SUPPLEMENTARY MATERIAL

Clinical and occupational history, diagnostic tests and procedures

A 30 years-old male, non-smoker, who has been working for 1 year in a company which produced pharmaceutical and herbal products, referred to our Occupational Medicine Unit. He experienced a work-related rhino-conjunctivitis symptoms after 6 months started the work in the production task of tablets containing Orange aroma, Apple, Lemon, Polycaptil gel, Opuntia ficus indica, Orphophallus konjac, Althea officinalis, Linum usitatissimun, Tilia platyphyllos, Cichorium intybus.

He referred nasal obstruction, rhinorrhea, sneezing, itching and ocular burning those improved or disappeared when he was out of the work. Skin prick test to common aeroallergens and apple were negative. Prick by prick tests were carried out using single components of the tablets and resulted highly positive with linseed extract (20 mm wheal lasted for about 2 hours as well as a transient exacerbation of oculo-rhinitis symptoms, histamine wheal 7 mm).

The control skin prick by prick tests carried out in three subjects as controls were negative. Nasal provocation test was performed with flaxseed powder resulted in a highly positive response. It was impossible to measure the nasal resistance after the provocation test because of evident clinical rhino-conjunctivitis symptoms, followed few minutes of flaxseed dust exposure. Total serum IgE resulted of 27,3 KU/l and specific IgE to *Linum usitatissimun* 0,54 KU/l. The basophil activation test (BAT) was also performed with a linseed extract at a dilution of 1:1000 and resulted positive with a 26% of basophils expressed CD63 receptor (**Figure 1S**). The patient did not refer asthma symptoms, also after specific nasal provocation test, spirometry showed normal lung function with a 116% of Forced Vital Capacity and 112% of Forced Expiratory Volume of predicted values. He underwent treatment with antihistamine and nasal corticosteroids and changed the task with a significant reduction of exposure and improvement of symptoms. Occupational rhinitis due to *Linum usitatissimum* allergy was diagnosed and patient received medical certification for compensation. The patient continued to be followed by the occupational physician and continued to work. Usually, occupational rhinitis precedes the asthma onset (1) in particular in the case of high molecular weight agents as *Linum usitatissimum* allergenic proteins. In this case report, we identified seven new possible allergenic proteins of flaxseed relevant also in occupational exposure (Supplemental Materials and Methods). Allergens identified were described previous as flaxseed proteins with specific role in growing and maturation of *Linum usitatissimun* and seed, some of those were also described for their nutritional and medical properties (2, 3, 4). There was only one flaxseed allergen included in the allergen

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nomenclature, the Lin u 1, a 2S albumin (conlinin) protein that is recognized as causative protein of food allergy cases (5). Lipid transfer protein (LTP) and malate dehydrogenase-1 were also reported as flaxseed allergen proteins (6, 7). In the **Table 1S** we summarized proteins identified, their molecular weight and similar/homologous allergen proteins reported in other allergenic source and included in the WHO/IUIS allergen nomenclature database and sometimes responsible of flaxseed allergy reaction due to cross-reactions (8).

Materials and methods of proteins identification

Flax seeds coming from biological crops were purchased from a local market. For protein extraction from flax seeds: Trichloroacetic acid (TCA), Acetone, Methanol, Ammonium acetate, Sucrose, Sodium Dodecyl Sulfate (SDS), Trizma base, 2-mercaptoethanol, and Phenol solution were purchased from Sigma-Aldrich (Saint Louis, MO, USA). For the determination of protein content, Quick Start™ Bradford 1× Dye Reagent was purchased from Bio-Rad (Hercules, CA, USA). Prestained Protein SHARPMASS^{TM} VI protein MW marker from Euroclone (Pero, Italy). For gel staining: Coomassie Blue R-250 was purchased from Bio-Rad (Hercules, CA, USA). For immunoblot analysis: polyvinylidene difluoride (PVDF) membrane and Pierce™ ECL Western Blotting Substrate were obtained from Thermo Fisher Scientific (Waltham, MA, USA) whereas HRP-linked anti-human IgE was obtained from Cell Signaling Technology (Danvers, MA, USA). For 2DE: 8 M urea, 2 M thiourea, 2% CHAPS, 30 mM Tris, 100 mM 1,4-dithiothreitol, 0.8% ampholytes were obtained from Bio-Rad whereas IPG strips where purchase from GE-Healthcare (Chicago, IL, USA).

Protein Extraction from Flax Seeds

Flax seeds were ground for 60 seconds in a blender to obtain a fine powder. The protein extraction was carried out using the method for protein extraction from recalcitrant plant tissues with a few modifications (9) Briefly, 150 mg of the flaxseed powder were transferred into a 2 mL microtube filled with 10% (*w/v*) TCA/Acetone. The microtube was intensely vortexed for 30 seconds and then centrifuged at 16,000 × g for 3 minutes at 4 °C (5418 R, Eppendorf, Hamburg, Germany). The supernatant was removed and the microtube was filled with 80% Methanol plus 0.1 M Ammonium acetate and vortexed for 30 seconds in order to resuspend the pellet, and then centrifuged at 16,000 × g for 3 minutes at 4 °C. The supernatant was discarded to proceed to the third and last washing of the pellet with 80% Acetone. Again, the tube was vortexed for 30 seconds and then centrifuged at 16,000 × g for 3 minutes at 4 °C. The pellet obtained was placed at 50 °C for 10 minutes to evaporate the residual Acetone and then resuspended in 0.45 mL of Phenol solution pH 8.0 and 0.45 mL of SDS buffer (30% (*w/v*) Sucrose, 5% 2-mercaptoethanol (*v/v*), and 2% SDS (*w/v*) in 0.1 M Tris-HCl pH

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8.0). The solution was strongly vortexed for 30 seconds and incubated at room temperature for 5 minutes, then the Phenol phase was separated by centrifugation at 16,000 × g for 3 minutes at 4 °C. The upper Phenol phase was collected in a new 2 mL microtube that was filled with 80% Methanol plus 0.1 M Ammonium acetate solution and stored at -20 °C overnight. The proteins precipitated like a white floc and were recovered at 16,000 × g for 3 minutes at 4 °C. The resulting pellet was first washed with 80% Methanol plus 0.1 M Ammonium acetate solution, and then with 80% Acetone. Finally, it was resuspended in 0.15 mL of Isoelectric focusing (IEF) buffer (8 M urea, 2 M thiourea, 2% CHAPS, 30 mM Tris, 100 mM 1,4-dithiothreitol, 0.8% ampholytes). The protein content was assessed using the Bradford method, based on the use of Quick Start™ Bradford 1× Dye Reagent (Bio-Rad, Hercules, CA, USA), using bovine serum albumin (BSA; Sigma-Aldrich, Saint Louis, MO, USA) for calibration curve.

Two-dimensional electrophooresis

Flax seed protein solubilized in IEF sample buffer were loaded on IPG strips (7 cm, pH 3–10) during active rehydration for 4 h at 50 V. Proteins were than focused by using a Protean IEF Cell (Bio-Rad) at 20 °C, until a total 25,000 V/h were reached, fixing limiting current of 50 μA/strip. After focusing, proteins were equilibrated by incubating the IPG strips in equilibration buffer (EB) (375 mM Tris–HCl pH 8.8, 6 M urea, 20% w/v glycerol, 2% w/v SDS) containing 2% DTT for 12 min and then for further 12 min in EB with 2.5% w/v iodoacetamide. The second dimension was carried out on SDS page (10%) covering the IPG strips on the top of gels with 0.5% agarose. Preparative gels (obtained loading 300 ug of total proteins) were stained O.N. with colloidal Coomassie blue G250 (0.12% G250, 20% v/v methanol, 10% v/v o-phosphoric acid, 10% w/v ammonium sulphate) whereas 2-DE gels for WB (obtained loading 30 ug of total proteins) were blotted on PVDF membrase as above described. The resulting film and gel images were acquired by using GS-800 imaging systems (Bio-Rad). Molecular mass and pI values were estimated by running a sample containing plasma proteins together with a mixture of protein standards (Bio-Rad). Gels were later stained with Coomassie Blue R-250 (Bio-Rad, Hercules, CA, USA).

Immunoblot Analysis

The proteins were transferred to a polyvinylidene difluoride (PVDF) membrane using the Trans-Blot Turbo Transfer System (Bio-Rad, Hercules, CA, USA). The blotted PVDF membrane was washed three times in PBS with 0.1% (*v/v*) Tween 20 detergent. Then, membrane was incubated in blocking solution (5% (*w/v*) milk powder in PBS + 0.1% (*v/v*) Tween 20) for 1 hour at room temperature and

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then incubated over/night with patient serum at 4 °C. After three washes in PBS + 0.1% (*v/v*) Tween 20, the membrane was incubated for 1 hour at room temperature in a PBS solution containing 1% (*w/v*) BSA and 0.1% (*v/v*) Tween 20 with human anti-IgE antibodies conjugated with horseradish peroxidase (HRP). Blots were analyzed by the ECL detection system.

Mass spectrometry protein identification by ESI-LC-MSMS

Eleven spots were cut and sent at 4 °C to the Proteomics Core Facility (UNIVERSITE' deGENEVE, Geneve, Switzerland). Gel spots were in-gel digested with trypsin and peptides were analysed by nanoLC-MSMS using an easynLC1000 (Thermo Fischer Scientific) coupled with a Q-Exactive HF mass spectrometer (Thermo Fischer Scientific). Database searches were performed with MASCOT (Matrix Science) using the *Linum usitatissimum* database from Phytozome, the Plant Comparative Genomics portal of the Department of Energy's Joint Genome. Data were analysed and validated with Scaffold (Proteome Software) with 95% of probability at both peptide and protein level and at least 2 unique peptides per protein.

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