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Extraction of kiwi-seeds oil: Soxhlet versus four different non-conventional techniques

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Kiwi-seeds oil has a nutritionally interesting fatty acid profile, but a rather low oxidative stability, which requires careful extraction procedures and adequate packaging and storage. For these reasons and with the aim to achieve a process intensification with shorter extraction time, lower energy consumption and higher yields, four different non-conventional techniques were experimented. Kiwi-seeds were extracted in hexane using classic Soxhlet as well as under power ultrasound (US), microwaves (MWs; closed vessel) and MW-integrated Soxhlet. Supercritical CO_2 was also employed and compared to the other techniques in term of yield, extraction time, fatty acids profiles and organoleptic properties. All these non-conventional techniques are fast, effective and safe. A sensory evaluation test showed the presence of off-flavours in oil samples extracted by Soxhlet and US, an indicator of partial degradation.

Keywords: kiwi seed oil**;** ultrasound**;** microwaves**;** supercritical CO_2**;** microwave integrated Soxhlet**;** GC/MS

1. Introduction

Current techniques of solid-liquid extraction of plant material are essentially based on diffusion and osmosis. The usual ways to shorten extraction times and improve yields are to increase the temperature of the treatment and/or to repeat it several times with fresh solvent. The simplest and cheapest technique is maceration, where the surface area of a solid matrix is increased and then the material is soaked in a liquid to which it releases its soluble components. At any rate, since local saturation at the matrix surface has to be avoided, stirring must also be applied to improve diffusion. Conventional extraction processes are time-consuming and laborious; they require large volumes of solvents and may cause thermal decomposition of some target molecules and partial loss of volatiles. Great improvements may be in the offing with non-conventional techniques and their combinations; each matrix however need careful optimization of operating conditions (Cravotto & Cintas, 2007). The application of ultrasound (US) to enhance extraction yields was first applied in the 1050s at the laboratory level. It has been shown to improve the extraction from plant materials of oils and bioactive compounds (Vinatoru, Toma, & Mason, 1999), mainly through the phenomenon of cavitation. The mechanical effect of US accelerates the release of organic compounds contained within the plant body by disrupting cell walls and enhancing mass transfer. The use of US to extract lipidic fraction (Cravotto, Binello, Merizzi, & Avogadro, 2004; Cravotto, Boffa, Mantegna, Perego, & Cintas, 2008) and flavours (Chemat et al., 2004b) from plants gives great advantages in terms of both time saving and quality of the extracts. Extraction time mainly depends on diffusion rates, in this respect US contributes to the speeding up of the process by breaking vegetal tissues as well as, with dried matrices, by re-hydrating and swelling them. Laboratory US extraction can be obtained either by simple baths or by the most effective probe systems, while, on an industrial scale either large cavitating tubs similar to giant US baths, with transducers applied underneath, or mechanically stirred cylindrical reactors with transducer plates assembled on the lower part of the wall, are usually adopted.

The use of dielectric heating in analytical laboratories started in the late 1970s and the food industry first applied this technology. Microwave (MW) heating of fresh plant material is a simple way to obtain a hydrodistillation-like product of essential oils. It can also strongly enhance extractions with organic solvents either MW-absorbing or transparent. MW-assisted liquid-phase extraction is based on the difference in MW absorbance by materials with different dielectric constants. The matrix to be extracted (usually water-rich) is mixed with a solvent with a low dielectric constant, so that most of the heating effect are concentrated on the plant material. MW-assisted extraction requires less solvent and less time, producing better products at lower costs.

Recently Virot et al., described the MW-integrated Soxhlet (MIS; Virot, Tomao, Colnagui, Visinoni, & Chemat, 2007). It is a new multi-mode/open vessel lab-station for a rapid and complete

extraction of fats and oils. The principle is relatively easy and involves the use of a polytetrafluoroethylene/graphite (Weflon®, Milestone, Italy) stir bar placed in the base vessel, able to absorb MW energy and thus heat transparent solvent such as *n*-hexane. The dried sample matrix is then placed on a Teflon® support inside the base vessel and covered with the extraction solvent. The base vessel is fitted with an extraction tube and a condenser. The extraction tube is fitted up with a three way valve allowing the solvent, after being condensed with the condenser, to either return in the base vessel or collected.

Supercritical Fluid Extraction (SFE), replaces common organic solvents with a gas (typically CO_2) that, under supercritical conditions (SC-CO_2), behaves like a non-polar solvent. In spite of the large outlay for apparatus, this method is widely being practised in the food and beverage industries. Many industrial plants use SC-CO_2 to extract caffeine from coffee and tea (de Azevedo et al., 2008; W. Kim, J. Kim, % Oh, 2007), beer flavouring agents from hops (Bravi, Perretti, Montanari, Favati, & Fantozzi, 2007) or nicotine from tobacco (Rincon et al., 1998) and separating oils and oleoresins from spices (Rout, Naik, Rao, Jadeja, & Maheshwari, 2007). Due to its good solvent and transport properties (low viscosity and high diffusivity), SC-CO_2 seems to be one of the best candidates for extraction of natural compounds. One of the greatest hurdles besetting SFE with raw plant materials is the very slow kinetics of the process (Brunner, 1994). The classical way to accelerate the process is by mechanical stirring or US transducers, the latter being technically complex.

A comparison between the above mentioned non-conventional techniques in the extraction of the oil from kiwi seeds is discussed here; in particular the following techniques are compared: US-assisted extraction, MW-assisted extraction (MAE), SC-CO_2-SFE, MIS and conventional Soxhlet extraction. The kiwi fruit is the edible berry of a cultivar group of the woody vine of several Actinidia species. The most common commercially available, green-fleshed kiwi fruit is the 'Hayward' cultivar, belonging to the *Actinidia deliciosa* species. Kiwi fruits are known to contain high amounts of protective phytochemicals, including vitamin C and E, flavonoids, phytosterols and ursolic acid. On the other hand, they are known to induce allergy: until now, seven allergens from green kiwi fruit have been identified and designated according to the guideline of the International Union of Immunological Societies Allergen Nomenclature Subcommittee (IUIS, http://www.allergen.org) (Oberhuber et al., 2008; Tanaka, Shan, Kasajima, & Shimoda, 2007). Several studies described the aroma of kiwi fruit that results from the combination of at least 26 different volatile compounds, among them ethyl butanoate, unsaturated C-6 aldehydes and alcohols (Talens, Escriche, Martinez-Navarrete, & Chiralt, 2003). About 90% of the volatile constituents are derived from lipid degradation. With the cell wall breakage, the lipolytic enzymes leak from the cell compartments and split the alkyl-lipids; the free fatty acids are then degraded to the aroma compounds (Pfannhauser, 1988). The kiwi fruit seeds do not show biological activities when

ingested with sarcocarps. The activity of the kiwi seed extract was successfully tested on inflammation and melanin production (Mills, Jenkins, Alocer, & Shewry, 2004). This oil is an interesting component for food supplements and cosmetic preparations owing to its high omega-3 fatty acids content.

2. Results and Discussion

In spite of a rich literature on kiwi fruit (Fiorentino et al., 2009), few data are reported on kiwi-seed oil. Recently Van Hoed et al. published a study on the composition and the oxidative stability of several seed oils (Van Hoed et al., 2009), among blackberry, blueberry, cranberry, strawberry, red raspberry and kiwi seed oils. The oxidative stability of kiwi seed oil was rather low (0.17 h) due to the high amount of polyunsatured fatty acids (PUFAs) (57% in linolenic acid) and the low content of tocols (tocopherol + tocotrienol 35 mg kg^{-1}).

In this study, kiwi seeds were extracted with hexane with four techniques: Soxhlet, MIS, MW irradiation and US irradiation. SFE with SC-CO_2 was also used.

Table 1 reports the fatty acid composition of the oil content obtained by MW, US and SC-CO_2 extractions compared to conventional Soxhlet extracts. The results provided by these techniques are similar to those obtained by Soxhlet. Oil composition was equivalent in term of the number of components and relative amounts for both extraction methods. A higher percentage in omega-3 fatty acids has been found in the kiwi-seed oil obtained by MW and US extractions compared to conventional Soxhlet and SC-CO_2 extractions. MW irradiation accelerated the extraction process (Table 2) without inducing noticeable changes in the kiwi seed oil composition (Pare & Belanger, 1997).

Table 1 Comparison of fatty acids compositions for kiwi extraction functions of each process used.

No.	Fatty acids	Soxhlet (%±SD)	Microwave (%±SD)	SC-CO_2 (%±SD)	Ultrasound (%±SD)	MIS (%±SD)
1	C16:0	8.02 ± 0.10	6.02 ± 0.09	8.46 ± 0.01	5.72 ± 0.03	6.08 ± 0.04
2	C18:0	4.55 ± 0.09	3.30 ± 0.07	3.09 ± 0.1	3.22 ± 0.09	3.20 ± 0.05
3	C18:1 *n*-9 *cis*	18.79 ± 0.12	14.46 ± 0.09	18.00 ± 0.17	14.09 ± 0.07	14.34 ± 0.02
4	C18:1 *n*-7	1.10 ± 0.05	0.72 ± 0.02	0.82 ± 0.02	0.70 ± 0.04	0.69 ± 0.01
5	C18:2 *n*-6	14.08 ± 0.04	14.91 ± 0.03	18.43 ± 0.08	14.86 ± 0.03	15.17 ± 0.02
6	C18:3 *n*-3	53.46 ± 0.16	60.59 ± 0.14	51.20 ± 0.36	61.41 ± 0.19	60.51 ± 0.02
	∑ SFAs	12.57	9.32	11.55	8.94	6.12
	∑ MUFAs	19.89	15.18	18.82	14.79	15.03

∑ PUFAs	67.54	75.50	69.63	76.27	75.68
MUFAs / PUFAs	0.29	0.20	0.27	0.19	0.20

Note: SFAs: saturated fatty acids; MUFAs: mono-unsaturated fatty acids.

Table 2 Extraction time, temperature, yield and sensory evaluation.

	Soxhlet	**MW**	**SC-CO_2**	**US**	**MIS**
Oil content (oil/seed w % ±SD)	28.3±1.0	27.8±1.0	26.8±0.5	28.9±1.0	28.0±1.0
Time	8 h	20 min	2.5 h	30 min	30 min
Temperature	69°C[a]	80°C	40°C	50°C	69°C[a]
Colour	Pale yellow	Yellow	Pale yellow	Yellow	Pale yellow
Olfactive note	1	4	5	2	3

Note: [a]Hexane boiling point.

With the aim to evaluate the oxidative degradation in kiwi-seed oil, sensory evaluation of five samples extracted by either MW, US, SC-CO_2, MIS or Soxhlet was conducted by 12 trained panellists who were graduate students and staff members in the laboratory UMR408, University of Avignon, France. Randomly coded samples were individually served to panelists. One sensory attribute (olfactive note in Table 2) was evaluated (generation of off-flavours) using a 5-point hedonic scale for each trait where 5 means absence of off-flavours and 1 corresponds to the greatest detectable presence of off-flavours. The sensory results show that kiwi seed oil extracted by Soxhlet and US were oils partially degraded. In contrast, no degradation of kiwi seed oil was detected by the panelists for oils extracted by SC-CO_2 or MW. The explanation for generation of off-flavours is probably related to the amount of unsaturated fatty acids in the edible oils, and more precisely to the presence of limonene typical indicators for lipid oxidation. The metallic and rancid odour detected after oil treatment by US was due to the formation of (*Z*)-hept-2-enal and (2*E*,4*E*)-deca-2,4-dienal which give a fishy-sweet and a deep-fried odour. These results extend the findings reported in literature (Chemat et al., 2004a) where, after sonication of sunflower oil with a titanium alloy rod, the authors identified limonene, aldehydes and 2-methylfuran in the resulting extracts.

3. Experimental

3.1. *Materials and methods*

In this study, *n*-Hexane and *n*-heptane were all analytical grade and purchased from Carlo Erba Reagents (Italy). BF_3-methanol reagent (20% solution methanol); analytical grade NaOH and NaCl

were supplied by VWR International. Kiwi seeds were kindly supplied by Rivoira Frutta (Piasco - CN, Italy). The peeled fruits were frozen and immediately placed in water at room temperature under mechanical stirring. After a few hours, the seeds were separated from the pulp by centrifugation and dried.

3.1.1. *Ultrasound-assisted extraction*

Twenty grams of dried and ground samples were weighed to the nearest 10 mg. Seed powder and *n*-hexane (400 mL) were placed in a cylindrical reactor (700 mL) where the US-titanium horn is dipped for about 2 cm below the level of the liquid. This equipment enables to work under modified atmosphere (Cravotto, Omiccioli, & Stevanato, 2005); a weak nitrogen stream has to be applied to extract PUFA-rich oils. The mixture was sonicated for 30 min at 80 W. The temperature was increased to 50°C in 10 min and kept constant by a cooling system. The mixture was then filtered on a paper filter and the filtrate concentrated to dryness under vacuum with a rotary evaporator and weighed to the nearest milligram.

3.1.2. *Microwave-assisted extraction*

Twenty grams of dried and ground samples were weighed to the nearest 10 mg. Six aliquots of seed powder and n-hexane (50 mL x 6) were placed in six closed vessels (90 mL, thermowells - Milestone) to which 50 mg of charcoal were then added. As hexane and the oil itself are transparent to MW radiation, the addition of charcoal (excellent MW adsorbent) was necessary to heat the samples. All vessels were irradiated with MW for 20 min at 80°C. After extraction, all samples were filtered on a paper filter and the filtrates collected in the same flask. The resulting solution was evaporated to dryness under vacuum with a rotary evaporator and weighed to the nearest milligram.

3.1.3 *Supercritical CO_2 extraction*

SFE was run with a high-pressure through-flow apparatus (SCE-100, Separeco - Italy) with supercritical carbon dioxide (CO_2). One hundred and fifty grams of dried and ground samples were weighed to the nearest 10 mg. Samples were placed in the extractor (300 mL) and hermetically sealed. Gaseous CO_2 was compressed and stored in a storage vessel. The SC-CO_2 was pumped into the extractor to soak the seeds powder at 400 bar and 35°C for about 1 h. After extraction, the oil mixture and CO_2 was pumped into the separator and depressurized. The oil was separated from the evaporating CO_2 and collected in a cooled container. The whole cycle entailed about 2.5 h.

3.1.4. *Conventional Soxhlet extraction*

Thirty grams of dried and ground samples were weighed to the nearest 10 mg of sample. The amount was transferred in a cellulose thimble and placed in the extraction chamber. The Soxhlet apparatus, fitted with a condenser, was placed on a distillation flask containing 300 mL of *n*-hexane. Samples were extracted under reflux for 4 h.

Thereafter, the cellulose cartridge was cooled to room temperature in a desiccator and its content milled before transferring it again in the thimble. The above procedure was thus repeated during 2 h until a total extraction time of 8 h (4+2+2h). The content of the distillation flask was then concentrated to dryness under vacuum with a rotary evaporator and the flask was dried in a desiccator and weighed to the nearest milligram.

3.1.5. *MIS extraction*

Thirty grams of dried and ground samples were weighed to the nearest 10 mg. Weflon® particles were placed in the base vessel and a Teflon® filter support was added. The sample was loaded onto the support and 300 mL of hexane was added to cover the sample. The base vessel was then assembled in the MW oven. The condenser was placed on the extraction tube and the system started.

The solvent was heated up to the boiling point by MW and stirred with a Weflon® magnetic stirrer under reflux. After 13 min, the level of the solvent is lowered below the sample thanks to opening the three-way valve. Repeated washing with only clean warm solvent followed the extraction from the sample. After extraction, the level of the solvent may be lowered again to concentrate the extracts. Each extraction has been performed at least three times and mean values have been reported. Results are expressed as a percentage of the weight of oil extracted during extraction relative to the total weight of the dry sample before extraction.

3.2. ***Fatty acid methyl esters derivatives (FAMEs)***

The fatty acid content of extracted oils was determined according to the official procedure (AOCS, 1989). Samples were then filtered through a cellulose-regenerated filter (Alltech, Deerfield, IL, USA) and then injected in a GC system.

3.2.1. *Gas chromatography–mass spectrometry*

FAMEs were separated and identified by gas chromatography coupled with mass spectrometry (GC/MS). GC/MS analyses were carried out using a Shimadzu QP2010 (Kyoto, Japan). The gas chromatograph was equipped with a CP-Wax capillary column 30 m x 0.32 mm, 0.5μm (Varian). The carrier gas (He) velocity was 47 cm s^{-1}. Samples were injected (2 μL) in split mode (ratio 1:15), injector temperature: 250 °C. The oven temperature increased from 60 ◦C (1min) to 180 ˚C at a rate

of 20 °C min^{-1}, then from 180 to 230 °C at 4 °C min^{-1}, and then it was held at 230 °C for 15 min. The mass spectra were recorded at 3 scan/s from *m/z* 50 to 400. The ionisation mode was EI (electronic impact) at 70 eV. Identification of common fatty acids was performed using the NIST'98 [US National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA] mass spectral database and when available versus reference standards (Sigma-Aldrich).

4. Conclusions

The intensification of extraction processes of oils, flavours or bioactive compounds, which means improving their efficiency and cutting down energy consumption, requires more automation and non-conventional energy sources, as well as new, efficient and scalable protocols to be implemented in semi-continuous flow reactors. We showed that the extraction of kiwi seed oil under US, MW, SC-CO_2 and MIS is fast, efficient and safe. The replication of the experiments and the accuracy of measurements were determined by the standard deviation (SD), as reported in both Tables 1 and 2.

The seasonal overproduction of green kiwi fruit offers a huge availability of seeds at a very low cost. Nutritional and cosmeceutical properties of kiwi seed oil are mainly due to its high content of omega-3 fatty acids of the oil (about 60%).

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