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Dossier

Varietal selection of oilseeds: the prospective nutritional and technological benefits
Perspectives offertes par la sélection variétale sur la qualité nutritionnelle et technologique des oléagineux

Characterization of sunflower oils obtained separately by pressing and subsequent solvent extraction from a new line of seeds rich in phytosterols and conventional seeds

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Abstract – In this study we evaluate the chemical composition of sunflower oils obtained separately by pressing and subsequent solvent extraction from a new seeds rich in phytosterols (IASP-18) and conventional seeds (HA-89). Results have shown that the total content of oil was much lower in the IASP-18 (18.1%) than in the conventional (37.5%) seeds. The extraction yield obtained by pressing was as low as 3% in the IASP-18 seeds and 37.5% in HA-89, while in the solvent extraction it was of the same order (~18 wt% on seeds extracted by pressing) for the two types of seeds. No significant changes in the fatty acid composition were found between the oils extracted by the two procedures, but the pressed oils presented significantly lower acidity and larger content of the unsaponifiable fraction. Expressed as free sterols, the total sterols were 37–38% more concentrated in the oils extracted with solvent, reaching amounts of 13 700 and 6500 mg/kg in the IASP-18 and HA-89 oils, respectively. No substantial differences were found in the composition of total sterols analysed as free sterols between the oils extracted with the two procedures, but the contents of free sterols and sterol glycosides were much higher in the oils extracted with solvent.

Keywords: Sunflower oil / pressing / solvent extraction / phytosterols

Résumé – Caractérisation des huiles de tournesol obtenues séparément par pressage et extraction ultérieure par solvant à partir des nouvelles graines riches en phytostérols et des graines conventionnelles. Dans cette étude, nous évaluons la composition chimique des huiles de tournesol obtenues séparément par pressage et ultérieure extraction par solvant, à partir de nouvelles graines riches en phytostérols (IASP-18) et des graines conventionnelles (HA-89). Les résultats ont montré que la teneur totale en huile était beaucoup plus faible dans l'IASP-18 (18,1 %) que dans les des graines conventionnelles (37,5 %). Le rendement d'extraction obtenu par pressage était aussi bas que 3 % dans les graines de l'IASP-18 et de 37,5 % dans les HA-89, tandis que dans le solvant d'extraction, il était du même ordre (~18 % en poids des graines extraites par pressage) pour la deux types de graines. Aucune modification significative de la composition en acides gras n'a été trouvée entre les huiles extraites par les deux procédures, mais les huiles pressées présentaient une acidité significativement inferieur et supérieure teneur de fraction insaponifiable. Exprimé en stérols libres, les stérols totaux étaient 37–38 % plus concentrés dans les huiles extraites avec solvant, atteignant, respectivement, les montants de 13 700 et 6500 mg/kg dans les huiles d'IASP-18 et de HA-89. Aucune différence substantielle n'a été trouvée dans la composition de stérols totaux analysés sous forme de stérols libres entre les huiles extraites avec les deux procédés, mais le contenu de stérols libres et les glucosides de stérols étaient beaucoup plus élevés dans les huiles extraites avec solvant.

Mots clés : Huile de tournesol / pressage / extraction par solvant / phytostérols

Expressed as free sterols, total sterols were 37–38% more concentrated in the solvent extracted oils compared to the pressed oils. The former showed higher contents of free sterols and sterol glycosides and the pressed oils were characterized by higher relative concentrations of sterol esters.

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1 Introduction

Vegetable oils are the richest natural sources of phytosterols, followed by cereal grains and nuts (Piironen *et al.*, 2000). Phytosterols and the related phytostanols are essential components of plant cell membranes. They are C28 and C29 steroidal alcohols and differ from the C27 sterols such as cholesterol, commonly found in animal cell membranes. Plants do also contain cholesterol but only in very small amounts, typically amounting only to 1–2% of the total sterol fraction. Phytosterols are found in plant tissues as free steroids and in a variety of conjugated forms. Through the C3 hydroxyl group the sterols can be found esterified with fatty or phenolic acids, or glycosylated with D-glucose or acyl-glucose (Plumb *et al.*, 2011).

Because of their structural similarity with cholesterol, dietary phytosterols reduce intestinal absorption of cholesterol and contribute to reducing serum cholesterol levels (Plat and Mensink, 2005). In plants, these compounds are involved in membrane fluidity and permeability (Hartmann, 1998; Schaller, 2003) and in embryogenesis (Schrick *et al.*, 2011). As plant hormone-precursors, they play a crucial role in plant growth and developmental processes such as cell division, polarity and morphogenesis (Merah *et al.*, 2012).

Sunflower oil is the fourth oil most produced in the world and its composition has a high potential to be improved for nutritional and industrial purposes through genetically selection and breeding of seeds (Mouloungui *et al.*, 2006; Merah *et al.*, 2012). The total sterol content of sunflower oil has been reported to range between 3000 and 4000 mg/kg, being the content of free sterols (62.4%) higher than that of the esterified forms (37.6%) (Verleyen *et al.*, 2002).

At present, a new sunflower oil characterized by a high content of phytosterols, ranging between 7660 and 14 980 mg/kg in the unrefined oil, can be found. These elevated levels of phytosterols are not produced in nature by sunflower plants. They have been obtained through a complex genetic process consisting of artificial induction of mutations and fixation of the identified new character (Velasco *et al.*, 2012).

The total content and composition of phytosterols in the oil depend not only on genetic and environmental factors of sunflower, but also on technological processes of oil extraction (Van Hoed *et al.*, 2010; Fernández-Cuesta *et al.*, 2014). Generally, the oil extraction is based on two main steps: pressing (solid-liquid expression) and solvent extraction. Mechanical and thermal pre-treatments preceded these processes and contribute to enhance their performances (Savoire *et al.*, 2013). After the first extraction by pressure with an expeller, the resulting solid matter has a relatively high fat content (approximately 15–18%) and it is subjected to a solvent extraction to exhaust the oil retained (Kovari, 2004). The pressed and solvent extracted oils are generally blended before storage and refining.

The growing interest of consumers for minimally processed products has recently encouraged the production of cold pressed oils (Rass *et al.*, 2008). Compared to oils obtained by solvent extraction, the oils obtained by mechanical procedures might present an added value that makes it reasonable to commercialize the two oils separately. In this regard, it is essential to get to know the chemical composition of the oils obtained separately by pressing and solvent extraction. The objective of this study was to evaluate possible changes in the chemical composition between sunflower oils obtained separately by mechanical pressing and subsequent solvent extraction from a new line of seeds rich in phytosterols and conventional seeds. The content and composition of phytosterols were studied in detail.

2 Materials and methods

2.1 Oil extraction

HA-89 and IASP-18 seeds, with conventional and high phytosterol content, respectively, were conditioned at 70 °C for 60 min. The oil was extracted using a small expeller press, Täby Pressen model 40a (Skeppsta Maskin AB, Örebro, Sweden), with a capacity of 3.5 kg h^{-1}. Whole sunflower seeds were used because it is known that husked seeds are plasticized in the screw press and the drainage canals can be blocked (Rass *et al.*, 2008). Moisture content of samples was 10% when expelled.

The oil retained in the pellets was further extracted with 2.5 L hexane during 4 h at 60 °C in a Soxhlet extractor with a capacity for solids of 2 kg. Then the solvent was removed under vacuum.

2.2 Analytical methods

The total content of oil in the seeds was determined from ground seeds using a Soxhlet extractor according to official method UNE 55-062 (AENOR, 1991). Free fatty acids were determined following method UNE 55-011 (AENOR, 1991).

Unsaponifiable fractions were extracted from 2 g of oil, adding 1 mg of α-cholestanol as internal standard, following the standard method UNE 55-004 (AENOR, 1991). The standard procedure was followed by a last extraction step using 50 ml chloroform to increase the extraction of polar compounds.

Free sterols and steryl glycoside quantification was performed following the method proposed by Aguirre *et al.* (2012). Sterols were determined in the lipid unsaponifiable matter. The extract was dissolved in pyridine so as to obtain a 5 mg/ml solution. An aliquot of 200 µL was derivatized with 200 µL of pyridin:hexamethyldisilazane: chlorotrimethylsilane (9:3:1). After 15 min at 70 °C, the sylanized unsaponifiable fraction was directly analysed by high-temperature gas-liquid chromatography using an Agilent 6850 Series chromatograph (Agilent, Avondale, PA, USA) equipped with an on-column injector, an HP-5 capillary column (15 m × 0.25 mm, 0.1 mm) (Agilent, Avondale, PA, USA) and a flame ionization detector, set at 360 °C. The analyses were run using hydrogen as carrier gas (1 mL/min) and with the following oven temperature program: 80 °C (held for 1 min), rising up to 220 °C at 20°C/min and then rising at 10 °C/min up to 350 °C (held for 15 min). A correction factor of 1.06 was applied in the quantification of sterol glycosides.

Sterol Esters were quantified following the Tuckey and Stevenson method (1979).

Table 1. Oil content (% w/w) extracted from a new line of sunflower seeds rich in phytosterols (IASP-18) and conventional seeds (HA-89) by pressing and subsequent solvent extraction compared to total oil determined by Soxhlet.

	IASP-18	HA-89
Pressing extraction	2.9	20.0
Solvent extraction[a]	17.6	18.4
Analytical results (Soxhlet)	18.1	37.5

Mean (n = 2). [a] Extraction of the oil retained in the pellets after pressing extraction.

Fatty acids were determined by GC after derivatization of the oil to fatty acid methyl esters with 2 N KOH in methanol, according to UNE 55-037 method (AENOR, 1991). An HP-7890 (Hewlett-Packard, Palo Alto, CA, USA) equipped with an SP-2380 (Supelco, Bellefonte, PA, USA) capillary column of fused silica (30 m × 0.32 mm I.D., 0.2 µm film thicknesses) and a flame ionisation detector (FID). Hydrogen was used as carrier gas with a linear rate of 28 cm s^{-1}. The oven temperature was maintained at 170 °C, and that of the injector and the detector at 220 °C. The injection split ratio was 1:50.

Tocopherols were determined by HPLC with fluorescence detection (excitation at 290 nm and emission at 330 nm), following the IUPAC Standard Method 2.432 (IUPAC, 1991). n-Hexane:isopropanol (99:1, by vol.) as mobile phase at a flow rate of 1 mL min^{-1} and a LiChrosorb Si 60 (250 × 4 mm) column packed with silica (5 µm particle size) (Merck, Darmstadt, Germany) were used. Oil solutions of 50 mg mL^{-1} were analysed.

The oil stability index (OSI) was determined following AOCS method number Cd 12b-92 (AOCS, 2008) using a Rancimat (Metrohm Ltd., Herisau, Switzerland) at 110 °C and an air flow of 20 L h^{-1}.

3 Results and discussion

The total content of oil was much lower in the IASP-18 (18.1%) than in the conventional (37.5%) seeds (Tab. 1). The extraction yield obtained by pressing was as low as 3% in the IASP-18 seeds and 37.5% in the HA-89 seeds, while the yield in the solvent extraction was of the same order (~18 wt% on seeds extracted by pressing) for the two types of seeds. In addition, the total content of oil obtained by the sum of both extraction procedures was similar to that obtained in the standard method with Soxhlet.

The oils obtained by the two extraction procedures presented similar fatty acid composition (Tab. 2). However the oils extracted by pressing showed significantly larger content of the unsaponifiable matter, being the differences more pronounced in the IASP-18 oils. On the contrary, the oils extracted by pressing showed significantly lower acidity and the acidity of the oils extracted with solvent was lower than 1%, which is typical of high quality seeds with low hydrolysis levels. The lower acidity found for the pressed oils are in agreement with studies of other authors (Fernández- Cuesta *et al.*, 2014; Van Hoed *et al.*, 2010).

The content of tocopherol was higher for the IASP-18 oil and slight differences were also found between the oils extracted with different extraction methods (Tab. 2). The oils extracted by pressing were approximately 10% more concentrated in tocopherol. The oxidative stability was lower for the IASP-18 oils and can be attributed in part to the larger content of linoleic acid. There were slight differences between the oxidative stability values of the oils extracted by pressing and solvent, although such differences were not consistent. While the stability of the oil extracted by pressing was higher in the IASP-18 seeds, the opposite was found in the HA-89 oils. Therefore the differences found in the oxidative stability can not be accounted by the slight differences in the content of tocopherols. Fernández-Cuesta *et al.* (2014) have also reported discrepancies between oil stability values of crude pressed oils and oils subsequently extracted by solvent from safflower seeds and that such varying results could be explained by small differences in minor components of antioxidant or prooxidant effects.

Table 3 lists results obtained for the total content of sterols and their composition. The total sterols were 37–38% more concentrated in the oils extracted with solvent, reaching amounts of 13 700 and 6500 mg/kg in the IASP-18 and HA-89 oils, respectively. In this regard, other authors have also reported higher contents of sterols in oils extracted by solvent from different seeds compared to cold-pressed oils (Fernández-Cuesta *et al.*, 2014; Van Hoed *et al.*, 2010; Li *et al.*, 2007). Unlike other similar studies on other oleaginous seeds (Fernández-Cuesta *et al.*, 2014; Van Hoed *et al.*, 2010; Li *et al.*, 2007), no substantial differences were found in the present study in the composition of total sterols analysed as free sterols between the oils extracted with the two procedures.

Significant differences were however found for the different classes of sterols (Tab. 4). In the IASP18 sample, 52.3% of sterols was found in the free form in the mechanically extracted oil, whereas 63.0% was obtained in the oil extracted by solvent. A higher relative concentration of free sterols in the oil extracted with solvent was also found in the conventional sunflower sample, being of 78% against 60% in the oil obtained by mechanical extraction. The relative concentration of complex sterols was obviously greater in the oils obtained by mechanical extraction. Thus the sterol esters constituted 44 and 39% (w/w, expressed as free sterols) of total sterols in the oils extracted by pressing and solvent, respectively, from the IASP-18 sample, and 41 and 25% (w/w, expressed as free sterols) in the oils from conventional seeds, respectively. The amount of sterol glycosides was very low but significantly much higher, in a factor of 8, in the oils extracted with solvent.

The larger total content of sterols and the higher proportion of those with larger polarity, *i.e.* free sterols and sterol glycosides, in the oils extracted with solvent can be attributed to a greater extractive capacity of the Soxhlet extraction compared to the pressing procedure. In the first step, *i.e.* pressing extraction, the solubility of minor components in the oil mainly depends on the content of oil released, which acts like a solvent. Once the oil available is saturated this can not incorporate more sterols. On the contrary, in the Soxhlet extraction, the sterols along with the neutral lipids are extracted with solvent, which is saturated in sterols in each extraction cycle and these are concentrated in the oil as a result. In addition, due to the greater solubility in the oil of those sterols with less

Table 2. Physical and chemical characteristics of crude sunflower oils extracted by mechanical press and subsequent solvent extraction from a new line of seeds rich in phytosterols (IASP-18) and conventional seeds (HA-89).

	IASP-18		HA-89	
	Pressing	Solvent	Pressing	Solvent
Acidity* (% on oleic)	0.56	0.72	0.18	0.85
Major Fatty acids* (%)				
C16:0	8.0	9.6	6.4	7.1
C16:1	0.3	0.4	0.2	0.2
C18:0	1.9	1.9	4.2	4.0
C18:1	16.2	16.4	45.6	44.3
C18:2	72.2	71.1	42.4	43.3
C20:0	0.2	0.1	0.3	0.3
C22:0	0.2	0.5	0.7	0.6
Others	1.0	0.1	0.3	0.2
Unsaponifiable Matter* (%)	2.89	2.13	2.53	2.37
Tocopherols Conc.* ($mg\ kg^{-1}$)	875	803	651	588
Stability* (Rancimat 110 °C, h)	6.2	8.0	14.9	11.7

* Mean ($n = 2$).

Table 3. Sterol content and composition in crude sunflower oils extracted by mechanical press and subsequent solvent extraction from a new line of seeds rich in phytosterols (IASP-18) and conventional seeds (HA-89).

	IASP-18		HA-89	
	Pressing	Solvent	Pressing	Solvent
Sterols Conc. ($mg\ kg^{-1}$)	9956 ± 15	13 703 ± 346	4757 ± 212	6520 ± 316
Composition (%)				
Campesterol	4.5	5.2	4.9	5.0
Stigmasterol	5.1	5.5	4.0	6.9
β-Sitosterol	68.9	69.6	67.7	68.5
Δ^5-Avenasterol	2.1	0.7	0.7	2.4
Δ^7-Stigmastenol	14.6	14.8	16.9	13.2
Δ^7-Avenasterol	2.3	2.8	3.0	1.9
Others	2.2	1.8	2.8	2.0

Table 4. Sterol composition by classes in crude sunflower oils extracted by mechanical press and subsequent solvent extraction from a new line of seeds rich in phytosterols (IASP-18) and conventional seeds (HA-89).

	IASP-18		HA-89	
	Pressing	Solvent	Pressing	Solvent
Free sterols* ($mg\ kg^{-}1$)	5512 ± 232	8636 ± 391	2856 ± 73	5086 ± 373
Sterol esters* ($mg\ kg^{-}1$)				
a	4414 ± 235	5402 ± 470	1971 ± 30	1639 ± 105
b	7327 ± 290	8967 ± 780	3272 ± 50	2721 ± 174
Sterol glycosides* ($mg\ kg^{-}1$)	32 ± 6	268 ± 55	16 ± 1	138 ± 28

* Mean ($n = 3$) ± SD. [a] Sterol esters expressed as free sterols. [b] Sterol esters calculated from the equation $b = a \times 1.66$.

polarity, *i.e*. sterol esters, compared to free sterols, the seeds that result after the pressing extraction have increased relative concentrations of free sterols and sterol glycosides. The same reasoning might explain the higher acidity values found in the oils extracted with solvent.

4 Conclusions

The results of this study have shown that the oils obtained by pressing from IASP-18 and HA-89 seeds do not differ from those by solvent extraction in terms of fatty acid composition, although the pressed oils showed significantly larger content of unsaponifiable material. The main differences were observed in the total content of sterols, being substantially much higher in the solvent extracted oils. Thus, total sterols were 37–38% more concentrated in the oils extracted with solvent, reaching amounts of 13 700 and 6500 mg/kg in the IASP-18 and HA-89 oils, respectively. No substantial differences were found in the composition of total sterols analysed as free sterols between the oils extracted with the two procedures, but significant differences were found for the different classes of sterols. The contents of free sterols and sterol glycosides were much higher in the oils extracted with solvent and the oils extracted by pressing were characterized by higher relative concentrations of sterol esters.

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