Economically Efficient Production of Hydro-Alcoholic Extracts from Medicinal Plants

Study for *Mentha Piperita* L.

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This work aims to define a set of operating conditions that ensure economic efficiency for the industrial production of hydro-alcoholic extracts by percolation. The importance of such studies is pointed out by data collected from S.C. Parapharm S.R.L., Brad, Romania, during preparation of BANO Swedish Drops. For cost reasons, the influence of various parameters on the extraction efficiency as well as on the dynamics of wearing out the extractable species from the vegetal is assessed by means of the Mentha Piperita L. extract and its solid dry residue mass percentage. Results are correlated with chromatographic and spectral findings. A more alcoholic solvent and higher temperatures favour the extraction of volatile rather than of mucilaginous species. Consecutive percolation steps follow first order kinetics in time, but the nature of extracted species shifts from light and volatile chemicals to more heavy ones. A well-grained raw material may be used efficiently without prior separation of leafs from strains or roots.

Keywords: Hydro-alcoholic extract, Mentha Piperita L., Economic efficiency

The Romanian market of dietary supplements obtained from medicinal and aromatic plants has dramatically increased over the last 10-15 years. One of the most popular items in terms of shelf price, wide range of therapeutic effects and employment easiness is represented by the hydro-alcoholic extracts. Local companies compete against imported products of well-known brands by trying to lower their overall costs. Since those generated by personnel, location, utilities, marketing, etc., are regulated either by legislation or by other market segments, they strive for production efficiency and therefore lower costs.

Extraction of therapeutically active species may be carried out in various ways. For example, the menthol rich essential oil of *Mentha Piperita* L. may be obtained by steam driven distillation [1,2], supercritical fluid extraction [1,3,4] (for example by carbon dioxide [1]), direct thermal desorption [3] or atomization [3]. On the other hand, the solid standardized extract is often gained by extraction with organic solvents such as methanol [3-6], ethanol [7-9] or methylene chloride [1].

Except the CO_2 driven extraction which is considered safe and environmentally "clean", almost all above listed procedures are either expensive, presume temperatures that might affect chemical structure of therapeutically active compounds or involve toxic chemicals. The "least" dangerous solvent on human health is readily available ethanol. Therefore, plain percolation, that is diffusion driven solid-liquid extraction, is the most widespread and inexpensive way of preparing hydro-alcoholic extracts on industrial scale.

Even so, high effectiveness of extraction is compulsory for economic competitiveness. A widely used parameter in the assessment of extraction efficiency but also in that of the quality of a hydro-alcoholic extract is its solute (dry residue) mass percentage (DR) [10]. Since it is affected by factors such as type of vegetal raw material (leaf, strain, root, fruit, etc.), its previous treatment, granulation, type of chemical species to be extracted, liquid phase composition, temperature - influenced by stability conditions [11] from and total contact time between solid and liquid phases, a set of optimal and economically feasible percolator operating guidelines are required for each product.

Scientific publications reflect the economic potential of hydro-alcoholic extracts on the market of therapeutic herbal products as well as the continuous drive for their cost-effective production. Some describe laborious kinetic studies [3-6,12-18] where the results are presented in the form of complex mathematical models [12,13], detailed characterization of solid-liquid mass transfer [17] or specific diffusion coefficient values [17]. Others deal with the optimization of the mass transfer during extraction [13,16,19,20] or with the recovery of species of economic interest (caffeine, proteins, antioxidants, etc.) from vegetal waste provided by food industry [13,17,18,21].

The present work has primarily an economic motivation. It seeks to improve the efficiency of the production line of the *BANO Swedish Drops* from *S.C. Parapharm S.R.L.*, Brad, Romania. The product is presented in the form of a hydro-alcoholic solution obtained by solid-liquid extraction from a mixture of seventeen to twenty plants [22]. The industrial scale percolators are operated completely manually in charges, at microclimate in this case - room temperature [23]. Depending on the season, this varies between 15 and 35^{0}C.

Trials for the efficiency assessment of the existing technology, although correlated with cost calculations [19], only revealed the necessity of a more detailed study [19,20]. This should evaluate, for example in the usual terms of dry residue mass percentage, the effects of various operating conditions such as size and type of vegetal grains, ethanol content of the solvent, temperature, number of successive extraction steps and total extraction time, respectively. Variation of these parameters will also provide information about the dynamics of wearing out the extractable species

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within the solid. Furthermore, the results ought to be correlated with those obtained by other experimental means.

For the reason of lowering experimental costs, this more detailed study was carried out by using only the readily available *Mentha Piperita* L. instead of the mixture of twenty plants, some of which very expensive (for example *Croci Stigma)*. Moreover, by using a single and well-described plant, chromatograms could be more easily interpreted and correlated with literature data as well as with own findings. Conclusions may be applied not only as specific technical guidelines for the industrial preparation of hydro-alcoholic extracts, but also as recipes for home-made tinctures.

Experimental part

Industrial preparation of BANO Swedish Drops and its quality assessment

Preparation of *BANO Swedish Drops* occurred in 160 L stainless steel percolators. These were loaded with a mixture of 18 Kg of dry, grained vegetal material [22] and 80 kg of slightly warm (35÷45°C) hydro-alcoholic solvent. The latter consisted of 52 Kg drinking water (as provided by the municipal water company Brad, Romania) and 28 Kg 96.5% ethanol (*PRODVINALCO*, Romania). The mixture was left to percolate at room temperature and occasionally stirred manually. Depending on the season, the resulted extract (5060 L) was emptied at the bottom of the percolator after 3 to 7 days. For the succeeding extraction steps, the solvent was renewed by purring a mixture of 41.5 Kg water and 21.5 Kg ethanol over the remaining wet plants.

Samples of 100 mL were taken from extracts resulted after each step, from 3 different simultaneously but independently operating percolators. For dry residue mass percentage (DR) determination [10], a 10 mL sample was evaporated to dryness from a weighing ampoule, at 105°C, by means of an *ECOCELL* oven. This operation lasted 3 h . The ampoules were weighed before and after evaporation by using a *PRECISA CB 220A* analytical balance. This permitted calculation of both extract DR and density.

Extracts obtained after all steps were gathered and mixed in a buffer stainless steel container of 1000 L, filtered and bottled for marketing. The final product was also characterized in terms of DR and density.

The hydro-alcoholic extract of Mentha Piperita L. and its quality assessment

This hydro-alcoholic extract was obtained in a manner that mimics the existing industrial procedure described above. The *Mentha Piperita* L. was provided by *S.C. Parapharm S.R.L.*, Romania, and used in the experiments as such, in the form and granulation employed by the company for the production of *BANO Swedish Drops*. Granulometric analysis of *Mentha Piperita* L. was carried out by means of a *RETSCH AS 200* shaker on sieves of 4.0, 3.15, 2.0, 1.4 and 0.71 mm, respectively.

The hydro-alcoholic solvent was prepared by using distilled water (*Stainless Steel GFL 2001/2* distillation device) and 96.5 % ethanol (*PRODVINALCO*, Romania) in various proportions.

The extracts were obtained by percolation in 100 mL lid equiped brown glass jars. Each extract was prepared by covering 2 g of *Mentha* with 20 mL of liquid. Mixtures were shortly shaken manually after preparation. At predetermined time intervals the liquid extract (12÷18 mL) was separated from the wet vegetal material. The latter was covered again with 20 mL of fresh solvent of the same composition. This operation corresponds to one extraction step. Depending on the temperature, it was repeated 3 to 5 times. Percolation temperature was controlled by keeping the jars in an *ECOCELL* oven.

DR and density of extracts obtained after each extraction step and for all employed experimental conditions were determined as described above.

For the qualitative and quantitative analysis of some hydro-alcoholic extracts a *GC-MS SHIMADZU QP 2010-Plus* gas-chromatograph coupled with mass spectrometry was employed. Its technical details were: DB - 5 ms column operated at 230°C, length of 30 m (0.25 μm x 0.25 mm), injected volums of 1 μL, a Spliter injector, helium as carrying gas at 1.2 μL/min. The MS detector operated at 230°C. The UV-Vis spectra were obtained by using quarz cells of 1 cm optical path length and a *JASCO 630* spectrophotometer. The reference cell contained the hydro-alcoholic solvent used to obtain the analysed extract.

Results and discussions

Efficiency assessment of existing technological procedure

Initial assessment of the efficiency of the *BANO Swedish Drops* industrial production line was carried out directly at the *S.C. Parapharm S.R.L.*, Brad, Romania. Samples of the obtained hydro-alcoholic extract were collected after each extraction step, that is before each solvent renewal for the same charge of solid vegetals. Since percolation occurs at room temperature, distinct sets of samples were gathered both during summer and winter, that is for the high and low temperature season, respectively. The average of 3 gravimetrically determined dry residue mass precentage as well as density values are presented in table 1 for all consecutive percolation steps S1÷S7.

For both seasons S1 is the most efficient because of DR over 10%. An other efficient step - S2 - follows in summer, after which DR drops significantly during the succeeding S3 to S7. In winter, extraction steps S2 ¸ S7 are less efficient than their correspondents in summer; they last longer and result in smaller DR values. The vegetals are usually worn out and DR values level off, depending on the temperature of the environment [24] - that is on the season - after six to eight consecutive percolations.

Table 1 also shows that the density of the extracts develops in agreement with DR values: it decreases towards the value of the solvent (~0.915 Kg/L) with increasing number of extraction steps.

The total contact (percolation) time necessary to accomplish comparable states of final extract quality (data for the bottled product in table 1) and remaining worn out vegetals is of 25 days during summer and 35 days during winter, respectively. Thus, mass transfer and such efficiency of extraction, depend heavily on temperature. On the other hand, ingredient cost calculations proved that economic efficiency is achieved only after 5 succesive extraction steps [19].

Since the final product, with an average DR of 2.6÷2.7%, is obtained by mixing and filtering extracts gathered from all extraction steps, it may be concluded that the lack of temperature control and regulation during percolation affects total productivity; the 7 succesive percolating steps of the same charge of raw material, that is obtaining the same amount of *Swedish Drops*, lasts in winter about 30% longer than in summer.

Yet, as it may be concluded from figure 1, winter is just the season where the market demand is higher for this product. It illustrates the evolution of total sales volume of *BANO Swedish Drops* during a period of five years for the downtown *Farmacia Naturista* Pharmacy from Campia-

Percolation step	Summer: - **high temperature** season -			Winter: - **low temperature** season -		
	Total extraction time (days)	**DR (%m/m)**	Density (Kg/L)	Total extraction time (days)	**DR (%m/m)**	Density (Kg/L)
S1	4	12.10	0.983	6	10.37	0.980
S2	7	8.52	0.996	10	2.72	0.994
S3	12	1.63	0.964	17	2.53	0.966
S4	18	0.52	0.953	24	1.92	0.958
S5	21	0.92	0.965	28	1.31	0.962
S6	24	0.61	0.949	32	0.37	0.955
S7	25	0.10	0.915	35	0.29	0.915
Bottled product	**Total: 25 days**	**2.65**	0.961	**Total: 35 days**	**2.61**	0.961

Table 1
HIGH AND LOW TEMPERATURE SEASON PERCOLATION DATA FOR THE INDUSTRIAL PRODUCTION OF *BANO SWEDISH DROPS*

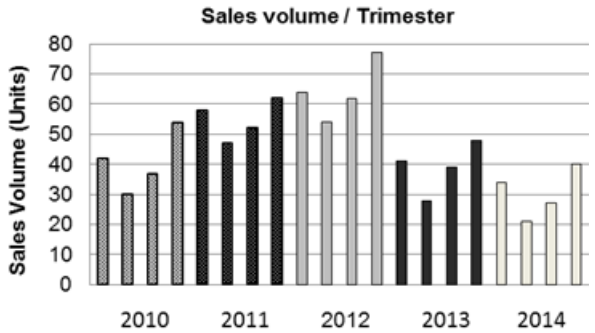

Fig.1. Evolution of total sales volume of BANO Swedish Drops, *Farmacia Naturistã* Pharmacy, Campia-Turzii, Romania

Turzii, Romania. It is obvious that trimesters 1 (January – March) and 4 (October – December) register each year best results in terms of saled units. (The decrease in sales during the last 2 years is due to the opening of a similar shop in the neighbourhood). Data gathered from other retailers located in various cities of Transylvania, Romania, show the same market tendency [19].

Neverthelss, if the company could meet the demands of the market even under existing circumstances, it might still save the costs of control and regulation equipment (aquisition, usage, maintenence, etc.). Economic data of recent years proved otherwise, productivity and hence efficiency of existing percolation procedures at *S.C. Parapharm S.R.L.* need to be improved by means of conclusions drawn from a more thorough study.

Hydro-alcoholic extract of Mentha Piperita L. - Effect of percolating conditions on the extract quality and extraction efficiency.

This chapter focuses on the hydro-alcoholic extract of *Mentha piperita L.*. It was prepared by diffusion driven solid-liquid extraction (percolation / maceration) of the bioactive components from the dry vegetal into a mixture of water and ethanol. It evaluates the effect of various parameters (dimensions and source of vegetal - leaf, strain or root -, alcohol content of the solvent, temperature, number of consecutive extraction steps, total extraction time) on the extract's quality as well as extraction efficiency in terms of dry mass percentages, chromatographic and photometric data.

Granulometric analysis of raw vegetal material

An amount of 300 g of dry grained *Mentha Piperita* L. was subject to granulometric analysis. The results are shown in figure 2. It is obvious that small fractions, with grain sizes under 2 mm, represent about 70% of the total vegetal mass. It has to be mentioned that these results ought to be regarded generously; since they describe a current state of the raw material, they are not strictly reproducible. It is obvious that the solid dry vegetal matter is very fragile and smashes easily, even when handled very carefully.

Figure 3 illustrates the differences between the mixture employed in the industrial production of *BANO Swedish Drops* – picture ***a*** - and the matter used in some of the hereby described experiments: fractions with grain sizes over 4 mm and in the range of 0.71¸1.4 mm – pictures ***b*** and ***c***, respectively. The retention of the 4 mm sieve corresponds to the un-grained big size leaf or strain parts.

Description of percolating conditions

In order to evaluate the effect of various percolating conditions on extraction efficiency, on extract quality as well as on the dynamics of wearing out the extractable species within the vegetal during several consecutive percolating steps, the following sets of experiments were carried out:

The effect of solvent composition on the extract quality was studied by preparing a set of extracts in ethanol - water mixtures of various volumetric ratios. These are presented in table 2 together with the corresponding calculated densities. Values at 20°C for H_2O and C_2H_5OH of 1.000 and 0.788 Kg/L respectively, were used for calculus. The extracts were prepared at constant 40°C with *Mentha Piperita* L. in it is provided as a mixture of leafs and strains of various sizes form (fig.3, ***a.***).

The effect of total percolation time and number of consecutive extraction steps on extraction efficiency and dynamics of wearing out the vegetal matter, was evaluated by separating the extract at predetermined time intervals: at 24, 48, 72, 96 and 117 hours when percolating at 30, 40 and 50°C, and at 38, 72 and 116 h when percolating at 20°C, respectively. *Mentha* depicted in figure 3, ***a.*** was employed.

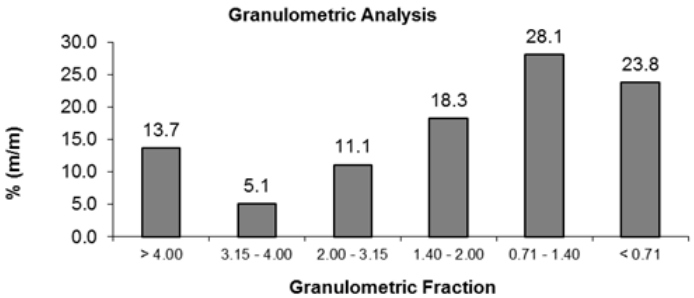

Fig. 2. Results of granulometric analysis of dry *Mentha Piperita* L.

Fig. 3. Aspect and granulometry of *Mentha Piperita* L. *a)* Mixture as delivered by *S.C. Parapharm S.R.L.*; *b)* Grain size over 4 mm; *c)* Grain size between 0.71 and 1.4 mm

Table 2
COMPOSITION AND DENSITY OF THE HYDRO-ALCOHOLIC SOLVENT AT 20°C

Ratio C_2H_5OH / H_2O (% V/V)	30	40	50	60	70
Density (Kg/L)	0.936	0.915	0.894	0.873	0.852

The effect of temperature on percolation dynamics and efficiency was assessed by preparing each set of extracts at 20, 30, 40 and 50°C, respectively. Again, *Mentha* depicted in figure 3, ***a.*** was employed.

The effect of the grain size on extraction effectiveness was evaluated by using two other *Mentha Piperita* L. fractions of different sizes: over 4 mm and within the range of 0.71¸1.4 mm, respectively (fig. 3, ***b.*** and ***c.***). In these cases, both temperature and solvent composition were kept constant at 40°C and 40% v/v C_2H_5OH / H_2O, respectively.

Effect of solvent composition on extract quality

Figure 4 illustrates the values of DR obtained at 40°C for different ethanol/water volumetric ratios in the solvent, for all five consecutive extraction steps (S1 to S5). On the scale of the abscissa, 0 and 100% mean pure water and ethanol, respectively. It may be observed that within the first three stages (S1¸S3), DR values drop significantly. The next steps (S4 and S5) bring about only slight changes, suggesting that the raw material seemingly runs out of extractable species.

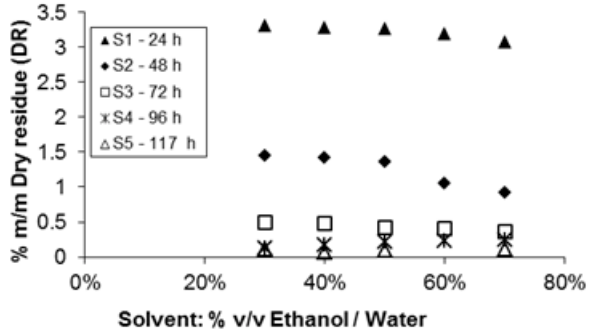

Fig. 4. The effect of solvent composition on DR values at 40°C

When increasing the ethanol content of the solvent to over 50%, DR drops during stages S1 to S3. This is due to the fact that the alcohol favours the solid to liquid diffusion of chemical species that are highly soluble in organic solvents. Examples are menthol, menthone and their isomers. These are also main components of the volatile *Mentha* oil. They degrade easily even at moderate temperatures [25], hence such species will not be present in the dry residue after evaporating the solvent for 3 h at 105°C [10]. As a result, the corresponding DR values drop.

These findings are in agreement with the results, presented in table 3, of the GC-MS analysis of some extracts obtained in first percolation steps: more alcohol in the solvent and elevated temperatures during percolation favour the diffusion of light and volatile species from the solid towards the liquid phase, in the detriment of the mucilaginous species [7,8,25]. Hence, the extract presented in the last column contains almost exclusively (95%) species with low retention times.

On the other hand, the last percolation stages S4 and S5 behave differently: DR values slightly increase with increasing ethanol content of solvent. This is due to the fact that the type of chemical species extracted from the solid modifies. Since the vegetal matter is already worn out in low mass, volatile species, other species with higher molecular mass will now migrate into the liquid phase, such as a-methyl-glucoside, palmitic acid, linolenic acid, pytol and others. These are more stable at elevated temperatures [25], do not degrade during solvent evaporation at 105°C and will be present in the dry residue. As a result, DR values are slightly elevated.

A visual comparison of the various extracts revealed that their greenish colour intensified with higher alcohol/water ratios in the solvent. This may be due to higher amounts of chlorophyll that migrated into the liquid phase. Since chlorophyll is soluble in organic solvents and insoluble in water, a liquid containing more ethanol will have an enhanced affinity towards it.

This fact is also sustained by the UV-VIS spectra of the extracts corresponding to first percolation steps at 40°C, for solvents containing 30 and 70% v/v ethanol in water. These are presented in figure 5. It is obvious that in the visible domain the spectrum of the more alcoholic extract exhibits higher absorption maxima corresponding to ***a*** and ***b*** pure chlorophyll [25,26] (at approximately 430 and 670 nm as well as 470 and 630, respectively). Other percolating temperatures resulted in similar extraction behaviour.

Dynamics of consecutive percolations of the same charge of raw material.

Because after each extraction step the solvent is renewed for the same charge of *Mentha*, the evolution in time of the dry residue mass percentage may not be described by the existing data in terms of classic kinetic studies. The concentration gradient, that is the driving force of the solid-liquid diffusion, is altered by both the renewal of the liquid phase and by wearing out of the vegetal matter. However, time resolved data can still provide valuable information for establishing economically efficient percolation conditions.

	30°C		50°C	
	30 % v/v ethanol/water	*70 % v/v ethanol/water*	*30 % v/v ethanol/water*	*70 % v/v ethanol/water*
Chemical species	**% m/m**	**% m/m**	**% m/m**	**% m/m**
Retention time < 10 min				
Menthone (154.25 g/mole)	2.40	9.76	13.64	10.28
Iso-menthone (154.25 g/mole)	1.62	2.12	2.81	3.86
Iso-menthol (156.27 g/mole)	0.05	0.46	0.44	0.65
Menthol (156.27 g/mole)	53.28	46.24	46.88	74.38
Other species (Total)	5.64	3.84	6.18	6.30
TOTAL	**51.71**	**62.42**	**69.95**	**95.47**
Retention time 10 ÷ 20 min				
α-methyl-glucoside (355.43 g/mole)	5.13	2.49	5.29	4.53
Palmitic acid (256.42 g/mole)	5.09	6.87	4.96	-
Other species (Total)	14.02	6.96	3.48	-
TOTAL	**25.25**	**16.32**	**13.73**	**4.53**
Retention time > 20 min				
Linoleic acid (278.43 g/mole)	6.03	11.07	13.89	-
Phytol (296.53 g/mole)	-	3.54	-	-
Other species (Total)	5.49	6.65	2.43	-
TOTAL	**11.52**	**21.26**	**16.32**	**0**

Table 3
RESULTS OF THE GC-MS ANALYSIS FOR SOME HYDRO-ALCOHOLIC EXTRACTS

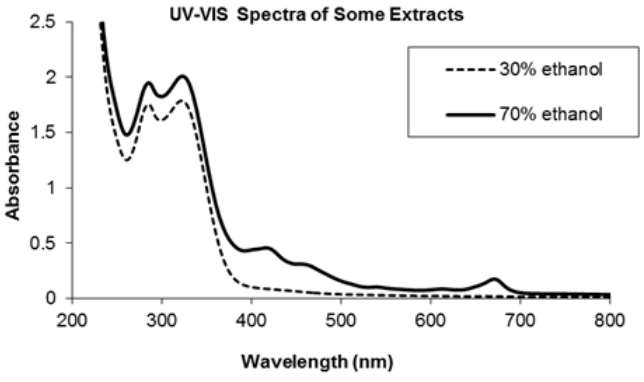

Fig. 5. UV-VIS spectra of some *Mentha Piperita* L. extracts obtained at 40°C

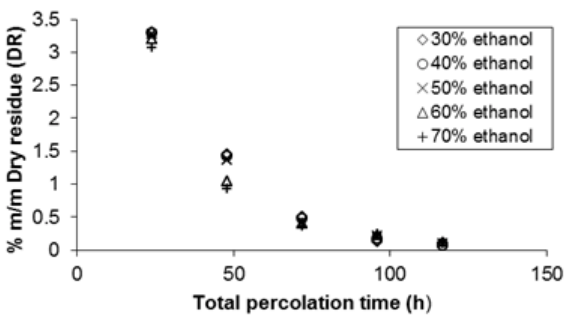

Fig. 6. DR values *vs* total extraction time (from S1 to S5) at 40°C

Solvent % V/V Ethanol / Water	**DR** = f (*t*) - equation (1)	n / R
30	**DR** = 8.73 exp (-0.040 *t*)	5 / 0.986
40	**DR** = 9.73 exp (-0.042 *t*)	5 / 0.999
50	**DR** = 8.09 exp (-0.038 *t*)	5 / 0.997
60	**DR** = 6.78 exp (-0.036 *t*)	4 / 0.989
70	**DR** = 5.76 exp (-0.034 *t*)	5 / 0.985

Table 4
EQUATIONS DESCRIBING DR EVOLUTION *VERSUS* PERCOLATION TIME AT 40°C.

% V/V Ethanol / Water	Temperature							
	20°C		30°C		40°C		50°C	
	DR_0 (%m/m)	100 k (h^{-1})	DR_0 (%m/m)	100 k (h^{-1})	DR_0 (%m/m)	100 k (h^{-1})	DR_0 (%m/m)	100 k (h^{-1})
30	10.04	3.3	5.70	3.5	8.74	4.0	7.23	3.9
40	7.19	2.6	4.90	3.4	9.73	4.2	6.51	3.6
50	6.61	2.5	4.99	3.3	8,09	3.8	5.74	3.2
60	6.52	2.4	4.68	3.2	6.78	3.6	4.59	2.9
70	6.54	2.5	3.74	2.8	5.76	3.4	4.70	2.9

Table 5
CALCULATED VALUES OF PARAMETERS DR_0 AND ***k***

Figure 6 presents DR values plotted against total extraction time at 40°C for all employed solvents, as they evolve from extraction step S1 to S5. It is obvious that, regardless of the ethanol content of the solvent, DR values follow the same decreasing pattern. The same is true for data gathered at all other temperatures. They correspond with good correlation coefficients ($R^2 > 0.971$) and in all cases to an exponential decay such as the first-order rate law in equation (1).

$$DR = DR_0 \exp(k t) \qquad (\%\, m/m) \qquad (1)$$

Table 4 contains the fittings of each set of experimental data in figure 6 with equation (1), along with the correlation coefficients (R) as well as the number of experimental points (n) used to obtain the presented values.

Calculated parameters in equation (1) are DR_0 and ***k***. In kinetic terms, DR_0 stands for an apparent initial available quantity of extractable species (present at percolation beginning). It is expressed in the units of DR. On the other hand, ***k*** corresponds to a first-order extraction rate coefficient and is expressed in h^{-1}. Values of DR_0 and ***k*** obtained at various temperatures and solvent compositions are summarized in table 5.

It may be observed that at all temperatures, as the ethanol content of the solvent increases, apparently, the initially available amount of species to be extracted, DR_0, decreases. These findings ought to be regarded with generosity and the expression *apparently* is to be emphasized because equation (1) describes the evolution of the dry residue precentage, a parameter whose decay in more alcoholic solvents was explained above. This does not mean that there is less available extractable solid mass able to migrate into the liquid phase, but rather that the nature of these species modifies, shifting from light and volatile chemicals to more heavy ones.

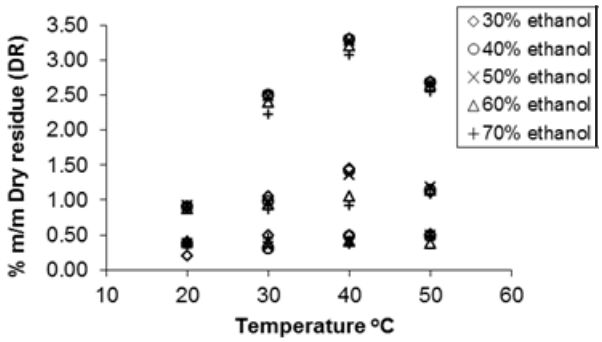

Fig. 7. DR values *vs* temperature for the first three extraction steps.(S1 to S3: decreasing DR values)

The same explanation is valid for the decreasing tendency of ***k*** values with growing solvent's alcohol content. The fact that ***k*** values at 50°C are lower than those at 40°C also proves that the solid-liquid extraction does not involve the same species.

Effect of temperature on percolation dynamics

Values of DR registered for the first three extraction steps (S1 to S3 from top to bottom) are plotted against percolation temperature in figure 7, for all solvent compositions. The conclusions to be drawn are in agreement with previous ones: at higher alcohol content in the liquid phase, the nature of extracted species will change. The efficiency of the first extraction step is most significantly influenced by temperature since it starts with fresh raw material. During the following percolations, the vegetal matter starts to wear out and influence by both temperature and solvent composition loose significance. These parameters still affect the effectiveness of extraction but not as strongly as for step 1.

Eventhough the dynamics of percolation for the same raw material during several consecutive steps can not be

Table 6
GLOBAL EXTRACTION ACTIVATION ENERGY *VS* SOLVENT COMPOSITION

% V/V Ethanol / Water	E_a (kJ/mole)	R
30	7.3	0.971
40	18.3	0.999
50	16.0	0.986
60	15.5	0.976
70	11.7	0.986

regarded and handled in the sense of classic kinetics, first-order extraction rate coefficient values (***k***) presented in table 5 place on Arrhenius type plots for temperatures under 50°C and may be used for the calculus of a global extraction activation energy E_a. Table 6 presents E_a values as a function of solvent composition, as well as the correlation coefficients (R) of the Arrhenius type linearisations obtained from 3 points: at 20. 30 and 40°C, respectively. It is again obvious that the nature of extractable species vary with the alcohol content of the solvent.

In the sense of dynamics of a global hydro-alcoholic extraction carried out in more than one step, E_a does not refer to the solid-liquid diffusion of a distinct chemical substance, but describes an overall process of simultaneous extraction of many species form the same charge during succesive percolations.

Effect of grain size and source of vegetal matter on extraction efficiency

Figure 8 illustrates dry residue precentages obtained under the same percolating conditions, for the the same number of consecutive extraction steps, but for *Mentha Piperita L.* in different grained conditions. The powder like fraction (grain size between 0.71 and 1.40 mm) brings about comparable results with the unseparated mixture. Since it accounts for approximatelly 70 % of the mixture, this finding is obvious. The fraction containing mainly un-grained big size leaf and strain parts, wears out seemingly much sooner than the other employed fractions. This may due to the fact that strains and roots have a different morphology as compared to leafs [14, 27]. This fact combined with the lower contact surface between the solid and the liquid phase [14], results in slower diffusion processes. Hence, DR values drop. The same conclusion was drawn at each employed temperature. Otherwise, data in figure 8 prove that it is no point in separating the leafs from strains or roots, but it is definitelly worthwhile to use well-grained (to sizes of ~ 1 mm) raw material.

Conclusions

The main goal was to define a set of operating conditions that ensure economic efficiency for the industrial production of hydro-alcoholic extracts by percolation. The importance of such detailed studies was proven by data collected from *S.C. Parapharm S.R.L.*, Brad, Romania, during preparation of *BANO Swedish Drops*. Yet, the influence of various parameters on the process efficiency was assessed by means of the *Mentha Piperita* L. extract.

The employment of diffusion driven solid-liquid extraction, as well as of a common, inexpensive and less toxic organic solvent such as ethanol, points out both the strive for economic effectiveness of industrial scale processes and the need for scientifically established guidelines. This is a seldom approach within a literature

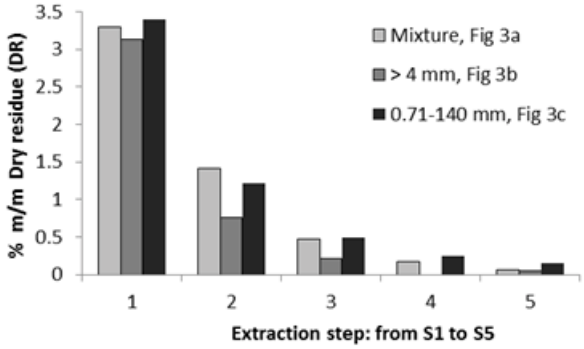

Fig. 8. DR values at different *Mentha* grain sizes (extraction steps S1 to S5)

focused rather on the research of *Mentha* essential oil [1,2,14,27-30]. Furthermore, the use of ethanol-water mixtures and moderate temperatures allows a large variety of bioactive species to diffuse into the liquid phase but avoids the structural changes that might occur during distillation.

Experiments targeted description of the effect of various percolating conditions on extraction efficiency, on extract quality and on the dynamics of wearing out the extractable species from the vegetal during several consecutive percolating steps. Assessment of these parameters was carried out gravimetrically by means of the dry solid residue (solute) mass percentage. Results were correlated with chromatographic and spectral findings.

The employment of a solvent containing more ethanol as well as of elevated temperatures will favour the extraction of volatile rather than that of mucilaginous species. Thus, these products will also contain more chlorophyll and will be more intensely green coloured. Consecutive percolation steps follow a first order kinetic pattern in time, but the nature of extracted species shifts from light and volatile chemicals to more heavy ones. A well-grained raw material may be used efficiently without prior separation of leafs from strains or roots.

The final conclusions (applicable even as a recipe for a home-made tincture) are to be summarized as the following recommendations for percolating conditions: a well and uniformly grained dry vegetal with grain sizes of ~ 1 mm; the employment of a 40% (V/V) ethanol – water mixture at temperatures that do not exeed 40°C but neither fall under 30°C. The same charge of vegetals ought to be subject to at least three consecutive percolation steps but with less solvent, yet of the same composition and at the same temperature.

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