Influence of instantaneous controlled pressure drop extraction conditions on composition and oil yield from Maritime Pine (Pinus Pinaster)

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Influence of instantaneous controlled pressure drop extraction conditions on composition and oil yield from Maritime Pine (*Pinus Pinaster*)

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Abstract

Experiments to extract the essential oil from maritime pine (*pinus pinaster*) were carried out using the instantaneous controlled pressure drop process: "Détente Instantanée Contrôlée" (D.I.C). This process involves subjecting the maritime pine needles for a short period of time to a steam pressure varying from 2 to 5 bar (120 to 150 °C) during a fixed processing time, followed by an instantaneous decompression towards a vacuum (about 50 mbar). In this contribution, we have studied the effect of processing pressure and processing time of *pinus pinaster* needles on the extraction yield and on the composition of the extracted oil. A detailed attention was attached two three important qualitative molecules namely: α-pinène, β-pinène and germacrene D. The processing pressure was varied from 2 to 5 bar and the processing time from 1 to 35 minutes. The variation of processing pressure from 2 to 5 bar was carried out at 4 minutes processing time. The extraction yield varied respectively from 0.38 to 2.02 g/100 g dry material. However, the optimum can be considered between 3 and 4 bar due to a certain degradation of α-pinene indicated by a diminution of the percentage of this molecule in the extracted oil when the processing pressure exceeds 4 bar. The effect of processing time was studied from 1 to 35 minutes at optimised processing pressure of 4 bar. At this processing pressure, the optimum essential oil extraction yield of 2 g/100 g dry material basis was obtained at 8 minutes processing time. For the three studied molecules, two behaviours were observed: for the less volatile one (α-pinene), 1 minute of processing time is sufficient to extract the totality of this compound present in the raw material. Increasing of processing time leads to a decrease of the yield of α-pinene. For the two others molecules (β-pinene and germacrene D), increasing of processing time from 1 to 35 minutes induced an increase of their percentages in the extracted oil from 16.4 and 1.37 to 27.3 and 2.61 respectively

Mots-clés : Maritime pine (*pinus pinaster*), extraction, vacuum, essential oil, D.I.C

1. Introduction

Essential oil is any class of volatile oil composed of a mixture of complex hydrocarbons (mainly terpenes) and other chemicals isolated from plants. One of their characteristics is the generation of flavour or aroma. Essential oils extracted from plants as pine are widely used as fragrances in cosmetics, flavouring additives of foods and beverages, scenting agents in a variety of household products as detergents, soaps or insect repellent. They are also used as intermediate in the synthesis of perfume chemicals and often sold for unconventional medicinal purposes as well as in aromatherapy (Lazutka et al., 2001). The conventional methods for the extraction of the essential oils have some disadvantages. For the steam distillation and hydrodistillation, the elevated temperatures can cause chemical modifications of the oil components and often a loss of the most volatile molecules (Khajeh et al., 1997). When using the solvent extraction, it is impossible to obtain a solvent-free products and this process usually results also in

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the loss of the highly volatile components. In contrast, extraction by supercritical fluids leads to high-quality and solvent-free extracts (Reverchon, 1997). However, according to Temelli et al., (1988) and to Oszagyan et al., (1996) the technological conditions required for the use of supercritical fluids are onerous and the high cost of producing specific products has limited its use. Moreover, several studies have shown that the CO_2 not only extracts the essential oil, but also other compounds such as vegetable waxes or resins.

The Instantaneous Controlled Pressure Drop process, known as "D.I.C", was developed in our laboratory some years ago (Rezzoug et al., 1998), initially for use in the field of the drying-texturation of various food products by improving the hydration capacity. This process is based on the thermo-mechanical processing induced by subjecting the product to a rapid transition from high steam pressure to a vacuum. In the case of food products such as vegetables, the aim was to improve the vaporization of water with a preservation of the fragrance coupled to an alveolate texture of dried product while in this paper, we used this process in order to separate the volatile oil from the solid material. The essential oil isolation based on this process, which was successfully tested for isolation of essential oil from orange peels (Rezzoug et al., 2000a) is an interesting alternative not only to standard techniques of essential oil extraction, such as extraction with solvents or steam distillation, but also to more effective processes such as those using the supercritical fluids. In fact, this extraction process does not require the use of any solvent and the induced cooling when the plant is rapidly transferred from a high steam pressure to a vacuum stops all thermal degradation of the essential oil components (Rezzoug et al., 2005). The processing by instantaneous controlled pressure drop increases the global diffusivity of the product and improves the availability of the liquid in the plant. In this contribution, this process was used to extract the volatile oil from maritime pine (*pinus pinaster*). Two processing parameters of this process were evaluated: the processing pressure and the processing time. The response parameters were the global extraction yield of essential oil as well as the variation of the yield of 3 important qualitative molecules namely α-pinene, β-pinene and germacrene D.

2. Materials and methods

2.1 Plant material

Maritime pine (*Pinus Pinaster*) was collected from plants growing in west southern France. The leaves were used at their residual moisture content (63.1 % by weight on dry basis). Some experiment were also performed on dried needles (40 % and 10 % by weight on dry basis)

2.2 Experimental set-up

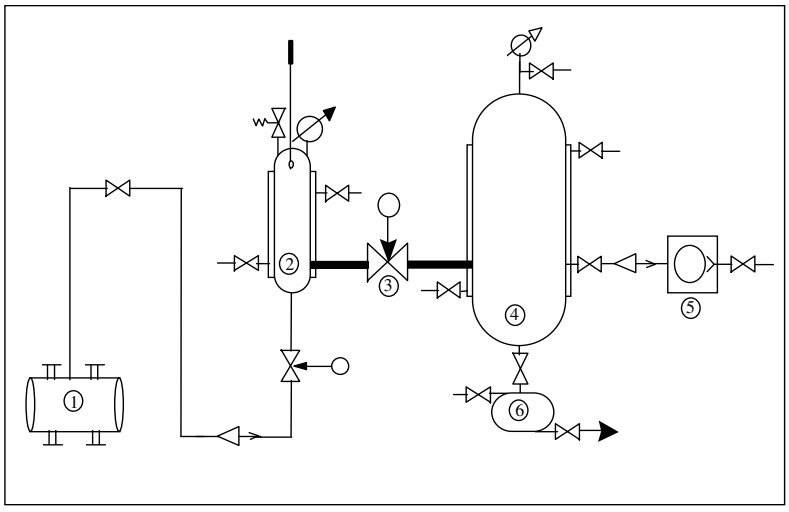

Figure.1 Schematic diagram of the apparatus used for the extraction of essential oil from rosemary leaves by Controlled Instantaneous Decompression. 1. Boiler - 2. Treatment vessel (made of stainless steel) - 3.Valve - 4. Vacuum container - 5. Vacuum pump - 6. Extract container.

The experimental set-up (fig. 1) was largely described in a previous study (Rezzoug et al., 2005); it is composed of three main elements

→ The processing vessel (2) where the samples were placed and treated.

→ The vacuum system which consists mainly from a vacuum tank (4) with a volume (360 l) 130 fold greater than the processing vessel (12 l), and a vacuum pump (IV). The initial vacuum pressure of the vacuum container was maintained at 50 mbar in all the experiments.

→ A pneumatic valve (3) that separate the processing vessel from the vacuum tank. It can be opened in less than 0.2 seconds; this ensures a rapid decompression within the reactor.

2.3 Protocol of extraction by the instantaneous controlled pressure drop process

The needles are firstly placed in the D.I.C vessel (fig.1-2) which is maintained under a vacuum (~ 50 mbar) through its connection to a vacuum container (fig.2a). The vacuum allows a better diffusion of the heating fluid through the plant and consequently heat transfer between the steam and wood is improved and the time to reach the desired processing pressure (or processing temperature) is shortened. After closing the electropneumatic valve (fig.1-3) which connects the reactor to the vacuum tank (Fig.1-4), an atmosphere of steam pressure (between 1 and 6 bar) is created within the D.I.C. reactor (fig.2c). After a processing time at fixed processing pressure (fig.2d), the thermal treatment is followed by a rapid decompression resulting in a rapid drop in pressure (fig.2e). The equilibrium pressure after decompression depends on the operating pressure: the higher the processing pressure, the higher the equilibrium pressure. The evaporation, which is effected in adiabatic conditions, induces a rapid cooling of the residual product. The final temperature must be commensurate with the final pressure. The maritime pine wood extract and the condensed steam are recovered in a specific container (Fig.1-6). The volume of the obtained mixture was about 400 ml for all experiments.

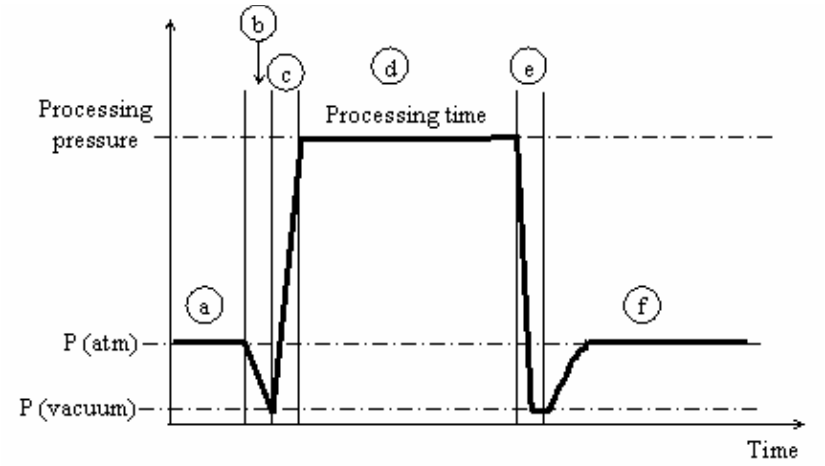

Figure 2: Typical pressure-time profile for DIC processing cycle. (a) sample at atmospheric pressure; (b) establishment of vacuum; (c) steam injection to reach selected pressure; (d) treatment time at selected processing pressure; (e) pressure drop; (f) atmospheric pressure for the sample recovery

2.4 Steam distillation

50 g of maritime pine needles chips were placed on stainless steel grid. This grid was disposed in a glass chamber containing boiling water. The steam crossed the grid during two hours and was recovered in condensate form with volatiles compounds after crossing a refrigerant. The condensates were separated into aqueous phase and organic phase (essential oil) by decantation.

2.5 GC/MS conditions

Varian 3900 gas chromatograph coupled to a Varian Saturn 2100T ion trap mass spectrometer (Varian, France) for characterizing the extracts was used. The column was a 30m× 0.25 mm, 0.25 µm CP-Sil 8 CB Low Bleed MS capillary column (Varian, France). Column temperature was 80°C (3min) - 3°C/min - 250°C (40 min). We used Helium as carrier gas at 1 ml/min. The extract samples were injected via a Varian CP-8400 autosampler fitted with a 5µl syringe. Transfer line temperature was 280°C. Electron

impact mass spectra were obtained at 70 eV ionization potential and peak identity was identified by NIST 2002 Data library.

3. Results and discussion

The compounds identified and their yield are shown in table 1. The yield of essential oil in fresh raw material was 0.82 % by mass (g of E.O/100 g dm). This value is in agreement with the value cited by authors like Kelkar et al., (2006) and Dob et al., (2005).

Table 1. Percentage composition of fresh pinus pinaster needles oil isolated by steam distillation

Constituents	g/100 g of E.O
Tricyclene	0.08
α-Pinene	40.50
camphene	0.75
β-pinene	25.42
β-myrcene	3.61
p-cymene	4.02
limonene	3.52
γ-terpinene	0.08
dehydro p-cymene	0.07
terpinolene	0.74
4-terpineol	0.21
α-terpineol	1.05
α-copaene	0.40
longifolene	1.92
β-caryophillene	6.30
α-humulene	1.08
germacrene-D	3.21
g-cadinene	1.09
Caryophillene oxyde	0.29

3.1 Effect of drying of maritime pine needles before Instantaneous Controlled Pressure Drop process on extraction efficiency

To evaluate the effect of drying (in an oven at 25 °C) on the composition of the essential oil extract, experiments of extraction by steam distillation were carried out on three samples of maritime pine needles. The first one on fresh product, the second one on needles dried to 40 % moisture content (d.b) and the third experiment on needles dried to 10 % moisture content (d.b). The results are grouped in table 2. It clearly appears that the global extraction yield diminishes with the moisture content as well as the concentration of the majority of molecules. For the most important one the yield decreased from 40.5 for fresh needles to 22.5 % (g of molecule / 100 g essential oil) for needles dried to 10 % moisture content (d.b). This can be explained by an evaporation of a part of the molecule during the drying. For some molecules as β-caryophillene, an increasing of the yield was observed. It can be explained by a total disappearance of some molecules which leads to an augmentation of the concentration of the identified molecules. So for the extraction by instantaneous controlled pressure drop process, the needles were treated at fresh state (~63 % d.b).

A parametric study was performed by varying two process parameters of the instantaneous controlled pressure drop process. The variations of process parameters are summarized in table 2. The processing pressure was varied from 1 to 5 bar, the initial and the processing time from 1 to 35 minutes. The validity of the results was confirmed by the low uncertainty limit in the extraction yield (based on 1.35 % error estimate) obtained from eight replications at processing pressure of 4 bar and processing time of 4 minutes.

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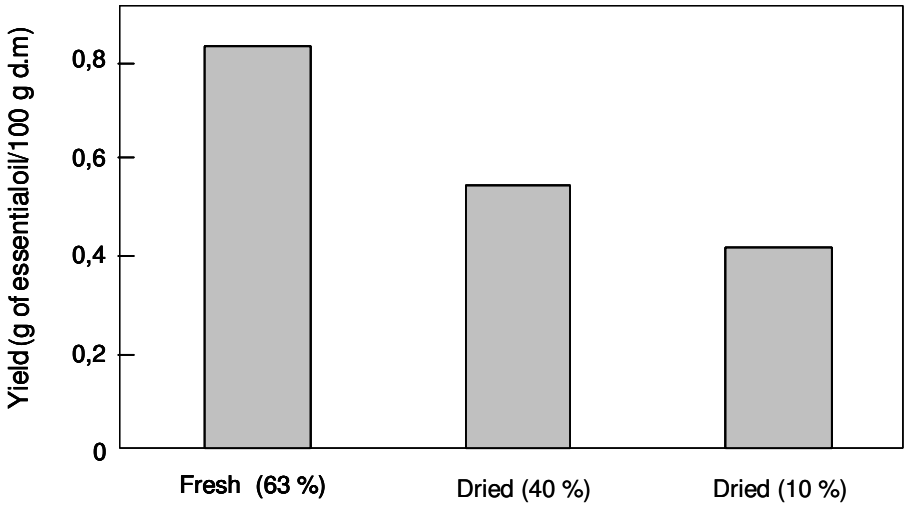

Figure 3: Effect of drying of maritime pine needles on the yield of essential oil extracted by steam distillation

Table 2. Percentage composition (in g of molecule 100 g of essential oil) of pinus pinaster needles oil dried at two different moistures contents: 10 % and 40 % (d.b)

Constituents/ Moisture content	40 %	10 %
Tricyclene	0.059	0.06
α-Pinene	28.91	22.52
camphene	0.863	0.640
β-pinene	15.52	13.32
β-myrcene	1.571	1.012
p-cymene	0.015	0.014
limonene	2.994	2.424
γ-terpinene	0.141	0.175
dehydro p-cymene	0.033	0.021
terpinolene	0.902	0.765
4-terpineol	0.085	0.064
α-terpineol	0.735	0.413
α-copaene	1.005	0.912
longifolene	3.114	4.853
β-caryophillene	6.748	9.882
α-humulene	1.496	1.801
germacrene-D	2.328	1.758
γ-cadinene	1.775	2.059
Caryophilene oxyde	1.503	1.295

Table 3. Processing parameters performed in the parametric study of maritime pine oil extraction by instantaneous controlled pressure drop process

Processing pressure (bar)	Processing time (min)
1	1
2	2
3	4
4	8
5	12
6	20
	35
processing time = 4 min*	processing pressure = 4 bar*

* Fixed processing parameters in each parametric study

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3.2 Effect of processing pressure on global extraction yield and on the yield of the three selected qualitative molecules

In the first variation, the processing time was fixed at an acceptable value of 4 minutes as contact time between the raw material and saturated steam at different temperatures corresponding to a processing pressure variation from 1 to 6 bar. It should be noted that steam distillation at industrial scale is performed in two from three hours. Chen and Spiro (1994) who worked on microwave extraction of essential oil from rosemary leaves reported that a long time at high temperatures could cause rearrangement or polymerization of some of rosemary oil constituents which are close to the constituents present in the needles of maritime pine oil. Figure 4 show that the processing pressure has a significant effect on extraction efficiency. When the processing pressure increases from 1 to 4 bar, the extraction yield increases from 0.25 to 1.75 %. Beyond 4 bar, a weak variation was observed and yield stabilize about 2 % (d.b) from 5 bar processing pressure.

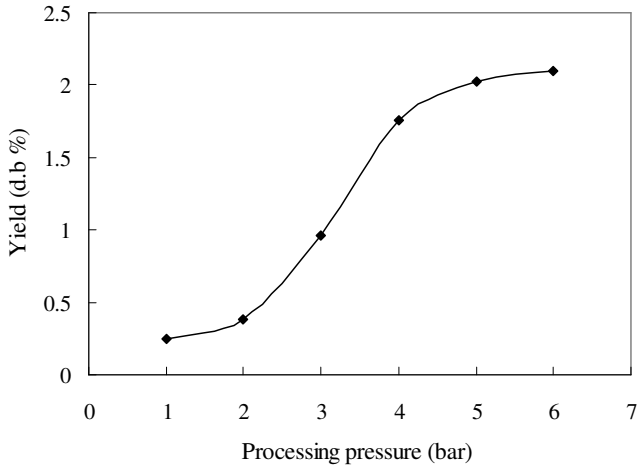

Figure 4: Effect of processing pressure in Instantaneous Controlled Pressure Process on the extract yield

The drop of steam pressure from 3-5 bar to vacuum caused a mechanical strain, which had as effect a degradation of the glands containing the volatile molecules. The same observation was cited by Chen and Spiro (1994), who reported that the oil synthesized in the secretory cells was not released unless an external factor damages the gland. This observation was also verified by Boutekedjiret et al., (2004), whom compared the isolation of rosemary oil by different extraction processes, including the Instantaneous Controlled Pressure Drop Process.

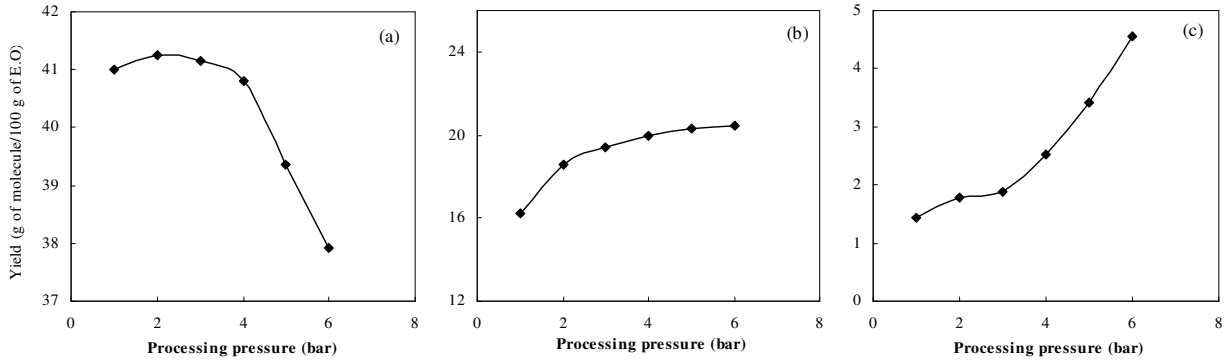

Figure 5: Effect of processing pressure in Instantaneous Controlled Pressure Process on the yield of 3 important molecules of maritime pine essential oil. (a): α-pinene; (b): β-pinene; (c) germacrene D. In all experiments, the processing time is fixed at 4 minutes.

The authors observed by scanning electron microscopy, that the microstructure of rosemary leaves after extraction were more expanded with appearance of cavities indicating a partial collapse of the leaf structure. The processing pressure was therefore maintained at 4 bar in processing time variation experiments. This results shows that compared with other "flash" extraction processes, extraction by D.I.C is more efficient and thus at industrial level, a subsequent steam distillation step is not necessary.

From industrial point of view, the qualitative criterion of maritime pine extracts is based on the presence of three molecules namely α-pinène, β-pinène and germacrene D, in defined percentage in the essential oil as commercial standards. The α-pinene must represent between 33 and 43 % of the extract, β-pinene between 22 and 32 % and germacrene D between 0.5 and 4 % of the oil. For this reason, the effect of processing pressure of DIC extraction process on the quantity of these molecules was studied.
From figure 5 (a), it can be seen that when the processing pressure increases from 1 to 2 bar, the yield of α-pinene slightly increase from 41 to 41.3 % and remains almost stable up to 4 bar. Beyond this value of processing pressure, an important diminution of α-pinene yield was observed, from 40.8 at 4 bar to 37.5 at 6 bar. This clearly indicates a certain degradation of this molecule occurs when the processing exceeds 4 bar. For β-pinene, which is also an important molecule in the maritime pine needles oil, it can be seen from figure 5 (b), that 90 % of this molecule is extracted at 2 bar (at 4 minutes processing time). Beyond this processing pressure, a very weak variation of β-pinene yield is observed.
Figure 5 (c) indicates the effect of processing pressure on the yield of germacrene D. It can be seen that the behavior is different to that of the two first molecules. In fact, the yield is stable about 1.5 and 1.8 g of germacrene D/100 g of essential oil, for processing pressures ranged between 1 and 3 bar. Over this value, the yield of germacrene D increases from 1.8 to 4.6 g of molecule /100 g of essential oil. We can therefore argue that the proposed process is selective. If we favor the presence of monoterpenes hydrocarbons in the maritime pine extract, it would be better to work at processing pressure included between 2 and 4 bar. In contrast, if we favor the presence of germacrene D, processing pressures more than 4 bar are more suitable.

3.3 Effect of processing time on global extraction yield and on the yield of the three selected qualitative molecules

For the variation of processing time, the processing pressure was maintained at 4 bar. It can be seen from figure 6 that at this processing pressure, 4 minutes are sufficient to extract more than 80 % of available essential oil. The maximum of extraction yield is almost reached after 8 minutes processing time. Beyond this value, a certain degradation of isolated oil, expressed by a more deepened coloring was observed. By looking figure 6 more closely, we can observe that the yield evolution versus processing time show a very rapid increase during the first minutes of the isolation process, then gradually levelled to the equilibrium value at the end of the process. The kinetic plot is similar to that obtained for rosemary leaves oil extracted by instantaneous controlled pressure drop process (Rezzoug et al 2000b).
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The extraction of the oil from maritime pine needles, by instantaneous controlled pressure drop process, seems to be regulated by two distinct phenomena corresponding to two steps. The first one is rapid compared to the second. It corresponds to a free diffusion phenomenon which takes place at the plant surface. On the other hand, the rapid decrease in the extraction rate observed from 5 min processing time may be due to endogenous stockage sites of the oil.

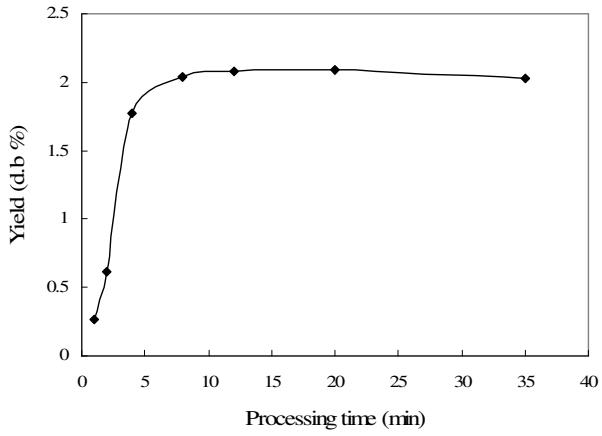

Figure 6: Effect of processing time in Instantaneous Controlled Pressure Process on the extract yield

The oil recovered in the second step is probably regulated by osmosis phenomena and slow diffusion through the plant cells towards the surface. However it can be seen that the oil collected in the last step (~6 %) is very low compared to the first step (~94 %). It is obvious that the major part of the oil is recovered by a simple process of free diffusion and evaporation. The proportions of these two parts for steam distillation extraction are generally lower for the first step and higher for the last one. This difference may be attributed to the presence of saturated steam under pressure in the case of instantaneous controlled pressure drop process that allows reaching more endogenous sites than at atmospheric pressure in the case of steam distillation.

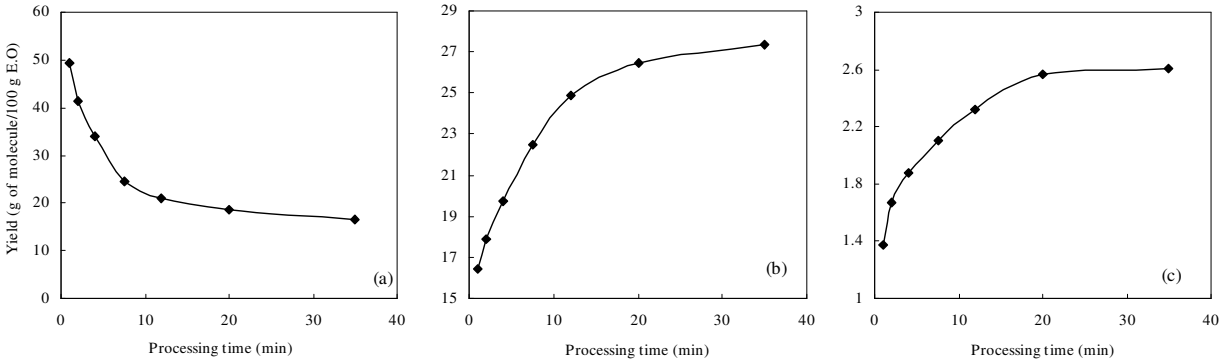

Figure 7: Effect of processing time in Instantaneous Controlled Pressure Process on the yield of 3 important molecules of maritime pine essential oil. (a): α-pinene; (b): β-pinene; (c) germacrene D. In all experiments, the processing pressure is fixed at 4 bar.

For the three studied molecules, the processing time have also a significant effect. The previous observation is clearly confirmed for one of the two monoterpene hydrocarbons i.e β-pinene as well as germacrene D (figures 7(b) and (c)). For these essential oil compounds increasing of processing time leads to an increasing of their yield, essentially between 1 and 20 minutes. For β-pinene, 1 minute is sufficient to extract more than 60 % from the total of this compound and to extract the totality; 10 to 12 minutes are required. The germacrene D, which is considered as a precursor of many sesquiterpene hydrocarbons, exhibit the same behaviour as β-pinene. When the processing time increases from 1 to 35 minutes, the yield varied from 1.37 to 2.61 g of germacrene D/100 g of essential oil. For these two molecules, the variation of the yield has two phases. The first one from 1 to 10-12 minutes and the second one from 12 to 35 minutes. As for the global extraction yield, the first phase is more rapid than the second one.

An inverse trend is observed for α-pinene. Increasing of processing time induced a decreasing in its yield, indicating also here a certain degradation of this molecule which is the more volatile. Commelli et al., (2006) reported a same behaviour for the isomerization reaction of α-pinene which produces bicyclic and monocyclic compounds and other products, in presence of catalyst and temperature.

3.4 Conclusion

The aim of this work was to provide an efficient and economically attractive process for extraction of oil from maritime pine (*pinus pinaster*) needles. This recent extraction process was also successfully tested on isolation of oils from other plants such as citrus. The developed extraction process is an interesting alternative not only to standard techniques of essential oil extraction, such as extraction with solvents or steam distillation, but also to more effective processes such as those using the supercritical fluids. This method doesn't require the use of a solvent and has the advantage of being both fast and selective. Among operative parameters of the instantaneous controlled process, we varied 2 parameters in this work: the processing pressure and the processing time. The conducted parametric study showed that the optimum extraction conditions in the studied ranges are a 4 bar steam pressure for 8 minutes. Unfortunately, for this value of processing time a certain degradation of α-pinene was observed and if the aim is to favor the presence of this molecule to the detriment of the global extraction yield, the processing time must be reduced to 4 minutes. The energy consumption and the flexibility of the proposed process can be favourably compared with other methods because the processing time was reduced from more than 2-4 hours hour to 4-8 minutes.

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