UNIVERSITY OF PADUA
DEPARTMENT OF INDUSTRIAL ENGINEERING
MASTER OF SCIENCE IN CHEMICAL AND PROCESS ENGINEERING

Master Thesis in Chemical and Process Engineering

PRODUCTION OF NUTRACEUTICALS RICH IN POLYPHENOLS AND ANTHOCYANINS BY SUPERCRITICAL ANTISOLVENT PRECIPITATION OF CHERRY EXTRACT

Supervisor: Prof. Alberto Bertucco
Assistent: Prof. Antonio Scipioni
Ing. Miriam Solana Siprés
Ing. Anna Mazzi
Ing. Sara Toniolo

Student: LUCREZIA BACCHIN

ACADEMIC YEAR 2015 – 2016

Lucrezia Bacchin

PRODUCTION OF NUTRACEUTICALS RICH IN POLYPHENOLS AND ANTHOCYANINS BY SUPERCRITICAL ANTISOLVENT PRECIPITATION OF CHERRY EXTRACT

University of Padua

July 2016

UNIVERSITY OF PADUA
DEPARTMENT OF INDUSTRIAL ENGINEERING
MASTER OF SCIENCE IN CHEMICAL AND PROCESS ENGINEERING

Master Thesis in Chemical and Process Engineering

PRODUCTION OF NUTRACEUTICALS RICH IN POLYPHENOLS AND ANTHOCYANINS BY SUPERCRITICAL ANTISOLVENT PRECIPITATION OF CHERRY EXTRACT

Supervisor: Prof. Alberto Bertucco
Assistent: Prof. Antonio Scipioni
Ing. Miriam Solana Ciprés
Ing. Anna Mazzi
Ing. Sara Toniolo

Student: LUCREZIA BACCHIN

ACADEMIC YEAR 2015 – 2016

To my parents

Riassunto

Negli ultimi anni il mercato degli ingredienti funzionali sta aumentando, come conseguenza dell'interesse dei consumatori nei prodotti naturali per la prevenzione di malattie. Numerosi studi supportano i benefici relazionati a una dieta ricca in frutta e verdura per la prevenzione del cancro e dei disturbi cardiovascolari. La ciliegia è stata riconosciuta essere una buona fonte di composti antiossidanti i quali mostrano effetti antibatterici, antiinfiammatori, analgesici, antitumorali e antivirali. La quantità di antiossidanti recuperati dalla ciliegia dipende principalmente dalla tecnica di estrazione e dal tipo di solvente. L'estrazione solido-liquido è la più usata poiché è la più semplice, economica e non richiede strumenti complicati. Il principale svantaggio dell'estrazione classica è dovuto alla degradazione termica dei composti, ma è anche richiesta una fase di rimozione del solvente, le cui tracce devono essere completamente rimosse per l'uso alimentare/farmaceutico.

Il processo di estrazione condotto mediante fluidi in condizioni supercritiche si pone come una buona alternativa ai sistemi classici di estrazione con solvente, garantendo numerosi vantaggi che riguardano l'assenza di solventi residui nel prodotto, la bassa temperatura di lavoro (evita la degradazione termica) e l'assenza di luce e ossigeno (evita la degradazione chimica). Un fluido è considerato supercritico quando la sua temperatura e la sua pressione sono superiori a quelle critiche. In questo stato non c'è distinzione tra fase liquida e gas e alcune delle sue proprietà sono simili a quelle dei liquidi, come la densità, mentre altre sono più somiglianti a quelle dei gas, come la viscosità e la diffusione molecolare. A temperatura costante, ad una variazione di pressione corrisponde una variazione di densità, che comporta cambiamenti di alcune proprietà come la solubilità e la costante dielettrica.

D'altra parte la CO_2 ha una capacità molto limitata di dissolvere i composti polari e ad alto peso molecolare presenti nelle ciliegie. La SFE ha quindi bisogno di un co-solvente per condurre l'estrazione che deve essere poi separato dal prodotto finale.

La *Supercritical anti-solvent precipitation* (SAS) è stata proposta per superare questa limitazione e per ottenere gli antiossidanti sotto forma di particelle di piccolissime dimensioni (dell'ordine del nanometro) senza la presenza di solvente. L'applicazione di questa tecnica per il recupero di antiossidanti dalla frutta è ancora sotto esame, e finora è stata provata con il lycium (Kuo-Li Lin *et al.*, 2014) e con il mango (Meneses M. *et al.*, 2015). Questa è la prima volta che la SAS è applicata alla ciliegia.

Lo scopo di questo lavoro è di ottimizzare le variabili operative di un processo SAS su estratto di ciliegie per ottenere la più alta resa in antiossidanti. In primo luogo sono state condotte alcune prove di estrazione dei composti fenolici dalle ciliegie con diversi solventi per valutare il migliore, presso il Dipartimento di Farmacia dell'Università degli studi di

Padova, dove le ciliegie denocciolate, macinate e liofilizzate sono messe in un matraccio con metanolo (91% w/w) e poste in un bagno ad ultrasuoni per 1h a 25°C. La soluzione estratta è stata centrifugata per separare i residui non dissolti prima di poter essere utilizzata. Le prove di precipitazione mediante il processo SAS sono state, condotte nel Laboratorio di alta pressione del Prof. Bertucco, all'interno del Dipartimento di Ingegneria Industriale, dove è stato messo a punto un impianto specifico allo scopo. La soluzione estratta e la SC-CO2 sono pompate nel precipitatore dell'impianto pilota ad una temperatura di 40°C per evitare degradazione termica del prodotto, la portata di soluzione è stata fissata a 0,2 mL/min mentre i parametri indagati sono essenzialmente la pressione (100-150 bar) e la portata di CO_2 (2-9 L/min). Il tempo di precipitazione è di circa 30 minuti mentre quello di lavaggio con sola CO_2 è di 20 minuti. Inoltre sono state condotte alcune prove in zona subcritica (35°C e 75bar) e in semi batch.

La SC-CO_2 si scioglie nel e modifica così le sue proprietà, facendo così precipitare gli antiossidanti, non più solubili nel solvente misto. Il precipitato cosi ottenuto è privo di solvente e non necessita quindi della fase di separazione. Il precipitato per essere analizzato è stato disciolto in metanolo, e l'analisi è stata condotta presso il Dipartimento di Farmacia dell'Università degli studi di Padova con un HPLC-MS. La resa di precipitazione è stata calcolata come rapporto tra la concentrazione finale rispetto a quella iniziale. Dalle prove eseguite si è visto come un aumento di pressione sfavorisca la resa di precipitazione mentre un aumento della portata di CO_2 la avvantaggi. Le prove in zona subcritica hanno mostrato bassa resa in antiossidanti nel prodotto finale mentre quelle in semi batch difficoltà nella rimozione del solvente e nella dimensione delle particelle. La migliore resa di precipitazione si è ottenuta a pressione e portata di CO_2 di 100 bar e 9 L/min rispettivamente. Da queste condizioni si è scalato l'impianto per una produzione di 1 kg di precipitato.

Mediante il software ASPEN Plus™ 7.3 si è simulata la separazione del metanolo dalla CO_2 a fine lavorazione.

La seconda parte del lavoro si basa su una valutazione ambientale del processo mediante l'utilizzo del software SimaPro™ 7.3.2. L'analisi di valutazione del ciclo di vita è un metodo che considera un insieme di interazioni che un prodotto o un servizio ha con l'ambiente nell'arco dell'intero ciclo di vita, che include le fasi di preproduzione (quindi anche estrazione e produzione dei materiali), produzione, distribuzione, uso (quindi anche riuso e manutenzione), riciclaggio e dismissione finale. La procedura LCA è standardizzata a livello internazionale dalle norme ISO 14040 e 14044. In accordo con tali norme il *Life Cycle Assessment* è suddiviso in quattro fasi di valutazione:

- Mete e obiettivi;
- Inventario del ciclo di vita;
- Valutazione dell'impatto del ciclo di vita;
- L'interpretazione dei risultati;

Nel caso in esame si è scelto un approccio *cradle to gate* (dalla culla al cancello) trascurando le fasi di distribuzione, uso e dismissione del prodotto in quanto non è nota la sua destinazione finale. L'intero processo è basato sulla produzione di un kg di precipitato. I dati di consumo energetico sono stati ricavati da schede tecniche dei vari macchinari scelti su scala industriale, e mediante il simulatore di processo ASPEN Plus™ 7.3. All'interno dei confini del sistema sono stati valutati la coltivazione delle ciliegie, la produzione di metanolo, la CO_2, l'elettricità a medio voltaggio e il vapore da gas naturale. Inoltre sono stati considerati i trasporti, il trattamento degli scarti e il riciclo delle materie prime (CO_2 e metanolo). Il metodo scelto per la valutazione degli impatti è il ReCiPe con approccio *midpoint.* Attraverso un'analisi d'incertezza sono state scelte le quattro categorie da studiare (*Climate change, Ozone depletion, particulate matter formation e Fossil depletion*). Inoltre è stata fatta un'analisi di sensibilità per vedere quale fase del processo sia maggiormente impattante nelle varie categorie d'impatto. Dai risultati ottenuti si è dimostrato che l'impatto più importante è dato dal consumo di elettricità del processo soprattutto nella fase di riciclo della CO_2.

Abstract

The market of functional ingredients/foods is increasing, as a consequence of the interest of customers in preventing illness and improving health using natural sources (Khan *et al*., 2013). Cherry (*Prunus avium*) fruit has been recognized as good source of bioactive compounds that show antiviral, antibacterial, analgesic, anti-inflammatory and immune modulatory activities (Kim *et al*., 2005). The objective of this study is the Production of antioxidants from cherries using the supercritical anti-solvent precipitation process (SAS). The antioxidant extract was obtained by solid–liquid extraction using methanol. The antioxidants extracted in the resulting solution were precipitated using the SAS technique, in which supercritical CO2 is used to precipitate selected, non soluble compounds. The pressure and CO2 flow rate were varied in the range 100-150 bar and 2-9 L/min. The content of the antioxidants in the different extracts was determined using HPLC-MS. The SAS process allowed the recovery of about 74% of the initial phenolic compounds, the best recovery being obtained at 40 °C, 100 bar and 9 L/min. The product was a dry powder. The analyses performed indicated that SAS processing of natural extracts is effective technique to recover a solvent-free precipitate rich in phenolic compounds without use of high temperature. A conceptual design of an industrial scale application of the process has been done to perform a Life Cycle Assessment analysis, using the software SimaPro™ 7.3.2. Some data about energy consumption and separation of methanol/carbon dioxide were obtained using process simulator ASPEN Plus™.

Acknowledgments

I’m very grateful to Professor Alberto Bertucco (Department of Industrial Engineering, University of Padua) for his advice and expert guidance. His constant good mood has always made me feel looked after and encouraged me to carry on my studies. I would like to express my sincere gratitude to my assistant supervisor, Ms Miriam Solana Ciprés by whom I have not only been taught how to work in a lab but I have been given unwearyingly support as well. A Thank to Professor Antonio Scipioni (Department of Civil, Environmental AND Architectural Engineering, University of Padua) for agreeing the collaboration with CESQA group (Centro Studi Qualità e Ambiente) and to his assistants Anna Mazzi and Sara Toniolo for having advised and assisted in the Life Cycle Assessment study. A thank to Stefano Dall’acqua (Department of Pharmaceutical and Pharmacological Science), for the collaboration during the phases of extraction and empirical analysis of results.

Finally but not least, I would like to thank my parents Dario e Onelia for their great help and support throughout the course of study underling that without their sustenance I could never finish my studies. A special thank to my partner Marco for the support in managing the home and the family and for encouraging me during the studies and to my two children Aurora and Christian that have given me the strength to go on with their unconditional love.

Padua, July 2016

Contents

List of figures

List of tables

Introduction

Nowadays, research in chemical engineering is mostly focused on new techniques that combine efficiency and safety, in regard to human health and environmental protection. Key principles in the development of alternative techniques are eco-sustainability, efficiency, minimization of environmental impact, low energy, water consumption and of course economic feasibility.

This work is about the supercritical anti solvent precipitation of phenolic compounds from cherries, a new technique developed in recent years whose purpose is to recover the solvent free product rich in nutraceutical compounds in order to avoid the thermal degradation of labile compounds.

A supercritical fluid is a substance over its critical temperature and pressure. In this state liquid and gas are not distinguishable and the fluid has some properties similar to those of the liquid, like density, and other properties similar to those of the gas, like viscosity. The solvent power of this fluid depends of density, and since density increases with pressure, solubility is enhanced when pressure is higher. Therefore, operating pressure and temperature can be varied to optimize thermo-physical properties like diffusivity, viscosity and especially solubility. Carbon dioxide is particular convenient as a supercritical fluid since its critical conditions are at quite lower pressure (73,8 bar) and temperature (31,3 °C), so that thermo sensible compound are not degradable. Furthemore it is cheap and easy available, and non-toxic non-flammable, being General Recognised As Safe Gas (GRAS).

With respect to extraction process, SC-CO_2 has a very limited capacity to dissolve polar and high molecular weight compounds, while it dissolves very well in solvent such as methanol. For these reason it can be used as anti-solvent to precipitate solid from a liquid solution. This technique has been recently developed to recover dry antioxidant from fruit since the market of functional ingredients/foods is increasing, as a consequence of the interest of customers in preventing illness and improving health using natural sources (Khan et al., 2013). Cherries are in this respect of particular interest since they have been recognized as good source of bioactive compounds that show antiviral, antibacterial, analgesic, anti-flammatory and immune modulatory activities which are used in food, pharmaceutical and cosmetic industries.

In this work the phenolic compounds contained in cherries have been extracted by methanol using an ultrasonic bath to improve the extraction yield. Then the solution extracted was pumped in a precipitator pressurized with SC-CO_2, that dissolves into methanol and caues the precipitation of phenolic compound in a filter. Finally, the mixture SC-CO_2 and methanol is separated from the powders by expansion. The aim of the work was to investigate operating conditions of SC-CO_2 precipitation in order to find the optimum values of pressure, temperature, solvents, carbon dioxide and solution flow rates. Furthermore a Life Cycle Assessment analysis has been considered in order to assess the main impacts due to the different phases of the process.

The thesis has been divided into five chapters.

Chapter 1 provides a description of supercritical fluids in general and the SAS method in particular. It also describes some characteristics about phenolic compounds contained in cherries.

Chapter 2 is about the materials and methods that were used to perform the SAS tests and the analysis of the powders.

In chapter 3 the results obtained in the precipitation of phenolic compounds from cherry solution are reported and discussed.

Chapter 4 describes the Life Cycle Assessment (LCA) methodology and the life cycle inventory (LCI) applied to the production of a precipitate rich in polyphenols and anthocyanins.

Chapter 5 explains the Life Cycle impact assessment and the interpretation of the results using an uncertainty and sensitivity analyses.

Chapter 1

Supercritical fluid and SAS method

Supercritical anti-solvent (SAS) particle precipitation is the method used in this thesis. The first part of this chapter describes supercritical fluids with their properties and applications, in particular carbon dioxide (SC-CO_2), which is the most used supercritical fluid. In our case, SC-CO_2 was used to precipitate chemical compounds such as polyphenols from cherries. The second and third part of this chapter present a literature review of the cherry's composition and of the method used.

1.1 Supercritical fluids and applications

A supercritical fluid (SFC) is any substance at a temperature and pressure above its critical point, where distinct liquid and gas phases do not exist. The versatility of SCFs in green technology has led to innovative approaches for the design of micro- and nanoparticles (Jung and Perrut, 2001). More specifically, supercritical fluid extraction (SFE) and supercritical anti-solvent (SAS) processing of natural matter are very effective techniques developed about 30 years ago.

1.1.1 Characteristics and definition of supercritical fluids

A pure component is considered to be at the supercritical state when its temperature and its pressure are higher than the critical values. The standard single-component phase diagram is shown in Figure1.1 [web site[1]] representing the regions of temperature and pressure where the substance is present as solid, liquid and gas.

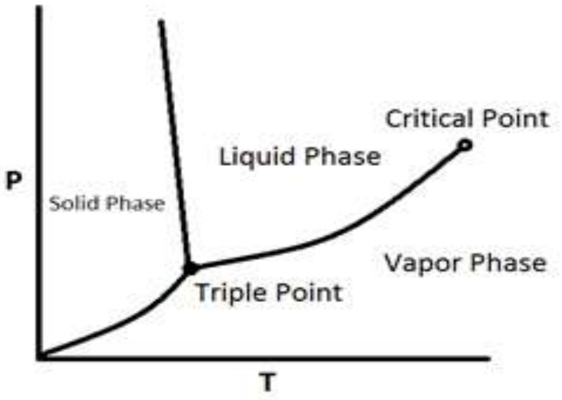


Figure 1.1 *Schematic p-T phase diagram of a pure.*

Equilibrium phase transition lines separate these regions. The three phase boundaries meet at the triple point, where the three phases coexist. The diagram shows that the supercritical fluid region is apart from the three standard aggregate states. Following the gas-liquid coexistence curve, both temperature and pressure increase. The liquid becomes less dense because of thermal expansion, and the gas becomes denser as pressure rises.

At critical conditions of pressure and temperature, P_c and T_c respectively, the densities of the two phases become identical and there is no distinction between the gas and the liquid. Above the conditions the substance can only be described as a supercritical fluid. The aspect of supercritical phase can be illustrated in Figure 1.2 [web site[2]], where carbon dioxide phases are represented. It can be seen the differences between the image of liquid-vapor equilibrium where the two phases are distinguishable, and the picture of supercritical state, where there is a unique phase.

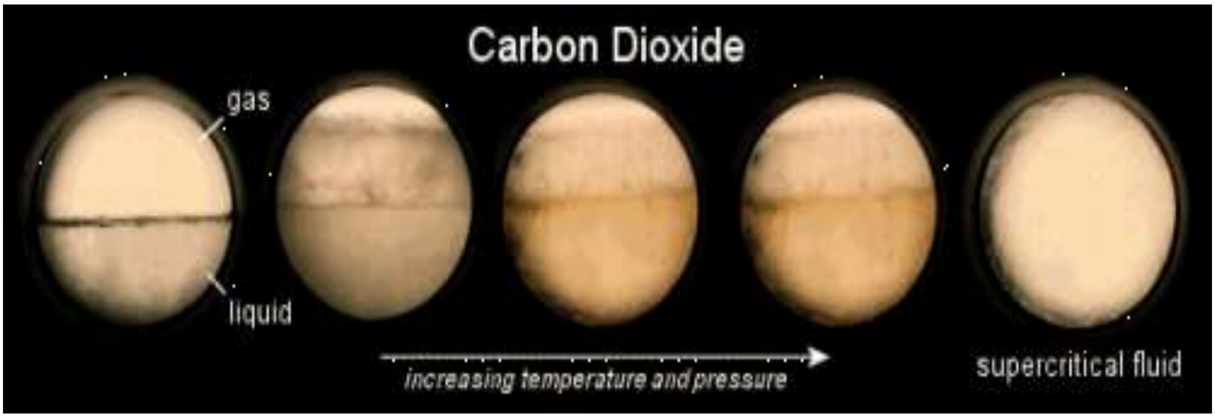


Figure 1.2 *Images of liquid-vapor equilibrium and supercritical state of carbon dioxide.*

A feature of supercritical fluids is their pressure-dependent density. If temperature is constant, density can be adjusted by pressure from that of a vapor to that of a liquid with no discontinuity. This can be observed in the density-pressure phase diagram for carbon dioxide, as shown in Figure 1.3 [web site[3]]. At well below the critical temperature, e.g., 280K, as the pressure increases, the gas compresses and eventually (just over 40 bar) it condenses into a much denser liquid, resulting in the discontinuity in the line (vertical dotted line). The system consists of 2 phases in equilibrium, a dense liquid and a low-density gas.

As the critical temperature is approached (300K), the density of the gas at equilibrium becomes higher, and that of the liquid lower. At the critical point, (304.1 K and 7.38 MPa), the difference in density disappears and the 2 phases become one single fluid phase. Thus, above its critical temperature a gas cannot be liquefied by pressure.

At slightly above the critical temperature (310K), in the vicinity of the critical pressure, the line is almost vertical, so that a small increase in pressure causes a large increase in the density of the supercritical phase.

Many other physical properties also show large gradients with pressure near the critical point, e.g. viscosity, relative permittivity and solvent strength, which are all closely related to density. At higher temperatures, the fluid starts to behave like a gas. For carbon dioxide at 400 K, the density increases almost linearly with pressure.

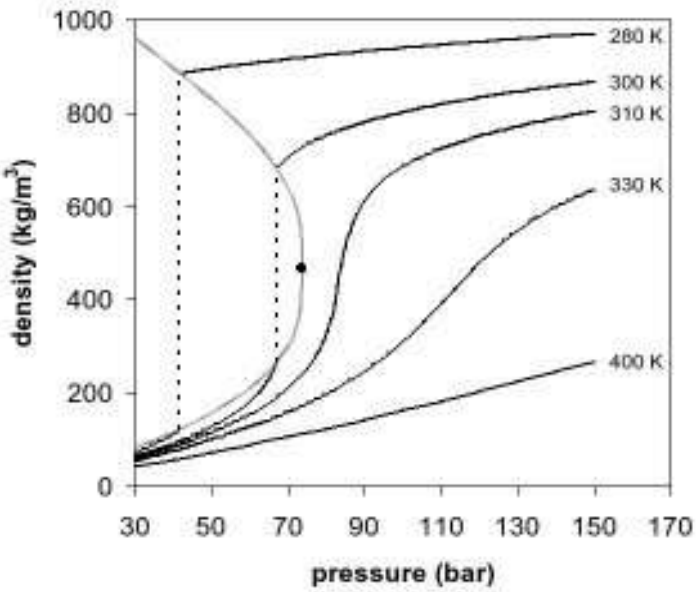


Figure 1.3 *Carbon dioxide density-pressure phase diagram.*

Within the supercritical region there is no phase boundary between the gas and the liquid phases, meaning that there is continuity in physical properties of the fluid between the gas and the liquid states. The most important consequence is that supercritical fluids have properties that are a curious hybrid of those associated to liquids and gases. The viscosities and diffusivities are similar to those of the gases, while the density is closer to that of a liquid. Comparison of selected physicochemical properties of liquids, gases and supercritical fluids is displayed in Figure 1.4 (Schneider G., 1980).

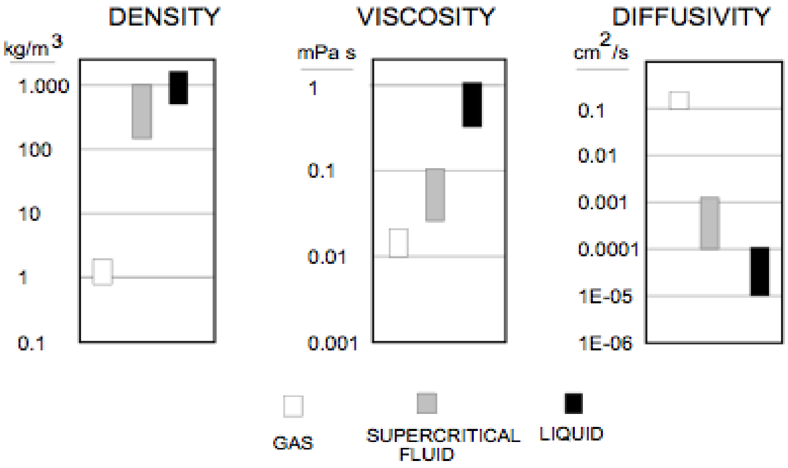


Figure 1.4 *Selected physicochemical properties of liquids, gases and supercritical fluids.*

1.1.2 Supercritical CO_2

The application of supercritical carbon dioxide has attracted interest in recent years, as it is inexpensive, innocuous, non-toxic, nonreactive, non-flammable, non-polluting and has low critical temperature (T_c = 31.1 °C) and moderate critical pressure (P_c=7.38 MPa) as shown in figure 1.5 (Nediljko Budisa and Dirk Schulze-Makuch, 2014).

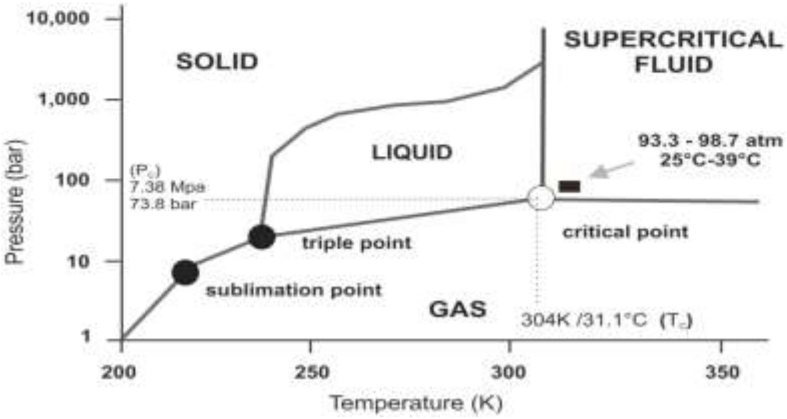


Figure 1.5 *CO_2 p-T diagram.*

All gases become SCFs above their critical point, but many of them have high critical temperature such as critical water (as shown figure 1.6 (Mirofci, 2014)). These gases are not suitable for pharmaceutical compounds. Safety and cost of SCFs are another relevant point in the selection of the supercritical fluid. For example, as indicated in table 1.1 (Pereira *et al.*, 2013), Xenon (Xe) and Su-fur hexafluoride (SF_6) have low critical temperature and pressure, but are very expensive (Esfandiari, 2014).

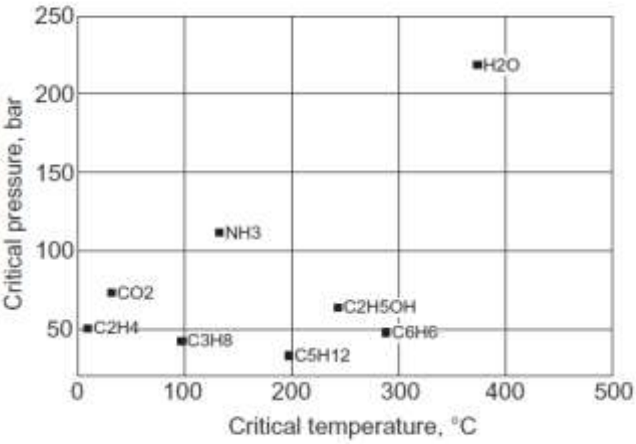


Figure 1.6 *Critical points of some fluids.*

CO_2 has a TLV (= threshold limit value for airborne concentration at 25°C to which it is believed that nearly all workers may be repeatedly exposed to day after day without adverse effects) of 5000 ppm. This value means that it is less toxic than many other organic solvents such as acetone, which has a TLV of 750 ppm, pentane (600 ppm), chloroform (10 ppm).

Table 1.1 *Critical point of some gases.*

Compound	Name	T_c (°C)	P_c(MPa)
CO_2	Carbon dioxide	31.1	7.38
Xe	Xenon	16.6	5.83
SF_6	Sulfur hexafluoride	45.5	3.76

Carbon dioxide is also relatively inert towards reactive compounds (however not completely inert), highly pure, gaseous at atmospheric pressure, cheap and defined "environmentally friendly" because it can be removed from the environment, employed in a process, then returned to the environment as 'clean', without environmental detriment accrues.

CO_2 is also a greenhouse gas, but it is a naturally abundant material that can be theoretically extracted from the atmosphere or collected as a by-product of some processes. However, while CO_2 could in theory be extracted from the atmosphere (or the stack gas of a combustion based power plant), most of the CO_2 employed in processes today is collected from the effluent of ammonia plants or derived from naturally occurring deposits (Beckman, 2004).

Supercritical CO_2 is the anti-solvent chosen in this work and its solubility parameter should be evaluated at this condition. The thermodynamic criteria of solubility are based on the free energy of mixing □G_M. Two substances are mutually soluble if G_M is negative. By definition:

$$G_M = H_M - T \cdot S_M \tag{1.1}$$

where H_M is the enthalpy of mixing and S_M is the entropy of mixing. Since S_M is generally positive there is a certain boundary positive value of H_M below which the dissolution is possible. Scatchard (1931) introduced the concept of "cohesive energy density", defining this property as the cohesive energy per unit volume. Hildebrand (1936) provided a comprehensive treatment of this concept and proposed the square root of the energy density as a parameter that can describe the behaviour of specific solvents. He proposed the term "solubility parameter" and the symbol "δ". The solubility parameter of a pure component is defined by Eq. (1.2):

$$\square\,\delta = {\frac{\Delta U_{vap}}{V_{liq}}}^{0.5} \tag{1.2}$$

Where U_{vap} is the energy of complete vaporization, which is the energy change upon isothermal vaporization of the saturated liquid to the ideal gas state, and V_{liq} is the molar volume.

The work of Giddings *et al.*, 1968 is the most widely referenced on the extension of the solubility parameter for supercritical fluids. Giddings *et al.*, 1968 suggested that the “solvent power” of a gas depends in part on its state relative to its critical condition. The influence of the state, that is, the reduced pressure and temperature, it was termed a “state effect” or “physical effect” by these authors. Besides, there is a “chemical effect”, unique for each chemical specie and dependent on its polarity, acid–base properties and hydrogen bonding tendencies. Classical solubility differences among liquids depend mainly on the “chemical effect” as most liquids are in similar states or can be simply reduced to such without much alteration of their solvent characteristics. With some manipulations, by correlating data from liquid and assuming the equivalence of gases and liquids in a common density, Giddings *et al.*, derived the following expression:

$$\delta = 0{,}326\sqrt{P_c} \;\; \rho_r \;\; \rho_{rliq} \tag{1.3}$$

Where δ is the solubility parameter of the gas in (cal/cm^3)$^{0.5}$, P_c the critical pressure in psia, ρ_r the reduced density, defined as $\rho \;\; \rho_c$, where ρ is the density and ρ_c the critical density, and ρ_{rliq} is the reduced density of the gas at its normal boiling point, considered constant and equal to 2.66. The “state effect” is represented by $\rho_r \;\; \rho_{rliq}$ and the term $0.326(P_c)^{0.5}$ is related to the “chemical effect”. As outlined by Eq. (1.1), calculation of the solubility parameter of the supercritical fluid requires the gas density ρ as an input.

Therefore, accuracy of the solubility parameter of the supercritical fluid is deeply dependent on the precision of the description of its pressure–volume–temperature behaviour. The Reynolds equation is suitable for CO_2:

$$P = \rho^2 \cdot M_1 + \rho^3 \cdot M_2 + \quad \rho^3 \cdot M_3 + \rho^5 \cdot M_4 \quad \cdot exp \; -A_{20} \cdot \rho^2 \quad + \rho \cdot R \cdot T + \rho^4 \cdot \; A_g \cdot T + A_{10} \quad + \rho^5 \cdot \; A_{11} \cdot T + A_{12} \quad + \rho^6 \cdot A_{13} \tag{1.2}$$

Where T is the temperature (°C), P is the pressure (atm), ρ is the density (g/cm^3) and

$$M_1 = A_1 \cdot T + A_2 + A_3 \;\; T + A_4 \;\; T^2 + A_5 \;\; T^3 \tag{1.3}$$

$$M_2 = A_6 \cdot T + A_7 + A_8 \;\; T \tag{1.4}$$

$$M_3 = A_{14}\ T^2 + A_{15}\ T^3 + A_{16}\ T^4 \tag{1.5}$$

$$M_4 = A_{17}\ T^2 + A_{18}\ T^3 + A_{19}\ T^4 \tag{1.6}$$

The constants A_i of the Reynolds equation of state for CO_2 are shown in Table 1.2 (Pereira *et al.*, 2013).

Table 1.2 *Constants of the Reynolds equation of state for.*

Constant		Constant	
A_1	2.2488×10^{-1}	A_{11}	1.2115×10^{-1}
A_2	-1.3718×10^{2}	A_{12}	1.0784×10^{-7}
A_3	-1.4430×10^{4}	A_{13}	4.3962×10^{-11}
A_4	-2.9631×10^{6}	A_{14}	-3.6505×10^{4}
A_5	-2.0606×10^{8}	A_{15}	1.9491×10^{7}
A_6	4.5554×10^{-5}	A_{16}	-2.9187×10^{9}
A_7	7.7043×10^{-2}	A_{17}	2.4359×10^{-2}
A_8	4.0602×10^{1}	A_{18}	-3.7546×10^{1}
A_9	4.0295×10^{-7}	A_{19}	1.1898×10^{4}
A_{10}	-3.9436×10^{-4}	A_{20}	5.0×10^{-6}

Fig. 1.7 (Pereira *et al.*, 2013) shows the temperature and pressure effect on the solubility parameter of CO_2.

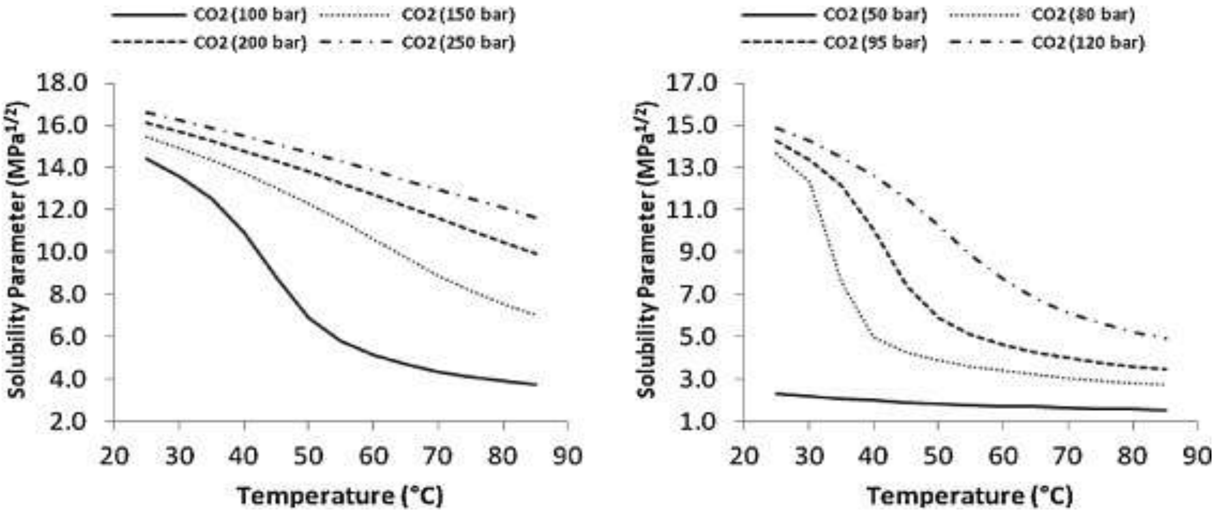


Figure 1.7 *Solubility parameter of CO2 calculated as function of temperature and pressure.*

To allow a good visualization of the difference in the values of the solubility parameter, the plots were separated into two pressure ranges. At constant temperature an increase in pressure corresponds to an increase in the solubility parameter. For isobaric conditions, a decrease in the solubility parameter occurs with an increase in temperature.

An important point to be emphasized is that at the highest-pressure levels (200 and 250 bar) there is almost a linear variation of the solubility parameter with temperature, which does not occur at lower pressure levels. Furthermore, at the lowest pressure (50 bar) there is a very small variation of the solubility parameter with temperature. A possible explanation to this behaviour is that at higher pressures the molecules are closer and due to this reason the temperature effect on the solubility is more pronounced. However, this effect decreases when pressure gets lower because the molecules stay further from each other (Pereira, *et al.*, 2013).

1.1.3 Applications of SC-CO_2

Supercritical CO_2 is becoming an important commercial and industrial solvent (or anti-solvent) due to its role in chemical processes in addition to its low toxicity and environmental impact. Also the relatively low temperature of the process and the stability of CO_2 allow most compounds to be extracted with little damage or denaturing. In addition, the solubility of many compounds in CO2 varies with pressure, permitting selective extractions. The main applications of SC-CO_2 are summarised below (Web site[4]):

- carbon dioxide has gained popularity among coffee manufacturers looking to move away from classic decaffeinating solvents, because of real or perceived dangers related to their use in food preparation. SC-CO_2 is forced through the green coffee beans, which are then sprayed with water at high pressure to remove the caffeine. The caffeine can then be isolated for resale (e.g. to the pharmaceutical or beverage manufacturers) by passing the water through activated charcoal filters or by distillation, crystallization and reverse osmosis;
- supercritical carbon dioxide is used to remove organochloride pesticides and metals from agricultural crops without adulterating the desired constituents from the plant matter in the herbal supplement industry;
- supercritical carbon dioxide can be used as a more environmentally friendly solvent for dry cleaning over traditional solvents such as hydrocarbons, including perchloroethylene;
- supercritical carbon dioxide is used as the extraction solvent for production of essential oils and other herbal distillates. Its main advantages over solvents such as hexane and acetone in this process are that it is non-toxic and non-flammable. Furthermore, separation of the reaction components from the starting material is much simpler than with traditional organic solvents. The CO_2 can evaporate into the air or

recycled by condensation into a cold recovery vessel. Its advantage over steam distillation is that it operates at a lower temperature, which can separate the plant waxes from the oils;

- in laboratories, SC-CO_2 is used as an extraction solvent, e.g., for determining total recoverable hydrocarbons from soils, sediments, fly ash and other media, and determination of polycyclic aromatic hydrocarbons in soil and solid wastes. Supercritical fluid extraction has been used in determining hydrocarbon components in water;
- processes that use SC-CO_2 to produce micro and nano scale particles, often for pharmaceutical uses, are under development. The gas anti-solvent process, rapid expansion of supercritical solutions (RESS) and supercritical anti-solvent precipitation (SAS) (as well as several related methods) are just example to process a variety of substances into particles.

1.2 Aim of the work

The market of functional ingredients/foods is increasing, as a consequence of the interest of customers in preventing illness and improving health using natural sources (Khan *et al.*, 2013). Cherry (*Prunus avium*) fruit has been recognized as a good source of bioactive compounds that show antiviral, antibacterial, analgesic, anti-inflammatory and immune modulatory activities (Kim *et al.*, 2005). In previous studies, it has been found that total phenolic in sweet cherries ranged from 92.1 to 146.8 mgGAE/100g (GAE=Gallic-acid-equivalent) with an average of 109.8 mgGAE/100g (Kim *et al.*, 2005).

The recovery of polyphenols from cherry mainly depends on the extraction technique and the solvents used. Most frequently, these are methanol, ethanol, ethyl acetate, acetone and mixtures of them solvents with water (Dorta *et al.*, 2012). Solid liquid extraction is the most used extraction technique because it is easy, cheaper and does not required sophisticated instruments. From a technological point of view this technique is easily transferred to agroindustry. Novel extraction techniques have been developed and applied for recovery of natural compounds. Nevertheless, in all cases when the solvent extraction is used as a preparative step, post-processing steps are required to fractionate or to purify the compounds from co-extracted material, and to eliminate the toxic organic solvent.

The main drawbacks related to classical extraction techniques are the thermal and chemical degradation of labile compounds and the residual of harmful solvent affecting the quality and safety of the extract. Indeed, direct use of cherry extracts in food or pharmaceutical industry is not allowed without quality warrantied of low residual of toxic solvents (Wijngaard *et al.*, 2012). Therefore, it is highly desired to develop non-organic-solvent-based-extraction methods with high extraction efficiency.

The use of carbon dioxide (CO_2) in supercritical fluid extraction (SFE) is a real alternative for extracting natural antioxidants from herbs and plants (De Melo *et al*., 2014; Della Porta *et al*., 1998; Reverchon, 1997; Reverchon *et al*., 1994; Zabot *et al*., 2014); it shows advantages in comparison with traditional extraction techniques, since degradation and decomposition of the active compounds is avoided operating at reduced temperatures, in the absence of light and oxygen. The process is non-toxic, no pollutant organic solvents are used and, as a consequence, the post-processing of extracts to separate organic solvents is not necessary (Reverchon and De Marco, 2006). Moreover it is also possible to modulate the solvent power of supercritical carbon dioxide (SC–CO2) to perform a selective extraction. However, CO2 has a very limited capacity to dissolve polar and high molecular weight compounds. Supercritical Antisolvent particles Precipitation (SAS) has been proposed to overcome these limitations (Martín *et al*., 2011; Reverchon and De Marco, 2006). So far, SAS has been extensively used to study the production of micronic and nanometric particles of pharmaceutical compounds with controlled particle size and distribution (Reverchon, 1999; Reverchon *et al*., 2008). This technique is used on natural matters containing several families of compounds, extracted using a polar solvent, like methanol. When added to liquid solutions of this type SC–CO2 extracts the liquid solvent forming the solution. Some compounds keep being soluble or partially soluble in the mixture formed (methanol–CO2) and can be recovered in the separation step, whereas the remaining compounds, that are mainly high molecular weight and polar compounds, are quantitatively and totally precipitated.

The possibility to obtain these precipitates, as micro particles is a further advantage. Indeed, it is known that the reduction of particle size is the first step for improving the dissolution rate of compounds for pharmaceutical or food applications, as demonstrated in the literature for phenolic compounds (Lee *et al*., 2012; Sahoo *et al*., 2011; Tzeng *et al*., 2011; Yen *et al*., 2010).

The application of SAS technique for the recovery of phenolic compounds from cherries has been never performed before and could overcome the drawbacks discussed for the conventional extraction techniques, leading to the complete elimination of the solvent and to the concentration of phenolic compounds in a single step, and with a final product, with the extra characteristic to be in the form of micronized particles. Therefore, in this work, SC-CO_2 was used as an anti-solvent to recover the phenolic compounds present in a cherry extract, without modification of their characteristics.

1.3 Literature review on cherries composition

The cherry is the fruit of cherry tree (*Prunus avium*), it is shown in figure 1.8 [web site[5]]. The domesticated plant was obtained by repeated hybridizations of the botanical species. The fruit, usually spherical, of 0,7-2 cm in diameter, can also assume a heart shape or slightly elongated sphere. The color, usually red, may range, depending on the variety, from light yellow to almost black red.

Figure 1.8 *Sweet cherries (Prunus avium).*

The fruit ripens in the spring-summer period and contains a single hard seed, wood coloured. Italy is the fourth largest producer of cherries in the world as shown in table 1.3 [web site[6]].

Table 1.3 *Top cherry producing nations – 2012 (in metric tons).*

Rank	Country	Production
1	🇹🇷 Turkey	480,748
2	🇺🇸 United States	384,646
3	🇮🇷 Iran	200,000
4	🇮🇹 Italy	104,766
5	🇪🇸 Spain	98,400

Sweet cherry is one of the most popular of the temperate fruits. Sweetness and skin colour influence consumer acceptance of cherry cultivars (Crisosto and Metheney, 2003) as well as fruit weight. Skin colour is the most important indicator of quality and maturity of fresh cherry, and depends on the anthocyanidin content (Esti *et al.*, 2002). In numerous fruit production areas sweet cherries are very widespread and appear on the market as the first fresh fruits among all.

Sweet cherries have been reported to contain various phenolics (Kim *et al.*, 2005), which contribute to cherry total antioxidant activity. Phenolics are a diverse group of aromatic compounds with at least one hydroxyl group, which include derivatives such as glycosides. The composition and concentration of phenolics in cherries are significantly influenced by the stage of maturity, cultivars, cultural practices, geographic origin, growing season, climatic conditions, postharvest storage conditions, and food-processing procedures. Many dietary phenolics are known to provide health-improving benefits due to their various biological activities. These possible health beneficial effects included antioxidant, antiallergic, anticarcinogenic, antimicrobial, antimutagenic, and anti-flammatory properties. Also, some phenolics protected neuronal cells from the oxidative stress induced by Reactive Oxygen Species (ROS) or amyloid protein, which may be related to the pathogenesis of Alzheimer's disease. Phenolics may have long-term human health benefits and may reduce the risk of some chronic diseases such as cancer and heart disease. A dietary intake of phenolics was epidemiologically found to have an inverse relationship with the risk of dementia, of which the most well known type is Alzheimer's disease. These beneficial effects have been related to the presence of natural polyphenolic compounds. In the last years, the researches started to focus on their possible health applications as nutritional supplements, functional food formulations, medicines, and cosmetics (Kim *et al.*, 2005).

The major phenolic antioxidants in sweet cherries are anthocyanins but sweet cherries also have significant amounts of phenolic acids and flavonols.

1.3.1 Anthocyanins

Cherries are known to have anthocyanins as the major phenolics. Anthocyanins belong to a parent class of molecules called flavonoids as shown in figure 1.9 [web site[7]]. They are responsible for the cyan and red colors of fruits. These were first used in food industry as natural colorants. Anthocyanins are glycosides of anthocyanidins, the basic chemical structure of which is shown in figure 1.10 [web site[8]].

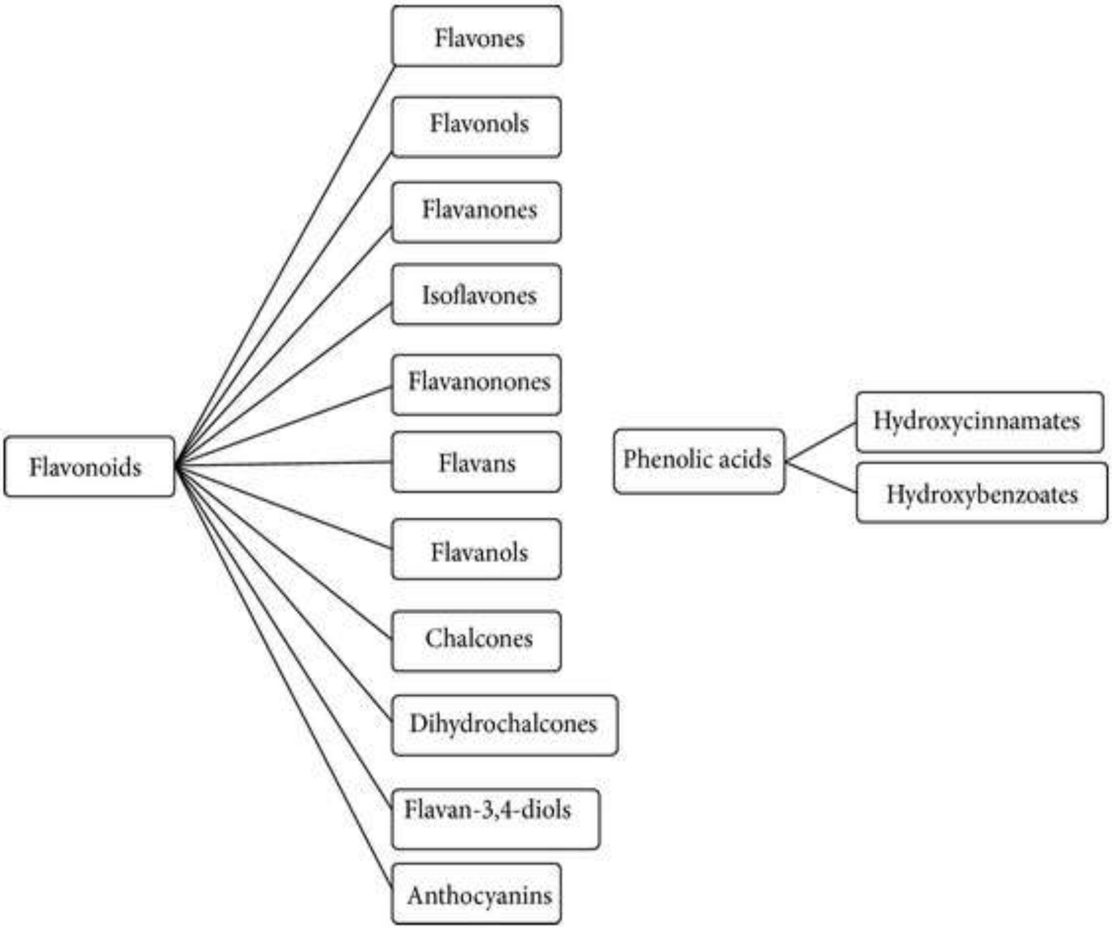


Figure 1.9 *Basic classifications of flavonoids and phenolic acids.*

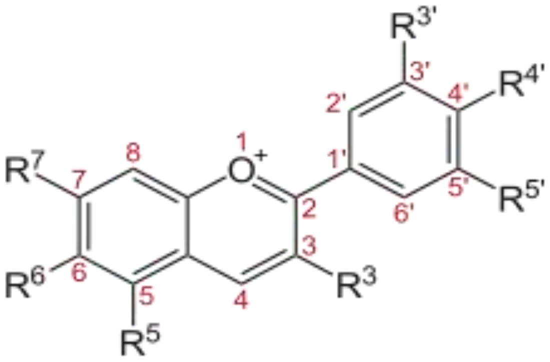
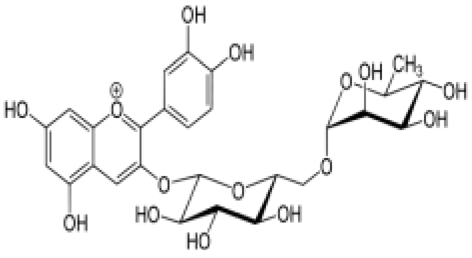


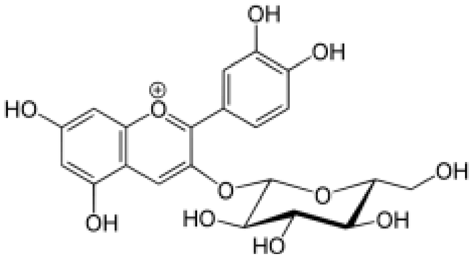
Figure 1.10 *Basical chemical structure of anthocyanins.*

Anthocyanins are polar compounds thus solvents used for their extraction are acidified aqueous mixtures of ethanol, methanol or acetone (Kim *et al*., 2005).

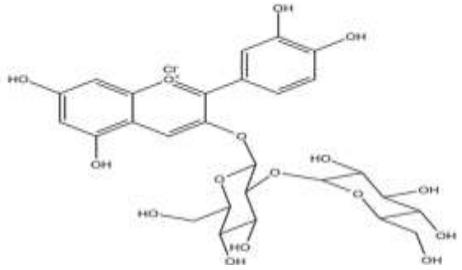
Cyanidin 3-glucoside, cyanidin 3-rutinoside, cyanidin 3-sophoroside, pelargonidin 3-glucoside, pelargonidin 3-rutinoside, peonidin 3-glucoside, and peonidin 3-rutinoside have been identified in sweet cherries. Their structures are illustrated in figure 1.11 [web site[9]].



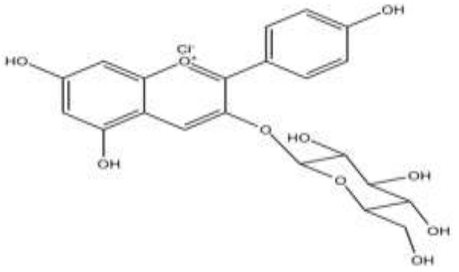
Cyanidin 3-rutinoside (Antirrhinin)



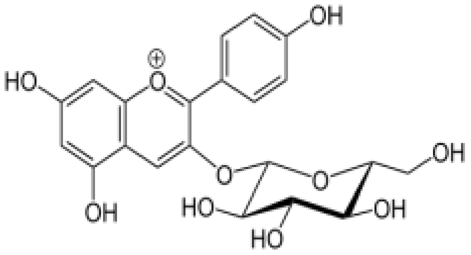
Cyanidin 3-glucoside (Chrysanthemin)



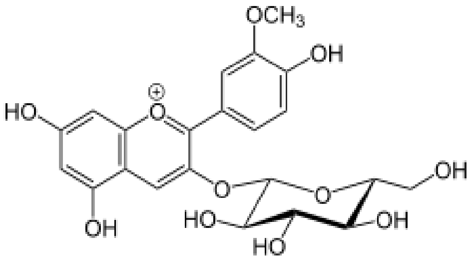
Cyanidin 3-sophoroside



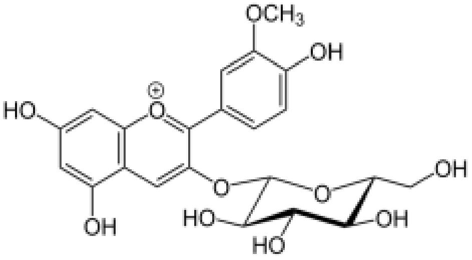
Pelargonidin 3-glucoside



Pelargonidin 3-rutinoside



Peonidin 3-glucoside



Peonidin 3-rutinoside

Figure 1.11 *Anthocyanins contained in sweet cherries.*

All these compounds are highly water-soluble and are easily degraded by hydrolysis and /or hydrogenation at temperatures >40°C.

1.3.2 Phenolic acids

Phenolic (or phenolcarboxylic) acids are types of aromatic acid compound. Included in that class are substances containing a phenolic ring and an organic carboxylic acid function. The major phenolic acids in sweet cherries are hydroxycinnamic acids (Jakobek *et al.*, 2007; Usenik *et al.*, 2008). Its basic chemical structure is shown in figure 1.12 [web site].

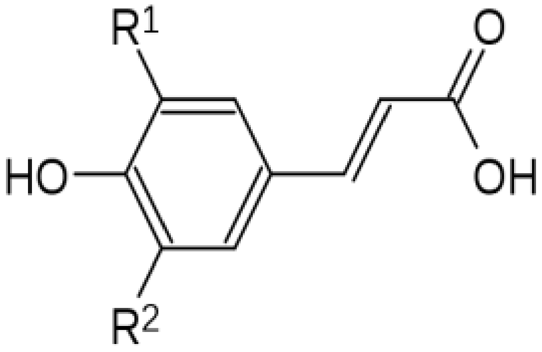


Figure 1.12 *Basical chemical structure of hydroxycinnamic acids.*

Among hydroxycinnamates, sweet cherries have neochlorogenic acid and p-coumarylquinic acid as the predominant compounds (Kim *et al.*, 2005). In addiction, small amounts of chlorogenic acid (Kim *et al.*, 2005) and ferulic acid (Matilla *et al.*, 2006) were found as well. Their chemical structures are illustrated in figure 1.13 [web site[10]].

Neochlorogenic acid

P-coumarylquinic acid

Chlorogenic acid

Ferulic acid

Figure 1.13 *Phenolic acids contained in sweet cherries.*

Hydroxybenzoic acids (p-hydroxybenzoic acid) were found in sweet cherries only in small amounts (Matilla *et al.*, 2006).

1.3.3 Flavonols and Flavanols

Flavonols are a class of flavonoids containing the 3-hydroxyflavone backbone (IUPAC name: 3-hydroxy-2-phenylchromen-4-one). Their diversity stems from the different positions the phenolic -OH groups as illustrated in figure 1.14 [web site[11]].

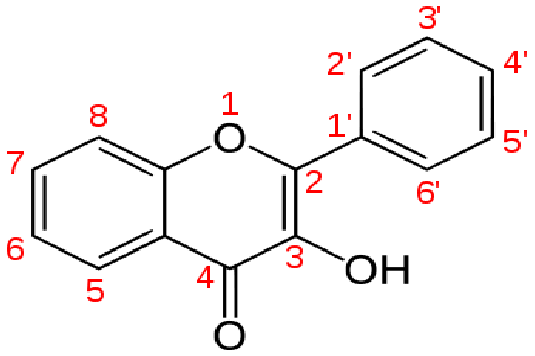
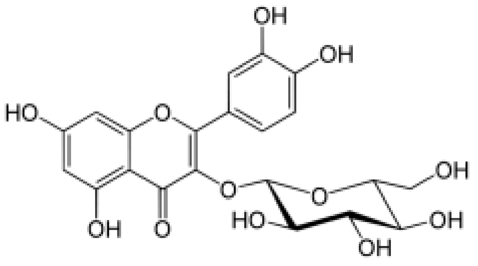
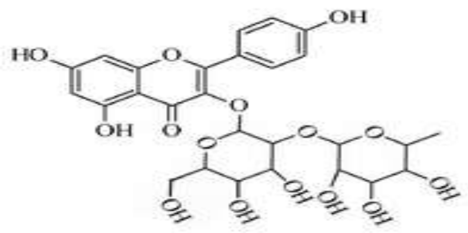


Figure 1.14 *Basical chemical structure of hydroxycinnamic acids.*

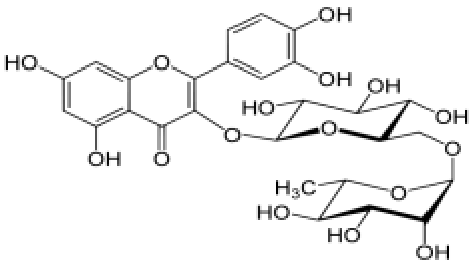
Flavonols detected in sweet cherries are quercetin-3-glucoside (isoquercetin), quercetin-3-rutinoside (rutin), and kaempferol 3-rutinoside (Kim *et al.*, 2005). Their structures are shown in figure 1.15 [web site[12]].



Quercetin-3-glucoside



Kaempferol 3-rutinoside



Quercetin-3-rutinoside

Figure 1.15 *Flavonols contained in sweet cherries.*

They are distinct from flavanols (with “a”) another class of flavonoids shown above in figure 1.9 [web site[13]].

Flavanols, (whose chemical structure is illustrated in figure 1.16), are contained in sweet cherries. They include catechin and epicatechin and their chemical structures are indicated in figure 1.17 [web site[13]].

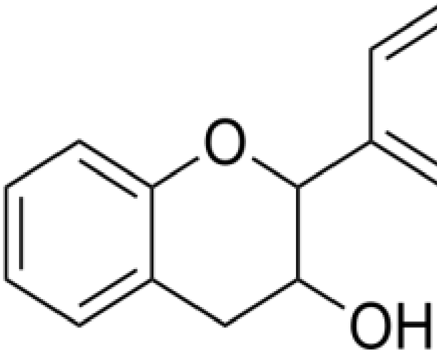
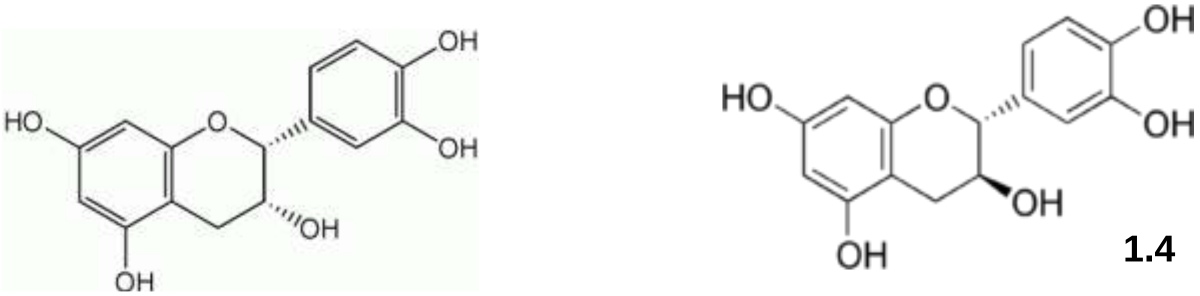


Figure 1.16 *Basical chemical structure of Flavanols.*



Epicatechin **Catechin**

Figure 1.17 *Flavanols contained in sweet cherries.*

Literature review on supercritical anti-solvent CO_2 precipitation of phenolic compounds from cherries

Serra, *et al.*, (2010) performed SFE of sweet cherry, finding that anthocyanins could not be extracted. So to obtein them a second step was necessary where different mixtures of CO_2 and ethanol were tested. All extractions were performed at 50 °C and 25 MPa during 1 plus 1.5 h. To avoid this problem and reduce temperature and pressure we performed the application of SAS technique for the recovery of phenolic compounds from cherries. Moreover the particles obtained are solvent free according to our knowledge the SAS technique for cherries was not applied before.

A review on the supercritical anti-solvent precipitation of phenolic compounds from other vegetables or fruit has been performed. Methods and results of these works are briefly listed in table 1.4.

Table 1.4 *Main characteristics of SAS process to precipitate phenolic compounds from fruit and vegetables.*

Fruit and vegetables	SAS conditions			References
	P (MPa)	T (°C)	Inlet solution and CO_2	
Mango by-products	8-10	35-40	X_{CO2}: 98%	Meneses, *et al.*, 2015
Grape seeds	8-15	30-60	X_{CO2}: 98%	Marqués, *et al.*, 2013
Persea indica	8-15	35-60	Solution: 5.7 mL/min CO_2: 2.38 kg/h	Martin, *et al.*, 2011
Rosemary	8-12	25-50	Solution: 1 mL/min CO_2: 0.7 kg/h	Visentin, *et al.*, 2012
Grape residues	Up to 20	40	Solution: 0,7 ml /min CO_2: 25 mL/min	Floris, *et al.*, 2010
Andrographis panicutala	5-24	35-55	Solution: 0.13 mL/min CO_2: 0.5/1.5 g/min	Imsanguan, *et al.*, 2010
Marigold flowers	8-12	55	Solution: 0.25-0.5 mL/min CO_2: 15-25 mL/min	Boonnoun, *et al.*, 2013
Lycium barbarum	12.5-17	55	Solution: 0.1-0.3 mL/min CO_2: 15 L/min	Lin, *et al.*, 2014
Olea europea leaves	10-20	35-60	Solution: 2-8 mL/min CO_2: 11-30 g/min	Chinnarasu, *et al.*, 2014
Eucalyptus globulus leaves	10-20	35-50	Solution: 10-30mL/min CO_2: 2-5 g/min	Chinnarasu, *et al.*, 2015

The method and solvent used for the extraction of antioxidants from raw materials change in each process listed in the table, while the time required to wash the precipitator is never less than 20 minutes. Table 1.4 was the starting point for the choice of operating conditions of precipitation of phenolic compounds from cherries, which will be discussed in Chapter 3.

Chapter 2

Materials and methods

Supercritical anti-solvent precipitation experiments were performed at laboratory scale, in a plant assembled for the purpose. Raw material samples have been freeze-dried before extraction tests, to reduce the water content. For solvent extractions an ultrasonic bath was used. The precipitates have been analysed using HPLC-MS.

In this chapter procedures and materials used to carry out the experimental runs are described.

2.1 Chemicals and cherries

Carbon dioxide was used for supercritical anti-solvent precipitation, while methanol and dimethylsulfoxide were utilized for the solvent extraction. Carbon dioxide, 4.0 type, with purity greater than 99,9% was provided by Rivoira. The solvents were purchased from Sigma Aldrich, with a purity of 99,8%. Cherry by-products have been bought in a local market.

2.2 Pre-treatment: Destoning, grinding and lyophilization of the cherries

Before extraction, cherries need to be destoned, milled and dried. Indeed, it is favourable to have low water content, since it could compromise the extraction diminishing the contact between solvent and solute. Traditional drying methods use high temperatures that can damage the internal structure of biological compounds. In order to keep the internal structure of the leaves intact, lyophilization seemed to be the most appropriate technique in our case.

This process, also known as freeze-drying is a dehydration method that works by freezing the material and then reducing the pressure to allow the frozen water in the material to sublimate directly from the solid to the gas phase. Freeze-drying does not usually cause shrinkage or toughening of the material being dried. In addition, flavours, smells and nutritional content generally remain unchanged, making the process popular for preserving food. However, water is not the only chemical capable of sublimation, and losses of other volatile compounds such as acetic acid and alcohols can occur.

Freeze-drying is mostly used in pharmaceutical and food industry to increase the shelf life of the products. Preservation is possible because the greatly reduced water content inhibits the action of microorganisms and enzymes that would normally spoil or degrade the substance.

Moreover, foods, which are lyophilized, can usually be stored without refrigeration, which results in a significant reduction of storage and transportation costs. Another advantage of using this technique of dehydration is that freeze-dried products can be rehydrated much more quickly and easily because the process leaves microscopic pores. The ice crystals that sublimate, leaving gaps or pores in their place, create the pores. Lyophilisation does not significantly reduce the volume; therefore water quickly regains its place in the molecular structure of the food or product. Generally, the first stage is a pre-treatment of the product before freezing. In our case, cherries were separated from the bones manually then they were ground with an electric food processor to increase the surface area. Now the cherries were ready to be dried.

The second stage is freezing. The sample was placed in a freeze-drying flask and rotated in a bath, called shell freezer cooled by liquid nitrogen. To allow the sublimation to occur rather than melting, in this step it is important to go below the triple point. This stage is the most critical of the freeze-drying process. The freezing temperature is about -50°C.

The third stage is primary drying. During this step the pressure is lowered to a few millibar and enough heat to allow water to sublime is supplied to the sample. The heat is brought by conduction or radiation. About 95% of the water is sublimated. This step is very slow and can require several days. Increasing the heat supplied to lower the time could lead to the degradation of the biological material. In this stage pressure is controlled through the application of partial vacuum. Moreover, a cold condenser chamber and condenser plates provide a surface for the water vapour to solidify on. Condenser temperatures are below minus 50°C.

2.3 Extraction of antioxidants from cherries

The extraction of antioxidant compounds was performed by solid–liquid extraction. Aliquots of lyophilized cherries were mixed with solvent into a flask to do a solution with 9% w/w of cherries. The flask was kept for 1 h into an ultrasonic bath at room temperature (25°C) to improve the extraction. It is illustrated in figure 2.2.

Figure 2.1 *Ultrasonic bath.*

The solid cherries inside the flask (figure 2.2) were separated with a centrifuge that worked at 4.000 rpm for 6 minutes.

Figure 2.2 *Extracted sample before the centrifugation.*

To choose the best solvent for extraction of antioxidants from cherries, the laboratory of Pharmaceutical and Pharmacological Science Department has analysed nine samples of extract solution. These have been prepared in the same manner described above. The solvent analysed are: water with 1% of HCl, Ethanol with 1% of HCl, methanol, methanol with 1% of HCl, dimethylsulfoxide (DMSO), DMSO/ethanol, 30% DMSO/ethanol and 70% DMSO/ethanol. The results will be reported in chapter 3.

2.4 Supercritical anti-solvent CO_2 precipitation plant

The supercritical precipitation plant has been constructed with high-pressure equipment supplied by Swagelok. The process flow diagram of the experimental setup is shown in figure 2.3. A picture of the pilot plant employed is illustrated in Figure 2.4.The CO_2 is stored in a tank, and then it passes in a container located before the pump to ensure a continuous flow rate. This container is equipped with a manometer and a vent valve. The pressure indicated at the manometer is an indication if the CO_2 is sufficient to be pumped.

Before the pump, a heat exchanger lowers the CO_2 temperature to subcooled liquid, in order to prevent the pump cavitation. The heat exchanger is a serpentine with concentric tubes where the CO_2 is the inner stream and ethylene glycol, cooled by a chiller, is the outer one. A temperature controller keeps the temperature before the pump at 5 °C. The supercritical temperature is achieved by an insulated electrical resistance, which is placed around the tube before the precipitator.

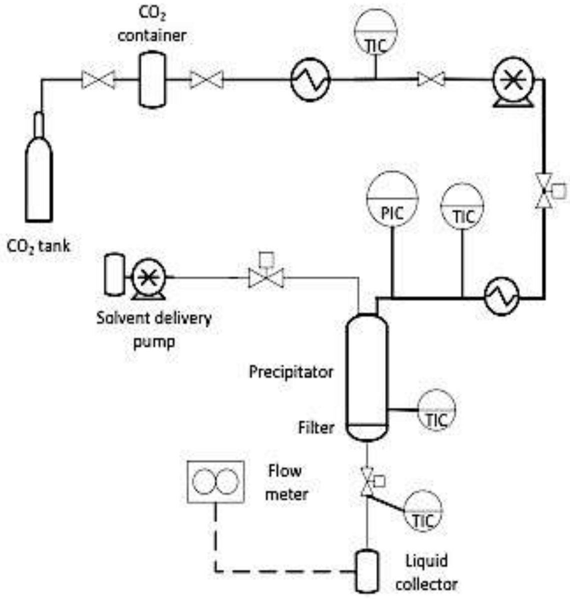


Figure 2.3 *Supercritical anti-solvent equipment used to perform the precipitation of antioxidants from extract solution of cherries.*

A temperature regulator ensures a constant temperature. After that, a manometer indicates the pressure right before the precipitator. The extracted solution is pumped with a HPLC pump that is connected to a little solution vessel. The solution line has an on-off valve before to be linked to precipitator.

Figure 2.4 *Picture of the pilot plant used to perform the precipitation of antioxidant from cherries extracted solution.*

The precipitator is characterized by three pieces made of stainless steel. The top and the bottom are screwed with the cylindrical vessel. It is illustrated in Appendix A.

The solution line continues inside the precipitator until the first filter to avoid the dispersion of precipitate on the wall of precipitator chamber, as shown in figure 2.5.

Figure 2.5 *Dispersion of the precipitate on the wall of precipitator chamber*

Two stainless steel filters frits (0,4 μm), located at the bottom, allowed the collection of the powder material as shown in figure 2.6.

a) *b)*

Figure 2.6 *a) Big steel filter located above the small steel filter represented in figure b).*

A resistance is linked to a water bath and it is placed around the precipitator to monitor the temperature value. An expansion valve (a Medium-Flow High-Pressure Metering Valve) follows the precipitator and regulates the CO_2 flow rate. A resistance placed around the valve body heats it, in order to avoid freezing of the solvent and CO_2 during depressurization. The separator located downstream the pressure reduction valve, operating at room pressure, was used to recover the liquid solvent while the CO_2 in its gaseous state is automatically separated. A flow meter and a volumetric counter measure the CO_2 flow rate and the passed volume.

2.4.1 Supercritical anti-solvent precipitation procedure (semi continuous process)

To perform Supercritical anti-solvent (SAS) semi continuous experiments, first CO_2 was pumped to the precipitator at fixed temperature until the desired condition of pressure were reached; then, the flow rate was regulated by a micrometric valve located between the precipitator and the separator. When a constant flow rate of CO_2 was established, the solution was sent to the precipitator. The fast extraction of the solvent by SC–CO_2 produced the precipitation of the solute. At the end of the methanol solution delivery, the precipitator was purged with pure SC–CO_2 at the process conditions, to wash away residual solvent solubilized in the precipitated solid. If the final purge with pure SC–CO_2 was not performed, the solvent contained in the anti-solvent condensed during depressurization, solubilizing or modifying the precipitate. Finally, the precipitation vessel was depressurized and the precipitated compounds were collected from the filters.

The powder was dissolved and stored in methanol. The particles were very sticky and it was difficult to collect them because they were too much dispersed as shown in figure 2.7.

Figure 2.7 *The photos show that the precipitate doesn’t disperse only on filter but on the bottom screwed part of precipitator too.*

For this reason it was necessary to wash the precipitator with methanol to recover the powder. Precipitate was stocked into a test tube to be analysed.

2.4.2 Supercritical anti-solvent precipitation procedure (batch process)

To implement Supercritical anti-solvent (SAS) batch experiments, first the precipitator chamber was filled with a prefixed volume of extracted solution. The precipitator was screwed on the top and the expansion valve was closed on the bottom: this must be done very quickly to avoid leakage of the solution from the bottom. Then CO_2 was pumped to the precipitator at fixed temperature until the desired condition of pressure. The precipitator was maintained at constant temperature while the pressure increased inside. After 30 minutes the expansion valve was opened and the precipitator was purged with pure SC–CO_2 at the process conditions, to wash away residual solvent solubilized in the supercritical anti-solvent. Finally, the precipitation vessel was depressurized and the precipitated compounds were collected from the filters. The powder was dissolved and stored into a test tube with methanol as shown in figure 2.8.

Figure 2.8 *Precipitate dissolved in methanol.*

2.4 Analysis of the precipitate

The samples were analysed in a laboratory of the Department of Pharmaceutical and Pharmacological Science. The identification and the content of polyphenols were measured with HPLC-MS technique. Liquid chromatography mass spectrometry LC-MS, or high performances liquid chromatography HPLC-MS, are analytical chemistry techniques that combine the physical separation abilities of liquid chromatography with the mass analysis capabilities of mass spectrometry (MS). It is generally used to separate, generally detect or to identify chemicals of particular masses in the presence of other chemicals. Principally, it is used to identify a natural product from a natural product extract or a pure substance from a mixture of other intermediates.

HPLC is a liquid chromatography separation technique that operates at relatively high pressures. As in liquid chromatography there is a mobile phase and a stationary phase. The sample is forced by a liquid at high pressures (mobile phase) through a column that is packed with a stationary phase composed by irregularly or spherically shaped particles depending on the separation target to achieve. Depending on the stationary phase and on the polarity of the mobile phase two methods of HPLC can be used. For reversed phase liquid chromatography (RP-LC) octadecylsyl (C18) is used as stationary phase with organic-water mixture as mobile phase (water-acetonitrile and water-methanol). For normal phase liquid chromatography (NP-LC) silica gel is used as stationary phase with organic mixtures. Usually, the former is applied as a mean to introduce samples into the mass spectrometry. The flow used in the standard column (4.6 mm bore diameter) has to be split before to be sent to the column (10:1). Mass spectrometry will give improved sensitivity at flow rates of 200 μL/min or less.

Mass spectrometry is an analytical technique that measures the mass to charge ratio of charged particles. MS works by ionizing chemical compounds to generate charged molecules or molecule fragments and measuring their mass-to-charge ratios. It is used for determining masses of particles for elucidating the composition of a sample or the structure of a molecule. A typical HPLC-MS coupling is shown in Figure 2.9 [web site[14]].

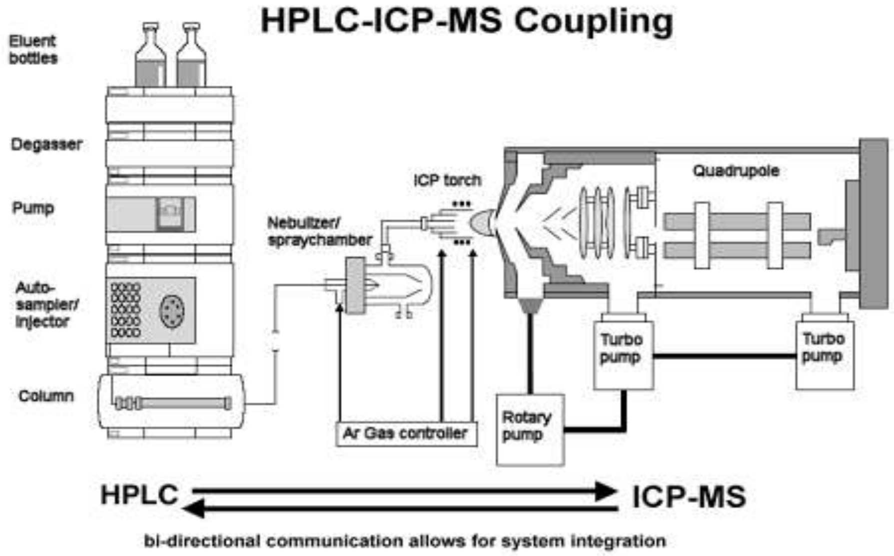


Figure 2.9 *Example of HPLC-MS coupling.*

The component of the sample separated in the HPLC is vaporized and then ionized by inductive coupled plasma (ICP) or other techniques, to produce ions. The ions are separated according to their mass-to-charge ratio in an analyzer by electromagnetic fields. The ions are detected by a quantitative method (e.g quadrupole) and the signal is processed into mass spectra (Mirofci, 2014). In this work, HPLC−MS measurements were obtained on a Varian 212 series chromatograph equipped with Prostar 430 autosampler and a MS-500 Ion Trap as detector. MS spectra were recorded in positive and in negative ion mode in the m/z range between (50 and 2000 Da). The ESI ion source was used for analysis of polyphenols. Fragmentations of the main $[M+H]^+$ (in positive mode) and $[M+H]^-$ (in negative mode) ionic species were obtained during the HPLC run using the turbo data depending scanning (tdds) function, yielding in fragmentation patterns of eluted compounds. As stationary phase an Agilent Eclipse C-18 (2.1 x 150mm) 2.7µm column was used. As mobile phases solvent A (water 0.1% formic acid) and solvent B (Acetonitrile 0.5% formic acid) were utilized. The flux was 0.2 mL/min. The solvent gradient used was: 0 min: 5% B; 2 min: 5% B; 38 min: 95% B; 39 min: 5% B; 45 min: 5% B.

Chapter 3

Results and discussion

This chapter of the work is divided into three parts. The first one regards the results of extraction of phenolic compounds from cherries. In the second part the results of supercritical anti-solvent process with batch and semi-continuous approaches are reported and the effects of the main parameters affecting the precipitation, such as pressure, temperature, SC–CO2 and liquid solution flow rates are studied. The third part shows the flowsheet of the process scale-up necessary to perform the life cycle analysis in the next chapter.

3.1 Extraction of antioxidants from cherries

To choose the best solvent for extraction of antioxidants from cherries, the laboratory of Pharmaceutical and Pharmacological Science Department has analysed nine samples of extracted solution. The solvents analysed are: water with 1% of HCl, Ethanol (EtOH), Ethanol with 1% of HCl, methanol, methanol with 1% of HCl, dimethylsulfoxide (DMSO) and three mixtures respectively at 30%, 50% and 70% of dimethylsulfoxide/ethanol.
The amounts of antioxidant extracted by each solvent are shown in table 3.1.

Table 3.1 *Amounts of antioxidants extracted by solvents.*

mg/g	HCl 1%	EtOH	EtOH/ HCl 1%	MeOH	MeOH/ HCl 1%	DMSO	DMSO /EtOH	70DMSO /30EtOH	30DMSO /70EtOH
Anthocyanins	1.00	0.15	0.18	0.91	1.03	0.95	1.05	0.89	0.86
Deriv. Daidzeina	0.00	0.00	0.04	0.01	0.00	0.01	0.01	0.00	0.00
Deriv. Ferulico	0.11	0.18	1.23	0.87	3.81	0.65	0.96	0.86	0.94
clorogenico	1.69	0.06	0.21	0.98	0.00	0.78	0.85	1.00	0.94
Rutina	0.15	0.03	0.12	0.23	0.00	0.23	0.23	0.19	0.16
	2.95	**0.42**	**1.78**	**3.00**	**4.84**	**2.62**	**3.10**	**2.94**	**2.90**

From the table 3.1 it can be seen how the best result is adopted methanol with HCl 1%. To avoid the use of HCl others two solvents were considered; methanol and a mixture 50%

ethanol and 50% dimethylsulfoxide (DMSO). It is also clear that anthocyanins contained in the methanolic solution are more abundant than polyphenols (*Rutina*).

3.1.1 Effect of solvent in the precipitation process

In order to select the solvent used to extract the phenolic compounds, some tests using two different cherries solution were performed. The first ones used a cherries solution extracted with a mixture of Ethanol and DMSO (50%w/w). The temperature was set to 40 °C to avoid the degradation of phenolic compounds, while the pressure was changed from 100 bar to 150 bar. The mole fraction of CO_2 was fixed at 95% so the flow rate of carbon dioxide and cherries solution were 3L/min and 0,45 mL/min respectively. These experiments did not allow the formation of particles since no solid phase was found when the precipitator vessel was opened. Other experiments used cherries solutions extracted with methanol as the solvent, were performed at the same condition of temperature, pressure and CO_2 mole fraction above, and were successful obtained powders as precipitate.

Therefore methanol was chosen as the solvent for the preparation of cherries solution. In the following paragraph the optimization of the precipitation parameters using a methanolic solution will be addressed.

3.2 Supercritical CO_2 anti-solvent precipitation process

SAS precipitation was carried out with two different approaches, the batch and semi continuous methods. In both processes the particle formation is based on the anti-solvent effect. Following the calculation for the precipitation yields are described respectively for the total, polyphenols and anthocyanins yield.

The total precipitation yield (*Y)* was calculated by:

$$Y[\%] = \frac{cV}{c_s V_s} * 100 \tag{3.1}$$

Where c is the total concentration of polyphenols and anthocyanins recovered after the *supercritical anti-solvent precipitation* (SAS) dissolved in methanol (ug / ml); V is the volume of methanol in which the precipitate was dissolved (ml); c_s is the total concentration of polyphenols and anthocyanins in the initial solution (ug / ml); V_s is the volume of solution injected during the test (ml).

The polyphenols precipitation yield (Y_p) was calculated by:

$$Y_p[\%] = \frac{c_p V}{c_{sp} V_s} * 100 \tag{3.2}$$

Where c_p is the total concentration of polyphenols recovered after the *supercritical anti-solvent precipitation* (SAS) dissolved in methanol for analysis (ug / ml); c_{sp} is the concentration of polyphenols in the initial solution (ug / ml).

The anthocyanins precipitation yield (Y_a) was calculated by:

$$Y_a = \frac{c_a V}{c_{sa} V_s} * 100 \tag{3.3}$$

Where c_a is the total concentration of anthocyanins recovered after the *supercritical anti-solvent precipitation* (SAS) dissolved in methanol for analysis (ug / ml); c_{sa} is the concentration of anthocyanins in the initial solution (ug / ml).

Anthocyanins contained in the initial methanolic solution are more abundant than polyphenols; these are 78,5% and 21,5% respectively.

3.2.1 Effect of operating variables

The influence of pressure, temperature and CO2 molar fraction, the main parameters of the supercritical anti solvent process, on the fractionation efficiency of phenolic compounds was studied. The process temperature is a relevant parameter when phenolic compounds are processed, since they are degraded by exposure to light and high temperatures; therefore, the maximum temperature applied was 40°C. The operating conditions were selected to work in correspondence of the supercritical region of the vapour-liquid equilibrium (VLE) binary diagram that is above the mixture critical point. The hypothesis that the possible solute solubilisation in the fluid phase does not significantly change the interaction between CO2 and methanol when many different compounds are present is very strong; therefore, the binary diagram, can give only approximate indications on the operating conditions that should be adjusted according with the real system used. The vapour-liquid equilibrium diagram (VLE) of the system CO_2–MetOH at 313,15 K is shown in figure 3.1(Chang et al. 1997). Phase diagrams are extremely useful to explain the precipitation results. For example, at low pressures, say 5bar, both xCO_2 and yCO_2 (the mole fraction of CO_2 dissolved in liquid and vapour methanol) are very small. As the pressure increases, xCO_2 increases while yCO_2 remains nearly unchanged. At mixture critical point (CP) where the saturated liquid and vapour curves meet, the interface between the liquid and gaseous phase vanishes and therefore, at this point and beyond the supercritical region occurs.

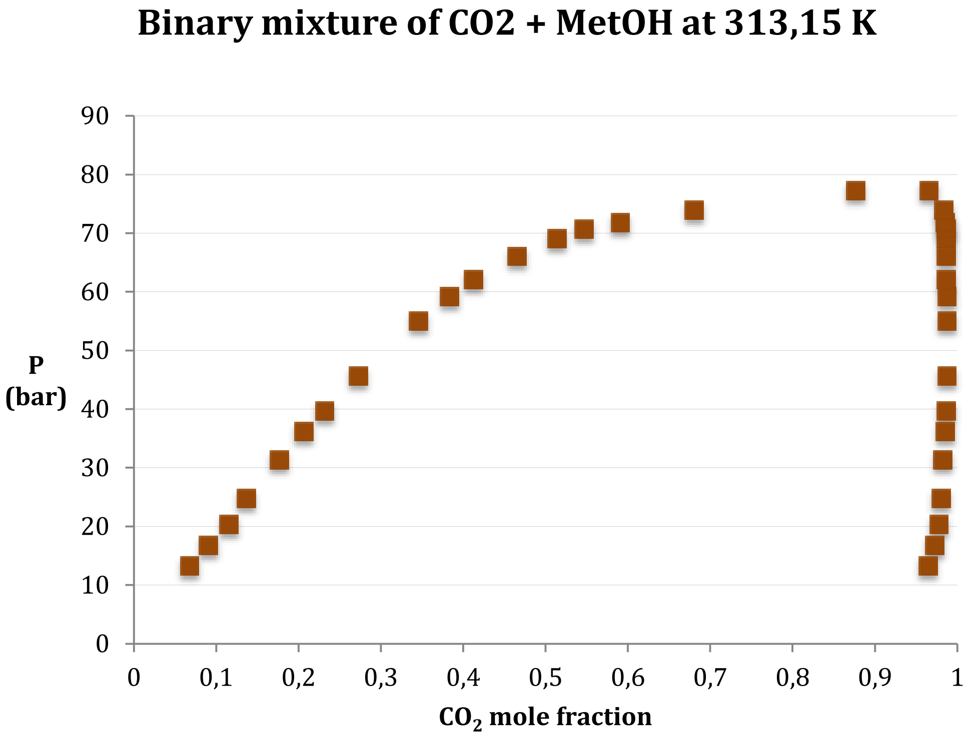


Figure 3.1 *CO_2 VLE experimental data at 313,15K.*

3.2.1 Batch process

The batch supercritical anti solvent process consists in the addition of SC-CO_2 into a vessel pre-filled with the solution containing the phenolic compounds to be precipitated until the desired pressure is reached and remained stable. Afterwards, the solution is drained under isobaric conditions and the CO_2 flow is remained constant until all the organic solvent is removed by stripping. The effect of pressure has been analysed with two sets of experiments. The temperature was fixed to 40°C to avoid the degradation, the time of the process and the volume of solution pre-filled were kept constant at 30 minutes and 40 ml respectively. In the first run the pressure was set to 100 bar, while in the second one it was set to 120 bar. During both experiments the pressure increases from 100 to 130 bar and from 120 to 150 bar respectively. The washing step takes 20 minutes, in which the flow of CO_2, the temperature and the pressure are held constant. The precipitation yields (calculated as written above) and the operating conditions are explained in table 3.3. The corresponding precipitation curve is plotted in Figure 3.2, while the pictures of the powders are shown in figure 3.2.

Table 3.2 *Experiments conducted with batch process at different pressure.*

Cherries solution (mL)	P (bar)	T (°C)	Time of batch process	Drying Time (min)	CO_2 Drying (L/min)	Yp average yield (%)	Ya average yield (%)	Y average yield (%)
40	100-130	40	30	20	2,0	40,2	30,0	33,8
40	100-130	40	30	20	2,0			
40	120-150	40	30	20	2,0	35,4	22,4	25,3
40	120-150	40	30	20	2,0			

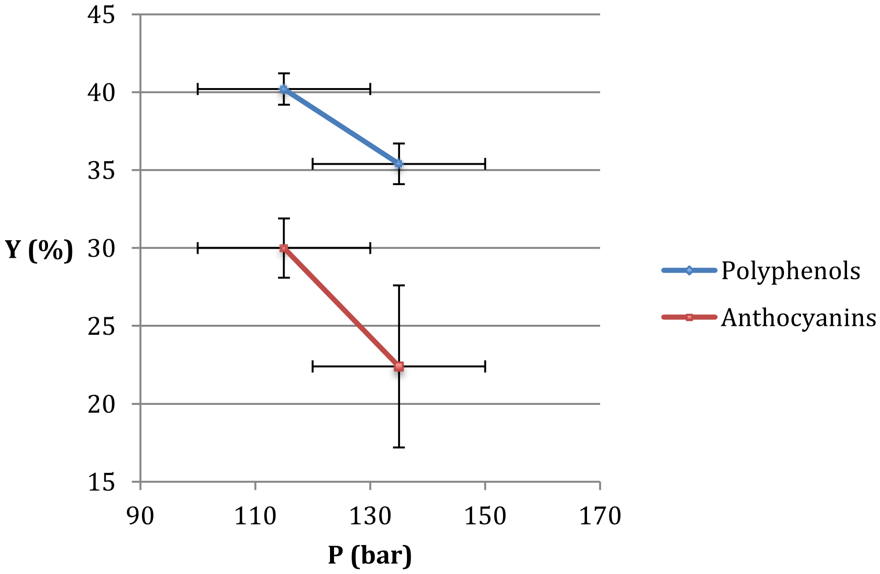


Figure 3.2 *Precipitation yield curve at constant T=40°C and different pressure (100-130,120-150 bar) in batch process.*

How can be seen from table 3.2 and figure 3.2, an increase in pressure corresponds to a decrease in anthocyanins and polyphenols yields.

a) b)

Figure 3.3 *Powders recovered with batch process at 40°C with different pressure. a)100-130 bar; b)120-150bar;*

The limiting factor of the GAS process is that it is essentially a batch operation and it is associated with problems of solvent removal. In the batch operation, the liquid phase cannot, in general, be completely removed as shown in figure 3.3, which calls for an additional processing step before a dry product can be recovered. Therefore semi continuous experiments have been developed.

3.2.2 Semi continuous process

The experiments were done at fixed condition of temperature while the mole fraction of CO_2 and pressure were varied. Furthermore some experiments in subcritical area were performed too.

3.2.2.1 Effect of pressure

In order to find the optimum value of the pressure, various runs in a range from 100 to 150 bar were performed. In these tests the operating parameters such as SC–CO_2 flow rate and liquid solution flow rate were fixed to obtain a 0.95M fraction (xCO_2) in the overal system CO_2–MeOH, the liquid cherries solution was injected at 0,18mL/min for 30 minutes while the time for drying the powder from the solvent residue was 20 minutes. The CO_2 flow rate to wash the powder was fixed at the same condition of the CO_2 necessary to obtain a 0,95M fraction (2L/min). The procedure for calculating CO_2 flow rate is described below, while in Table 3.3 the properties of methanol and carbon dioxide at room temperature (20 ° C) are reported. Pressures tested were 100 bar, 125 bar and 150 bar.

Table 3.3 *Density and molecular weight of CO2 and methanol at room temperature (25°C).*

	Density (ρ) g/cm^3	**Molecular weight (PM) g/mol**
CO_2	1,98	44,01
Methanol	0,79	32,04

At fixed liquid solution flow rate (L_v= 0,18 mL/min) the molar flow rate (L_M) is:

$$L_M = L_v * \rho_m * \frac{1}{PM_m} \tag{3.4}$$

Where ρ_m and PM_m are reported to methanol.

The molar flow rate of CO_2 (V_M) is:

$$V_m = L_M * x * \frac{1}{1-x}$$

(3.5)

Where x is the CO_2 mole fraction.

Then the volumetric flow rate of CO_2 (V_v) is:

$$V_v = V_m * PM_c * \frac{1}{\rho_c}$$

(3.6)

The fixed operating conditions of the experiments and their precipitation yield (calculated as written above) are summarised in table 3.4.

Table 3.4 *Experiments conducted at different pressure and constant temperature (40°C) and constant CO_2 molar fraction (95%) in a semi continuous process.*

Cherries solution flow rate (mL/min)	**CO_2 flow rate (L/min)**	**x CO_2 (%)**	**T (°C)**	**P (bar)**	**Drying Time (min)**	**Yp average yield (%)**	**Ya average yield (%)**	**Y average yield (%)**
0,2	2,0	95	40	100	20	44,1	38,5	39,8
0,2	2,0	95	40	100	20			
0,2	2,0	95	40	125	20	34,2	25,2	27,7
0,2	2,0	95	40	125	20			
0,2	2,0	95	40	150	20	30,9	17,7	21,1
0,2	2,0	95	40	150	20			

The corresponding precipitation curve is plotted in Figure 3.4. From the behaviour of the curves at different pressure and constant temperature and x CO_2 (40°C), it can be seen that the highest yields of anthocynins and polyphenols were obtained at 100 bar. From 100 bar to 150 bar an increase of pressure corresponds to a decreasing yield. The operating pressure influences the solubility in SC–CO_2 of the liquid solution. But, when pressure is increased, also the solubility of compounds in the solution SC–CO_2–MetOH is increased, and less precipitation of solid particles can be observed.

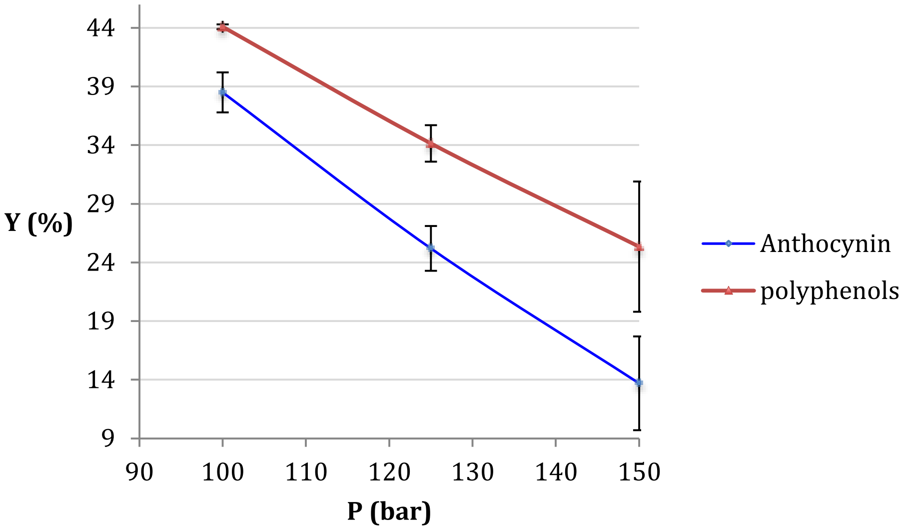


Figure 3.4 *Precipitation curve at constant T=40°C; constant xCO2=95% and different pressure (100,125,150 bar) in continuous process.*

In summary the most selective precipitation was the one carried out at 100 bar, in which the quantity of phenols precipitated is the highest one.

The picture in figure 3.5 shows the powders recovered after the continuous precipitation process.

a) **b)** **c)**

Figure 3.5 *Powders recovery at 40°C and xCO$_2$ =95% with different pressure in continuous process. a)150 bar; b)125bar; c)100bar.*

3.2.2.2 Effect of CO_2 mole fraction

In order to study the effect of CO_2 flow rate in the yield, various experiments in a range from 2 to 9 L/min were performed while temperature and pressure were kept constant at 100 bar and 40°C respectively. The CO_2 flow rate influenced the precipitation yield (calculated as written above) as can be seen in Table 3.5, while the corresponding precipitation curve is plotted in Figure 3.6.

Table 3.5 *Experiments conducted at different CO_2 flow rate at constant temperature (40°C) and pressure (100 bar).*

Cherries solution (mL/min)	CO_2 (L/min)	x CO_2 (%)	T (°C)	P (bar)	Drying Time (min)	Yp average yield (%)	Ya average yield (%)	Y average yield (%)
0,2	2,0	95,0	40	100	20	44,1	38,5	39,8
0,2	2,0	95,0	40	100	20			
0,2	6,0	98,2	40	100	20	42,6	59,9	54,7
0,2	6,0	98,2	40	100	20			
0,2	9,0	98,8	40	100	20	52,2	82,2	73,6
0,2	9,0	98,8	40	100	20			

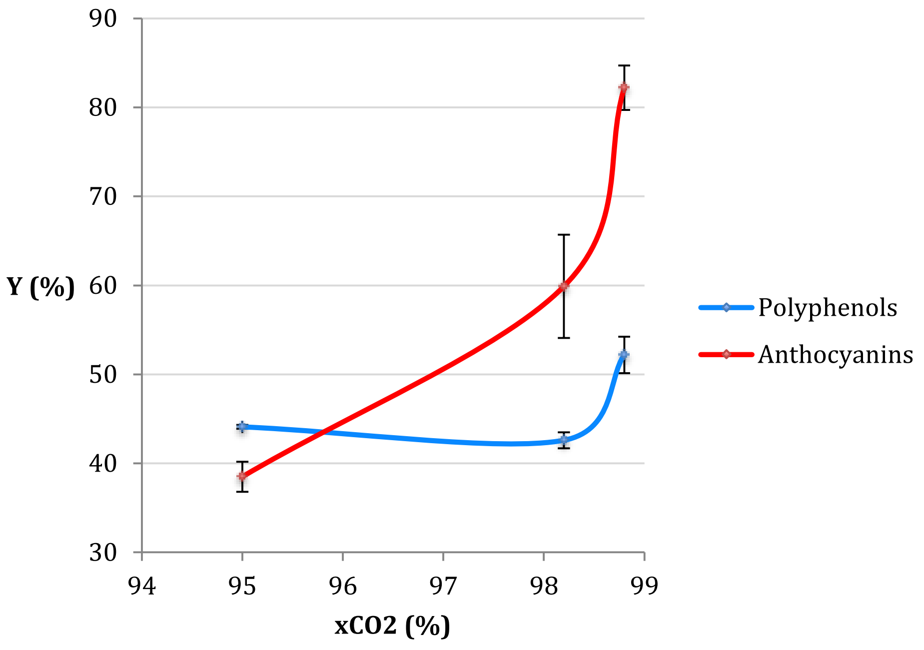


Figure 3.6 *Precipitation curve at constant T=40°C and P=100bar and different xCO_2 (95,0; 98,2; 98,8 %) in semi continuous process.*

When CO_2 flow rate is increased, the kinetic energy of dense gas atomization and mass transfer between CO_2 with solution droplet are also increased causing high mass transfer resulting in small droplets, high supersaturation, small particles (Imsanguan *et al.*, 2010). The change in CO_2 flow rate not only affects kinetic energy of SC–CO_2, but also the composition of fluid phase. When the ratio of supercritical fluid to organic solution is decreased by decreasing CO_2 flow rate (fixed solution flow rates), the fugacity coefficient also decreases leading to higher solubility of phenolic compound in SC-fluid and a lower precipitation yield, as in particular can be seen for the anthocyanins in Table 3.5. The pictures of powders recovered are displayed in figure 3.7.


a) **b)** **c)**

Figure 3.7 *Powders recovered at 40°C and P=100bar with different xCO_2 in semi continuous process. a) xCO_2 =98,8 %; b) xCO_2 =98,2 %; c) xCO_2 =95,0 %.*

3.2.2.3 Experiment conducted in subcritical region

In order to see the precipitation behaviour near to the critical point, two tests were done. The operating conditions were selected to work in correspondence of the subcritical region of the vapour-liquid equilibrium (VLE) binary diagram that is just above the mixture critical point (see figure 3.6). It was chosen to work at T=35 °C, P=75 bar and xCO_2=95%. The operating conditions are shown in the VLE diagram in figure 3.8 (Chang *et al.* 1997). The precipitation yields (calculated as written above) are summarised in table 3.7.

Table 3.7 *Experiments conducted in the subcritical zone in a semi continuous process.*

Cherries solution (mL/min)	CO_2 (L/min)	x CO_2 (%)	T (°C)	P (bar)	Drying Time (min)	Yp average yield (%)	Ya average yield (%)	Y average yield (%)
0,2	2,0	95,0	35	75	20	8,8	9,3	9,1
0,2	2,0	95,0	35	75	20			

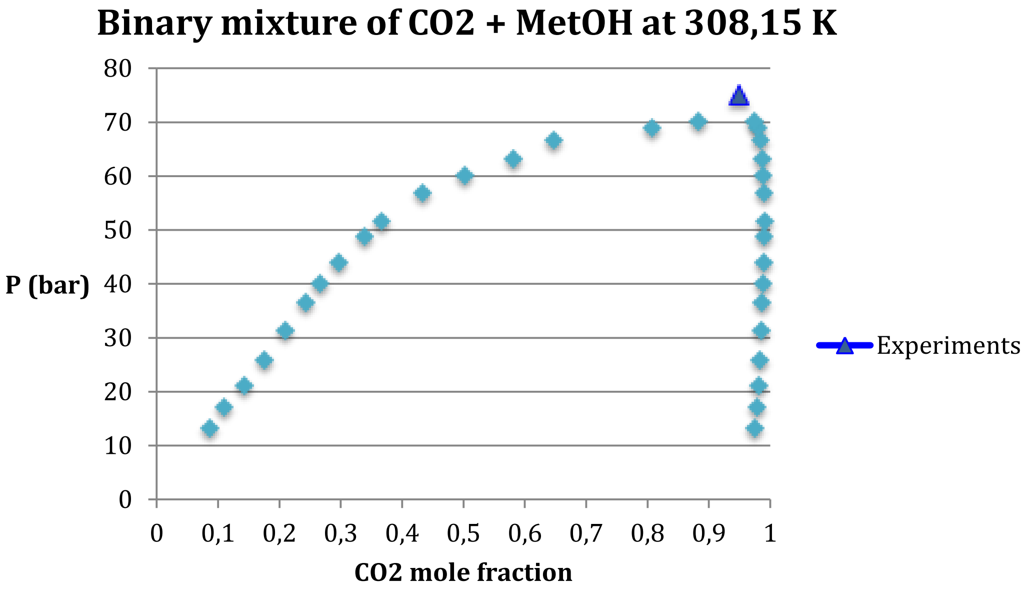


Figure 3.8 *CO_2 VLE experimental data at 308,15 K.*

How can be seen from table 3.7 the yields in polyphenols and anthocyanins are very low, due to the proximity to the two-phase region.

The results indicated that the precipitation of phenolic compounds from cherries extract is successfully performed using SAS process. The semi continuous SAS process produced solvent free particles of small size. The operating pressure and CO_2 flow rate are important variables affecting the precipitation process. It was found that total yield decrease with increasing pressure in the range of 100–150 bar and increase with increasing CO_2 flow rate in the range of 2-9 L/min. 0.5–1.5 g/min. On the other hand, the subcritical anti solvent process (P=75 bar, T=35°C and xCO2 =95%) lead to low precipitation yields.

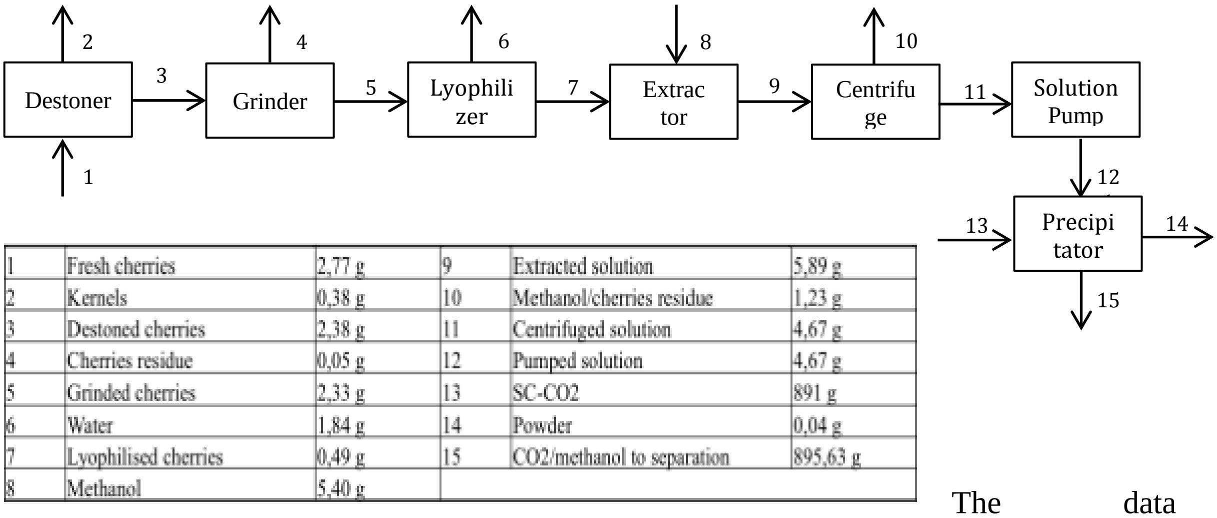
3.3 Scale-up of the process

In order to perform the life cycle analysis, (see chapter 4), the best operating conditions for the semi continuous process were chosen, in according to the results above, as reported in table 3.8.

Table 3.8 *Operating conditions and total yield of the best SAS precipitation experiment.*

Pressure (bar)	**Temperature (°C)**	**CO_2 flow rate (L/min)**	**Cherries solution (mL/min)**	**Time of injecting solution and CO_2 (min)**	**Time of drying (min)**	**Total Yield (%)**	**Powder (g)**
100	40	9	0,2	30	20	73,6	4,0E-02

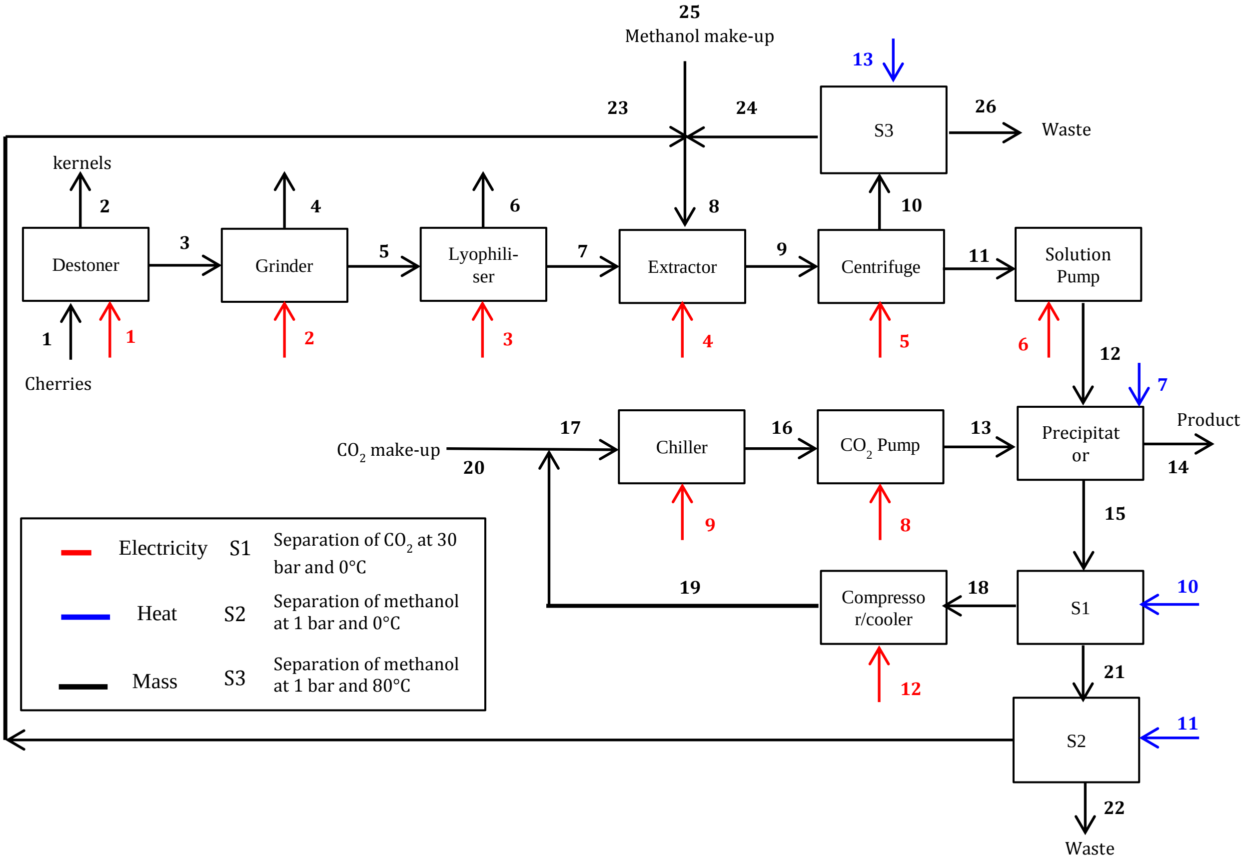
The data about the amounts consumed to produce the powder were obtained from direct measurements in the laboratory during the phase of extraction and precipitation, and are reported in figure 3.9. The data about energy consumption were recovered from technical data sheets and from process simulation. This latter was done by ASPEN Plus™ 7.3 simulator using as model the predictive Soave–Redlich–Kwong Equation of State (PSRK). The PSRK-EOS connects the group contribution model UNIFAC for the calculation of the excess free energy with Soave–Redlich–Kwong Equation of State (SRK-EOS). The parameters of the SRK-EOS are calculated with the aid of UNIFAC, they making the EOS predictive.



1	Fresh cherries	2,77 g	9	Extracted solution	5,89 g
2	Kernels	0,38 g	10	Methanol/cherries residue	1,23 g
3	Destoned cherries	2,38 g	11	Centrifuged solution	4,67 g
4	Cherries residue	0,05 g	12	Pumped solution	4,67 g
5	Grinded cherries	2,33 g	13	SC-CO2	891 g
6	Water	1,84 g	14	Powder	0,04 g
7	Lyophilised cherries	0,49 g	15	CO2/methanol to separation	895,63 g
8	Methanol	5,40 g			

Figure 3.9 *Scheme of the laboratory processes to produce the precipitate.*

The data reported in the scheme of figure 3.8 were scaled up to produce 1 kg of precipitate. Recycle of carbon dioxide and methanol have been included to improve the process performances. The data about separation of mixture methanol-carbon dioxide were obtained from ASPEN Plus™ simulation. The Block flow diagram represented in figure 3.10 shows the scale up of the process to produce 1kg of precipitate. All the relevant amounts of input/output are also reported.



1	Fresh cherries	47,38 kg	14	Powder	1,00 kg	1	Electricity	8,78 E-2 kWh
2	Kernels	6,58 kg	15	CO2/methanol to separation	15,34 kg	2	Electricity	2,99 E-2 kWh
3	Destoned cherries	40,8 kg	16	CO2 to 5°C	15,26 ton	3	Electricity	11,19 kWh
4	Cherries residue	0,87 kg	17	CO2 to 60 bar and 20°C	15,26 ton	4	Electricity	11,79 kWh
5	Grinded cherries	39,93 kg	18	Recycle CO2 to 30 bar	15,09 ton	5	Electricity	10,01 kWh
6	Water	31,53 kg	19	Recycle CO2 to 60 barand 20°C	15,09 ton	6	Electricity	9,47 E-1 kWh
7	Lyophilised cherries	8,41 kg	20	Make-up CO2	168,60 kg	7	Heat	373,26 kWh
8	Methanol	92,58 kg	21	Methanol/CO2 from S1	247,49 kg	8	Electricity	67,63 kWh
9	Extracted solution	100,89 kg	22	Waste from S2	182,62 kg	9	Electricity	54,62 kWh
10	Methanol/cherries residue	20,99 kg	23	Recycled methanol from S2	64,88 kg	10	Heat	520,15 kWh
11	Centrifuged solution	79,89 kg	24	Recycled methanol from S3	13,78 kg	11	Heat	69,17 kWh
12	Pumped solution	79,89 kg	25	Make-up methanol	13,83 kg	12	Electricity	343,64 kWh
13	SC-CO2	15,26 kg	26	Waste from S3	7,22 kg	13	Heat	5,21 kWh

Figure 3.10 *Flow chart of the process to produce 1 kg of precipitate with recycle of methanol and* CO_2

Chapter 4

Application of Life cycle assessment to the production of a precipitate rich in phenolic compounds

This chapter reports the study of Life Cycle Assessment (LCA) applied to the production of a precipitate rich in polyphenols and anthocyanins. It is divided into three parts. The first one defines the Life Cycle Assessment (LCA) methodology and the guidelines that analysts should follow, while the second and third parts are application phases. These latters describe respectively the goal and scope definition and the Life Cycle Inventory (LCI).

4.1 Life Cycle Assessment methodology

LCA, life cycle analysis, cradle to grave analysis are all different names for a single methodology born in the late sixties that watched the entire life cycle of products and materials, with particular attention to energy efficiency, the consumption of raw material and also for the disposal of scraps and wastes. This methodology grew quickly over the next thirty years becoming before the end of the twentieth century an emerging family of tools and techniques designed to help the long-term environmental management and sustainable development (EEA, 1997).

Nowadays, the increased awareness of the importance of environmental protection has prompted companies to assess how their activities have effect on the environment. The environmental impact of products and processes has thus become the key issue; so many companies are studying how to minimize their effects on the environment. A tool to do this it is represented by LCA. In the early nineties it was made an inappropriate use of LCA to promote product in a misleading way so it was necessary to standardize this methodology to consolidate the procedures and methods. Guidelines for this procedure are described in the ISO 14040 series; the first ISO standards were published in 1997 and have been the subject of subsequent updates, the last one in 2006 (EPA, 2006).

4.1.1 Methodological framework

According to the ISO 14040 standards, a Life Cycle Assessment, as illustrated in the figure 4.1 (ISO, 2006 a), is carried out in four distinct phases:

1. Goal and scope definition
2. Inventory analysis
3. Impact assessment
4. Interpretation

The phases are often interdependent; the results of one phase will inform how other phases are completed.

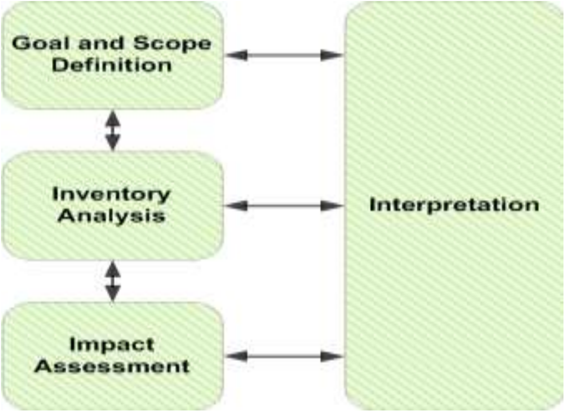


Figure4.1.*Four phase of LCA.*

LCA is an iterative technique and the depth of detail and time frame depends on the goal and scope definition.

4.1.1.1 Goal and scope definition

The first phase of the LCA is to describe the goal and scope of the study, which sets out the context of the study and explains how and to whom the results are to be communicated. This is a key step and the ISO standards require that the goal and scope of an LCA have to be clearly defined and consistent with the intended application. The goal and scope document therefore includes technical details that guide subsequent work:

- the functional unit defines what precisely is being studied and quantifies the service delivered by the product system. It provides a reference to which the inputs and outputs can be related. Further, the functional unit is an important basis that enables alternative goods or services, to be compared and analysed;
- the system boundaries;
- any assumptions and limitations;

The allocation methods used to partition the environmental load of a process when several products or functions share the same process;

- the impact categories chosen.

It is important to underline that LCA is an iterative technique, so the data and information collected, as well as the different aspects of application field, can be modified to fulfil the original goal of the study (ISO, 2006a).

4.1.1.2 Inventory Analysis

Life Cycle Inventory (LCI) analysis involves creating an inventory of flows from and to nature for a product system. Inventory flows include inputs of water, energy, and raw materials, and releases to air, land, and water. To develop the inventory, a flow model of the technical system is constructed using data on inputs and outputs. The flow model is typically illustrated with a flow chart that includes the activities that are going to be assessed in the relevant supply chain and gives a clear picture of the technical system boundaries. The input and output data needed for the construction of the model are collected for all activities within the system boundary, including from the supply chain (referred to as inputs from the techno-sphere). The data must be related to the functional unit defined in the goal and scope definition. Data can be presented in tables and some interpretations can be made already at this stage. The results of the inventory is an LCI which provides information about all inputs and outputs in the form of elementary flow to and from the environment from all the unit processes involved in the study.

The four steps for the inventory analysis are: the definition of the process flow diagram, the development of a data collection plan, the data acquisition and the presentation of the results (ISO, 2006a).

The *process diagram* is a graphical representation of the life cycle of the product concerned. In this way, it is possible to get an overview of the processes involved, including consumption and releases on the environment the more complex the flow diagram, the greater the accuracy and utility of results. Unfortunately, increased complexity also means more time and resources must be devoted to this step, as well as the data collecting and analysing (EPA, 2006).

The second stage, *the development of a data collection* plan, is to ensure the quality and accuracy of the data. The data have to be site-specific, cover a defined time period and refer to the technology required by the study.

These are divided into:

- Primary: collected directly at the production site
- Secondary: data from literature, other studies, technical manuals.
- Tertiary: data obtained from estimates, laboratory tests, environmental statistics.

Data collection (third stage) involves a combination of research, site-visits and direct contact, which generate large quantities of data. Alternatively, it is possible to use database of software that implement the LCA methodology.

Finally, *the presentation of the final results* of the life cycle inventory should describe the methodology used during the analysis.

In this phase it is necessary to consider the allocation procedures: the inputs and outputs should be assigned correctly to the product under study (ISO, 2006b).

4.1.1.3 Impact assessment

Inventory analysis is followed by impact assessment. This phase of LCA is aimed at evaluating the significance of potential environmental impacts based on the LCI flow results. Classical life cycle impact assessment (LCIA) consists of the following steps:

- *Selection and definition of impact categories,* it is an integration of the initial goal and scope definition phase. The potential impacts have as target human health, ecological health, and resource depletion.
- *Classification:* is the organisation of the LCI results into impact categories.
- *Characterization:* using characterization factors the LCI results are converted and combined into characteristic indicators of impacts categories. These factors are also called equivalency factors. With characterization is possible to directly compare the LCI results within each impact category: this means that these factors translate different inventory inputs into directly comparable impact indicators (EPA, 2006).
- *Normalization:* (optional step), the results of the impact categories from the study are usually compared with the total impacts. This procedure normalizes the indicator results that are divided by a selected reference value (EPA, 2006).
- *Grouping:*(optional step), the impact categories are assigned in one or more specific areas of concern to facilitate the interpretation of the results (EPA, 2006).
- *Weighting:*(optional step), this step provides the assignment of weights to impact based on their perceived importance or relevance (EPA, 2006).

4.1.1.4 Life cycle interpretation

Life Cycle Interpretation is a systematic technique to identify, quantify, check and evaluate information from the results of the life cycle inventory and/or the life cycle impact assessment. The results from the inventory analysis and impact assessment are summarized during the interpretation phase. The outcome of the interpretation phase is a set of conclusions and recommendations for the study.

According to ISO 14040:2006, the interpretation should include:

- Identification of significant issues based on the results of the LCI and LCIA phases of an LCA.
- Evaluation of the study considering completeness, sensitivity and consistency checks;
- Conclusions, limitations and recommendations.

4.1.2 Different approaches for the conduction of LCA

According to the different stages of the life cycle, different approaches can be identified for a LCA study:

- "from cradle to grave": this is the complete approach to assess life cycle. It considers phases from extraction of raw materials (cradle) to disposal (grave).
- "from cradle to gate": it is a partial assessment that considers phases from extraction of raw materials to input of product on market (gate), without take into account use and disposal of product.
- "from gate to gate": it is another partial approach that considers only phases about manufacturing and assembling of the product.
- "from cradle to cradle": this is a complete approach to assess the life cycle of the product. It adds at "cradle to gate" approach the recycle of product at end-life.

In addition to the approaches listed above there is another division that can be made and which has based on the level of detail of the study (EEA, 1997), as summarized below.

4.1.2.1 Simplified LCA

It is an application of LCA methodology for a comprehensive evaluation and selection. It covers the entire life cycle but it uses generic data, standard modules for transportation or energy production. It is followed by a simplified evaluation that focuses on the most important environmental aspects and/or potential environmental impacts and/or phases of the cycle of life (EEA 1997).

4.1.2.2 Conceptual LCA

This is a qualitative discussion to identify those are the phases of life cycle and/or the potential environmental impacts more significant. The greatest benefit of this approach is that it helps to focus on the entire life cycle of a product or system. The data used are qualitative and very general.

4.1.2.3 Detailed LCA

This is pure LCA analysis that follows the guidelines of the legislation. The results obtained can be used for public statements and for this reason the data should be specific and fully satisfy the quality criteria listed in the definition of the goal and scope (EEA, 1997).

4.2 Goal and scope definition

The objective of this study is to assess environmental impacts related to the production of a powder from a solution cherry extract, by supercritical anti-solvent process. In particular, the study aims to identify the most impactful phase of the life cycle of the product.
The configuration of this phase is divided according to the stage established.

4.2.1 Functional unit

The functional unit taken as reference is one kilogram of precipitate product. This is in according to Rodrìguez-Meizoso *et al*. (2012), which have studied the Life cycle assessment of supercritical fluid extraction process to obtain antioxidants from rosemary leaves. Rodrìguez-Meizoso *et al*. (2012) have taken 10 g of powder as reference. In this study the functional unit is bigger because it have been simulated an industrial- scale production.

4.2.2 System boundary

According to ISO standards, in the LCA study the process units that are included in the study must be specified. In this case, in agreement with a “from cradle to gate” approach, the process unit taken into account are:

- extraction and processing of primary materials: CO_2, methanol;
- cultivation of cherries;
- extraction and production of auxiliary materials and utilities needed to produce primary materials and cherries;
- transport of primary and auxiliary materials to the manufacturing plant;
- production process
- waste treatment of refuse production;

This work does not take into account the impacts associated to industrial equipment building and the end life of product, because it is not known the final use of it. The system boundary is shown in the flow chart of figure 4.2

4.2.3 Allocation procedure

In order to ensure the correct distribution of the inputs, such as energy, auxiliary materials and waste among the phase of process, it was assumed an allocation of cherry kernels as co-product, and this kind of allocation is based on mass. The kernels allocation is 13,9% of the total mass flow rate.

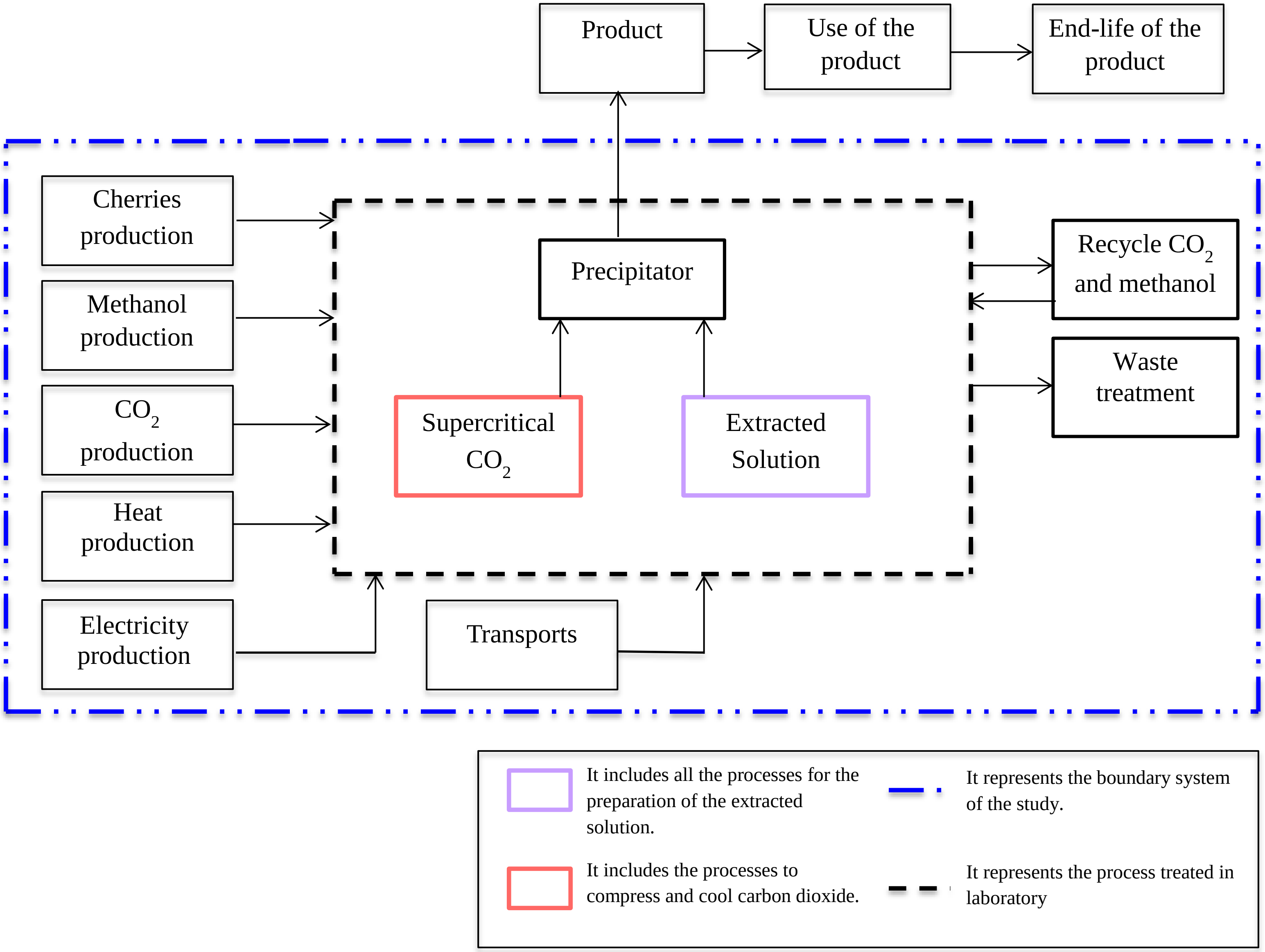


Figure 4.2 *System boundaries (personal elaboration).*

4.2.4 Method and impact categories chosen

To assess the environmental impact the software SimaPro^{TM} 7.3.2 was used. The method applied to this work is ReCiPe 2008 with approach midpoint and Hierarchistkind perspective. This perspective was chosen because it is based on more common politic principles, and it does not consider a time frame of short or long end (Goedkoop *et al.*, 2010).

The midpoint approach permits to divide the results into 18 categories of impact: *Ozone depletion, Human toxicity, Ionising radiation, Photochemical oxidant formation, Particulate matter formation, Climate change, Terrestrial ecotoxicity, Terrestrial acidification, Agricoltural land occupation, Urban land occupation, Natural land transformation, Marine ecotoxicity, Marine eutrophication, Fresh water eutrophication, fresh water ecotoxicity, Water depletion, Metal depletion, Fossil fuel depletion.* In this study four categories of impact will be analysed, which are: *Climate change, Ozone depletion, Particulate matter formation* and *fossil depletion.* This choice will be discussed later in chapter 5.

4.3 Life cycle inventory (LCI)

The second phase of the study of LCA, according to ISO standards, is the inventory analysis, which comprises data collection for the creation of an accurate and realistic model. Therefore it is convenient to develop a flow chart to identify the various stages and processes involved. The block diagram in figure 4.3 allows to highlight inputs and outputs in terms of energy, primary material and waste. Subsequently, starting from the block diagram the model is developed and implemented through SimaPro^{TM}. This organizes the model in blocks that are inter linked among them. The blocks considered in this study are subdivided into two categories:

- “processes”
- “product stages”

The category “product stages” includes two groups: “Assemblies” and “Life cycle”, while the category “Processes” is subdivided into seven groups that are created to divide better the parts of model and to easily find information. These seven groups are:

- “material”, which considers materials that will be used such as methanol, cherries, carbon dioxide and others;
- “energy”, which considers the kind of energy as electric or heat, and include the origin of it;
- “transport”, where, the blocks regarding transport can be inserted;
- “processing”, where, the blocks concerning the processes can be included;
- “use”, which is related to the usefulness of the product;

- “waste scenario”, including all blocks regarding possible scenarios for treating refuse;
- “waste treatment”: accounting for the blocks, where the kind of treatment is modeled and how it is done.

Every block is composed of different entries, which are filled or not, depending on the information that the block should contain. In these items, others existing blocks within the software are recalled so that it is possible to create the relationships between the blocks themselves. The entries that will be used inside the model are divided into “products”, “inputs” and “outputs”. These last are:

- “known outputs to techno sphere. Products and co-products”, to reports which product is referred to inputs and outputs inside the block;
- “known outputs to techno sphere. Avoided products”, to indicate which inputs and outputs of that block can be considered as production avoided. For example, the recycled methanol is avoided production of virgin methanol, as well as, carbon dioxide.

Type “inputs” entries are:

- “known inputs from nature. Resources”, accounting for the consumption of natural resources;
- “known inputs from techno sphere. Materials/fuel” related to the building materials, reactans, and fuels. We have not considered this type;
- “known inputs from techno sphere. Electricity/heat”, where the consumption of electricity, heat and transport are take into account;

Type “outputs” entries are:

- “emission to air”, which considers emissions to air;
- “emission to water”, which considers emissions to water;
- “emission to soil”, which considers emissions to soil;
- “final waste flows” includes waste, that are not specified on *Emission to air/water/soil*;
- “known outputs to techno sphere. Waste and emissions to treatment”: it regards treatment of materials at end of life.

In agreement with the standards ISO the accuracy of the study depends on the type of data chosen.

In our case data were mainly collected through literature review, empiric experiments, technical sheets for machineries, online sources and by simulation with ASPEN Plus™ software. Specific assumptions were made in case of unreliable or missing sources. Processes from the Ecoinvent 2.2 database (Frischnecht *et al.,* 2007) were used. The detailed inventory is described in the following paragraphs.

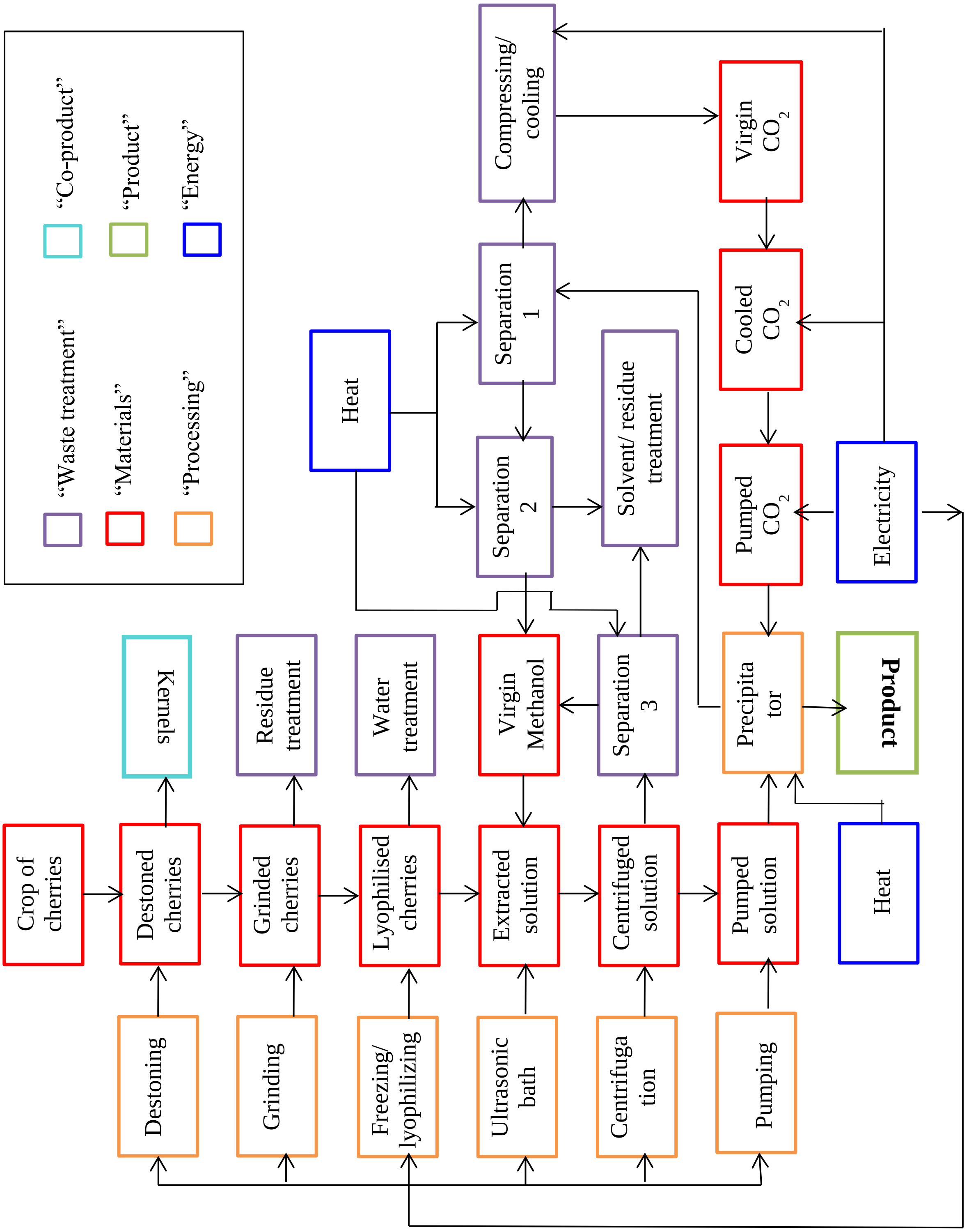


Figure 4.3 *Block diagram of the process (personal computing).*

4.3.1 Description of the Process

The process is structured in three parts: the first one to prepare cherry extract and the second one to prepare of Supercritical CO_2, linked by a unit called precipitator where supercritical carbon dioxide solubilises methanol and the product precipitates on a filter. Methanol and supercritical carbon dioxide go to the third part, called waste treatment, where methanol and carbon dioxide are separated and recycled.

The block diagram (sketched figure 4.3) shows the blocks of the process, which are divided according to colour. The red, orange, blue, and violet blocks represent respectively "Materials", "Processing", "Energy" and "Waste treatment". The green block "Product" connects among them all the blocks that have been created to describe the process and it represents the life cycle that will be investigated, while the light blue block is the co-product.

Table 4.1 shows the list of modules and their data approximation and collection.

Table 4.1 *Modules and their data collection (Personal computing from SimaPro code).*

Module	**Data collection**
Cherries	Data about cultivation of cherries are approximately compared to cultivation of peaches and are collected from literature (De Manna, *et al,* 2014). Data about production of fertilizers, pesticides and electricity belong to Ecoinvent 2.2 database (Frischnecht *et al.,* 2007). Data about consumption of cherries derived from empirical experiments.
Methanol	Data about production of methanol derived from Ecoinvent 2.2 database (Frischnecht *et al.,* 2007) while the data about consumption is collected from empirical experiments.
CO_2	Data about production of carbon dioxide belong from Ecoinvent 2.2 database (Frischnecht *et al.,* 2007) while the data about consumption is collected from empirical experiments.
Electricity of process	Data about production of electricity derived from Ecoinvent 2.2 database (Frischnecht *et al.,* 2007).
Heat of process	Data about production of heat belong to ELCD database (JRC, 2011)
Destoning	Data about consumption of electricity is collected from generic data sheet for destoning equipment. (web site[1])
Grinding	Data about consumption of electricity is collected from generic data sheet for grinding equipment. (web site[2])
Freezing and lyophilizing	Data about consumption of electricity is collected from generic data sheet for lyophilizing equipment. (web site[3])

Ultrasonic bath	Data about consumption of electricity is collected from generic data sheet for ultrasonic bath equipment. (web site[4])
Centrifugation	Data about consumption of electricity is collected from generic data sheet for centrifuge equipment. (web site[5])
Pumping 1	Data about consumption of electricity is collected by ASPEN plus simulation
Cooling	Data about consumption of electricity is collected by ASPEN plus simulation
Pumping 2	Data about consumption of electricity is collected by ASPEN plus simulation
Precipitation	Data about consumption of heat is collected by ASPEN plus simulation
Water treatment	Data about process of water treatment are collected from ELCD database (JRC, 2011)
Residues cherries treatment	Data about process of cherries residues treatment are collected from Ecoinvent 2.2 database (Frischnecht *et al.*, 2007).
Destoned cherries	Data about mass flow are collected by empirical experiments
Grinded cherries	Data about mass flow are collected by empirical experiments
Freeze and lyophilized cherries	Data about mass flow are collected by empirical experiments
Extracted solution	Data about mass flow are collected by empirical experiments
Centrifuged solution	Data about mass flow are collected by empirical experiments
Pumped solution	Data about mass flow are collected by empirical experiments
Cooled CO_2	Data about mass flow are collected by empirical experiments
Pumped CO_2	Data about mass flow are collected by empirical experiments
Precipitate	Data about mass flow are collected by empirical analysis
Separation 1	Data about mass flow and heat consumption are collected by ASPEN plus simulation
Separation 2	Data about mass flow and heat consumption are collected by ASPEN plus simulation
Separation 3	Data about mass flow and heat consumption are collected by ASPEN plus simulation
CO_2 recycle	Data about mass flow and electricity consumption are collected by ASPEN plus simulation
Transports	Data about distance is estimated, while data about vehicles are collected from Ecoinvent 2.2 database (Frischnecht *et al.*, 2007).
Residues methanol treatment	Data about treatment of solvent residues are collected from Ecoinvent 2.2 database (Frischnecht *et al.*, 2007).

4.3.2 "Energy" modules

In the blocks inside the "Energy" group, the information about electric and heat consumption are collected. The electricity module is shown in table 4.2.

Table 4.2 *Model of process: "Electricity of process" module (personal computing from SimaPro code)*

PRODUCTS			
Known outputs to techosphere. Products and co-products	**Amount**	**Unit**	**Comment**
Electricity of process	1,0	kWh	Electricity for processing
INPUTS			
Known inputs fromtechosphere. Electricity/heat	**Amount**	**Unit**	**Comment**
Electricity: medium voltage,production IT,at grid/IT S	1,0	kWh	Medium voltage electricity produced in Italy

All the electricity consumption was assumed to be medium voltage electricity, and produced in Italy. The data about production of electricity come from to Ecoinvent 2.2 database inside the software. They include the electricity production in Italy, the transmission network and direct SF6 emissions to air. Electricity losses during medium-voltage transmission and transformation from high-voltage are accounted for (Frischnecht *et al*., 2007). The other form of energy used in the process is heat. All the heat was assumed to be supplied by steam produced with natural gas combustion. The data belongs to The European Reference Life Cycle Database (ELCD), a database established by the European commision's Joint Research Centre (JRC) and integrated in the SimaPro™ software. The information about heat consumption can be seen in table 4.3.

Table 4.3 *Model of process: "Heat of process" module (personal computing from SimaPro code)*

PRODUCTS			
Known outputs to techosphere. **Products and co-products**	**Amount**	**Unit**	**Comment**
Heat of process	1,0	kWh	Heat for processing
INPUTS			
Known inputs fromtechosphere. **Electricity/heat**	**Amount**	**Unit**	**Comment**
Process steam from natural gas, heat plant, consumption mix, at plant, MJ IT S	1,0	kWh	Production of steam.

The data set of heat is calculated with an efficiency of 90% as a default value. The technology description includes the background system: the process steam is produced in a boiler fuelled by natural gas. The Italian specific fuel supply (share of resources used by import and / or domestic supply) including the energy carrier properties (e.g. element and energy content) are accounted for. In addition, Italian specific technology standards of heat plants regarding efficiency, firing technology, flue-gas desulphurisation, NO_x removal and dedusting were considered. The data set embraced the whole supply chain of the fuels from exploration over extraction and preparation to transport of fuels to the heating plants. Furthermore the data set comprises the infrastructure as well as the end-life of the plant. The background system is addressed including: all relevant and known transport processes. Overseas transports, as rail and truck transportation to and from major ports for imported bulk resources are taken into account. Furthermore all relevant and known pipeline and / or tanker transport of gases and oil imports are included. Energy carries (coal, crude oil, natural gas and uranium) are modelled according to the specific import situation. Refinery products (diesel, gasoline, technical gases, fuel oils and residues) are modelled via a country–specific, refinery parameterized model. The refinery model represents the current national standard in refinery techniques as well as the individual country-specific product output spectrum, which can be quite different from country to country. So the refinery products used show the individual country-specific use of resources. Also the supply of crude oils is modelled according to the country-specific crude oil situation with the respective properties of the resources (JRC, 2011).

4.3.3 "Processing" modules

This section shows "Processing" blocks used to model the process. The modules represent the equipment to work the flow of material, so they will be recalled inside the "material" modules. The "Processing" modules for preparation of cherry extract are:

- "Destoning": represents destoner machine to reel off cherries. Table 4.4 shows the "destoning" module, where the consumption of electricity is specified. The input to this module is the block called "electricity of process" that it was described in the section above within the "energy" group. The data about the machine electricity consumption were found in the literature [web site[15]], where data sheet specifies the consumption of electricity for working 98400 pieces of cherries. The weight of one cherry was found by direct weighed average of 100 cherries. It was calculated to be 8,2 g, so the total amount worked is 809,1 kg with a consumption of 1,5 kWh.

Table 4.4 *Model of process: "Destoning" module (personal computing from SimaPro code)*

PRODUCTS			
Known outputs to techosphere. Products and co-products	**Amount**	**Unit**	**Comment**
Destoning	809,1	kg	Cherries to destoner machine
INPUTS			
Known inputs fromtechosphere. Electricity/heat	**Amount**	**Unit**	**Comment**
Electricity of process	1,5	kWh	Electricity needed to work 98400 pieces of cherries.

- "Grinding": represents the grinder machine to obtain a puree of cherries and its input is "electricity of process". The data about the machine electricity consumption were found in literature [web site[16]], where data sheet specified the consumption of electricity for milling 1500 kg of cherries with a consumption of electricity equal to 1,1 kWh. These data are reassumed in table 4.5

Table 4.5 *Model of process: "Grinding" module (personal computing from SimaPro code)*

PRODUCTS			
Known outputs to techosphere. **Products and co-products**	**Amount**	**Unit**	**Comment**
Grinding	1500,0	kg	Cherries to grinder machine
INPUTS			
Known inputs fromtechosphere. **Electricity/heat**	**Amount**	**Unit**	**Comment**
Electricity of process	1,1	kWh	Electricity needed to grind 1500 kg of cherries.

- "Freezing and lyophilizing": represents freezer and lyophilize machines into a single block, its input is "electricity of process".
  The data in this block were found in literature [web site[17]], where the consumption of electricity was given. This equipment consumes 28 kWh to work 100 kg of cherries. The data are summarised in table 4.6.

Table 4.6 *Model of process: "Freezing and lyophilizing" module (personal computing from SimaPro code)*

PRODUCTS			
Known outputs to techosphere. **Products and co-products**	**Amount**	**Unit**	**Comment**
Freezing and lyophilizing	100,0	kg	Cherries to work
INPUTS			
Known inputs fromtechosphere. **Electricity/heat**	**Amount**	**Unit**	**Comment**
Electricity of process	28,0	kWh	Electricity needed to lyophilized 100 kg of cherries.

- "Ultrasonic bath": represents the machine used to help the extraction of antioxidants from cherries by methanol. Table 4.7 shows the "Ultrasonic bath" module, where the consumption of electricity is specified. The input of this module is the block called "electricity of process" that was described in the section above within the "energy" group. The data about the machine electricity consumption were found in literaute [web site[18]], and it is 3,7 kWh for working 40L of solution. To convert units volume to mass, it is necessary to know the density of the solution, which was assumed to be equal to methanol density (0,8 g/mL). This value underestimates real value because inside the solution there is an amount of cherries that has a higher density than methanol. Thus the environmental impacts associated with energy consumption are overestimated. For working 31,6 kg of solution the machine uses 3,7 kWh.

Table 4.7 *Model of process: "Ultrasonic bath" module (personal computing from SimaPro code)*

PRODUCTS			
Known outputs to techosphere. Products and co-products	**Amount**	**Unit**	**Comment**
Ultrasonic bath	31,6	kg	Cherries to work
INPUTS			
Known inputs fromtechosphere. Electricity/heat	**Amount**	**Unit**	**Comment**
Electricity of process	3,7	kWh	Electricity needed to extract 40 L of solution.

- "Centrifugation": represents the machine to separate solid residues of cherries from solution. It recalls "electricity of process". The data about the machine electricity consumption were found in literature [web site[19]], where a centrifuge 140 L of solution consumes to 11 kWh of electricity. At the same manner of "ultrasonic bath" the density is assumed to be equal to methanol. Therefore the consumption of electricity is 11 kWh to centrifuge 110,7 kg of solution. These data are shownin table 4.8.

Table 4.8 *Model of process: "Centrifugation" module (personal computing from SimaPro code)*

PRODUCTS			
Known outputs to techosphere. Products and co-products	**Amount**	**Unit**	**Comment**
Centrifugation	110,7	kg	Cherries to work
INPUTS			
Known inputs fromtechosphere. Electricity/heat	**Amount**	**Unit**	**Comment**
Electricity of process	11,0	kWh	Electricity needed to extract 140 L of solution.

- "Pumping 1": represents the machine to pump the solution up to 100 Bar. The input of this block is the "electricity of process" as blocks above. Data about electricity consumption were simulated by ASPEN Plus™. The table 4.9 shows the data of energy consumption calculated.

Table 4.9 *Model of process: "Pumping 1" module (personal computing from SimaPro code)*

PRODUCTS			
Known outputs to techosphere. Products and co-products	**Amount**	**Unit**	**Comment**
Pumping 1	79,9	kg	
INPUTS			
Known inputs fromtechosphere. Electricity/heat	**Amount**	**Unit**	**Comment**
Electricity of process	9,5E-1	kWh	Electricity needed to pump solution from 1 to 100 bar

The "Processing" blocks for the passage from liquid CO_2 to supercritical CO_2 are:

- "Cooling 1": represents the machine to cool the CO_2 to 5 degrees to avoid cavitation of pump. The data about the energy consumption have been obtained by ASPEN plus simulation. As shown in table 4.10, the input of this block is the "electricity of process" block within the "energy" group.

Table 4.10 *Model of process: "Cooling" module (personal computing from SimaPro code)*

PRODUCTS			
Known outputs to techosphere. Products and co-products	**Amount**	**Unit**	**Comment**
Cooling	15256,0	kg	Amount of carbon dioxide cooled
INPUTS			
Known inputs fromtechosphere. Electricity/heat	**Amount**	**Unit**	**Comment**
Electricity of process	54,6	kWh	Electricity needed to cool carbon dioxide from 20 to 5 degrees

- "Pumping 2": is the machine to pump liquid carbon dioxide up to 100 Bar. The entire in this block is the "Electricity of process" as blocks above. ASPEN plus has simulated the data about electricity consumption, as illustrated in table 4.11.

Table 4.11 *Model of process: "Pumping 2" module (personal computing from SimaPro code)*

PRODUCTS			
Known outputs to techosphere. Products and co-products	**Amount**	**Unit**	**Comment**
Pumping 2	15256,0	kg	Amount of carbon dioxide pumped
INPUTS			
Known inputs fromtechosphere. Electricity/heat	**Amount**	**Unit**	**Comment**
Electricity of process	67,6	kWh	Electricity needed to pump solution from 60 to 100 bar

- "Precipitation": recalls the "heat of process" module needed to warm constantly the mixture of methanol and supercritical carbon dioxide at 40 degrees. The source of data is the same of the three blocks above, obtained by simulation with ASPEN plus™. The consumption of energy is shown in the following table 4.12.

Table 4.12 *Model of process: "Precipitation" module (personal computing from SimaPro code)*

PRODUCTS			
Known outputs to techosphere. Products and co-products	**Amount**	**Unit**	**Comment**
Precipitation	15336,0	kg	Amount of mixture of carbon dioxide and solution warmed
INPUTS			
Known inputs fromtechosphere. Electricity/heat	**Amount**	**Unit**	**Comment**
Heat of process	373,3	kWh	Electricity needed to maintain warming the mixture at 40 degrees

Table 4.13 summarised the data related to the consumption of energy of all equipment. These are referred to production of the functional unit (one kilogram of precipitate).

Table 4.13 *Energy needed to equipment for producing the functional unit.*

Processing	Electricity kWh	Heat kWh
Destoning	8,8 E-02	-
Grinding	3,0 E-02	-
Freezing and lyophilizing	11,2	-
Extraction	11,8	-
Centrifugation	10,0	-
Pumping 1	9,5 E-01	-
Cooling 1	54,6	-
Pumping 2	67,6	-
Precipitation	-	373,3

4.3.4 "Materials" modules

This section describes all the materials involved in the process; each of them recalls its "processing" module to define the consumption of energy and the "waste treatment" module for describing the end-life of waste.

These blocks are:

- "Cherries": represents the raw material used by process; it takes into account data about the cultivation of cherries. In literature data about cherry's cultivation were not found. For this reason it has been assumed similar to cultivation of peach, which is discussed by De Manna *et al.*, (2014). Both cultivations have the same characteristics such as seasonality and method of treatment. The data are obtained from cultivation of 1 ha of peach orchard, for a period of 19 years, plus 1 year of nursery (De Manna, *et al*, 2014). The lifetime yield was estimated in 240 ton, with the following assumptions:
    - Years 1, 2 and 3 (nursery and orchard): null production;
    - Years 4 and 5 (orchard): increasing production from 0 to16 t y^{-1} (average of 8 t y^{-1});
    - Years 6 -18: constant production of 16 t y^{-1}
    - Years 19 and 20: decreasing production from 16 to 0 t y^{-1}(average of 8 t y^{-1})

  The nursery was included as an input to the orchard. All the productive inputs from pot manufacturing, electric irrigation and water, laces and supports, fertilization and application of plant products, to transport of inputs, were considered. The impact of nursery was allocated according to the estimated tree density of 1 ha orchard. The number of trees was calculated assuming a vase-shaped pruning method, with a 5x3 m distance between rows and trees. The CO_2 uptake was estimated from the carbon content of dry wood. Land occupation was considered to be equal to the life cycle of the orchard (19 ha y). Land use change was modelled basing on the assumption of the conversion from pasture (29%) and arable irrigated land (71%) to the cultivation of a permanent fruit crop.

Main agricultural operations and input materials, as well as their transport to the farm, were inventoried using Ecoinvent processes. In particular for transports it was assumed the distance of 300 km. For fertilizers use it was assumed the application of the yearly maximum amount of ammonium nitrate, triple superphosphate, and potassium chloride.

The amount of pesticides was estimated according to the following method. The lifecycle spraying volume was calculated consistent with the limit foreseen by the Regional Integrated Production Guidelines of the Emilia Romagna region. The maximum spraying volume per product was derived and multiplied by the dose suggested by the producer. The energy consumption (electricity) of a small shed of 36 m^3 was also included. The amount of electricity was estimated assuming the consumption of 1 kWh day^{-1} y^{-1}.

Onground disposal was not assumed as waste treatment for both pruning and wasted fruit, then the theoretical energy available in wood biomass has not been considered. The data for the production of 0,7 kilograms of cherries are shown in table 4.14.

Table 4.14 *Model of process: "Cherries" module (personal computing from SimaPro code)*

PRODUCTS			
Known outputs to techosphere. Products and co-products	**Amount**	**Unit**	**Comment**
Cherries	0,7	kg	Amount of cherries produced from cultivation
Known outputs to techosphere. Avoided products			
Avoided CO_2	0,1	kg	CO_2 taken from to air by plants
INPUTS			
Known inputs from nature (resources)			
Occupation, permanent crop, fruit	5,8E-4	ha a	Land occupation ha y
Transformation, from arable	2,2E-5	ha	Transformation from arable 71% of single farming
Transformation, to permanent crop, fruit	3,0E-5	ha	Transformation to crop fruit (single farming)
Transformation, from pasture and meadow	8,8 E-6	ha	Transformation from pasture 29% of single farming

Known inputs from techosphere. Materials and fuels			
Pesticide unspecific, at regional storehouse/CH S	0,4	g	Production of pesticides
Ammonium nitrate, as N, at regional storehouse/RER S	5,5	g	Production of N
Triple superphosphate, as P2O5, at regional storehouse/RER S	2,2	g	Production of P2O5
Potassium chloride, as K2O, at regional storehouse/RER S	2,8	g	Production of K2O
Irrigating/m3/CH S	0,03	m3	
Known inputs from techosphere. Electricity/heat	**Amount**	**Unit**	**Comment**
Transport, lorry 3.5-7.5t, EURO5/RER S	0,1	kgkm	Amount of kilograms of pesticide multiplied for 300 kilometres
Transport, lorry 3.5-7.5t, EURO5/RER S	1,6	kgkm	Amount of kilograms of Ammonium nitrate multiplied for 300 kilometres
Transport, lorry 3.5-7.5t, EURO5/RER S	0,7	kgkm	Amount of kilograms of triple superphosphate multiplied for 300 kilometres
Transport, lorry 3.5-7.5t, EURO5/RER S	0,8	kgkm	Amount of kilograms of potassium chloride multiplied for 300 kilometres
Electricity of process	0,02	kWh	Electricity needed to small shell

In table 4.14 some blocks are recalled, such as, production of pesticide, ammonium nitrate, K_2O, P_2O_5, irrigations, transports and avoided CO_2. These blocks are described below:

- “Avoided CO_2 “ represents the avoided carbon dioxide from air by the trees.
- “Pesticide unspecific, at regional storehouse/CH S” takes into consideration production of pesticides: including materials, energy uses, infrastructure and emissions. Data are collected in Switzerland (Frischnecht *et al.*, 2007).
- “Ammonium nitrate, as N, at regional storehouse/RER S” includes data regarding the production of ammonium nitrate from ammonia and nitric acid. Transports of the intermediate products to the fertiliser plant as well as the

  transport of the fertiliser product from the factory to the regional storehouse are accounted for, whereas, production and waste treatment of catalysts, coating and packaging of the final fertilizers product are not. Infrastructure was included by means of a proxy module (Frischnecht *et al.*, 2007).
  - "Triple superphosphate, as P2O5, at regional storehouse/RER S" unit process takes into account the production of triple superphosphate from phosphoric acid and rock phosphate. Transport of raw materials and intermediate products to the fertiliser plant as well as the transport of the fertiliser product from the factory to the regional department store were included. Production and waste treatment of catalysts, coating and packaging of the final fertilizers product were not. Infrastructure was included by means of a proxy module (Frischnecht *et al.*, 2007).
  - "Potassium chloride, as K2O, at regional storehouse/RER S": Starting from mining of potash alts, the processes of concentration of the potassium chloride, conditioning, drying and transport to the regional storage were included. The use of the resource sylvnite was accounted for, as well as the disposal of the salt residues on heaps and to rivers, and the treatment of wastewater, while coating and packaging of the final fertiliser products were not. Infrastructure was considered by means of a proxy module (Frischnecht *et al.*, 2007).
  - "Irrigating/m3/CH S" the inventory of this block deals with electricity and diesel consumption, the amount of agricultural machinery, the tool shed and further infrastructures like pump or water hose, which have to be attributed to the irrigation. It is also taken into consideration the amount of emissions to the air from combustion and the emission to the soil from tyre abrasion during the work process. The amount of water irrigated was also accounted for (Frischnecht *et al.*, 2007).
  - "Transport, lorry 3.5-7.5t, EURO5/RER S" takes into account: operation, production, maintenance and disposal of vehicles, as well as construction, maintenance and disposal of road. The data for vehicle operation, road infrastructure and vehicle disposal reflect Swiss conditions while data for vehicle manufacturing and maintenance represents generic European data (Frischnecht *et al.*, 2007).
- "Destoned cherries": represents the flow of cherries worked by "Destoning" module. The "cherries" module in input is separated from kernels; this latter was assumed to be a co-product, using an allocation procedure. In the table 4.15 are shown the data related to functional unit.

Table 4.15 *Model of process: "Destoned cherries" module (personal computing from SimaPro code)*

PRODUCTS				
Known outputs to techosphere. **Products and co-products**	**Amount**	**Unit**	**Quantity**	**Allocation %**
Destoned cherries	40,8	kg	Mass	86,1
Kernels	6,6	kg	Mass	13,9
INPUTS				
Known inputs fromtechosphere. **(Materials and fuel)**				
Cherries	47,4	kg	Amount of cherries needed to produce the functional unit	
Known inputs fromtechosphere. **Electricity/heat**	**Amount**	**Unit**	**Comment**	
Destoning	47,4	kg	It recalls the consumption of energy needed to work 47,4 kilograms.	

The data about the weight of kernels was obtained from empirical experiments, from a sample of cherries.

- "Grinded cherries": contains the information about how much cherries remain inside the equipment (table 4.16), and has to be considered as a waste to send to treatment. This data was collected by empirical experiment on laboratory scale (as the difference between weight of input and output).

Table 4.16 *Model of process: "Grinded cherries" module (personal computing from SimaPro code)*

PRODUCTS			
Known outputs to techosphere. **Products and co-products**	**Amount**	**Unit**	**Comment**
Grinded cherries	39,9	kg	
INPUTS			
Known inputs fromtechosphere. **(Materials and fuel)**	**Amount**	**Unit**	**Comment**
Destoned cherries	40,8	kg	Amount of destined cherries needed to produce the functional unit
Known inputs fromtechosphere. **Electricity/heat**	**Amount**	**Unit**	**Comment**
Grinding	40,8	kg	It recalls the consumption of energy needed to work 40,802 kilograms.
Known outputs to technosphere. **Waste and emissions to treatment**	**Amount**	**Unit**	**Comment**
Disposal, biowaste, 60% H2O, to municipal incenerition, allocation price/CH S	0,9	kg	Treatment of residual of cherries inside the machinery.

The waste was sent to treatment. The data about treatment process come from Ecoinvent system processes and includes the information about transport of incineration, water and air emissions from incineration, the auxiliary material consumption for flue gas cleaning, the short–term emissions to river water and long-term emissions to ground water from slag compartment and residual material landfill.

- "Freeze and lyophilised cherries": this module (table 4.17) is similar to the previous block. There is an amount of water separated from "grinded cherries", that was sent to waste water treatment.

  The data about this process of treatment were collected from ELCD database; they include mechanical, biological and chemical treatment steps for the wastewater and treatment steps for the sludge.

Table 4.17 *Model of process: "Freeze and lyophilised cherries" module (personal computing from SimaPro code)*

PRODUCTS			
Known outputs to techosphere. Products and co-products	**Amount**	**Unit**	**Comment**
Freeze and lyophilised cherries	8,4	kg	
INPUTS			
Known inputs fromtechosphere. (Materials and fuel)	**Amount**	**Unit**	**Comment**
Grinded cherries	39,9	kg	Amount of Grindedcherries needed to produce the functional unit
Known inputs fromtechosphere. Electricity/heat	**Amount**	**Unit**	**Comment**
Freezing and lyophilizing	39,9	kg	It recalls the consumption of energy needed to work 39,937 kilograms.
Known outputs to technosphere. Waste and emissions to treatment	**Amount**	**Unit**	**Comment**
Waste water- untreated, slightly organic contaminated EU-27-S	31,5	kg	Treatment of residual water from cherries

The amount of water contained into cherries was calculated by empirical experiment at laboratory scale. It was the difference among input ("grinded cherries") and output ("Freeze and lyophilised cherries"). The content of water in destoned cherries was calculated to be 78,95% in mass.

- "Methanol": this block describes the production of methanol and the data were found in Ecoinvent system processes (production of methanol from natural gas by steam reforming). They include raw materials, processing energy, estimate on catalyst use, emissions to air and water from process, plant infrastructure (Frischnecht *et al.*, 2007). The "methanol " module includes the transport of the methanol that, as in the previous module, required 300 kilometres. The block is shown in table 4.18.

Table 4.18 *Model of process: "Methanol" module (personal computing from SimaPro code)*

PRODUCTS			
Known outputs to techosphere. Products and co-products	**Amount**	**Unit**	**Comment**
Methanol	1,0	kg	
INPUTS			
Known inputs fromtechosphere. (Materials and fuel)	**Amount**	**Unit**	**Comment**
Methanol, at plant/GLO S	1,0	kg	Data are collected from various plants of different locations. They refers to steam reforming of natural gas.
Known inputs fromtechosphere. Electricity/heat	**Amount**	**Unit**	**Comment**
Transport, lorry 16-32t, EURO5/RER S	300,0	kgkm	Amount of methanol (1 kg) transported for 300 kilometres

- "Extracted solution": represents the module where "methanol" and "Freeze and lyophilised cherries" are mixed. The input of energy comes from the "ultrasonic bath" block. In the "extracted solution" there is not waste to send to treatment. Table 4.19 shows consumption of methanol and freeze and lyophilised cherries to produce the functional unit.

Table 4.19 *Model of process: "extracted solution" module (personal computing from SimaPro code)*

PRODUCTS			
Known outputs to techosphere. Products and co-products	**Amount**	**Unit**	**Comment**
Extracted solution	100,9	kg	
INPUTS			
Known inputs fromtechosphere. (Materials and fuel)	**Amount**	**Unit**	**Comment**
Methanol	92,5	kg	Amount of methanol to produce the solution needed to have 1 kilogram of precipitate
Freeze and lyophilised cherries	8,4	kg	Amount of Freeze and lyophilised cherries to produce the solution needed

			to have 1 kilogram of precipitate
Known inputs fromtechosphere. Electricity/heat	**Amount**	**Unit**	**Comment**
Ultrasonic bath	100,9	kg	It recalls the consumption of energy needed to work 100,9kilograms.

The amount of methanol and cherries aims to produce a solution containing 8,33% of solids, and 91,67% of methanol.

- "Centrifuged solution": in this module (shown in table 4.20) the "extracted solution" was separated in "centrifuged solution"(output) and wet methanol residues. This latter was sent to waste treatment to separate methanol from residues, which will be described in the next paragraph, "waste treatment" modules. The amount of residues was found by empirical experiments in the laboratory scale resulting in a massive percentage of refuse is 20,8% of the input.

Table 4.20 *Model of process: "Centrifuged solution" module (personal computing from SimaPro code)*

PRODUCTS			
Known outputs to techosphere. Products and co-products	**Amount**	**Unit**	**Comment**
Centrifuged solution	79,9	kg	
INPUTS			
Known inputs fromtechosphere. (Materials and fuel)	**Amount**	**Unit**	**Comment**
Extracted solution	100,9	kg	
Known inputs fromtechosphere. Electricity/heat	**Amount**	**Unit**	**Comment**
Centrifugation	100,9	kg	It recalls the consumption of energy needed to work 100,886 kilograms.
Known outputs to technosphere. Waste and emissions to treatment	**Amount**	**Unit**	**Comment**
Separation 3	21,0	kg	Separation of methanol to send to recycle

- "Pumped solution": this module simply recalls the consumption of energy to pump the amount of solution needed to produce the functional unit. Data are shown in table 4.21.

Table 4.21 *Model of process: "Pumped solution" module (personal computing from SimaPro code)*

PRODUCTS			
Known outputs to techosphere. Products and co-products	**Amount**	**Unit**	**Comment**
Pumped solution	79,9	kg	
INPUTS			
Known inputs fromtechosphere. (Materials and fuel)	**Amount**	**Unit**	**Comment**
Centrifuged solution	79,9	kg	
Known inputs fromtechosphere. Electricity/heat	**Amount**	**Unit**	**Comment**
Pumping 1	79,9	kg	It recalls the consumption of energy needed to work 79,9 kilograms.

- "CO_2": represents liquid carbon dioxide assumed to be at 60 bar and 20 °C. This module recalls the block to produce it called "Carbon dioxide liquid, at plant/RER S". It was created by Ecoinvent system processes within SimaPro™ and contains the information about material and energy input and emissions for the production of liquid carbon dioxide out of waste gases from different production processes. Water consumption and infrastructure have been estimated. Data are based on a Swiss study, but they are assumed to be valuable for European conditions (Frischnecht *et al.,* 2005). Moreover "CO_2" module contains transport to the process site. As in the previous modules a distance of 300 kilometres was considered. Table 4.22 shows "CO_2" module.

Table 4.22 *Model of process: "CO_2" module (personal computing from SimaPro code)*

PRODUCTS			
Known outputs to techosphere. Products and co-products	**Amount**	**Unit**	**Comment**
CO_2	1,0	kg	
INPUTS			
Known inputs fromtechosphere. (Materials and fuel)	**Amount**	**Unit**	**Comment**
Carbon dioxide liquid, at plant/RER S	1,0	kg	It recalls the data about production of liquid carbon dioxide.
Known inputs fromtechosphere. Electricity/heat	**Amount**	**Unit**	**Comment**
Transport, lorry 16-32t, EURO4/RER S	300,0	kgkm	Amount of CO_2 (1 kg) transported for 300 kilometres

- "Cooled CO_2": the input of this module is only the energy needed to cool the amount of carbon dioxide to produce the functional unit. It is shown in table 4.23.

Table 4.23 *Model of process: "Cooled CO_2" module (personal computing from SimaPro code)*

PRODUCTS			
Known outputs to techosphere. Products and co-products	**Amount**	**Unit**	**Comment**
Cooled CO_2	15256,0	kg	
INPUTS			
Known inputs fromtechosphere. (Materials and fuel)	**Amount**	**Unit**	**Comment**
CO_2	15256,0	kg	
Known inputs fromtechosphere. Electricity/heat	**Amount**	**Unit**	**Comment**
Cooling 1	15256,0	kg	It recalls the consumption of energy needed to work 15256 kilograms.

- "Pumped CO_2": as the module above, it recalls only the energy needed to pump the amount of carbon dioxide to produce the functional unit (shown in table 4.24).

Table 4.24 *Model of process: "Pumped CO_2" module (personal computing from SimaPro code)*

PRODUCTS			
Known outputs to techosphere. Products and co-products	**Amount**	**Unit**	**Comment**
Pumped CO_2	15256,0	kg	
INPUTS			
Known inputs fromtechosphere. (Materials and fuel)	**Amount**	**Unit**	**Comment**
Cooled CO_2	15256,0	kg	
Known inputs fromtechosphere. Electricity/heat	**Amount**	**Unit**	**Comment**
Pumping 2	15256,0	kg	It recalls the consumption of energy needed to work 15256 kilograms.

- "Precipitate": this module (shown in the table 4.25) is the final product of the process, assembly all the modules earlier considered. It links the two part of the process: the production of cherry's solution and the production of supercritical CO_2.

Table 4.25 *Model of process: "Precipitate" module (personal computing from SimaPro code)*

PRODUCTS			
Known outputs to techosphere. Products and co-products	**Amount**	**Unit**	**Comment**
Precipitate	1,0	kg	Functional unit
INPUTS			
Known inputs fromtechosphere. (Materials and fuel)	**Amount**	**Unit**	**Comment**
Pumped CO_2	15256,0	kg	
Pumped Solution	79,9	kg	
Known inputs fromtechosphere. Electricity/heat	**Amount**	**Unit**	**Comment**
Precipitation	15335,9	kg	It recalls the consumption of energy.
Known outputs to technosphere. Waste and emissions to treatment	**Amount**	**Unit**	**Comment**
Separation 1	15334,9	kg	Separation of CO_2 to send to recycle

In this module it happens that supercritical CO_2 is dissolved in methanol solution, which releases the precipitate that is collected on a filter. On the countrary the “waste solution” containing methanol, supercritical CO_2 and compounds not precipitated, exits from the precipitator. Waste solution is sent to “Separation 1” that belongs to “Waste Treatment” group and will be described in the following.

4.3.5 “Waste Treatment” modules

After the separation of precipitate, the “waste solution” goes to section of waste treatment where the flows are separated to be recycled. The “Waste Treatments” modules are:

- “Separation 1”: in this block there is a release of pressure from 100 to 30 Bar at 0 degrees. Two phases are created a gas one with almost all CO_2 and a liquid one containing methanol, CO_2 and compounds not precipitated. This latter is sent to “separation 2”, while the gas phase is fed back as to ”recycled CO_2”. This module contains the energy needed to warm the equipment, as shown in table 4.26.

Table 4.26 *Model of process: “separation 1” module (personal computing from SimaPro code)*

PRODUCTS			
Waste specification	**Amount**	**Unit**	**Comment**
Separation 1	15334,9	kg	
INPUTS			
Known inputs fromtechosphere. Electricity/heat	**Amount**	**Unit**	**Comment**
Heat of process	520,1	kWh	It recalls the consumption of energy needed to worm
Known outputs to technosphere. Waste and emissions to treatment	**Amount**	**Unit**	**Comment**
CO_2 recycle	15087,4	kg	Recycled CO_2
Separation 2	247,5	kg	Separation of methanol to send to recycle

- “CO_2 recycle”: this module contains the amount of energy needed to compressing and cooling carbon dioxide to return to the initial condition of 60 Bar and 20 degrees. This recycled CO_2 reduces the use of virgin CO_2. So this block recalls “CO_2” module as avoided products, as shown in table 4.27.

Table 4.27 *Model of process: "CO_2 recycle" module (personal computing from SimaPro code)*

PRODUCTS			
Waste specification	**Amount**	**Unit**	**Comment**
CO_2 recycle	15087,4	kg	
Known outputs to technosphere. **Avoided products.**	**Amount**	**Unit**	**Comment**
CO_2	15087,4	kg	Amount of CO_2 avoided.
INPUTS			
Known inputs fromtechosphere. **Electricity/heat**	**Amount**	**Unit**	**Comment**
Electricity of process	178,1	kWh	Consumption of energy needed to cool
Electricity of process	165,6	kWh	Consumption of energy needed to compress

- "Separation 2": in this block there is a depressurisation from 30 to 1 bar to 0 °C. Two phases are created, a liquid one with almost all methanol and a gas one containing methanol, CO_2 and compounds not precipitated. In this block it is assumed that these wastes are separable. Methanol and residues are sent to treatment, while CO_2 is released to air. Furthermore this block contains the energy needed to warm the equipment at 0 degrees, as shown in table 4.28.

Table 4.28 *Model of process: "Separation 2" module (personal computing from SimaPro code)*

PRODUCTS			
Waste specification	**Amount**	**Unit**	**Comment**
Separation 2	247,5	kg	
INPUTS			
Known inputs fromtechosphere. **Electricity/heat**	**Amount**	**Unit**	**Comment**
Heat of process	69,2	kWh	Consumption of energy needed to warm
Emissions to air	**Amount**	**Unit**	**Comment**
Carbon dioxide	179,0	kg	Carbon dioxide loss in air
Known outputs to technosphere. **Waste and emissions to treatment**	**Amount**	**Unit**	**Comment**

Methanol recycle	64,9	kg	Recycled CO_2
Disposal, solvent mixture, 16.5% water, to hazardous waste incineration/ CH S	3,6	kg	Treatment of waste

The data about the process of waste treatment ("Disposal, solvent mixture, 16.5% water, to hazardous waste incineration/ CH S") were collected from Ecoinvent system processes database. The data include waste-specific air and water emissions from incineration, auxiliary material consumption for flue gas cleaning, short-term emissions to river water and long-term emissions to ground water from residual material landfill and energy demand for the process.

- Separation 3: the waste from "centrifuged cherries" contains cherry's residues and methanol. These are separated, methanol is sent to "methanol recycle", while cherry's residues are sent to treatments. It has been assumed that cherry's residues go to "Disposal, biowaste, 60% H2O, to municipal incineration, allocation price/CH S" (described in "grinded cherries" module), and methanol's residues go to "Disposal, solvent mixture, 16.5% water, to hazardous waste incineration/ CH S" (described in "separation 2"). "Separation 3" block contains the energy needed to warm the equipment up to 80 degrees, as shown in table 4.29.

Table 4.29 *Model of process: "Separation 3" module (personal computing from SimaPro code)*

PRODUCTS			
Waste specification	**Amount**	**Unit**	**Comment**
Separation 3	21,0	kg	
INPUTS			
Known inputs fromtechosphere. Electricity/heat	**Amount**	**Unit**	**Comment**
Heat of process	5,2	kWh	Consumption of energy needed to warm
Known outputs to technosphere. Waste and emissions to treatment	**Amount**	**Unit**	**Comment**
Methanol recycle	13,8	kg	Recycled CO_2
Disposal, solvent mixture, 16.5% water, to hazardous waste incineration/ CH S	0,2	kg	Treatment of solvent waste
Disposal, biowaste, 60% H2O, to municipal incineration, allocation price/CH S	7,0	kg	Treatment of cherry's residues

- "Methanol recycle": in this module methanol from "separation 3" and "separation 2" is recycled. This block specifies the amount of virgin methanol avoided as shown in table 4.30.

Table 4.30 *Model of process: "Methanol recycle" module (personal computing from SimaPro code)*

PRODUCTS			
Waste specification	**Amount**	**Unit**	**Comment**
Methanol recycle	78,7	kg	13,8 from "Separation 3" and 64,9 from "Separation 2"
Known outputs to technosphere. Avoided products.	**Amount**	**Unit**	**Comment**
Methanol	78,7	kg	Amount of Methanol avoided.

Chapter 5

Life cycle impact assessment and interpretation

This chapter is divided into two parts. The first one describes the choice of the impact categories selected and the results of characterization and normalization. The second one explains the sensitivity analysis in order to evaluate some improvements in the process.

5.1 Impact assessment

The third phase specified by ISO 10140:2006 consists in the evaluation of the potential impacts due to consumption of resources and energy and emissions reported in LCI. The impact assessment (LCIA) objective is the correlation between the product system and the impact on the environment.

The elements in the impact assessment are: selection and definition of impact categories, classification, characterization, normalization, aggregation, weighting, evaluation and preparation of the final report. In this case only the first three phases, classification, characterization and normalization, have been considered. The SimaPro™ software provides several methods for impact assessment, among which ReCiPe 2008 has been chosen (Goedkoop *et al.,* 2010). According to the selected approach (midpoint) only the first two phases are mandatory, while the others are optional. To evaluate the results easier, it has been chosen to assess the impacts through some groups, which contain the blocks modelled above. In this manner the impact linked to a specific section can be evidenced. With the "Group analysis" command it is possible to research the processes used by the model and to choose the most important to consider. The groups created are six and they include following blocks:

- *Cherries:* contains the block "Cherries".
- *Methanol*: includes the blocks where the use of Methanol is considered such as "Methanol recycle", "Methanol"
- *CO_2*: includes the blocks where the use of CO_2 is considered such as "CO_2recycle", "CO_2"
- *Electricity*: contains the block "Electricity of process"
- *Heat*: includes the block "Heat of process"

- *Waste*: contains all blocks where there is a treatment of waste and emissions, such as “grinded cherries”, “freeze and lyophilised cherries”, “separation 3” and “separation 2”.

5.1.1 Selection of the impact categories

For the selection of impact categories that have to be examined, ILCD handbook guidelines (JRC, 2011) have been taken into account. In these guidelines, the recommended characterisation models and associated characterisation factors are classified, according to their quality, into three levels: “I” (recommended and satisfactory), level “II” (recommended but in need of some improvements), level “III” (recommended, but to be applied with caution). We decided to assess categories at level “I”, they are: *Climate change, Ozone depletion and Particulate matter formation.*

In the next paragraph the uncertainty analysis will be done in order to ensure that the choices made are consistent.

5.1.1.1 Uncertainty analysis

It is a procedure to determine how the uncertainties of data and the assumptions if the calculations affect the reliability of the results (ISO, 2006b). In general a study of Life Cycle Assessment has at least two types of uncertainty: the first one is the normal uncertainty associated with the determination of a parameter in a given system, the second one consists in choosing the value of a given parameter to represent a similar system (Steen *et al.*, 1997). Often the data used are given as average values, without any indication of relative uncertainty. Recently it was developed a procedure in order to quantify the uncertainty of the data, using a qualitative assessment of data quality (Scipioni *et al.,* 2009). This assessment is made on the basis of a pedigree matrix, developed by Pedersen & Weidema Wesnae (Weidema *et al.*, 1996), so called because the quality indicators (data) refer to the data source. In particular, the sources of these data are evaluated according to the following features: reliability, completeness, temporal correlation, geographical correlation, further technological correlation and the sample size, in agreement with what reported in figure 5.1. The indicators, so identified, are transformed into the uncertainty factors, based on the values reported in table 5.1. Then, the uncertainty factors are inserted in the equation 5.1 in order to determine the square of the geometric standard deviation, equal to 95% range.

$$SD_{g95} = \sigma_g^2 = exp\ \overline{[\ln(U1)]^2 + [\ln(U2)]^2 + [\ln(U3)]^2 + [\ln(U4)]^2 + [\ln(U5)]^2 + [\ln(U6)]^2} \quad (5.1)$$

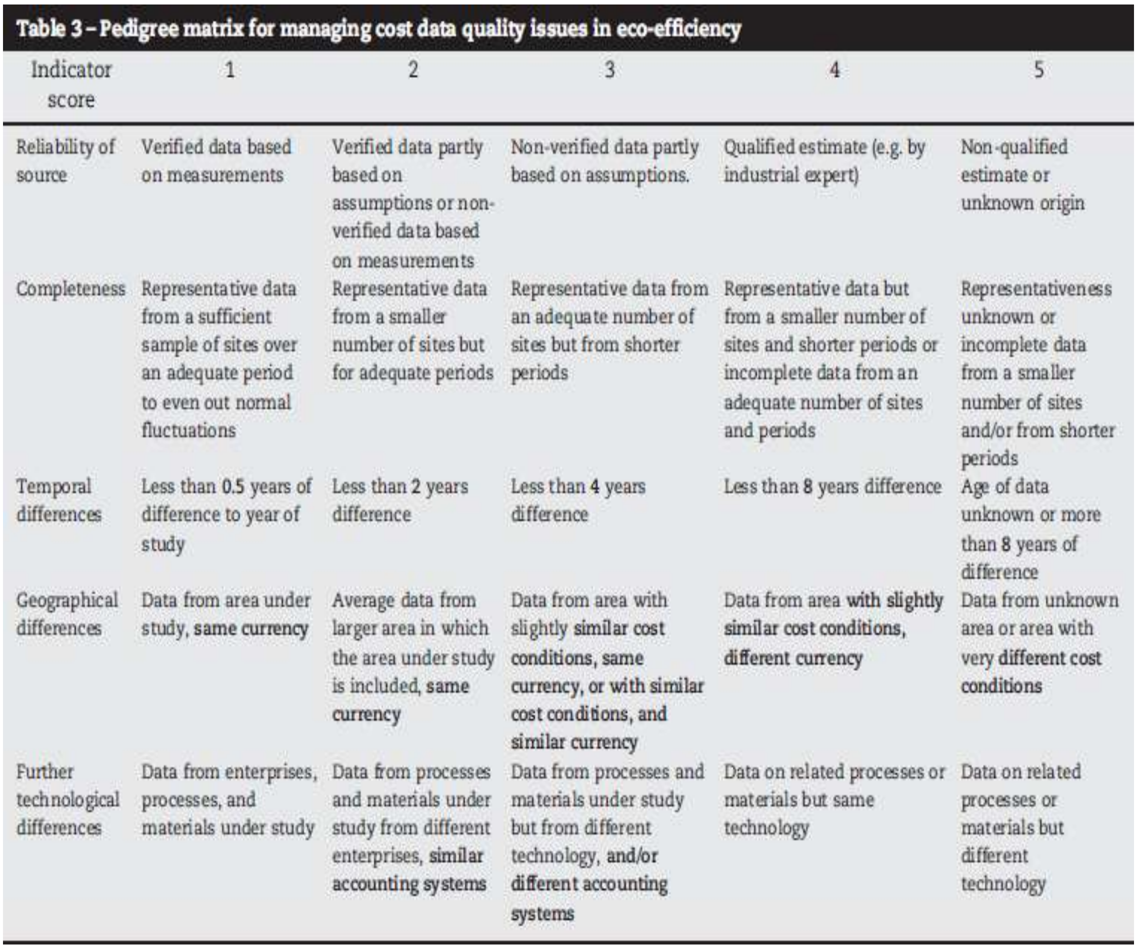


Table 3 – Pedigree matrix for managing cost data quality issues in eco-efficiency

Indicator score	1	2	3	4	5
Reliability of source	Verified data based on measurements	Verified data partly based on assumptions or non-verified data based on measurements	Non-verified data partly based on assumptions.	Qualified estimate (e.g. by industrial expert)	Non-qualified estimate or unknown origin
Completeness	Representative data from a sufficient sample of sites over an adequate period to even out normal fluctuations	Representative data from a smaller number of sites but for adequate periods	Representative data from an adequate number of sites but from shorter periods	Representative data but from a smaller number of sites and shorter periods or incomplete data from an adequate number of sites and periods	Representativeness unknown or incomplete data from a smaller number of sites and/or from shorter periods
Temporal differences	Less than 0.5 years of difference to year of study	Less than 2 years difference	Less than 4 years difference	Less than 8 years difference	Age of data unknown or more than 8 years of difference
Geographical differences	Data from area under study, **same currency**	Average data from larger area in which the area under study is included, **same currency**	Data from area with slightly **similar cost conditions, same currency, or with similar cost conditions, and similar currency**	Data from area **with slightly similar cost conditions, different currency**	Data from unknown area or area with very **different cost conditions**
Further technological differences	Data from enterprises, processes, and materials under study	Data from processes and materials under study from different enterprises, **similar accounting systems**	Data from processes and materials under study but from different technology, **and/or different accounting systems**	Data on related processes or materials but same technology	Data on related processes or materials but different technology

Figure 5.1 *Pedigree matrix used to evaluate the quality relative to the data sources (Ciroth, 2009).*

Table 5.1 *Default uncertainty factors (contributing to the square of the geometric standard deviation) applied together with the pedigree matrix (Frischknecht et al., 2005).*

Indicator score	1	2	3	4	5
Reliability	1.00	1.05	1.10	1.20	1.50
Completeness	1.00	1.02	1.05	1.10	1.20
Temporal correlation	1.00	1.03	1.10	1.20	1.50
Geographical correlation	1.00	1.01	1.02		1.10
Further technological correlation	1.00		1.20	1.50	2.00
Sample size	1.00	1.02	1.05	1.10	1.20

Uncertainly factors in the formula 5.1 are briefly defined (Weidema *et al.*, 1996).

- U1: is linked to the reliability of the data, which corresponds to “Reliability” (R) in the Matrix Pedigree, as well as in Figure 5.1. This factor is based on sources, the methods of acquisition and employed on the verification procedures for obtaining such data.

- U2 is concerning the completeness of the data, which is “Completeness” (C) in the Matrix Pedigree, as well as in Figure 5.1. This factor examines the properties of the data statistics, their representativeness, if the sample contains a sufficient number of data and finally, if the period is adjusted to take into account fluctuations.
- U3: is about the temporal correlation, which is “Temporal correlation” (T) in the Matrix Pedigree, as well as in Figure 5.1. This refers to the temporal correlation between the year of the study (according to what is specified in the target definition) and the year in which data refer.
- U4: is the geographical correlation factor of uncertainty, which is “Geographic correlation” (G) in the Matrix Pedigree, as well as in Figure 5.1. It evaluates the geographical correlation between the defined area in the study and the data actually used.
- U5: is related to other technological correlations, which correspond to “Other technological correlation” (TC) in the Matrix Pedigree, as well as in Figure 5.1. This factor considers the technological correlations related to the processes and materials.
- U6: refers to the size of the sample considered, which corresponds to “Sample size” (S) in the Matrix Pedigree, as well as in Figure 5.1.

To perform an uncertainty analysis, there are two different methods: one through a statistic sampling and the other one by analytical formulas based on error propagation. A well known random sampling method is represented by the analysis of Monte Carlo, whose basic procedure is the following (Heijungs *et al.*, 2005):

- Each input parameter is regarded to as a stochastic variable, to which a specific probability distribution is assigned;
- The LCA model is built with a particular configuration on the basis of the stochastic parameters;
- The LCA study results are calculated with this specific configuration;
- The two previous stages are repeated according to the number of assigned iterations;
- The results have been analysed based on the statistical properties such as average, median, standard deviation and confidence intervals related to the sample of the LCA results.

5.1.1.2 Uncertainty analysis applied to the case study

The uncertainty analysis was performed through the application of ReCiPe 2008 methodology, at midpoint level. It was chosen the lognormal distribution, according to reports in SimaPro manual (PRé Consultants, 2010).

The standard deviation values should not be calculated for the data belonging to Ecoinvent database because these are already indicated in the computer code. A single value of standard deviation ($SD_{g\ 95}$) was calculated for all the other data. The indicators identified in figure 5.1

were transformed into the uncertainty factors shown in table 5.2. Then, the uncertainty factors obtained were used in equation 5.1. The value of $SD_{g\ 95}$ obtained in this way is equal to 1.60.

Table 5.2 *Default uncertainty factors chosen (contributing to the square of the geometric standard deviation)*

	Value
Reliability	1.10
Completeness	1.10
Temporal correlation	1.20
Geographical correlation	1.01
Further technological correlation	1.50
Sample size	1.05

Then the Monte Carlo simulation was started: accordingly SimaPro™ samples at random a set of values on the basis of the distribution assigned to the significant inventory data, recalculates the LCA for each parameter, stores its result and depicts the distribution of these results. Figure 5.2 shows the results of the analysis of uncertainty.

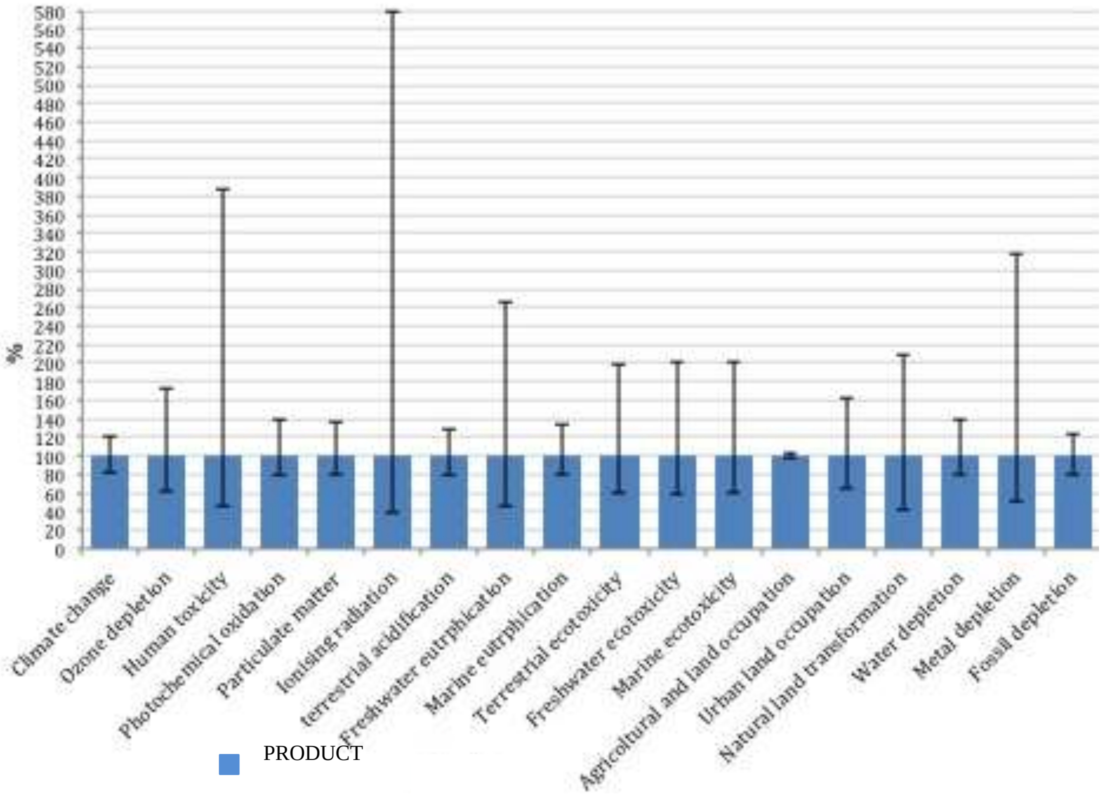


Figure 5.2 *Uncertainty analysis carried out using the methodology ReCiPe 2008 at midpoint level*

From figure 5.2 it is clear that the categories recommended by the ILCD handbook guidelines (JRC, 2011) (i.e. *Climate change, Ozone depletion and Particulate matter formation)* are consistent with the results obtained from the analysis of uncertainty, which is lower enough. Also Fossil depletion was chosen to be assess, since from the results will be displayed in paragraph 5.1.2, it will be seen the largest impact caused from energy consumption. Moreover, the result obtained from the analysis of uncertainty for category *fossil depletion* is lower enough.

So the four impact categories chosen in according to ILCD handbook guidelines and uncertainty analysis are:

- *Climate change*: considers the effects caused by the issue of greenhouse gases in the atmosphere, in addition to human activities, which affect their atmospheric concentration. These gases have the ability to absorb infrared radiation reflected from the earth (radiative forcing), which is modelled by determining the change in concentration of the test substance and the consequent absorption of the infrared radiation. Furthermore, it is also considered the residence time of the substance. For this category of impact it has been developed a model, by the IPCC and recognized worldwide, which determines the radiative forcing of each greenhouse gas, by expressing it in terms of Global Warming Potentials (GWP). Specifically, the GWP is used to express the contribution to the greenhouse effect, by date gaseous emissions into the atmosphere, by calculating for a specific time interval. All refer to the CO2 molecules, whose GWP value was assumed to be equal to 1 (IPCC, 2007). The model used in the ReCiPe 2008 for the midpoint assessment is the GWP 100, Climate Change 2007 IPCC that are used to midpoint characterization factors belonging to the IPCC list of 2007, calculated considering a time horizon of 100 years. The reference substance used is carbon dioxide and the unit appears to be increasingly expressed in kg CO_2 eq.
- *Ozone depletion*: This impact category considers the reduction of the ozone layer, which is dramatically enhanced by substances emitted by human activities, and persistent in the atmosphere. Ozone is a gas that is continuously created and destroyed by sunlight and chemical reactions in the stratosphere. This gas is essential for life because it prevents UV-B ultraviolet radiation, but it is harmful to human health, to aquatic ecosystems and the plants belonging terrestrial ecosystem, to achieve the lowest levels of the atmosphere. The substances responsible for the depletion of the ozone layer, known as ozone depletion substances (ODS), are the chlorofluorocarbons and halogens, and which, contain elements such as chlorine and bromine, which, thanks to their persistence, manage to get into the stratosphere and act as catalysts in a series of reactions of ozone degradation, as follows.

$$\mathrm{Cl} + \mathrm{O_3} \rightarrow \mathrm{ClO\cdot} + \mathrm{O_2} \tag{5.2}$$

$$\mathrm{Br} + \mathrm{O_3} \rightarrow \mathrm{BrO\cdot} + \mathrm{O_2} \tag{5.3}$$

$$\mathrm{ClO\cdot} + \mathrm{O\cdot} \rightarrow \mathrm{Cl\cdot} + \mathrm{O_2} \tag{5.4}$$

$$\mathrm{ClO\cdot} + \mathrm{BrO\cdot} \rightarrow \mathrm{Cl\cdot} + \mathrm{Br\cdot} + \mathrm{O_2} \tag{5.5}$$

The methodology ReCiPe 2008 as the midpoint evaluation model uses the template WMO 2003 (WMO, 2003). It classifies the ODS as follows:

  - Chlorofluorocarbons (CFCs);
  - Hydrochlorofluorocarbons (HCFCs) and hydrobromofluorocarbons (HBFCs);
  - Halons (brominated CFCs);
  - Carbon tetrachloride (CCl_4);
  - Methyl chloroform (CH_3CCl_3);
  - Methyl bromide (CH_3Br).

The unit used to evaluate this category is kg of CFC-11 equivalent.

- *Particulate matter formation*: according to the content of the ILCD Handbook (JRC, 2010b), this category considers the impact on human health, generated by fine particles (Particulate Matter (PM)), for which there are two types of emissions: primary (primary PM) and secondary ones (secondary PM). In particular, the secondary emissions result from emissions of SO_2 and NO_x, which create aerosols of sulphates and nitrates. The fine particles can be measured in different ways, such as:
  - Total suspended particulate matter (total suspended particulates (TSP);
  - Powders with smaller than 10 microns in diameter (PM 10);
  - Powders with less than 2.5 microns in diameter (PM 2.5);
  - Powders with less than 0.1 microns in diameter (PM 0.1).

Recipe 2008 uses as a reference the dust with a diameter smaller than 10 microns (PM10). As a midpoint model, the European air transport model EUTREND is applied (Van Jaarsveld, 1995; Van J. *et al.*, 1997), to calculate the fraction PM10 low as SO_4^{2-}, NH_4^+, NO_3^- and to determine the relative fraction of the aerosol PM10 associated with the emission of SO_2, NH_3 , NO_x and PM10 (Gooedkoop *et al.*, 2009). This category is evaluated in kg of equivalent PM10.

Fossil depletion: As reported in the manual ILCD (JRC, 2010b) this impact category examines the depletion of resources. In particular, fossil depletion considers the effects of the use of non-renewable resources (fossil fuels). The characterization factors used, to midpoint level, for this category are calculated as the ratio between the energy content (EDC) of the substance in question and that of the reference substance. The factors used refer to Ecoinvent database (Frischknecht *et al*., 2007). This category is evaluated in kg of equivalent fuel oil.

5.1.2 Characterization

Using characterization factors the LCI results are converted and combined into characteristic indicators of impact categories. With characterization it is possible to directly compare the LCI results within each impact categories: it means that these factors translate different inventory inputs into directly comparable impact indicators (EPA, 2006).

Within the software the results for each category are expressed with positive or negative values. Positive values correspond to an environmental damage, while negative impact avoids damage. Following, the results of the characterization are shown in tabular form (in table 5.3a and 5.3b) and graphically (figure 5.3).

To help extrapolation of the significant values of each category some commands were used as "specification for groups" and "specification for substances".

- *Climate Change*: 941 kg CO_2 eq.

  The major contribution to this category is given by electricity production (329 kg CO_2 eq.) that is due to its consumption in the phases of CO_2 transformation from liquid to supercritical and CO_2 recycles. The others contributions come from: heat production (276,3 kg CO_2 eq.), waste (188 kg CO_2 eq.), CO_2 production (138 kg CO_2 eq.) and methanol production (11 kg CO_2 eq.). Regarding the waste, the emission on air of carbon dioxide impacts for 95,2%. Cherries have an avoided impact (-0,3 kg CO_2 eq.) that is due to uptake CO_2 by trees. The mainly substances that cause the total impact are carbon dioxide and methane (emitted principally in the production of carbon dioxide), corresponding to 90% and 9% respectively. The remaining 1% comes from others substances such as Sulfur hexafluoride, ethane, Dinitrogen monoxide and others.
- *Ozone Depletion*: 4,2E-05kg CFC-11 eq.

  The production of electricity contributes most to this category for 69% (2,9E-05kg CFC-11 eq.). The production of carbon dioxide gives an impact of 21,7% (9,1E-06 kg CFC-11 eq.). The others can be considered negligible. In particular the impact is due to some substances, the most abundant are Bromotrifluoromethane (also called Halon 1301) and Bromochlorodifluoromethane (also called Halon 1211).
- *Particulate Matter Formation*: 0,6 kg PM10 eq.

  The largest impact is given by consumption of energy, i.e from its production. It is 64,8% of total (0,4 kg PM10 eq.) and is due to sufur dioxide and nitrogen oxides. The other contributions are production of carbon dioxide for (16,9%) and the consumption of heat for (15,5%).

- *Fossil Depletion*: 260 kg oil eq.

  The major contributions to this category are given by the production of electricity and heat and these have the same impacts, which are 42,3% of total (110 kg oil eq.). Then the productions of carbon dioxide and methanol have a minor impact in this category. These are respectively 13,2% and 4,8%.

  The impact to the production of electricity is due to natural gas, crude oil, and hard coal in ground while that of the production of heat is mostly caused from natural gas.

Table 5.3a *Results of the characterization phase for the life cycle of the precipitate (personal computing from SimaPro)***.**

Impact category	Unit	Total	Cherries	Methanol	CO2
Climate change	kg CO2 eq.	9,4E+02	-3,0E-01	1,1E+01	1,4E+02
Ozone depletion	kg CFC-11 eq.	4,2E-05	6,6E-07	2,4E-06	9,1E-06
Particulate matter formation	kg PM10 eq.	6,1E-01	5,3E-03	6,3E-03	1,0E-01
Fossil depletion	kg oil eq.	2,6E+02	7,6E-01	1,2E+01	3,4E+01

Table 5.3b *Results of the characterization phase for the life cycle of the precipitate (personal computing from SimaPro)***.**

Impact category	Unit	Total	Electricity	Heat	Waste
Climate change	kg CO2 eq.	9,4E+02	3,3E+02	2,8E+02	1,9E+02
Ozone depletion	kg CFC-11 eq.	4,2E-05	2,9E-05	3,4E-07	1,4E-07
Particulate matter formation	kg PM10 eq.	6,1E-01	4,0E-01	9,4E-02	3,1E-03
Fossil depletion	kg oil eq.	2,6E+02	1,1E+02	1,1E+02	4,3E-01

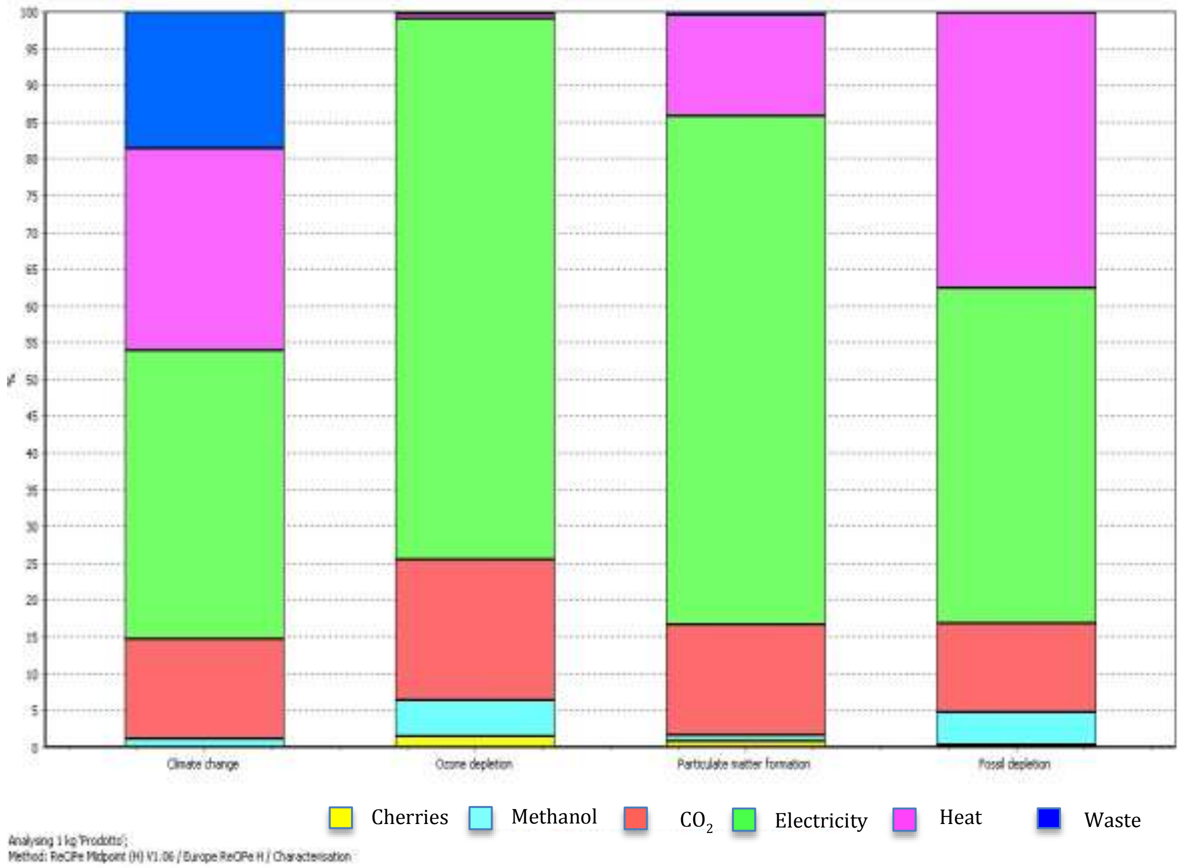


Figure 5.3 *Results of the characterisation phase for the life cycle of the precipitate (personal computing from SimaPro)***.**

From the results above it can be seen how the consumption of electricity is the most important factor in all categories. It is mainly associated with carbon dioxide recovery processes. This recycle is very important because without it there would be more impacts related to carbon dioxide emission to air. In particular in the climate change category, in the absence of recycle the impact related to the “waste” group would increase from 188 (see table 5.3b) to 15.256 kg CO_2 eq (kilograms of CO_2 used to produce the functional unit). This happens because 1kg of carbon dioxide corresponds to an impact of 1kg of CO2 eq (IPCC, 2007). The results obtained from this study are consistent with those proposed by from Rodriguez-Meizoso *et al.*, (2012), who found that the major contribution on supercritical fluid extraction process is due to consumption of energy.

5.1.3 Normalization

“Normalization” is an optional step within Life Cycle Impact Assessment (LCIA) that may be used to assist both in the interpretation of life cycle inventory data and in life cycle impact assessment results. Normalization transforms the magnitude of LCI and LCIA results into relative contributions by substance and by life cycle impact category. The results obtained from the characterization operations are normalized and then they are processed in order to compare the result obtained for a given category with a reference value. These can be the overall impact, regional and local level, the impact per inhabitant or the scenario alternative. This step is optional since the normalization parameters are related to subjective and debatable evaluations, and then it is often not correct to relate the entire environmental profile to a single reference parameter. On the other hand, the normalization is useful as it allows to check possible inconsistencies or errors. It also highlights the relative importance of the results and provides a support to the following stages of aggregation, weighting and interpretation of the results. The results of the characterization are shown in tabular form (in table 5.4a and 5.4b) and graphically (figure 5.4).

Table 5.4a *Results of the normalization phase for the life cycle of the precipitate (personal computing from SimaPro)*.

Impact category	Unit	Total	Cherries	Methanol	CO2
Climate change		8,4E-02	-2,7E-05	9,8E-04	1,2E-02
Ozone depletion		1,9E-03	3,0E-05	1,1E-04	4,1E-04
Particulate matter formation		4,1E-02	3,6E-04	4,2E-04	6,9E-03
Fossil depletion		1,6E-01	4,6E-04	7,5E-03	2,1E-02

Table 5.4b *Results of the normalization phase for the life cycle of the precipitate (personal computing from SimaPro)*.

Impact category	Unit	Total	Electricity	Heat	Waste
Climate change		8,4E-02	2,9E-02	2,5E-02	1,7E-02
Ozone depletion		1,9E-03	1,3E-03	1,5E-05	6,3E-06
Particulate matter formation		4,1E-02	2,7E-02	6,3E-03	2,1E-04
Fossil depletion		1,6E-01	6,4E-02	6,3E-02	2,6E-04

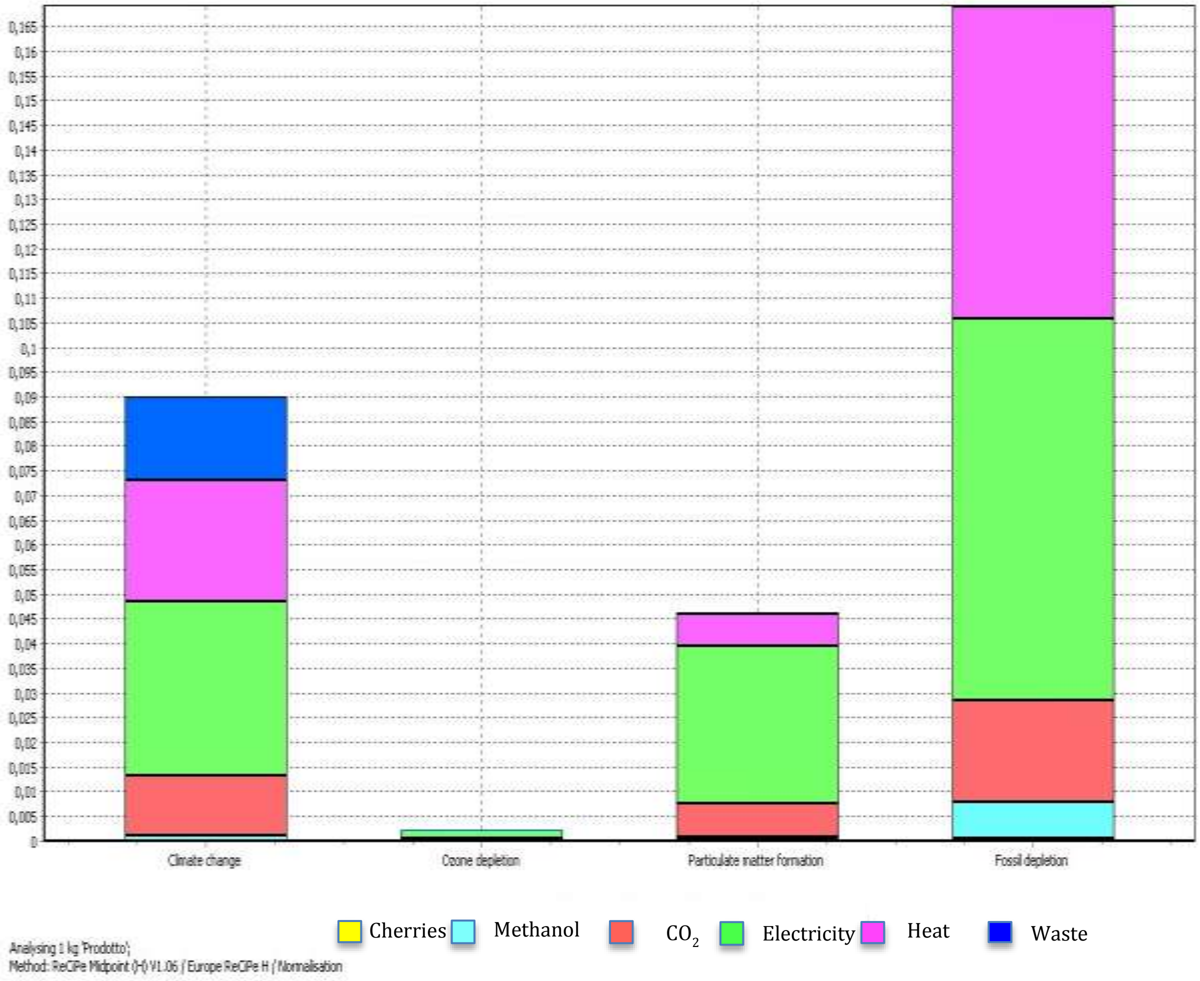


Figure 5.4 *Results of the normalisation phase for the life cycle of the precipitate (personal computing from SimaPro)***.**

5.2 Interpretation of results

Interpreting the results of an LCA starts with understanding the inaccuracy and ensuring they meet the goal of the study. This is accomplished by identifying the data elements that contribute significantly to each impact category, evaluating the sensitivity of these significant data elements, assessing the completeness and consistency of the study, and drawing conclusions and recommendations based on a clear understanding of how the LCA was performed.

5.2.1 Uncertainty analysis

This step was done in the section 5.1.2 to select the impact categories. It shows that the impact categories analysed were not affected by large uncertainties in accordance to ILCD handbook guidelines (JRC, 2010). It can be observed how the LCA phases are iterative. In fact, the uncertainty analysis that is done according to different steps definition should be performed during interpretation phase. While in this case study it was done at the impact assessment phase.

5.2.2 Sensitivity analysis

The sensitivity analysis was done to see how the environmental impacts vary with a hypothetical improvement of the process. Three cases were considered; they represent reduction of 30% of electricity, heat consumption and CO_2 loss respectively. Only one variable was changed in each sensitivity analysis. Using the ReCiPe method midpoint the three cases were compared to the base case.

1. *Electricity*: in this case it was assumed a theoretical reduction of electricity equal to 30% of total amount. To do this, in all blocks, which contain consumption of energy, this has been reduced by 30%, heat, CO_2 and waste were kept constant. Table 5.5 shows the values of energy consumption in each block.

Table 5.5 *Electricity reduction of 30%.*

Blocks	**Electricity case base kWh**	**Electricity -30% kWh**
Destoning	8,8 E-02	6,2 E-02
Grinding	3,0 E-02	2,1 E-02
Freezing and lyophilizing	11,2	7,8
Extraction	11,8	8,3
Centrifugation	10,0	7,0
Pumping 1	9,5 E-01	6,6 E-01
Cooling 1	54,6	38,2
Pumping 2	67,6	47,3
CO_2 recycle	343,6	240,6

2. *Heat:* it has been done a hypothetical reduction of 30% of consumption of heat in all blocks containing the heat module, such as precipitator, separation 1, separation 2 and separation 3. The oter variables were kept as in the base case. The values of heat reduction are shown in table 5.6.

Table 5.6 *Heat reduction of 30%.*

Blocks	**Heatcase base kWh**	**Heat -30% kWh**
Precipitation	373,3	261,3
Separation 1	520,2	364,1
Separation 2	69,3	48,4
Separation 3	5,2	3,8

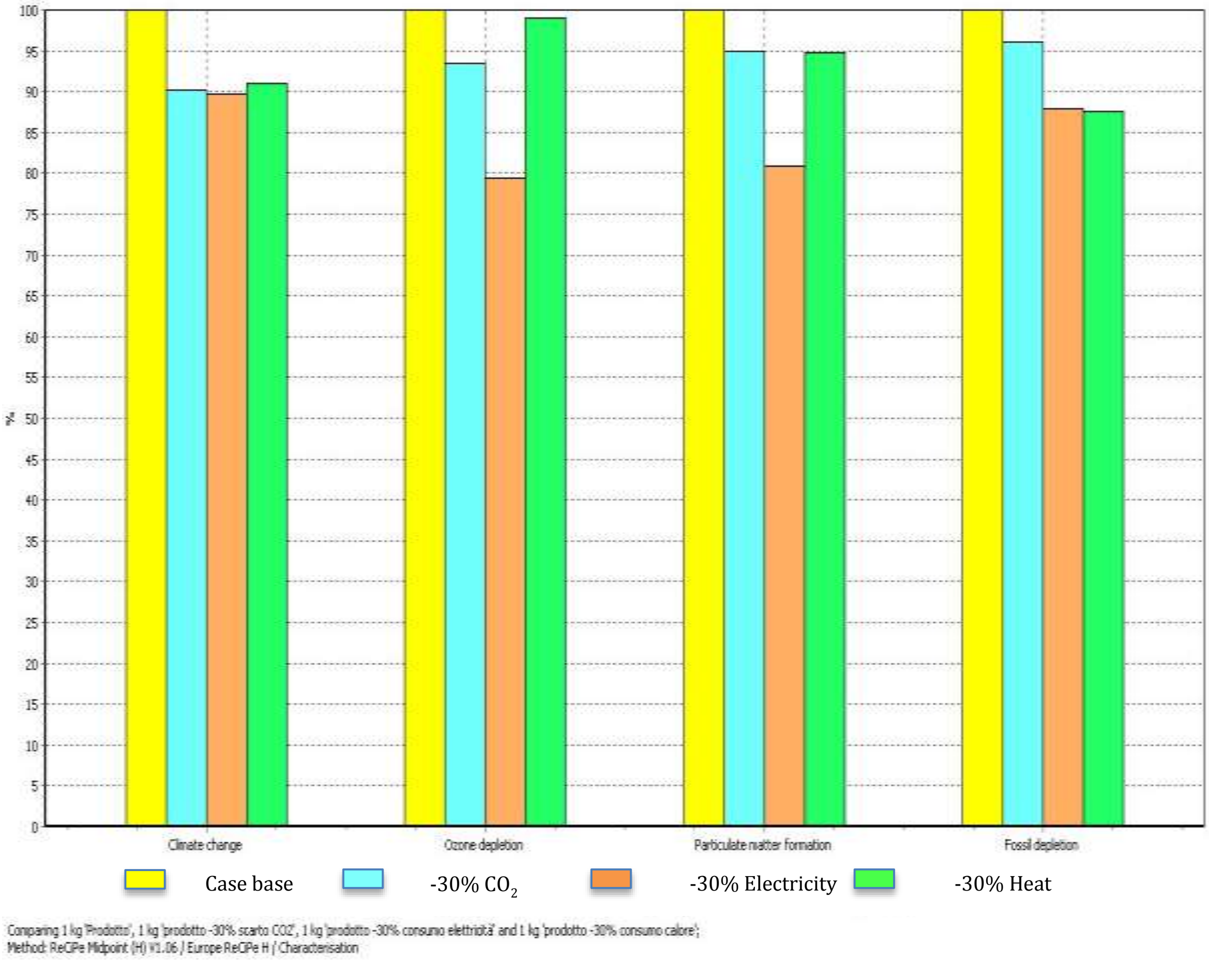
3. CO_2: in this case it was assumed a reduction of 30% of carbon dioxide make-up from 168,6 kg to 118,0 kg. In this manner it was decreased the amount of CO_2 emission on air and the production of carbon dioxide, keeping the equipment energy consumption unchanged.

5.2.3 Comparison of cases

The three cases above and the base case are now compared to see how the reductions of variable considere improve the impact. The comparison was done with ReCiPe 2008 midpoint method. The results are shown in tabular form (table 5.7) and graphically (figure 5.5).

Table 5.7 *Results of the characterization phase for the comparison of the altered cases and base case (personal computing from SimaPro).*

Impact category	Unit	Base case	CO2 case	Electricity case	Heat case
Climate change	kg CO2 eq.	9,4E+02	8,5E+02	8,5E+02	8,6E+02
Ozone depletion	kg CFC-11 eq.	4,12E-05	3,9E-05	3,3E-05	4,1E-05
Particulate matter formation	kg PM10 eq.	6,1E-01	5,8E-01	4,9E-01	5,8E-01
Fossil depletion	kg oil eq.	2,6E+02	2,5E+02	2,3E+02	2,3E+02



Comparing 1 kg 'Prodotto', 1 kg 'prodotto -30% scarto CO2', 1 kg 'prodotto -30% consumo elettriotà' and 1 kg 'prodotto -30% consumo calore';
Method: ReCiPe Midpoint (H) V1.06 / Europe ReCiPe H / Characterisation

Figure 5.5 *Results of the comparison of the three altered cases and base case (personal computing from SimaPro)***.**

As it can be seen in figure 5.5 a reduction of electricity has a better improvement of impact than both heat and CO_2 reduction. This improvement is higher especially in the Ozone depletion and Particulate matter formation categories where electricity consumption is almost 20% less than the case base. While in the climate change category, the three cases considered show similar impact reductions, which is almost about to 10% with respect to the case base.

5.2.3 Concluding remarks and recommendations

This study showed that the largest contribution in all impact categories is given by electricity. This is due to the electricity consumption in the phases of recycle, in particular in the cooler and compressor. The sensitivity assessment demonstrated the importance of energy sources in the production of powders, especially in the fossil depletion category. Moreover the results of normalisation showed that the fossil depletion category has the biggest impact in this study.

However there are some limitations due to approximations done. In order to consider the full process and to estimate the cultivation impacts, it was necessary to consider peach cultivation data because cherries are absent in both literature and SimaPro database. To include the transport inside the system boundaries it was necessary to estimate the distance covered for raw materials transportation because the origin location of them was unknown. Anyway the consumption of cherries and the transports do not have a relevant impact.

As a conclusion the electricity consumption has a fundamental role in this study. Aspen plus simulation was used to calculate most of the data such as recycle of CO_2, recycle of methanol and preparation of supercritical CO_2. The data concerning the consumption of electricity of equipment to prepare cherry's solution belong to generic data sheet, for which, in order to get a more reliable study it would be recommended to use primary data or more certain sources. Anyway, the consumption of electricity to prepare the cherry's solution is only the 6,6% of the total electricity for the entire process. To conclude the results obtained in this case study are consistent with Rodriguez-Meizoso *et al.* (2012), who found how the total impact of a supercritical CO_2 extraction process is mainly due to electricity duties.

Conclusions

The aim of this work was to investigate the operating conditions to obtain the best precipitation yield and to assess the environmental impact associated to this process. Different experimental tests have been carried out in order to extract phenolic compounds, polyphenols and anthocyanins from cherries (*Prunus Avium*) using different solvents. From extraction results it was seen as the best yields in phenolic compounds are obtained by using methanol and mixture ethanol / dimethyl sulfoxide (DMSO) (50%w/w) as solvent. The SAS experiments were performed in a laboratory plant assembled for the purpose. Only methanol allowed to obtain solid particles as a product.

The first group of SAS experiments were focused on finding the optimal operative conditions. The temperature was set to 40°C to avoid the degradation of compounds and only the pressure since the temperature was fixed at 40°C to avoid the degradation of compounds only the pressure affects the density and, therefore, the solubility. The SAS precipitations were carried out at pressures of 100, 125 and 150 bar also CO_2 and solution flow rates were kept constant. The precipitates were analysed to determine the value of pressure that brings the higher precipitation yield. It was observed that an increase in pressure leads to an increase of solubility of the compounds and thus a decrease of the precipitation yield; therefore, the optimum value was set to 100 bar. A second group of experiments was considered to understand how the flow rate of the solution and the CO_2 influences the precipitation yield. The tests were performed at fixed value of pressure (100 bar), 40°C and solution flow rate of 0,2 mL/min while the CO_2 flow rates varied from 2-9 L/min. The results obtained indicate that a higher CO_2 flow rate, i.e. a higher CO_2 molar fraction, leads to a better precipitation yield. So, the best operating conditions were found to be 100 bar, 40°C, 0,18 mL/min of solution and 9L/min of CO_2.

At these conditions the process proposed is effective for the recovery of valuable compounds from cherries. The product obtained is a dry powder formed by particles containing about 71% of the total phenolic compounds present in the methanolic extract of cherries and no methanol residuals.

In the second part of the work, the environmental impact caused by the entire process to produce 1 kg of precipitate was evaluated at the best operating conditions.

The Life Cycle Assesment methodology was applied according to ISO 14040:2006 and ISO 14044:2006.

SimaPro software was used to implement the model and the impact assessment has been carried out by adopting the ReCiPe 2008 method.

The study was performed according to a “from cradle to gate” approach; from the extraction of raw materials to the final product also considering the transports and energy supply for the production stage. The recycle of methanol and carbon dioxide was considered. The LCI results were organized in eighteen impact categories with a hierarchies weighting method. Four of these categories were selected and checked with respect to uncertainty analysis and ILCD guidelines. These are *Climate change, Ozone depletion, Particulate matter formation and fossil depletion.* The study of the base case showed that the largest contribution to all categories is given by electricity. This is due to the greater consumption in the phases of recycle, in particular in the cooler and compressor. A sensitivity analysis was eventually performed to see how the environmental impacts vary with a hypothetical improvement of the process: reduction of 30% of electricity consumption, heat duty and CO_2 loss were addressed. The sensitivity assessment demonstrated the importance of energy sources in the production of powder especially in the fossil depletion category. Moreover the results of normalisation showed that the fossil depletion category has the biggest impact.

Appendix A

The main characteristics of precipitator used in SAS precipitation are defined in table A.1.

Table A.1*:* Main characteristics of precipitator used in SAS plant.

Vessel function	Precipitator
Construction material	Stainless steel
Shape	Cylindric made up of three screwed parts
Orientation	Vertical
Volume	350 cm^3
Inside diameter	5 cm
Height	25 cm
Net height	18 cm
Threated height	1.9 cm
Thickness of cylinder	0.6 cm

In Figure A.1 is represented a picture of the three screwed parts of the precipitator (a) and of the total precipitator (b)

a) **b)**

Figure A.1 *a) inside of precipitator b) three screwed parts of precipitator.*

Bibliography

Beckman E. J. (2004). Supercritical and near-critical CO_2 in green chemical synthesis and processing. *Journal of Supercritical Fluids,* (Pittsburgh, USA), 28, 121–191.

Boonnoun P., Nerome H., Machmudah S., Goto M., Shotipruk A., 2013. Supercritical anti-solvent micronization of chromatography purified marigold lutein using hexane and ethyl acetate solvent mixture. *J. of Supercritical Fluids,* 80, 15–22.

Chinnarasu C., A. Montes, Fernández- Ponce M.T., Casas L., Mantell C., Pereyra C., Martínez de la Ossa E.J., 2014. Precipitation of antioxidant fine particles from Olea europaea leaves using supercritical antisolvent process. *J. of Supercritical Fluids*, 97, 125–132.

Chinnarasu C., Montes A., Fernandez-Ponce M.T., Casas L., Mantell C., Pereyra C., Martinez de la Ossa E.J., Pattabhi S., 2015. Natural antioxidant fine particles recovery from Eucalyptus globulus leaves using supercritical carbon dioxide assisted processes. *J. of Supercritical Fluids*, 101, 161–169.

Ciroth, A (2009) Cost data quality considerations for eco-efficiency measures, *Ecological Economics* 68(6) 1583-1590.

Crisosto, C. H., Crisosto, G. M., & Metheney, P. (2003). Consumer acceptance of ‘Brooks’ and ‘Bing’ cherries is mainly dependent on fruit SSC and visual skin colour. *Postharvest Biology and Technology*, 28(1), 159–167.

De Melo, M.M.R., Silvestre, A.J.D., Silva, C.M., 2014. Supercritical fluid extraction of vegetable matrices: applications, trends and future perspectives of a convincing green technology. *Journal Supercritical Fluids.* 92, 115–176.

De Menna F.,Molari G., Vittuari M. (2014). Impact evaluation of integrated food-bioenergy systems: A comparative LCA of peach nectar. *Biomass and bioenergy* 73 (2015) 48e61

Della Porta, G., Taddeo, R., D’Urso, E., Reverchon, E., 1998. Isolation of clove bud and star anise essential oil by supercritical CO_2 extraction. *LWT – Food Sci. Technol.,* 31 (5), 454–460.

Dorta, E., Lobo, M.G., Gonzalez, M., 2012. Reutilization of mango byproducts: study of the effect of extraction solvent and temperature on their antioxidant properties. *Journal Food Sci.* 77 (1), C80–C88.

EEA, Electricity production by fuel (ENER 027)- assessment published (Apr. 2012). Data refers to 2009.

EPA/600/R-06/060, 2006. Life cycle assessment: principles and practice.

Esfandiari N., 2015. Production of micro and nano particles of pharmaceutical by supercritical carbon dioxide. *Journal of Supercritical Fluids,* 100, 129–141.

Esti, L., Cinquanta, F., Sinesio, E., Moneta, E., & Di Matteo, M. (2002). Physicochemical and sensory fruit characteristics of two sweet cherry cultivars after cool storage. *Food Chemistry,* 76, 399–405.

Floris T., Filippino G., Scrugli S., Pinna M.B., Argiolas F., Argiolas A., Murru M., Reverchon E., 2010. Antioxidant compounds recovery from grape residues by a supercritical antisolvent assisted process. *J. of Supercritical Fluids,* 54, 165–170

Frischknecht R., Braunschweig A., Hofstetter P. and Suter P. (2000). Human Health Damages due to Ionising Radiation in Life Cycle Impact Assessment. In Review Environmental Impact Assessment, 20(2), pp. 159-189

Frischknecht R., Jungbluth N., Althaus H.J., Bauer C., Doka G., Dones R., Hischier R., Hellwag S., Humbert S., Köllner T., Loerincink Y., Margni M., Nemecek T. (2007). Implementation of Life Cycle Impact Assessment Methods, Ecoinvent report No. 3, Swiss Centre for Life cycle Inventories, Dübendorf, CH.

Frischknecht R., Jungbluth N., AlthausH.J., Doka G., Dones R., Heck T., Helleweg S., Hischer R., Nemecek T., Rebitezer G., Spielmann M. (2005). The EcoinventDabase: Overview and Methodological Framework, *International Journal of Life CycleAssessement* 10 (1), 3-9.

Frischknecht, R., Steiner, R., Jungbluth, N. (2008). Methode der ökologischen Knappheit – Ökofaktoren 2006, ö.b.u. und Bundesamt für Umwelt, Bern.

Goedkoop M., De Schryver A., Oele M., Durksz S., De Roest D. (2010). Introduction to LCA with SimaPro 7

Goedkoop M., De Schryver A., Oele M., Vieira M., Hegger S. (2010). SimaPro Methods Manual: Methods Manual, Prè consultants.

Greco, S.L., Wilson, A.M., Spengler J.D., and Levy J.I. (2007). Spatial patterns of mobile source particulate matter emissions-to-exposure relationships across the United States. *Atmospheric Environment* (41), 1011-1025.

Guinée J.B., Gorrée M., Heijungs R., Huppes G., Kleijn R., van Oers L., Wegener Sleeswijk A., Suh S., Udo de Haes H.A., de Bruijn H., van Duin R. e Huijbregts MAJ (2002). Life Cycle Assessment: An Operational Guide to the ISO Standards. *Kluwer Academic Publishers,* Dordrecht (NL)

Hildebrand J.H., Solubility of Non-Electrolytes, Chapman & Hall Ltd, London, 1936.

Heijungs R., Suh S., Kleijn R. (2005). Numerical Approaches to Life Cycle Intepretation. The case of the ecoinvent '96 database, *International Journal of Life CycleAssessment* 10 (2) 103-112

Imsanguan P., Pongamphai S., Douglas S., Teppaitoon W., Douglas P. L., 2010. Supercritical antisolvent precipitation of andrographolide from Andrographis paniculata extracts: Effect of pressure, temperature and CO2 flow rate. *Powder Technology*, 200, 246–253

IPCC, 2007. Climate Change (2007). The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovenmental Panel on Climate Change. [Solomon S., Qin D., Manning M., Chen Z., Marquis M., Averyt K.B., Tignor M. and Miller H.L. (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp 996.

ISO, (2006a), UNI EN ISO 14040: Environmental management, Life CycleAssessment – Principles and Framework, International Organization for Standardization (ISO), Ginevra.

ISO, (2006b), UNI EN ISO 14044: Environmental management, Life CycleAssessment – Requirements and Guidelines, International Organization for Standardization (ISO), Ginevra.

Jakobek, L., Sˇeruga, M., Medvidovic´-Kosanovic´, M., Novak, I., 2007a. Anthocyanin content and antioxidant activity of various red fruit juices. Dtsch. Lebensm.- Rundsch. 103, 59–64.

J.C. Giddings, M.N. Meyers, S. Mclaren, R.A., 1968. Kelfer, High pressure gas chromatography of non volatile species. *Science,* 162, 67–73.

J. Jung, M. Perrut, 2001. Particle design using supercritical fluids: literature and patent survey. *Journal of Supercritical Fluids,* 20, 179–219.

JRC (2011). ILCD Handbook (International Reference Life Cycle Data System Handbook) – Recommendation for Life Cycle Impact Assessment in the European context – based on existing environmental impact assessment models and factors. European Commission, Joint Research Centre, Institute for Environmental and Sustainability.

Khan, R.S., Grigor, J., Winger, R., Win, A., 2013. Functional food product development–Opportunities and challenges for food manufacturers. *Trends Food Sci. Technol.* 30 (1), 27–37.

Kim Dae-Ok, Ho Jin Heo, Young Jun Kim, Hyun Seuk Yang and Chang Y. Lee, 2005. Sweet and Sour Cherry Phenolics and Their Protective Effects on Neuronal Cells**.** *Journal of Agric. Food Chem.,* 53, 9921−9927

Kuo-Li Lin, Lee-Muei Chng, Justin Chun-Te Lin, Shih-Lan Hsu, Chiu-Chung Young, Chwen-Jen Shieh, Chieh-Ming J. Chang, 2014. Effect of Anti-solvent Conditions on Low Density Supercritical Fluids Precipitation of Zeaxanthin Palmitates from Lycium Barbarum Fruits. *J. of Supercritical Fluids*, 87, 104–110

Lee, C.W., Yen, F.L., Huang, H.W., Wu, T.H., Ko, H.H., Tzeng, W.S., Lin, C.C., 2012. Resveratrol nanoparticle system improves dissolution properties and enhances the hepatoprotective effect of resveratrol through antioxidant and anti- inflammatory pathways. *J. Agric. Food Chem.* 60 (18), 4662–4671.

Marqués J.L., Della Porta G., Reverchon E., Renuncio J.A.R., Mainar A.M., 2013. Supercritical antisolvent extraction of antioxidants from grape seeds after vinification. *J. of Supercritical Fluids,* 82, 238–243

Martín, L., González-Coloma, A., Adami, R., Scognamiglio, M., Reverchon, E., Porta, G.D., Urieta, J.S., Mainar, A.M., 2011. Supercritical antisolvent fractionation of ryanodol from Persea indica. *J. Supercrit. Fluids* 60, 16–20.

Matilla, P., Hellstro □m, J., To □rro □nen, R., 2006. Phenolic acids in berries, fruits and beverages. *J. Agric. Food Chem.* 54, 7193–7199.

Miguel A. Meneses, Giuseppe Caputo, Mariarosa Scognamiglio, Ernesto Reverchon, Renata Adami, 2015. Antioxidant phenolic compounds recovery from Mangifera indica L. by-products by supercritical antisolvent extraction. *Journal of Food Engineering* ,163, 45–53.

Milà i Canals L., Romanyà J., Cowell SJ. (2007b). Method for assessing impacts on life support functions (LSF) related to the use of ‘fertile land’ in Life Cycle Assessment (LCA). J Clean Prod 15 1426-1440.

Mirofci S. 2014. A supercritical fluid extraction process to obtain valuable compound from eruca sativa leaves. *Master degree in chemical engineering*, Department of Indrustrial engineering, university of Padua.

Nediljko Budisa and Dirk Schulze-Makuch, 2014. Supercritical Carbon Dioxide and Its Potential as a Life-Sustaining Solvent in a Planetary Environment. *Life*, 4(3), 331-340.

Pereira V.J., Matos R.L., Cardoso S.G., Soares R.O., Santana G.L., Costa G.M.N., Vieira de Melo S.A.B. 2013. A new approach to select solvents and operating conditions for supercritical antisolvent precipitation processes by using solubility parameter and group contribution methods. *J. of Supercrit. Fluids*, 8,1128–146.

Posch, M., Seppälä, J., Hettelingh, J.P., Johansson, M., Margni M., Jolliet, O. (2008). The role of atmospheric dispersion models and ecosystem sensitivity in the determination of characterisation factors for acidifying and eutrophying emissions in LCIA. *International Journal of Life Cycle Assessment* (13) pp.477–486.

PRé Consultants, (2010). Introduction to LCA with SimaPro 7, PRé Consultants, the Netherlands.

Rabl, A. and Spadaro, J.V. (2004). The RiskPoll software, version is 1.051 (dated August 2004).

Reverchon, E., Sesti Osseo, L., Gorgoglione, D., 1994. Supercritical CO_2 extraction of basil oil: Characterization of products and process modeling. *J. of Supercrit. Fluids,* 7 (3), 185–190.

Reverchon, E., 1997. Supercritical fluid extraction and fractionation of essential oils and related products. *J.l of Supercrit. Fluids,* 10 (1), 1–37.

Reverchon, E., 1999. Supercritical antisolvent precipitation of micro- and nano- particles. *J. Supercrit. Fluids* 15 (1), 1–21.

Reverchon, E., De Marco, I., 2006. Supercritical fluid extraction and fractionation of natural matter. *Journal of Supercritical Fluids,* 38 (2), 146–166.

Reverchon, E., Adami, R., Caputo, G., De Marco, I., 2008. Spherical microparticles production by supercritical antisolvent precipitation: Interpretation of results. *J. Supercrit. Fluids* 47 (1), 70–84.

Rodríguez-Meizoso I., Castro-Puyana M., Börjesson P., Mendiola J. A., Turner C., Ibánez E. (2012). Life cycle assessment of green pilot-scale extraction processes to obtain potent antioxidants from rosemary leaves, *Journal. of Supercritical Fluids* 72 205–212.

Rosenbaum, R.K., Bachmann, T.M., Gold, L.S., Huijbregts, M.A.J., Jolliet, O., Juraske, R., Köhler, A., Larsen, H.F., MacLeod, M., Margni, M., McKone, T.E., Payet, J., Schuhmacher, M., van de Meent, D., Hauschild, M.Z. (2008): USEtox - The UNEP- SETAC toxicity model: recommended characterisation factors for human toxicity and freshwater ecotoxicity in Life Cycle Impact Assessment. *International Journal of Life Cycle Assessment,* 13(7): 532-546.

Sahoo, N.G., Kakran, M., Shaal, L.A., Li, L., Muller, R.H., Pal, M., Tan, L.P., 2011. Preparation and characterization of quercetin nanocrystals. *J. Pharm. Sci.* 100 (6), 2379–2390.

Scatchard G., Equilibria in nonelectrolyte solution in relation to vapor pressures and densities of components, Chemical Reviews 8 (1931) 321–333.

Schneider, G., Physicochemical Principle of Extraction with Supercritical Gases, in *Extraction with Supercritical Gases*, E. Stahl, G. Wilke (Eds.), Verlag Chemie, Weinheim, 1980, 45-81.

Scipioni A., Mazzi A., Niero M., BoattoT. (2009). LCA to choose among alternative design solutions: The case study of a new Italian incineration line. *Waste management* 29, 2462-2474.

Seppälä, J., Posch, M., Johansson, M., Hettelingh, J.P. (2006). Country-dependent Characterisation Factors for Acidification and Terrestrial Eutrophication Based on Accumulated Exceedance as an Impact Category Indicator. *International Journal of Life Cycle Assessment* 11(6): 403-416.

Serra A.T., Seabra Inês J., Mara E.M. Braga, Bronze M.R., de Sousa Hermínio CDuarte., Catarina M.M., 2010. Processing cherries (*Prunus avium*) using supercritical fluid technology. Part 1: Recovery of extract fractions rich in bioactive compounds. *Journal of Supercritical Fluids, 55(1),* 184–191.

Steen B. (1997). On uncertainty and sensitivity of LCA-based priority setting, *Journal of Cleaner Production* 5 (4), 255-262.

Struijs, J., Beusen, A., van Jaarsveld, H. and Huijbregts, M.A.J. (2009b). Aquatic Eutrophication. Chapter 6 in: Goedkoop, M., Heijungs, R., Huijbregts, M.A.J., De Schryver, A., Struijs, J., Van Zelm, R. (2009). ReCiPe 2008 A life cycle impact assessment method which comprises harmonised category indicators at the midpoint and the endpoint level. Report I: Characterisation factors, first edition.

Tzeng, C.W., Yen, F.L., Wu, T.H., Ko, H.H., Lee, C.W., Tzeng, W.S., Lin, C.C., 2011. Enhancement of dissolution and antioxidant activity of kaempferol using a nanoparticle engineering process. *J. Agric. Food Chem.* 59 (9), 5073–5080.

Usenik, V., Fabcˇicˇ, J., Sˇtampar, F., 2008. Sugars, organic acids, phenolic composition and antioxidant activity of sweet cherry (Prunus avium L.). *Food Chem.,* 107, 185–192.

Van Jaarsveld J.A. (1995). Modelling the long-term atmospheric behavior of pollutants on various spatial scales. *PhD Thesis,* University of Utrecht, Utrecht.

Van Jaarsveld J.A, Van Pul WAJ., De Leeuw FAAM. (1997. Modelling transport and deposition of persistent organic pollutants in the European region.) *Atmospheric Environment* 7, 1011-1024

Van Zelm, R., Huijbregts, M.A.J., Den Hollander, H.A., Van Jaarsveld, H.A., Sauter, F.J., Struijs, J., Van Wijnen, H.J., Van de Meent, D. (2008). European characterization factors for human health damage of PM10 and ozone in life cycle impact assessment. *Atmospheric Environment* 42, 441-453.

Visentin A., Rodríguez-Rojo S., Navarrete A., Maestri D., M.J. Cocero, 2012. Precipitation and encapsulation of rosemary antioxidants by supercritical antisolvent process. *Journal of Food Engineering,* 109, 9–15.

Weidema B., WesnaesM.S. (1996). Data Quality Management for life cycle Inventories – an example of using data qualityindicators, *Journal of Cleaner Production* 4 (3-4), 167-174.

Wijngaard, H., Hossain, M.B., Rai, D.K., Brunton, N., 2012. Techniques to extract bioactive compounds from food by-products of plant origin. *Food Res. Int.* 46 (2), 505–513.

WMO (World Meteorological Organization) (2003). Scientific Assessment of Ozone Depletion: Global Ozone Research and Monitoring Project – Report No. 47.

Yen, F.-L., Wu, T.-H., Tzeng, C.-W., Lin, L.-T., Lin, C.-C., 2010. Curcumin nanoparticles improve the physicochemical properties of curcumin and effectively enhance its antioxidant and antihepatoma activities. *J. Agric. Food Chem.* 58 (12), 7376– 7382.

Zabot, G., Moraes, M., Meireles, M.A., 2014. Supercritical fluid extraction of bioactive compounds from botanic matrices: experimental data, process parameters and economic evaluation. *Recent Patents Eng.* 6 (3), 182–206.

Web site

---

[1] http://perminc.com/resources/fundamentals-of-fluid-flow-in-porous-media/chapter-5-miscible-displacement/fluid-phase-behavior/pressure-temperature-diagram-p-t-diagram/(30/04/2016)

[2] http://www.nasa.gov/vision/earth/technologies/harvestingmars_prt.htm(30/04/2016)

[3] https://commons.wikimedia.org/wiki/File:Carbon_dioxide_density-pressure_phase_diagram.jpg(30/04/2016)

[4] https://en.wikipedia.org/wiki/Carbon_dioxide(30/04/2016)

[5] https://en.wikipedia.org/wiki/Cherry(30/04/2016)

[6] https://en.wikipedia.org/wiki/Cherry(30/04/2016)

---

[7] http://www.hindawi.com/journals/jchem/2013/289392/fig1/(30/04/2016)

[8] https://en.wikipedia.org/wiki/Anthocyanin(30/04/2016)

[9] http://www.polyphenols.com/cyanidin-products/(30/04/2016)

[10] http://www.genome.jp/dbget-bin/(30/04/2016)

[11] https://en.wikipedia.org/wiki/Flavonols(30/04/2016)

[12] https://en.wikipedia.org/wiki/ (30/04/2016)

[13] https://en.wikipedia.org/wiki/Flavanols (30/04/2016)

[14] http://www.speciation.net/Public/Document/2007/06/21/2914.html (30/04/2016)

[15] http://www.alibaba.com/product-detail/multifunction-cherry pitter _684826242.html?spm=a2700.7724838.0.0.DTJpgg (29/04/2016).

[16] http://italian.alibaba.com/product-gs/industrial-fruit-and-vegetable-chopper-60081785276.html (29/04/2016).

[17] http://italian.alibaba.com /product-gs/ce-certification-vacuum-freeze-drying-equipment-prices-low-1934686687.html (29/04/2016).

[18] http://www.globusextraction.com/it/DiaSonic_5_250lt.pdf (29/04/2016).

[19] http://www.alibaba.com/product-detail/SGZ-PGZ-Liquid-Solid-Bottom-Discharge_60328928316.html?spm=a2700.7724838.0.0.w5Oi87 (29/04/2016).