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Vegetable and Nut Oils Extraction by d-limonene as Alternative Solvent

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Abstract

Objective: The objective of this study was to test the possibility of using a by-product of vegetable origin for the extraction of vegetable oils. An opportunity to find a substitute for n-hexane for the extraction of vegetable oils is studied. The d-limonene is an agricultural by-product that is used in various fields. It can also be used as a green solvent, replacing n-hexane, which is responsible for environmental problems. Extraction yields of 8 vegetable oils and their fatty acid profiles were studied. **Materials and Methods:** Oils were extracted from oilseeds with a Soxhlet apparatus, fatty acid methyl esters (FAMES) were prepared according to AOCS Official method Ce 2-66 (1989). The GC-MS analyses were carried out in a Shimadzu QP2010 gas chromatography coupled with an ion trap mass spectrometer. **Results:** The findings of this study were that d-limonene allowed to obtain higher yields without changing the fatty acid composition of different oils compared to n-hexane extraction, the d-limonene is proposed as an alternative to n-hexane for the extraction of vegetable oils. **Conclusion:** The d-limonene has been verified as an efficient solvent for the extraction of vegetable oil using 8 different natural sources. This makes the use of d-limonene as an extraction solvent of vegetable oils, an attractive alternative to n-hexane in accordance with new regulations related to the use of organic solvents.

Key words: GC-MS analysis, extraction, fatty acids, lipids, n-hexane, d-limonene, vegetable and nuts, yield

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Soxhlet extraction is a method initially used to extract the fat in milk and it is more accurate method than those previously proposed¹. Franz von Soxhlet's motivation was to introduce this innovation to determine the quantitative amount of fat contained in organic matter². The n-hexane is a solvent widely used for the industrial lipids extraction from seeds. It extracts oil from flattened or crushed seeds or from the remaining of the cake, waste from the pressure step³.

The replacement of n-hexane is motivated by regulatory constraints. Three regulations have now a direct impact on reducing consumption of petroleum solvents. Directive 1999/13/EC, which aims to reduce emissions of volatile organic compounds (VOCs) from industrial solvents, guidelines on dangerous substances (67/548/EEC) and the hazardous products (1999/45/EC) revised in 2002, mainly concern aromatic and chlorinated solvents⁴. The best solvent to replace n-hexane would have to be abundant and cheap, non-flammable and would not be an environmental hazard. Several requirements must be considered in choosing a new solvent, including: The compatibility of the solvent with existing equipment or requiring little and cheap modifications, with at least no effects on profitability of operations which result from changes in extractor capacity, solvent and energy costs, product yield and market value⁵.

The use of agricultural byproducts is an attractive alternative. Being directly related to agricultural production and respecting environmental constraints, agro-solvents are perfectly fitted for this task. Among agro-solvents, terpenes are unsaturated hydrocarbons, some of which are extracted from pine (α and β pinene) or citrus (d-limonene). The d-limonene is the most concentrated terpene in citrus essential oil. It is used in anticancer therapy, as a cleaning agent or in the preparation of plastic⁴.

In this study, we focused on the use of d-limonene as a potential solvent for vegetables or nuts oils soxhlet extraction. It is reported that d-limonene effect on yield and on the fatty acid composition, comparatively to n-hexane to confirm the use of terpenes for the lipids extraction.

MATERIALS AND METHODS

Materials and chemicals: Almond, peanut, corn, hazelnut, walnut and cashew were purchased commercially in Algeria. Olive and pomace olive were purchased from a traditional oil mill situated in Tizi-Ouzou (Algeria). The d-limonene, n-hexane, n-heptane, sodium chloride, sodium hydroxide,

BF₃-methanol reagent (20% solution in methanol) used for experiments or derivatization were of analytical grade and were purchased from VWR International (Darmstadt, Germany).

Oil extraction protocol: Oils were extracted from oilseeds with a Soxhlet apparatus, according to the standardization procedure using two solvents, n-hexane or d-limonene⁶, according to the method described by Virost *et al.*⁷.

Values are the average of three extractions; the extraction yield was calculated according to the following method:

$$\text{Oil content (\%)} = \frac{\text{Weight of extracted oil} \times 100}{\text{Weight of dry sample}}$$

Gas Chromatography-Mass Spectrometry (GC-MS) analysis:

Fatty acid methyl esters (FAMES) were prepared according to AOCs Official method Ce 266⁸. Samples were then filtered through a 0.2 μm cellulose regenerated filter (Alltech associates, Deerfield, IL, USA) before injection.

The GC-MS analyses were carried out in a Shimadzu QP2010 gas chromatography coupled with an ion trap mass spectrometer. The gas chromatograph was equipped with CP-Wax (52 CB) capillary column 30 m \times 0.32 mm \times 0.5 μm (Varian, Walnut Creek, CA, USA). The velocity of the carrier gas (He) was at 47 cm sec⁻¹. Flow rate was held at 1.69 mL min⁻¹ and the column head pressure was 20 kPa. Samples were injected (2 μL) with a split mode (ratio 1:15) and the injector temperature was set at 250°C. The oven temperature increased from 60°C (1 min) to 18°C at a rate of 20°C min⁻¹, increased from 180-230°C at a rate of 4°C min⁻¹ and held at 230°C for 15 min. The mass spectra were recorded at 3 scan sec⁻¹ between 50-400 amu. The ionization mode was electron impact (EI) at 70 eV. Identification of common fatty acids was performed using the NIST'98 [US National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA] mass spectral database.

RESULTS

Quantitative determination: The extraction of vegetable oils by d-limonene allowed having a performance comparable with that obtained by n-hexane and that found in the literature, the results are shown in Table 1.

The researchers noted that d-limonene has the same ability to extract vegetable oils as n-hexane. This is related to the Hansen solubility parameters that explain the mechanisms of dissolution of vegetable oils in solvents. It appears that the d-limonene and n-hexane have similar HSP values. Furthermore, d-limonene has a dielectric constant, a polarity

Table 1: Extraction yields of vegetable oils in percentage on dry matter (DM%)

Oilseeds	Extraction yields (%)		
	n-hexane	d-limonene	According to the literature
Almond	52.25	54.61	52.10-60.40*
Peanut	51.30	52.96	49.40-53.8*
Corn	03.85	04.04	05.00**, 02.36-04.15***
Hazelnut	61.80	64.89	55.90-67.10*
Walnut	63.40	65.01	63.2-65.20*
Cashew	46.70	48.61	44.80-49.10*
Olive	40.60	43.58	30.00-70.00**, 40.3#
Pomace olive oil	05.80	07.24	04.50-09.00##

*Kornsteiner *et al.*⁹, **Enig¹⁰, ***Ali and Ashraf¹¹, #Virot *et al.*¹², ##Conseil Oleicole International¹³

Table 2: Profile of fatty acids (%) by GC-MS of different oils extracted using d-limonene or n-hexane as solvents

Oils	C14:0	C16:0	C16:1	C18:0	C18:1a	C18:1b	C18:2	C18:3	C20:0	C20:1	C22:0
Almond H	0.0	6.3	0.4	1.5	62.4	1.3	27.8	0.0	0.1	0.1	0.0
Almond L	1.3	6.9	0.5	1.6	65.2	1.6	22.6	0.0	0.3	0.0	0.0
Peanut H	0.3	11.5	0.0	4.1	44.7	0.5	33.7	0.0	1.7	0.9	2.7
Peanut L	0.4	12.5	0.2	4.5	49.2	0.5	26.7	0.1	1.9	1.0	2.9
Corn H	0.0	9.0	0.0	2.0	29.6	0.8	56.4	1.0	0.5	0.3	0.4
Corn L	0.6	10.5	0.8	2.7	29.6	1.2	51.8	1.1	0.8	0.5	0.5
Walnut H	0.0	6.3	0.0	2.3	23.0	1.0	59.1	8.2	0.0	0.2	0.0
Walnut L	0.1	6.4	0.1	2.2	23.2	1.0	58.6	8.1	0.1	0.2	0.0
Cashew H	0.0	8.1	0.3	7.5	64.7	0.5	18.3	0.0	0.5	0.0	0.0
Cashew L	0.2	8.7	0.3	9.1	65.4	0.4	14.5	0.2	0.7	0.3	0.1
Hazelnut H	0.0	5.6	0.0	2.6	82.3	1.4	8.2	0.0	0.0	0.0	0.0
Hazelnut L	0.0	6.7	0.3	2.9	80.0	1.4	8.1	0.0	0.2	0.2	0.2
Olive H	0.1	12.9	1.2	1.9	65.8	3.0	13.6	0.6	0.4	0.4	0.1
Olive L	0.0	18.9	0.0	0.0	54.4	0.0	8.3	0.0	18.4	0.0	0.0
Pomace olive H	0.0	13.2	1.0	2.7	63.8	2.7	14.7	0.6	0.6	0.4	0.3
Pomace olive L	0.0	13.8	1.4	2.4	65.3	3.2	12.5	0.5	0.5	0.3	0.0

H: Oil extracted by n-hexane. L: Oil extracted by d-limonene

and a power of dissociation, higher than that of n-hexane. Moreover, d-limonene has a higher flash point than n-hexane, therefore it is less flammable and less hazardous^{14,15}.

Qualitative determination: Vegetable oils obtained were converted to fatty acids methyl esters and the fatty acid profile were determined by GC-MS.

The fatty acid profile of obtained vegetable oils is summarized in Table 2.

DISCUSSION

Table 1 shows researchers noted that oil extraction performed with d-limonene resulted in higher yields than those extracted by n-hexane and those extraction yields increase with time. This may be due to the greater triglycerides solubility in d-limonene and to the d-limonene higher boiling point compared to n-hexane, as higher temperatures facilitate extraction⁵. It may also be due to the decrease of both d-limonene and oil viscosities at high temperatures, which increase the rate of diffusion of the

solute from the solid phase to the solvent. Another feature possibly involved for this result is the slightly polar nature of d-limonene when compared to n-hexane^{16,17}. The results of this study were similar to those found by Mamidipally and Liu¹⁶, Liu and Mamidipally¹⁷ and Virot *et al.*^{17,18} proved that whatever the oilseed and the operating conditions, the extraction yield by d-limonene is always higher than that of n-hexane.

Table 2 shows that researchers noted for almond oil, that palmitic (C16:0), oleic (C18:1) and linoleic (C18:2) acids, represent 96.50% of total oil extracted by n-hexane and 94.70% for the extracted d-limonene. These results were consistent with those found in the previous studies^{19,20}, which were between 91.93 and 97.83%.

For peanut oil, palmitic (C16:0), oleic (C18:1) and linoleic (C18:2) acids represent 89.90% for oil extracted by n-hexane and 88.40% for the extracted d-limonene. These results confirm those reported in the previous studies^{12,21-23}, which were between 87.64 and 96%.

For corn oil, palmitic (C16:0), oleic (C18:1) and linoleic (C18:2) acids represent 95.00% for oil extracted by n-hexane

and 91.90% for that extracted by the d-limonene. These results were comparable to those found in the previous studies^{11,12,22-24}, which were between 43.00 and 97.28%.

For walnut oil, palmitic (C16:0), oleic (C18:1) and linoleic (C18:2) and linolenic (C18:3) acids represent 96.70% for oil extracted by n-hexane and 96.30% for the extracted d-limonene. These results were consistent with those found in the previous studies^{20,25-27}, which were between 92.00 and 97.1%.

For cashews oil, palmitic (C16:0), stearic (C18:0), oleic (C18:1) and linoleic (C18:2) acids represent 98.60% for oil extracted by the n-hexane and 97.70% for the extracted d-limonene. These results were consistent with the results of several studies that have found values between 88.20 and 99%²⁸⁻³⁰.

For hazelnut oil, palmitic (C16:0), stearic (C18:0), oleic (C18:1) and linoleic (C18:2) acids represent 98.70% for oil extracted by n-hexane and 97.70% for the extracted d-limonene. These results were consistent with several studies^{20,24,27,30} that have found values between 97.20, 98.93% and 99.10%.

For olive oil, palmitic acid (C16:0), oleic (C18:1) and linoleic (C18:2) acids represent 92.30% for oil extracted by n-hexane and 81.60% for the extracted d-limonene. These results were consistent with the literature, being valued between 79.43 and 96.35%. These rates vary depending on the treatment received, the extraction method (pressure, solvent and solvent nature) and cultivar^{7,18,23,31-37}.

It also appears from the olive pomace oil, that the palmitic (C16:0), oleic (C18:1) and linoleic (C18:2) acids represent 91.70% for oil extracted by n-hexane and 91.60% for the extracted d-limonene. These results were consistent with other studies they found a range between 66.00 and 99%, these results were based on the extraction method and solvent^{13,38,39}.

These results are very interesting insofar as they imply that d-limonene is potentially interesting in the field of extraction of vegetable oils and makes it possible to generalize its use in this field.

The obvious question that should be answered as to the potential widespread use of d-limonene in the process of extraction instead of n-hexane, is how to reduce energy consumption associated with solvent recovery of d-limonene, due to its high boiling point (175°C) compared with n-hexane (69°C).

Several studies have found interesting solutions, Liu and Mamidipally¹⁷ proposed a power-saving technology represented by a membrane separation. Virot *et al.*³² have developed a device for recovering d-limonene using distillation by Clevenger assisted by microwaves. These can

make the use of d-limonene as an extraction solvent of vegetable oils an attractive alternative to n-hexane.

CONCLUSION

In terms of this study, it is clear from the obtained results that the extraction ability of vegetable oils by d-limonene has been verified. The extraction with d-limonene as solvent gave a higher yield compared to that of n-hexane. The d-limonene did not alter the fatty acid profile and composition of extracted oils. It can be considered as a new solvent for the extraction of vegetable oils.

SIGNIFICANCE STATEMENTS

- The d-limonene is the most concentrated terpene in citrus essential oil. Algeria is a producer of citrus and citrus essential oils
- The valorization capacity of citrus essential oils is linked to the possibility of using them as extraction solvent for vegetable oils in place of hexane, the most used solvent currently in this field
- It would be interesting for Algeria to valorize its by-products and co-products of vegetable origin in the field of green chemistry and thus reduce the emission of volatile organic compounds (VOCs)

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