OIL FROM THE WILD ALMOND *Amygdalus scoparia* OBTAINED BY GREEN PROCESSES. COMPARATIVE LIPIDOMIC PROFILING

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Introduction

- ✓ *Amygdalus scoparia* is a wild species of almond that occupies large areas in many parts of Iran and its neighbouring countries. The extraction and use of the oil from the *A. scoparia* kernel could be of interest due to their fatty acids composition that is comparable to those of olive oil. Thus, oil from *A. scoparia* can be seen as a nutritionally healthy edible oil with high stability, considering the high levels of monounsaturated fatty acids and the presence of natural antioxidants.
- ✓ Traditionally is extracted using hexane. Pressurized Liquid Extraction and Supercritical Fluid Extraction can be green alternative techniques for its extraction.

Objective

The goal of the present work was to **optimize the extraction of lipids from *A. scoparia* using environmentally clean procedures**. The effect of sub and supercritical extraction parameters such as extraction time, extraction pressure and type of solvent on oil yield and on the lipidomic profile were evaluated.

Supercritcal Fluid Extraction

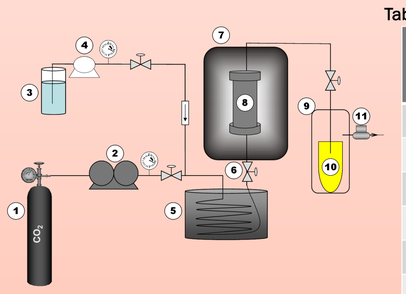
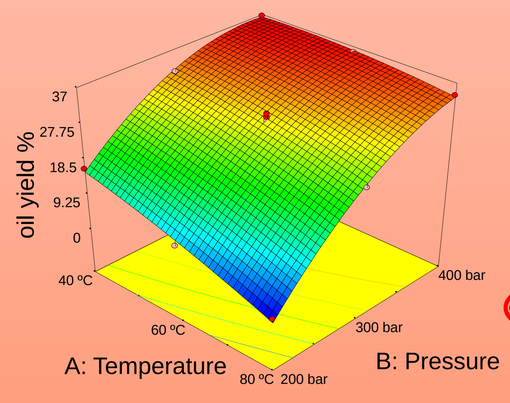


Fig 1. CO_2 extractor device. (1) CO_2 tank, (2) CO_2 pump, (3) cosolvent reservoir, (4) pump, (5) heater bath, (6) on/off controller, (7) oven, (8) extraction cell with sample, (9) expansion cell, (10) extract recovered, (11) mass flowmeter.

Table 1. Experimental design and yield obtained

Pressure (bar)	Temp. (ºC)	Oil Yield %
200	40	16.3
200	60	7.7
200	80	1.6
300	40	31.2
300	60	26.8
300	80	22.0
400	40	36.6
400	60	35.6
400	80	33.9

The effect of extraction pressure and temperature on extraction yield was studied simultaneously. **Response Surface Methodology** (RSM) was used and for this purpose a two-variable, **three-level Miscellaneous Design** was applied to find the best extraction conditions for obtaining the highest oil yield from almond powder. The two independent variables were extraction temperature, and extraction pressure. Extraction time was fixed in 160 min.



The **optimal conditions** obtained using (RSM) were Temperature; **44.5 °C**, and Pressure; **391.5 bar.** Oil yield (%) at these conditions was **36.6 % oil.** The addition of **10% ethanol** to CO_2 increased the yield up to **42.3%.**

Lipidomic profiling

Lipid classes were analyzed by HPLC-ELSD using a normal phase separation method.
Fatty acids composition was analyzed by GC-MS after derivatization to form ethyl esters.

For comparison purposes, classical Solid Liquid Extractions (SLE) were performed at 50 ºC for 24 hours under agitation. Those extractions provided lower yields than PLE and SFE

Pressurized Liquid Extraction

The effects of extraction temperature and time were examined using a **Full Factorial Experimental Design** at three levels (3^2). Temperatures tested ranged from 50 to 150 ºC, and extraction time between 5 and 15 minutes.

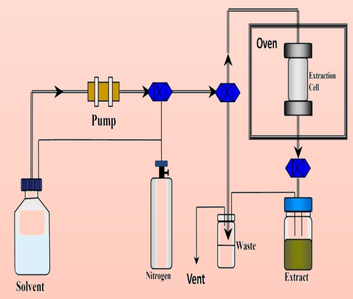


Fig 2. Pressurized Liquid Extractor Scheme

This experimental design was repeated with three different solvents: n-hexane, acetone and ethanol

Table 2. Experimental design and yields obtained in PLE

Temp (ºC)	Time (min)	Hexane Yield %	Ethanol Yield %	Acetone Yield %
50	5	41.13	40.33	41.08
50	10	41.21	41.05	41.13
50	15	41.58	41.01	41.45
100	5	43.23	50.16	45.77
100	10	43.42	50.89	46.18
100	15	43.27	50.24	45.93
150	5	44.51	54.43	48.12
150	10	43.42	55.04	48.16
150	15	44.55	55.26	47.71

Ethanol provided the highest yields. Temperature was the main factor, while the effect of extraction was not significant. Therefore the optimal PLE extraction temperature was set at **150 ºC**.

Table 3. Lipidomic profile of extracts obtained at different conditions. Results expressed as % of each lipid class. Supercritical extracts used for comparison were obtained at optimal conditions of pressure and temperature, along with extracts obtained with 5 and 10 % of ethanol as polar modifier

		PLE 150 ºC-15 min			Classical SLE extrations*				Supercritical CO_2 extracted						
		Hexane	Ethanol	Acetone	Hexane	Acetone	Ethanol	Water	40 MPa - 80ºC	20 MPa - 40 ºC	40 MPa -40 ºC	30 MPa - 40 ºC	40 MPa - 60 ºC	40 MPa -40 ºC + 5% Ethanol	40 MPa -40 ºC + 10% Ethanol
Lipid Classes	CE	0,01	0,01	0,01	0,01	<0,01	0,01	0,00	0.01	0.01	0.01	0.01	0.01	0.01	0.02
	TAG	99,03	99,12	99,01	99,03	98,93	92,24	98,64	98.75	98.38	98.83	98.87	98.78	98.78	98.67
	DAG	0,27	0,25	0,29	0,29	0,33	2,67	0,40	0.47	0.78	0.42	0.41	0.41	0.42	0.42
	STEROLS+FFA	0,06	0,05	0,06	0,05	0,06	0,67	0,05	0.08	0.18	0.07	0.09	0.08	0.07	0.07
	MAG	<0,01	<0,01	<0,01	n.d.	<0,01	0,02	n.d.	0	0.01	0	0.01	0	0	0
Fatty Acids	C16	12.11	13.42	12.31	12.58	12.12	14.87	12.13	12.43	12.45	9.40	10.14	9.33	9.44	9.32
	C16:1	1.48	1.41	1.56	1.52	1.52	1.38	1.57	1.60	1.57	0.52	0.64	0.51	0.55	0.53
	C18	7.36	6.31	6.77	7.50	7.62	3.86	7.54	7.66	7.04	3.18	2.78	3.20	3.02	3.13
	c18:1	59.50	59.15	58.88	59.32	59.71	58.03	58.84	59.77	58.63	70.78	69.66	71.15	70.84	71.22
	c18:2	18.99	18.93	18.99	18.69	18.46	20.36	19.43	18.51	18.63	16.04	16.72	15.73	16.07	15.70
	c18:3	0.12	0.13	0.11	0.13	0.11	0.13	0.12	0.11	0.12	0.09	0.06	0.08	0.07	0.09
	Yield %	45.5	55.3	47.7	26.7	25.0	6.3	32.5	33.9	16.3	36.6	31.2	35.6	40.4	42.3

The HPLC-ELSD method developed by Rodríguez-Alcalá & Fontecha (2010, *J. Chromatogr. A, 1217*(18), 3063-3066) was used for measuring the main lipid classes in the extracted oil at different conditions. The main lipids detected by HPLC-ELSD were triacylglycerols (TAG), diacylglycerols (DAG), monoacylglycerols (MAG), and free fatty acids (FFA).
The main lipid class extracted were TAG (above 98%), being oleic acid, C18:1, their main fatty acid (around 58% in PLE and 70 % in SFE), followed by palmitic acid, C16.
The SC-CO2 extracted almond oil was rich in unsaturated fatty acids (more than 70 % mono-unsaturated fatty acids and 16% poly-unsaturated fatty acids).Oleic acid was the obtained at highest concentration in all the samples while palmitic acid was the most important saturated fatty acid. The lipidomic profile of the extracted oils was similar except in terms of fatty acid composition at low CO2 densities (low press. or high temp.), meaning that pressure has a significant effect on fatty acid profile of oil (Femenia, et al, 2001, *J. Agric. Food Chem.*, 49(12), 5828-5834.).

Green extraction processes, such as SFE with **SC-CO_2** and **PLE** with **ethanol**, have proved to be **highly valuable techniques** for extracting of oil from the wild almond *A. scoparia*. Extraction yield can increase **above 50%** (~1.5 times more compared with classical extraction methods)

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