

Supercritical Carbon Dioxide Processing of Pyrethrum Oleoresin and Pale

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Possible refining of crude hexane extract (CHE) from pyrethrum flowers and further refining of Pyrethrum Board of Kenya (PBK) pale product is investigated with both liquid and supercritical carbon dioxide. The experiments were carried out in a small pilot plant with a 200 mL extractor and three cyclonic separators in series. To understand the dynamics of pyrethrin extraction, CHE was extracted in a single step; pyrethrin concentration was found to be improved from 0.16 to 0.50 g/g. The effects of temperature and pressure on the quality of the extract were studied at 29 °C and 80 bar and at 40 °C and 100 bar. Liquid CO₂ processing (29 °C, 80 bar) yielded slightly better product quality. A comparison study of CHE and PBK pale processing with supercritical CO₂ (40 °C, 100 bar) showed that the final products were similar in terms of pyrethrin content. Extraction of both PBK pale and CHE in two steps with different operating conditions improved their purity.

KEYWORDS: Supercritical carbon dioxide; pyrethrin extraction; oleoresin; pale; pyrethrum

INTRODUCTION

Pyrethrins, which are the most widely used natural domestic insecticides, are extracted from pyrethrum flowers. This extract contains six active compounds, which are classified as pyrethrins I and pyrethrins II. Pyrethrins I include pyrethrin I, jasmolin I, and cinerin I, whereas pyrethrins II include pyrethrin II, jasmolin II, and cinerin II. The most valuable compounds in pyrethrum extract are pyrethrin I, which is very toxic to insects, and pyrethrin II, which has a very high knockdown effect on insects. In general, pyrethrins are nontoxic to warm-blooded animals, and they are unstable in the presence of air and light. In the conventional commercial process, pyrethrins are extracted with organic solvents from pyrethrum flowers to obtain oleoresin concentrate. Oleoresin is a crude extract, which contains waxes, chlorophyll pigments, and fixed oils from the flowers. The most commonly used solvent to obtain oleoresin concentrate is hexane. Methanol is then used to precipitate nonpolar compounds from oleoresin to obtain methanol micelle, which is evaporated to produce methanol concentrate. The methanol concentrate is diluted with SST, a Shell proprietary solvent of the family of petroleum ether, to remove the so-called green oils after sedimentation. The SST solution is winterized at around -8 °C to precipitate a great part of the waxes. Activated carbon is added at ambient temperature to remove the color pigments. SST is evaporated to obtain the final concentrate called "pale product", which is ~50% pyrethrin content (by weight).

Direct extraction from pyrethrum flowers with liquid and supercritical carbon dioxide has been already reported. Wynn et al. (1) and Stahl et al. (2) observed that, by operating a two-step depressurization after extraction, fractionation was possible and yielded a final product with a pyrethrin content of ~60%. Otterbach et al. (3) observed that CO₂ extraction yielded better extracts in terms of color and pyrethrin content compared to those obtained by ultrasonic and Soxhlet extraction. Bunzenberger et al. (4) noticed that with supercritical carbon dioxide, pyrethrins I were extracted more quickly than pyrethrins II from pyrethrum flowers. In our previous works (5), we obtained a light yellow extract, which changes very quickly to brown with 20% pyrethrins (by weight). An alternative development of such products could be to use carbon dioxide to refine the conventional crude extract. To our knowledge, this was mentioned only by Sim (6) in his patent, in which use of liquid carbon dioxide was proposed and in an unpublished report from the Archimex Co. (7), in which a pre-industrial study of such a process was proposed. Therefore, there is no available detailed information on the extraction or purification of pyrethrin from oleoresin and pale product using supercritical and liquid carbon dioxide. In this work, using carbon dioxide we have studied the extraction of pyrethrins from crude hexane extract (CHE) (from our own batch extraction experiments) and from a commercial pale product from the Pyrethrum Board of Kenya (PBK pale).

MATERIALS AND METHODS

Pyrethrum flowers were bought from local farms in Kenya, and the Pyrethrum Board of Kenya donated the conventional purified extract (PBK pale) at 0.48 g/g pyrethrin content. A refined pyrethrin sample

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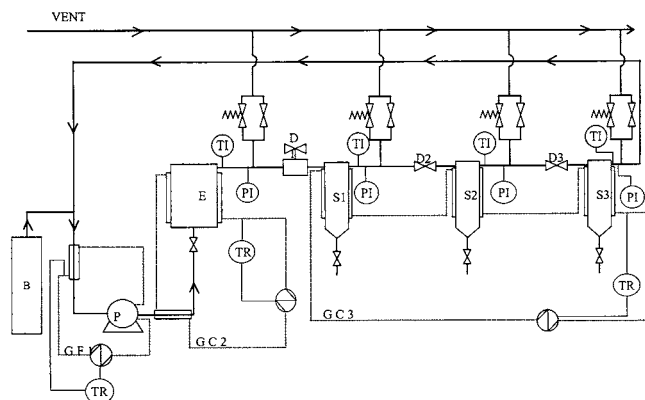


Figure 1. Scheme representation of the pilot: B, CO₂ bottle; E, extractor; S1, S2, S3, separators; P, pump; GF1, cooling group; GC2, GC3, heating groups; PI, pressure indicators; TR, temperature regulators; D1, D2, D3, depressurization valves.

was bought from RdH laborchemikalien and Co. KG for standardization of the analytical method. Its pyrethrin content was claimed to be 21.1% (by weight).

Batch extraction of pyrethrins from ground pyrethrum flowers with hexane was conducted at ambient temperature, in an agitated vessel. The batch process was repeated several times, until the color of the solvent in the vessel was clear. CHE was then obtained by evaporation of hexane from the cumulative extracts of all batches. We obtained a CHE with a pyrethrin content of 0.16 g/g.

The CO₂ processing was performed with a pilot plant from Separex Chimie Fine (series 3417 type SF 200), having a contacting capacity of 200 mL (extractor, diameter of 42 mm and length of 145 mm) and a maximum CO₂ flow rate of 5 kg/h. The extractor is connected with three cyclone separators in series of 15 mL each as patented by Perrut (8). The scheme of the pilot plant is shown in **Figure 1**. The extractor and separators are jacketed to maintain constant temperature. Pressure in all separators was set to 50 bar, whereas their temperatures were set to 40 °C, because at lower temperature the extract in the separators was solid and not easily removable. In the extractor, temperature was set either to 29 °C or to 40 °C.

The different liquid loads (CHE or PBK pale) were poured in the extractor cylinder (internal diameter of 35 mm and length of 145 mm), filled with glass beads of 1 mm diameter, and the liquid remained as a film on the beads. Filter mesh screens were placed at both ends of the cylinder. The cylinder was then introduced into the temperature-controlled contactor. Care was taken to ensure that air was purged before processing was begun. The CO₂ was pumped at a constant flow rate and directed into the bottom of the extractor. The fluid phase leaving the extractor passed through valves, where the pressure was throttled via the three separators in series. Then the CO₂ was cooled and recycled again into the system. The extracts were found and collected only in the first separator at regular interval. Samples were weighed and analyzed. In all experiments, the CO₂ flow rate was kept constant at 0.3 kg/h.

Analyses of the extracts were performed using a high-performance liquid chromatograph (HPLC) Hewlett-Packard series 1050 equipped with a 250 × 4.6 mm column Lichrosorb SI60 5 μm, as proposed by Marr et al. (5). Elution was conducted with a mixture of acetyl acetate and hexane, in a ratio of 1:10 at a constant flow rate of 1.5 mL/min, leading to a 15 min analysis. The UV detector was set at a wavelength of 242 nm.

RESULTS

Purification of CHE at 40 °C and 100 bar. In **Figure 2**, we observe that the pyrethrin mass fraction in samples decreases with time. This indicates that, at the beginning of extraction, samples had a high pyrethrin content (which was as high as 0.6 g/g) and toward the end of extraction more undesired products were extracted.

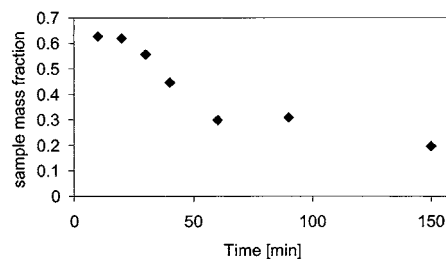


Figure 2. Pyrethrin mass fraction of samples extracted from CHE at 40 °C and 100 bar.

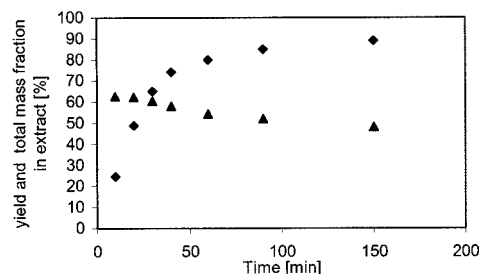


Figure 3. Pyrethrin extraction yield and pyrethrin percentage mass fraction from CHE extract at 40 °C and 100 bar: ◆, yield; ▲, percentage mass fraction.

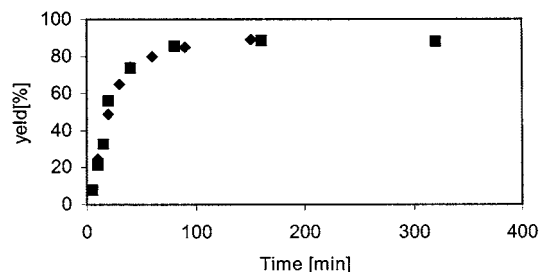


Figure 4. Pyrethrin extraction yield curves from CHE at (■) 80 bar and 29 °C and at (◆) 100 bar and 40 °C.

Figure 3 shows the extraction yield of pyrethrins at 40 °C and 100 bar. It took ~50 min to extract 80% of the total pyrethrin content of the load and 150 min to reach 90%. The product was light brown, unlike the initial CHE load, which was dark green or black. At the end, the residual product in the contactor had a pyrethrin content of 0.0171 g/g. From **Figure 3**, it can be observed that after 50 min, the cumulated samples had a pyrethrin content of >0.55 g/g and the final CO₂ processed extract had a pyrethrin content of 0.5 g/g. This is much more concentrated than the initial CHE load (0.16 g/g) and also more concentrated than in the case of direct CO₂ extraction from flowers, which yielded ~0.2 g/g [from our previous work (5)].

Influence of Operating Conditions for CHE Processing. We investigated two conditions: liquid CO₂ (80 bar, 29 °C) and supercritical CO₂ (100 bar, 40 °C). In **Figure 4**, we observe that yield curves were similar at both conditions. At the beginning of processing, the curve is a straight line, which indicates that the process is controlled by solubility. This is followed by a curved part that probably reveals the effects of pyrethrin concentration decrease on its liquid–fluid equilibrium.

Figure 5 shows pyrethrin mass fraction in extracted samples of CHE, using liquid CO₂ (density = 731 kg/m³) and supercritical CO₂ (density = 519 kg/m³). In both cases it is observed that the sample mass fraction variations with time are similar, whereas the final samples had pyrethrin contents of ~0.2 g/g. Residual pyrethrin content from the initial load after liquid processing was 0.0152 g/g, as compared to 0.0171 g/g obtained

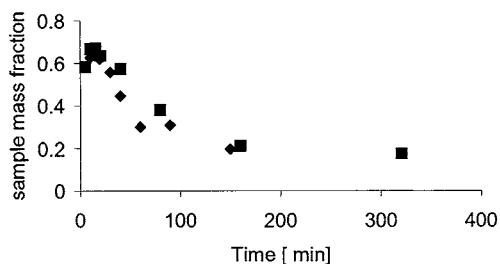


Figure 5. Pyrethrin mass fraction in extracted samples from CHE at (■) 80 bar and 29 °C and at (◆) 100 bar and 40 °C.

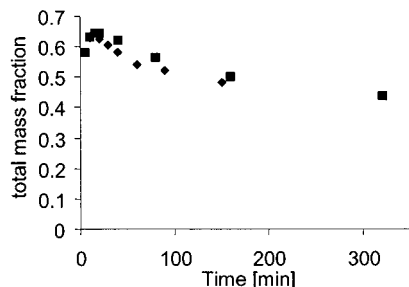


Figure 6. Pyrethrin mass fraction in cumulative extract from CHE at (■) 80 bar and 29 °C and at (◆) 100 bar and 40 °C.

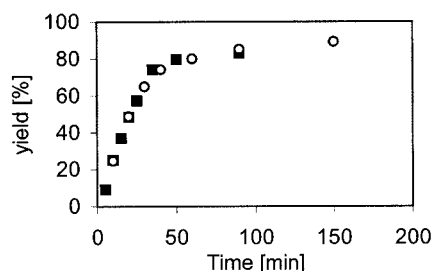


Figure 7. Extraction yield curves of pyrethrins extract from CHE and PBK at 40 °C and 100 bar: ○, CHE; ■, PBK.

after supercritical CO₂ processing. Figure 6 shows pyrethrin mass fraction in cumulative extract and indicates that up to 100 min, liquid CO₂ processing (80 bar, 29 °C) yielded slightly higher pyrethrin content than supercritical CO₂ processing (100 bar, 40 °C). Because we know from yield curves of Figure 4 that, at 100 min, almost all pyrethrins have been extracted, it can be said that better quality is obtained by liquid CO₂ processing provided that the processing is stopped at 100 min. Indeed, at low temperature, the amount of undesired products in the extract is lower because the solubility of these undesired product decreases with decrease in temperature. Nevertheless, when pursuing extraction, the final pyrethrin content in both cases is the same.

Comparison of Processing of PBK Pale and CHE by Supercritical CO₂ (100 bar, 40 °C). We notice that the yield curves are very similar as shown in Figure 7 but that the evolution pathways of sample mass fractions are very different (see Figure 8). Indeed, at the beginning of PBK pale extraction, volatile solvents that are very soluble in CO₂ caused the observed low pyrethrin content of the extracts shown in Figure 8. After extraction of these volatile solvents, the pyrethrin content increased because the PBK pale is a refined product and does not contain undesired product, such as soluble waxes, which are present in CHE. In this latter case, the slower extraction of these waxes explained the decrease of extract pyrethrin content. Surprisingly, after extraction, the pyrethrin mass fractions in the cumulative extract of PBK pale and CHE were the same (see Figure 9).

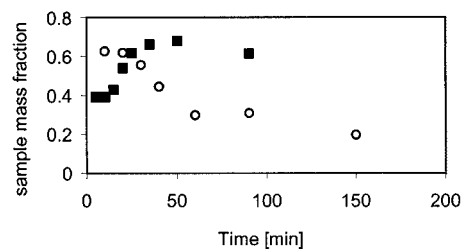


Figure 8. Pyrethrin mass fraction in extracted sample from CHE and PBK at 40 °C and 100 bar: ○, CHE; ■, PBK.

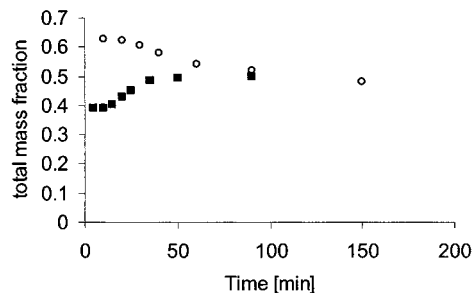


Figure 9. Pyrethrin mass fraction in cumulative extract from CHE and PBK at 40 °C and 100 bar: ○, CHE; ■, PBK.

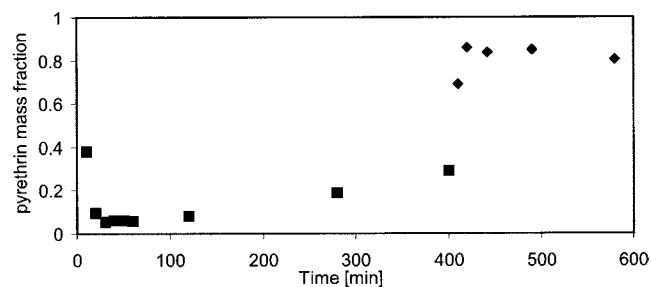


Figure 10. Pyrethrin mass fraction of extracted samples from PBK at 40 °C and a pressure of 80 bar then followed by 100 bar: ■, 80 bar; ◆, 100 bar.

Table 1. Results Obtained from Extraction of PBK at 40 °C and a Pressure of 80 bar Then Followed by a Pressure of 100 bar

step	step 1	step 2
initial mass (g)	7.0400	5.4627
mass extracted (g)	1.5773	2.7543
mass of pyrethrin extracted (g)	0.1934	2.2732
initial pyrethrin mass fraction (g)	0.4800	0.5388
residual pyrethrin mass fraction (g)	0.5388	0.2387
pyrethrin mass fraction in extract (g)	0.1226	0.8253
% of pyrethrin yield	5.720	77.23
% of mass extracted	22.40	50.42

Two-Step Processing of PBK Pale Using Supercritical CO₂. From these results, we can imagine a procedure for additional refining of the PBK pale, to obtain a new product with better quality. This is done in the two-step processing proposed in Figure 10. It shows the mass fraction of extracted samples from PBK pale with supercritical CO₂ at 80 bar and 40 °C for 400 min (step1), followed by a pressure increase up to 100 bar at the same temperature, for 180 min (step 2). Results are shown in Table 1. It is observed that in step 1, the cumulative mass extracted was 22.4% of the initial mass of the load, whereas the pyrethrin yield is 5.72% (see Figure 11) and the pyrethrin mass fraction in the cumulative extract is 0.1226 g/g (see Figure 12). The mass fraction of pyrethrin after this

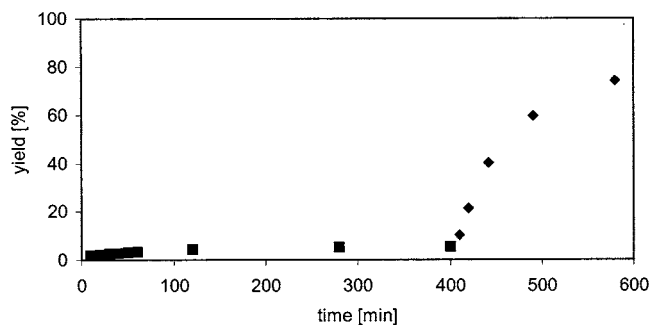


Figure 11. Extraction yield curve of pyrethrin extract from PBK at 40 °C and a pressure of 80 bar then followed by 100 bar: ■, 80 bar; ◆, 100 bar.

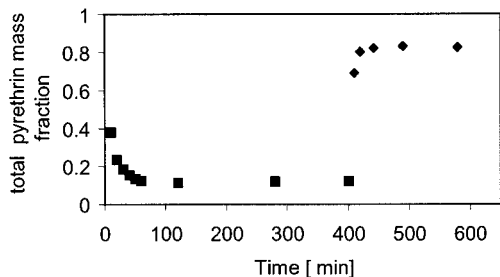


Figure 12. Pyrethrin mass fraction in cumulative extract from PBK at 40 °C and a pressure of 80 bar then followed by 100 bar: ■, 80 bar; ◆, 100 bar.

first step is 0.5388 g/g, which indicates that the pyrethrin content in the residual load was increased by 5.88%.

In step 2, extracted samples rapidly yield a pyrethrin content of >0.8 g/g (see Figure 10). The cumulative mass extracted in this step was 50.42% of the initial mass, in which the pyrethrin yield was 77.23%, whereas the mass fraction of pyrethrins in the cumulative extract increased to 0.8253 g/g (see Figure 12). The pyrethrin mass fraction of the residual load after this step was 0.2387 g/g.

In step 1 of this experiment, we were able to first extract volatile undesirable product. Indeed, at 80 bar and 40 °C the density of CO₂ is 260 kg/m³, leading to very low solubility of pyrethrins and fixed oils, whereas the solubility of the volatile product, which includes the extracting solvents, is high. The color of the extract of step 1 was orange.

In step 2, we obtained a high pyrethrin content extract. In contrast to step 1, at 100 bar and 40 °C, the CO₂ density is 520 kg/m³ and the solubility of pyrethrins is very high, whereas the solubility of fixed oils remains low. The final product was light yellow, unlike the initial PBK pale, which is orange.

In this experiment, we investigated the ratio of pyrethrins I to pyrethrins II, and we observed its variation with time, as shown in Figure 13. In step 1 (40 °C, 80 bar), an increase of this ratio is observed, indicating that pyrethrins I were extracted more quickly than pyrethrins II. In the initial PBK pale, the pyrethrin ratio was 1.45, and in the last sample of step 1, after 400 min, it was 4.44. The ratio in the cumulative extract of step 1 was 2.68, whereas the ratio in the corresponding residue was 1.23. In step 2 (40 °C, 100 bar), the pyrethrin ratio decreases with time, to 1.34 in the last sample of this step. The ratio in the cumulative extract of all the extractions was 1.73 and 0.69 in the residual load. It is then possible, with supercritical carbon dioxide, to obtain fractions with different pyrethrin ratios, allowing tuneable insecticide formulation, from a high killing effect to a high knockdown effect.

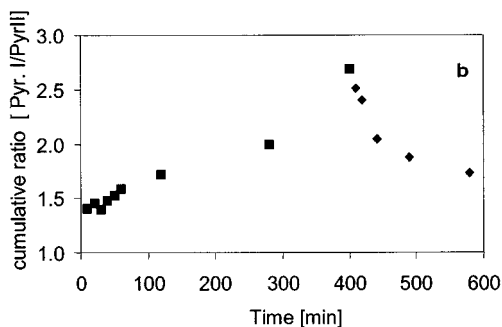
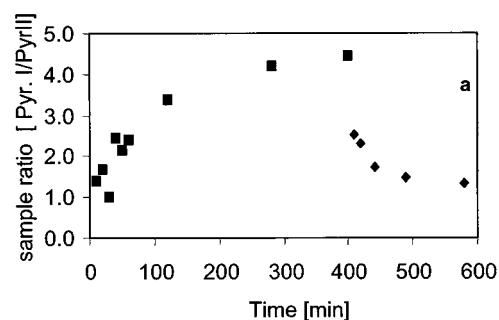


Figure 13. Pyrethrin ratios in (a) sample extract and (b) cumulative extract at 40 °C and a pressure of 80 bar then followed by 100 bar: ■, 80 bar; ◆, 100 bar.

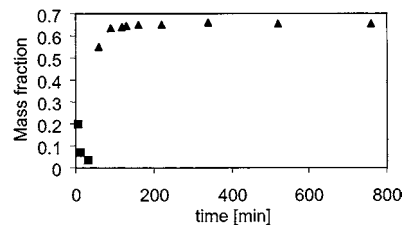


Figure 14. Pyrethrin mass fraction in cumulative extract from CHE at 40 °C and a pressure of 70 bar then followed by 100 bar: ■, 70 bar; ▲, 100 bar.

Extraction of CHE in Two Steps. A sample of CHE with a pyrethrin mass fraction of 0.16 kg/kg was cooled below 0 °C for 24 h, and the wax and solid extract settled at the bottom of the container. The upper part, which contained less wax and solids, was filtered out and subjected to CO₂ refining using two extraction steps. Unlike in the PBK extraction, in this extraction no glass bead packing was used and the CO₂ was bubbled in the extractor. The first extraction step was done at 70 bar and 40 °C. At this condition, most of volatile solvents including hexane was extracted. It was observed that in this step, 73.11% of the total initial sample was extracted and the pyrethrin mass fraction of this extract was 0.034 kg/kg (Figure 14). After 30 min, the second step was started; pressure was increased to 100 bar, and temperature was maintained at 40 °C. In this step 26.89% of the initial sample was extracted, and we obtained a brown viscous organic liquid with a pyrethrin mass fraction of 0.6534 kg/kg. The residue in the extractor was only a few black spots on the walls of extractor. Figure 15 shows the pyrethrin yield in each step and the combined pyrethrin yield with respect to initial pyrethrin content in the extractor. It was noted that the pyrethrin yield in the first step was 12.37%, whereas in the second step it was 87.00%.

Conclusion. The idea of processing conventional pyrethrum crude hexane extract with supercritical CO₂ is probably the more realistic proposal to obtain a new product with a better quality, in terms of concentration or lower organic solvent content. We

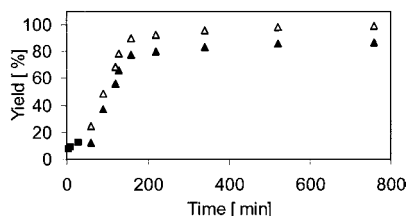


Figure 15. Extraction yield curve of pyrethrin extract from CHE at 40 °C and a pressure of 70 bar then followed by 100 bar: ■, 70 bar; ▲, 100 bar; △, total yield.

established here that the refining of CHE in two extraction steps and refining by fractionation seem to be the best choice on the criteria of pyrethrin concentration. Of course, this product is not as satisfactory as the one obtained by a direct extraction from the flowers with carbon dioxide [see our previous works (5)] provided that, in this direct extraction, no organic solvent is used subsequently to refine the extract. Indeed, an attractive method could be to operate the “on-line” purification in the separators of the postextraction section, using only changes in pressure and temperature. No report has yet clearly assessed such a process, although it is the only hope for any cost-effective applicability.

It is common opinion that the economics of solid–fluid extraction is always greatly handicapped by the batch operation of a solid load. Conversely, and as was the aim of this work, CO₂ refining of the already extracted liquid products (like CHE or PBK pale) can be done in a continuous way, for instance, in counter-current columns. Besides, this refining involves much smaller quantities to process. In this work, the comparison of the processing of CHE and PBK pale gave some explanations and led us to propose the principle of a two-step process. In this case, additional refining of the PBK pale yielded a very high quality product, that is, a product with high concentration and which is likely to have a low organic solvent content;

however, this point was not clearly assessed in this work. Indeed, we have seen that operating in two steps may allow the selective removal of these solvents. Nevertheless, yields must still be improved by optimization of the operating conditions. This preliminary study made us also aware of the interest of having a modeling tool (even very simple in the description of the mass transfer mechanisms) capable of accurately describing the solubility equilibria of the pyrethrins, the oils, and the waxes, in liquid or supercritical CO₂. In this case an optimal parameter profile for the processing could be easily proposed.

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