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Extraction of fatty acids from grape seed by superheated hexane

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Abstract

Superheated hexane extraction has been tested for obtaining fatty acids from grape seed and compared with conventional Soxhlet and hot hexane extractions. Seeds from grape residues from a winery were dried for 46 h at 105 °C, milled and sieved by particle size ($d < 0.42$ mm, $0.42 < d < 0.84$ mm and $d > 0.84$ mm). An optimization study of influential variables on superheated hexane extraction (namely extraction time, temperature, pression, particle size and sample amount) was carried out by a multivariate approach. All the extracts were concentrated in a rotary evaporator and dried by adding 1 g of Na_2SO_4. Then, 2 ml of the dried extract were subjected to reaction with 1 ml of a 0.5 M solution of sodium methylate in methanol to obtain fatty acid methyl esters (FAMEs). After derivatization, FAMEs were quantified by GC-FID. The results show that the optimal conditions for superheated hexane extraction are: time extraction, 10 min; temperature, 80 °C; pressure, 40 bar; particle size, $d < 0.42$ mm; amount of sample, 0.4 g. Under these conditions, around 84% of the fatty acids (out of the amount obtained by Soxhlet extraction) is extracted. Comparison with Soxhlet and hot hexane extractions showed that the percentages of FAMEs are similar in all the extracts and they agree with the data in the bibliography.

Keywords: Grape seed oil; FAMEs; Superheated fluids extraction

1. Introduction

Grape seeds make up around 15% of the solid waste produced in wine industries. They are generally burnt and sometimes used for cattle feed, despite of they are the source of an excellent oil for human consumption. Oil content of grape seeds strongly depends on grape variety, though the usual range is 10–16% of dry weight. It consists mainly of triglycerides and the fatty acids composition is adjusted to the following values: 0–0.2% myristic acid ($C_{14:0}$), 7–13% palmitic acid ($C_{16:0}$), 3–6% stearic acid ($C_{18:0}$), 0–0.9% palmitoleic acid ($C_{16:1}$), 14–25% oleic acid ($C_{18:1}$), 61–73% linoleic acid ($C_{18:2}$), 0–0.6% linolenic acid ($C_{18:3}$). The high content in unsaturated fatty acids (around 85–90%) makes it a high-quality nutritional oil, which exhibits properties for prevention of thrombosis, inhabitation of cardiovascular diseases, reduction of cholesterol in serum, dilation of blood vessels and regulation of autonomic nerves [1]. Particularly, the linoleic acid percentage is higher than in any other oil, included safflower, sunflower and corn oils [2]. Also, it is an important source for the production of conjugated linoleic acid (CLA), a mixture of positional and configurational isomers of $C_{18:2}$ fatty acid. It has been reported that synthetic CLA is an effective agent for inhibiting mammary, colon, forestomach and skin carcinogenesis in experimental models [2], and CLA-feeding has demonstrated to reduce body fat in several animal models, independent of the type or quantity of dietary fat consumed [3].

In addition, grape seed oil contains tannins at levels higher than other seed oils [4] and 0.8–1.5% unsaponifiable lipids, mainly esterols as β-sitosterol, campesterol and stigmasterol [5]. The antioxidant activity of these compounds makes this oil very resistant to peroxidation and suitable for using as cosmetic ingredient. In this sense, it is used for the treatment of dry skin and protection against aging. It helps to balance the skin pH, is hypoallergenic, does not irritate skin, and helps irritated skin to become smooth and calm [6].

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The traditional methods for extracting grape seed oil consist of pressing the whole seeds in discontinuous-hydraulic press or the milled and heated seeds in screw press, but both have low cost-effectiveness [7]. At present, they have been replaced almost totally by solvent (hexane, generally) extraction after crushing seeds in roller mills and heating. Then, the crude oil is neutralised, bleached with activated carbon and clay and finally deodorized under vacuum. Yield of this extraction method is high but several hours are necessary to complete the extraction step.

In recent years, alternative methods without organic solvents for oil extraction have been proposed, as hot water extraction [5] and supercritical fluid extraction (SFE) [8–11]. The first has not problems concerning both security and cost, but it is very ineffective and requires deemulsification and evaporation steps. On the other hand, similar yield and oil quality as compared to conventional solvent extraction have been reported for SFE [11], and, moreover, solvent removal by distillation and several steps of the subsequent oil refining process are deleted. However, its very high acquisition and maintenance costs make its application to large scale very difficult.

The aim of this work is to show that superheated hexane extraction could be a viable alternative to industrial conventional extraction of oil from grape seeds.

2. Experimental

2.1. Reagents

n-Hexane (LiChrosolv, Merck, Darmstadt, Germany), methyl palmitate, methyl stearate, methyl oleate, methyl linoleate, methyl linolenate and methyl eicosanoate (chromatographic purity, Fluka, Buchs, Switzerland) and sodium methylate 0.5 mol l^{-1} (PA-ACS quality, Panreac, Barcelona, Spain) were used.

All gases were of 95% purity or higher and supplied by Carburos Metálicos (Barcelona, Spain).

2.2. Sample preparation

Grape residues were obtained from a winery in Manzanares (Ciudad Real, Spain). They were a mixture of several grape varieties preserved below −10 °C until use. Seeds were separated from stems and skins manually and dried for 46 h at 105 °C. Then, they were milled, sieved by particle size ($d < 0.42$ mm, $0.42 < d < 0.84$ mm and $d > 0.84$ mm) and kept in a desiccator until use.

2.3. Instruments and apparatus

Grape seeds were milled with a grinder (Moulinex D56, Barcelona, Spain).

Superheated hexane extractions were performed with a system composed of the following elements, depicted in Fig. 1: (a) an extractant reservoir; (b) a high-pressure pump (Shimadzu LD-AC10) which propels the extractant through the system; (c) a selection valve (V_1) located next to the pump, which allows flushing the extract with dry N_2 after extraction; (d) a stainless steel cylindrical extraction chamber (200 mm × 10 mm i.d., 16 ml internal volume), where the sample is introduced. This chamber is closed at both ends with screws whose caps contain stainless steel filter plates (1 mm thick, 12 mm d) to ensure the sample is not carried away by the extractant; (e) a restriction valve (V_2) to maintain the preset pressure in the system; (f) a cooler made from stainless steel tubing (1 m length, 0.4 mm i.d.) and refrigerated with water; (g) a gas chromatograph oven (Konix, Cromatix KNK-2000) used as heating source where the extraction chamber is placed.

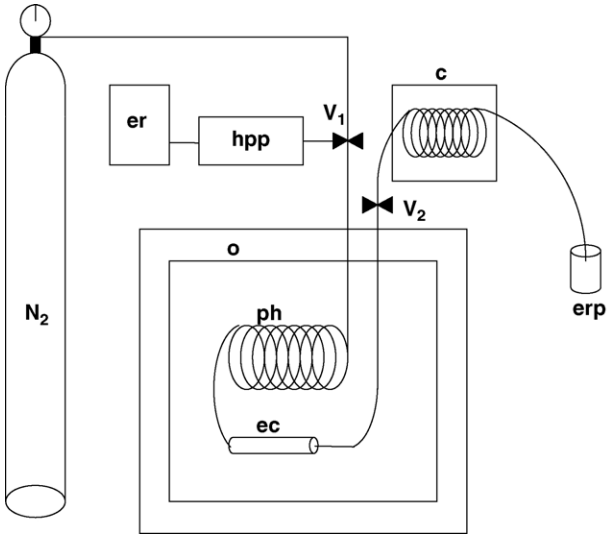

Fig. 1. Superheated fluids extraction system. hpp: high pressure pump, er: extractant reservoir, ph: preheater, ec: extraction cell, o: oven, c: cooler, V_1: selection valve, V_2: restriction valve, erp: extract recipient.

Shaking and centrifuging of the extracts during the derivatization process were carried out by means of an MS2 Minishaker (IKA, Germany) Vortex and a Mixtasel (Selecta, Barcelona, Spain) centrifuge, respectively.

The extracts were analyzed by a Varian 3400CX gas chromatograph equipped with a Supelco Omegawax 250 fused–silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) and a flame ionization detector (FID).

Statgraphics plus v.2.1 for Windows was employed for the optimization study.

2.4. Procedures

2.4.1. Superheated hexane extraction

The required amount of sample was placed in the extraction cell mixed with 2 g of sand (previously washed with diethyl ether to eliminated organic material, dried and sieved), for avoiding compaction. After assembling the cell to the oven, the pump was connected. To ensure air absence

Table 1
Ranges tested in the screening study

Variable	Range tested	Optimal conditions
Time (min)	10–30	10
Temperature (°C)	80–100	80
Pressure (bar)	20–40	40
Particle size (mm d)	<0.42 to > 0.84	<0.42
Amount of sample (g)	0.4–1.0	0.4

in the system, valve V_2 was maintained in the open position until the first drop of extractant appeared; then valve V_2 was closed. The hexane flow-rate was fixed at 4 ml min^{-1} to allow cell filling in 5 min, approximately. After this, the oven was heated to the working temperature while pumping the extractant. When the system reached the preset pressure, valve V_1 was also closed and static extraction was performed for a preset time. Finally, the chamber was cooled below the boiling point of the extractant to avoid evaporation (as depressurisation happens in openning valve V_2). Then, valve V_1 was switched to enable dried nitrogen to flow through the cell and flush the extract, which was concentrated in a rotary evaporator (final volume 5 ml) and then dried by adding 1 g of Na_2SO_4. The tested conditions are shown in Table 1.

2.4.2. Soxhlet extraction

Soxhlet extraction was employed to know the total oil content of the grape seed. With this aim, three extractions were carried out using for each 80 ml of *n*-hexane and 3 g of the smallest particle size milled seeds ($d < 0.42$ mm), mixed with 6 g of sand. After extraction for 24 h, the extracts were concentrated and dried as described above.

2.4.3. Hot hexane extraction

Hot hexane extraction was performed in the same system as superheated hexane under the optimal conditions, but at 60 °C.

2.4.4. Preparation of fatty acid methyl esters (FAMEs)

A preparation step was necessary prior to introduction of the extracts into the gas chromatograph for the individual determination of fatty acids. FAMEs were obtained by transesterification with sodium methylate [12]. 1 ml of a 0.5 M solution of sodium methylate in methanol and 2 ml of the dried extract were mixed and shaken vigorously for 5 min in a vortex-mixer. After centrifugation for 5 min at 4000 rpm, 1 ml of the hexane fraction was taken and 52.5 μl of methyl eicosanoate solution in hexane (external standard) was added. The extracts for the kinetics study were treated in the same manner, but all the volumes were half.

2.4.5. Chromatographic separation and detection

After derivatization, 0.5 μl of the extract was injected into the gas chromatograph in the split mode. The split ratio was 1:100 and the flow-rate of carrier gas (helium) 2 ml min^{-1}. The injector and detector temperatures were fixed at 280 °C and that of the oven at 200 °C. Under these conditions, the separation of FAMEs took 17 min.

3. Results and discussion

3.1. Optimization of variables

The factors affecting significantly the superheated hexane extraction were studied. The optimal values found are shown in Table 1. A multivariate method based on a Plackett–Burman experimental design, which required 17 experiments, was chosen for this study. The results obtained appear in Table 2. As can be seen, none of the variables has a significant influence, except the particle size, the influence of which on the amount of extracted oil is enormous. The differences between the percentages of FAMEs (on a sample weight basis) for each particle size were within the repeatability and reproducibility values, as checked later in the precision study.

The pressure has a slightly positive non-significant effect on extraction. This behaviour is in agreement with previous works about oil extraction with superheated fluids, in which the use of pressure in high excess over the minimum value to guarantee supercritical state of the extractant does not improve the extraction yield [13–15]. On the other hand, time, temperature and amount of sample have a negative non-significant effect, which indicates that the extracted oil yield increases when these variables have lower values. Thus, the lowest values of all variables, but pressure, were selected as optimal.

The negative non-significant influence of the amount of sample could be explained by the higher extractant volume/solid mass ratio when 0.4 g is used, though it is not significant because of the high solubility of fatty compounds in the extractant.

Concerning time, the small differences found in the extraction efficiency could be explained by the concept "extraction time", which is referred to the time during which the extraction chamber stays in superheated conditions, but the extractant is really in contact with the sample a longer time: while the cell is filled (5 min), acquires the preset temperature and pressure (2–3 min) and the extract is cooled below the boiling point of solvent when the extraction is finished (5 min), before collecting the extract. Therefore, assuming that the particle size is very small, even the shortest time can be enough to practically extract the whole fat.

The decrease in the amount of extracted fat when the extraction time increases can be explained as a joint effect of both, time and temperature. It is known that high values of the latter results in the formation of free fatty acid from triglycerides during extraction [11]. These acids are not esterified by sodium methylate, which only produces transesterification [16], so these free acids are not quantified. Therefore, the lowest values of these variables must be employed in order to avoid hydrolysis, though temperatures lower than 80 °C are

Table 2
Results of the screening experiments

Sample	t[a]	T[b]	P[c]	P.S.[d]	S.Am.[e]	MP[f]	ME[g]	MO[h]	ML[i]	MLN[j]	Total[k]	%S.W.[l]
EP-38	20	90	30	Intermediate[m]	0.7	8.1	3.8	15.8	72.2	0.0	41.8	4.2
EP-39	30	80	20	<0.42	0.4	7.4	3.8	15.5	73.0	0.3	119.9	12
EP-40	30	100	20	<0.42	1	7.1	3.7	15.4	73.4	0.4	97.5	9.8
EP-41	30	80	40	>0.84	0.4	8.9	4.4	16.1	70.6	0.0	42.8	4.3
EP-42	10	80	40	<0.42	0.4	7.3	3.8	15.4	73.1	0.3	115.9	11.6
EP-43	30	80	40	<0.42	1	7.1	3.8	15.4	73.4	0.4	103.5	10.4
EP-44	10	100	40	>0.84	0.4	9.1	4.0	15.9	71.0	0.0	39.7	4
EP-45	20	90	30	Intermediate[m]	0.7	7.9	3.8	15.9	71.9	0.0	41.6	4.2
EP-46	10	100	40	<0.42	1	7.1	3.8	15.5	73.3	0.4	104.8	10.5
EP-47	30	100	40	<0.42	0.4	7.4	3.8	15.5	72.8	0.5	115.6	11.6
EP-48	30	100	20	>0.84	0.4	11.7	4.5	16.2	67.6	0.0	17.9	1.8
EP-49	30	80	20	>0.84	1	7.9	4.1	16.1	71.9	0.0	31.7	3.2
EP-50	10	80	20	<0.42	1	7.1	3.7	15.5	73.3	0.4	98.8	9.9
EP-51	10	80	20	>0.84	0.4	9.0	4.1	16.1	70.7	0.0	41	4.1
EP-52	10	80	40	>0.84	1	7.9	3.7	16.0	72.2	0.0	38.1	3.8
EP-53	20	90	30	Intermediate[m]	0.7	7.9	4.0	15.8	71.9	0.0	40.5	4.1
EP-54	10	100	20	>0.84	1	7.9	3.9	16.0	71.9	0.0	40.6	4.1
EP-55	30	100	40	>0.84	1	7.7	3.9	15.9	72.1	0.0	44.1	4.4
EP-56	10	100	20	<0.42	0.4	7.5	3.8	15.6	73.1	0.5	112.9	11.3

[a] *t*: extraction time (min).
[b] *T*: temperature (°C).
[c] *P*: pressure (bar).
[d] P.S.: particle size (mm d).
[e] S.Am.: sample amount (g).
[f] MP: methyl palmitate (%).
[g] ME: methyl stearate (%).
[h] MO: methyl oleate (%).
[i] ML: methyl linoleate (%).
[j] MLN: methyl linolenate (%).
[k] Total: total amount of methyl esters (mg/g sample).
[l] %S.W.: percentage on sample weight.
[m] Intermediate size between 0.42 and 0.84 mm d.

not advisable to guarantee the liquid state of hexane (boiling point 69 °C).

Taking into account all these facts, the best extraction conditions are: time extraction, 10 min; temperature, 80 °C; pressure, 40 bar; particle size, $d < 0.42$ mm; amount of sample, 0.4 g.

3.2. Precision of the extraction process

The precision of the superheated hexane extraction process, expressed as repeatability and reproducibility, was calculated for the total amount of FAMEs. Within assays (intra-day) and between days (inter-day) were developed over a 7-day period. Two extractions under the selected working conditions were carried out every day, one in the early morning and other in the evening. The intra-day assay variability (RDS) was 3.0% and that of the inter-day assay, 5.5%.

3.3. Comparison of superheated hexane extraction with Soxhlet and hot hexane extractions

The results obtained after 24-h Soxhlet extraction are shown in Table 3. The total amount of FAMEs found was 13.9%. Therefore, in only 10 min (approximately 23 min if the total time is considered), superheated hexane is able of extracting around 84% of seed fatty acids. Moreover, the FAMEs percentages are similar and in agreement with the data in the bibliography.

Also, the fact that the extraction efficiency is higher with superheated hexane than with hexane heated below its boiling temperature has been verified by hot hexane extraction (Table 3).

Table 3
Percentages of FAMEs on the amount of sample obtained using different techniques

Extraction method	MP[a]	ME[b]	MO[c]	ML[d]	MLN[e]	Total[f]
Superheated hexane (optimal conditions)	7.3	3.8	15.4	73.1	0.3	11.6
Soxhlet 24 h	8.5	3.9	15.6	71.7	0.3	13.9
Hot hexane (optimal conditions, but at 60 °C)	7.2	3.8	15.5	72.9	0.6	9.6

[a] MP: methyl palmitate.
[b] ME: methyl stearate.
[c] MO: methyl oleate.
[d] ML: methyl linoleate.
[e] MLN: methyl linolenate.
[f] Total: total amount of methyl esters.

On the other hand, the extraction time with superheated hexane is also much shorter than with supercritical carbon dioxide (over 3 h) [11].

4. Conclusion

The discontinuous superheated hexane extraction of grape seed fatty acids has been studied. This technique has one substantial advantage over the conventional industrial extraction method: the very much shorter extraction time, which involves a drastic cost reduction. From the point of view of small industries, which use the conventional extraction method, superheated hexane extraction could be an economically feasible improvement, because the extraction system is quite simple and its acquisition and maintenance costs are relatively low, in opposition to a supercritical fluid extractor system.

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References

[1] Y. Liu, J.X. Yin, M.S. Zhao, K.M. Yan, H.M. Wang, Food Oil 10 (2001) 36.
[2] C. Ghisalberti, PCT International Application WO 2001018161 A2 (15 March 2001).
[3] G.S. Kelly, Altern. Med. Rev. 6 (2001) 4.
[4] P.U. Rao, Food Chem. 50 (1994) 379.
[5] J. Sineiro, H. Domínguez, M.J. Nuñez, Alimentación, equipos y tecnología Abril 1995.
[6] J. Hojerova, Vinohrad 41 (6) (2003) 6.
[7] C. Flanzy, Enología: Fundamentos Científicos y Tecnológicos, 1ª Edición. Ed., AMV Ediciones-Mundi Prensa, Madrid, 2000.
[8] M. Palma, L.T. Taylor, J. Agric. Food Chem. 47 (1999) 5044.
[9] T.A. Pan, L. Peng, Sci. Technol. Food Ind. 4 (2001) 22.
[10] X. Cao, Y. Ito, J. Chromatogr. A 1021 (2003) 117.
[11] A. Molero Gómez, C. Pereyra López, E. Martínez de la Ossa, Chem. Eng. J. 61 (1996) 227.
[12] D. Pretch, J. Molkentin, Nahrung 44 (4) (2000) 222.
[13] M.M. Jiménez Carmona, J.L. Ubera, M.D. Luque de Castro, J. Chromatogr. A 855 (1999) 625.
[14] L. Gámiz-Gracia, M.D. Luque de Castro, Talanta 51 (2000) 1179.
[15] R. Soto Ayala, M.D. Luque de Castro, Food Chem. 75 (2001) 109.
[16] W.W. Christie, in: W.W. Christie (Ed.), Advances in Lipid Methodology II, Oily Press, Dundee, 1993.