone (set up after CO\(_2\) expansion to separate the solvent from the extract -Figure 5.7-) solving the internal surface of the cyclone and making it rough. The modifier was replaced by a mixture of Isopropanol-Ethanol, at the tested rates, showing similar results.
The use of a stainless steel cage in the super critical extractions was also avoided, because the paper filter containing the substrate showed to be stable enough during the process and considering that the cage parts were seemly oily at the end of every experiment. The process was further improved by reducing volume from 1 L to 0.515 L, by the installation of metal blocks to reduce the CO₂ consumption from 700 g to about 300 g per extraction.

Results on the load of oil per mass of substrate by SCFE have been satisfactory either with acetone or with Isopropanol, compared to the traditional extraction methods (ultrasound extraction) shown in Table 6.1.

Figure 6.1 Peanut peeling (left), grinding (center) and filtering of extract (right) where it is possible to see the filter impregnated with oil once the modifier was evaporated.

There were two former extractions without modifier, where the yield was rather low. It was important to see the reduction of solvent and/or co-solvent in further experiments, analyzing the interaction of the modifier as direct solvent.
6 RESULTS

M. Sc. Gerardo Bermejo Acosta

6.2 Maize corn tests (*Zea mays*).

Another abundant material in Mexico (concerning the nationality of the author) and with a good presence in edible oils within the market, is the well known maize. Different tissues from the corn were prepared to be extracted by supercritical conditions. The maize grains were removed from the rachis and the extractions were done by separating from the rachis the same as from the grains.

![Image of maize corn, rachis, and grains powder](image1)

Figure 6.2 Top: Fresh maize corn (left), maize rachis (center) and maize rachis and grains powder (right). Bottom: a broken extraction pack (left), shake autoclave (center) and the shaking device in movement (right).

By performing the extractions in the shaking device instead of the stationary device (Figure 6.2), the CO$_2$ consumption was reduced from 700 g to about 50 g. It is interesting that the difference in duration between the ‘fix’ process and the ‘shaken one’ achieves almost the same yield at roughly 1/4 of the time, 25% of the CO$_2$ consumption as well as the concentration of the modifier within the solvent (Table 6.2). The rachis yielded the higher amount of oil per unit of substrate.
Table 6.2 Summary of maize extractions showing sort (‘S’ “shaking device”), modifier substances, ratio of substances in co-solvent, experiment duration, pressure and temperature averages, CO₂ consumption, β-sitosterol and oil mass per mass of substrate.

<table>
<thead>
<tr>
<th>Sort</th>
<th>Modifier</th>
<th>Ratio</th>
<th>t (h)</th>
<th>P bar</th>
<th>T °C</th>
<th>kg CO₂</th>
<th>mg BS/g subst</th>
<th>Oil Load %</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC-CO₂</td>
<td>Isop-EtOH</td>
<td>3:1</td>
<td>23:38</td>
<td>103.6</td>
<td>41.6</td>
<td>0.32</td>
<td>3.259</td>
<td>5.0</td>
</tr>
<tr>
<td>SC-CO₂</td>
<td>Isopropanol</td>
<td>—</td>
<td>15:48</td>
<td>87.6</td>
<td>38.0</td>
<td>0.32</td>
<td>0.483</td>
<td>1.8</td>
</tr>
<tr>
<td>SC-CO₂(S)</td>
<td>Isop-EtOH</td>
<td>3:1</td>
<td>06:15</td>
<td>101.8</td>
<td>39.7</td>
<td>0.07</td>
<td>0.962</td>
<td>4.7</td>
</tr>
<tr>
<td>SC-CO₂(S)</td>
<td>Isop-EtOH</td>
<td>3:1</td>
<td>29:30</td>
<td>146.5</td>
<td>39.7</td>
<td>0.08</td>
<td>0.470</td>
<td>3.2</td>
</tr>
<tr>
<td>SC-CO₂(S)</td>
<td>Isop-EtOH</td>
<td>3:1</td>
<td>30:30</td>
<td>99.0</td>
<td>39.7</td>
<td>0.05</td>
<td>0.160</td>
<td>0.4</td>
</tr>
<tr>
<td>SC-CO₂(S)</td>
<td>Isop-EtOH</td>
<td>3:1</td>
<td>31:30</td>
<td>147.5</td>
<td>39.7</td>
<td>0.08</td>
<td>0.000</td>
<td>0.8</td>
</tr>
</tbody>
</table>

The material had a mechanical pretreatment mentioned by different authors [130] permitting a more rapid mass diffusion due to the damage or breakage of the oily cells within the seed tissues. The first step mentioned by Freese [52], namely the desorption process is also affected by this mechanical pretreatment by the properties of the substrate mentioned in equation (2.8).

6.3 Walnut tests (*Juglans regia*).

Walnut is also world wide utilized for extracting edible oil, its nutritional values are published and next we can find the results of the oil extraction by supercritical fluids. The change in modifier substances didn’t affect adversely, according to the walnut oil yield: from 26.1% acetone vs. 38.4% Isopropanol. Walnut has been the most oil productive seed from all with almost 39% of oil content per mass of substrate. The next closer value is held by peanut with about 24%. Published results don’t mention high sterolic fraction values for both sources of edible oil.

Table 6.3 Summary of walnut extractions showing sort, modifier substances, ratio of substances in co-solvent, experiment duration, pressure and temperature averages, CO₂ consumption, β-sitosterol and oil mass per mass of oil and substrate.

<table>
<thead>
<tr>
<th>Sort</th>
<th>Modifier</th>
<th>Ratio</th>
<th>t (h)</th>
<th>P bar</th>
<th>T °C</th>
<th>kg CO₂</th>
<th>mg BS/g oil</th>
<th>mg BS/g subst</th>
<th>Oil %</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC-CO₂</td>
<td>Ac-EtOH</td>
<td>2:1</td>
<td>11:25</td>
<td>109.4</td>
<td>41.5</td>
<td>0.32</td>
<td>4.63</td>
<td>1.21</td>
<td>26.1</td>
</tr>
<tr>
<td>SC-CO₂</td>
<td>Isop-EtOH</td>
<td>3:1</td>
<td>23:30</td>
<td>127.2</td>
<td>40.5</td>
<td>0.32</td>
<td>3.97</td>
<td>1.52</td>
<td>38.4</td>
</tr>
<tr>
<td>Commercial</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.24</td>
<td>—</td>
</tr>
</tbody>
</table>
6.4 Cherry pit tests (*Prunus caeresum*).

Regarding the processing of the cherry seed, the pulp or flesh was consumed (eaten) and the stones were washed one part with soap solution and the second part with only water before crushing the seed shell. The soap solution seems to have covered the shell somehow, or to have collapsed the pores avoiding a dehydration of cotyledons. However, there was not plenty of material, so it was necessary to mix both groups of seeds: washed with soap or only water flushed.

![Image of cherry pit tests](image)

Figure 6.3 Shell removing (left) and difference of washing processes (right) where can be seen the big cotyledons of the seeds washed with soap at the left Petri box.

The same grinding process as mechanical pretreatment was adopted for cherry pits extractions. Milling the substrate samples the radius \( r \) in the substrates agglomerates is also reduced increasing the diffusion coefficient through the diminution of the particle radius, where when the particles get smaller contact surface or the border film (Figure 2.10) increase for a higher mass transfer coefficient as included mathematically in equation (2.12).

It was obtained from the milling process a strong cherry smelling substrate sample. This substrate was the matrix for the results in Table 6.4.

Table 6.4 Summary of cherry pit extractions showing sort, modifier substances, ratio of substances in co-solvent, experiment duration, pressure and temperature averages, CO\(_2\) consumption, \(\beta\)-sitosterol and oil mass per mass of substrate and oil load.

<table>
<thead>
<tr>
<th>Sort</th>
<th>Modifier</th>
<th>Ratio</th>
<th>t (h)</th>
<th>P bar</th>
<th>T °C</th>
<th>(\text{kg CO}_2)</th>
<th>(\text{mg BS/g}_{\text{subst}})</th>
<th>Oil Load %</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC-CO(_2)</td>
<td>Isop-EtOH</td>
<td>3:1</td>
<td>20:58</td>
<td>100.8</td>
<td>41.5</td>
<td>0.30</td>
<td>5.778</td>
<td>10.9</td>
</tr>
</tbody>
</table>
6.5 ‘Prickly pear’ tests (*Opuntia ficus-indica*).

Extractions from raw material were done: first from the whole (complete: without breaking) seeds obtaining the results presented in Table 6.5:

<table>
<thead>
<tr>
<th>mg BS/g seed</th>
<th>mg BS/g oil</th>
<th>mg BS/g CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.02</td>
<td>1.31</td>
<td>0.525</td>
</tr>
</tbody>
</table>

It was necessary to sieve the samples to remove this hard material Figure 6.4 and to be able this way to compare it to other substrates. It was found, during pilot extractions of *Opuntia ficus-indica* that the oil yield varies depending on the grain size Figure 6.5. Thus the oil amount obtained from the seed coat (shell) which is more abundant in the large fractions $>500\,\mu$m of the sieved material was neglected.

![Figure 6.4](image)

*Figure 6.4 Left: material after the grinding which is empty after the sieving. Right: overview of the trepidation sieving system, giving better results to get the soft-oily material.*

Dividing the substrate mass by the volume the absolute density ($\rho$) was calculated

$$\rho = \frac{m}{V}$$ (6.1)

Different tests yielded the mean value

$$\rho = 1.298\,\text{g mL}^{-1}$$
Figure 6.5 Grain distribution of the substrate after the 3 mm sieve grinding.

Due to the non polarity of the CO$_2$, different polar co-solvents have been used to test them at supercritical conditions. Next are shown the most common substances used as modifier, form major to minor polarity [68].

![Grain Distribution Diagram]

6.5.1 Control process

The control process was the ultrasonic extraction and it was performed using 0.150 g$_{\text{subst}}$/g$_{\text{solvent}}$, the solvent used was n-heptane with the yields shown in Table 6.6.

Table 6.6 Yields of control experiments performed by ultrasonic extraction.

<table>
<thead>
<tr>
<th></th>
<th>mg$<em>{\beta$-sit}$/g$</em>{\text{n-heptane}}$</th>
<th>mg$<em>{\beta$-sit}$/g$</em>{\text{subst}}$</th>
<th>mg$<em>{\beta$-sit}$/g$</em>{\text{oil}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrasound n-Heptane</td>
<td>1.31</td>
<td>5.422</td>
<td>98.850</td>
</tr>
</tbody>
</table>
6.5.2 Experimental process

6.5.2.1 Oil load

Results of the mass of oil per mass of substrate extracted by pure CO$_2$, are summarized in Table 6.7. Additional experiments at high pressure (225 bar) either at low temperature (25 °C) didn’t improve the results obtained by the extractions performed at planned conditions (Figure 6.6).

Table 6.7 Summary of the oil yields by pure CO$_2$ [g$_{oil}$/g$_{subs}$].

<table>
<thead>
<tr>
<th>CO$_2$ T (°C)</th>
<th>125</th>
<th>150</th>
<th>175</th>
<th>* 225</th>
</tr>
</thead>
<tbody>
<tr>
<td>55</td>
<td>0.01</td>
<td>0.05</td>
<td>0.03</td>
<td>–</td>
</tr>
<tr>
<td>45</td>
<td>0.01</td>
<td>0.03</td>
<td>0.05</td>
<td>–</td>
</tr>
<tr>
<td>35</td>
<td>0.03</td>
<td>0.04</td>
<td>0.32</td>
<td>0.10</td>
</tr>
<tr>
<td>* 25</td>
<td>–</td>
<td>–</td>
<td>0.08</td>
<td>–</td>
</tr>
</tbody>
</table>

Figure 6.6 Summary of oil yield per mass of substrate obtained by pure CO$_2$. 
The use of a modified solvent, namely CO$_2$ with Ethanol, showed an average improvement in the oil yield, regarding that reached by using pure CO$_2$ (Table 6.8).

Table 6.8 Summary of the oil yields by modified CO$_2$ [g$_{oil}$/g$_{subs}$].

<table>
<thead>
<tr>
<th>CO$_2$ EtOH</th>
<th>Pressure (bar)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T (°C)</td>
<td>125</td>
</tr>
<tr>
<td>55</td>
<td>0.08</td>
</tr>
<tr>
<td>45</td>
<td>0.18</td>
</tr>
<tr>
<td>35</td>
<td>0.21</td>
</tr>
<tr>
<td>* 25</td>
<td>–</td>
</tr>
</tbody>
</table>

Contrary to the extraction by pure CO$_2$, the oil yield shows in general higher oil amount values at low pressures. However, the highest oil yield values were found also at low temperature as seen in the pure CO$_2$ extraction (Figure 6.7).

Figure 6.7 Summary of oil yield per mass of substrate obtained by modified CO$_2$.

Additional experiments were performed to confirm the action of the solvent using nitrogen instead CO$_2$ at the same conditions which gave the highest β-sitosterol yield. Nevertheless, the yield was even lower than in the extraction by pure CO$_2$, showing that the CO$_2$ functioned really as solvent and it was not only the direct dilution by ethanol.
The experimental process was the continuous flow extraction; a 2 min sample was taken every 7 minutes as already explained. And after a treatment whether it has a modifier or not, the extract is solved in chloroform and analyzed by gas chromatography.

![Figure 6.8 Example of chromatogram where the β-sitosterol peak can be found at 30.252 min, hexadecanoic (palmitic) acid at 16.827 min and 9,12 octadecadienoic (linoleic) acid at 18.470 min.](image)

The analysis in the GC (Figure 6.8) lasts about 1.5 h and it is possible to print the automatic reference suggested by the device according to the loaded library. The other two high peaks were hexadecanoic (palmitic) acid and 9,12 octadecadienoic (linoleic) acid.
The values from the GC integrations were analyzed in a database for the three main components: β-sitosterol, n-hexadecanoic acid (Palmitic Acid) and octadecadienoic acid (Linoleic Acid).

Figure 6.9 Example of β-sitosterol yield plot in a mass–extraction time graphic.

The curve projected by the extraction values is analyzed with *Origin pro 7.5* to find an equation which matches with the behavior of our extraction curve (Figure 6.10). Once the best equation is found, it can be integrated yielding the mass of the analyzed substance in the whole extraction period.

Figure 6.10 Example of the correlation between the extraction (dots) and fitted (continuous line) graphics.
Three different temperatures were matched with three different pressures giving a set of nine ‘planned’ extraction conditions for pure CO₂ and the same set of conditions for CO₂ made flow at a rate of 2 L/min, modified with ethanol at 0.50 mL/min. Next, a description of the extraction velocity based on the former curves (Figure 6.10, Figure 6.11) is shown. As described by Sovová [130], while working with extraction of natural substances from ground natural substrates the first part of the extraction is mostly governed by the solution of ‘easy extractable oil’ followed by a ‘hard to extract oil’. A simple extraction velocity ($v_{xt}$) model was utilized where the accumulated mass between the beginning of the extraction period (‘hard to extract’ compound) with coordinates $(x_i,y_i)$, in the Figure 6.11 from the intersection of $M-1$ and $M-2$, and the beginning of the yield–end period is divided by the time between this point and the one where the extraction curve KF076 (extraction example) deviates from the kinetic model line $M-2$ related to the area I described by Herdegen [68] (Figure 2.11).

$$v_{xt} = \frac{y_s - y_i}{x_s - x_i}$$  \hspace{1cm} (6.2)

Figure 6.11 Extraction curve with a simple kinetic model $M-1$ at the origin and a second phase $M-2$ at the circle ending at $\triangle$, with the deviation point of the extraction curve ◇ (experiment code) from the kinetic model.

These will be the considerations to evaluate the extraction’s performance: the amount of the substance of interest and the time to reach this point.
6.6 Load of β-sitosterol

6.6.1 Pure CO₂ β-sitosterol yields

6.6.1.1 Pure CO₂ β-sitosterol yields 35 °C

Two experiments were performed in addition to the planned extraction set: the *225 bar*, to analyze the influence of a higher pressure during the extraction period, and the *175 bar-25 °C* because a better extraction performance was assumed while using the highest extraction pressure and the lowest planned temperature.

![Pure CO₂ - 35 °C](image)

Figure 6.12 Integration of the β-sitosterol at different pressures as an Isotherm overview at 35 °C. Asterisk variations refer additional experiments.

According to the ideal gases equation, all variables but the pressure $p$ remain stable during the supercritical extraction. The variation of $p$ at constant volume and temperature is possible only by a matter increase within the system, in this case, solvent molecules which are responsible for a faster diffusion of the solute [30], which means: higher extraction velocity, slopes with great inclinations and higher $v_{st}$ values. The second zone (according to the Figure 2.11) lasts longer in the extraction curve at low pressures to reach the third zone of the extraction curve.
Yield values are also directly related to the $p$ values increasing the solvent absorption in the particles of the packed bed. At *225 bar* was seen the same described effect despite the lower temperature (Figure 6.12).

At the 35 °C isotherm the planned parameters reached the minute 90 of extraction with the highest yield at 175 bar. Nevertheless, a variation in the lowest planned temperature from 35 to 25 °C affected positively the maximum yield value getting above all of the planned pressures for this isotherm, because the reduction of the temperature increases the CO$_2$ density and thus the diffusion [90][147].

The maximum yield value is not that obvious as the extraction kinetic rate which shows a higher inclination or a faster extraction. This is also due to the drastic density change at critical pressures for CO$_2$ going ‘colder’ than the $T_c$ of 31.1 °C getting into the subcritical region.

The reduction of the temperature at the highest pressure value accelerated the extraction reaching the maximum shortly after the 40 minutes of extraction.

The ‘hard to extract oil’ [131] or the first part of the extraction curve [68] began rather soon after the beginning of the extraction. Nevertheless the additional high pressure (225 bar) didn’t seem to be a determinant factor reducing the extraction time or increasing the β-sitosterol amount.

Similar extraction yields were reached working at the three planned pressures, but the extraction times were quite different between them varying from 40 minutes at the lowest pressure, 40 minutes at 150 bar and less than 15 minutes at 175 bar.
6.6.1.2 Pure CO₂ β-sitosterol yields 45 °C

Pressure values 175, 150, and 125 bar, were analyzed at 45°C. Notice that even for the highest yield, at extraction pressure 175 bar, it was not possible to reach the 2 mg achieved at lower temperatures and the time for the extraction took about one third additional, compared to the isotherm 35 °C (Figure 6.13).

Figure 6.13 Integration of β-sitosterol at different pressures as an isotherm overview at 45 °C.

The 45 °C isotherm was characterized by lower extraction velocities in comparison to 35 °C, this is reasonable due to the decrease in the density as the temperature increases, offering this low density CO₂, less diffusivity especially for the relative heavier compounds being more difficult to carry out. The fastest was found at the highest pressure (175 bar) taking about 40 minutes but beginning at 45 of the whole extraction time! It is also possible to notice a relation between high pressure, the amount of β-sitosterol yielded and the extraction velocity, since the slopes of β-sitosterol extraction are more inclined (faster extraction) when the pressure was higher, showing a clear relationship between the diffusivity and the molar concentration $n$ of the solvent within the system. This middle temperature value didn’t show an exceptional effect on the β-sitosterol extraction but shows the extraction’s speed late and slower compared to a lower temperature (35 °C).
6.6.1.3 Pure CO₂ β-sitosterol yields 55 °C

Since the 150 bar curve showed an increase even after 120 min (Figure 6.14), a new experiment was performed at the same extraction conditions showing a late yield end after 150 minutes apparently because of a difference in the storage time. The original 150 bar extraction (in squares) was performed with material dried and stored while the one with crosses was dried and used immediately without giving time to oxidation, decomposition or denaturalization of the β-sitosterol.

Figure 6.14 Integration of the β-sitosterol yields at different pressures as an Isotherm overview at 55 °C. Asterisk variations refer additional experiments.

The 175 bar curve had also at 55 °C the highest β-sitosterol yield with a similar value than at 45 °C and in a shorter time, but lower than that at 35 °C though since at 35 °C the low temperature propitiated higher diffusion values. Although the n-hexadecanoic acid and the octadecadienoic acid appear in all the experiments, the high temperature (55 °C) at low pressure (125 bar, triangles curve in Figure 6.14) did not yield any β-sitosterol since reduced CO₂ density by high temperature and low pressure seems to offer a low diffusion power, not enough for high weight substances yielding zero for β-sitosterol at these extraction conditions. The addition of 30 minutes to the 150 bar experiment presented a slight ‘constant yield’ production between the minute 42 and 53 of extraction.
Table 6.9 Synoptic table showing coordinates from \( i \) intersection, to \( sep \) separation of the \( \beta \)-sitosterol extraction model regarding the yield curve by pure CO\(_2\) from the model at different pressures and temperatures, and a extraction velocity \( v_{BS,xt} \) in mg/min.

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>P (bar)</th>
<th>( x_i )</th>
<th>( y_i )</th>
<th>( x-sep )</th>
<th>( y-sep )</th>
<th>( v_{BS,xt} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>175</td>
<td>15.05</td>
<td>0.19</td>
<td>33.00</td>
<td>1.86</td>
<td>0.0932</td>
</tr>
<tr>
<td>35</td>
<td>225</td>
<td>16.22</td>
<td>0.20</td>
<td>40.00</td>
<td>1.17</td>
<td>0.0408</td>
</tr>
<tr>
<td>35</td>
<td>175</td>
<td>18.50</td>
<td>0.45</td>
<td>47.00</td>
<td>1.97</td>
<td>0.0536</td>
</tr>
<tr>
<td>35</td>
<td>150</td>
<td>31.14</td>
<td>0.23</td>
<td>75.00</td>
<td>1.36</td>
<td>0.0258</td>
</tr>
<tr>
<td>35</td>
<td>125</td>
<td>55.39</td>
<td>0.62</td>
<td>89.00</td>
<td>1.32</td>
<td>0.0206</td>
</tr>
<tr>
<td>45</td>
<td>175</td>
<td>45.80</td>
<td>0.32</td>
<td>89.00</td>
<td>1.33</td>
<td>0.0235</td>
</tr>
<tr>
<td>45</td>
<td>150</td>
<td>69.14</td>
<td>0.58</td>
<td>110.00</td>
<td>1.28</td>
<td>0.0172</td>
</tr>
<tr>
<td>45</td>
<td>125</td>
<td>86.24</td>
<td>0.23</td>
<td>117.00</td>
<td>0.43</td>
<td>0.0064</td>
</tr>
<tr>
<td>55</td>
<td>175</td>
<td>29.96</td>
<td>0.30</td>
<td>54.00</td>
<td>1.19</td>
<td>0.0373</td>
</tr>
<tr>
<td>55</td>
<td>150</td>
<td>3.20</td>
<td>0.00</td>
<td>124.00</td>
<td>0.72</td>
<td>0.0060</td>
</tr>
<tr>
<td>55</td>
<td>150 +35'</td>
<td>42.78</td>
<td>0.42</td>
<td>54.00</td>
<td>0.62</td>
<td>0.0180</td>
</tr>
</tbody>
</table>

Additional to a general tendency towards high pressures (Table 6.9 and Figure 6.15), it is possible to find the highest extraction velocity at low temperature during the additional experiments (25 °C).

Figure 6.15 Extraction velocities for \( \beta \)-sitosterol by pure CO\(_2\).
6.6.2 Modified CO\textsubscript{2} \(\beta\)-sitosterol yields

The CO\textsubscript{2} was made flow at a rate of 2 L/min as well as the pure CO\textsubscript{2} extractions with the difference of the ethylic alcohol addition at a rate of 0.5 mL/min. At the first experiments with ethanol apparently the humidity (of the alcohol) and the pressure provoked a “cake effect” on the substrate, and the CO\textsubscript{2} couldn’t flow any more after 10 minutes of extraction. Hence, it was necessary to use stainless steel bearings.

6.6.2.1 Modified CO\textsubscript{2} \(\beta\)-sitosterol yields 35 °C

The use of a modifier benefits the general extraction performance \textit{i.e.} the amount of \(\beta\)-sitosterol as well as the extraction time. Two of the planned pressures were already above the 2 mg of \(\beta\)-sitosterol and the three of these planned pressures reached their maximum not later than 50 minutes of extraction.

![Figure 6.16 Integration of the \(\beta\)-sitosterol yields extracted by modified CO\textsubscript{2} at different pressures at 35 °C. Asterisk variations refeer additional experiments: WS without sieving and N using nitrogen instead CO\textsubscript{2}.](image)

Since the lowest planned temperature showed the highest yield values, two additional conditions were tested, namely: the highest planned pressure value with seeds without sieving and extractions using nitrogen instead of CO\textsubscript{2}. 

60
The polarity addition to CO\(_2\) by means of EtOH gave a plus value to the solvency at 35 °C. The diffusion in terms of CO\(_2\) density plays a main role at 175 bar offering the maximum yield despite the inversion of the values in middle and low pressure.

Extractions in the last graphic (Figure 6.16) *175 bar-WS* are referred to those extractions performed with seeds (whole seeds) without sieving as substrate. Here it was not necessary to add the stainless steel bearings. The use of nitrogen instead of CO\(_2\) was due to the risk of having a “modifier extraction” instead a CO\(_2\) extraction, and with these experiments it was possible to notice a real CO\(_2\) action along the extraction time compared to *175 bar-N* with nitrogen, which reaches the maximum after 90 minutes of extraction while the normal modified CO\(_2\) extraction does it at 30 minutes of extraction.

The ‘hard to extract’ oil is yielded sometimes from the origin (the beginning) or mostly shortly after the beginning of the extraction and except the two additional performed extractions, the maximum yield values are reached before 40 minutes. Modeling in a simple way the extraction kinetics for the β-sitosterol, the graphic reaches the maximum point before 20 minute of extraction.
6.6.2.2 Modified CO$_2$ β-sitosterol yields at 45 °C

45 °C extractions showed a very similar behavior between them, it exhibit a slight difference where the higher the pressure, the shorter the extraction time to the maximum yield value, but also for this temperature, the lower the pressure, the higher the mass of β-sitosterol yielded (Figure 6.17).

![Figure 6.17 Integration of the β-sitosterol yields extracted by modified CO$_2$ at different pressures at 45 °C.](image)

A larger solvent mol amount supported by a higher polarity through the addition of EtOH increased the yield media between the experiments at 45 °C compared to those performed by pure CO$_2$. Moreover, the extraction process showed higher velocities thanks to polarity assisted diffusion (cf. Figure 6.13).

The three pressure variations at the same temperature reached the maximum β-sitosterol yield by 50 minutes of extraction. A more similar performance between them was shown by the three different pressures at the middle temperature modified with ethanol compared to the performance at low temperature (cf. Figure 6.16). Despite the low yield compared to the low temperature extractions using modified solvent, the time required to reach the maximum (50 minutes) was much shorter than without the use of modifier (about 90 minutes).
6.6.2.3 Modified CO₂ β-sitosterol yields at 55 °C

The variation in the pressure values exhibit in comparison with the 45 °C, a wider range of variation in the yield of β-sitosterol between the extraction pressures (Figure 6.18).

Figure 6.18 Integration of the β-sitosterol yields extracted by modified CO₂ at different pressures at 55 °C.

Despite the similarity between the extractions yields at 55 °C with pure and modified CO₂, the velocities increased by means of the modified solvent showing also more inclined slopes. Although the high pressure condition (175 bar) didn’t yield so much as the two other variables, it reached the maximum also during the first 40 minutes of extraction.

High pressures at low temperatures seem to affect the same way as high temperatures at low pressures since the extractions reach the maximum yield point inversely to their pressures. In addition, the low and the middle pressures reach a similar maximum β-sitosterol value while high pressures hardly exceeded 1 mg of β-sitosterol.
Table 6.10 Synoptic table showing coordinates from $i$ intersection, to $sep$ separation of the $\beta$-sitosterol extraction velocity model $v_{BS,xt}$ in mg/min, regarding the yield curve by modified CO$_2$ from the model at different pressures and temperatures.

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>P (bar)</th>
<th>$x_i$</th>
<th>$y_i$</th>
<th>$x-Sep$</th>
<th>$y-Sep$</th>
<th>$v_{BS,xt}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>175</td>
<td>0.0</td>
<td>0.0</td>
<td>18.0</td>
<td>2.8</td>
<td>0.1581</td>
</tr>
<tr>
<td>35</td>
<td>150</td>
<td>3.9</td>
<td>0.0</td>
<td>19.0</td>
<td>1.2</td>
<td>0.0825</td>
</tr>
<tr>
<td>35</td>
<td>125</td>
<td>5.4</td>
<td>0.0</td>
<td>19.0</td>
<td>1.8</td>
<td>0.1343</td>
</tr>
<tr>
<td>45</td>
<td>175</td>
<td>4.6</td>
<td>0.0</td>
<td>19.0</td>
<td>1.3</td>
<td>0.0936</td>
</tr>
<tr>
<td>45</td>
<td>150</td>
<td>5.0</td>
<td>0.0</td>
<td>24.0</td>
<td>1.6</td>
<td>0.0848</td>
</tr>
<tr>
<td>45</td>
<td>125</td>
<td>9.6</td>
<td>0.1</td>
<td>26.0</td>
<td>1.5</td>
<td>0.0862</td>
</tr>
<tr>
<td>55</td>
<td>175</td>
<td>4.6</td>
<td>0.0</td>
<td>26.0</td>
<td>1.2</td>
<td>0.0563</td>
</tr>
<tr>
<td>55</td>
<td>150</td>
<td>4.8</td>
<td>0.0</td>
<td>26.0</td>
<td>1.6</td>
<td>0.0739</td>
</tr>
<tr>
<td>55</td>
<td>125</td>
<td>0.1</td>
<td>0.0</td>
<td>12.0</td>
<td>1.5</td>
<td>0.1284</td>
</tr>
</tbody>
</table>

The addition of polarity by the use of ethanol as modifier increased in a general overview the extraction velocity average among the different conditions (Table 6.10 and Figure 6.19). Between middle and high temperatures there were inverted tendencies, showing the highest velocity at low temperature and high pressure.

Figure 6.19 Extraction velocities for $\beta$-sitosterol by modified CO$_2$. 
6.7 *Opuntia* β-sitosterol Extractions Overview

6.7.1 Load of β-sitosterol in the Substrate

The mass of β-sitosterol per mass of substrate showed a trend to yield higher values at higher pressures at 55 °C and 45 °C, while at 35 °C this behavior was not that defined (Table 6.11).

Table 6.11 β-sitosterol concentration per mass of substrate (mg/g) by pure CO2.

<table>
<thead>
<tr>
<th>CO2 Pressure (bar)</th>
<th>CO2 Pressure (bar)</th>
<th>CO2 Pressure (bar)</th>
<th>CO2 Pressure (bar)</th>
<th>CO2 Pressure (bar)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T (°C)</td>
<td>125</td>
<td>150</td>
<td>175</td>
<td>* 225</td>
</tr>
<tr>
<td>55</td>
<td>0.00</td>
<td>0.14</td>
<td>0.24</td>
<td>—</td>
</tr>
<tr>
<td>45</td>
<td>0.06</td>
<td>0.19</td>
<td>0.22</td>
<td>—</td>
</tr>
<tr>
<td>35</td>
<td>0.25</td>
<td>0.22</td>
<td>0.27</td>
<td>0.19</td>
</tr>
<tr>
<td>* 25</td>
<td>—</td>
<td>—</td>
<td>0.30</td>
<td>—</td>
</tr>
</tbody>
</table>

Additional extractions confirmed that at 150 bar and 55 °C the extraction time favored the maximum extraction yield. At 225 bar (and 35 °C) the yield was lower. However, 25 °C and 175 bar suggest the use of lower temperatures instead of high pressures (Figure 6.20).

Figure 6.20 Summary of β-sitosterol yield per mass of substrate by pure CO$_2$.
The use of ethanol as modifier, elicited more similar β-sitosterol mass yields between the different extraction conditions, compared to those extractions by pure CO$_2$ (Table 6.12).

Table 6.12 β-sitosterol concentration per mass of substrate (mg/g) by modified CO$_2$.

<table>
<thead>
<tr>
<th>CO$_2$ Pressure (bar)</th>
<th>T (°C) 125</th>
<th>150</th>
<th>175</th>
<th>225</th>
</tr>
</thead>
<tbody>
<tr>
<td>55</td>
<td>0.24</td>
<td>0.22</td>
<td>0.17</td>
<td>—</td>
</tr>
<tr>
<td>45</td>
<td>0.23</td>
<td>0.22</td>
<td>0.18</td>
<td>—</td>
</tr>
<tr>
<td>35</td>
<td>0.30</td>
<td>0.19</td>
<td>0.34</td>
<td>—</td>
</tr>
<tr>
<td>25</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

As well as for the whole β-sitosterol mass analysis, the highest yield values are found at low temperatures with low or high pressure, in this case the middle pressure value didn’t yield values comparable to higher temperatures (Figure 6.21).

Figure 6.21 Summary of β-sitosterol yields per mass of substrate by CO$_2$-EtOH.
6.7.2 Efficiency of solvent for β-sitosterol

Considering the total β-sitosterol amount obtained, the pure CO₂ shows higher values at high pressures according to the three planned isotherms (55, 45 and 35 °C) (Table 6.13).

Table 6.13 Yield of β-sitosterol pro mass of pure CO₂ (molₜₚ/мол CO₂).

<table>
<thead>
<tr>
<th>CO₂ Pressure (bar)</th>
<th>T (°C)</th>
<th>125</th>
<th>150</th>
<th>175</th>
<th>225</th>
</tr>
</thead>
<tbody>
<tr>
<td>55</td>
<td>0.0</td>
<td>2.3E-07</td>
<td>4.7E-07</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>1.1E-07</td>
<td>3.5E-07</td>
<td>4.1E-07</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>7.9E-07</td>
<td>3.8E-07</td>
<td>7.8E-07</td>
<td>5.5E-07</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>—</td>
<td>—</td>
<td>1.2E-06</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

The additional experiments performed at 225 bar didn’t yield higher β-sitosterol values than the isotherm at 175 bar, while low temperature (25 °C) yield slightly more than twice the value at 35 °C (Figure 6.22).

![CO₂ Consumption](image)

Figure 6.22 Summary of β-sitosterol yields per mass of solvent by pure CO₂.

The use of modifier increased the efficiency of CO₂ yielding in a general view more than the maximum yield obtained without modification (Table 6.14).
Table 6.14 Yield of $\beta$-sitosterol pro mass of modified CO$_2$ (mg$_{BS}$/g$_{CO2}$).

<table>
<thead>
<tr>
<th>CO$_2$-EtOH Pressure (bar)</th>
<th>T (°C) 125</th>
<th>150</th>
<th>175</th>
<th>225</th>
</tr>
</thead>
<tbody>
<tr>
<td>55</td>
<td>1.6E-06</td>
<td>1.5E-06</td>
<td>1.3E-06</td>
<td>—</td>
</tr>
<tr>
<td>45</td>
<td>1.1E-06</td>
<td>1.9E-06</td>
<td>1.2E-06</td>
<td>—</td>
</tr>
<tr>
<td>35</td>
<td>1.1E-06</td>
<td>1.6E-06</td>
<td>4.5E-06</td>
<td>—</td>
</tr>
<tr>
<td>25</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

At the additional experiment performed with nitrogen instead CO$_2$, the efficiency of the modifier working alone hardly reached the 0.01 mg of $\beta$-sitosterol per gram of solvent.

Figure 6.23 Summary of $\beta$-sitosterol yields per mass of solvent by modified CO$_2$.

Once it was found that the parameter combination with higher yield was 35 °C and 175 bar by modified CO$_2$ (Figure 6.23), additional experiments under different conditions were performed to confirm this values using whole seeds, nitrogen instead of carbon dioxide, 10wt% moisture resembling the pretreatment mentioned by Rout et al. [125], increasing of the solvent mass flow and the modifier flow (Figure 6.24).
Figure 6.24 Variation highest yield conditions (35°C-175 bar) per mass of substrate by modified CO₂. Codes: WS, whole seeds; N2, (modified) nitrogen extraction; +Wet, substrate with 10 % moisture; N2+wet, nitrogen extraction by substrate with 10 % moisture; 4X1, 4 L CO₂/min and 1 mL EtOH/min; 2X1, 2 L CO₂/min and 1 mL EtOH/min.

Also the oil amount was affected by these experimental changes (Figure 6.25) based as well in the original values for the highest β-sitosterol yield at 35 °C and 175 bar.

Figure 6.25 Variation of highest yield conditions (35°C-175 bar) per mass of solvent by modified CO₂. Codes: WS, whole seeds; N2, (modified) nitrogen extraction; +Wet, substrate with 10wt% moisture; N2+wet, nitrogen extraction by substrate with 10wt% moisture; 4X1, 4 L CO₂/min and 1 mL EtOH/min; 2X1, 2 L CO₂/min and 1 mL EtOH/min.
Table 6.15 shows a summary of the results beginning with the amount of oil obtained per mass of seed leaded by the continuous extraction with 287.0 \( \text{mg}_{\text{oil}}/\text{g}_{\text{subst}} \). The highest \( \beta \)-sitosterol yield per mass of substrate was obtained by SCF Stationary extraction with 6.4 \( \text{mg}_{\beta \text{-sitosterol}}/\text{g}_{\text{substrate}} \). The highest concentration of \( \beta \)-sitosterol per mass of oil was reached by SCFE, also with the stationary device 168.9 \( \text{mg}_{\beta \text{-sitosterol}}/\text{g}_{\text{oil}} \).

Table 6.15 Summary of maximal results from nopal seed oil extractions (\( \text{ws} \) subindex refers to experiments performed with \textit{whole seeds} ‘no-sieved’ material).

<table>
<thead>
<tr>
<th>Source</th>
<th>Yield ( \text{mg}<em>{\text{oil}}/\text{g}</em>{\text{subst}} )</th>
<th>( \beta )-sitosterol ( \text{mg}<em>{\beta \text{-sitosterol}}/\text{g}</em>{\text{oil}} )</th>
<th>( \text{mg}<em>{\beta \text{-sitosterol}}/\text{g}</em>{\text{substrate}} )</th>
<th>( \text{mg}<em>{\beta \text{-sitosterol}}/\text{g}</em>{\text{oil}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrasound/2 h/n-Heptane( \text{ws} )</td>
<td>83.7</td>
<td>0.903</td>
<td>5.4</td>
<td>98.9</td>
</tr>
<tr>
<td>SCF Stationary/\text{Iso-EtOH}/5 h</td>
<td>37.8</td>
<td>0.420</td>
<td>\textbf{6.4}</td>
<td>\textbf{168.9}</td>
</tr>
<tr>
<td>SCF Shaker/\text{EtOH}/8 h</td>
<td>11.0</td>
<td>0.064</td>
<td>1.0</td>
<td>92.9</td>
</tr>
<tr>
<td>SCF Continuous-\text{XT}/\text{EtOH}/1 h</td>
<td>\textbf{287.0}</td>
<td>0.106</td>
<td>0.7</td>
<td>6.0</td>
</tr>
<tr>
<td>\textit{Published Values}_{\text{ws}}/\text{hexan}/12 h</td>
<td>91.4</td>
<td>–</td>
<td>0.5</td>
<td>4.30</td>
</tr>
</tbody>
</table>
6.8 Load of Linoleic Acid

6.8.1 Pure CO₂ Linoleic Acid yields

6.8.1.1 Pure CO₂ Linoleic Acid yields at Isotherm 35 °C

Difference beginning with the scale can be seen in the results of Linoleic acid, while for β-sitosterol the maximum scale reached 0.4 mg, for Linoleic acid it went over 300 mg (Figure 6.26).

![Graph of Pure CO₂ 35 °C](image)

Figure 6.26 Integration of the Linoleic acid yields extracted by modified CO₂ at different pressures and 35 °C. Asterisk variations refer additional experiments: 225 bar higher pressure and 25 °C with lower temperatures.

At 35 °C, higher yields were shown at high pressures and according to the results of additional extractions, the low temperature has more influence than high pressure on yielding higher amount of Linoleic acid. Except the low pressures from the planned set (namely 150 and 125 bar) the Linoleic acid yield began from the origin reaching the values already mentioned. The importance of this graphic is the kinetic parameter that we can obtain; being possible to notice a very similar extraction velocity between the different pressure conditions, nevertheless, the additional experiment at low temperature (25 °C) yielded the highest Linoleic acid values.
6.8.1.2 Pure CO₂ Linoleic Acid yields Isotherm 45 °C

This middle temperature elicited in general low values for Linoleic acid mass; nevertheless, it is possible to notice a correlation between the pressures, the maximum yield value and the extraction velocity for every parameter (Figure 6.27).

![Figure 6.27 Integration of the Linoleic acid yields extracted by pure CO₂ at different pressures at 45 °C.](image)

Extraction at 45 °C did not yield high Linoleic acid values or high extraction velocities. The extraction velocities began not from the origin as at 35 °C and only the highest pressure (175 bar) helped to reach the highest yield value within one hour of extraction and the rest exceeded this period. As rather slow, it is possible to describe the extraction process at 45 °C due to the time that it lasts to reach the maximum yield points at 150 and 125 bar. Besides this, the maximum yield was lower than 200 mg of Linoleic acid.
6.8.1.3 Pure CO₂ Linoleic Acid yields at Isotherm 55 °C

Higher yield values of Linoleic acid were found throughout the experiments at 55 °C reaching a maximum yields at 150 bar (Figure 6.28).

Figure 6.28 Integration of the Linoleic acid yields extracted by pure CO₂ at different pressures at 55 °C.

Experiments at 55 °C showed a positive correlation between the pressure and the extraction velocity, supported with the maximum yield values reached at the end of the extraction time.

Low pressure -125 bar- began the linoleic acid yield rater late beginning by 60 min.
Table 6.16 Synoptic table showing coordinates from $i$ intersection, to $sep$ separation of the Linoleic acid extraction velocity model $v_{LA,xt}$ in mg/min, regarding the yield curve by pure CO$_2$ from the model at different pressures and temperatures.

<table>
<thead>
<tr>
<th>$T$ (°C)</th>
<th>$P$ (bar)</th>
<th>$x_i$</th>
<th>$y_i$</th>
<th>$x-sep$</th>
<th>$y-sep$</th>
<th>$v_{LA,xt}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>175 *25 °C</td>
<td>5.0</td>
<td>31.0</td>
<td>26.0</td>
<td>334.4</td>
<td>14.5</td>
</tr>
<tr>
<td>35</td>
<td>225</td>
<td>0.3</td>
<td>0.0</td>
<td>19.0</td>
<td>182.9</td>
<td>9.8</td>
</tr>
<tr>
<td>35</td>
<td>175</td>
<td>0.7</td>
<td>0.0</td>
<td>19.0</td>
<td>261.8</td>
<td>14.3</td>
</tr>
<tr>
<td>35</td>
<td>150</td>
<td>5.3</td>
<td>0.0</td>
<td>26.0</td>
<td>69.4</td>
<td>3.4</td>
</tr>
<tr>
<td>35</td>
<td>125</td>
<td>18.9</td>
<td>50.2</td>
<td>33.0</td>
<td>114.1</td>
<td>4.5</td>
</tr>
<tr>
<td>45</td>
<td>175</td>
<td>7.5</td>
<td>0.0</td>
<td>26.0</td>
<td>127.6</td>
<td>6.9</td>
</tr>
<tr>
<td>45</td>
<td>150</td>
<td>3.8</td>
<td>0.0</td>
<td>47.0</td>
<td>159.7</td>
<td>3.7</td>
</tr>
<tr>
<td>45</td>
<td>125</td>
<td>9.9</td>
<td>0.0</td>
<td>82.0</td>
<td>145.4</td>
<td>2.0</td>
</tr>
<tr>
<td>55</td>
<td>175</td>
<td>7.7</td>
<td>3.4</td>
<td>26.0</td>
<td>187.0</td>
<td>10.0</td>
</tr>
<tr>
<td>55</td>
<td>150</td>
<td>4.8</td>
<td>0.0</td>
<td>61.0</td>
<td>235.5</td>
<td>4.2</td>
</tr>
<tr>
<td>55</td>
<td>150</td>
<td>7.7</td>
<td>19.7</td>
<td>33.0</td>
<td>218.5</td>
<td>7.9</td>
</tr>
<tr>
<td>55</td>
<td>125</td>
<td>54.4</td>
<td>8.3</td>
<td>68.0</td>
<td>50.9</td>
<td>3.1</td>
</tr>
</tbody>
</table>

For LA it is possible to see a tendency at 55 and 45 °C towards high pressure showing also the highest velocity value at low temperatures at 175 instead 225 bar.

![Figure 6.29 Extraction velocities for linoleic acid by pure CO$_2$.](image-url)
6.8.2 Modified CO$_2$ Linoleic Acid yields

6.8.2.1 Modified CO$_2$ Linoleic Acid yields at Isotherm 35 °C

Effects of modified CO$_2$ on the extraction performance could be described rather as poor since the experiments at the same temperature without modifier yielded values higher than 150 mg while the currently described did not (Figure 6.30).

![Figure 6.30 Integration of the Linoleic acid yields extracted by Modified CO$_2$ under different pressures at 35 °C. Asterisk variations refeer additional experiments: WS without sieving and N using nitrogen instead CO$_2$.](image)

Experiments performed with whole seeds (ground but with shell during the extraction) yielded very low Linoleic acid values, while the Nitrogen extractions yielded good Linoleic acid values but quite late based on the extraction time, compared to the rest of the experiments. Middle pressure showed the highest extraction velocity followed by the minor pressure, while neither the major planned, nor the parallel extraction at this pressure (175 bar) had a similar or higher extraction velocity.

In Figure 6.30, the low yield reached by the extraction performed with whole seeds, and the late yield beginning of the extraction by Nitrogen is more obvious.
6.8.2.2 Modified CO$_2$ Linoleic Acid yields at Isotherm 45 °C

Better Linoleic acid yields were obtained rather at low or high pressure. At middle pressure the results were quite low (Figure 6.31).

![Figure 6.31 Integration of the Linoleic acid yields extracted by modified CO$_2$ at different pressures at 45 °C.](image)

Values above the 200 mg of Linoleic acid can be remarkable from these extraction conditions beside the relative short time required to reach the maximum yield values for all of them. Low pressure extractions reached the maximum yield value at a higher extraction velocity according to the simple kinetic modeling, followed by the high pressure extractions.

Although the middle pressure extraction (150 bar) didn’t exceed the 30 minutes of extraction, the maximum yield value before the extraction curve left the extraction model reached only 6.4 mg of Linoleic acid, quite low compared to the next value at this temperature by the 175 bar extraction (120.1 mg$_{LA}$).
6.8.2.3 Modified CO₂ Linoleic Acid yields at Isotherm 55 °C

High extraction values were reached during the experiments at high temperature especially combined with low pressures (Figure 6.32).

![Figure 6.32 Integration of the Linoleic acid yields extracted by modified CO₂ at different pressures at 55 °C.](image)

It is also possible to notice a negative correlation between the Linoleic acid extraction yield and the extraction pressure, while all of the experiments reached the maximum Linoleic acid values before the 20 minutes of extraction.

Extraction velocity parameters were also inverse proportional to the extraction pressure. All three kinetic models departed from the origin reaching different extraction velocities as already mentioned, being highly remarkable the 329.5 mg of Linoleic acid reached short after 12 minutes at 125 bar.
Table 6.17 Synoptic table showing coordinates from \( i \) intersection, to \( sep \) separation of the Linoleic acid extraction velocity model \( v_{LA,xt} \) in mg/min, regarding the yield curve by modified CO\(_2\) from the model at different pressures and temperatures.

<table>
<thead>
<tr>
<th>( T ) (°C)</th>
<th>( P ) (bar)</th>
<th>( X/i )</th>
<th>( Y/i )</th>
<th>( x-sep )</th>
<th>( y-sep )</th>
<th>( v_{LA,xt} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>175</td>
<td>0.0</td>
<td>0.0</td>
<td>12.0</td>
<td>78.9</td>
<td>6.6</td>
</tr>
<tr>
<td>35</td>
<td>150</td>
<td>0.0</td>
<td>0.0</td>
<td>12.0</td>
<td>134.3</td>
<td>11.2</td>
</tr>
<tr>
<td>35</td>
<td>125</td>
<td>0.1</td>
<td>0.0</td>
<td>12.0</td>
<td>109.8</td>
<td>9.2</td>
</tr>
<tr>
<td>45</td>
<td>175</td>
<td>0.0</td>
<td>0.0</td>
<td>12.0</td>
<td>120.1</td>
<td>10.0</td>
</tr>
<tr>
<td>45</td>
<td>125</td>
<td>0.2</td>
<td>0.0</td>
<td>12.0</td>
<td>197.7</td>
<td>16.7</td>
</tr>
<tr>
<td>55</td>
<td>175</td>
<td>0.0</td>
<td>0.0</td>
<td>12.0</td>
<td>62.4</td>
<td>5.2</td>
</tr>
<tr>
<td>55</td>
<td>150</td>
<td>0.0</td>
<td>0.0</td>
<td>19.0</td>
<td>186.9</td>
<td>9.8</td>
</tr>
<tr>
<td>55</td>
<td>125</td>
<td>0.0</td>
<td>0.0</td>
<td>12.0</td>
<td>329.5</td>
<td>27.5</td>
</tr>
</tbody>
</table>

Quite high velocities were found at low pressures and high temperatures (Table 6.17 and Figure 6.33), while at low temperature (35 °C) the highest extraction velocity was found at 150 bar.

Figure 6.33 Extraction velocities for linoleic acid by modified CO\(_2\).
6.8.3 *Opuntia* sp. Linoleic acid results overview

Regarding the substrate exposed to supercritical conditions by pure CO₂, the Linoleic acid highest concentration was yielded at 150 bar and 55 °C (Figure 6.34).

![Load of Lineleic Acid in Substrate](image)

Figure 6.34 Summary of Linoleic acid yields per mass of substrate by pure CO₂.

It was also found that extractions at a reduced temperature increased the gain at high pressure extraction (175 bar) (Table 6.18).

<table>
<thead>
<tr>
<th>CO₂ T (°C)</th>
<th>Pressure (bar)</th>
</tr>
</thead>
<tbody>
<tr>
<td>55</td>
<td>125 8.81</td>
</tr>
<tr>
<td></td>
<td>150 55.36</td>
</tr>
<tr>
<td></td>
<td>175 34.06</td>
</tr>
<tr>
<td>45</td>
<td>125 21.30</td>
</tr>
<tr>
<td></td>
<td>150 24.71</td>
</tr>
<tr>
<td></td>
<td>175 25.17</td>
</tr>
<tr>
<td>35</td>
<td>125 20.81</td>
</tr>
<tr>
<td></td>
<td>150 12.15</td>
</tr>
<tr>
<td></td>
<td>175 39.35</td>
</tr>
<tr>
<td>25</td>
<td>125 –</td>
</tr>
<tr>
<td></td>
<td>150 –</td>
</tr>
<tr>
<td></td>
<td>175 43.38</td>
</tr>
</tbody>
</table>

Table 6.18 Yield of Linoleic acid pro mass of substrate (mg/g) by pure CO₂.
The summary of results during the extraction by modified CO$_2$ is shown in Figure 6.35.

Figure 6.35 Summary of Linoleic acid yields per mass of substrate by modified CO$_2$.

In a general tendency towards the low pressure with high temperature, the highest concentrations of Linoleic acid per mass of substrate were seen (Table 6.19).

Table 6.19 Yield of Linoleic acid pro mass of oil (mg/g) by pure CO$_2$.

<table>
<thead>
<tr>
<th>CO$_2$-EtOH</th>
<th>Pressure (bar)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T (°C)</td>
<td>125</td>
</tr>
<tr>
<td>55</td>
<td>44.04</td>
</tr>
<tr>
<td>45</td>
<td>28.06</td>
</tr>
<tr>
<td>35</td>
<td>15.14</td>
</tr>
<tr>
<td>* 25</td>
<td>–</td>
</tr>
</tbody>
</table>
The optimal conditions for $\beta$-sitosterol are not the same as for Linoleic acid as it is possible to see next (Figure 6.36) by the variations at 35 °C-175 bar conditions.

Figure 6.36 Variation of LA at the highest $\beta$-sitosterol yield conditions (35°C-175 bar) per mass of solvent by modified CO$_2$. Codes: WS, whole seeds; N2, (modified) nitrogen extraction; +Wet, substrate with 10wt% moisture; N2+wet, nitrogen extraction by substrate with 10wt% moisture; 4X1, 4 L CO$_2$/min and 1 mL EtOH/min; 2X1, 2 L CO$_2$/min and 1 mL EtOH/min.
6.9 Load of Palmitic Acid

6.9.1 Pure CO\textsubscript{2} Palmitic Acid yields

6.9.1.1 Pure CO\textsubscript{2} Palmitic Acid yields at Isotherm 35 °C

Palmitic acid (PA) has the lower molecular weight among the three main extracted substances. PA showed also high extraction velocities in correlation with the extraction pressure providing a higher PA density and contributing this way to a faster transport by solute diffusion supplying more solvent molecules surrounding the substrate particles of the packed bed.

Figure 6.37 Integration of the Palmitic acid yields extracted by pure CO\textsubscript{2} at 35 °C. Asterisk plots refer to additional experiments at higher pressure and lower temperature.

To yield from the origin, was the tendency for all the extraction pressure except the additional extraction at 25 °C and the planned extraction at middle pressure (150 bar). High pressure extractions yielded the highest yield values for the planned experimental conditions, but higher values were yielded during extractions at lower temperature (25 °C) while increasing the pressure up to 225 bar yielded only slightly above 125 bar.
Extraction kinetic was not found to be proportional to the extraction pressure especially at 150 bar, showing an extraction velocity lower than the rest. Besides this, it didn’t yield from the origin but from short before the 5 min of extraction (Figure 6.37).

Additional experiments at minor temperature than planned (25 °C) yielded with an extraction velocity similar to that of the best results at planned conditions although its kinetic model began to yield near to the minute 3 of extraction but maintained the yield rate until the 26 min. Although according to the kinetic model, the extraction at 225 bar yield Palmitic acid from the origin, it appeared slightly slower than the best performance curves and reached constant the 31 mg yielding Palmitic acid.

6.9.1.2 Pure CO₂ Palmitic Acid yields at Isotherm 45 °C

Extraction curves showed that the higher the pressure, the higher the yield of Palmitic acid, reaching above 35 mg (Figure 6.38).

![Pure CO₂ 45 °C](image_url)

Figure 6.38 Integration of the Palmitic acid yields, extracted by pure CO₂ at 45 °C.

Shorter times to reach the maximum Palmitic acid yield point can be seen in Figure 6.38 as the extraction pressure increased. It is possible to confirm, through the extraction velocities, best performances at higher pressures. The viscosity of the fluids is inversely proportional to the temperature conferring faster pore imbibitions, thinner boundary
layers at the substrate particles surface making diffusion mechanisms easier for the solvent. On the other hand, 45 °C extractions compared to 35 °C extractions have a higher molecular thermal energy reflected in the solvency due to the vibration, thus collision increase at molecular level.

6.9.1.3 Pure CO₂ Palmitic Acid yields at Isotherm 55 °C

High pressure helped to reach the maximum yield value faster, slightly above 52 mg\(_{\text{PA}}\) (Figure 6.39)

![Integration of the Palmitic acid yields extracted by pure CO₂ at 55 °C.](image)

According to Gupta and Shim [63] PA has the lower solubility \(y\) of 170 X \(10^6\) in CO₂ at 125 bar (\(y = \text{mole fraction, } \frac{\text{mol}_\text{solute}}{\text{mol}_\text{mixture}}\)) increasing to 535 X \(10^6\) and 1013 X \(10^6\) at 150 and 175 bar respectively. Thus the extraction velocity was directed by the different pressures through their solubility coefficients.

\[5 \quad \text{* Note: Precise values for the main fatty acids represented by Linoleic acid and Palmitic acid (in abundance order) are estimated in a range of 5% of inaccuracy after the specific calibration for the individual substances, since the abundances were calculated initially according to the } \beta\text{-sitosterol calibration by the gas chromatograph.}\]
Table 6.20 Synoptic table showing coordinates from $i$ intersection, to $sep$ separation of the Palmitic Acid extraction velocity model $v_{PA,xt}$ in mg/min, regarding the yield curve by pure CO$_2$ from the model at different pressures and temperatures.

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>P (bar)</th>
<th>$x_i$</th>
<th>$y_i$</th>
<th>x-sep</th>
<th>y-sep</th>
<th>$v_{PA,xt}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>175</td>
<td>2.8</td>
<td>0.0</td>
<td>26.0</td>
<td>62.9</td>
<td>2.7</td>
</tr>
<tr>
<td>35</td>
<td>225</td>
<td>0.2</td>
<td>0.0</td>
<td>18.0</td>
<td>31.0</td>
<td>1.7</td>
</tr>
<tr>
<td>35</td>
<td>175</td>
<td>0.4</td>
<td>0.0</td>
<td>12.0</td>
<td>34.2</td>
<td>2.9</td>
</tr>
<tr>
<td>35</td>
<td>150</td>
<td>4.5</td>
<td>-0.5</td>
<td>23.0</td>
<td>18.2</td>
<td>1.0</td>
</tr>
<tr>
<td>35</td>
<td>125</td>
<td>0.6</td>
<td>-0.5</td>
<td>36.0</td>
<td>30.1</td>
<td>0.9</td>
</tr>
<tr>
<td>45</td>
<td>175</td>
<td>5.2</td>
<td>-0.6</td>
<td>26.0</td>
<td>38.1</td>
<td>1.9</td>
</tr>
<tr>
<td>45</td>
<td>150</td>
<td>3.7</td>
<td>-0.5</td>
<td>38.0</td>
<td>35.6</td>
<td>1.1</td>
</tr>
<tr>
<td>45</td>
<td>125</td>
<td>8.3</td>
<td>-0.3</td>
<td>61.0</td>
<td>28.7</td>
<td>0.5</td>
</tr>
<tr>
<td>55</td>
<td>175</td>
<td>9.7</td>
<td>1.0</td>
<td>26.0</td>
<td>34.5</td>
<td>2.1</td>
</tr>
<tr>
<td>55</td>
<td>150</td>
<td>5.4</td>
<td>0.0</td>
<td>47.0</td>
<td>44.8</td>
<td>1.1</td>
</tr>
<tr>
<td>55</td>
<td>125</td>
<td>54.7</td>
<td>3.9</td>
<td>68.0</td>
<td>17.9</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Extraction velocity show tendencies towards high pressures at 35 and 45 °C (Table 6.20 and Figure 6.40), finding the highest velocity value at 25 °C and 175 bar.

Figure 6.40 Extraction velocities for palmitic acid by Pure CO$_2$. 

![Figure 6.40 Extraction velocities for palmitic acid by Pure CO$_2$.](image)
6.9.2 Modified CO₂ Palmitic Acid yields

6.9.2.1 Modified CO₂ Palmitic Acid yields at Isotherm 35 °C

Low Palmitic acid yields were found during the extractions at 35 °C with maximum yield values above 20 mg (Figure 6.41).

Figure 6.41 Integration of the Palmitic acid yields extracted by modified CO₂ at 35 °C. Asterisk plots refer to additional extractions WS without sieving and N with nitrogen instead of CO₂.

Low temperature elicited a negative correlation between the pressure and the Palmitic acid yield during the planned extractions set in agreement with the low solubility at 35 °C reported by Gupta and Shim [63]. Low pressure had a higher extraction velocity being the fastest (more inclined in the graphic) of the investigated pressure variations at 125 bar.

As well as for the Linoleic acid, the Palmitic acid didn’t get good results by using the whole seeds during the extraction. Regarding the use of nitrogen instead of CO₂ to see the effect of the modifier, although it showed an extraction velocity similar to the planned extractions the yield began quite late in the extraction, from the minute 19.
6.9.2.2 Modified CO₂ Palmitic Acid yields at Isotherm 45 °C

Low and high pressures showed the best performance regarding Palmitic acid yield, between round about 30 and 39 mg (Figure 6.42).

![Graph of Palmitic acid yields](image)

Figure 6.42 Integration of the Palmitic acid yields extracted by modified CO₂ at 45 °C.

Middle pressure, meanwhile, showed quite low yield values, getting hardly above 1.5 mg. Extraction velocities had a similar pattern than the yield values, getting higher Palmitic acid yields at low and high pressures while the middle pressure (150 bar) was quite low even though all of them showed yield from the origin.

Not really as an advantage but as a characteristic (because of the low value at 150 bar), the three planned pressures at 45 °C reached the maximum extraction yield values within the first hour of extraction, deriving from the extraction curve about the minute 12 of extraction.
6.9.2.3 Modified CO₂ Palmitic Acid yields at Isotherm 55 °C

Higher Palmitic acid values were reached at 55 °C with major difference between the results at every pressure (Figure 6.43).

Figure 6.43 Integration of the Palmitic acid yields extracted by modified CO₂ at 55 °C.

At this isotherm it was seen a higher Palmitic acid yield value by lower extraction pressures. These values were reached before 20 min of extraction. As well as the yield values, the extraction rates were higher for the lowest pressures reaching, as already mentioned, higher Palmitic acid yield values. According to the kinetic model, all the experiments yielded from the origin and derived from the extraction graph after 12 min of extraction.
Table 6.21 Synoptic table showing coordinates from \( i \) intersection, to \( sep \) separation of the palmitic acid extraction velocity model \( v_{PA,xt} \) in mg/min, regarding the yield curve by modified CO\(_2\) from the model at different pressures and temperatures.

<table>
<thead>
<tr>
<th>( T (°C) )</th>
<th>( P ) (bar)</th>
<th>( xi )</th>
<th>( yi )</th>
<th>( x-sep )</th>
<th>( y-sep )</th>
<th>( v_{PA,xt} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>175</td>
<td>0.1</td>
<td>0.0</td>
<td>12.0</td>
<td>11.7</td>
<td>0.99</td>
</tr>
<tr>
<td>35</td>
<td>150</td>
<td>0.0</td>
<td>0.0</td>
<td>12.0</td>
<td>20.8</td>
<td>1.74</td>
</tr>
<tr>
<td>35</td>
<td>125</td>
<td>0.1</td>
<td>0.0</td>
<td>12.0</td>
<td>22.5</td>
<td>1.89</td>
</tr>
<tr>
<td>45</td>
<td>175</td>
<td>0.1</td>
<td>0.0</td>
<td>12.0</td>
<td>28.3</td>
<td>2.36</td>
</tr>
<tr>
<td>45</td>
<td>125</td>
<td>-0.1</td>
<td>-0.8</td>
<td>12.0</td>
<td>36.2</td>
<td>3.04</td>
</tr>
<tr>
<td>55</td>
<td>175</td>
<td>0.0</td>
<td>0.0</td>
<td>12.0</td>
<td>12.7</td>
<td>1.06</td>
</tr>
<tr>
<td>55</td>
<td>150</td>
<td>0.3</td>
<td>0.0</td>
<td>12.0</td>
<td>28.2</td>
<td>2.42</td>
</tr>
<tr>
<td>55</td>
<td>125</td>
<td>0.0</td>
<td>0.0</td>
<td>12.0</td>
<td>59.9</td>
<td>4.99</td>
</tr>
</tbody>
</table>

Under modified CO\(_2\), palmitic acid yields show a general tendency towards low pressures and high temperatures (Table 6.21 and Figure 6.44)

Figure 6.44 Extraction velocities for palmitic acid by modified CO\(_2\).
6.9.3 *Opuntia* sp. Palmitic acid results overview

Since values as CO₂ consumption and oil load were already mentioned, in this summary only the concentration per substrate mass and oil mass is going to be mentioned.

![Load of Palmitic Acid in Substrate](image)

Figure 6.45 Summary of Palmitic acid yields per mass of substrate by pure CO₂.

The summary Figure 6.45 and Table 6.22 suggest as the integration curves a higher Palmitic acid yield (regarding the substrate mass extracted) at low temperatures than at high pressures (additional experiment at 25 °C vs 225 bar), showing as well different trends depending on the isobar and/or the isotherm.

<table>
<thead>
<tr>
<th>CO₂ (°C)</th>
<th>Pressure (bar)</th>
<th>Load of Palmitic Acid in mgPA/gsubst</th>
</tr>
</thead>
<tbody>
<tr>
<td>55</td>
<td>125</td>
<td>3.1</td>
</tr>
<tr>
<td>45</td>
<td>150</td>
<td>6.4</td>
</tr>
<tr>
<td>35</td>
<td>175</td>
<td>5.5</td>
</tr>
<tr>
<td>25</td>
<td>225</td>
<td>6.6</td>
</tr>
<tr>
<td>35</td>
<td>225</td>
<td>6.4</td>
</tr>
<tr>
<td>25</td>
<td>4.7</td>
<td>8.1</td>
</tr>
</tbody>
</table>

Table 6.22 Yield of Palmitic acid by pure CO₂ (values in mgPA/gsubst).
To finish the Palmitic acid results, the yield from the extractions by modified CO$_2$ are shown next (Figure 6.46).

**Figure 6.46 Summary of Palmitic acid yields per mass of substrate by modified CO$_2$.**

Palmitic acid yield as a concentration in the substrate by modified CO$_2$ showed a marked tendency for high temperatures and low pressures (Table 6.23).

**Table 6.23 Yield of Palmitic acid by pure CO$_2$ (values in mg$_{PA}/g_{substrate}$).**

<table>
<thead>
<tr>
<th>CO$_2$-EtOH</th>
<th>Pressure (bar)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T (°C)</td>
<td>125</td>
</tr>
<tr>
<td>55</td>
<td>7.99</td>
</tr>
<tr>
<td>45</td>
<td>4.97</td>
</tr>
<tr>
<td>35</td>
<td>3.09</td>
</tr>
<tr>
<td>25</td>
<td>—</td>
</tr>
</tbody>
</table>
As well as for LA, for PA there were considerable differences at the highest yield conditions for β-sitosterol extraction (Figure 6.47).

Figure 6.47 Variation of PA per mass of substrate at 35°C-175 bar by modified CO2. Codes: WS, whole seeds; N2, (modified) nitrogen extraction; +Wet, substrate with 10wt% moisture; N2+wet, nitrogen extraction by substrate with 10wt% moisture; 4X1, 4 L CO2/min and 1 mL EtOH/min; 2X1, 2 L CO2/min and 1 mL EtOH/min.

6.10 Suitability for a fractionated extraction

Next will be explained an example of the suitability for a fractionated extraction which in a first instance, it is usually recommended to be done by time, based in the extraction time differences shown in the Figure 6.48.
Figure 6.48 Extraction curve for the three main substances, according to the time.

From the extraction curve, the higher BS yield values are selected falling in the range between 12 and 47 min. After integration of such range of values the results are as shown in Table 6.24. By means of time fractionation, at 35 °C and 175 bar, from the minute 12 to 47 it can be achieved a reduction of 42 wt% of BS, 88 wt% PA and 86 wt% LA, increasing BS concentration from 0.6 wt% to 2.6 wt%.

Table 6.24 Integration of the main components in original value vs. fractionation.

<table>
<thead>
<tr>
<th>Integrated mass</th>
<th>BS</th>
<th>PA</th>
<th>LA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original 131 min</td>
<td>2.0 mg</td>
<td>50.0 mg</td>
<td>268.1 mg</td>
</tr>
<tr>
<td>Fraction 12-47 min</td>
<td>1.1 mg</td>
<td>6.2 mg</td>
<td>38.3 mg</td>
</tr>
<tr>
<td>Removed</td>
<td>42 %</td>
<td>88 %</td>
<td>86 %</td>
</tr>
</tbody>
</table>

Another fractionation method is crystallization at supercritical conditions, however, it would not be advisable as a purification process for BS according to the results of Michael and Zacchi [103], since it only increases 7 % the BS content from the oil to the crystal form.

A theoretical example of fractionation by solubility will depart from the solubility data published by Gupta and Shim [63] for stigmasterol (SS) since its chemical structure (C_{29}H_{48}O) is very similar to that of β-sitosterol (BS: C_{29}H_{46}O).
Since the solubility data of the main compounds in modified CO₂ at low pressure are quite limited, the theoretical considerations are done based on a projected line from the last data at low pressure to the estimated ‘near to zero’ solubility value at the critical pressure point for pure CO₂ (73.8 bar) (Figure 6.49). On the other hand, exemplifying at 40 °C offers the advantage of a relative data abundance hardly to find at any other temperature. Considering interpolated extraction values between those obtained at 35 and 45 °C, the pressure is dropped down from the original extraction conditions of 40 °C and 175 bar, as the highest yield parameters for β-sitosterol to a cyclone at 82 bar and constant temperature.

**Figure 6.49** Fractionation of BS and fatty acids based on solubility data from Gupta and Shim [63] where the experimental values (‘E’) are represented by the filled symbols.

In the solvency plots shown by Gupta and Shim [63] between 35 to 60 °C there is a crossover region but mostly between PA and LA while at 40 °C solubility plots show no crossover region. At 82 bar BS will hardly reach the estimated equilibrium point while LA and PA will have walked down the equilibrium line reducing in 98 % and 99 % of the original PA and LA concentrations respectively (Table 6.25 and Figure 6.49).

<table>
<thead>
<tr>
<th>Table 6.25 Solubility in CO₂, original vs. pressure fractionation.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Original (mol/molCO₂)</td>
</tr>
<tr>
<td>Fractionated (mol/molCO₂)</td>
</tr>
<tr>
<td>Removed</td>
</tr>
</tbody>
</table>
In the flow diagram modified for the fractionation it is suggested to reduce the pressure slightly under $P_c$, collecting the BS in a second cyclone and saving energy by avoiding high recompression steps.

Figure 6.50 Flow diagram of a theoretical fractioned extraction from a cactus seed sample by pure supercritical CO$_2$ 40 °C-175 bar 2 L/min.
7 CONCLUSIONS

Green Solvent

Supercritical CO₂ as a green solvent offers a clean and easy-to-handle option to get valuable natural products from vegetable matrixes. The experiments performed by n-heptane required more safety procedures attention during the extraction and during the rotation at the end to recover the solutes and the solvent confinement. The cheapness and the easy safety procedures to handle CO₂ confirmed by several authors make the CO₂ a friendly to use solvent. The risk of solvent contamination in the samples is negligible by SC-CO₂ due its no toxicity and/or flammability, important especially in the food industry.

Oil yield

The predominant fatty acids are linoleic acid and palmitic acid. Fractions minor than 500 μm according to sieve tests (Appendix 1) gave an average of 30.5 % of the total sample. The oil yield 287.0 mg oil/g subst (Table 6.15) from this fraction represents 87.46 mg oil/g subst of the whole seed sample: slightly beneath the oil concentration reported in publications: 91.4 mg oil/g subst. However the oil extracted by SCFE in the stationary device contains about 13 times the β-sitosterol content than the values published (6.4 vs. 0.5 mg BS/g subst) in agreement with publications mentioning the higher oil yields but with less selectivity by the TSE beside the presence of relevant compounds not observed in conventional extraction methods. Although the yields by pure CO₂ were not low compared to nitrogen extraction or the published values, due to the polarity of the extracted compounds, higher amounts of all of the main solutes were gathered while using a co-solvent to add polarity to the CO₂.
Reducing wastes: holistic use of the natural resources

The avoidance of large amounts of organic solvents required by TSE is also part of the waste reduction achieved by these kinds of supercritical extractions.

The use of a sub-product like the prickly pear seeds shows to be an affordable solution for the holistic harvesting of the nopal resource. Investments in infrastructure to recover the material, and scale up of the laboratory procedures here described, are worthy to be continued since the O. ficus-indica cacti crops are already established and expanding to the international market. Part of the extracted substrate was donated to the agglomeration laboratory to realize further pelletizing and calorimetry tests of this resource fraction.

Stability and homogeneity of the results

Out of the differences in physiological structures due to the oil reserves within the seeds depending on the fertility or infertility, besides the crude and ground storage time the results between the different treatments showed defined trends and peaks under certain conditions more accentuated by the use of modifier in the solvent where the solvent density determined the solubility for every extracted substance.

Extraction Processes

It is really possible to reduce or eliminate the use of hazardous substances; these experiments confirm the convenience in $T_c$ and $P_c$ among other SC-CO$_2$ properties avoiding the thermal degradation of the relevant solutes. Regarding the hypothesis, it exists a difference between conventional methods and the advantages of using SCF’s, since although the amount of $\beta$-sitosterol per mass of seed is similar to the concentration by ultrasound, the concentration per mass of oil obtained by the static device is roughly about 1.7 times the one obtained by means of ultrasonic extraction. Concerning the load, only the stationary device was able to reach 46 % of the value reached by the control test: 0.903 Control vs. 0.420 SCF Stationary (mg$_{BS}$/g$_{solvent}$).
The solvent-solute ratio shall be considered due to the volume of the stationary device, the opposite happens to the continuous flow device, which working at a higher pressure (above 125 bar while shaken at 100 bar) offers a more dense solvent increasing the efficiency of the extraction through the diffusion of the oil components in unsaturated media. The selectivity was surely affected by the use of modifiers, as mentioned by several authors agreeing with the function of the modifier in the selectivity of the solutes.

Regarding the aims

According to the continuous flow process, the parameter set with highest $\beta$-sitosterol values has been the high pressure (175 bar) combined with a low temperature (35 °C) under modified CO₂ conditions and a flow of 4 L/min 1 mL EtOH/min, yielding 0.7 mgBS/g subst.

Linoleic acid yield per mass of substrate was higher at low temperatures and high pressures confirmed by additional extractions at 25 °C during experiments with pure CO₂, reaching 45 mgLA/g subst. While the yield by modified CO₂ was found higher at the opposite conditions: low pressure–high temperatures, reaching 44 mgLA/g subst.

Palmitic acid, similar to Linoleic acid showed the highest value at 25 °C and 175 bar, nevertheless it was under pure CO₂ conditions, where, besides this, the yield values were also more even between them. While working with modified CO₂, yields showed a trend towards the low pressure and high temperatures.

Since nutrition scientists look for unsaturated fatty acids to avoid atherosclerosis problems, the advantage of high $\beta$-sitosterol and Linoleic acid yields at low temperatures and high pressures additional to a low concentration of palmitic acid while working under modified CO₂, represents according to our results the best set of variables or conditions for an oil enriched in $\beta$-sitosterol and low fatty acids, i.e. linoleic acid.
Extraction kinetics

Meanwhile with pure CO\textsubscript{2} the maximum extraction velocity was about 0.09 mg\textsubscript{BS}/min, by the modified CO\textsubscript{2} experiments the extraction velocity reached rates of 0.16 mg\textsubscript{BS}/min. Moreover these extractions rates were found at the previously mentioned conditions: low temperature and high pressure.

The development of these extraction processes should offer an even more attractive horizon to that mentioned in references about the energy (time and solvent) costs for substances recovering due to the high extraction rates achieved by SCF’s, reaching maximum yields within 30 min. Improvements in the extract quality are theoretically possible by a time fractionation removing 42 wt% of BS 88 wt% PA and 86 wt% LA, increasing BS concentration from 0.6 wt% to 2.6 wt%. Also a solubility fractionation is possible adjusting the density of the solvent by dropping the pressure at constant temperature of 40 °C until the BS concentration matches with the equilibrium plot, that means about 82 bar, removing 98 % and 99 % of the original PA and LA concentrations respectively.
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## APPENDIX

Appendix 1: Sieving form from Agglomeration laboratory in the TU Bergakademie Freiberg.

<table>
<thead>
<tr>
<th>Kornklasse (um)</th>
<th>Massenanteil (g)</th>
<th>Massenanteil (%)</th>
<th>Häufigkeitsdichte q² (%/um)</th>
<th>Kumulativ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 500</td>
<td>20.50</td>
<td>28.9</td>
<td>0.058</td>
<td>28.9</td>
</tr>
<tr>
<td>500 - 630</td>
<td>3.91</td>
<td>5.5</td>
<td>0.042</td>
<td>34.5</td>
</tr>
<tr>
<td>630 - 800</td>
<td>6.33</td>
<td>8.9</td>
<td>0.053</td>
<td>43.4</td>
</tr>
<tr>
<td>800 - 1000</td>
<td>8.63</td>
<td>12.2</td>
<td>0.061</td>
<td>55.6</td>
</tr>
<tr>
<td>1000 - 1250</td>
<td>9.39</td>
<td>13.3</td>
<td>0.053</td>
<td>68.8</td>
</tr>
<tr>
<td>1250 - 1600</td>
<td>13.62</td>
<td>19.2</td>
<td>0.055</td>
<td>88.1</td>
</tr>
<tr>
<td>1600 - 2000</td>
<td>7.32</td>
<td>10.3</td>
<td>0.026</td>
<td>98.4</td>
</tr>
<tr>
<td>2000 - 2500</td>
<td>1.14</td>
<td>1.6</td>
<td>0.003</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Rueckwaage (g): 70.84  
Siebverlust (g): -0.03  
Unterschrift:

SP1000, Copyright Retzsch GmbH, 1993 All rights reserved
GERARDO BERMEJO ACOSTA

Doctorand, TU Bergakademie Freiberg
Leipziger Str.28, Karl Kaegel Bau, ZI, 73
19596 Freiberg/Sachsen, Alemania.

En atención a su solicitud vía correo electrónico recibida el día 10 de Julio, para el envío de 8 Kg de semilla de nopal (Opuntia ficus-indica) variedad cristina con el fin de ser utilizado en sus trabajos de investigación de Doctorado en el proyecto “Evaluación de características químicas y medios de extracción de aceite de semilla de Opuntia ficus-indica”.

Una vez analizado el proyecto de investigación, se consideran satisfechas las condiciones establecidas en el Capítulo II, Art. 26 del Acuerdo de Transferencia de Materiales (ATM) del Sistema Nacional de Recursos Fitogenéticos para la Alimentación y la Agricultura, por lo que se considera procedente continuar con los trámites necesarios para el envío de muestra conforme lo establecido en el ATM.

En virtud de lo anterior, se deberá gestionar el Contrato de Transferencia de Materiales con el Dr. Antonio Reyes Aguirre del Instituto de Investigaciones Desérticas de la Universidad Autónoma de San Luis Potosí, en el cual deberán contemplar lo establecido en el Título Segundo Capítulo I y III, haciendo énfasis en el Art. 17 incisos: g) La obligación de poner en conocimiento de los donantes y del SNICS los resultados de las investigaciones realizadas; h) Disposiciones respecto de la distribución de beneficios derivados del estudio o aprovechamiento del RFAA; e) En su caso, los términos para la transferencia a terceros del material accedido.

Lo anterior en el entendido que el material proporcionado será empleado exclusivamente para el objeto del proyecto planteado y que no podrá reclamar ningún derecho de propiedad intelectual o de otra índole que limite el acceso al material o a la información conexa.

Esperando haber atendido su petición y en espera de recibir copia del contrato firmado por las partes, y en su oportunidad los resultados del proyecto aprovecho la ocasión para enviarle un cordial saludo.

Atentamente
La Directora del SNICS

Ing. Enriqueta Molina Macías

12 JUL 2006

Appendix 2 Permission for extraction material exchange from the Mexican Government.
10 ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>BS</td>
<td>Beta sitosterol</td>
</tr>
<tr>
<td>EPA</td>
<td>Environmental Protection Agency</td>
</tr>
<tr>
<td>FA</td>
<td>Fatty Acids</td>
</tr>
<tr>
<td>kgf</td>
<td>Kilogram-force (1 kgf = 9,80655 kg·m/s² or Newtons)</td>
</tr>
<tr>
<td>LA</td>
<td>Linoleic acid</td>
</tr>
<tr>
<td>PA</td>
<td>Palmitic acid</td>
</tr>
<tr>
<td>P&lt;sub&gt;c&lt;/sub&gt;</td>
<td>Critical pressure</td>
</tr>
<tr>
<td>RTECS</td>
<td>Registry of toxic effects of chemical substances</td>
</tr>
<tr>
<td>SC</td>
<td>Supercritical</td>
</tr>
<tr>
<td>SCF</td>
<td>Supercritical Fluids</td>
</tr>
<tr>
<td>SCFE</td>
<td>Supercritical Fluids Extraction</td>
</tr>
<tr>
<td>SS</td>
<td>Stigmasterol</td>
</tr>
<tr>
<td>T&lt;sub&gt;c&lt;/sub&gt;</td>
<td>Critical temperature</td>
</tr>
</tbody>
</table>