Green extraction of hemp seeds cake (Cannabis sativa L.) with 2-methyloxolane: A response surface optimisation study

Christian Cravotto, Anne-Sylvie Fabiano-Tixier, Mickaël Bartier, Ombeline Claux, Silvia Tabasso

Sustainable Chemistry and Pharmacy 39 (2024) 101509

Contents lists available at ScienceDirect

Sustainable Chemistry and Pharmacy

journal homepage: www.elsevier.com/locate/scp

Green extraction of hemp seeds cake (*Cannabis sativa* L.) with 2-methyloxolane: A response surface optimisation study

Christian Cravotto [a, *], Anne-Sylvie Fabiano-Tixier [a], Mickaël Bartier [b], Ombeline Claux [a], Silvia Tabasso [c, **]

[a] GREEN Extraction Team, INRAE, UMR 408, University of Avignon, F-84000, Avignon, France
[b] Pennakem Europa (EcoXtract ®), 224 Avenue de la Dordogne, F-59944, Dunkerque, France
[c] Department of Drug Science and Technology, University of Turin, Via P. Giuria 9, 10125, Turin, Italy

ARTICLE INFO

Handling Editor: Vania Zuin

Keywords:
Hemp seed oil
2-Methyloxolane
Bio-based solvent
Green extraction
Sustainable food processing
Green recovery of bioactive compounds

ABSTRACT

The extraction of hemp seed oil (HSO) with 2-methyltetrahydrofuran, also known as 2-methyloxolane (2-MeOx), was investigated as an alternative to hexane. Hemp seeds (*Cannabis sativa* L.) have a high nutrient content with an excellent source of lipids (25–35%) and easily digestible proteins (20–25%). HSO is generally obtained by cold pressing and/or solvent extraction. Commercial hexane is still the reference solvent for the oilseeds extraction industry. However, there is an urgent need to find alternatives, as hexane is highly toxic. 2-MeOx is a bio-based solvent that was added to the list of permitted solvents for foodstuffs and food ingredients production in Europe in January 2023. This study investigated the extraction of HSO with 2-MeOx and the influence of solvent water content (1.1–4.2% water), liquid/solid ratio (2–4 g/g) and temperature (50–65 °C) on extraction efficiency, solvent retention index, residual oil content, total phenolic content, carotene and chlorophyll content. Extraction conditions were optimised using response surface methodology and Box-Behnken design. Optimal conditions were obtained using a solvent with a water content of 1.1%, a liquid/solid ratio of 3, and a temperature of about 60 °C. The predicted optimum values were an oil yield of 12.15 oil/100 g DM, a solvent retention index of 0.205 g/g, a residual oil content of 0.319 g oil/100 g DM, a total phenolic content of 4735 mg GAE/kg oil, a carotene content of 474 μg/g oil and a chlorophyll to carotene ratio of 3.55.2-MeOx proved to be a valuable solvent for the extraction of high-quality HSO.

1. Introduction

The oldest knowledge about the use of *Cannabis sativa* L. comes from Asia, where hemp was already cultivated 10,000 to 12,000 years ago (Montero et al., 2023). Hemp is a versatile and environmentally friendly crop that is suitable for various applications, from agriculture and phytoremediation to the food and feed, cosmetic, construction and pharmaceutical industries (Farinon et al., 2020). Industrial hemp, a hemp variety with low tetrahydrocannabinol content, is mainly grown for fibre and/or seed production (Burton et al., 2022). According to market size estimates, the global market for industrial hemp will grow from USD 4.6 billion in 2019 to USD 9.4 billion in 2025, at an annual growth rate of 12.8% (Burton et al., 2022). Hemp seeds (HS) are commonly considered one of the most nutritionally complete food sources. Various HS products are available on the market, including whole and dehulled HS, oil ob-

* Corresponding author.
** Corresponding author.
E-mail addresses: christian.cravotto@alumni.univ-avignon.fr (C. Cravotto), anne-sylvie.fabiano@univ-avignon.fr (A.-S. Fabiano-Tixier), mickael.bartier@minakem.com (M. Bartier), oclaux.papers@gmail.com (O. Claux), silvia.tabasso@unito.it (S. Tabasso).

https://doi.org/10.1016/j.scp.2024.101509
Received 21 December 2023; Received in revised form 9 February 2024; Accepted 25 February 2024
Available online 8 March 2024

tained by mechanical pressing or solvent extraction, cake (the by-product after mechanical pressing of the oil), meal (the by-product after solvent extraction), hulls and HS protein concentrates/isolates (Burton et al., 2022). HS typically contain 25–35% lipids with a unique and perfectly balanced fatty acid composition, 20–25% easily digestible protein containing all essential amino acids, 20–30% carbohydrates, mainly insoluble fibre, vitamins and about 5% minerals (Chen et al., 2023). More than 90% of the fatty acids in hemp seed oil (HSO) are unsaturated fatty acids with an excellent ω-6/ω-3 ratio (Blasi et al., 2022). Up to 70–80% are polyunsaturated fatty acids (PUFAs), consisting mainly of linoleic acid (LA, 55.1–63.7%), followed by α-linolenic acid (ALA, 15.2–26.2%), while the predominant monounsaturated fatty acid is oleic acid (OA, 9–22.5%) (Montero et al., 2023). According to EFSA, the optimal ω-6/ω-3 ratio is between 3:1 and 5:1. The values reported in the literature for HSO are 2.5–3.5:1, and these values are associated with a lower risk of chronic diseases and mortality (Leonard et al., 2020).

The plant protein market is growing rapidly, and in 2020, the two main protein sources were soy and wheat, contributing 57.6% and 36.8% of the total market, respectively (Burton et al., 2022). HS are a very rich source of nutritionally high-quality protein (25–30%) as they contain all the essential amino acids required by the human body in a balanced ratio. According to FAO/WHO, the amino acid score of HS meets the recommendations for children aged 2–5 years (Chen et al., 2023). In general, the amino acid profile of HS is comparable to that of egg white and soy, with a high concentration of arginine, glycine and histidine (Leonard et al., 2020). However, both soy and wheat are among the top eight food allergens responsible for 90% of all allergic reactions to food. Therefore, novel protein sources like hemp, which are not known to trigger food allergies or affect coeliacs, are well positioned to meet the growing global demand for plant-based proteins. In addition to their nutritional value, HS are also rich in natural antioxidants such as phenolic compounds, tocopherols and phytosterols, which may play a role in reducing the risk of chronic diseases (Irakli et al., 2019).

HSO is generally obtained by cold pressing and solvent extraction. Due to the relatively low oil yield obtained by mechanical extraction, oilseed cakes are generally further extracted with solvents. Mechanical extraction reduce the oil content in the cake to 5–10% by weight, while solvent extraction reduces the oil content to less than 1% in the meal (Cravotto et al., 2023). Hexane is still the solvent of choice for the extraction of vegetable oils, although this substance has been known for more than 50 years for its neurotoxicity and reproductive toxicity, and new evidence has shown that it is also a potential endocrine disruptor (Ruiz-García et al., 2020).

To obtain a high-quality oil, studies have investigated the extraction of HSO using new extraction techniques such as ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), enzyme-assisted extraction, liquefied gases, and supercritical fluid extraction (Table 1).

However, these technologies are still limited to the laboratory scale, except for supercritical CO_2. This is mainly due to the different design of these extraction plants compared to the hexane process. Therefore, it is not possible to convert plants that use hexane to exploit novel technologies. In addition, the installation and operating costs are still comparatively high. A green solvent that can be

Table 1
Novel technologies and solvents for the pre-treatment and extraction of HSO.

	Technology	Hemp fraction	Extraction technique	Optimised extraction condition	Extraction yield (%)	Ref.
Pre-treatment	Pulsed electric field (PEF)	Seed	Mechanical pressing	PEF intensity 2.33 kV/cm; press speed 28.69 rpm	25.72	Haji-Moradkhani et al. (2019)
	Enzyme hydrolysis	Ground seed	Cold pressing	Viscozyme L; 6 h at 40 °C	32.8	Latif and Anwar (2009)
Solvent extraction intensification	Microwave-assisted extraction	Ground seed	Solvent extraction (hexane/isopropanol)	Hexane/isopropanol 3:2; MW power 450 W; 1 min	25.76	Soroush et al. (2021)
	Microwave-assisted extraction	Ground seed	Solvent extraction (*n*-hexane)	MW power 450 W; 7.19 min	33.91	Rezvankhah et al. (2019)
	Ultrasound-assisted extraction	Ground seed	Solvent extraction (hexane/isopropanol)	Hexane/isopropanol 3:2; 40 °C; US treatment 55 min	32.10	Esmaeilzadeh Kenari and Dehghan (2020)
	Ultrasound-assisted extraction	Ground seed	Solvent extraction (*n*-hexane)	Duty cycle 0.2s; amplitude 60%; time as, 60% US treatment 45 min	35.72	Devi and Khanam (2019)

	Solvent	Hemp fraction	Temp (°C)	Time (min)	Pressure (MPa)	Extraction yield (%)	Ref.
Supercritical fluid extraction	$scCO_2$ + 10% ethanol	Ground seed	40	240	35	36.26	Devi and Khanam (2019)
	$scCO_2$	Ground seed	40	n/r	30	22.1	Da Porto et al. (2012a)
	$scCO_2$	Ground seed	40	60	30	21.5	Da Porto et al. (2012b)
	$scCO_2$	Dehulled seed	40	240	40	40.9	Grijó et al. (2019)
Liquefied gases	Dimethyl ether (DME)	Ground seed Unground seed	25	40	0.055	25 31	Subratti et al. (2019)
	n-Propane	Dehulled seed	60	30	10	37.81	Grijó et al. (2019)
Green solvent	2-Methyloxolane	Mechanically pressed pellets	60	60	0.1	32.9 [a]	This study

[a] Calculated as the sum of the HSO yield by mechanical pressing (20.80%) and solvent extraction under optimised conditions with 2-MeOx (12.09%); n/r, not reported.

used in already existing hexane extractors with only partial changes to the equipment would allow easier and faster introduction into production processes.

In recent years, 2-methyltetrahydrofuran, also known as 2-methyloxolane (2-MeOx), has emerged as a viable alternative to hexane in the extraction of vegetable oils (Rapinel et al., 2020). 2-MeOx is a bio-based solvent obtained from the conversion of lignocellulosic biomass (e.g., corncobs and sugarcane bagasse). It has interesting properties that are technically comparable to hexane. Replacing hexane with 2-MeOx on an industrial scale does not require significant changes of the extraction plants (Rapinel et al., 2020). 2-MeOx has a boiling point of 80 °C, and a density (0.855 g/mL) and viscosity (0.6 cP at 25 °C) that are within an acceptable range for efficient diffusion through solid particles (Rapinel et al., 2020). 2-MeOx is predominantly lipophilic, but a small percentage of water can be dissolved, with the saturated 2-MeOx/H_2O mixture reaching 95.5/4.5% w/w at 55 °C. In addition, 2-MeOx has a much safer toxicological profile than hexane, and the use of this solvent in industrial production could lead to a 97% reduction in CO_2 emissions compared to petroleum-based solvents (Rapinel et al., 2020). 2-MeOx has already been approved for the extraction of organic and natural cosmetic ingredients (COSMOS label) and pharmaceutical products. Moreover, 2-MeOx (EcoXtract®) was recently added to the list of permitted solvents for production of foodstuffs and food ingredients in Europe (Directive 2009/32/EC) (Commission Directive (EU), 2023). To the best of our knowledge this is the first study dealing with extraction of HSO with 2-methyloxolane. In addition, the typical industrial process of mechanical pressing with subsequent solvent extraction was reproduced and the quality of the products characterised. The aim of this study was to investigate the effects of parameters such as solvent water content (% w/w of water in the solvent), L/S ratio (g of solvent per g of HS) and temperature on the extraction of HSO to increase extraction efficiency and improve product quality.

2. Materials and methods

2.1. Raw material preparation

Hemp seeds (HS) were purchased from a local supplier and stored at 18 °C until further analysis. The oil content was determined according to a reference method (ISO 659, 2009). To reproduce the industrial extraction process, whole HS with an oil content of 33.62 ± 0.43% of dry matrix (DM) were first mechanically pressed using a Komet oil screw-press CA59G (IBG Monforts Oekotec GmbH & Co. KG, Germany) to obtain an HS cake. Press parameters: screw-head was pre-heated for 5 min with a removable heating element, nozzle size of 7 mm in diameter, rotational speed 7. HS cake in form of pellets was stored at 5 °C in airtight plastic bags. HS cake was then extracted with solvent. The proximate composition of HS cake was determined using standard protocols. The proximate values were crude protein (AOCS Official Method Ac 4, 2009): 30.9 ± 0.2 g/100 g DM; oil content (ISO 659, 2009): 11.0 ± 0.2 g oil/100 g DM; moisture: 6.3 ± 0.6 g/100 g DM.

2.2. Solvents, standards, and reagents

2-Methyloxolane (reagent grade) and hexane (technical grade) were purchased from VWR international (Darmstadt, Germany). Before extraction, 2-MeOx (butylated hydroxytoluene, BHT, stabilised) was distilled to remove BHT. To obtain 2-MeOx at the desired water content (% w/w) appropriate amount of water was added. The accurate water content was confirmed by Karl-Fischer method using a 917 Coulometer (Metrohm, Switzerland). The three water contents were: 1.11 ± 0.03%, 2.63 ± 0.08%, 4.18 ± 0.04%. These values were approximated to 1.10%, 2.60%, 4.20% to fit the Box-Behnken design.

2.3. Extraction procedure

Approximately 50 g of HS cake were extracted in a double jacket reactor connected to the CORIO CD-200F circulating thermocryostat (Julabo Italia Srl, Italy). The solvent was weighed and heated to the desired temperature in the double-jacket reactor for 10 min before the solid was immersed. The HS cake was extracted for 20 min and then drained on a coarse sieve for exactly 1 min. Two further extractions were carried out under the same conditions with fresh solvent at the desired water content, so that the total time of extraction was 1 h. At the end of the third cycle, after draining, a meal sample was taken to determine the solvent retention index. The three miscellas (1st, 2nd, 3rd cycle) were collected, and evaporated under reduced pressure (up to 30 mbar) in a Rotavapor (R-300 Büchi, Flawil, Switzerland). The crude oils were cooled under a stream of nitrogen for 10 min and stored at −20 °C before analysis. The meals were desolventised under reduced pressure (up to 30 mbar) in a Rotavapor (R-300 Büchi, Flawil, Switzerland) and then stored at 5 °C prior to analysis.

2.4. Volatile content

The volatile content (VC) of various solid samples was determined directly by gravimetry at 130 °C to constant weight using the OHAUS MB35 moisture analyser (OHAUS, US). Dry matter (DM) content of samples was calculated using the following relationship (Eq. (1)):

$$DM\ (g/100g) = 100 - VC\,(g/100g) \qquad \text{Eq. 1}$$

DM, dry matter; VC, volatile content.

2.5. Solvent retention index

After extraction, the volatile content of the drained wet meal was determined, as shown in equation (2). Since the insoluble solid mass is known by the characterization of the raw material, as well as the residual oil content, it is possible to determine the mass of solution that was adhered to the inert solid by difference, defined as solvent retention index (Kocatas and Cornell, 1954).

$$SRI\ (w/w) = VC\ (g/100g) / (100 - ROC\ (g/100g)) \quad \text{Eq. 2}$$

SRI, solvent retention index; VC, volatile content; ROC, residual oil content.

2.6. Oil total phenolic content

Briefly, 1 g of oils were diluted in 1 mL of HPLC grade *n*-hexane, and the phenolic compounds extracted with 5 mL ethanol/water (60:40, v/v). The tube was shaken vigorously for 10 min and after centrifugation (8750 G, 10 min) the lower phase was collected. The upper layer was extracted two more times by the same procedure. Finally, the combined alcoholic phases were washed with a small volume of *n*-hexane and then transferred to a volumetric flask (Cravotto et al., 2022). Twenty microlitres of the corresponding dilutions of the extracts or gallic acid (standard) were placed in a 96-well microplate and 80 μL of a 7.5% Na_2CO_3 solution (m/v) was added and equilibrated at room temperature for 5 min. Then 100 μL of 1 N Folin-Ciocalteu reagent was added and absorbance was measured at 750 nm after incubation for 60 min with a FluOstar Omega microplate reader (BMG LABTECH, Germany). Results were calculated as mg of gallic acid equivalents (GAE) per kg of sample.

2.7. Residual oil content

Approximately 10 g of meal were extracted with technical hexane in an automatic Soxhlet extractor B-811 (BÜCHI, Switzerland). After 8 h, hexane was evaporated, and the residual oil content (ROC) was weighed. The ROC was calculated with the following equation (Eq. 3):

$$ROC\ (g\ oil/100g\ DM) = m\ oil\ residue\ (g) / m\ dry\ meal\ (g) \times 100 \quad \text{Eq. 3}$$

2.8. Protein content

The crude protein content of raw HS and defatted samples was determined with Kjeldahl method according to AOCS official methods Ac 4–91 (AOCS Official Method Ac 4, 2009).

2.9. Determination of chlorophyll a, chlorophyll b, and total carotene content

For pigment determination, the method of Aladić (2015) was used. The weighed sample was dissolved with ethyl ether (50 mL per gramme) for 1 min in an ultrasonic bath. It was then shaken for 30 s and again in the ultrasonic bath for 1 min. The sample was centrifuged at 3000 rpm for 10 min. The supernatant was recovered, and the absorbance values were measured at 400–700 nm in a UV spectrophotometer. Chlorophyll *a* showed the highest absorbance at 660 nm, chlorophyll *b* at 642 nm and total carotene at 470 nm. The amount of these pigments was calculated according to the following formulae (Eq. 4–6):

$$\text{Chlorophyll } a = 9.93 \times \text{Abs660} - 0.78 \times \text{Abs642} \quad \text{Eq. 4}$$

$$\text{Chlorophyll } b = 17.60 \times \text{Abs642} - 2.81 \times \text{Abs660} \quad \text{Eq. 5}$$

$$\text{Carotene} = (1000 \times \text{Abs470} - 0.52 \times \text{Chlorophyll } a - 7.25 \times \text{Chlorophyll } b) / 226 \quad \text{Eq. 6}$$

Pigment concentration was expressed as micrograms of pigment per gram of oil (μg/g oil).

2.10. Experimental design

The Box-Behnken design consisted of 15 experiments with three variables at three levels (−1, 0 and 1) and three repetitions at the midpoint. The ranges of the variables, namely solvent water content (1.1–4.2% w/w of water), temperature (50–65 °C) and L/S ratio (2–4 g/g), were selected to assess the optimal extraction conditions. For further comparisons, extractions with hexane and dry 2-MeOx were carried out. Statistical analysis was performed using Response Surface Methodology (RSM) and Design-Expert version 13 (Stat-Ease, Inc., Minneapolis, MN, USA). The levels of the independent variables and their codes are shown in Table 2. The model used in the RSM was the quadratic equation shown below (Eq. (7)):

Table 2
Range and variables used for the experimental designs.

Independent variables	Unit	Symbol	Levels		
			Low (−1)	Middle (0)	High (1)
Water	% w/w	A	1.1	2.6	4.2
L/S ratio	g/g	B	2	3	4
Temperature	°C	C	50	57.5	65

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i<j=1}^{3} \beta_{ij} X_i X_j \quad \text{Eq. 7}$$

where Y is the estimated response and the expressions β_0, β_i, β_{ii} and β_{ij} are the equation constant (y-intercept), linear effect coefficient, quadratic effect coefficient and interaction coefficient, respectively. X_i and X_j are the levels of independent variables. For each response, the regression coefficients of the variables with *p*-values <0.1 were selected in the second-order polynomial equations.

3. Results and discussion

In this work, 2-MeOx was investigated as a green, food-grade solvent for the extraction of HSO. The effects of three key parameters such as solvent water content, L/S ratio and temperature were evaluated. Controlling these parameters can have a significant impact on process efficiency and product quality. Hexane and dry 2-MeOx were chosen for comparison.

3.1. Extraction yield

As shown in Table 3, the extraction yield varied between 11.24% and 12.84%. The highest yield was achieved with the following conditions: 2.6% water, L/S ratio of 4, and a temperature of 65 °C. Obtained second-order polynomial equation (Table 4) was found well to represent the experimental data ($R^2 = 0.9626$). The results of the analysis of variance showed that the linear and quadratic effects of the studied parameters, such as solvent water content (A and A^2), L/S ratio (B and B^2) and temperature (C and C^2), on extraction yield were significant ($p < 0.05$), while only one interaction effect (BC) was significant ($p < 0.05$) (Table 5).

Factors with high coefficients indicate a higher level of influence on the variable. According to Table 4, the solvent water content (0.37) and the extraction temperature (0.33) had the highest coefficient and consequently a higher effect on the extraction yield compared with the L/S ratio. Oil extraction yield with dry 2-MeOx was higher than with hexane, in the same experimental conditions. Fig. 1 shows that increasing the solvent water content increases the extraction yield, which is presumably due to the extraction of further polar compounds. This is consistent with previous studies that showed higher extraction yields with water-saturated 2-MeOx (4.5% w/w water) than with dry solvent (Cravotto et al., 2022; Claux et al., 2021). In another study on HS, it was found that the addition of

Table 3
Experimental parameters of the Box-Behnken design and the responses for oil yield, SRI, ROC, TPC, carotene, and chlorophylls to carotene ratio.

Run	Independent variables			Responses					
	Water (% w/w)	L/S ratio (g/g)	Temperature (°C)	Yield (g oil/100 g DM)	SRI (w/w)	ROC (g oil/100 g DM)	TPC (mg GAE/kg oil)	Carotene (μg/g oil)	Chl *a* + *b*/Carotene
1	4.2	4	57.5	12.63	0.310	0.521	6028	401	3.75
2	2.6	3	57.5	12.67	0.246	0.429	4559	399	3.78
3	4.2	2	57.5	11.85	0.308	0.695	6735	423	3.79
4	2.6	3	57.5	12.73	0.273	0.444	4749	399	3.84
5	2.6	4	50	11.33	0.244	0.816	3057	482	3.28
6	2.6	2	65	11.68	0.229	0.627	6213	477	3.86
7	4.2	3	50	12.34	0.304	0.733	5657	427	3.91
8	1.1	3	50	11.24	0.216	0.572	4065	519	3.32
9	1.1	2	57.5	11.76	0.199	0.495	4296	502	3.62
10	4.2	3	65	12.73	0.289	0.651	7631	414	3.78
11	2.6	4	65	12.84	0.241	0.469	4354	407	3.80
12	2.6	2	50	11.59	0.248	0.665	5173	443	3.88
13	1.1	4	57.5	11.70	0.214	0.341	4801	506	3.41
14	1.1	3	65	11.89	0.207	0.315	5084	486	3.63
15	2.6	3	57.5	12.79	0.245	0.486	4939	395	3.85
Hexane	0	3	57.5	10.38	0.133	0.273	120	311	2.93
2-MeOx	0	3	57.5	11.79	0.197	0.303	3617	457	3.38

SRI, solvent retention index; ROC, residual oil content; TPC, total phenolic content.

Table 4
Regression coefficients of second-order polynomial equations showing relationships among response variables and independent variables.

Responses	Equation	R^2	R^2 adjusted
Yield	$Y = 12.73 + 0.37A + 0.2025B + 0.33C + 0.21AB + 0.355BCE - 0.2775A^2 - 0.4675B^2 - 0.4025C^2$	0.9626	0.9127
SRI	$Y = 0.2515 + 0.0469A$	0.9061	0.8989
ROC	$Y = 0.4479 + 0.1096A - 0.0419B - 0.0905C - 0.0772BCE + 0.0683B^2 + 0.1236C^2$	0.9164	0.8537
Polyphenols	$Y = 4720.57 + 975.62A - 522.13B + 666.25C + 816.55A^2$	0.8594	0.8031
Carotene	$Y = 397.57–43.70A - 6.10B - 10.72C - 6.49AB + 4.54AC - 27.28BCE + 35.26A^2 + 25.26B^2 + 29.26C^2$	0.9975	0.9929
Chl *a* + *b*/Carotene	$Y = 3.76 + 0.1546A - 0.1146B + 0.0844C - 0.1102AC + 0.1372BCE - 0.1A^2$	0.8765	0.7838

SRI, solvent retention index; ROC, residual oil content; TPC, total phenolic content.

Table 5
The analysis of variance (ANOVA) for the fitted models.

	Yield (g extract/100 g DM)		SRI (w/w)		ROC (g oil/100 g DM)		Polyphenols (mg GAE/kg oil)		Carotene (μg/g oil)		Chl *a* + *b*/Carotene	
	SS	*p*-value	SS	*p*-value	SS	*p*-value	SS	*p*-value	SS	*p*-value	SS	*p*-value
Model	4.47	0.0036*	0.0187	0.0038*	0.2778	0.0122*	1.649E+07	0.0507	28, 519	<0.0001*	0.5495	0.0210*
A-Water	1.10	0.0020*	0.0176	<0.0001*	0.0961	0.0030*	7.612E+06	0.0068*	15, 273	<0.0001*	0.1913	0.0050*
B- L/S ratio	0.3280	0.0229*	0.0001	0.4791	0.0140	0.0950	2.182E+06	0.0635	298	0.0062*	0.1054	0.0166*
C-Temperature	0.8712	0.0032*	0.0003	0.2186	0.0655	0.0068*	3.552E+06	0.0290*	920	0.0005*	0.0570	0.0482*
AB	0.1764	0.0635	<0.0001	0.5983	0.0001	0.8690	3.675E+05	0.3743	168	0.0190*	0.0069	0.4073
AC	0.0169	0.4951	9.00E-06	0.8056	0.0077	0.1893	2.277E+05	0.4774	83	0.0624	0.0486	0.0615
BC	0.5041	0.0102*	0.0001	0.5198	0.0239	0.0438*	16499.40	0.8445	2976	<0.0001*	0.0753	0.0304*
A^2	0.2843	0.0295*	0.0003	0.2267	0.0003	0.7942	2.442E+06	0.0536	4590	<0.0001*	0.0473	0.0640
B^2	0.8070	0.0038*	0.0001	0.4263	0.0172	0.0719	34800.64	0.7762	2356	<0.0001*	0.0164	0.2212
C^2	0.5982	0.0072*	0.0003	0.1967	0.0559	0.0093*	8188.15	0.8900	3161	<0.0001*	0.0095	0.3376
Residual	0.1563		0.0007		0.0166		1.933E+06		72		0.0421	
Lack of Fit	0.1491	0.0683	0.0002	0.8787	0.0149	0.1535	1.861E+06	0.0555	62	0.2124	0.0395	0.0917
Pure Error	0.0072		0.0005		0.0017		72162.01		11		0.0026	
Core Total	4.63		0.0194		0.2944		1.843E+07		28, 591		0.5916	

SRI, solvent retention index; ROC, residual oil content; TPC, total phenolic content; SS, sum of square. *The values are significant at $p < 0.05$.

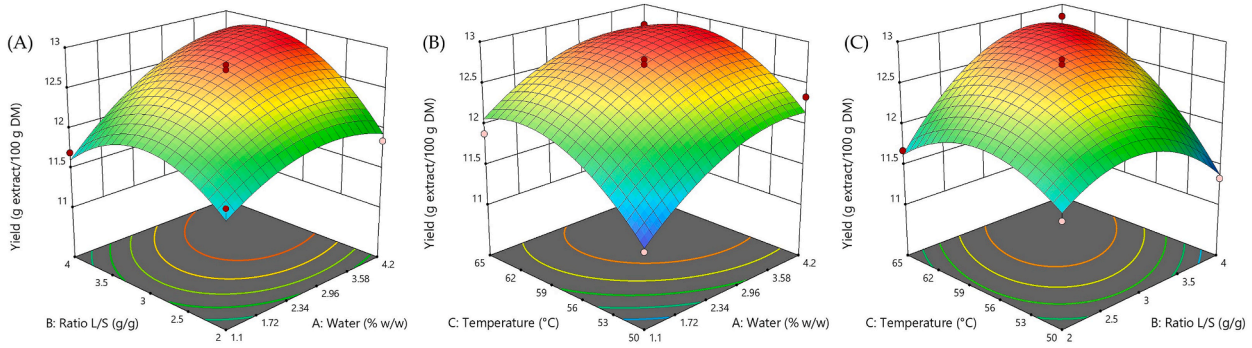

Fig. 1. Effect of process variables on extraction yield (g extract/100 g DM): (A) L/S ratio versus water, (B) temperature versus water, (C) temperature versus L/S ratio.

polar solvents (isopropanol) to non-polar solvents (hexane) increased the recovery rate of polar lipids like phospholipids and lipoproteins and thus the total fat content (Esmaeilzadeh Kenari and Dehghan, 2020). A similar effect could be achieved by increasing the water content of 2-MeOx and thus its polarity. The improvement in oil yield by increasing the temperature is possibly due to the increased solubility of the oil in the solvent (Senrayan and Venkatachalam, 2019). When the temperature rises from 50 to about 62 °C, the extraction yield gradually increases. This phenomenon can be considered as an effect of a better diffusion of the oil in the solvent (Sicaire et al., 2016). However, a further increase in temperature leads to a decrease in yield.

Indeed, a higher temperature can have a negative effect on bioactive compounds such as the tocopherol content of the oil, which are sensitive to heat (Senrayan and Venkatachalam, 2019). Increasing the L/S ratio increases the extraction yield, which is probably due to a higher mass transfer (Senrayan and Venkatachalam, 2019).

3.2. Solvent retention index

The solvent retention index (SRI) is an important parameter that predicts the number of stages (extractor volume) required for complete oil extraction and the amount of energy needed for meal desolventisation (Da Costa Rodrigues and Oliveira, 2010). SRI is related to the physical properties, density and viscosity of the extract solution (Bessa et al., 2017). More viscous extracts lead to higher SRIs, lower extraction rates and consequently a higher number of theoretical stages required to fully exhaust the oil matrix. For example, the SRI for hexane is lower than for alcohols such as ethanol and isopropanol, which have a greater affinity for the fibres due to their higher polarity (Da Costa Rodrigues and Oliveira, 2010; Araújo et al., 2018; Zhang et al., 2002; Wlsnlak et al., 1987). The results of the analysis of variance showed that only the linear effect of the solvent water content (A) on SRI was significant ($p < 0.05$), as shown in Table 5. The SRI varied between 0.199 and 0.310, and the lowest SRI was achieved with the following extraction conditions: 1.1% water, L/S ratio of 2, and a temperature of 57.5 °C. Linear regression equation (Table 4) was found well to represent the experimental data ($R^2 = 0.9061$). Hexane resulted in the lowest SRI, also compared to dry 2-MeOx. This could be explained by the higher viscosity of 2-MeOx (0.6 cP at 25 °C) compared to hexane (0.3 cP at 25 °C) (Rapinel et al., 2020). As the water content of the solvent increases, the SRI gradually increases, as shown in Fig. 2. This behaviour agrees with the results obtained with alcohols (Da Costa

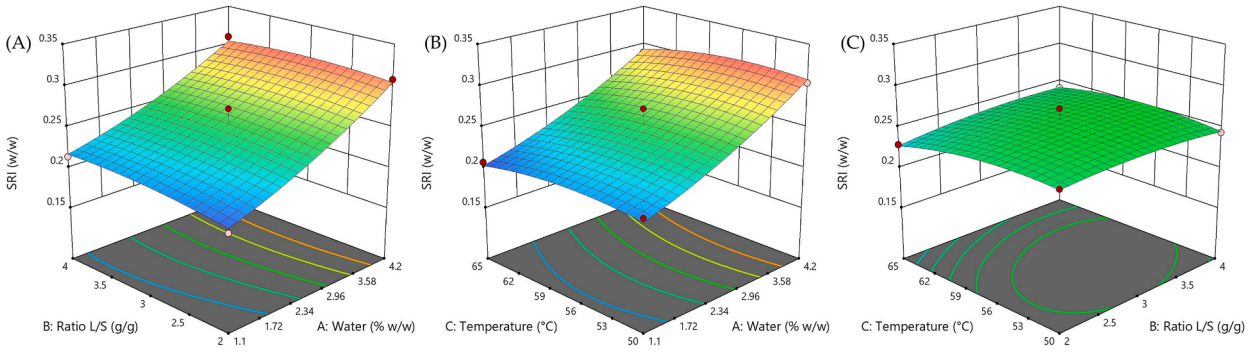

Fig. 2. Effect of process variables on SRI (w/w): (A) L/S ratio versus water, (B) temperature versus water, (C) temperature versus L/S ratio.

Rodrigues and Oliveira, 2010; Kashaninejad et al., 2021). Since the polarity of the solvent increases with higher moisture content, it can be inferred that the attractive forces between solvent and solid increase, leading to a higher SRI. With decreasing L/S ratio, the SRI decreased, but the effect was not significant. Araújo et al. (2018) studied the effect of temperature on SRI during extractions with ethanol and isopropanol. While the SRI for ethanol tended to increase with increasing temperature from 35 to 45 °C or up to 55 °C, a decrease in SRI was observed for isopropanol with increasing temperature from 35 to 45 °C, followed by an increase with increasing temperature up to 55 °C. With 2-MeOx, increasing the temperature resulted in a decrease of SRI, and extraction at 65 °C resulted in the lowest SRI; however, the effect of temperature was not significant.

3.3. Residual oil content

The residual oil content (ROC) is an important indicator of the extraction efficiency of the process. The ROCs resulting from the extraction with hexane (0.273 w/w) and dry 2-MeOx (0.303 w/w) were the lowest recorded. The results of the analysis of variance showed that the linear and quadratic effects of the extraction temperature (C and C^2), the effect of the solvent water content (A), and the interaction effect between ratio and temperature (BC), on ROC were significant ($p < 0.05$), as shown in Table 5. The ROC varied between 0.315 and 0.816 g oil/100 g DM, and the lowest ROC was achieved with the following extraction conditions: 1.1% water, L/S ratio of 3, and a temperature of 65 °C. Obtained second-order polynomial equation (Table 4) was found well to represent the experimental data ($R^2 = 0.9164$). With increasing solvent water content, the ROC content increases, while an increase in temperature leads to a decrease in ROC (Fig. 3). The effect of solvent water content and temperature on ROC is probably related to the change in oil solubility in the solvent. A similar behaviour was described for extraction with ethanol and isopropanol, as the oil solubility decreases with increasing alcohols water content and decreasing temperature (Cravotto et al., 2023; Toda et al., 2016; Sawada et al., 2014). The increase in the L/S ratio resulted in a reduction in ROC, however the effect was not significant.

3.4. Polyphenols

Several studies have reported that 2-MeOx is also an efficient solvent for the extraction of phenolic compounds from plant (black cumin seeds, basil seeds, olive pomace, and soybean flakes) (Cravotto et al., 2022; Claux et al., 2021; Bourgou et al., 2021) and animal (black soldier fly larvae) matrices (Ravi et al., 2019), yielding extracts richer in polyphenols than those obtained with hexane and with higher antioxidant activity. In agreement with previous studies, all extracts obtained with 2-MeOx (both dry and hydrated) were found to contain a significantly higher concentration of polyphenols than those obtained with hexane, as the apolar nature of hexane does not allow efficient extraction of this compounds. Lignanamides, hydroxycinnamic acids, hydroxybenzoic acids, and flavonoids are characteristic phenolic compounds in HS (Montero et al., 2023). Farinon et al. (2020) reviewed the studies reporting values for the total phenolic content of cold-pressed HSO or whole HS and reported values of 0.44–2.68 mg GAE/g oil and 0.77–51.60 mg GAE/g seeds. Since polyphenols are polar and water-soluble compounds, only part of them is recovered during oil extraction, while the ma-

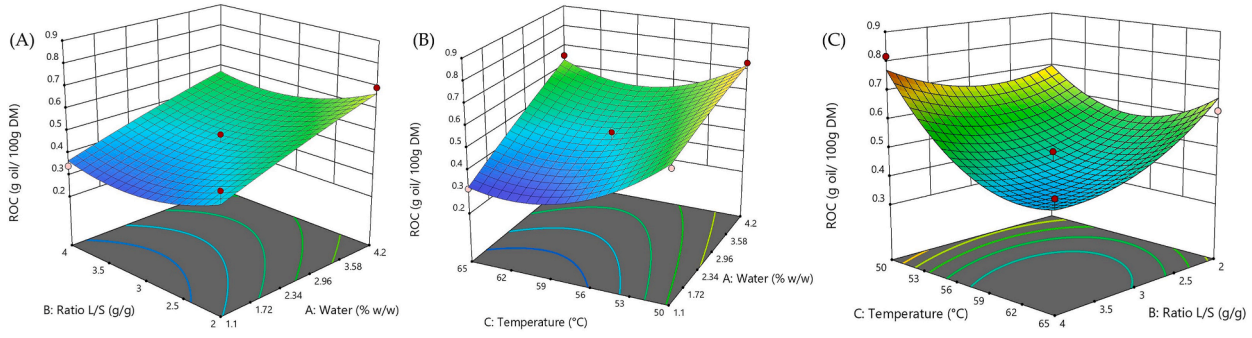

Fig. 3. Effect of process variables on ROC (g oil/100 g DM): (A) L/S ratio versus water, (B) temperature versus water, (C) temperature versus L/S ratio.

jority remains in the hemp cake. This agrees with the results of Siano et al. (2018) and Moccia et al. (2020) who analysed and compared the TPC and radical scavenging activity of whole HS, HS flour and HSO and found that the TPC and antioxidant activity were significantly lower in HSO.

As shown in Table 5, only the linear effects of solvent water content (A) and extraction temperature (C) significantly influenced the total content of the extracted polyphenols ($p < 0.05$). The polyphenols content varied between 3056.6 and 7630.6 mg GAE/kg oil, and the highest concentration was achieved with the following extraction conditions: 4.2% water, L/S ratio of 3, and a temperature of 65 °C. Obtained second-order polynomial equation (Table 4) was found well to represent the experimental data ($R^2 = 0.8594$). Previous studies have optimised the recovery of phenolic compounds during mechanical and solvent extraction of HSO. Haji-Moradkhani et al. (2019) studied the influence of pulsed electric fields (PEF) and screw press speed during oil extraction. Under optimised extraction conditions, the polyphenols content in the oil was 1946 mg GAE/kg oil. In two other studies, the extraction of HSO was optimised with a mixture of hexane/isopropanol using microwaves (Soroush et al., 2021) and ultrasound (Esmaeilzadeh Kenari and Dehghan, 2020), respectively. The optimised extraction conditions (RSM) resulted in a content of phenolic compounds in the oil of 3910 and 3210 mg GAE/kg oil for microwave- and ultrasound-assisted extraction, respectively. In both studies, it was found that the extraction of phenolic compounds increased with the increase in isopropanol content, which can be attributed to the greater polarity of isopropanol compared to the non-polar solvent hexane. In this study, the solvent water content had the highest coefficient (975.62) and consequently the highest effect on the polyphenols content. A sharp increase in the content of phenolic compounds was observed when the water content of 2-MeOx was increased from 2.6 to 4.2% (Fig. 4). Furthermore, the increase in temperature has led to a progressive increase in the diffusion of the polyphenols into the extraction solvent and thus to their recovery in the oil.

3.5. Carotenoids and chlorophylls

Other bioactive compounds in HSO are represented by carotenoids. While xanthophylls are only considered yellow pigments in oils, carotenes, especially β-carotene, can act as an antioxidant and reduce the risk of degenerative diseases (Liang et al., 2015). Another type of pigments are chlorophylls, fat-soluble pigments found in many raw vegetable oils. The high total chlorophylls content, consisting mainly of the two isomers, chlorophyll *a* and chlorophyll *b*, is responsible for the intense dark green colour and can have many negative effects on HSO (Blasi et al., 2022). Chlorophylls are susceptible to photooxidation when exposed to light, causing the oil to turn from dark green to yellow (Liang et al., 2015). In addition, the high chlorophylls content can trigger the oxidation of PUFAs, which accelerates rancidity and reduces the shelf life of the HSO (Izzo et al., 2020). However, the presence of carotenoids in HSO can protect chlorophylls from degradation and prevent colour change during storage, but minimising chlorophylls content is imperative to avoid photooxidation (Blasi et al., 2022). Some studies reported the concentrations of carotenoids and chlorophylls in HSO obtained by cold mechanical pressing. Blasi et al. (2022) analysed eight commercial HSO samples from Italy and from non-European countries and reported average values of 76.4 μg/g and 34.8 μg/g of chlorophyll $a + b$ and 2.61 μg/g and 1.78 μg/g of carotenoids in the two groups, respectively. In the study by Izzo et al. (2020), the total chlorophyll content varied considerably among the thirteen samples analysed, with an average value of 1.46 μg/g (0.41–4.81 μg/g) for all samples. In addition, carotenoids were detected in a concentration range between 0.18 and 1.73 μg/g, with an average value of 0.52 μg/g. Aladić (2015) reported a total chlorophylls content of 98.6 μg/g (59.22 μg/g chlorophyll *a* and 39.45 μg/g chlorophyll *b*) for cold-pressed HSO. In comparison, supercritical fluid extracted HSO had twice the total chlorophylls content (228.79 μg/g). The carotene content was also significantly higher after supercritical CO_2 extraction (125.37 μg/g) compared to mechanical cold pressing (31.46 μg/g). In this study, the oil obtained by mechanical pressing, before solvent extraction, had a total chlorophylls content of 50.5 μg/g (30.8 μg/g chlorophyll *a* and 19.7 μg/g chlorophyll *b*), and a carotene content of 14.9 μg/g. The solvent-extracted oils contained significantly more chlorophylls (1501.2–1838.3 μg/g) and carotene (394.9–519.1 μg/g) than the mechanically extracted oil. Furthermore, the oils extracted with dry and hydrated 2-MeOx had a higher concentration of carotene and chlorophylls than the oil extracted with hexane.

As shown in Table 3, the carotene content varied between 394.9 and 519.1 μg/g oil. The highest content was achieved with the following extraction conditions: 1.1% water, L/S ratio of 3, and a temperature of 50 °C. Obtained second-order polynomial equation (Table 4) was found well to represent the experimental data ($R^2 = 0.9975$). Only one interaction effect (AC) had no significant influence on the total carotene content ($p > 0.05$). According to Table 4, the solvent water content (43.70) had the highest coefficient and

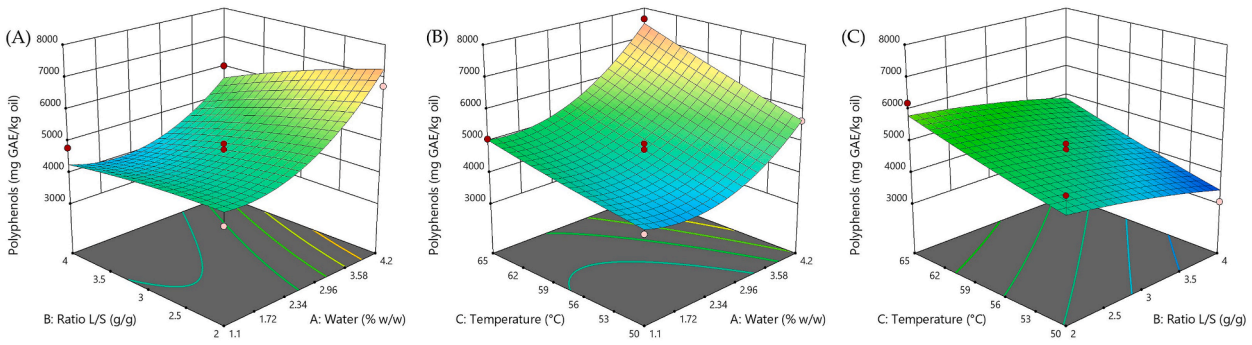

Fig. 4. Effect of process variables on TPC (mg GAE/kg oil): (A) L/S ratio versus water, (B) temperature versus water, (C) temperature versus L/S ratio.

consequently the highest effect on the HSO carotene content. Due to the lipophilic nature of carotenoids, their content gradually decreases with increasing the water content of the solvent, as shown in Fig. 5.

Other studies have shown that the ratio between the chlorophyll fraction and the carotenoid fraction differs significantly, suggesting that in HSO the green and yellow fractions are not in balance (Izzo et al., 2020). In other edible oils, such as virgin olive oils, the ratio between the two fractions seems to be constant at a value close to one, regardless of the variety (Roca and nguez-Mosquera, 2001). Due to the preferential loss of chlorophyll during the extraction process of olive oil, the ratio of chlorophyll to carotenoids drops significantly from 2.5 to 3.7 in the fruit to 0.8–1.4 in the oils (Roca and nguez-Mosquera, 2001). In this study, the extraction was optimised to obtain the lowest chlorophylls to carotenoids ratio. As shown in Table 3, the chlorophylls to carotenoids ratio varied between 3.278 and 3.907. Fig. 6 shows that the best conditions to achieve the highest carotene content and the lowest chlorophyll/carotene ratio were to work with low solvent water content and low temperature and a high L/S ratio. Obtained second-order polynomial equation (Table 4) was found well to represent the experimental data (R^2 = 0.8765).

3.6. Meal protein content

HS are a very rich source of protein (25–30% DM) with a high nutritional value. The proteins are mainly found in the inner layer of the seed, while the proportion of proteins in the hull is very low (Montero et al., 2023). Defatted HS meal contains up to 44% protein, protein concentrates have up to 50–75% protein, and HS protein isolates can reach protein concentrations of over 85%. HS proteins contain the nine essential amino acids necessary for the proper functioning of the human body. The most important proteins in HS are storage proteins such as albumin (25–37%) and the legumin protein called edestin (67–75%) (Montero et al., 2023). Unlike other plant proteins, HS proteins contain very low levels of antinutritional factors and are therefore more digestible (Burton et al., 2022). In this study, the percentage of protein in the defatted meals was determined. The results showed that all meals extracted with 2-MeOx had a protein concentration between 32.6 and 34.7%, similar to the meals extracted with hexane (34.7%) and dry 2-MeOx (34.5%). Surface responses are not reported as the results did not fit the second-order model equation.

3.7. Optimal extraction conditions

To obtain the highest extraction yield, TPC and carotene content, and lowest SRI, ROC and chlorophyll/carotene ratio, the optimisation of oil extraction from HS cake with 2-MeOx was investigated by varying the solvent water content (1.1–4.2% water), temperature (50–65 °C) and L/S ratio (2–4 g/g). Since in some cases the responses compete and the improvement of one response may have opposite effects on another, the optimised conditions were chosen by solving the problem of multiple responses using a desirability function that combines all responses into a single measure (Haji-Moradkhani et al., 2019). Other studies on the optimisation of HSO extraction with innovative technologies reported optimised model with desirability values between 0.62 and 0.70 (Haji-Moradkhani

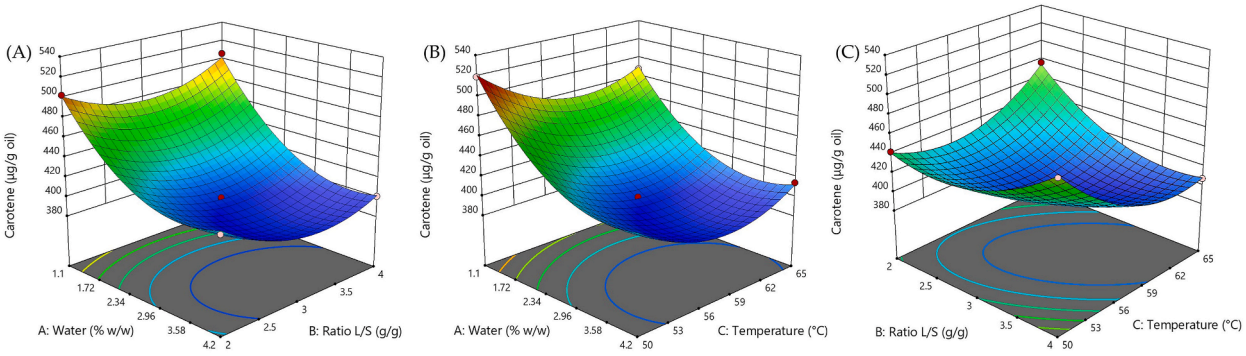

Fig. 5. Effect of process variables on carotene content (µg/g oil): (A) L/S ratio versus water, (B) temperature versus water, (C) temperature versus L/S ratio.

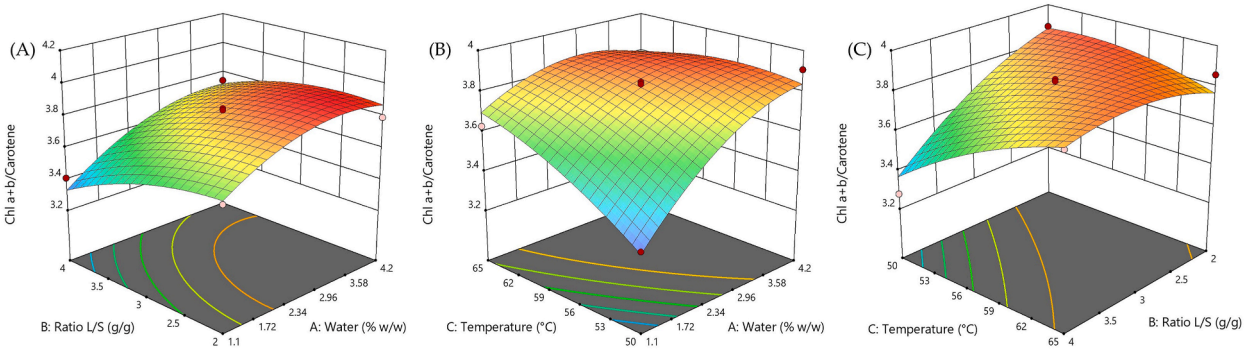

Fig. 6. Effect of process variables on chlorophylls/carotene ratio: (A) L/S ratio versus water, (B) temperature versus water, (C) temperature versus L/S ratio.

Table 6
Predicted response values for the optimum extraction conditions.

Water (w/w)	L/S ratio (w/w)	Temperature (°C)	Yield (g oil/ 100 g DM)	SRI (w/ w)	ROC (g oil/ 100 g DM)	Polyphenols (mg GAE/kg oil)	Carotene (μg/g oil)	Chl *a* + *b*/ Carotene	Desirability
1.1	3.0	60	12.15	0.205	0.319	4735	474	3.55	0.64

et al., 2019; Soroush et al., 2021; Esmaeilzadeh Kenari and Dehghan, 2020). From the optimisation results proposed by the software, the most desirable solution with the lowest temperature and L/S ratio was selected. With the same desirable level, the reduction of these two factors brings an energy advantage to the process. The results showed that by setting the solvent water content to 1.1, using an L/S ratio of 3 and setting the extraction temperature to 60 °C, a desirability of 0.64 was achieved (Table 6). In the industrial extraction of seed oils, after the distillation of the miscella (oil-rich solvent) and the desolventisation of the meal, the solvent is condensed and recovered to be fed back into the extractor. After distillation, the 2-MeOx is in the saturated form with a water content of 4.5% w/w. Since the solvent with the lowest water content (1% water) gave the best results, the energy cost for partial solvent dehydration must be further evaluated.

4. Conclusion

2-MeOx is a promising green alternative to petrochemical solvents in the extraction of hemp seed oil (HSO). The extraction was investigated and optimised using the response surface methodology and the Box-Behnken design. The parameters studied were solvent water content (1.1–4.2% water), L/S ratio (2–4 g/g) and temperature (50–65 °C). The optimised extraction conditions to obtain the highest extraction yield, the highest TPC and carotene content, and the lowest SRI, ROC and chlorophyll/carotene ratio were a solvent water content of 1.1%, an L/S ratio of 3 and an extraction temperature of about 60 °C. Notably, compared to hexane, the unsaponifiable profile was remarkably improved and enriched in polyphenols and carotenoids. The results obtained in this study provide important insights for the future optimisation of HSO extraction on an industrial scale. Given the differences in the industrial extraction process compared to the laboratory scale, especially continuous countercurrent extraction versus batch cross-current extraction, further pilot-scale studies will be useful to confirm these results.

CRediT authorship contribution statement

Christian Cravotto: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Anne-Sylvie Fabiano-Tixier:** Writing – review & editing, Supervision, Data curation. **Mickaël Bartier:** Writing – review & editing, Validation, Supervision, Investigation. **Ombeline Claux:** Writing – review & editing, Validation, Methodology, Formal analysis, Conceptualization. **Silvia Tabasso:** Writing – review & editing, Validation, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

Aladić, K., 2015. Cold pressing and supercritical CO2 extraction of hemp (Cannabis sativa) seed oil. Chem. Biochem. Eng. Q. 28, 481–490. https://doi.org/10.15255/CABEQ.2013.1895.

AOCS official method Ac 4-91 - nitrogen and protein in soybeans, modified Kjeldahl method. In: Official Methods and Recommended Practices of the AOCS. 2009. AOCS Press, Urbana, IL, US.

Araújo, A.C.M.A., Resende De Oliveira, É., Menezes, E.G.T., Dias, B.O., Terra, A.W.C., Queiroz, F., 2018. Solvent effect on the extraction of soluble solids from murici and pequi seeds. J. Food Process. Eng. 41, e12813. https://doi.org/10.1111/jfpe.12813.

Bessa, L.C.B.A., Ferreira, M.C., Rodrigues, C.E.C., Batista, E.A.C., Meirelles, A.J.A., 2017. Simulation and process design of continuous countercurrent ethanolic extraction of rice bran oil. J. Food Eng. 202, 99–113. https://doi.org/10.1016/j.jfoodeng.2017.01.019.

Blasi, F., Tringaniello, C., Verducci, G., Cossignani, L., 2022. Bioactive minor components of Italian and extra-European hemp seed oils. LWT (Lebensm.-Wiss. & Technol.) 158, 113167. https://doi.org/10.1016/j.lwt.2022.113167.

Bourgou, S., Bettaieb Rebey, I., Ben Kaab, S., Hammami, M., Dakhlaoui, S., Sawsen, S., Msaada, K., Isoda, H., Ksouri, R., Fauconnier, M.-L., 2021. Green solvent to substitute hexane for bioactive lipids extraction from black cumin and basil seeds. Foods 10, 1493. https://doi.org/10.3390/foods10071493.

Burton, R.A., Andres, M., Cole, M., Cowley, J.M., Augustin, M.A., 2022. Industrial hemp seed: from the field to value-added food ingredients. J. Cannabis Res. 4, 45. https://doi.org/10.1186/s42238-022-00156-7.

Chen, H., Xu, B., Wang, Y., Li, W., He, D., Zhang, Y., Zhang, X., Xing, X., 2023. Emerging natural hemp seed proteins and their functions for nutraceutical applications. Food Sci. Hum. Wellness 12, 929–941. https://doi.org/10.1016/j.fshw.2022.10.016.

Claux, O., Rapinel, V., Goupy, P., Patouillard, N., Vian, M.A., Jacques, L., Chemat, F., 2021. Dry and aqueous 2-methyloxolane as green solvents for simultaneous production of soybean oil and defatted meal. ACS Sustainable Chem. Eng. 9, 7211–7223. https://doi.org/10.1021/acssuschemeng.0c09252.

Commission Directive (EU), 2023. 2023/175 of 26 January 2023. Amending Directive 2009/32/EC of the European Parliament and of the Council as Regards 2-Methyloxolane (Text with EEA Relevance), vol. 25.

Da Costa Rodrigues, C.E., Oliveira, R., 2010. Response surface methodology applied to the analysis of rice bran oil extraction process with ethanol: rice bran oil extraction using ethanol. Int. J. Food Sci. Technol. 45, 813–820. https://doi.org/10.1111/j.1365-2621.2010.02202.x.

Cravotto, C., Fabiano-Tixier, A.S., Claux, O., Rapinel, V., Tomao, V., Stathopoulos, P., Skaltsounis, A.L., Tabasso, S., Jacques, L., Chemat, F., 2022. Higher yield and

polyphenol content in olive pomace extracts using 2-methyloxolane as bio-based solvent. Foods 11, 1357. https://doi.org/10.3390/foods11091357.
Cravotto, C., Claux, O., Bartier, M., Fabiano-Tixier, A.-S., Tabasso, S., 2023. Leading edge technologies and perspectives in industrial oilseed extraction. Molecules 28, 5973. https://doi.org/10.3390/molecules28165973.
Devi, V., Khanam, S., 2019. Comparative study of different extraction processes for hemp (Cannabis sativa) seed oil considering physical, chemical and industrial-scale economic aspects. J. Clean. Prod. 207, 645–657. https://doi.org/10.1016/j.jclepro.2018.10.036.
Esmaeilzadeh Kenari, R., Dehghan, B., 2020. Optimization of ultrasound-assisted solvent extraction of hemp (Cannabis sativa L.) seed oil using RSM: evaluation of oxidative stability and physicochemical properties of oil. Food Sci. Nutr. 8, 4976–4986. https://doi.org/10.1002/fsn3.1796.
Farinon, B., Molinari, R., Costantini, L., Merendino, N., 2020. The seed of industrial hemp (Cannabis sativa L.): nutritional quality and potential functionality for human health and nutrition. Nutrients 12, 1935. https://doi.org/10.3390/nu12071935.
Grijó, D.R., Piva, G.K., Osorio, I.V., Cardozo-Filho, L., 2019. Hemp (Cannabis sativa L.) seed oil extraction with pressurized n-propane and supercritical carbon dioxide. J. Supercrit. Fluids 143, 268–274. https://doi.org/10.1016/j.supflu.2018.09.004.
Haji-Moradkhani, A., Rezaei, R., Moghimi, M., 2019. Optimization of pulsed electric field-assisted oil extraction from Cannabis seeds. J. Food Process. Eng. 42, e13028. https://doi.org/10.1111/jfpe.13028.
Irakli, M., Tsaliki, E., Kalivas, A., Kleisiaris, F., Sarrou, E., Cook, C.M., 2019. Effect of genotype and growing year on the nutritional, phytochemical, and antioxidant properties of industrial hemp (Cannabis sativa L.) seeds. Antioxidants 8, 491. https://doi.org/10.3390/antiox8100491.
ISO 659, 2009. Oilseeds. In: Determination of Oil Content (Reference Method). https://www.iso.org/standard/43169.html. (Accessed 13 March 2023).
Izzo, L., Pacifico, S., Piccolella, S., Castaldo, L., Narváez, A., Grosso, M., Ritieni, A., 2020. Chemical analysis of minor bioactive components and cannabidiolic acid in commercial hemp seed oil. Molecules 25, 3710. https://doi.org/10.3390/molecules25163710.
Kashaninejad, M., Blanco, B., Benito-Román, O., Beltrán, S., Niknam, S.M., Sanz, M.T., 2021. Maximizing the freeze-dried extract yield by considering the solvent retention index: extraction kinetics and characterization of moringa oleifera leaves extracts. Food Bioprod. Process. 130, 132–142. https://doi.org/10.1016/j.fbp.2021.09.008.
Kocatas, B.M., Cornell, D., 1954. Holdup and residual saturation of hexane in gravity-drained soybean flake beds. Ind. Eng. Chem. 46, 1219–1224. https://doi.org/10.1021/ie50534a038.
Latif, S., Anwar, F., 2009. Physicochemical studies of hemp (Cannabis sativa) seed oil using enzyme-assisted cold-pressing. Eur. J. Lipid Sci. Technol. 111, 1042–1048. https://doi.org/10.1002/ejlt.200900008.
Leonard, W., Zhang, P., Ying, D., Fang, Z., 2020. Hempseed in food industry: nutritional value, health benefits, and industrial applications. Compr. Rev. Food Sci. Food Saf. 19, 282–308. https://doi.org/10.1111/1541-4337.12517.
Liang, J., Appukuttan Aachary, A., Thiyam-Holländer, U., 2015. Hemp seed oil: minor components and oil quality. Lipid Technol. 27, 231–233. https://doi.org/10.1002/lite.201500050.
Moccia, S., Siano, F., Russo, G.L., Volpe, M.G., La Cara, F., Pacifico, S., Piccolella, S., Picariello, G., 2020. Antiproliferative and antioxidant effect of polar hemp extracts (*Cannabis sativa* L., Fedora *Cv* .) in human colorectal cell lines. Int. J. Food Sci. Nutr. 71, 410–423. https://doi.org/10.1080/09637486.2019.1666804.
Montero, L., Ballesteros-Vivas, D., Gonzalez-Barrios, A.F., Sánchez-Camargo, A. del P., 2023. Hemp seeds: nutritional value, associated bioactivities and the potential food applications in the Colombian context. Front. Nutr. 9. https://doi.org/10.3389/fnut.2022.1039180.
Da Porto, C., Decorti, D., Tubaro, F., 2012a. Fatty acid composition and oxidation stability of hemp (Cannabis sativa L.) seed oil extracted by supercritical carbon dioxide. Ind. Crop. Prod. 36, 401–404. https://doi.org/10.1016/j.indcrop.2011.09.015.
Da Porto, C., Voinovich, D., Decorti, D., Natolino, A., 2012b. Response surface optimization of hemp seed (Cannabis sativa L.) oil yield and oxidation stability by supercritical carbon dioxide extraction. J. Supercrit. Fluids 68, 45–51. https://doi.org/10.1016/j.supflu.2012.04.008.
Rapinel, V., Claux, O., Abert-Vian, M., McAlinden, C., Bartier, M., Patouillard, N., Jacques, L., Chemat, F., 2020. 2-Methyloxolane (2-MeOx) as sustainable lipophilic solvent to substitute hexane for green extraction of natural products. Properties, applications, and perspectives. Molecules 25, 3417. https://doi.org/10.3390/molecules25153417.
Ravi, H.K., Vian, M.A., Tao, Y., Degrou, A., Costil, J., Trespeuch, C., Chemat, F., 2019. Alternative solvents for lipid extraction and their effect on protein quality in black soldier fly (Hermetia illucens) larvae. J. Clean. Prod. 238, 117861. https://doi.org/10.1016/j.jclepro.2019.117861.
Rezvankhah, A., Emam-Djomeh, Z., Safari, M., Askari, G., Salami, M., 2019. Microwave-assisted extraction of hempseed oil: studying and comparing of fatty acid composition, antioxidant activity, physiochemical and thermal properties with soxhlet extraction. J. Food Sci. Technol. 56, 4198–4210. https://doi.org/10.1007/s13197-019-03890-8.
Roca, M.A., nguez-Mosquera, M.I.M., 2001. Change in the natural ratio between chlorophylls and carotenoids in olive fruit during processing for virgin olive oil. J. Am. Oil Chem. Soc. 78, 133–138. https://doi.org/10.1007/s11746-001-0233-z.
Ruiz-García, L., Figueroa-Vega, N., Malacara, J.M., Barrón-Vivanco, B., Salamon, F., Carrieri, M., Jiménez-Garza, O., 2020. Possible role of N-hexane as an endocrine disruptor in occupationally exposed women at reproductive age. Toxicol. Lett. 330, 73–79. https://doi.org/10.1016/j.toxlet.2020.04.022.
Sawada, M.M., Venâncio, L.L., Toda, T.A., Rodrigues, C.E.C., 2014. Effects of different alcoholic extraction conditions on soybean oil yield, fatty acid composition and protein solubility of defatted meal. Food Res. Int. 62, 662–670. https://doi.org/10.1016/j.foodres.2014.04.039.
Senrayan, J., Venkatachalam, S., 2019. Optimization of ultrasound-assisted solvent extraction (UASE) based on oil yield, antioxidant activity and evaluation of fatty acid composition and thermal stability of Coriandrum sativum L. Seed oil. Food Sci. Biotechnol. 28, 377–386. https://doi.org/10.1007/s10068-018-0467-1.
Siano, F., Moccia, S., Picariello, G., Russo, G.L., Sorrentino, G., Di Stasio, M., La Cara, F., Volpe, M.G., 2018. Comparative study of chemical, biochemical characteristic and ATR-FTIR analysis of seeds, oil and flour of the edible fedora cultivar hemp (Cannabis sativa L.). Molecules 24, 83. https://doi.org/10.3390/molecules24010083.
Sicaire, A.-G., Vian, M.A., Fine, F., Carré, P., Tostain, S., Chemat, F., 2016. Ultrasound induced green solvent extraction of oil from oleaginous seeds. Ultrason. Sonochem. 31, 319–329. https://doi.org/10.1016/j.ultsonch.2016.01.011.
Soroush, D.R., Solaimanimehr, S., Azizkhani, M., Kenari, R.E., Dehghan, B., Mohammadi, G., Sadeghi, E., 2021. Optimization of microwave-assisted solvent extraction of hemp (Cannabis sativa L.) seed oil using RSM: evaluation of oil quality. Food Meas. 15, 5191–5202. https://doi.org/10.1007/s11694-021-01087-2.
Subratti, A., Lalgee, L.J., Jalsa, N.K., 2019. Liquified dimethyl ether (DME): a green solvent for the extraction of hemp (Cannabis sativa L.) seed oil. Sustain. Chem. Pharm. 12, 100144. https://doi.org/10.1016/j.scp.2019.100144.
Toda, T.A., Sawada, M.M., Rodrigues, C.E.C., 2016. Kinetics of soybean oil extraction using ethanol as solvent: experimental data and modeling. Food Bioprod. Process. 98, 1–10. https://doi.org/10.1016/j.fbp.2015.12.003.
Wlsnlak, J., Hlllel, J., Katz, O., 1987. Holdup and extraction characteristics of jojoba meal. J. Am. Oil Chem. Soc. 64. https://doi.org/10.1007/BF02540796.
Zhang, F., Rhee, K.C., Koseoglu, S.S., 2002. Isopropyl alcohol extraction of cottonseed collets: efficiency and performance. J. Food Lipids 9, 147–160. https://doi.org/10.1111/j.1745-4522.2002.tb00215.x.