

Sesamin and sesamol as unexpected contaminants in various cold-pressed plant oils: NP-HPLC/FLD/DAD and RP-UPLC-ESI/MSⁿ study

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Thirteen cold-pressed oils (Japanese quince seed, black caraway, flaxseed, rapeseed, hemp, peanut, sunflower, pumpkin, hazelnut, poppy, walnut, almond and sesame oil) manufactured by the same company over a 2-year period (2011–12) were assessed for lipophilic compounds. The presence of sesamin and sesamol, two characteristic lignans of sesame oil, were detected in all tested plant oils. Both lignans were identified by NP-HPLC/FLD/DAD and confirmed by a RP-UPLC-ESI/MSⁿ method. The lowest amount of sesamin and sesamol was found for Japanese quince seed oil (0.10 and 0.27 mg/100 g), and the highest, excluding sesame oil, for almond oil (36.21 and 105.42 mg/100 g, respectively). The highly significant correlation between sesamol and sesamin concentrations was found in all samples tested ($r = 0.9999$; $p < 0.00001$). These results indicate contamination of cold-pressed oils from the same source. This investigation highlights the fact that increasing the range of products manufactured by the same company can contribute to a lesser regard for the quality of the final product. Moreover, less attention paid to the quality of final product can be related to the health risks of consumers especially sensitive to allergens. Therefore, proper cleaning of processing equipment is needed to prevent cross-contact of cold-pressed oils.

Keywords: authentication; quality; lignans; sesamol; sesamin; cold-pressed oil; NP-HPLC/FLD/DAD; RP-UPLC-ESI/MSⁿ

Introduction

Currently, in an era of globalisation, we are facing a great challenge to ensure safe food for the whole population across the world. Consumers are increasingly interested in the quality, authenticity and especially safety of purchased food products. Therefore, it is important to develop new appropriate quality control procedures that are rapid, reliable, accurate, sensitive, less labour intensive and, at the same time, that protect against sample contamination and analyte loss.

Recently an increase in the variety and popularity of cold-pressed oils has been observed due to their higher nutritional value compared with refined oils, as well as the ecologically more friendly extraction methods used to prepare them (Siger et al. 2008). As an example can be noted new cold-pressed Japanese quince seed oils obtained from by-products of the fruit industry (Górnaś, Siger, Segliņa 2013). Presently, an extended market of plant oils raises the need for verification of their authenticity and quality, which is important for commercial and health reasons (García-González et al. 2008). Mostly the authentication of edible oils is performed by characterisation and quantification of their well-known functional compounds such as fatty acids or tocopherols (Aparicio & Aparicio-Ruiz 2000). Nonetheless, some

plant oils contain unique components characteristic only of this oil, which facilitates their identification. For instance, sesame seed oil contains sesamin and sesamol – two major well-studied oil-soluble lignans (Hemalatha & Ghafoorunissa 2007; Sukumar et al. 2008; Konsoula & Liakopoulou-Kyriakides 2010; Rangkadilok et al. 2010; Schwertner & Rios 2012).

The production of the sesame seed in the last decade, between 2002 and 2011, almost doubled from 2.8 million to 4.8 million tons (FAOSTAT 2013). The popularity and consumption of sesame oil increased due to the presence of three major oil-soluble lignans: sesamol, sesamin and sesamol, and their proven health benefits (Ahmad et al. 2012). However, Stevens et al. (2002) observed anaphylaxis in patients sensitive to allergens contained in sesame seeds after ingesting products containing sesame seed oil. Therefore, it is recommended that consumers who have sesame allergy should avoid unrefined sesame oil.

During production in some cases there is insufficient attention to the quality and safety practices and/or replacing of expensive components by cheaper ones to reduce the costs of production that can negatively affect the quality of the final product (Boutrolle et al. 2009). Replacing expensive components by cheaper ones is well known, for instance, in the adulteration of extra virgin

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olive oil with hazelnut oil (Arlorio et al. 2010). Therefore, the importance of food analysis with regard to quality and authenticity has to be underlined, especially due to increasing consumer demand for high-quality, nutritionally valuable and safe food products as well the larger assortment and competition in the market. Due to the above reasons, and also as a result of a very surprising and unexpected finding, the routine control of 13 cold-pressed oils produced by the same company within a 2-year period (2011–12) was performed in this study.

Materials and methods

Cold-pressed oil samples and solvents

Twelve samples of cold-pressed plant oils (black caraway, flaxseed, rapeseed, hemp, peanut, sunflower, pumpkin, hazelnut, poppy, walnut, almond and sesame oil) were purchased twice, the first time in 2011 and the second in 2012, from the Latvian market. All oils were produced by the same company, as well as the 13th oil (the control sample) obtained from Japanese quince seeds provided by the Latvia State Institute of Fruit-Growing.

All used solvents were of HPLC grade purchased from Sigma-Aldrich (Steinheim, Germany).

The oil extraction and cleaning procedure of equipment after pressing

Due to the very surprising and unexpected finding in oil samples, a detailed description of the process for obtaining the cold-pressed oils and the cleaning procedures used were provided by the producer. Cold-pressed oil was obtained by pressing nuts and seeds by hydraulic press Farnet Duo (Farnet a.s., Česká Skalice, Czech Republic). The temperature recorded in the pressing room was $15 \pm 2^\circ\text{C}$ and $60 \pm 10^\circ\text{C}$ inside the press. The pressing time was from 15 min to 1 h, depending on the raw materials. The oils obtained at a temperature of $38\text{--}40^\circ\text{C}$ were immediately cooled $10\text{--}14^\circ\text{C}$ and filtrated using filters D18 20/20 (Filtrex, Svitavy, Czech Republic) of $5\text{ }\mu\text{m}$ for linseed oil and of $3\text{ }\mu\text{m}$ for the others. The oils were closed under vacuum in small dark bottles (100 ml) and stored at 4°C . Between pressings of different seeds/nuts a small amount of rapeseeds at the end of the shift are pushed through the oil pressing machine in order purge the previous raw material. If the break between oil pressings is planned to be more than 1 day, the pressing machine is dismantled, cleaned, washed with biological detergent, rinsed with warm water and dried.

Sesamin and sesamol standard preparation

Sesamin and sesamol were isolated and purified from sesame seed oil by an NP-HPLC. For this purpose, sesame seed oil (1 g) was dissolved in *n*-hexane (10 ml) and

injected (100 μl) into the column LiChrosorb Si 60 ($250 \times 4.6\text{ mm}$, $5\text{ }\mu\text{m}$) (Merck, Darmstadt, Germany). The mobile phase was a mixture of *n*-hexane with 1,4-dioxane (97:3 v/v) at a flow rate of 1.5 ml min^{-1} . Sesamin and sesamol were monitored by photodiode array detector. Each lignan was collected during several chromatographic runs, dried under vacuum and dissolved in isooctane. The concentrations of purified lignans were calculated by the optical density of each compound in isooctane solution using the extinction coefficients reported by Cooney et al. (2001): sesamin $\lambda_{\text{max}} = 287\text{ nm}$ ($E_{1\text{cm}}^{1\%} = 230.3$) and sesamol $\lambda_{\text{max}} = 288.5\text{ nm}$ ($E_{1\text{cm}}^{1\%} = 212.7$). To determine the standard calibration curves, the working solution of sesamin and sesamol was prepared by diluting purified lignans with *n*-hexane to obtain concentrations ranging from 0.1 to $10\text{ }\mu\text{g ml}^{-1}$ for each compound. The purity of each lignan (sesamin $\geq 99.5\%$ and sesamol $\geq 97\%$) was established using HPLC and by calculating the area of peaks after the injection of prepared standards (data not shown).

For the MSⁿ identification of isolated standards of lignans, the organic solvents were evaporated and the obtained residues dissolved in methanol. This same procedure for the isolation sesamin and sesamol as well as their identification was carried out for all samples of oil to confirm the results obtained by the NP-HPLC/DAD method.

NP-HPLC/FLD/DAD lignans identification and quantification

The oils to be tested ($0.20 \pm 0.01\text{ g}$) were dissolved in *n*-hexane (10 ml) and transferred to vials for analyses. Lignans were qualitatively and quantitatively identified using a Shimadzu HPLC system (Shimadzu Corporation, Kyoto, Japan) consisting of a pump (LC-10ADvp), degasser (DGU-14A), low-pressure-gradient unit (FCV-10ALvp), system controller (SCL-10Avp), auto-injector (SIL-10AF), column oven (CTO-10ASvp), photo-diode array detector (SPD-M20A), fluorescence detector (RF-10AXL) and column LiChrosorb Si 60 ($250 \times 4.6\text{ mm}$, $5\text{ }\mu\text{m}$) (Merck, Darmstadt, Germany). The mobile phase was a mixture of *n*-hexane and 1,4-dioxane (97:3 v/v). The flow rate was 1.5 ml min^{-1} . For fluorescence detection the excitation wavelength was set at $\lambda = 295\text{ nm}$ and emission at $\lambda = 330\text{ nm}$. The identification of the lignans in the cold-pressed oils was achieved on the basis of the ultraviolet (UV) spectra of the peaks compared with pure standards of sesamin and sesamol isolated from sesame oil and confirmed by the ESI/MSⁿ technique.

RP-UPLC-ESI/MSⁿ sesamin and sesamol identification

As reported by Struijs et al. (2008), sesamin originating from the sesame oil gave comparable ions with that of the

commercial standard of sesamin. Therefore, the lignans were isolated from sesame oil by the NP-HPLC/DAD method and identified by the RP-UPLC-ESI/MSⁿ technique. The identification of ions was performed in accordance with Struijs et al. (2008), Wang et al. (2009) and Yan et al. (2007) by using an Acquity Ultra Performance LC system (Waters, Milford, MA, USA) and KinetexTM C18 (50 × 3.0 mm, 1.7 µm; Phenomenex, Torrance, CA, USA) column at 30°C. The mixture of methanol and water (7:3 v/v) was used as the mobile phase at a flow rate of 0.4 ml min⁻¹. A QTRAP5500 mass spectrometer (AB SCIEX) equipped with an ESI source in positive-ion mode was under the following operating conditions: 30 psi curtain gas (CUR), 4500 V ion spray voltage, 400°C temperature, 40 psi ion source gas (GS1), 40 psi ion source gas (GS2), collision gas (CAD) high, 10 V entrance potential (EP) and 30 V declustering potential (DP). Table 1 outlines a summary of the most intensive fragmentation pathways and compound-dependent values obtained with the QTrap 5500. Marked ions were used for development of the method.

Statistical analysis

The results are presented as the mean ± SD from three independent experiments for each sample of oil. Multiple linear regression, analysis of variance (ANOVA) and Pearson correlation were conducted using Excel 2007 (Microsoft). A *p*-value < 0.05 was used to denote significant differences between mean values determined by the ANOVA using a Tukey HSD test with the assistance of Statistica 8 (StatSoft, Inc., Tulsa, OK, USA) software.

Results and discussion

There are no reports of the presence of sesamin and sesamol in plant oils other than sesame, except for the intentional addition to other oils with the aim to increase their oxidative

stability (Hemalatha & Ghafoorunissa 2007; Konsoula & Liakopoulou-Kyriakides 2010). In the present study both lignans were detected in various cold-pressed plant oils. The chromatograms obtained for selected six samples of oil (pumpkin, hazelnuts, poppy, walnuts, almonds, and sesame) are presented in Figure 1. Moreover, to prove the presence of these lignans in all oil samples, the MSⁿ technique was applied. Generally, for the detection of lignans by MS ESI with positive ionisation mode is more often used than the negative mode or atmospheric pressure chemical ionisation (APCI) due to easier detection (Song et al. 2012, Struijs et al. (2008); Yan et al. 2007; Wang et al. 2009). In order to develop and optimise the MS² and MS³ technique for the identification of sesamin and sesamol, the optimal ionisation parameters and ions with the highest intensity and low background noise were chosen (Table 1). The detected ions for the sesamin and sesamol were comparable with those reported by other researchers (Struijs et al. 2008; Yan et al. 2007; Wang et al. 2009). The peak with the retention time *R*_t = 26.8 min corresponded to sesamol (Figure 1). The MSⁿ showed the positively charged molecular ion at *m/z* 371 and its subsequent fragmentation resulted in ions at *m/z* 233 and 173 (CE 10 V). The second peak with the retention time (*R*_t) = 33.0 min corresponded to sesamin. The MSⁿ showed a positively charged molecular ion at *m/z* 355 and its subsequent fragmentation resulted in ions at *m/z* 233 and 173 (CE 10 V). The chromatograms obtained by MS² and MS³ allowed it to be concluded that both identification techniques are suitable and comparable (Figure 2). However, in many case the MS³ has advantages over MS² due to its ability to avoid possible matrix effects (Górnaś, Siger, Polewski, et al. 2013). Therefore, the MS³ method was chosen. Moreover, the method that was developed was very sensitive and allowed the rapid determination of the sesamin and sesamol as separate peaks (Figure 2). The presence of both sesame oil lignans, already detected and quantified by the NP-HPLC/FLD/DAD method, was confirmed by RP-UPLC-ESI/MSⁿ in all samples of oil.

Table 1. Developed operation parameters of the MSⁿ.

Lignan	<i>m/z</i> MS [<i>M</i> + <i>H</i>] ⁺	<i>m/z</i> MS ² [<i>M</i> + <i>H</i>] ⁺	<i>m/z</i> MS ³ [<i>M</i> + <i>H</i>] ⁺	CE (V)	AF2 (V)
Sesamin	355	355→135 ^a		20	
		355→173 ^a	355→173→143	22	0.12
		355→233	355→233→173 ^a	10	0.08
			355→233→203	10	0.08
		355→337	355→337→289	10	0.12
			355→337→319	10	0.12
			355→337→267	10	0.12
			371→233→173 ^a	10	0.12
Sesamol	371	371→233 ^a		30	
		371→135 ^a			
		371→355	371→355→353	15	0.12
			371→355→267	15	0.12
		371→203	371→203→135	15	0.10

Note: ^aTransitions used in the developed method. CE, collision energy; AF2, auxiliary radio frequency 2 used only for MS³.

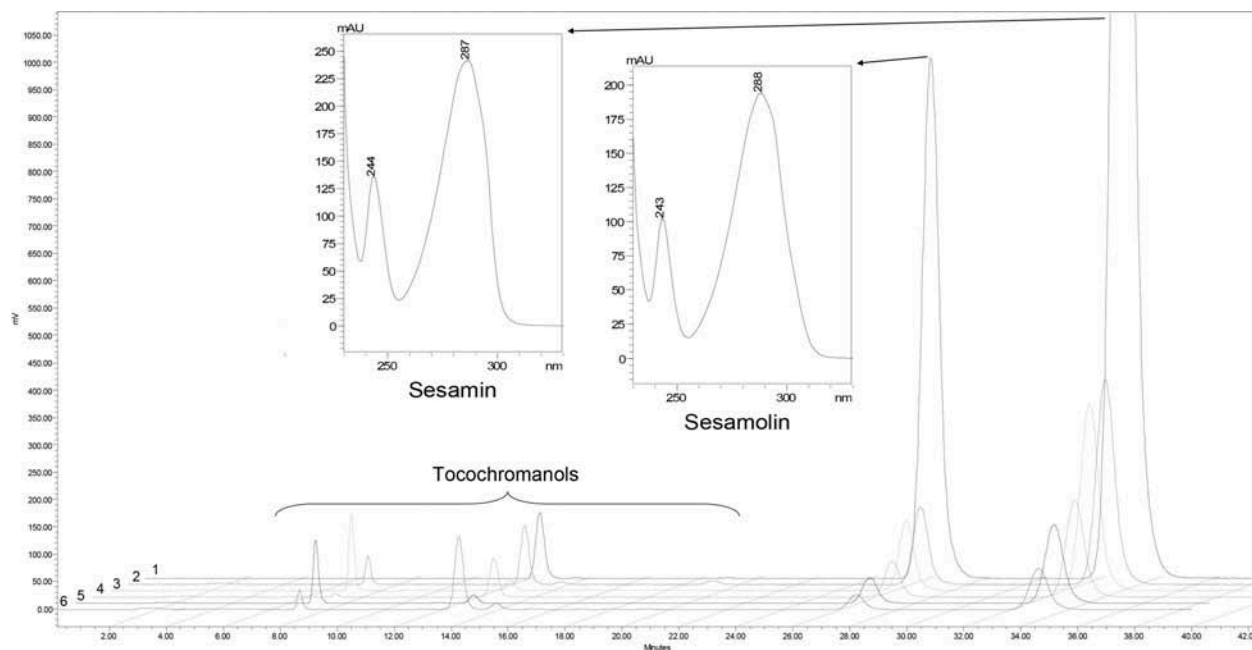


Figure 1. NP-HPLC-FLD chromatograms of six selected oils and ultraviolet (UV) spectra of sesamin and sesamolin. Oils: 1, sesame; 2, walnut; 3, almond; 4, poppy; 5, hazelnut; and 6, pumpkin.

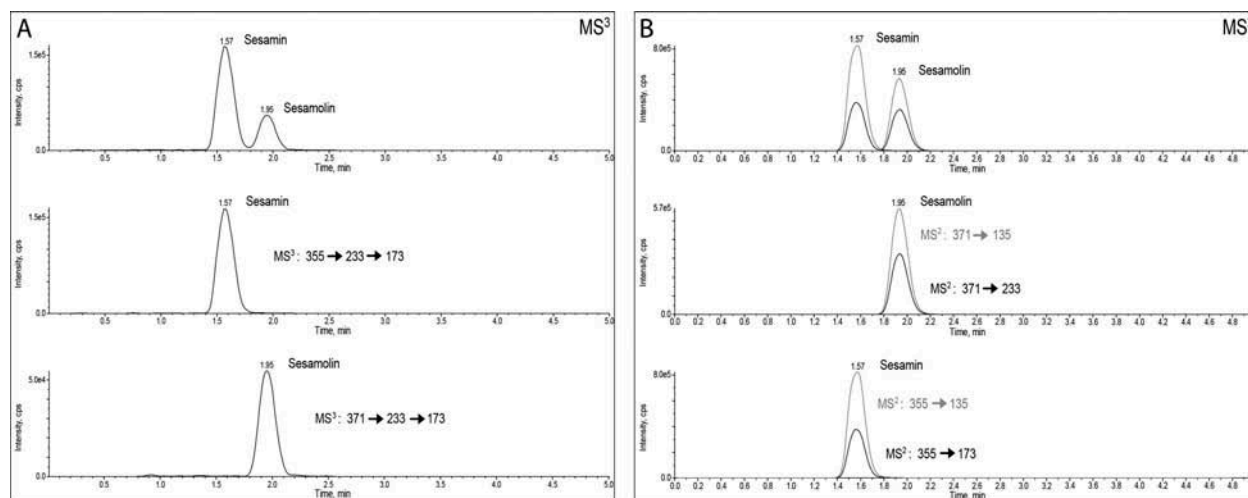


Figure 2. ESI/MS² and ESI/MS³ chromatograms of the sesamin and sesamolin isolated from sesame oil: A, MS³; and B, MS².

The concentrations of sesamolin and sesamin in all cold-pressed oils analysed, with the exception of sesame oil, were in the ranges of 0.10–36.21 and 0.27–105.42 mg/100 g, respectively. In sesame oil levels were 289.31–843.72 and 342.53–988.26 mg/100 g in 2011 and 2012, respectively. However, the differences noted in the content of lignans for the same oils in a period of 2 years (2011–12) demonstrated that we were dealing with a situation of inadequate attention to quality than deliberate addition (Table 2). In all samples studied the ratio between sesamolin and sesamin was approximately 1:3. In a study

performed by Rangkadilok et al. (2010), where 58 sesame oils was tested, the concentration of sesamolin and sesamin was in the ranges of 0.30–0.74 and 0.93–2.89 mg g⁻¹ of oil, respectively, which gave a ratio about 1:3. The same ratios obtained in the present study suggest that the identified lignans in all oils tested originated from sesame oil. This finding was confirmed by a significant correlation coefficient ($r = 0.9999$, $p < 0.00001$) between the concentration of sesamolin and sesamin (Figure 3). Due to the above observation, the cleaning procedures for the oil press and filtration equipment were considered as a critical step for quality

Table 2. Content of sesamin and sesamol in various cold-pressed plant oils (mg/100 g).

Oil	2011			2012		
	Sesamol	Sesamin	Total	Sesamol	Sesamin	Total
Japanese quince seed	0.17 ± 0.01 ^a	0.49 ± 0.01 ^a	0.66 ± 0.02 ^a	0.10 ± 0.01 ^a	0.27 ± 0.01 ^a	0.37 ± 0.02 ^a
Black Caraway	0.70 ± 0.02 ^b	1.97 ± 0.03 ^b	2.67 ± 0.05 ^b	0.53 ± 0.03 ^b	1.53 ± 0.05 ^b	2.06 ± 0.08 ^b
Flaxseed	1.20 ± 0.02 ^c	3.50 ± 0.04 ^c	4.70 ± 0.06 ^c	0.55 ± 0.01 ^b	1.59 ± 0.03 ^b	2.14 ± 0.04 ^b
Rapeseed	2.61 ± 0.03 ^d	7.28 ± 0.12 ^d	9.89 ± 0.15 ^d	1.41 ± 0.02 ^c	4.03 ± 0.06 ^c	5.44 ± 0.08 ^c
Hemp	2.76 ± 0.07 ^d	7.89 ± 0.19 ^d	10.65 ± 0.26 ^d	2.30 ± 0.04 ^d	6.58 ± 0.11 ^d	8.88 ± 0.15 ^d
Peanut	3.79 ± 0.06 ^e	11.15 ± 0.29 ^e	14.94 ± 0.35 ^e	2.92 ± 0.05 ^e	8.36 ± 0.23 ^e	11.28 ± 0.28 ^e
Sunflower	4.17 ± 0.08 ^e	11.97 ± 0.17 ^e	16.14 ± 0.25 ^e	3.82 ± 0.06 ^f	11.03 ± 0.14 ^f	14.85 ± 0.20 ^f
Pumpkin	7.17 ± 0.12 ^f	20.54 ± 0.23 ^f	27.71 ± 0.35 ^f	2.49 ± 0.04 ^d	7.22 ± 0.18 ^d	9.71 ± 0.22 ^d
Hazelnut	12.81 ± 0.21 ^g	37.03 ± 0.52 ^g	49.84 ± 0.73 ^g	10.84 ± 0.21 ^g	31.34 ± 0.45 ^h	42.18 ± 0.66 ^h
Poppy	15.05 ± 0.35 ^h	43.90 ± 0.70 ^h	58.95 ± 1.05 ^h	31.13 ± 0.38 ^h	89.78 ± 1.02 ⁱ	120.91 ± 1.40 ⁱ
Walnut	33.88 ± 0.47 ⁱ	98.38 ± 1.19 ⁱ	132.26 ± 1.66 ⁱ	1.14 ± 0.02 ^c	3.34 ± 0.05 ^c	4.48 ± 0.07 ^c
Almond	36.21 ± 0.54 ^j	105.42 ± 1.05 ^j	141.63 ± 1.59 ^j	4.31 ± 0.06 ^f	12.33 ± 0.22 ^g	16.64 ± 0.28 ^g
Sesame	289.31 ± 2.34 ^k	843.72 ± 6.21 ^k	1133.03 ± 8.55 ^k	342.53 ± 2.64 ⁱ	988.26 ± 7.34 ^j	1330.79 ± 9.98 ^j

Note: Results are expressed as mean ± SD ($n = 3$). Different superscript letters in the same column are significantly different at $p < 0.05$.

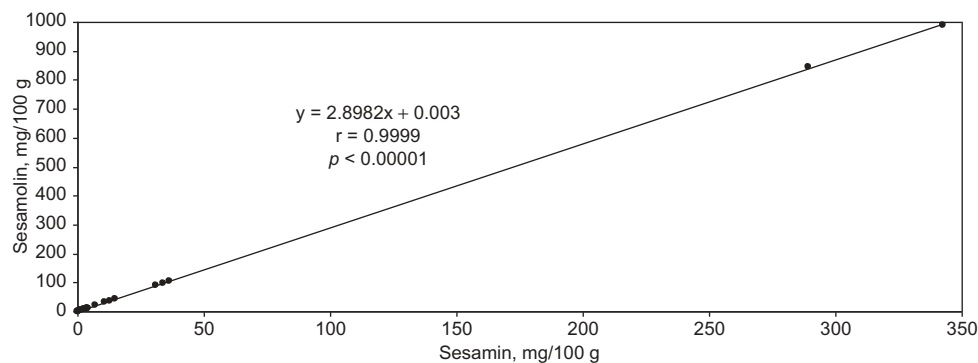


Figure 3. Correlation between the sesamin and sesamol content in cold-pressed plant oils.

assurance of the final products. To put more attention on this case, the company was asked to obtain cold-pressed oil from the Japanese quince seed (without filtering) and to provide detailed information concerning the cold-pressing process and methods used for cleaning of the press between the processing of various plant raw materials. According to the information provided, and based on the results obtained, it can be concluded that the cleaning procedures were ineffective due to the detection of sesamol and sesamin in all samples tested. However, probably the most critical point was filtering, since in Japanese quince seed oil (not filtered) the concentration of lignans was significantly lower than in other types of oil that were subjected to filtration treatment.

The detected presence of sesamol and sesamin as a contamination in various commercial oils can be considered from two aspects. As a positive, there is the proven beneficial effects of these lignans on human health. For instance, increasing the effectiveness of bioavailability of gamma-tocopherol (Cooney et al. 2001), antioxidative and

antihypertensive properties (Lee et al. 2009), inhibition of the proliferation of tumour cells (Harikumar et al. 2010), and neuroprotective effect (Ahmad et al. 2012). Sesamol and sesamin can also improve the storage stability of vegetable oils (Hemalatha & Ghafoorunissa 2007; Konsoula & Liakopoulou-Kyriakides 2010), probably due to the antioxidant activity of lignans and their synergism between alpha-tocopherol and alpha-tocotrienol (Ghafoorunissa et al. 2004). On the contrary we should be concerned about the quality and safety of commercial unrefined oils due to compounds that do not occur naturally in specific seeds and/or nuts used for oil production. They should not be present in products or the producer must inform customers about it. It is very important due to the health consequences for customers with allergies to sesame seeds and their products (Stevens et al. 2002; Alonzi et al. 2011). For instance, Stevens et al. (2002) observed an anaphylaxis reaction in patients with allergy to sesame seeds after ingestion of products containing sesame seed oil. Although the processing of cold pressed oils is becoming

increasingly popular due to the high content of bioactive compounds, the presence of proteins in those oils is usually not considered (Hidalgo & Zamora 2006).

Due to those reasons, the producers of oils from many different plant materials, which are produced on shared processing lines, are struggling with a major challenge to ensure the high quality of cold-pressed oils and especially their safety. This contributes to the necessity to find and develop not only rapid and reliable, but also more accurate, sensitive and less labour-consuming analytical methods for the detection of proteins that may cause allergenic responses in sensitive individuals or other compounds which may serve as surrogates for the detection of contaminant proteins. Balasubramanian and Panigrahi (2011) highlight that each additional step in the analytical method during the sample preparation can contribute to the increase in the contamination probability of the sample and analyte loss, which finally could lead to an analytical error. This suggests the need to reduce to a minimum the number of steps required to prepare the sample, and at the same time not affecting the quality of the analysis.

Conclusion

The NP-HPLC/FLD/DAD and RP-UPLC-ESI/MSⁿ methods used in this study allowed for the positive identification of both lignans (sesamin and sesamolin) in all analysed oil samples. Particularly rapid RP-UPLC-ESI/MSⁿ separation and identification (below 3 min) can be a very useful tool. The presence of the sesamin and sesamolin in all tested cold-pressed plant oils was very surprising and an unexpected finding. The detailed analysis allowed identification of the sources of contamination, which were the result of improper cleaning procedures for equipment between pressing and filtering of various oils.

As demonstrated in the current study, increasing the variety of oils produced by the same company can be associated with less attention paid to the quality of the final product. Therefore, due to possible cross-contact of cold-pressed oils, which can be a concern for consumer health, especially for people with allergies, finding new tools for the assessment of the quality and safety of cold-pressed oils is required.

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