Extraction of essential oil and oleoresin from chamomile (*Chamomila recutita* [L.] Rauschert) by steam distillation and extraction with organic solvents: a process design approach

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RESUMO: Extração de óleo essencial e oleoresina de camomila (*Chamomilla recutita* [L.] Rauschert) por destilação por arraste a vapor e extração com solventes orgânicos : Um projeto de processos. Camomila (*Chamomilla recutita* [L.] Rauschert) é uma planta medicinal bastante cultivada no estado do Paraná. Por este motivo, o propósito deste trabalho foi a identificação das melhores condições de processo para maximizar o rendimento em óleo essencial e oleoresina, bem como os teores de α-bisabolol e camazuleno, do ponto de vista do projeto de processos. A pressão de operação, a vazão de condensado e o tempo de destilação foram as variáveis estudadas para o processo de destilação por arraste a vapor. Os resultados indicam que estas variáveis exercem um efeito significativo no rendimento. O rendimento foi, também, fortemente afetado pela interação entre o tempo de destilação e a vazão de condensado. O maior rendimento (0,11%, m/m) foi obtido a 0.98 bar, destilação por 60 minutos e vazão de condensado de 30 ou 60 mL/min. O mesmo resultado foi obtido decrescendo o tempo de destilação para 45 minutos e usando uma vazão de condensado de 60 mL/min. A oleoresina foi obtida por extração com etanol e isopropanol, usando um extrator com agitação. Os efeitos da temperatura, razão solvente/alimentação, tempo de extração e tipo de solvente no rendimento em oleoresina foram significativos. O maior rendimento (4,10%, m/m) foi obtido usando etanol a 40°C com uma razão solvente/alimentação igual a 6 e tempo de extração de 4 horas. Os principais compostos identificados no óleo essencial e na oleoresina de camomila foram β-farneseno, acetato de linalila (dihidro), γ-cadineno, α-farneseno, óxido de bisabolol B, α-bisabolol, camazuleno, óxido de α-bisabolol A, trans-dicicloeter (PM 200) e ftalato de butila.

Palavras-chave: Camomila, óleos voláteis, destilação, extratos vegetais, solventes.

ABSTRACT: Extraction of essential oil and oleoresin from chamomile (*Chamomilla recutita* [L.] Rauschert) by steam distillation and extraction with organic solvents : a process design approach. Chamomile (*Chamomilla recutita* [L.] Rauschert) is a medicinal plant largely grown in the state of Paraná, Brazil. The aim of this work was to identify the best process conditions to maximize the yields of essential oil and oleoresin, as well as their content of α-bisabolol and chamaluzene. Operating pressure, condensation flow rate, and distillation time, were the variables studied for the steam distillation process. The results showed that these variables exerted a significant effect on the yield. The yield was also strongly affected by the interaction between distillation time and condensation flow rate. The highest yield (0.11% by wt.) was obtained at 0.98 bar, distillation time of 60min., and condensation flow rate of 30 or 60 ml/min. The same result was obtained decreasing the distillation time to 45min using a condensation flow rate of 60ml/min. The oleoresin was obtained both by extraction with ethanol and with isopropyl alcohol, using an agitating extractor. The effects of temperature, ratio of solvent to feed, time of extraction, and type of solvent, on the oleoresin yield were all significant. The largest yield (4.10% by wt.) was obtained using ethanol at 40°C with a solvent/feed ratio of 6, and an extraction of 4 hr. The major compounds of essential oil and of the oleoresin were β-farnesene, linaool acetate, (dihydro), γ-cadinene, α-farnesene, bisabolol oxide B, α-bisabolol, chamazulene, α-bisabolol oxide A, trans-dicycle-ether (MW 200) and butyl phthalate.

Key words: Chamomile, volatile oil, distillation, plant extracts, solvents.

INTRODUCTION

In spite of the spread use of medicinal plants by the Brazilian population, there is a consensus about the lack of process designing data related to the production of natural extracts. This is the information required for optimization studies in order to reach the desired economical viability of such industries. Because chamomile (*Chamomilla recutita* [L.] Rauschert) is one of the plants selected by the Paraná's Health Clerkship Phytotherapy Project - SUDS, among 21 species, for medicinal use in the public health network (Barata, 1998), it is the subject of the present work.

The essential oil extracted from the chamomile flowers is a mixture formed by several compounds and the most well known are: chamazulene, α-bisabolol, besides flavonoids, proteins, sugars, lipids, mineral elements, etc. The dark blue color of the chamomile essential oil is due to the presence of chamazulene. This substance is formed from matricine during the steam distillation process in a reaction catalyzed by temperature (Sousa *et al*, 1991). The

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applications of the α-bisabolol and chamazulene in the phytotherapy, according to the literature, are due to the anti-inflammatory, anti-spasmodic (Marderosian-Der and Liberti, 1988), anti-flogistic, sedative, anti-allergic (Della Loggia *et al.*, 1990), and vermifuge properties (Isaac & Kristen, 1980), among others (Redaeli *et al.*, 1981).

A series of processes was investigated to obtain the essential oil and/or oleoresin from chamomile. Among the conventional processes we can mention the extraction with organic solvent and the steam distillation, which is the most used in commercial installations in Brazil. For small-scale installations, particularly for portable units, water distillation or water and steam distillation offers the advantage of simplicity of equipment. For larger and fixed installations, steam distillation unquestionably offers the greatest advantages. In such plants the necessary control can be readily installed and under these conditions the quality, yield, and rate of oil are superior. Plant materials containing either low or high boiling oils can be handled in the same equipment with equal ease. However, the yield obtained in the extraction of essence from flowers is very low. To increase the yield of active principle the alternative is to employ extraction with organic solvents (Guenther, 1972).

Considering the above, the objective of this work was to study, from the process design point of view, the variables that affect the processes of steam distillation and extraction with organic solvents, with regard to the yield and composition of the extracts. For the steam distillation process the operating variables studied were the operating pressure (OP), the condensate flow rate (CFR), and the distillation time (DT). For the extraction with organic solvents the following variables were considered: solvent to feed ratio (SFR), extraction time (ET), solvent type (ST), and temperature (T). Factorial designs were used to assess the effects of these variables on the yield of the chamomile extracts. The chemical compositions of the extracts were evaluated by GC/MS. The quantification of chamazulene and the α-bisabolol were conducted by GC-FID. With respect to the solvent type just ethanol and isopropyl alcohol were tested due to both: *i*) the severe restrictions in the food industry to various organic solvents, and *ii*) the potential of using chamomile extracts in nutraceutical formulations. In addition, it is worthwhile considering that the process of extraction with organic solvents will require a desolventization step that will add up to the total manufacturing cost.

MATERIAL AND METHOD

1- Raw Material

Dried chamomile flowers from the 1999 crop were acquired directly from the producer (Mandirituba, Paraná, Brazil). The material was maintained under refrigeration (- 8ºC) prior to its usage.

2- Raw material characterization

The humidity of the chamomile flowers was determined periodically using the Jacobs's (1958) method. For the steam distillation process 300 g of chamomile flowers were used without any further treatment. For the solvent extraction tests, the chamomile flowers were triturated in an electric mill (TECNAL, model TE 340, Brazil). The particle size distribution of the triturated solid was determined using sieves of the Tyler series, with an agitator (BELTER, Brazil).

3- Steam Distillation

The steam distillation apparatus used is shown in Figure 1 (Povh *et al.*, 1985). The vapor was generated in an autoclave with pressure control. It was injected in the still containing an expansion camera, a perforated plate used to hold the basket with the raw material and a condenser. The mixture of vapor and essential oil was condensed, using water at ambient temperature, and collected in a separation funnel. The mixture was allowed to rest for one day; afterwards, the essential oil was collected in small flasks closed with rubber lids wrapped up with Teflon, weighted in a analytical scale (Mettler, model AE 200 S, 200 g and precision of 0.0001 g), and kept under refrigeration for posterior chromatographic analysis. The assays were done at three operating pressures (OP = 0.49 bar, 0.72 bar, and 0.98 bar), two distillation times (DT = 45 min and 60 min), and two flow rates of condensate (CFR = 30 mL/min and 60 mL/min). A factorial experimental design was used to assess the effects of the operating conditions on the steam distillation yield.

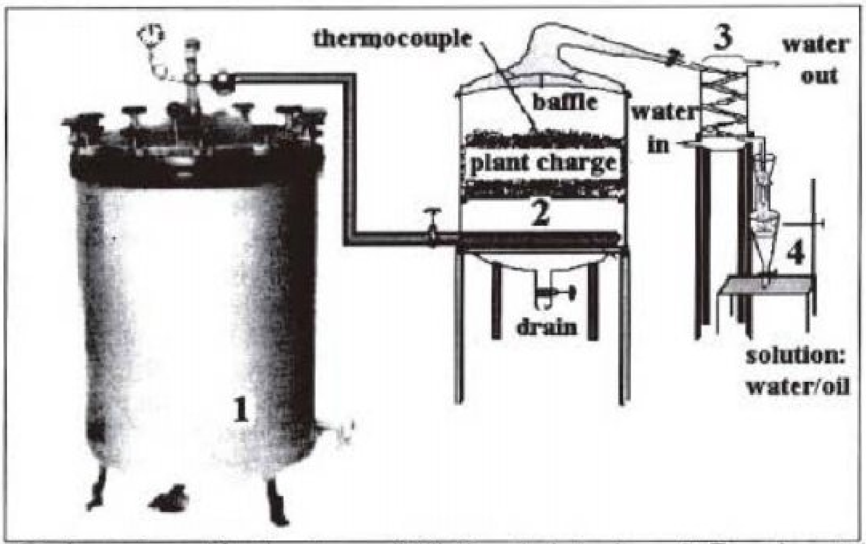

FIGURE 1. Equipment used for the steam distillation: 1: Autoclave, 2: Experimental still, 3: Condenser, and 4: Separation funnel.

4- Extraction with Organic Solvents

The extraction of chamomile oleoresin with organic solvents was made using a shaker (Marconi, model MA 830, Brazil). The extractions were done in two batches, one at 10ºC and the other at 40ºC. The flasks (Erlenmeyer), covered with rubber corks, containing 30 g of triturated chamomile and the required amount of solvent, were weighted before and after the extraction. The mixture of solid and solvent was kept under agitation for the preset extraction time. Afterwards, the mixture was vacuum filtered. The micelle (solvent + oleoresin + pigment) was placed in a dark flask, covered, weighted, and kept under refrigeration for posterior analyses. The amount of water originally present in the raw material dissolved by the solvent was determined gravimetrically: The washed solid was allowed to rest for 48 hours in a room with the air conditioning set at 18°C to evaporate the solvent, and afterwards the humidity was determined by Jacob's method. The oleoresin quantification was made gravimetrically (Spricigo, 1998): Twenty milliliters of the filtered micelle was weighted in Petri plates and allowed to rest for 48 hours in a room with the air conditioning set at 18ºC. The amount of solvent evaporated was then determined. The Petri plates were taken to an oven with air circulation and temperature set at 165°C for 6 hours. Afterwards, the plates were allowed to cool in a desiccator (30 minutes) and weighted. The Petri plates returned to the oven for additional periods of 3 hours up to constant mass. A factorial experimental design was used to assess the effects of the following operating variables on the extraction yield: solvent to feed ratio (SFR = 120 mL/30g and 180 mL/30 g), solvent type (ST = ethanol and isopropyl alcohol), extraction time (ET = 4 hours and 8 hours), and temperature (T = 10ºC and 40ºC).

5- Chemical composition of the essential oil and oleoresin

The chemical composition of both the essential oil and the oleoresin were determined using a gas chromatograph (GC mark VARIAN model 3300) equipped with a capillary column DB-1 (30 m x 0.25 mm x 0.25 μm). Temperature was held at 50°C for 5 min, raised to 180°C at 5°C/min, and to 280°C at 3°C/min. The injector temperature was 240ºC and that of the detector was 230ºC. The carrier gas was hydrogen (1.2 mL/min) and 1 μL of sample was injected. The steam distillation samples were diluted using ethyl acetate: $\cong$ 0.005 g of essential oil in 1 mL of solvent (Mallinckrodt, HPLC degree, Lot 3442 KTSA, 99.9%). For the solvent extracts 1 μL of the filtrate was injected directly. The split/splitless technique (1:21) was used and duplicated samples were injected.

Some samples were also analyzed using a GCMS system (Shimadzu, QP-5000), equipped with a capillary column of fused silica DB-1 (30 m x 0.25 mm x 0.25 μm). The electron impact technique (70 ev) was used. The carrier gas was helium (1.7 mL/min) and 1μL of sample was injected. The injector temperature was 240ºC and that of the detector was 230ºC. The temperature programming was 50ºC (5 min), 180ºC (4ºC/min) and 280ºC (15ºC/min). Identification of the chemical constituents was based on: *i*) comparison of substance mass spectrums with the GCMS system data bank (Nist 62 Library); *ii*) comparison of mass spectrums with data in literature (McLafferty & Stauffer, 1989; Adams, 1995); *iii)* Retention indices.

The contents of chamazulene and of α-bisabolol in the extract were calculated using the area percentage obtained in the GC analyses.

RESULT AND DISCUSSION

1- Process variables and the total yields

The humidity of the dried material was 12.03%, and did not vary during storage. Table 1 shows the operating conditions and the total essential oil yields obtained by steam distillation. An ANOVA was done using the essential oil yield as the response variable. The results showed that the process variables OP, DT, and CFR exerted a significant effect on the yield (p = 0.0060, p = 0.0001, p = 0.0002, respectively). The yield was also strongly affected by the interaction between DT and CFR (p = 0.0002) while the interactions of OP with those two variables were not significant (p = 0.8443 and p = 0.4120). These results can be more appreciated in Figure 2. At low CFR and low DT the effect of the operating pressure is negligible. On the other hand, the increase in DT appreciably increased the yield. At a CFR of 60 mL/min the effect of the pressure in the yield is evident at the higher operating pressure. At the lower level of both DT and CFR, the yield was marginally affected by the OP. Nevertheless, the highest yield was obtained at 0.98 bar depending on the selected combination of DT and CFR.

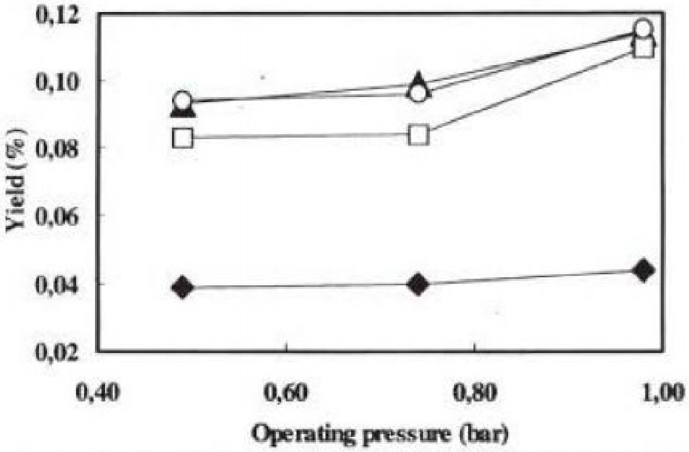

FIGURE 2. Total yield of essential oil as a function of the operational conditions: u CFR = 30 mL/min and DT = 45 min; o CFR = 60 mL and DT = 45 min; ¢ CFR = 60 mL/min and DT = 60 min; ? CFR = 30 mL/min and DT = 60 min

The particle size distribution used in the solvent extraction apparatus is shown in Figure 3, the triturated solid Sauter's mean diameter was 0.280 ± 0.035 mm (Foust, 1982). Thirty five percent of the water originally present in the chamomile flowers was dissolved by the ethanol and 20% by the isopropyl alcohol. Table 2 presents the operating conditions along with the yield for the extraction with ethanol and isopropyl alcohol. The statistical analysis (ANOVA) showed that temperature (T), solvent type (ST), solvent to feed ratio (SFR), and extraction time (ET) significantly affected the extraction yields (p = 0.0001, p = 0.0001, p = 0.0210, p = 0.0735, respectively). The interaction between SFR and T was also significant (p = 0.0478).

TABLE 1. Operating conditions and essential oil yield

Run No.	OP (bar, ±0.02)	DT (min)	CFR (mL/min, ± 2)	Yield* (wt%)
1	0.49	45	30	0.039
2	0.72	45	30	0.040
3	0.98	45	30	0.044
4	0.49	60	30	0.093
5	0.72	60	30	0.099
6	0.98	60	30	0.114
7	0.49	45	60	0.083
8	0.72	45	60	0.084
9	0.98	45	60	0.110
10	0.49	60	60	0.094
11	0.72	60	60	0.096
12	0.98	60	60	0.115

* Mass of essential oil / mass of feed

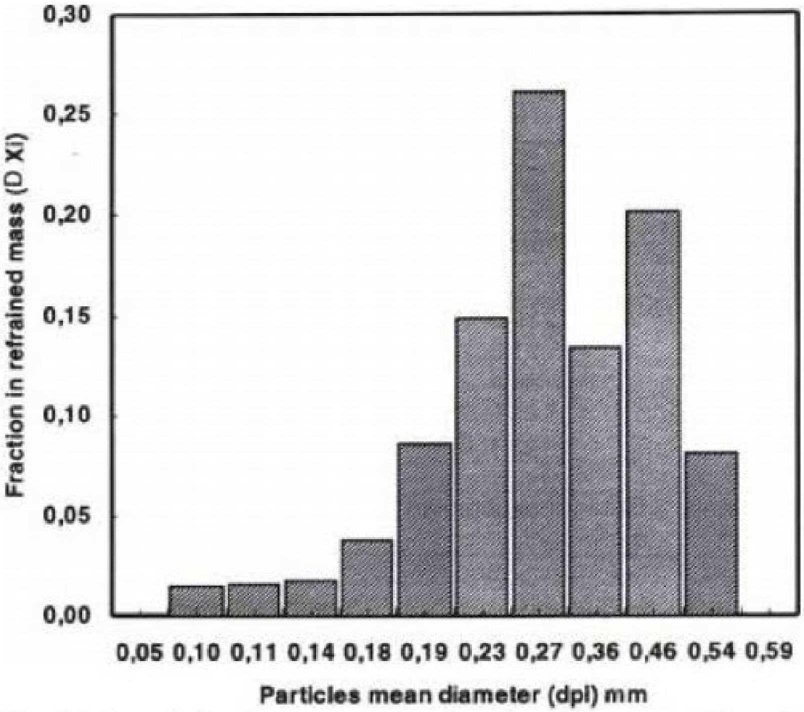

FIGURE 3 - Fraction in refrained mass as a function of particles mean diameter (dpi) mm

The essential oil yield was smaller for the steam distillation process as compared to the solvent extraction. Nevertheless, total operational times involved in each process were quite different. The steam distillation required 1 hour while 4 hours were necessary for the solvent extraction. Another difference between the processes was the color and the odor of the extract. The oleoresin was dark green while the essential oil was dark blue. In spite of that, the oleoresin odor was closer to the characteristic flower aroma than the essential oil.

TABLE 2. Operating conditions and yields for extraction with organic solvent

Run No.	ST	SFR*	ET (hours)	T (°C)	Yield (wt%)**
1	Ethanol	4	4	10	2.34
2	Ethanol	6	4	10	2.69
3	Isopropyl alcohol	4	4	10	1.77
4	Isopropyl alcohol	6	4	10	1.64
5	Ethanol	4	8	10	2.67
6	Ethanol	6	8	10	2.62
7	Isopropyl alcohol	4	8	10	1.72
8	Isopropyl alcohol	6	8	10	1.70
9	Ethanol	4	4	40	3.49
10	Ethanol	6	4	40	4.10
11	Isopropyl alcohol	4	4	40	2.42
12	Isopropyl alcohol	6	4	40	2.79
13	Ethanol	4	8	40	3.71
14	Ethanol	6	8	40	3.95
15	Isopropyl alcohol	4	8	40	2.89
16	Isopropyl alcohol	6	8	40	3.23

Volume of solvent/mass of dried solid; ** mass of extract/mass of feed

2- Characterization of the chamomile essential oil and oleoresin

Samples of extracts of the various experiments were analyzed by GC-FID, selected samples were also analyzed by GC-MS, Table 3 shows the major substances found in the chamomile essential oil (experiment 8) and the oleoresin (experiment 9).

TABLE 3. Major substances found in the chamomile essential oil and oleoresin

Substances	Steam distillation*		Solvent extraction*	
	Retention time	Area %	Retention time	Area %
β-farnesene	30.108	52.30	29.933	13.48
Linalool acetate (dihydro)	30.408	3.39	--	--
γ-cadinene	30.992	2.25	31.325	0.72
α-farnesene	31.408	3.13	35.400	0.78
Bisabolol oxide B	35.933	4.64	36.808	5.54
α-bisabolol	36.717	6.07	36.742	14.74
Chamazulene	37.867	1.71	37.742	0.75
α-Bisabolol oxide A	38.442	1.31	38.317	5.53
Trans-dicycloether MW200	41.592	3.20	41.642	29.36
Butyl phthalate	45.150	15.10	--	--
Non identified	--	6.90	--	29.10

*Data for experiment 8; ** Data for experiment 9.

The chemical profile for the extracts obtained in the various process conditions was similar. Using this information and the total mass of essential oil the relative content of α-bisabolol and chamazulene were estimated. Figure 4 presents the content of α-bisabolol and chamazulene in the essential oil. The results showed that the relative amount of these two substances varied similarly to the observed variation of the total yield, therefore, subject to the same effects with respect to the operating conditions as discussed previously.

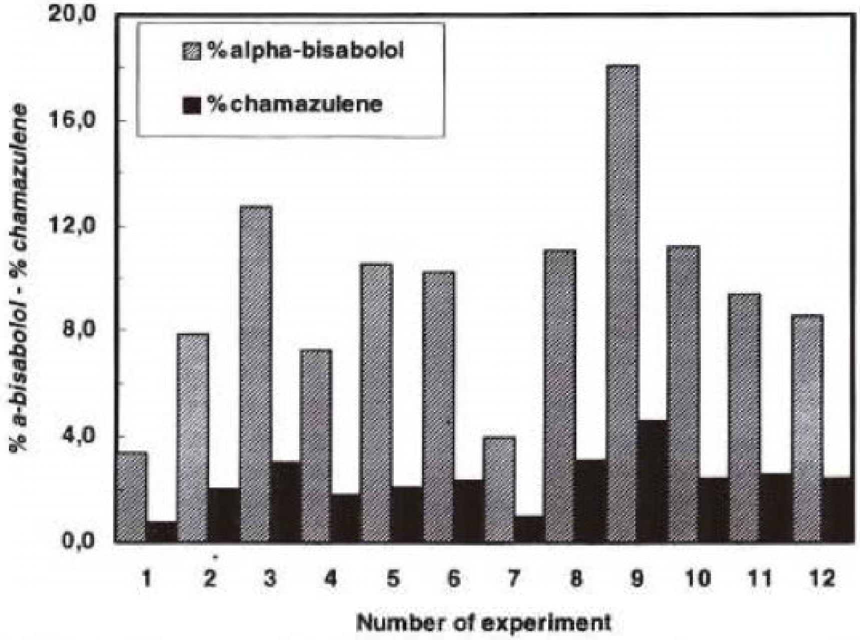

FIGURE 4 - Percentage of α-bisabolol and chamazulene in the essential oil.

Figure 5 shows the amount of waxes present in the oleoresin as a function of temperature. As can be observed the relative amounts of oil and waxes were significantly affected by the temperature but not by the solvent. Figure 6 show that the amounts of α-bisabolol and chamazulene in the solvent extracts were smaller compared to the steam distillation products (Figure 4). While, the steam distillation relative proportion between α-bisabolol and chamazulene varied from 3.5 to 4.9 for the solvent extraction the variation was from 20 to as high as 55, although, the amount of essential oil in the oleoresin was approximately constant at about 50%.

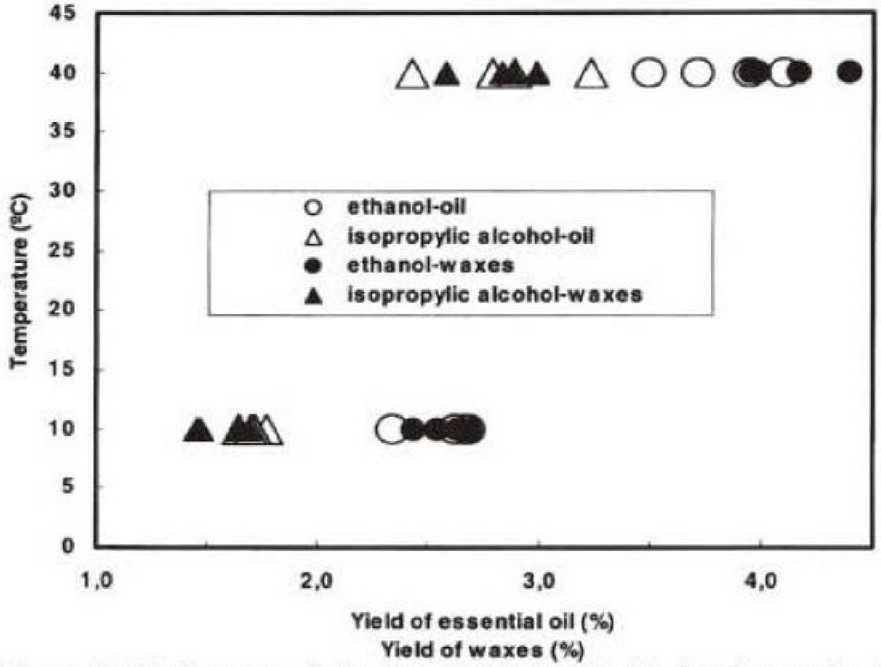

Figure 5. Relative amount of waxes and essential oil in the chamomile oleoresin.

The essential oil is of dark blue color while the oleoresin is dark green. The blue color is due to the presence of chamazulene in the essential oil. Ethanol and/or isopropyl alcohol are both capable of extracting waxes and pigments resulting in the dark green color of the oleoresin. Povh (2000) obtained a light yellow chamomile extracts by supercritical extraction with carbon dioxide. Upon heating the oleoresin became dark blue due to the transformation of matricine in chamazulene as expected (Sousa *et al*, 1991). Chamazulene was identified in the oleoresin obtained with both ethanol and isopropyl alcohol. Therefore, considering the oleoresin color and the use of relatively low temperatures for the solvent extraction process, matricine was present in the oleoresin and was convert during the analytical procedure (CG-MS and CG-FID) to chamazulene.

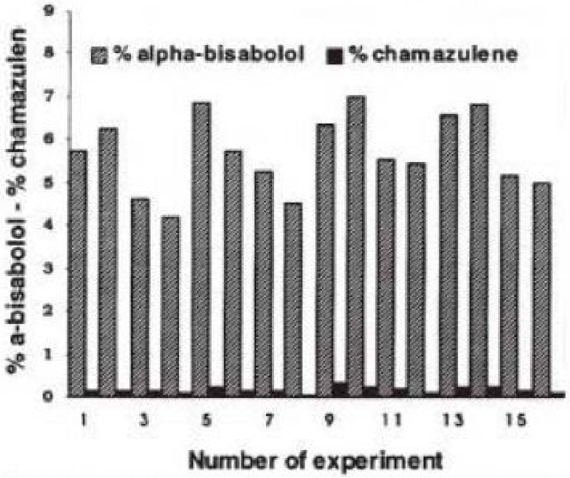

Figure 6 - Percentage of α-bisabolol and chamazulene in the essential oil in the solvent extract.

From the previous discussion, considering solely the total yield for the steam distillation process, the choice would be to conduct the process at 0.98 bar and condensate flow rate of 60 mL/min for a period of 45 minutes. A similar analysis for the solvent extraction process would lead one to choose the ethanol as the solvent, and run the process at 40º C using a SFR of 6 for a period of 4 hours. Although, it must be remembered that solvent extraction process produced an extract richer in α-bisabolol as compared to the steam distillation process.

Comparing the advantages and disadvantages of each process, the following can be listed: (i) Steam distillation requires low cost equipments but uses high temperature, therefore the process may not be recommended for thermo-sensitive compounds; (ii) Also, some substances present in the raw material such as phenol derivatives, alcohols, and acids are lost with the condensation water; (iii) In addition, the temperature used in the process catalyzes some reactions, such as the transformation of matricine to chamazulene; (iv) Conversely, the solvent extraction process can be performed at relatively low temperatures, although to get the final product another step is required to eliminate the solvent; (v) Also, the yields are higher but some fractionation will certainly be required in order to separate the essential oil from the waxes, pigments, resins, and other contaminants.

From the above, it is easily concluded that the total yield cannot be the single criterion used to select a process and its corresponding conditions. Other variables must be known before a sound decision can be made. To clear up the scenario, the criterion could, for instance, include both the content of the target substances α-bisabolol or chamazulene and the content of the waxes in the final product. Therefore, the characterization of the extracts is ultimately a very important information. Another question to be considered is that even though solvent extraction increases the yield, this process requires a further purification step, which would include the desolventization of both the extract and the solid residue in order to get a purified product and byproduct. This certainly increases the production costs. Therefore, all this must be taken under consideration to make a sound decision on the process best suited to produce chamomile extract for each specific application.

CONCLUSION

For the steam distillation process the results showed that the operating pressure, distillation time, and condensate flow rate exert a significant effect on the yield. The same is true for the interaction between distillation time and condensate flow rate. The highest yield that was obtained was 0.11%. The solvent (ethanol or isopropyl alcohol), the solvent to feed ratio, the extraction time, and the temperature affected the yield for the solvent extraction. The interaction between solvent to feed ratio and temperature was also significant. The major compounds of the essential oil and of the oleoresin were β-farnesene, linalool acetate (dihydro), γ-cadinene, α-farnesene, bisabolol oxide B, α-bisabolol, chamazulene, α-bisabolol oxide A, trans-dicycloether (MW 200) and butyl phthalate.

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