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Research Article

Dry and Aqueous 2-Methyloxolane as Green Solvents for Simultaneous Production of Soybean Oil and Defatted Meal

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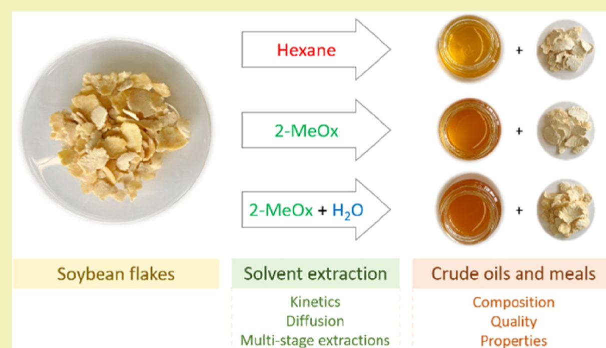
ABSTRACT: Edible oils are currently largely obtained by solvent extraction using hexane. Despite the fact that this petroleum solvent is known to be neurotoxic, hexane extraction remains the accepted standard process for seed oil extraction and particularly, but not exclusively, for soybean oil extraction. This study evaluates an alternative replacement of hexane with a bio-based and safe solvent, 2-methyloxolane (2-MeOx), either in its dry or in its water-saturated (4.5%w) aqueous form, referred to as 2-MeOx 95.5% in this paper. The analyses focused on extraction yields, composition, and quality of the extraction products. This study also evaluates the feasibility to substitute hexane in industrial extraction processes. Therefore, a kinetic study and multistage cross-current extractions were performed. The work concluded that both 2-MeOx and 2-MeOx 95.5% are good candidates to replace hexane in industrial processes for the extraction of soybean oil, as they gave similar compositions for oil and defatted meal. Hexane and 2-MeOx also gave similar results in terms of extraction rate and performances. 2-MeOx 95.5% exhibited a slightly different behavior during extraction, which was attributed to water diffusion from the solvent to the solid matrix due to its high water activity.



KEYWORDS: *2-methyloxolane, hexane, extraction, soybean oil, proteins, polyphenols*

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INTRODUCTION

Vegetable oils are our main source of lipids. They are one of the three macronutrients essential for human life. As world population continues to grow each year, there is an increasing demand for food supply. Consequently, world vegetable oil consumption increased from 147 million metric tons (Mt) in 2010 to 205 Mt in 2019.[1] This number is expected to reach nearly 240 Mt in 2028, of which 164.8 Mt (69%) will be used for food and 30.2 Mt (13%) for biofuel production.[2] Soybean oil is the second most consumed vegetable oil worldwide (28% of the total world consumption in 2019), and by far the first one in the United States, representing 56% of the country's total food oil consumption.[1]

Industrially, the oil production can be performed using two different techniques, depending on the plant material oil content. Solids containing more than 25% oil can be mechanically pressed to extract the majority of the oil, but this generally leads to 7−20% of residual oil in the press cake.[3] For a matrix with less than 25% of oil content (including soybeans), direct solvent extraction with extraction-grade hexane is a common practice. The obtained miscella (mixture of oil and solvent) is then evaporated to obtain a crude oil that can be further refined to remove phospholipids (lecithin production), free fatty acids, waxes, or undesirable color or odor. In the case of oilseeds, the marc (mixture of defatted seeds, called defatted meal in the rest of this paper, and solvent) is desolventized to be valorized for use as animal feed or protein-enriched ingredients for food. In particular, soybeans are considered a good source of protein because they contain important amounts of essential amino-acids, even if their use is controversial due to the presence of antinutritional factors such as isoflavones (phytoestrogens).[4−6]

The use of hexane as solvent for oil extraction is problematic and goes against some fundamental principles of Green Chemistry (5, 7, and 10) as defined by Anastas and Warner.[7] Indeed, extraction-grade hexane is a petroleum fraction (66−70 °C) composed of C6 isomers, with 48 to 98% of *n*-hexane,[3] which was shown to be toxic either by ingestion or by inhalation on rats with reprotoxic and neurotoxic effects (see Table 1), and

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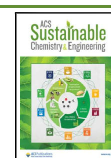

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Table 1. Comparison of Hexane and 2-MeOx: Specificities, Toxicological Profile and Guidelines[a]

		n-hexane	2-MeOx
solvent specificities	selectivity	affinity with lipids	affinity with lipids and slightly more polar compounds
	BP (°C)	68	80
	water miscibility	not miscible	slightly miscible
	vaporization enthalpy (kJ/kg)	334	364
sourcing		petroleum	bio-based
cost[17]	average solvent cost (€/ton)	900	8000
bioaccumulation potential	log $P_{o/w}$	4.00	1.85
ingestion toxicology[18,19]	NOAEL (mg/kg bw/day)	23	250
	effects	not detailed	no CMR effect
inhalation toxicology[20,21]	NOAEC (ppm)	122	2843
	Effects	neurotoxic and reprotoxic	no CMR effect
environmental toxicology[22,23]	96 h EC50 or NOEC on fishes (mg/L)	12.51	>100
	21d NOEC on *Daphnia* (mg/L)	4.89	>120
	48 h NOEC on algae (mg/L)	2.08	>104
pharmaceutical guidelines[24,25]	PDE (mg/day)	2.9	50
	calculated ADI (mg/kg bw/day)	0.058	1.000
	classification	class 2: solvent to be limited	class 3: low toxicity solvent

[a]log $P_{o/w}$, octanol−water partition coefficient; NOAEL, no-observed-adverse-effect level; NOAEC, no-observed-adverse-effect concentration; NOEC, no-observed-effect concentration; EC50, half maximal effective concentration; PDE, permitted daily exposure for an average human of 50 kg; ADI, admissible daily Intake (calculated from PDE value).

was classified as CMR2 (carcinogenic, mutagenic, and reprotoxic substance) under the REACH Regulation. Recent studies highlighted that *n*-hexane could act as an endocrine disruptor as well.[8,9] Also, *n*-hexane is known to be toxic for aquatic species at low concentrations and its high octanol−water partition coefficient traduces an important bioaccumulation potential.[10]

To tackle this issue, studies have been conducted since the 1980s with the aim of finding a suitable alternative solvent for oil extraction.[11,12] A wide range of alternatives to hexane have been investigated over the past years, such as enzyme-assisted aqueous extraction (EAAE), supercritical fluid extraction, or the use of bio-based solvents such as ethanol, isopropanol, acetone, or methoxy-cyclopentane (CPME).[3] Among them, 2-methyloxolane (2-MeOx), also known as 2-methyltetrahydrofuran (2-MeTHF), has recently appeared to be a promising solution.[13−15] This bio-based solvent actually has a high affinity for lipids and a boiling point (80 °C) compatible with existing extraction processes. It was shown to be safer than *n*-hexane by ingestion and inhalation, without CMR effect. It has a lower bioaccumulation potential and a good eco-toxicological profile (see Table 1). Pharmaceutical guidelines of the International Council for Harmonization (ICH) Q3C recently proposed 2-MeOx to be listed into Class 3 "Solvents with low toxic potential" (Permitted daily exposure (PDE) higher or equal to 50 mg/day), while *n*-hexane is listed into Class 2 "Solvents to be limited" with a much lower PDE (2.9 mg/day).

The economic viability of hexane replacement by 2-MeOx for the extraction of vegetable oil (rapeseeds) has already been studied twice in the literature.[16,17] The first element to consider is the solvent selling price. The price of 2-MeOx is currently in the 7−9 €/kg range, which is clearly higher than the price of food-grade hexane, which is around 0.8−1.0 €/kg. However, the impact of this higher price can be reduced through solvent recovery in the extraction process, which lowers the actual consumption (100 to 500 g/ton oilseed). The higher boiling point and vaporization enthalpy of 2-MeOx is also a potential drawback as it necessarily leads to higher energy consumption (steam) during desolventization steps, from either the miscella or the marc. However, in both studies, the authors indicated that these incremental costs could almost be completely compensated by using a more efficient oil extraction with 2-MeOx, resulting in a productivity gain. Rapinel et al.[17] concluded that the overall extraction process extra cost should be limited to 0.47€ per ton of seeds extracted, in the case of rapeseeds.

Oil production plants performing solvent extraction usually have to spread the equipment cost across very large production volumes, typically several hundred to a few thousand tons of seeds per day.[3] Such large continuous processes obviously require solvent recycling, by distillation or evaporation either from the miscellas or the marcs. If hexane recycling is quite straightforward, 2-MeOx will require slight recycling adjustments as it forms an azeotrope with water (10.6%w water at 71 °C) combined with a small water miscibility. Therefore, after condensation and decantation, the mixture separates into an organic phase saturated with 4.5% water (at 55 °C) and an aqueous phase containing 2-MeOx (7% at 55 °C).[17] Another distillation stage (drying step) would be necessary to recover dry 2-MeOx, increasing the energy consumption and global cost of the process. To be in line with principle 6 of Green Chemistry that recommends limiting energy consumption, and considering that gross margins in the oil extraction industry are usually very low (3−10% depending on the plant size for conventional soybean oil production[26]), it would be preferable to perform extraction directly with water-saturated 2-MeOx (4.5%w H_2O), instead of dry 2-MeOx. This water-saturated mixture will be referred to as 2-MeOx 95.5% in the remainder of this paper. To the best of our knowledge, it is the first laboratory study in which 2-MeOx 95.5% is considered as a full-fledged solvent for extraction.

Ideally, the replacement of hexane by a bio-based solvent in large-scale oil extraction processes should be as straightforward as possible, with minimal product and process impacts, and to be able to use existing process installations. Thus, this study was conducted to answer the following questions:

- Can 2-MeOx or 2-MeOx 95.5% extract the same compounds that hexane does?

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- Can 2-MeOx 95.5% be used to extract oil despite the presence of water?
- Would the replacement of hexane by 2-MeOx or 2-MeOx 95.5% for oil extraction have an impact on the resulting oil composition, quality or properties?
- Would it have an influence on the defatted meal proteins and antinutrients?
- Would the extraction rates be equivalent?
- Would 2-MeOx and 2-MeOx 95.5% be as efficient as hexane in industrial extractions?

■ MATERIALS AND METHODS

COSMO-RS Evaluation. Predicted solubilities of some selected solutes usually found in soybean oil were determined in 2-MeOx, 2-MeOx 95.5%, and *n*-hexane, using the conductor-like screening model for real solvents (COSMO-RS). Briefly, COSMO-RS is a calculation method developed by Klamt[27] using a quantum chemistry model, which has been applied since 2000 for solubility prediction in the context of natural products extraction.[28,29] More detailed explanations are presented in our previous studies.[30,31] Calculations were performed using the COSMOThermX software (Version 18.0.2, COSMOlogic GmbH & Co., Leverkusen, Germany), at 55 °C, using the "iterative" mode, considering that solutes and solvents are pure and in a liquid state.

Ternary Phase Diagram of Soybean Oil, Water, and 2-MeOx. The ternary phase diagram was established at 55 °C. To do so, appropriate amounts of 2-MeOx and commercial soybean oil were precisely weighed into glass tubes. Tubes were then closed with PTFE/silicone septa caps and put into a temperature-controlled water bath. Water was added dropwise through the septum while stirring until the solution became turbid. Tubes were weighed again to assess the new mixture composition. These compositions were reported on the ternary diagram to establish the limit separating the monophasic and biphasic regions.

Raw Material. Both dehulled soybean (*Glycine max* (L.) Merr.) seeds and flakes were provided by OLEAD (Pessac, France, 2019). Seeds were ground just before extraction using a microfine grinder MF 10 basic (IKA, Germany) equipped with a 2.0 mm sieve.

Soxhlet Extraction Procedure. Before each extraction, 2-MeOx (VWR, BHT stabilized) was distilled to remove BHT and dried with anhydrous sodium sulfate. 2-MeOx 95.5% was obtained by dissolving the corresponding mass of water in distilled 2-MeOx. The extractions were made in an automatic Soxhlet extractor B-811 (BÜCHI, Switzerland) in the "standard" mode, with a chamber volume of 175 mL, using hexane (VWR, technical grade), 2-MeOx, and 2-MeOx 95.5% as solvents.

For each solvent, soybean oil was quantitatively extracted during an 8 h timeframe, following standard procedure ISO 659.[32] The corresponding extraction yield was expressed as the percentage of the mass of crude oil obtained relative to the mass of dry soybean seeds used for extraction.

$$\text{Extraction Yield (g/100g DM)} = \frac{\text{Mass of crude oil (g)}}{\text{Mass of dry soybean seeds (g)}} \times 100 \quad (1)$$

Crude oils and defatted meals used for analysis were obtained with the same method but shortening the extraction time to 2 h 30 min. The shorter time was chosen to preserve the quality of the products and to be more representative of the industrial conditions. Once the extraction step was over, the defatted raw materials were placed into small aluminum cups and desolventized at approximately 50 °C for 3 h in a ventilated Biosec dehydrator (Tauro Essiccatori, Italy). Then, solids were ground into a fine powder and stored at 5 °C before analysis. Each liquid extract was collected, and the solvent was evaporated using a rotary evaporator. Crude oils were allowed to cool under a nitrogen flow for 10 min and were stored at −20 °C before analysis.

Crude Oil Analyses. *Saponifiable Compounds. Fatty Acid Profile.* Fatty acid methyl esters (FAMEs) were prepared from soybean oil samples by acid-catalyzed transmethylation. First, 1 mL of methanolic sulfuric acid (5%, *v/v*) solution was added to 20 to 30 mg of oil in a glass tube. Then, 500 μL of triheptadecanoin (C17:0; TAG) in *n*-hexane (2 mg/mL) was added as an internal standard. The mixture was then heated for 90 min at 85 °C in a heating block. Once at room temperature, 1.5 mL of 0.9% (*m/v*) NaCl solution and 1 mL of *n*-hexane were added into the tube and the mixture was vortexed for 30 s. The organic layer was collected and subjected to further analysis. Analyses were performed using an Agilent (Japan) 7820A gas chromatograph coupled with a flame ionization detector (GC-FID). The instrument was equipped with a BD-EN14103 capillary column (30 m × 320 μm × 0.25 μm). Velocity of the carrier gas (He) was set at 33 cm/s. Injection of 2 μL of sample was carried out in the split mode (split ratio 1:20), and injector temperature was set at 250 °C. Oven temperature was set at 50 °C for 1 min and then increased at a constant rate of 20 °C/min to 180 °C, and then up to 230 °C at a rate of 2 °C/min. Once it reached 230 °C, the temperature was held for a period of 10 min. FAMEs were identified based on their retention times after comparison with FAMEs standards.

Neutral Lipids. Identification and relative quantification of neutral glycerides in soybean oils was performed via high performance thin layer chromatography (HPTLC). Standard solutions of palmitic acid (free fatty acid, FFA), DL-α-palmitin (monoacyl glyceride, MAG), glyceryl 1,3-dipalmitate (diacyl glyceride, DAG), and glyceryl tripalmitate (triacyl glyceride, TAG) were prepared in chloroform (0.2 mg/mL), as well as crude oil sample solutions (1−15 mg/mL). Silica gel 60 F254 HPTLC plates (20 × 10 cm) were prewashed by complete elution with isopropanol and dried at 110 °C for 20 min. The Automatic TLC Sampler 5 (ATS 5, CAMAG, Switzerland) was used for application of samples and standards as 6 mm bands on plates. Plates were then developed in the automatic developing chamber (ADC2, CAMAG). Neutral glycerides were separated with a mixture of n-hexane/diethyl ether/glacial acetic acid (70:30:2, *v/v/v*). Eluent was allowed to reach a height of 7 cm from the origin. Each plate was then dipped in a primuline dye reagent (0.005% (*m/v*) in acetone/water, 4:1, *v/v*) for derivatization and allowed to dry for 10 min. Pictures of the plates were captured with the TLC visualizer (CAMAG) under UV 366 nm illumination. Finally, the plates were scanned in fluorescence mode at UV 366/>400 nm (mercury lamp) by the TLC Scanner 3 (CAMAG).

Phospholipids. Identification and quantification of phospholipids in soybean oils was performed by HPLC. The analysis was conducted by ITERG analytical laboratory (Canéjan, France) using an internal method.

Unsaponifiable Compounds. Determination of unsaponifiable matter was carried out according to standard method AOCS Ca 6a-40.[33] Sterols were determined following ISO 12228, and tocopherols and tocotrienols following ISO 9936.[34,35]

Quality Parameters. Determination of the following parameters in crude oils was carried out according to the standard AOCS methods: acid value (AV), Cd 3d-63; peroxide value (PV), Cd 8−53; conjugated dienes(CD) level, Cd 7−58; *p*-anisidine value (*p*-AV), Cd 18−90.[33] Iodine value (IV) was obtained following the analytical method ISO 3961.[36]

Crude Oil Properties. Antioxidant Activity. The antioxidant activity of soybean crude oils in MeOH was evaluated by the DPPH assay[37] using a FluOstar Omega microplate reader (BMG LABTECH, Germany). Exactly 100 μL of sample, reference (pure MeOH), or Trolox standard solution were placed in eight wells of a 96-well microplate. Then 100 μL of a 0.5 mM DPPH methanolic solution were added in each well. The microplate was kept in the dark for 1 h and absorbance was read at 520 nm. The antioxidant activity of samples was expressed as mg Trolox equivalents (TE) per kg of fat.

Oxidative Stability. Oxidative stability of soybean crude oils was evaluated by the Rancimat method, based on official standard NF EN 14112.[38]

Seeds and Defatted Meal Analyses. *Proteins. Crude Protein Content, Protein Dispersibility Index, KOH Protein Solubility, and*

Amino Acid Content. Crude protein content and protein dispersibility index (PDI) of soybean seeds and meals were determined according to AOCS official methods Ac 4-91, Ba 4d-90, and Ba 10b-09.[33] KOH protein solubility values were obtained following the procedure described by Van Eys et al.[39] Nitrogen content of both solid and liquid fractions was analyzed by the Kjeldahl method using BÜCHI SpeedDigester K-425 and distillation unit K-350. Determination of amino acid content and composition of raw material and defatted soybean was conducted according to ISO 13903.[40]

Protein Molecular Weight Distribution. Molecular weight distribution of water-soluble proteins from soybeans seeds and meals was obtained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Water-soluble proteins were extracted following the PDI procedure described in AOCS Ba 10b-09.[33] Extracts were dialyzed (cutoff value of 12−14 kDa) and the dialyzed fractions were freeze-dried. Protein solutions (2 mg/mL in water) were denatured by the addition of equal volume of Laemmli sample buffer and heating at 90 °C for 5 min. The analysis was then run in a Mini-PROTEAN Electrophoresis system (Bio-Rad, USA) using Mini-PROTEAN TGX precast gels.

Antinutritional Factors. Determination of urease activity and trypsin inhibitors (TI) activity were carried out according to AOCS official methods Ba 9-58 and Ba 12-75.[33] Phytic acid content analysis by spectrophotometry was conducted by ITERG (Canéjan, France) using an internal method.

Phenolic Compounds. *Extraction Procedure.* Approximately, 1 g of crude oil was placed into a centrifuge tube and diluted in 1 mL of *n*-hexane. Then, 5 mL of methanol/water (80:20, *v/v*) were added, and the tube was shaken vigorously for 10 min, before centrifugation (21,000 g, 5 min). The lower phase was collected, and the upper layer was extracted two more times repeating the same procedure. Then, the combined lower phases were transferred into a 25 mL volumetric flask and diluted to volume with solvent.

In the case of soybean seeds or meals, 1.5 g of fine powder was placed into a centrifuge tube before addition of 15 mL of methanol/water (80:20, *v/v*). The mixture was stirred vigorously at 10,000 rpm for 10 min using a T25 digital ULTRA-TURRAX (IKA, Germany). The mixture was centrifuged (21,000 g, 5 min), the supernatant collected, and the solid residue was extracted two more times repeating the same procedure. Then, the combined supernatants were transferred into a 50 mL volumetric flask and diluted to volume with solvent.

Ultra-Performance Liquid Chromatography Analysis. Identification of phenolic compounds in the extracts was performed by ultra-performance liquid chromatography (UPLC) using an ACQUITY UPLC system (Waters Corp., USA) linked to both a diode array detector 190−800 nm (UPLC DAD, Waters, USA) and a Bruker Daltonics HCT Ultra Ion Trap MS equipped with an electrospray ion source (UPLC DAD/ESI-MS^n). Compass software (Bruker Daltonics, Germany) was used for mass spectrometric instrument control and data processing. Quantitative analysis of the same extracts was then carried out using an ACQUITY UPLC system (Waters Corp., USA) linked to a diode array detector 190−800 nm (UPLC DAD, Waters, USA). Empower 2 software (Waters) was used for instrument control and integration of chromatograms.

Separation of soybean phenolic compounds was performed on an ACQUITY UPLC HSS T3 column (100 mm × 2.1 mm, i.d. 1.7 μm; Waters Corp., USA), with temperature set at 35 °C. Acidified H_2O (0.05% formic acid, *v/v*, solvent A) and MeOH (solvent B) were used as solvents and gradient conditions were: A, 80%, 0−2 min; A, 80−72%, 2−4 min; A, 72−40%, 4−9 min; A, 40%, 9−10 min; A, 40−0%, 10−12 min; A, 0%, 12−14 min; and return to the initial conditions (solvent A, 80%) for a 3 min re-equilibration period. Flow rate was 0.2 mL/min. The system operating pressure was 390 bars at initial conditions. Injection volume was 2 μL with partial loop with needle overfill injection. Chromatographic profiles were recorded between 190 and 800 nm and isoflavones were detected at 260 nm.

For the UPLC DAD/ESI-MS^n, the ion trap was operated in Ultra Scan mode from *m/z* 120 to 1400. The ICC target was set to 100,000 with a maximum accumulation time of 100 ms. Nitrogen (99.99% purity) was used as desolvation gas. Ionization was achieved using an ESI source in positive mode. Ionization source parameters were set as follows: dry temperature 365 °C, nebulizer pressure 50 psi, dry gas flow 8 L/min, capillary voltage was −2.5 kV for flavonols and hydroxycinnamic acids analysis, and −2.0 kV for isoflavones analysis.

Individual phenolic contents in the extracts were determined by UPLC DAD analysis at 260 nm against a calibration curve obtained from dilution series of genistein. A stock solution of standard (1 mg/mL) was prepared in methanol/water (80:20, *v/v*) and 10 dilutions from 0.25 to 80 μg/mL were prepared in the same solvent mixture and injected in the same conditions than samples. Results were expressed as mg genistein equivalents (GE) per kg of fat, or per kg of dry and defatted material (DDM).

Kinetic Study. Kinetic studies were conducted with the three solvents at 25 and 55 °C, and with 2-MeOx and 2-MeOx 95.5% at 65 °C. Indeed, the higher boiling point of 2-MeOx (80 °C) compared to hexane (68 °C) enables work at higher temperatures without risk of boiling. Approximately 40 g of soybean flakes were enclosed in a paper filter and plunged into a double jacketed reactor filled with 400 mL of solvent. The solution was continuously stirred, and 1 mL of clear solvent was collected from the extraction flask at regular interval times (every minute until 10 min, every 5 min until 45 min, every 15 min until 2 h and then every 30 min until 3 h). Collected samples were heated at 55 °C for at least 30 min under a flow of nitrogen using a block heater SBH200D/3 (Stuart, UK) equipped with a sample concentrator SBHCONC/1 (Stuart, UK) to evaporate the solvent. Residues were precisely weighed, data were normalized according to the mass of dry soybean flakes introduced and the normalized solute concentration (mg/mL) in the solvent was monitored over time.

Diffusion Model. Solid−liquid extraction generally occurs in several steps involving (i) the dissolution of the solute in the solvent within the solid matrix, (ii) its migration toward the surface, and (iii) its transfer from the solution in contact with the matrix to the main bulk of the solution.[41] In the particular case of a liquid solute such as oil, the whole process is driven by the transfer of the solute through nearly impermeable cell membranes. The extraction rate directly depends on this internal diffusion. However, industrial processes of soybean oil extraction involve both mechanical and thermal pretreatment of raw material that are made to facilitate solvent extraction. In particular, a step of flaking is used to reduce the distance that solvent needs to penetrate during extraction and distort the cells, making more oil available. Then, the flakes are cooked to coagulate the proteins of the fat-cell-walls and cause the walls to rupture.[3]

Fick's second law gives a relation between the solute concentration $C(x,t)$ in the solid, the global diffusion coefficient D of the process, the time t and the space coordinate measured normal to the section x:

$$\frac{\partial C(x,\,t)}{\partial t} = -D\frac{\partial^2 C(x,\,t)}{\partial x^2} \tag{2}$$

The resolution of this equation first requires several hypotheses regarding the system:

- Soy flakes are considered as thin plate with a thickness of 2*L, evaluated at 1 mm.
- The diffusion coefficient is supposed to be constant during the extraction process.
- The initial concentration $C(x \in]\text{-}L;\, +L[, t = 0)$ of solute is uniform in the solid matrix.
- The concentration at the interface $C(x = \pm L, t)$ is kept constant.

A solution of eq 2 for a plane sheet with the same hypotheses is given by Crank,[42] with M_t the total amount of oil which has left the solid at time t, and M_∞ the corresponding quantity after infinite time:

$$\frac{M_t}{M_\infty} = 1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2\pi^2}\exp\left(\frac{-(2n+1)^2\pi^2}{4L^2}\mathrm{Dt}\right) \tag{3}$$

The other terms of the series of eq 3 can be considered negligible in regard to the first one. Therefore, eq 3 can be simplified to the following equation:[43]

Table 2. COSMO-RS Predicted Solubilities for Some Target Solutes Found in Soybean Crude Oils in *n*-Hexane, 2-MeOx and 2-MeOx 95.5% at 55 °C[a]

	genistein	genistin	α-tocopherol	β-phytosterol	PL	FFA	TAG
2-MeOx	0.00	0.00	0.00	0.00	−1.09	0.00	0.00
2-MeOx 95.5%	0.00	0.00	0.00	0.00	0.00	0.00	0.00
n-hexane	−6.65	−9.22	0.00	−0.10	−6.06	−1.76	0.00

[a]Gray: reference solvent, green: better than the reference. PL, Phosphatidylcholine (R1 = C18:2; R3 = C16:0); FFA, Linoleic Acid (C18:2); TAG, Triglyceride (R1 = C18:1; R2 = C18:2; R3 = C18:2).

$$\ln\left(\frac{M_\infty}{M_\infty - M_t}\right) = -\ln\left(\frac{8}{\pi^2}\right) + \frac{\pi^2}{4L^2}\text{Dt} \quad (4)$$

Thus, the diffusion coefficient D can be obtained by plotting $\ln\left(\frac{M_\infty}{M_\infty - M_t}\right)$ over time.

Multistage Cross-Current. Multistage cross-current extractions were performed to compare the performances of the solvent through several extraction stages. Approximately 50 g of soybean flakes were introduced into a 250 mL double jacketed reactor filled with 100 g of solvent. The 1:2 solid-to-solvent ratio (*w*/*w*) was chosen to mimic industrial conditions, as well as the extraction temperature, set at 55 °C. Solids were placed in contact with the solvent for 1 h to ensure that thermodynamic equilibrium was reached. Then, the miscella (mixture of solvent and oil) was collected and the solvent evaporated to get the crude oil. Flakes were further extracted until the oil extraction yield reached 95% of the initial oil content. For fair comparison with hexane, 2-MeOx crude oils—containing additional nonlipidic compounds—were diluted in hexane, filtered (0.45 μm) and evaporated to access the "hexane-like oil" weight.

RESULTS AND DISCUSSION

COSMO-RS Evaluation. COSMO-RS predicted solubilities of some target soybean oil components in *n*-hexane (main isomer of the extraction-grade hexane), 2-MeOx and 2-MeOx 95.5% are presented in Table 2. These solutes were chosen because they are major constituents of the soybean oil (triglycerides, phospholipids, free fatty acids) or minor valuable constituents, mainly antioxidants (tocopherol, sitosterol, isoflavones). The results, expressed as $\log_{10}$ (x_{solub}), the logarithm of the molar fraction of solute in the solvent, show a much higher theoretical solubility of all the solutes in 2-MeOx (either dry or saturated with water). COSMO-RS calculations (see Table 2) logically predicted much higher solubilities of oxygenated compounds, such as isoflavones (genistein, genistin), phosphatidylcholines (PL), free fatty acids (FFA), and phytosterols but also equal solubilities for triglycerides (TAG), which constitute >95% of the oil. It must be also noted that all the target solutes were predicted to be fully soluble in both 2-MeOx and 2-MeOx 95.5%, except for phosphatidylcholine, which is more soluble in the presence of water.

In conclusion, COSMO-RS calculations predicted that 2-MeOx (with or without water) offers a similar solubilization than *n*-hexane for the major oil components but is also able to solubilize more polar compounds such as phospholipids or antioxidants. Therefore, higher extraction yields are expected in comparison with hexane, with crude oils containing more polar compounds. Those compounds can be easily removed from the oil during refining and then be purified to obtain value added chemicals such as phospholipids, sold as "lecithin", or phenolic compounds. This first study in silico represents an important first step in determining whether 2-MeOx can successfully substitute hexane for soybean oil extraction.

Ternary Phase Diagram of Soybean Oil, Water, and 2-MeOx. The ternary phase diagram of soybean oil (refined), water, and 2-MeOx at 55 °C is shown in Figure 1. The experimental points giving the limit between the monophasic

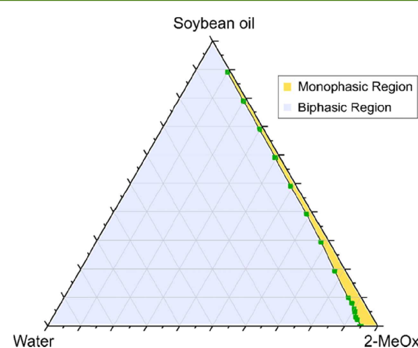

Figure 1. Ternary phase diagram soybean oil−water−2-MeOx, at 55 °C.

region (in yellow) and the biphasic region (in light blue) at 55 °C are represented by green squares. To the best of our knowledge, it is the first time that such a diagram is published. Interestingly, the diagram shows a small monophasic region on the right part. This corresponds to mixtures with moderate water contents (i.e. below 4.5%). The existence of such a monophasic region makes it possible to consider 2-MeOx 95.5% as a full-fledged solvent for oil extraction. It is also interesting to note that the more oil is contained in the solvent, the less water it can contain. For example, for miscellas containing 30% of soybean oil the maximum water content is around 2.5%.

Crude Oil Analyses. ***Extraction Yields, Lipid Composition, and Quality.*** After quantitatively extracting soybean seeds for 8 h with hexane, 2-MeOx, and 2-MeOx 95.5%, extraction yields were determined gravimetrically. As shown in Table 3, the extraction yields are within the 18.8−23.7% range, which is in accordance with the average lipid content of soybean seeds, around 20%.[4,44] Additionally, both 2-MeOx and 2-MeOx 95.5% gave higher crude yields than hexane, most likely due to the extraction of additional compounds, as suggested by the COSMO-RS predictions.

The crude oils produced after 2.5 h Soxhlet extraction were used for composition and quality analysis. Their fatty acid profile, phospholipids composition, and quality parameters are reported in Table 3. The three crude oils have similar fatty acid profiles with mainly linoleic acid (C18:2), oleic acid (C18:1), palmitic acid (C16:0), linolenic acid (C18:3, and stearic acid (18:0), accounting for more than 98% of total fatty acids, in agreement with the literature.[44] Lipid compositions obtained by HPTLC confirmed that the extracts are mainly composed of TAGs as other neutral lipids (FFAs, MAGs, and DAGs) were not detected. Regarding phospholipids, their proportion in hexane extract is in line with previous descriptions.[44,45] However, phospholipid content is higher in dry 2-MeOx crude

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Table 3. Extraction Yields, Lipid Composition and Quality of Soybean Crude Oils Extracted with Hexane, 2-MeOx and 2-MeOx 95.5%[a]

items	crude oilhexane	crude oil2-MeOx	crude oil2-MeOx 95.5%
extraction yields (g/100 g DM)	18.8 ± 0.1	23.5 ± 0.1	23.7 ± 0.1
saponifiable compounds			
Fatty acids (relative %)			
C16:0	9.55 ± 0.01	9.79 ± 0.01	9.80 ± 0.01
C18:0	4.38 ± 0.01	4.38 ± 0.01	4.31 ± 0.01
C18:1 *n*-9	23.12 ± 0.08	22.25 ± 0.05	22.34 ± 0.01
C18:2 *n*-6	53.90 ± 0.15	54.46 ± 0.05	54.48 ± 0.01
C18:3 *n*-3	7.62 ± 0.01	7.85 ± 0.01	7.83 ± 0.01
Others	1.43 ± 0.01	1.27 ± 0.01	1.24 ± 0.01
∑ SFAs	14.93 ± 0.05	15.07 ± 0.05	14.99 ± 0.05
∑ MUFAs	23.45 ± 0.10	22.54 ± 0.07	22.63 ± 0.03
∑ PUFAs	61.62 ± 0.16	62.39 ± 0.06	62.38 ± 0.02
phospholipids (g/100 g fat)			
PG	0.07 ± 0.02	0.13 ± 0.03	0.16 ± 0.04
PE	0.57 ± 0.12	1.02 ± 0.21	1.22 ± 0.25
PI	0.23 ± 0.05	0.60 ± 0.12	0.72 ± 0.15
PC	0.49 ± 0.10	1.49 ± 0.30	1.89 ± 0.38
SM	<0.1	<0.1	<0.1
LPE + PS + PA	0.23 ± 0.05	0.27 ± 0.06	0.40 ± 0.08
Total	1.58 ± 0.32	3.52 ± 0.71	4.39 ± 0.88
quality parameters			
AV (mg KOH/g fat)	2.3 ± 0.2	4.4 ± 0.1	5.9 ± 0.1
IV (g I_2/100 g fat)	125 ± 8	128 ± 8	127 ± 8
PV (meq/kg fat)	4.31 ± 0.04	23.5 ± 0.12	15.25 ± 0.01
CD level (%)	0.214 ± 0.004	0.329 ± 0.005	0.390 ± 0.004
p-AV	3.92 ± 0.52	1.84 ± 0.12	0.49 ± 0.08

[a]DM, dry matter; SFAas, saturated fatty acids; MUFAs, mono-unsaturated fatty acids; PUFAs, poly-unsaturated fatty acids; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PC, phosphatidylcholine; SM, sphingomyelin; LPE, lysophosphatidylethanolamine; PS, phosphatidylserine; PA, phosphatidic acid; AV, acid value; IV, iodine value; PV, peroxide value; CD, conjugated dienes; *p*-AV, *p*-anisidine value.

oil and even higher in 2-MeOx 95.5% crude oil, reaching 4.39%, as predicted by COSMO-RS. The ability of 2-MeOx to provide lipid extracts with a higher phospholipid content than hexane has already been reported for different matrix.[15,28] It is interesting to note that these molecules are known to have emulsifying properties, and can be separated from the neutral oil during the refining (water degumming step) to produce lecithin, especially from soybean or sunflower.[3] Nevertheless, a higher amount of phospholipids may also increase neutral oil losses during oil refining. This point should be addressed in further studies.

As 2-MeOx can generate peroxides and is partially miscible with water, it was important to check the oil quality parameters (see Table 3) to know if the oil was more deteriorated than with hexane. In the presence of water, TAGs can undergo hydrolysis and break down into FFAs and DAGs, MAGs or glycerol. Generally, the FFA content of an oil can be evaluated rapidly by measuring the acid value (AV).

The AVs of hexane, 2-MeOx, and 2-MeOx 95.5% crude oils were respectively 2.3, 4.4, and 5.9. However, it appears that the higher AVs cannot be directly associated with the presence of FFAs as they were not detected by HPTLC. It is more likely that these AVs are due to other species such as polar lipids that can interfere with the alkalimetric determination, as mentioned by Zhou and Ackman.[46]

More than hydrolysis, vegetable oils are particularly sensitive to oxidation, especially for highly unsaturated oils.[47] Auto-oxidation of unsaturated fatty acids occurs in different stages, involving the formation of several compounds.[48] First, there is a progressive loss of double bonds as primary oxidation compounds (conjugated dienes(CD) and hydroperoxides) are formed. Then, hydroperoxide content reaches a maximum before they break down into secondary oxidation compounds (ketones, aldehydes, etc.). Iodine values (IVs) of all three samples were similar, meaning that the same amount of unsaturated acids was present in each extract. Peroxides values (PVs) of hexane, 2-MeOx, and 2-MeOx 95.5% crude oils were respectively 4.3, 23.5, and 15.3. This could mean that the oils extracted with 2-MeOx are in an advanced deterioration stage than the hexane-extracted oils, but this could also be explained by the presence of residual 2-MeOx or its peroxides. CD levels increase in the following order: hexane < 2-MeOx < 2-MeOx 95.5%. Interestingly, the *p*-anisidine values (*p*-AVs) followed the opposite trend, indicating that more secondary oxidation products were present in the crude oil extracted with hexane than with the bio-based solvents. Overall, the results seem to indicate that oils obtained with bio-based solvents are initially slightly more deteriorated than the conventionally extracted oil. However, crude oils analyzed in this study are complex mixtures of different compounds that can produce interferences during analysis. This shows that standard methods for oil quality evaluation might not be suitable for crude oils extracted with 2-MeOx or 2-MeOx 95.5%. Further studies need to be performed to give a better understanding of results and evaluate potential interferences. Also, this only reflects the state of degradation of crude oils right after their production and they could exhibit different oxidative stability over time.

Unsaponifiable Compounds and Crude Oil Properties. Unsaponifiable content and composition of the three crude extracts are given in Table 4. Total unsaponifiable matter was slightly higher in 2-MeOx and 2-MeOx 95.5% crude oils compared to hexane. Considering the higher extraction yields obtained with the green solvents, these contents were expected to be even higher. However, the official method used is mainly based on saponification of the sample in ethanol and recovery of unsaponifiable matters by extraction with petroleum ether. Therefore, it does not consider polar unsaponifiable matters that cannot be solubilized in petroleum ether, such as phenolic compounds. All the extracts exhibited similar total and individual sterol contents, with β-sistosterol, campesterol, and stigmasterol, representing more than 90% of total sterols. Total and individual tocopherol and tocotrienol contents were also alike, with γ and δ tocopherols being predominant and tocotrienols mostly not detected. These individual and total contents are in accordance with the literature on soybean oil.[44] The data also reveal higher antioxidant activity and oxidative stability for 2-MeOx and 2-MeOx 95.5% crude oils, compared to hexane. This particularity can be explained by coextraction of phenolics compounds that will be described later in this paper.

Seeds and Defatted Meal Analyses. The protein contents and quality factors of the soybeans were evaluated before and after 2.5 h of Soxhlet extraction, as shown in Table 5. For each meal, crude protein content and total and individual amino acid contents were similar and in agreement with literature on soybeans.[4,39] Also, the molecular weight distribution of water-soluble proteins given by SDS-PAGE (see Figure 2) was the

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Table 4. Unsaponifiable Compounds and Properties of Soybean Crude Oils Extracted with Hexane, 2-MeOx, and 2-MeOx 95.5%

items	crude oil hexane	crude oil2-MeOx	crude oil2-MeOx 95.5%
unsaponifiable compounds			
unsaponifiable matter (g/100 g fat)	1.08 ± 0.03	1.15 ± 0.13	1.35 ± 0.13
sterol content (mg/kg fat)			
brassicasterol	<4	<4	<4
cholesterol	12 ± 1	11 ± 1	11 ± 1
24-Me-cholesterol	39 ± 4	38 ± 4	22 ± 2
campesterol	835 ± 84	783 ± 78	804 ± 80
campestanol	16 ± 2	15 ± 2	15 ± 2
stigmasterol	738 ± 74	712 ± 71	711 ± 71
δ-7-campesterol	16 ± 3	19 ± 4	15 ± 3
D5,23-stigmastadienol	23 ± 2	26 ± 3	22 ± 2
clerosterol	8 ± 1	11 ± 1	7 ± 1
β-sistosterol	1969 ± 197	1934 ± 193	1891 ± 189
sitostanol	39 ± 4	45 ± 5	41 ± 4
δ-5-avenasterol	74 ± 15	76 ± 15	71 ± 14
δ-5,24-stigmastadienol	16 ± 2	19 ± 2	15 ± 1
δ-7-stigmasterol	58 ± 12	57 ± 11	48 ± 10
δ-7-avenasterol	43 ± 9	38 ± 8	37 ± 7
total	3884 ± 777	3785 ± 757	3723 ± 745
tocopherol and tocotrienol content (mg/kg fat)			
α-tocopherol acetate	<5	<5	<5
α-tocopherol	113 ± 17	108 ± 17	104 ± 16
β-tocopherol	26 ± 5	27 ± 5	27 ± 5
γ-tocopherol	842 ± 127	872 ± 131	873 ± 131
δ-tocopherol	410 ± 62	413 ± 62	417 ± 63
α-tocotrienol	<2	<2	<2
β-tocotrienol	<2	<2	<2
γ-tocotrienol	3 ± 2	<2	<2
δ-tocotrienol	<2	<2	<2
total	1394 ± 209	1420 ± 213	1421 ± 213
properties			
antioxidant activity (mg TE/kg fat)	76 ± 3	218 ± 11	300 ± 15
oxidative stability (h)	14.1 ± 2.1	18.3 ± 2.7	15.9 ± 2.4

same for soybeans and defatted meals, indicating that the solvents did not altered protein chain length.

The PDI of soybeans and defatted meal were evaluated as it is an important indicator in the industry. Generally, for further use in food products, the extracted soybeans should have a PDI higher than 80%, which indicates a good preservation of protein functionalities. For feed, however, the PDI is generally below 40% as a result of the treatment (high temperature and toasting) applied to destroy or disactivate the antinutritional factors (ANFs).[49] The PDI of soybeans was 73.9%, which is quite low for raw material. This is certainly due to deterioration during storage. Values for meals defatted with hexane and 2-MeOx were only slightly lower, as they were desolventized at gentle temperature without steam. However, the PDI of 2-MeOx 95.5% defatted meal was much lower (47.4%). This is probably due to the presence of free water in the solvent, combined with a long drying time (3 h) that induced higher protein deterioration during desolventization. Indeed, the PDI is known to be sensitive to moisture and heat.[50] It is important to note that the experimental desolventization conditions used in this study are drastically different from what is done at larger scale; therefore, results are likely to be very different with industrial equipment.

KOH protein solubility was close to 90% for soybean seeds and around 80% for the three defatted meals. These numbers are within the 73−85% range, which is generally recommended for soybean meals.[39] Nevertheless, it is worth mentioning that KOH protein solubility is considered to be a good indicator of over processing of soybean meal, but it is not sensitive enough to detect under processing.

Even if they exhibit high protein and lipid content, soybeans also contain antinutrient or antinutritional factors (ANFs) that can interfere with nutrient digestibility by humans or animals. The most abundant and best known ANF in soybeans are trypsin inhibitors (TI), which reduce the digestibility of dietary proteins and cause pancreatic hypertrophy.[5] They can be inactivated by proper heating of the product. Soybean seeds also contain phytates or phytic acid, that bind to essential minerals and make them unavailable for absorption and utilization. Urease index (UI) is a common indicator of the efficacy of destruction, or inactivation, of many ANFs present in raw soybeans. Typical recommendation for correctly processed meal are between 0.01 and 0.35 pH unit rise.[39] The UI values obtained for soybean seeds and defatted meals were within the range 2.35−2.39, meaning that samples were not heated enough to inactivate ANFs. This is not surprising as products were not toasted at all. Accordingly, the same trend was observed regarding phytic acid content and TI activity.

Again, desolventization conditions used in the industry are drastically different from what was done in this study. Indeed, for feed industry, the use of a desolventizer-toaster with steam injection enables solvent removal and cooking at 100−105 °C for short time, in the range of 15−30 min.[51] For the food industry, flash desolventizers are commonly used, in which solvent is also removed but without toasting. Toasting time, temperature, and moisture conditions directly affect protein quality of the resulting meal. Therefore, desolventization conditions are a crucial point to consider with the objective of replacing hexane by an alternative solvent for lipid extraction.

Phenolic Compounds. Phenolic compounds of soybean seeds, defatted meals, and crude oils have been identified and quantified by UPLC DAD/ESI-MS^n. Individual and total phenolic contents of samples were expressed as GE per kg of fat, or per kg of dry, and defatted solid sample. Results are reported in Table 6. Phenolic compounds identified in samples were isoflavones that are typically found in soybeans and soybean products, derived from the three aglycones daidzein, genistein, and glycitein.[5,52] The major isoflavones detected in defatted meals were the same as those found in soybean seeds, that is, daidzin, genistin, daidzein malonyl glucoside, and genistein malonyl glucoside. Total isoflavone contents (TICs) of solid samples were found to increase in the following order: defatted meal 2-MeOx 95.5%, defatted meal 2-MeOx, defatted meal hexane, and soybeans. TICs of crude oils logically increase in the reverse order: hexane < 2-MeOx < 2-MeOx 95.5%. These results show that 2-MeOx and 2-MeOx 95.5% extracted more phenolic compounds than hexane. This can be explained by a higher solubility of isoflavones in these solvents, as predicted by COSMO-RS. The major compounds detected in 2-MeOx and 2-MeOx 95.5% crude oils were the same as those found in soybean seeds. Aglycones, daidzein and genistein, were also found at significant levels in these oils, whereas their level was negligible in soybean seeds. Interestingly, the hexane crude oil contained only traces of a few compounds, but not genistein malonyl glucoside, the most abundant in soybeans.

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Table 5. Composition and Quality of Soybean Seeds and Meals Defatted with Hexane, 2-MeOx and 2-MeOx 95.5%[a]

	soybean seeds	defatted meal hexane	defatted meal2-MeOx	defatted meal2-MeOx 95.5%
		proteins		
crude protein (g/100 g DDM)	54.07 ± 0.06	55.48 ± 0.37	56.60 ± 0.19	55.94 ± 0.32
PDI (%)	73.9 ± 1.1	70.7 ± 0.4	67.9 ± 1.2	47.4 ± 0.6
KOH protein solubility (%)	89.8 ± 1.2	80.3 ± 0.4	82.8 ± 0.8	78.6 ± 0.9
amino acid content (g/100 g DDM)				
tryptophan	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	1.1 ± 0.2
threonine	2.1 ± 0.3	2.2 ± 0.3	2.4 ± 0.3	2.2 ± 0.3
aspartic acid	6.3 ± 0.9	6.4 ± 0.9	7.0 ± 1.0	6.7 ± 0.9
serine	2.8 ± 0.4	2.8 ± 0.4	3.1 ± 0.4	2.9 ± 0.4
lysine	3.6 ± 0.5	3.5 ± 0.5	3.7 ± 0.5	3.5 ± 0.5
valine	2.5 ± 0.3	2.5 ± 0.3	2.7 ± 0.3	2.7 ± 0.3
proline	2.8 ± 0.4	2.7 ± 0.4	2.9 ± 0.4	2.9 ± 0.4
alanine	2.3 ± 0.3	2.3 ± 0.3	2.5 ± 0.4	2.4 ± 0.3
phenylalanine	2.8 ± 0.4	2.8 ± 0.4	3.0 ± 0.4	2.8 ± 0.4
isoleucine	2.3 ± 0.3	2.3 ± 0.3	2.6 ± 0.4	2.4 ± 0.3
glycine	2.3 ± 0.3	2.3 ± 0.3	2.5 ± 0.3	2.4 ± 0.3
tyrosine	2.1 ± 0.3	2.2 ± 0.3	2.1 ± 0.3	1.9 ± 0.3
arginine	4.3 ± 0.6	4.3 ± 0.6	4.5 ± 0.6	4.5 ± 0.6
leucine	4.0 ± 0.6	4.1 ± 0.6	4.5 ± 0.6	4.2 ± 0.6
histidine	1.5 ± 0.2	1.5 ± 0.2	1.5 ± 0.2	1.5 ± 0.2
glutamic acid	10.1 ± 1.4	10.4 ± 1.4	10.7 ± 1.5	10.6 ± 1.5
methionine	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1
cysteine + cystine	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1
total	54.2 ± 7.6	54.9 ± 7.7	58.2 ± 8.1	56.4 ± 7.9
		antinutritional factors		
urease index (pH unit rise)	2.36 ± 0.01	2.35 ± 0.02	2.39 ± 0.02	2.35 ± 0.01
phytic acid content (g/100 g DDM)		1.5 ± 0.3	1.6 ± 0.3	1.5 ± 0.3
TI activity (TI units/mg DDM)		51.9 ± 18.2	50.7 ± 17.7	52.2 ± 18.3

[a]DDM, totally defatted and dry material; PDI, protein dispersibility index; TI, trypsin inhibitors.

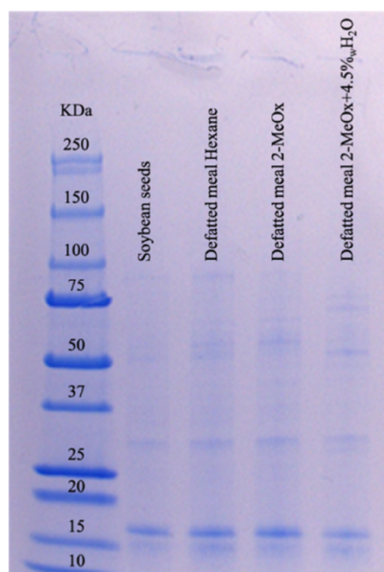

Figure 2. Molecular weight distribution of water-soluble proteins of soybean seeds and meals defatted with hexane, 2-MeOx, and 2-MeOx 95.5%.

It is worth mentioning that isoflavones are quite controverted species because of their estrogen-like activity. On the one hand, they have various beneficial health effects for menopausal women and seem to play a role in preventing certain cancers, such as breast, prostate, and colon cancer.[5] On the other hand, they have already been connected with negative health effects on animals because of their anti-estrogenic properties.[4,6] Therefore, a defatted meal with reduced isoflavone content could be considered to be of higher quality for feed use. Also, crude oils enriched in isoflavones could be easily refined to isolate phenolic compounds, which could be further valorized separately.

Kinetic Study. After quality evaluation of the extraction products, it is important to measure the impact of replacing hexane by 2-MeOx on the extraction process to determine if this replacement would be feasible at industrial scale. Extraction kinetics are of particular importance, as they directly affect residence times inside the extractors and therefore plant productivity.

Extraction kinetics were evaluated with hexane, 2-MeOx, and 2-MeOx 95.5% at 25 and 55 °C, and with 2-MeOx and 2-MeOx 95.5% at 65 °C. Plots of both solute concentration in the solvents and $\ln\left(\frac{M_\infty}{M_\infty - M_t}\right)$ over time (see Figure 3) reveal two different steps during extraction. First, there is a fast stage (0–20 min) with a high diffusion coefficient. This can be associated with a washing step of the oil readily available at the surface of the matrix, and where the extraction is only limited by the transfer from the interface to the solvent (bulk). Once this washing step is over, a second stage takes place at a much slower pace, with lower diffusion coefficient. The limiting step for diffusion is now oil diffusion through the cell walls. A similar pattern for extraction of vegetable oil from milled vegetable material in two steps has already been described by Sovóv.[53] The corresponding diffusion coefficients for each period are represented in Figure 3.

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Table 6. Peak Assignments, Phenolic Composition, and TPC of Soybean Crude Oils, Soybean Seeds and Meals Defatted with Hexane, 2-MeOx, and 2-MeOx 95.5%[a]

				concentration in samples (mg GE/kg DDM or Fat)						
					defatted meals			crude oils		
compounds/content	t_R (min)	$[M + H]^+$ (m/z)	MS fragment ion (m/z)	soybean seeds	hexane	2-MeOx	2-MeOx 95.5%	hexane	2-MeOx	2-MeOx 95.5%
daidzin	6.3	417.1	255.1	220 ± 8	201 ± 21	95 ± 8	53 ± 4	tr	240 ± 4	228 ± 12
glycitin	6.6	447.2	285.0	14 ± 2	17 ± 2	12 ± 5	tr	n.d.	32 ± 1	15 ± 2
daidzein malonyl glucoside	7.1	503.2	255.0	32 ± 5	42 ± 3	44 ± 3	35 ± 3	n.d.	4 ± 1	tr
genistin	7.3	433.2	271.0	469 ± 33	454 ± 51	172 ± 21	91 ± 4	tr	577 ± 8	567 ± 50
daidzein malonyl glucoside	7.9	503.2	255.1	572 ± 17	667 ± 34	655 ± 8	539 ± 32	tr	119 ± 2	166 ± 13
glycitein malonyl glucoside	8.1	533.2	285.1	27 ± 9	30 ± 9	35 ± 5	34 ± 5	tr	tr	tr
genistein acetyl glucoside	8.3	475.3	271.0	n.d.	n.d.	n.d.	n.d.	tr	tr	tr
daidzein acetyl glucoside	8.5	459.2	255.0	n.d.	n.d.	tr	n.d.	tr	16 ± 1	14 ± 2
genistein malonyl glucoside	8.6	519.2	271.0	1152 ± 17	1321 ± 42	1137 ± 14	921 ± 10	n.d.	519 ± 4	805 ± 106
daidzein	9.3–9.4	255.0		16 ± 2	16 ± 2	26 ± 1	16 ± 1	n.d.	98 ± 3	116 ± 17
glycitein	9.6	285.1		tr	tr	tr	tr	n.d.	tr	7 ± 2
genistein	10.2	271.0		15 ± 2	15 ± 2	tr	12 ± 1	tr	68 ± 1	116 ± 10
total				2519 ± 91	2762 ± 163	2192 ± 63	1710 ± 58	14 ± 2	1684 ± 26	2044 ± 213

[a]n.d., not detected; tr, traces (<10 mg GE/kg DDM or fat).

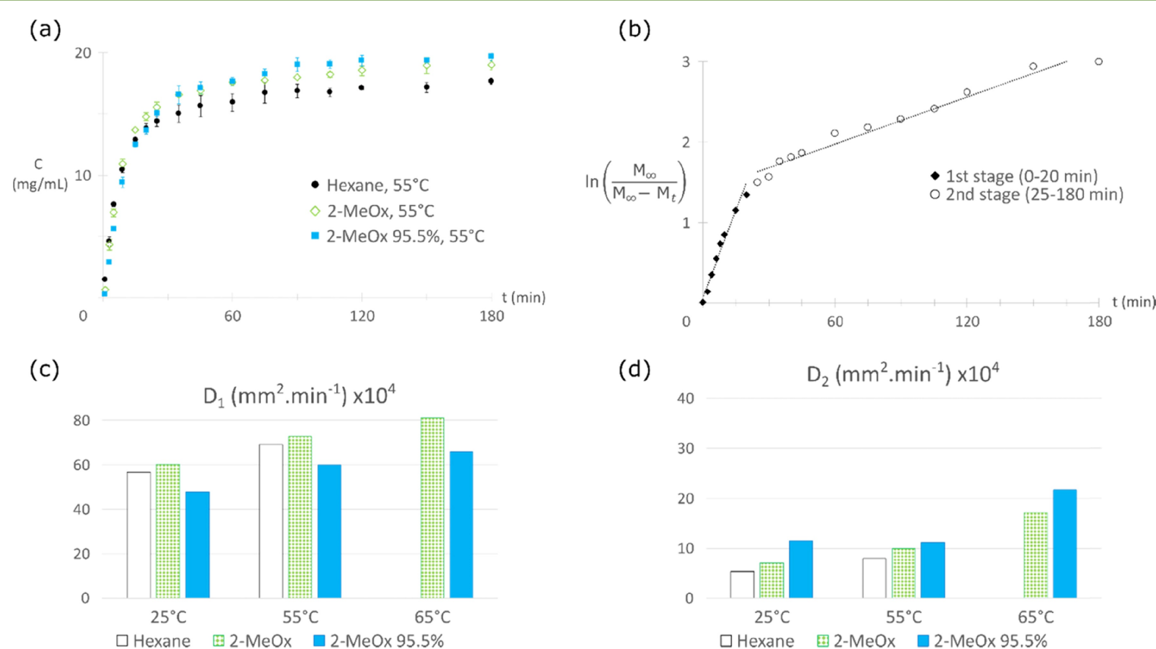

Figure 3. (a) Evolution of solute concentration in the solvent at 55 °C; (b) example of plot of $\ln\left(\frac{M_\infty}{M_\infty - M_t}\right)$ over time obtained with the data of 2-MeOx at 55 °C; (c) diffusion coefficients of the first stage of extraction; (d) diffusion coefficients of the second stage of extraction.

As the temperature rises, diffusion coefficient logically increases. The data also indicate that 2-MeOx allows slightly faster diffusion than hexane at any temperature and for both stages. Interestingly, 2-MeOx 95.5% gave lower values during the first stage, and higher values during the second stage. One could assume that during the first stage, the water contained in the solvent slows the extraction process by transferring within the solid matrix, the opposite way that the oil transfers from the solid to the solvent. Once water equilibrium is reached, the oil diffusion continues with still a lot of fat to extract from the matrix, which explains the higher second-stage coefficient compared to the other solvents. This theory is strengthened by the observation of a swelling of the solid matrix after extraction with this solvent (Figure 5). This swelling could reduce the extraction rate by increasing the distance that the solvent needs to penetrate, but it could be compensated by increasing pore size in the matrix.

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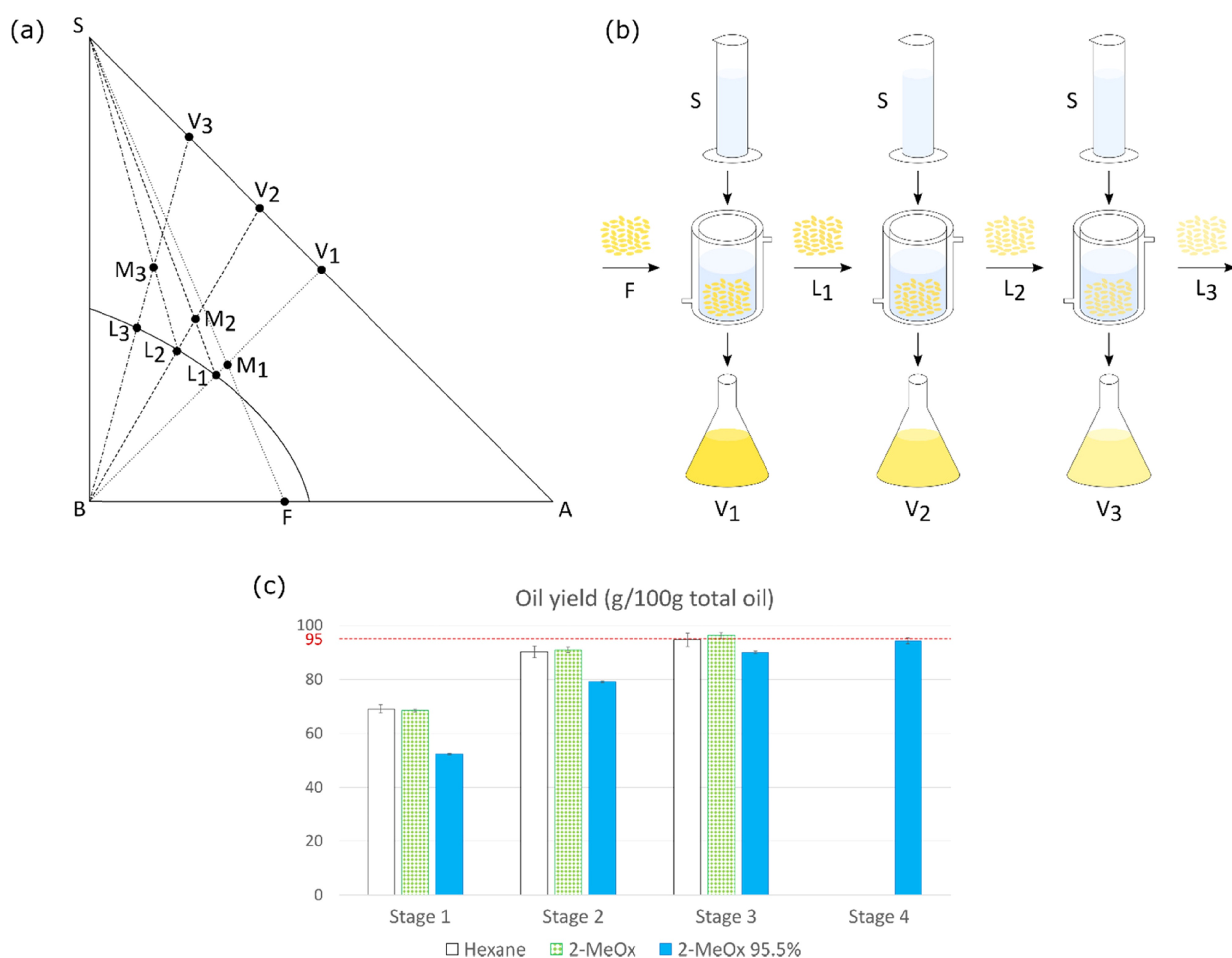

Figure 4. (a) Right-angle triangle diagram of solid/liquid extraction; (b) scheme of multistage cross-current extraction of soybean seeds; (c) cumulated oil yields obtained in multistage cross-current extraction.

It is also interesting to note that 2-MeOx and 2-MeOx 95.5% show, respectively, higher and similar diffusion coefficients at 65 °C than hexane does at 55 °C. Therefore, the industrial extraction times could be improved using 2-MeOx at 65 °C instead of hexane that cannot be used above 55 °C to avoid evaporation or even boiling.

Multistage Cross-Current. The previous kinetic study was conducted considering one stage of extraction. Nevertheless, industrial processes for vegetable oil extraction generally involve several extraction stages. Therefore, it is essential to compare performances of solvents through several stages of extraction. Even if counter-current extractors are mainly used industrially for continuous oil production, multistage cross-current extractions were chosen for practical reasons at lab scale. Also, this extraction design is industrially performed during small production campaigns of precious vegetable oils for cosmetic uses.

Graphical Representation of Solid−Liquid Extraction. Solid−liquid extraction equilibrium can be represented on a right-angle triangle diagram as seen in Figure 4. The three vertices represent the pure compounds: the solvent S, the solute (oil) A, and the inert solid (soybeans) B. The three sides stand for binary mixtures and the inside of the diagram represents ternary mixtures.

During cross-current extractions, the initial soybean feed (F = B + A) was put in contact with solvent (S) for a period of time judged long enough to reach thermodynamic equilibrium (1 h). Then, the overflow (V = S + A), also called miscella, was weighed and evaporated to evaluate the solute content. The underflow (L = B + A + S) was also weighed to determine the solution uptake by the inert material. Then, the solid was further extracted with similar stages of extraction with clean solvent, as represented in Figure 4.

For a better understanding of the process, the diagram was constructed stage after stage. First, the feed F was placed on the diagram and the segment $\overline{FS}$, representing the first stage inflows, was plotted. The point M_1 can be defined from the material balance of this stage:

$$F + S = L_1 + V_1 = M_1\# \tag{5}$$

Graphically M_1 is an equilibrium point, situated at the intersection of $\overline{FS}$ and $\overline{L_1V_1}$. As the mass quantity of feed and solvent were known, M_1 was found based on the following formulas:

$$F \times \overline{FM_1} = S \times \overline{M_1S}\# \tag{6}$$

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$$\bar{FS} = \bar{FM_1} + M_1\bar{S\#} \quad (7)$$

These equations lead to:

$$\bar{FM_1} = \frac{S}{F + S} \times \bar{FS\#} \quad (8)$$

Then, the intersections of $B\bar{M_1}$ with the underflow curve and the hypotenuse should respectively give the points L_1 and V_1, outflows of the first stage. The equilibrium (F, M_1, L_1, V_1) is then replaced by (L_1, M_2, L_2, V_2) for the second stage, that becomes (L_2, M_3, L_3, V_3) for the third stage, and so on and so forth.

Experimental Results. Multistage cross-current extractions were performed on soybean flakes using hexane, 2-MeOx, and 2-MeOx 95.5% at 55 °C. The extractions were continued until the oil yield reached 95% of the initial oil content of the solid matrix. The cumulated oil yields are presented in Figure 4. Hexane and 2-MeOx gave similar results: 3 stages were necessary to extract the desired quantity of solute with these solvents, when 4 were necessary for 2-MeOx 95.5%. This difference is likely to be due to the quantity of solution absorbed by the flakes, which was more important in the case of 2-MeOx 95.5%. Indeed, it was observed that the solid matrix tends to swell during extraction with this solvent. In the light of these results, dry 2-MeOx could be used in replacement of hexane at industrial scale without impact on oil yield for the same number of stages, while the use of 2-MeOx 95.5% would require further optimization to take into account the swelling effect.

CONCLUSIONS AND PERSPECTIVES

The potentials of 2-MeOx and 2-MeOx 95.5% were compared to that of hexane for the extraction of soybean oil and the coproduction of defatted soybean meal.

As a preliminary step, solubilities of some target molecules found in soybean oils were evaluated using COSMO-RS, and the ternary phase diagram soybean oil−water−2-MeOx was established to make sure that 2-MeOx 95.5% could be used for oil extraction.

Experimentally, both 2-MeOx and 2-MeOx 95.5% gave higher crude extraction yields compared to hexane with 23.5 ± 0.1 and 23.7 ± 0.1 vs 18.8 ± 0.1 g/100 g DM, respectively.

A complete analysis of the composition of crude oils revealed that the main constituents were the same, and yield differences were attributed to the coextraction of more polar additional compounds such as phospholipids and isoflavones. Those experimental results were in line with COSMO-RS predictions. As explained earlier, production of crude soybean oils with higher phospholipids content may or may not be an added value, depending on how they are valorized. Crude oils extracted with 2-MeOx and 2-MeOx 95.5% contained significant amounts of isoflavones, that is, 1684 ± 26 and 2044 ± 213 mg GE/kg fat respectively, while only traces were found in the hexane-extracted oil. The presence of these additional compounds is likely to be responsible for the enhanced antioxidant activity and oxidative stability exhibited by 2-MeOx and 2-MeOx 95.5% crude oils. The quality of the crude extracts was also evaluated through determination of standard quality parameters for oil samples (iodine, acid, peroxide and *p*-anisidine values and conjugated dienes level). However, it is hard to draw conclusions upon the deterioration state of samples as interferences are suspected. Nevertheless, this highlights the fact that standard methods for oil quality assessment might not be suitable, and it could be relevant to define new quality standards for samples extracted with 2-MeOx and 2-MeOx 95.5%.

Regarding the defatted meals, no significant difference was observed in terms of protein, amino acid content, and ANFs. The PDI value of hexane and 2-MeOx samples were similar, while the 2-MeOx 95.5% sample gave a much lower PDI value (70.7 ± 0.4, 67.9 ± 1.2 and 47.4 ± 0.6, respectively). This difference was attributed to the desolventization conditions and emphasized the difficulty to mimic the drying conditions at lab scale versus the drying conditions at larger commercial scale.

The kinetic study and multistage cross-current extractions showed that hexane and 2-MeOx gave similar results. However, 2-MeOx 95.5% gave a slower extraction rate and generated a higher solution uptake by the vegetable matrix, requiring a few more stages.

Overall, results of this study reveal that 2-MeOx has a good potential to replace hexane for the extraction of soybean oil, as this alternative solvent provide extracts with similar composition and exhibits a similar behavior during extraction.

However, 2-MeOx 95.5% would be more convenient and cost-effective to use industrially in a continuous extraction. Moreover, the composition and quality of the extracts were also similar to those of hexane. However, the use of 2-MeOx 95.5% generates more swelling of flakes (see Figure 5) and induces a more complex diffusion phenomenon. Further investigation is needed to better mimic the industrial process (percolation instead of immersion, counter-current instead of cross-current extractions, much shorter drying time) and assess which modifications are needed to adapt the extraction process, which has been optimized with hexane for 70 years, to this new bio-based solvent.

Figure 5. Cellulose cartridges initially filled with the same quantity of soybean seeds after a 2-h Soxhlet extraction with 2-MeOx (left) and 2-MeOx 95.5% (right).

The Agenda 2030 issued by the United Nations (UN) in 2015 emphasized the importance of sustainability, whether through

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Figure 6. 2-MeOx chain value and its potential positive contributions to SDGs.

its ecological, economic, or social dimensions. With the aim of addressing the daunting challenges that the world is currently facing, 17 Sustainable Development Goals (SDGs) were adopted. The SDGs represent global guidelines for both public and private actors that should enable overcoming poverty, hunger, and inequalities, mitigating environmental degradation and climate change and boosting economic growth and global development. As illustrated in Figure 6, 2-MeOx can contribute to meet 11 of the 17 SDGs all along its value chain.[17]

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Notes

The authors declare no competing financial interest.

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