Recovery of Essential Oils from Carobs through various Extraction Methods

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Abstract: The carob tree *Ceratonia siliqua* is abundant in the Mediterranean countries, thrives in dry climates and its fruit contains important essential oils with antioxidant properties. In the present work, various methods of extraction have been applied on carob powder in order to recover the essential oils. The results of the experiments help in to optimize the appropriate method of recovery, among ultrasonic bath, Soxtec extraction, and conventional (hexane) extraction and hydrodistillation..

Key-words: antioxidant activity, Carob tree, Carobs, essential oils, extraction, hydrodistillation, Soxtec

1 Introduction

The carob tree *Ceratonia siliqua* (Fig.1) is abundant in the Mediterranean countries, thrives in dry climates, and presents resistance to salinity and adaptation to poor soils. Carob tree requires little maintenance and it is considered to be an important component of vegetation for environmental, economic and social reason. The fruits are used for diverse purposes. They are more important in food industry and are an excellent source of many products such as gum, sugar and alcohol [1]. In addition, its fruit contains important essential oils with antioxidant properties [2].

Figure 1 The carob tree (*Ceratonia siliqua)* and its fruit

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Essential oils are aromatic substances that are widely used in the perfume industries, in the pharmaceutical sector and in the food and human nutrition field. They are mixtures of more than 200 compounds, that can be grouped basically into two fractions, a volatile fraction, that constitutes 90-95% of the whole oil and contains monoterpenes and sesquiterpene, hydrocarbons and their oxygenated derivatives, along with aliphatic aldehydes, alcohols and esters, and a non-volatile residue, that constitutes from 5 to 10% of the whole oil and contains hydrocarbons, fatty acids, sterols, carotenoids, waxes, coumarins, and flavonoids. The isolation, concentration and purification of essential oils have been important processes for many years, as a consequence of the widespread use of these compounds. The common methods used so far are mainly based on solvent extraction and steam distillation [3].
The aim of this study was to compare different extraction methods of essential oil from carobs in order to evaluate the most advantageous for food industry application in terms of bioactive compounds and antioxidant activity. Furthermore, the multi-factorial analysis was used to determine the effective factors on the recovery of essential oil in order to optimize the extraction procedures.

2 Materials and Methods

2.1 Plant material, reagents and standards

The medium milled carobs were obtained by the Cyprus Co-operative Carob Marketing Federation Ltd. Reagents and standard compounds were purchased by Sigma (St. Louis, MO), while all common solvents were provided by Scharlau (Barcelona, Spain).

2.2 Extraction methods

The carobs were triturated and extracted with four common methods. The recovery of essential oil from carobs was carried out using (i) solid-liquid extraction, (ii) ultrasonic assisted extraction, (iii) Soxtec extraction and (iv)hydrodistillation. The yields of essential oils were determined by gravimetric method after complete solvent removal.
(i) Solid-liquid extraction: Fifty (50) g of carobs were placed in a glass screw-topped vial and hexane (100, 150, 250 and 500 ml) was added to the sample. The mixture was thoroughly agitated 30, 60, 120 and 240 min. The hexane was removed in a rotary evaporator at 35 °C and the essential oils were stored at -20 °C until use. [4]
(ii) Ultrasonic assisted extraction: An amount of 50 g of carobs was placed into hexane (100, 150, 250 and 500 ml) into glass screw-topped vial. The mixture was allowed to extract for 30, 60, 120 and 240 min in an ultrasonic bath at room temperature. The ultrasonic assisted extraction was carried out using an ultrasonic cleaner (UCI-50, 35 KHz, Raypa-R. Espinar, Barcelona, Spain). Then, the hexane was removed in a rotary evaporator at 35 °C and the essential oils were stored at -20 °C until use [4].
(iii) Soxtec extraction: The recovery of essential oil from carobs was carried out on a semi-automated Soxtec system 2055 (Foss® Analytical, Hilleroed, Denmark) according to Sundram and co-workers [5] with slight modifications. Fifteen (15) grams of carobs were weighed into extraction thimbles and 60, 80 100 and 120 mL of hexane were transferred into each Soxtec extraction cup. Essential oils were extracted by setting the unit at the boiling position for 30 min and then at the rising position for 1 hour. The hexane was removed in a rotary evaporator at 35 °C and the essential oils were stored at -20 °C until use.
(iv) Hydrodistillation: The essential oils were isolated from grounded carobs (~20 g) by conventional hydrodistillation for 30, 60, 120 and 240 min. The obtained essential oil was dried with anhydrous Na_2SO_4 and was stored at -20 °C until needed [6].

2.3 Determination of total phenolics

The amount of total phenolics of essential oils was determined using the Folin-Ciocalteu assay [7]. The absorbance was measured at 725 nm using an UV-Vis- spectrophotometer (Jenway 6505, Keison Products, Essex, England). Each measurement was repeated in triplicate and the total phenolic content was expressed as equivalents of mg gallic acid Kg^{-1} carob.

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2.4 Determination of total terpenes

Total terpenic content was evaluated according to Doneva-Saspseka and co-workers [8]. Five ml of 2% vanillin-H_2SO_4 were mixed with five ml of diluted essential oil (1:10) in screw cap tube. The mixture was thoroughly vortexed and allowed to cool in an ice bath. The blue-green color was developed by heating in water-bath at 60 °C for 20 min. The absorbance of mixture was read at 608 nm. Each measurement was repeated in triplicate and the total terpenic content was expressed as equivalents of mg linalool Kg^{-1} carob.

2.5 Determination of radical scavenging activity

Two mL of diluted essential oil were mixed with 1 mL of 0.3 mmol L^{-1} solution of DPPH in methanol, incubated in the dark for 30 min and the absorbance of the mixture was monitored at 517 nm. Different concentrations of each sample were tested and the % of free radical scavenging activity was determined by the following equation:

$$\%scavenging\,activity = 100 - \frac{(Ab_{sample} - Ab_{blank})100}{Ab_{control}} \quad (1)$$

EC_{50} values are referred to the essential oil concentration at the 50 % of the antioxidant activity [9]

2.6 Determination of total antioxidant activity by Ferric Reducing/Antioxidant Power (FRAP) assay

A sample containing 3 mL of freshly prepared FRAP solution (0.3 mol L^{-1} acetate buffer (pH 3.6) containing 10 mmol L^{-1} TPTZ and 40 mmol L^{-1} $FeCl_3 \cdot 10H_2O$) and 100 μL of essential oil was incubated at 37°C for 4 min and the absorbance was measured at 593 nm. A standard solution of 500 μmol L^{-1} L-ascorbic acid in distilled water was prepared. The absorbance change was converted into a FRAP value, by relating the change of absorbance at 593 nm of the test sample to that of the standard solution of L-ascorbic acid and results were expressed as μmol ascorbic acid/ g d.m. [7]

2.7 Multi-factorial analysis

Multi-factorial analysis was performed on the experimental results, by using Matlab (Version 7.11,2010, The MathWorks Inc, Natick, Massachusetts) as described by Gekas and co-workers [10] in order to evaluate the effect of the two parameters solvent quantity (A) and time (B) and possible synergy effects for each method of recovery, on the response variables Essential Oil Yield (Y_1), Terpenes (Y_2), Phenols (Y_3) and antioxidant potential expressed as FRAP (Y_4) and DPPH (Y_5).

3 Results

The recovery of essential oil from carobs was carried out using different extraction methods and the results were presented in the Table 1. The efficiency of extraction terms was expressed in terms of essential oil yield, bioactive content (terpenenic and phenolic content) and antioxidant activity (FRAP, DPPH). Results indicated that the extraction method affects the yield of essential oils and their bioactive composition. The essential oils (yield, quality) were also influenced significantly by the ratio sample/ solvent and the extraction. The first number in the multi-factorial analysis row indicates the effect of solvent quantity (A), the second the effect of time (B) and the third their combined effect, if any. The multi-factorial analysis demonstrated that the ratio sample-solvent was the most critical factor for the recovery of essential oils, while synergism was monitored occasionally.

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Table 1. Essential oil yield, terpenic and phenolic content and antioxidant activity (FRAP, DPPH) of different extraction method used; multifactorial analysis for the effect of solvent quantity and time

CONVENTIONAL EXTRACTION							
Solvent Quantity (ml)	Mass Ratio (g carob/ ml solvent)	Time (h)	EO Yield (mg/kg carob)	Terpenes (mg linalool / kg carob)	Phenols (μmol gallic acid / kg carob)	FRAP (μmol Ascorbic acid/ ml EO)	DPPH EC_{50} (mg/ml)
100	0,5122	60	203±6	54±4	211±3	3,4±0,3	35,3±1,8
100	0,4936	240	253±11	62±5	249±8	4,6±0,2	26,1±1,2
250	0,2051	60	792±25	223±9	411±5	14,2±0,8	21,0±2,1
250	0,2011	240	995±37	187±8	514±9	21,1±0,9	13,8±1,1
Multifactorial Analysis (MA)			133 253 153	294 -28 -44	465 141 65	27.3 8.1 5.7	-26.6 -16.4 2
ULTRASONIC ASSISTED EXTRACTION							
Solvent Quantity (ml)	Mass Ratio (g carob/ ml solvent)	Time (h)	EO Yield (mg/kg carob)	Terpenes (mg/kg carob)	Phenols (μmol gallic acid / kg carob)	FRAP (μmol Ascorbic acid/ ml EO)	DPPH EC_{50} (mg/ml)
100	0,5186	60	689±12	185±6	415±6	20,5±1,0	19,8±0,8
100	0,5224	240	744±17	201±9	429±8	22.0±1,1	17.9±1,0
250	0,2077	60	1211±29	269±6	796±12	34,6±0,8	10,2±0,4
250	0,2016	240	1301±33	241±9	814±13	35,8±1,9	10,4±0,3
Multifactorial Analysis (MA)			1079 145 35	124 -12 -44	766 32 4	27.9 2.7 -0.3	-17.1 -1.7 2.1
SOXTEC EXTRACTION							
Solvent Quantity (ml)	Mass Ratio (g carob/ ml solvent)	Time (h)	EO Yield (mg/kg carob)	Terpenes (mg/kg carob)	Phenols (μmol gallic acid / kg carob)	FRAP (μmol Ascorbic acid/ ml EO)	DPPH EC_{50} (mg/ml)
60	0,2594	60	1291±22	147±5	651±15	26,8±1,2	22,1±1,5
60	0,2368	240	1174±28	151,0±8	669±22	25,9±0,9	21.5±1,0
100	0,1496	60	902±24	130±4	542±10	20,8±0,6	20,6±0,5
100	0,1153	240	895±17	171±5	549±14	23,1±0,9	18,9±1,0
Multifactorial Analysis (MA)			-668 -124 110	3 45 37	-229 25 -11	-8.8 1.4 3.2	-4.1 -2.3 -1.1
HYDRODISTILLATION							
Solvent Quantity (ml)	Mass Ratio (g carob/ ml solvent)	Time (h)	EO Yield (mg/kg carob)	Terpenes (mg/kg carob)	Phenols (μmol gallic acid / kg carob)	FRAP (μmol Ascorbic acid/ ml EO)	DPPH EC_{50} (mg/ml)
200	0,1197	---	689±20	188±4	417±10	17,9±0,7	23,6±1,1
300	0,0704	---	768±21	231±7	458±8	19,9±0,6	21,5±1,0
400	0,0492	---	597±14	244±9	444±6	15,2±0,4	28,9±1,4
500	0,042	---	605±11	225±11	460±7	15,5±0,4	27,4±2,1

4 Discussion and Conclusions

4.1 Absolute values of response variables

Between the four methods, hydrodistillation is highly differentiated from the others since it was the only one where the time variable was stable, hence multifactorial analysis was not conducted for it. It can be seen from the results that it is the only method where the increase in solvent results in comparable results of the response variables. On the other hand, the increase of solvent leads to descending results for the Soxtec extraction, with the exclusion of Terpene concentration.

Comparing the hydrodistillation method to the other three methods where the solvent is hexane, it can be seen that the obtained results are comparable to the ones obtained in the lower range of the ultrasonic assisted extraction and the middle range of the conventional extraction; therefore this method should be preferred after careful consideration of the costs, especially the ones deriving from the solvent.

It is obvious that the ultrasonic assisted extraction method gives better results of the response variables compared to the conventional method, but similar results to the Soxtec extraction. The Soxtec extraction should be considered in the cases where time is a decisive factor, since the required results (even though lower than the ones from Ultrasonic extraction) are obtained by the use of less solvent and less time.

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4.2 Effect of factors to the response variables

The effect of solvent quantity (A) on the EO yield (Y_1) is in descending order (absolute values): ultrasonic extraction>Soxtec extraction>conventional extraction. This can be seen both from the experimental results and the multifactorial analysis. The effect of solvent which is shown by the first number of the MA row (Table 1) is in the range of the results in the case of Ultrasonic extraction ($Effect_A$=1079 for range 689 to 1301), which means that by the change in solvent quantity, the EO yield is affected greatly. The solvent quantity has a large effect (although negative) for the Soxtec extraction and a substantial synergy effect between solvent and time is seen in the conventional extraction.

Solvent quantity also affects the terpene concentration (Y_2) and the DPPH (Y_5) in the order of Conventional extraction>Ultrasonic Extraction>Soxtec extraction

The effect of time (B) on the EO yield (Y_1), Phenols (Y_3) and FRAP (Y_4) is in descending order (absolute values): Conventional extraction>Ultrasonic extraction>Soxtec extraction. The effect of time is considerably lower than the one of solvent quantity, especially for the range of results obtained.

Significant synergy effects are found in the cases of the EO yield for the conventional extraction and the Terpene concentration for the Soxtec extraction. This is seen from the third number of the MA row (Table 1), compared to the range of results and the individual effect of the two factors.

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References

1. El Hajaji H, Lachkar N, Alaoui K, Cherrah Y, Farah A, Ennabili A, El Bali, Lachkar M. Antioxidant activity, phytochemical screening from three genders of carob tree barks growing in Marocco. *Arabian Journal of Chemistry,* 4, 2011, pp. 321-324.
2. Meikle R.D. *Flora of Cyprus Vol.1*, Bentham-Moxon Trust, Royal Botanic Garden, Australia, 1977, pp.589.
3. De Castro MDL, Jimenez-Carmona MM, Fernandez-Perez V. Towards more rational techniques for the isolation of valuable essential oils from plants. *Trends in Analytical Chemistry,* 18, 1999, pp. 708-716.
4. Abbasi H, Razaei K, Rashidi L. Extraction of essential oils from seeds of pomegranate using organic solvents and supercritical CO_2. *Journal of American Oil Chemists´s Society,* 85, 2008, pp. 83-89.
5. Syndram J, Kandala CV, Holser RA, Butts CL, Windham WR. Determination of in-shell peanut oil and fatty acid composition isung Near-Infrared Reflectance spectroscopy. *Journal of American Oil Chemists´s Society,* 87, 2010, pp.1103-1114.
6. Xie Y, Wang J, Yang F, Lei C. Comparative analysis of essential oil components of two *Cryptomeria* species from China. *Industrial Crops and Products,* 34, 2011, pp.1226-1230.
7. Goulas V, Manganaris GA. The effect of postharvest ripening on strawberry bioactive composition and antioxidant potential. *Journal of the Science of Food and Agriculture,* 91, 2011, pp.1907-1914.
8. Doneva-Sapceska D, Dimitrovski A, Bojadziev T, Milanov G, Vojnovski B. Free and potentially volatile monoterpenes in grape varieties from the Republic of Macedonia. *Bulletin of the Chemists and Technologists of Macedonia,* 25, 2006, pp.51-56.

9. Goulas V, Exarchou V, Troganis AN, Psomiadou E, Fotsis T, Briasoulis E, Gerothanassis IP. Phytochemicals in olive-leaf extracts and their antiproliferative activity against cancer and endothelial cells. *Molecular Nutrition and Food Research,*53, 2009, pp.600-608.

10. Gekas V., Katsivela. E, Frantzeskaki, N., *Toxic and hazardous waste treatments*, Tziola Publishing, Thessaloniki (in Greek), 2002, pp.46-50.