

Study of Tunisian “*Myrtus Communis L*”: Extraction process by supercritical CO₂

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Abstract— supercritical fluid extraction, in particular using the carbon dioxide, presents more advantages than classical extraction methods such as saving of time, selectivity improvement and absence of degradation of extracted substances. In this paper, we present the CO₂ extraction, which the use is very appreciated in the essential oils production. The extraction has been realized on “*Myrtus Communis L*” leaves finely crushed from the “Sejnène” area, Tunisia. In this study loaded many parameters play a key role. Thus, we have fixed some parameters such as the contact time, fluid flow, the load amount in the extractor as well as the pressure and temperature conditions in the separator. We have studied the extraction temperature and pressure effect of crushed leaves. Therefore, the extract solubility in CO₂ was studied in function of the experimental parameters illustrated previously. Sampling of the extracts at various the moments allowed in the time made it possible to establish the extraction kinetics as well as to calculate extraction output.

Terms—Extract, “*Myrtus Communis L*”, supercritical CO₂, solubility.

I. INTRODUCTION

Tunisia, processes more than 2160 plants species of which more than 350 are recognized as medicinal virtues (Gatté-Ditch, 1921; Floc' H, 1983; Nabli, 1991; Boussaid and Al, 1998). The current demand for these species, often developing in the form of small populations in fragile mediums, is more and more increased for their use in many fields such as traditional medicine, agro-alimentary, cosmetic, pharmacy and phytopharmacology (Heywood, 1991; De Silva, 1997). The effective exploitation of this natural heritage of Tunisia, remains limited to a reduced number of species such as Rosemary, the “*Myrtus Communis L*”, the “*Pistacia lentiscus*” and the Berberie Thuja [1].

The *Myrtus* kind comprises of 16 which, grows spontaneously primarily in the Mediterranean areas but also in the tropical zones and in Australia (Emberger, 1960). “*Myrtus communis L*” is a shrub with persistent leaves which can reach until 5m height (Pouttier-Alapetite, 1979; cross et al., 2001).

The plant is a tree with a dense ramification (height from 30cm to 50cm) according the sites [2]. Flowering, in Tunisia, start in “March” and lasts until “August”. The fruits fructification and maturation begin in “April”. The first type of fruit is of green color then yellow blade and becomes blue dark when mature. The second type of fruit remains yellow blade or becomes darker but never turns towards blue (Traverset and al., Messaoud...).

In Tunisia, the Myrtle is confined primarily in the west-north of the country (Kroumirie primarily) where the climate is humid. It is associated to the forests of oak cork, which occupy the region the north of the country and certain areas of the Cap Bon and the Tunisian Dorsal (Schoenenberger et al., 1995; Nabli 1995). This species can be met but in a less

abundant way, in others areas. It develops under bio climates from the sub humid to the semi-arid [5].

The use of the “*Myrtus Communis L*” myrtle in the pharmacopeia was realized by Matthioli in 16th century. The species has the capacity to tone up and contract muscles. It acts against the acne, the psoriasis, the fatty skins, the bruises and leucorrhées (Paris and Moyse, 1967). Essential oil extracted from the leaves can fighting help against the rodents (Uehleke and Brinkschulte-Freitas, 1979). In the inspiration problem, this oil is useful in the fight against the bronchial infections and of the higher respiratory tracts (painful throats) and can cure asthma and coughs (Lawless, 1995 [1,3]).

The flowers are used against the venous congestions and the disorders of blood circulation (Bouquet, 1921). They can be employed, in powder, mixed with some other aromatic herbs such as the origan (*Origanum majorana L*), to prepare the hair lotions (Trotter, 1915). Traditionally, the leaves infusion (30g/l of water) is useful to treat dry eczemas and psoriasis, ulcers and wounds. The roots bark is considered as astringent and the whole plant, in infusion, is anti diarrheic (Bouquet, 1921; Gattefossé, 1921). The fruits often considered as a cosmetic product (anti-wrinkle), can be used against variola, the hemorrhoids and the skin diseases [54]. Essential oil extracted from bays is simulating and raffermissante hardening (Ducros, 1930; Hussein, 1979).

The literature provides only the performance values of essential oil of myrtle obtained by conventional techniques. These ranged between 0.1% and 0.5% depending on origin [4].

In the mining sector of solid matrices where the solvent power variable geometry offers opportunities in terms of yield and selectivity of extraction that can make the traditional techniques of extraction using organic solvents. Moreover, the use of supercritical carbon dioxide, leaving no trace of residual solvent in the extracts is also an important asset in studies of therapeutic efficacy.

For these reasons in this work we study the performance extracts depending on the temperature and pressure and the variation of solubility of these extracts in CO₂ according to the parameters cited proceeded.

II. MATERIALS AND METHODS

In this section, we present the experimental set-up, used for extraction of the “*Myrtus communis L*” by supercritical CO₂, the extractor characteristics and the operation modes used during the process control, the receptacle filling and the sampling technique of the vegetable matter.

II.1. Experimental dispositive

The set up used in this work was designed by LRGP(Laboratoire Réactions et Génie des Procédés) at Nancy-France. It's about extraction dynamic system where the extractor is a metal cylinder, placed in series with three separators. The setup is shown in figure1.

The extractor is related to a thermostated bath ($\pm 0.1^{\circ}\text{C}$), who allows us to go up in temperature, and to a metal diaphragm pump, which makes it possible to reach the extraction pressure (Dosparo Milton Roy-MILROYAL D). Its maximum capacity is $3.2.\text{kg}.\text{h}^{-1}$. A cold bath is used to liquefy CO₂ before the pump head. The CO₂ flow is measured at the extraction column entry using a flow-meter through Coriolis (MICROMOTION: $\pm 1\text{g}$) who allows to know the CO₂ quantity used during the extraction period.

Separation CO₂/extract is done at a temperature T=30°C. These separators are also the containers which are used to recover essential oils. Then CO₂ is evacuated to the atmosphere.

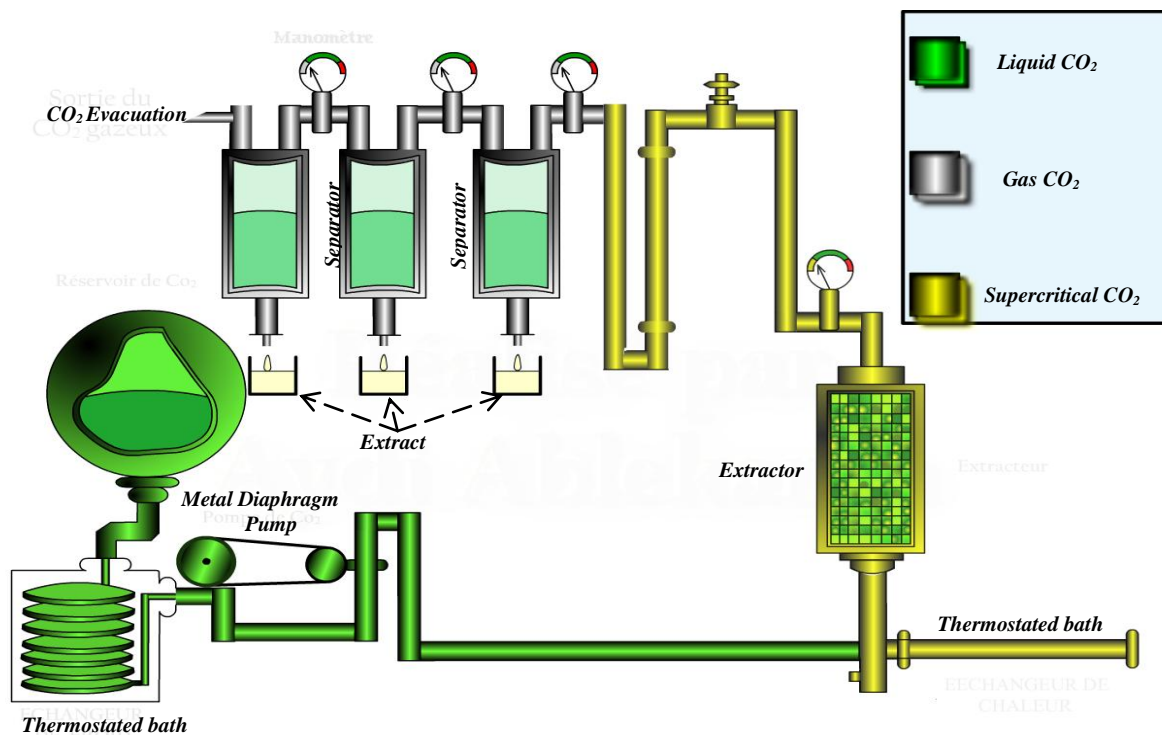


Fig.1. Setup of the dynamic extraction (extraction limit conditions T=80°C, P=250bars)

II.2. Principle of “Myrtus Communis L” extraction

Generally, the essential oil solubility increases with the pressure at constant temperature, thus, it is interesting to work with the highest possible pressures [5] However, the experimental installation can function only with pressures up to 200 bars.

The extract recovery must be realized, in the separators, with neither high pressures nor low temperatures to avoid a significant cooling during the relaxation, which would result the ice formation and a great products recovery difficulty.

In this study, the extractions were carried out on “Myrtus Communis L” Leaves gathered of Sejnène-Tunisia area in March 2008. The leaves used are finely crushed (30g of leaves are ground with Moulinex of type 980).

III. RESULTS AND DESCUSSION

III.1. Pressure influence on the global output and the extract solubility in supercritical CO₂

We have realized the extraction at various pressures in order to determine the various stages of extraction of Tunisian “Myrtus Communis L” and to determine the “Myrtus Communis L” extract solubility in supercritical CO₂.

The tests are realized using 30g of the leaves of this plant at a time. The experimental parameters are given follow:

<i>Vegetable matter :</i>	<i>Leaves of Tunisian "Myrtus Communis L" finely crashed</i>
<i>Mass vegetable matter :</i>	<i>30g</i>
<i>Mass flow</i>	<i>1.3kg h⁻¹</i>
<i>Size of the particles</i>	<i>2.5mm ≥ Ø ≥ 250µm</i>
<i>Extraction pressure :</i>	<i>12, 15, 16.5 and 18MPa</i>
<i>Extraction temperature :</i>	<i>40°C</i>
<i>Contact period of the vegetable matter with CO₂ :</i>	<i>0.5h</i>
<i>The vegetable matter/CO₂ :</i>	<i>0.43</i>
<i>Co-solvent :</i>	<i>No</i>
<i>Content of matter dries :</i>	<i>87.7279%</i>
<i>Temperature of separation :</i>	<i>30°C</i>
<i>Height of bed :</i>	<i>22cm</i>
<i>Volume of bed :</i>	<i>81.31 cm³</i>
<i>Density of "Myrtus Communis L" :</i>	<i>415kg.m⁻³</i>
<i>Fraction of vacuum of extractor :</i>	<i>0.11</i>

Several studies [6-7] on the influence of pressure and temperature were showed that increasing pressure increases the yield of extraction, which is due to an increase in the density of the solvent by a constant temperature increase does not give the same result, because of the influence of either the density of the solvent or the vapor pressure of compounds extracted.

Figure 2 shows the cumulated mass as a function of time for different pressures; it can be noted that as the pressure increases, the density of the solvent (CO₂) increases. On the other hand the vapor pressure of the aqueous solution certainly will decrease. With high pressures the variation of the density becomes less significant, whereas that of the CO₂ vapor pressure becomes more significant. This makes it possible to mitigate the effects of the density variation of the fluid on the extraction rate. Consequently, the rise in the pressure at a constant temperature has a positive effect on the extraction rate, which is controlled by a balance between the density variations of the solvent and that of the vapor pressure of supercritical fluid [8].

Thus, we can conclude that an increase in the pressure involves the reduction in the molar volume, which ends an increase in density thus inducing an improvement of the capacity of "Myrtus Communis L" extract in supercritical CO₂.

The car be explained by evolution of extract at the beginning of extraction process would be allotted to the extract recovery contained in the surface sites. The second slowest stage corresponds to the extract stored in the internal cavities.

The mechanism governing this kinetics comprises the four following stages:

- The solvent penetration in vegetable tissue,
- The dissolution of the solute in the vegetable,
- The solute transfer across cell membranes,
- The transfer of solute from the surface of the plant to the remaining mass of the solvent.

The penetration of solvent into the plant tissue causes the dissolution of the surface extract it contains; this step is rapid and regulated by the phenomena of mass transfer. This stage is fast and is governed by the matter transfer phenomena in supercritical phase.

The third phase is characterized by the diffusion of solute inside the pores. At this stage, the input speed of the solvent in the pores and higher than the rate of diffusion of the solute, so it's the latter that governs the extraction process.

Finally the last stage described the transfer of solute to the mass of remaining solvent; the kinetics of this step is imposed by the velocity of solvent through the pore spaces and not by the diffusion rate of solute [6].

This is consistent with results reported in Table 2 when comparing the overall performance of the extraction is recovered or extract the maximum is 18 MPa and 40 ° C.

This behavior is not similar if we studied the extraction during the first hour, an elevation in the pressure generates a clear improvement of the percentage of “*Myrtus Communis L*” extract until reaching an optimal value ((80% of total extract was recovered at a pressure of 15MPa in the first hour of extraction)) then this ratio decreases further the pressure (see table1).

In the quantitative study of the influence of pressure on yield of extract, it was found that the best yields were obtained at high pressures. To deepen our study on the influence of this parameter, it was interest to study the solubility of extracted in the supercritical CO₂ as a function of pressure. The results showed that the extract solubility increases with pressure and reach a value of 0.19g of extract/kg of CO₂. This can be explained by the fact that increased pressure causes the decrease in molar volume resulting in increased density and promoting the improvement of the power of solvation of myrtle extract in supercritical CO₂.

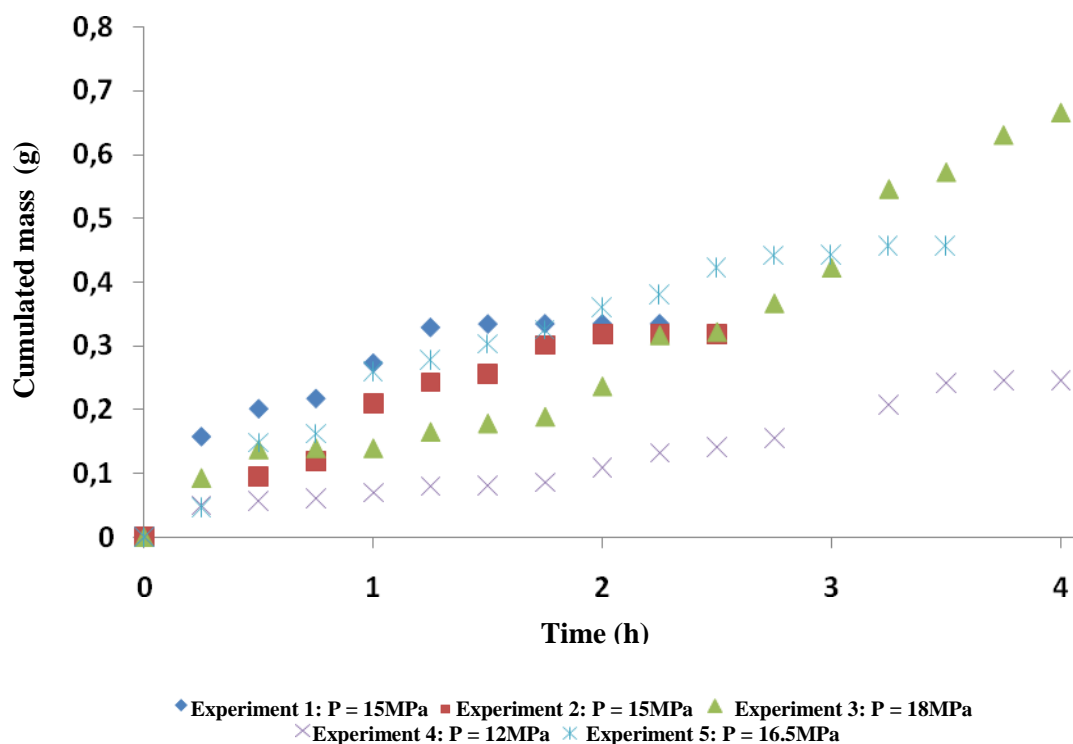


Figure 2: Extraction kinetics of «*Myrtus Communis L*» extract for various pressures (T=40°C)

Pression (MPa)	Output (%)	V_{molar} ($\text{cm}^3 \text{mol}^{-1}$)	ρ_{CO_2} (Kg. m^{-3})	Solubility (g of extract/ kg of CO_2)	R_1 (%) during the first hour of extraction
12	0.93	69.48	633.25	0.07	10
15	1.23	61.95	710.21	0.14	80
16.5	1.67	59.62	738.00	0.16	57
18	2.69	57.75	761.84	0.19	65

Table 1: Summary of the output variation of “Myrtus communis L” extract, the solubility and the output during the first hour in function of the pressure with T=40°C.

The molar volume values are determined by DIAGSIM software and using the state equation of SRK (Soave-Redlich-Kwong).

III.2. Temperature influence on the global output and the extract solubility in supercritical CO_2

In order to study the temperature effect on the extraction output, we fix the operating conditions used during the preceding experiments equal to those with are optimal pressure value equal to 18MPa since it proved to be the optimal value in terms of extraction output.

We now rang the temperature influence on the solubility of a substance in a supercritical solvent is more difficult to access than that of the pressure. At high pressures, the fluid is not easily compressible and thus solubility increases with the temperature.

On the other hand, at intermediate pressures, the fluid is highly compressible and a weak increase in the temperature involves a strong reduction in the density. In parallel the solubility of the compound decreases in the supercritical fluid. The pressure, to which the change of the temperature influence is carried out on the solubility of the compound, was defined like the pressure of crossing named "Crossover". The solubility of the compound is favored by an increase of the temperature [6]. This is checked during the time of the temperature influence study on the “Myrtus Communis L” extract solubility at $P = 18\text{MPa}$.

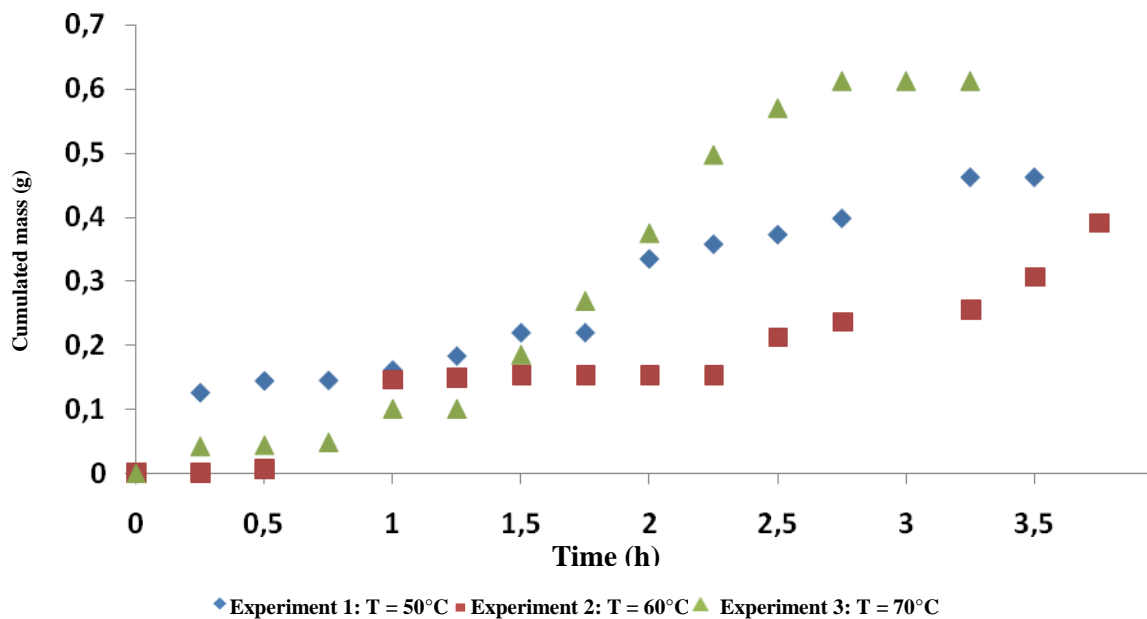


Figure 3: Extraction kinetics of “Myrtus Communis L” extract for various temperatures (P=18 MPa)

Figure 3 represents the temperature influence on the extraction kinetics of the extraction process by supercritical CO₂. First, we noticed that the rise in the temperature does not read inevitably an increase in the extraction output.

This could be explained by the competition between the density variation of solvent and the viscosity of the medium. This behavior is similar to that found in the study of the pressure effect. This indicates that there is an optimal temperature value leading to the best quantity of extract.

According to the results shown in Table 2, we note two temperature values, which could proceed to knowing 40°C and 70°C. Nevertheless, in order to preserve the quality of produced extract, we must choose the lower temperature value since extract are degraded more at higher temperature.

This choice is reversed if we wish to limit duration of the extraction and to gather the highest quantity of the produced extract. In this case, the most adequate temperature would be about 50°C since it corresponds to the fastest kinetics.

Temperature (°C)	Output (%)	V _{molar} (cm ³ mol ⁻¹)	ρ_{CO_2} (Kg.m ⁻³)	Solubility (g of extract/kg of CO ₂)	R ₁ (%) during the first hour of extraction
40	2.70	57.75	761,84	0.19	20
50	1.75	63.51	692,84	0.49	35
60	1.68	70.83	621,14	0.23	35
70	2.22	79.85	550,98	0.14	15

Table 2: Summary of the output variation of “Myrtus communis L” extract, the solubility and the output during the first hour in function of the temperature with P=18MPa

IV. CONCLUSION

The last years prove a very significant essential oils extraction insofar as the people are oriented towards the vegetable therapies and natural esthetics.

New technologies could lead to new extraction processes and such as, the extraction by supercritical CO₂ enjoys a dash interest because it makes it possible to lead to a pure product, absence of residual solvent and selectivity. This work made it possible to lead to the following results:

- The increase in the pressure generated a clear improvement of extraction rate which is controlled by a balance between the density variations of the solvent and that of the aqueous solution vapor pressure.
- We recover the extract maximum at P=18MPa.
- There is an optimal pressure (15MPa) for which we obtained the best extraction rate (80%) during the first hour.
- The increase in the temperature does not involve the extraction rate. This was explained by the competition between the variation of the density of the solvent and its viscosity;

We conclude that there are two optimal values of temperature (40°C and 70°C) for which we have the best extraction output. In addition, it's preferable to work with the low temperature if we wish to preserve the extract quality, but if we wish to recover the major part of the extract in an optimum time (the first hour) we must work with 50°C.

REFERENCES

1-L. Peyron, «Les essences de myrte, Plantes médicinales et phytothérapie». Tome IV, N°4, P.279-285, 1970.

2-Messaoud, Youssr Zouali, Abderraouf Ben Salah, Naoufel Souayah, Mohamed Labri Khoufa et Mohamed Boussaïd, «Analyse de quelques marqueurs isoenzymatiques chez le myrte (myrte communis.L) en Tunisie». Annales de l'INGREF(2002), 5, 43-56, 2002.

3-Monah.Boelens and rafael Jimenez, «The chemical composition of Spanish myrtle leaf oils». Part I, J.Ess.Oil Res.3, 173-177, 1991.

4-Bassem Jamoussi, Mehrez Romdhane, Abdelmanef Abderraba, Bechir Ben Hassine and Abdellatif Gadri, «Effect of harvest time on the yield and composition Tunisian myrtle oil». Flavour and Fragrance Journal, 2004.

5-J.P. FRIEDRICH and E.H.PRYDE, «Supercritical CO₂ Extraction of Lipid Bearing Materials and Characterization of the products». JAOCS, vol 61, N°2, 1984.

6-Nguyen, K., Barton, P., Spencer, J.S., supercritical carbon dioxide extraction of vanilla, Supercrit. Fluids. J, 1991, 4, 40.

7-Langa, E., Della porta, G., Palavra, J.S., Urieta, A., Mainar, M., Supercritical fluid extraction of Spanish sage essential oil: optimization of the process parameters and modelling, Supercrit. Fluids 2009, 49, 174-181.

8-Chrastil, j., Solubility of solids and liquids in supercritical gases, phys.chem.J, 1982,86, 3016- 3021