

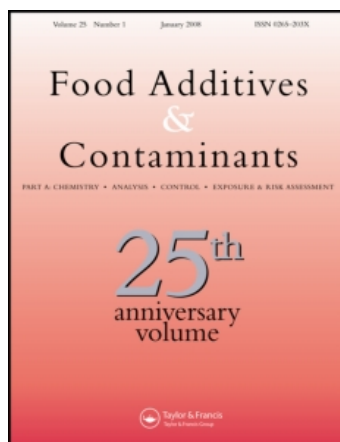
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Olive oil adulterated with hazelnut oils: simulation to identify possible risks to allergic consumers

M. Arlorio^{a*}, J.D. Coisson^a, M. Bordiga^a, F. Travaglia^a, C. Garino^a, L. Zuidmeer^b, R. Van Ree^b,
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According to European Union Regulation EC 1531/2001, olive oil labelled as “extra-virgin” should be cold-pressed and contain no refined oil or oil from other oleaginous seeds or nuts. Adulteration of extra virgin olive oil (EVOO) with hazelnut oil (HAO) is a serious concern both for oil suppliers and consumers. The high degree of similarity between the two fats complicates the detection of low percentages of HAO in EVOO. Many analytical approaches have been developed in recent years to trace HAO in EVOO, principally based on chromatographic analyses, differential scanning calorimetry or nuclear magnetic resonance. In addition adulteration of EVOO with HAO may introduce hazelnut-derived allergens. The aim of this work was to analyse the protein and allergen content of EVOO intentionally spiked with raw cold-pressed HAO or solvent-extracted HAO. SDS-PAGE analysis confirmed the presence of hazelnut proteins in solvent-extracted HAO with molecular masses ranging 10–60 kDa. In contrast, cold-pressed HAO showed no traces of protein. In spiked EVOO, solvent-extracted HAO was still detectable at a 1% contamination level. Several bands on SDS-PAGE migrated at apparent molecular masses coinciding with known allergens, such as Cor a 1 (~17 kDa), Cor a 2 (~14 kDa), Cor a 8 (~12 kDa), oleosin (~17 kDa) and Cor a 9 (~60 kDa). MALDI-TOF MS analysis confirmed the presence of two oleosin isoforms and of Cor a 9. Immunoblotting demonstrated that an allergic patient with known reactivity to Cor a 1 and Cor a 2 recognized a 17-kDa band in solvent-extracted HAO. In conclusion, we have shown that adulteration of extra virgin olive oil with solvent-extracted hazelnut oil can be traced by simple SDS-PAGE analysis, and that adulteration introduces a potential risk for hazelnut allergic patients.

Keywords: chromatography; electrophoresis; immunoassays; allergens; olive oil

Introduction

Due to its nutritional and commercial value, the consumption of olive oil has increased considerably in recent years, representing 15.2% of total oil utilization in the European Union (Firestone et al. 1985). Production of olive oil represents ~3% of the world oils and fat markets, with 78% of global production taking place in Mediterranean countries where it is important in sustaining the agricultural economy. The cultivation, harvesting and extraction of olive oil is a difficult and time-consuming task, which adds to its relatively high price. Olive oil is either classified as “virgin olive oil” (i.e. cold-pressed using physical means without chemical treatment), as “olive oil” (i.e. a mixture of virgin and refined oil) or “refined oil” (i.e. olive oil chemically treated to neutralize a strong taste and, in particular, acidity) (Reg. EC 1531/2001). Oils labelled as “extra-virgin olive oil” (EVOO) must contain no refined olive oil; oils labelled as “olive oil”

must not contain oil from other oleaginous nuts or seeds. To increase profits, unscrupulous dealers may be tempted to add lower-priced vegetable or hazelnut oils to fresh extra virgin olive oils. This fraudulent practice, in addition to being unfair to the consumer in terms of cost (Chiavaro et al. 2008a), may also cause severe health and safety problems, such as the Spanish toxic oil syndrome in 1981 (Blanch et al. 1998; Gelpi et al. 2002).

Hazelnut oil (HAO), obtained from *Corylus avellana* L. seeds, is often used in EVOO adulteration, due to the similar chemical composition with respect to both major and minor compounds (Vichi et al. 2001). The high degree of similarity masks the detection of HAO in EVOO, especially at concentrations below 20% (Parcerisa et al. 1998; Ozen and Mauer 2002). The seriousness of this situation has increased over the last twenty years. In 1993/94 1,829,000 tonnes of olive oil were consumed world wide. In 1995, it was reported

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that quantities of Turkish hazelnut oil was illegally introduced into the European community without proper declaration to customs and excise to adulterate olive oil (Aparicio 2004). The loss to European consumers was estimated at €40 million as hazelnut oil is three times cheaper than olive oil. In 1997, the European Commission Unit for the Coordination of Fraud Prevention (UCLAF) estimated that more than 20,680 tonnes of hazelnut oil was used for producing 103,400 tonnes of adulterated olive oil (UCLAF 1997; European Union Research Committee 2001).

A number of analytical approaches have been developed lasting recent years to detect HAO in EVOO, principally based either on chromatographic analyses of free and esterified sterols or on the detection of other minor secondary compounds, such as filbertone ((*E*)-5 methylhept-2-en-4-one) (Parcerisa et al. 2000; Flores et al. 2006a, 2006b). Other advanced approaches, such as differential scanning calorimetry, nuclear magnetic resonance and isotopic ratio coupled with mass spectrometry, were also been applied (Remaud et al. 1997; Sacchi et al. 1997; Chiavaro et al. 2008b). To date, no validated PCR-based methods to identify fraudulently spiked HAO in EVOO have been reported.

HAO, like other edible oils, is largely considered a “protein-free” food, but solvent-assisted extraction for “industrial” oil can potentially (and probably) influence the presence of residual hazelnut proteins, as has been shown for other nut and leguminous oil preparations (Crevel et al. 2000). There is some evidence that peanut oil (and soybean oil) can not always be considered a safe product for peanut-allergic patients (Hourihane et al. 1997; Crevel et al. 2000; Ring and Möhrenschrager 2007). Apart from a recent paper focusing on the use of a biosensor (Bremer et al. 2009), no specific data are available for the protein content of either raw or refined HAO.

Hazelnut allergy is often the result of primary sensitization to birch pollen, more particular to the major birch pollen allergens Bet v 1 and Bet v 2. Cross-reactivity of IgE antibodies to their homologues Cor a 1 and Cor a 2 in hazelnut is the origin of hazelnut allergy (Hirschwehr et al. 1992). Other hazelnut proteins that can elicit an allergic response are non-pollen-related seed storage proteins, such as Cor a 11 (7S globulin) (Lauer et al. 2004) and Cor a 9 (11S globulin) (Beyer et al. 2002) and the non-specific lipid transfer protein (nsLTP) Cor a 8 (Schocker et al. 2004). More recently, two oleosin isoforms, now designated Cor a 12 and Cor a 13, were identified as new allergens in hazelnut (Akkerdaas et al. 2006). Moreover, we very recently isolated and cloned 2S albumin from hazelnut (Zuidmeer et al. 2009; GenBank FJ358504; release date 1 November 2009) and demonstrated its allergenicity. This allergen is now listed by the IUIS Allergen

Nomenclature Committee as Cor a 14 (available at: <http://www.allergen.org/allergen.aspx>).

The problem of “hidden allergens” in food is an important issue in the assessment of food safety. These hidden ingredients can originate from cross-contamination during food processing or from fraudulent use of non-declared ingredients (Steinman 1996). Fraudulent introduction of “hidden allergens” is a serious risk to sensitized consumers as the producer wants the adulteration to go unnoticed. Cases of death from anaphylactic shock in children, adolescents and adults after ingestion of foods capable of triggering an allergic reaction in sensitive individuals have been reported. These deaths are often caused by the presence of “hidden” ingredients, sometimes not even declared. Peanuts and nuts, in particular, are implicated in this type of severe responses, provoking asthmatic crisis in sensitive patients (Sampson et al. 1992).

The lipophilic protein fraction containing oleosins and non-specific lipid transfer proteins (nsLTPs), considered allergens in several foods (Ballmer-Weber 2002; Fernandez-Rivas et al. 2002, 2006; Pons et al. 2002; Schocker et al. 2004), could be present as hidden residual ingredients either in HAO and, consequently, in adulterated EVOO. To date, the potentially risk of such products to hazelnut-allergic consumers has received little attention.

The primary aim of this study was to evaluate by SDS-PAGE the presence of residual protein in EVOO spiked with hazelnut oil obtained by cold-pressing or solvent-extraction and to prove via mass spectrometry the presence of relevant hazelnut allergens. A secondary goal was, via immunoblotting, to establish whether serum IgE of hazelnut allergic individuals with known reactivity to Cor a 1 and oleosin confirmed the presence of allergens and if, in fact, it is a real concern for sensitized consumers.

Materials and methods

Samples

Five commercial samples of raw HAO (cold-pressed) and six commercial samples of olive oil (three labelled “extra virgin olive oil” EVOO (cold-pressed) and three “olive oil” OO) were purchased in local stores (Piedmont, Italy). Soremartec (Alba, Italy) kindly provided a series of six cold-pressed HAO samples (three from unroasted and three from roasted hazelnut seeds, *Corylus avellana* L.). Solvent-extracted HAO was obtained from raw hazelnuts (“Nocciola Piemonte” PGI, certified from Piedmont, Italy) using the following procedure. Before oil extraction, 20 g of hazelnuts were crushed in a mortar using liquid nitrogen and transferred to the extractor paper vessel. Extraction was carried out in a semi-automated extractor (Büchi Extraction System B.811) for 9 h

using dichloromethane as solvent. Oil samples were filtered using a 0.4- μ m syringe filter.

Preparation of reference olive oil sample spiked with different percentage of hazelnut oil

Solvent-extracted hazelnut oil was added to olive oil aliquots (commercial sample of EVOO) at 0.5, 1, 2, 3, 4 and 5% (v/v).

Extraction of protein from oils

Diethylether (100 ml) was added to 100 g of oil (after gently mixing of the bottle, to obtain a homogeneous surfactant phase mixed with the oil dregs, typical of EVOO) and the mixture was maintained at 18°C for 30 min. Then, 20% w/v trichloroacetic acid (100 ml), previously cooled at 4°C, was added and the mixture was transferred to a separating funnel. The trichloroacetic acid extraction step was repeated three times. The three aqueous fractions were collected and washed twice with diethylether (2 $\times$ 100 ml) to remove all lipid material. The aqueous fraction was partially concentrated using a rotary evaporator at <30°C and centrifuged at 20,800 *g* for 20 min at 4°C. The supernatant containing trichloroacetic acid was then discarded. The pellet was washed twice with acetone (2 $\times$ 10 ml) to remove all trichloroacetic acid traces and dried in a vacuum oven for 2 h at room temperature. The dried pellet, obtained as an average of three separate extractions, was quantified as mg/albumin equivalent according to Bradford (1976). The pellet was stored at -20°C.

SDS-PAGE

Extracted proteins were dissolved in Laemmli sample buffer without 2-mercaptoethanol (Laemmli 1970) and separated, 10 μ g of protein per lane, by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE; Mini-PROTEAN II Cell, Bio-Rad, Richmond, CA, USA) with 12% acrylamide resolving and 4% acrylamide stacking gel. Following electrophoresis, the gel was fixed in 7% acetic acid and 40% methanol for 1 h, then stained with brilliant blue G-colloidal (Sigma Chemical Co., Milan, Italy) for 1 h, and destained with 25% methanol for 48 h (Laemmli 1970). In the preliminary phase of work, in addition to Coomassie blue, a silver staining procedure was also applied (Switzer et al. 1979).

Patient sera

For the immunoblotting, one in-house reference serum with known IgE-reactivity to Cor a 1 and profilin was used. In addition, four patient sera with a positive

clinical history to hazelnut were used. Known specific IgE (sIgE) are listed (IU/ml). 1: Hazelnut (8.8), Cor a 1 (7.2), Cor a 2 (0.3), oleosin (0.1). 2: Hazelnut (8.1), Cor a 1 (0.3), oleosin (21.9). 3: Hazelnut (15), Cor a 14 (15.6), oleosin (0.8). 4: Hazelnut (2.1), Cor a 1 (0.9), Cor a 8 (0.2) and oleosin (7.1). Total IgE content was not determined.

SDS-PAGE/immunoblotting

Proteins were separated by SDS-PAGE (NuPAGE® 4–12% Bis-Tris gel, protein: 10 μ g/cm) and Western blotting was performed by transferring the semi-dry proteins to nitrocellulose on a Novablot electrophoretic transfer apparatus, according to the manufacturer's protocol (Invitrogen, Carlsbad, CA, USA). After blocking with PBS/5% ELK/0.02% Tween-20 for a minimum of 10 min, the blots were cut into strips and incubated overnight with 150 μ l human serum (1 : 3000, v/v) in-house polyclonal rabbit antisera (to hazelnut extract, extracted at neutral and low pH), Cor a 8 or natural hazelnut oleosin) or 1 : 30,000 (v/v) in-house monoclonal mouse antiserum (to the major birch pollen allergen Bet v 1, shown to be cross-reactive to Cor a 1) in 3 ml of PBS/0.02% Tween-20/0.5% ELK. After washing five times (PBS/0.02% Tween-20), blots were incubated (4 h) with 1 : 30,000 (v/v) HRP-labelled goat anti-human IgE (KPL, Gaithersburg, MD, USA) and washed as before. Detection was performed with ECL according to the manufacturer's protocol (GE Healthcare, Little Chalfont, Buckinghamshire, UK). Blots were exposed to X-ray film (Fuji Super RX, Fujifilm Corp., Tokyo, Japan).

MALDI-TOF/TOF MS analysis of protein extracts

For “in gel” reduction and trypsin digestion, seven bands, chosen for their intensity, were cut out (Figure 1), reduced with 100 mM DTT (56°C, 1 h), alkylated by adding iodoacetamide to a final concentration of 200 mM, and incubated in the dark at room temperature for 45 min. The proteins were then “in gel” digested with trypsin (Promega), as previously described (Lauer et al. 2004). For MALDI-TOF mass spectrometry, 0.5 μ l of each peptide mixture was applied to a target disk and allowed to air-dry. Subsequently, 0.5 μ l of matrix solution (1% w/v α -cyano-4-hydroxycinnamic acid in 30% acetonitrile, 0.1% TFA) were applied to the dried sample and again allowed to dry. Spectra were obtained using a Bruker Autoflex II MALDI-TOF/TOF spectrometer (Bruker Daltonik, Bremen, Germany). The MS data were searched against the UniProt database with the Mascot search engine (<http://www.matrixscience.com>) following the peptide mass fingerprinting (PMF) method (Pappin et al. 1993). Mass tolerance of

30 ppm, hydrolysis with trypsin and two missed cleavages, carbamidomethylation of cysteine as fixed modification, oxidation of methionine as variable modification were specified; no post-translational modification were included in the search. To confirm the protein identification, the most intense signal of each spectrum underwent MS/MS analysis. The MS/MS spectra were automatically analysed by Flex Analysis software (Bruker Daltonik).

Results and discussion

Several studies assessing the authenticity of olive oil based on analytical techniques have been carried out (Ruiz del Castillo et al. 1998; Crevel et al. 2000; Parcerisa et al. 2000; Vichi et al. 2001; Baeten et al. 2005; Flores et al. 2006a). Our aim was to isolate and evaluate the concentration of putative residual proteins from EVOO, OO and HAO, and, via SDS-PAGE, estimate the presence of residual proteins in EVOO spiked with hazelnut oil. Retail samples of EVOO, OO and cold-pressed HAO were used. In addition, samples of HAO were obtained by solvent extraction with dichloromethane. A method for isolating and concentrating protein based on diethylether and trichloroacetic acid was used on all these samples. For EVOO and cold-pressed HAO, visible solid residue (dregs) at the bottom of the bottle was carefully mixed with the oil prior to protein isolation to obtain a representative extraction of the “whole” protein residue from the oil sample. OO did not contain such residues. After

extraction, the oil was filtered, avoiding any other type of refining process.

We chose not to consider any additional refining steps following filtration (e.g. deacidification, deodorisation, etc.), to allow a clear comparison of residual protein using two extraction procedures, i.e. solvent and cold-pressed extraction. Moreover, solvent-extracted HAO is very likely to be used in fraudulent adulteration of EVOO as it is a simple and cheap one-step procedure. Any additional refining steps would make the process of adulteration less attractive financially. The extraction method used in our laboratory (accelerated solvent extraction) is comparable to the industrial-solvent extraction of seed oils, allowing us to use a good model system to evaluate the residual presence of proteins secondary to tissue disruption.

Solvent-extracted hazelnut oil was the only analyzed sample, subjected to the extraction-concentration procedure, to have a protein precipitate (160 mg/albumin equivalent from 100 g of oil); cold-pressed HAO and olive oil did not show the presence of protein. Therefore, only the solvent-extraction procedure is capable of extracting proteins from hazelnut. We also increased 3-fold the initial amount of cold-pressed oil (300 g) and tried a SDS-PAGE separation followed by a very sensitive silver staining technique with a detection level of 0.3–10 ng (Switzer et al. 1979). With this technique we were unable to visualize any band. Thus, for gel staining, we choose to apply the protocol of colloidal Coomassie brilliant blue (G-250), which is more sensitive (~30 ng per band) than Coomassie brilliant blue (R-250) (~100 ng per band) and fully compatible with mass spectrometry analysis (Neuhoff et al. 1985). We found that the sensitivity of this staining technique was suitable for the purpose of this study.

Protein isolation from EVOO, OO and cold-pressed HAO showed no detectable bands on SDS-PAGE (Figure 1). The same analysis of solvent-extracted HAO clearly demonstrated the presence of different proteins with apparent molecular masses ranging from 10 to 60 kDa (Figure 1). The visible solid residue in EVOO and cold-pressed HAO is most likely be explained by the presence of phenolics and fibre compounds. Solvent-extracted HAO showed the clear presence of residual proteins as the solvent extracted the protein more efficiently from hazelnut seeds as well as dispersing residual protein-coated oil bodies. The use of different solvents in Soxhlet extraction is chosen for lipid extraction at inter- or intra-cellular level; hence, we can justify the presence of proteins in solvent-extracted and micro-filtered HAO according to “technological” differences between the different oils employed in our analysis.

For the EVOO samples spiked with solvent-extracted HAO, a conventional SDS-PAGE was able to show two bands down to 1% (v/v) solvent-extracted HAO.

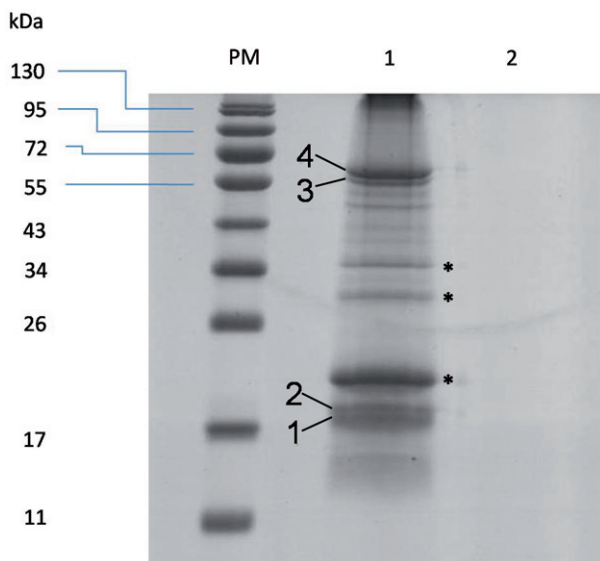


Figure 1. SDS-PAGE of total proteins isolated from solvent-extracted HAO (1) and EVOO (2) (colloidal Coomassie brilliant blue staining). Numbers highlight the proteins as “putative allergens” and (*) the bands analyzed but not identified.

Hazelnut oil was added to olive oil aliquots at 0.5, 1, 2, 3, 4 and 5% (v/v) corresponding to 0.8, 1.6, 3.2, 4.8, 8 mg of hazelnut protein, respectively (Figure 2). The result highlights how this simple method is a good tool for the discrimination of HAO-adulterated EVOOs, even though more advanced analytical chemical techniques have been suggested previously (Ruiz del Castillo et al. 1998; Baeten et al. 2005; Flores et al. 2006a; Chiavaro et al. 2008b). In our case, sensitivity was higher than that recorded for the monitoring of filbertone using an on-line hyphenated HPLC–GC technique, allowing the detection of adulterated olive oil with 5–10% of virgin and refined hazelnut oils (Flores et al. 2006b). A constraint in the suggested technique could be that the presence of hazelnut proteins is correlated only with some categories of HAO (solvent-extracted), as clearly shown in this study. When we applied the method to commercial samples of HAO (undeclared as to whether cold-pressed or refined), no protein contamination was detected, indicating the need of a more accurate and enlarged study in this field.

The detection of protein in adulterated oils is a major concern for allergic consumers. The allergenicity of different oils processed by different methods was previously investigated in peanut (*Arachis hypogaea* L.) and soybean (*Glycine max* L.) oils (Crevel et al. 2000). The presence of hazelnut protein in HAO has been demonstrated in several studies on nut oils; hazelnut gourmet oil was reported to contain up to $62.0 \pm 1.1 \mu\text{g}$ of protein in 1 ml of oil (Teuber et al. 1997). To date the possible allergenicity of nut oils is still under discussion (Peeters et al. 2004); understanding this safety issue is clearly affected by the different oil extraction and refining processes. The detection of residual hazelnut protein in adulterated olive oil (as well as the qualitative description and identification)

was never been thoroughly investigated. Two isoforms of oleosin with molecular masses of 16.7 and 14.7 kDa have recently been identified as new hazelnut allergens (Akkerdaas et al. 2006). Oleosins isolated from seed- or nut-derived oil bodies were reported to be contaminated with small but significant quantities of albumins and globulins. Albumins and globulins in seeds and nuts are major sources of allergenicity. Taken together, oil extraction from hazelnuts may result in residual protein content with a high likelihood of including allergens.

Considering the presence of allergenic proteins in solvent-extracted HAO, we aimed at identifying proteins using MALDI–TOF MS. This analysis clearly demonstrated the presence of two oleosin isoforms (UniProt ref. Q84T91, Q84T21) (Figure 1, bands 1 and 2), newly described by Akkerdaas et al. 2006) (http://expasy.org/uniprot/Q84T91_CORAV; http://expasy.org/uniprot/Q84T21_CORAV) and of two bands both containing 11S globulin-like protein (UniProt ref. Q8W1C2) (Figure 1, bands 3 and 4) (Beyer et al. 2002). Fifteen peptides belonged to the 11S globulin-like protein in band 4, whereas there were only nine in band 3, probably because of the different amount of the protein in the two bands. The presence of 11S globulin-like protein in two distinct bands is not surprising and it is probably due to post-translational modifications occurring on the protein and responsible for the slight differences in molecular weight compared to the nominal weights reported in Table 1. No specific investigation on post-translational modifications was performed because MS/MS data were obtained only for one signal and MS data are unreliable without MS/MS confirmation. Evidence of the presence of other allergens, such as Cor a 1 or Cor a 8, was not found. A more accurate bi-dimensional analysis should be carried out to improve the selectivity of protein fingerprint separation, permitting a clear identification of unknown proteins.

As oleosin and Cor a 9 were detected in hazelnut oil, immunoblotting was applied to confirm the presence of possible allergens by testing sera from both patients and sensitized animals (rabbit and mouse). We analyzed sera from four patients with a positive clinical history to hazelnut and an in-house serum with IgE reactivity to Cor a 1 and 2. The four patient sera (Figure 3, lanes 12–15) did not show any IgE reactivity; however, the in-house serum showed IgE reactivity to a protein with an apparent molecular mass of 17–18 kDa (Figure 3, lane 1). The main difference between the reference serum and the other sera is that the former has IgE-reactivity to profilin, while the four others do not. Profilin was not one of the proteins recognized in the MALDI–TOF analysis, which may be due to the low levels present in the extract.

Two rabbit antisera against hazelnut extracts confirmed the presence of hazelnut-derived protein

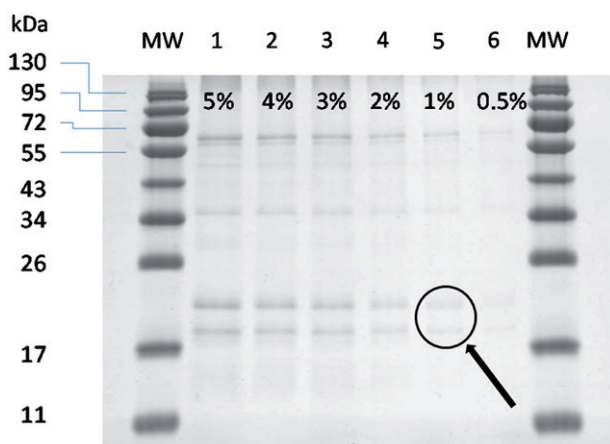


Figure 2. SDS–PAGE of total protein extract isolated from EVOO, artificially spiked with different percentage of solvent-extracted HAO (from 5 to 0.5% v/v). The arrow highlights hazelnut oleosins identified by MS/MS analysis.

Table 1. Identification by PMF and MS/MS of proteins selected from the total protein extract of solvent-extracted HAO (used to spike EVOO).

Band	Species	Protein name	UniProt KB/TrEmbl Entry	Nominal MW	Number of peptides	Sequence coverage	MS/MS (sequence)
1	<i>Corylus avellana</i>	Oleosin	Q84T91	14,723 Da	4/47	27%	HPPGADQLDHAR
		Oleosin	Q84T21	16,688 Da			
2	<i>Corylus avellana</i>	Oleosin	Q84T91	14,723 Da	4/84	27%	HPPGADQLDHAR
3	<i>Corylus avellana</i>	11S globulin-like protein	Q8W1C2	59,614 Da	9/86	22%	AESEGFWEVAFK
4	<i>Corylus avellana</i>	11S globulin-like protein	Q8W1C2	59,614 Da	15/75	28%	AESEGFWEVAFK

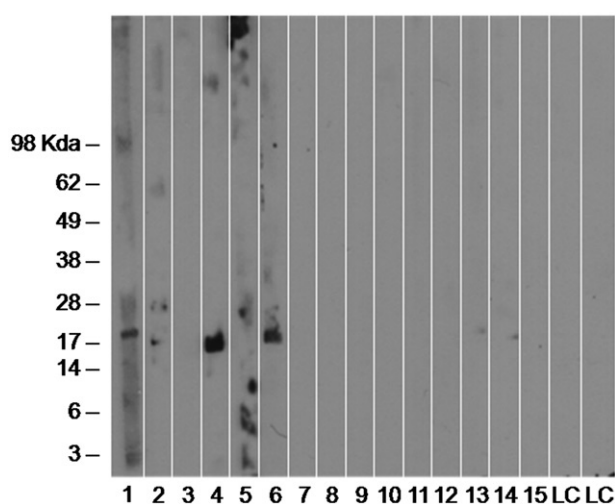


Figure 3. Immunoblotting profiling of the total protein fraction isolated from hazelnut oil. Lane 1: Bet v 1/Bet v 2 – and homologues – recognizing serum; lane 2: rabbit anti-hazelnut oleosin; lane 4: rabbit anti-hazelnut; lane 6: rabbit anti-hazelnut (pH 2.5 performed extraction); lane 8: rabbit anti-LTP; lane 10: mouse anti-Bet v 1 (Cor a 1); lanes 3, 5, 7, 9, 11: pre-immune sera (taken previously to immunization with the allergen or extract, thus before the animal develops specific antibodies); lanes 12–15: hazelnut positive human sera; lanes LC: label controls. Spots in lanes 2 and 5 are caused by a background phenomenon.

(Figure 3, lanes 4 and 6). According to these preliminary results, although only one in-house serum from an allergic patient gave a positive response in the immunoblot, the initial assumption of a potential risk to consumers can be considered plausible. The in-house serum was from a patient with known inhalant allergies; however, since this serum was from a retrospective patient, we have no history on the clinical reactivity to hazelnut. Although we do know the IgE-reactivity to Cor a 1 and 2 on blotting and in RAST/CAP experiments, we cannot claim that the reactivity will lead to clinical reactions on hazelnut. However, the purpose of the paper was to detect

allergens in adulterated olive-oil and, thus, this serum can be used.

The fraudulent adulteration of olive oil with hazelnut oil, already a problem in terms of commercial cost for the consumer, appears to exacerbate health and food safety-related problems with an additional risk associated to its allergenic component. This new issue, as highlighted in our preliminary study, should be considered for very severe cases of reported allergy in future research.

Conclusions

There is a potential risk for hazelnut-sensitized patients when exposed to fraudulently spiked virgin olive oil, especially in the case of solvent-extracted unrefined HAO. This preliminary observation confirms previous studies concerning the putative presence of allergens in oils of different origin. We conclude that our method represents a step toward the difficult task of detecting hazelnut oil in other olive oils, a fraudulent practice that not only causes economic damage but introduces a potential health risk for sensitized patients.

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