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# Prospective study of sensitization and food allergy to flaxseed in 1,317 subjects

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## KEY WORDS

Flaxseed allergy, industrial processing, cross-reactivity, CCD, extruded flaxseed

## SUMMARY

**Background:** Foods containing flaxseed proteins rich in polyunsaturated fatty acids are new on the market. **Objectives:** In a population of patients attending the allergology department, we evaluated the frequency of sensitization to flaxseed, characterized allergens and looked for modifications related to industrial processing. **Methods:** Natural, heated and extruded flaxseeds were tested using prick-in-prick tests (PIP using the fresh seed), SDS PAGE, immunoblots, immunoblot inhibition and Fourier Transform Infrared (FTIR) spectroscopy. **Results:** PIP tests to natural flaxseed were positive in 5.8% of the 1,317 patients. 73 of 77 PIP-positive patients were atopic. There was cross-reactivity with five seeds: peanut, soybean, rapeseed, lupine and wheat, and with rape pollen. Immunoblot inhibition by bromelain confirmed the presence of specific IgE to cross-reactive carbohydrate determinants (CCD). 0.15% of this population presented with food allergy to flaxseed and positive PIP to heated and extruded flaxseed. Two sera showed that clinically relevant allergens in industrial products had MW between 25 and 38 kDa. Sensitization to processed flaxseed characterized only the allergic subjects. FTIR spectroscopy showed major modifications in  $\beta$  and  $\alpha$  structures following industrial processing. **Conclusion:** Positive prick tests to natural flaxseed were mainly due to cross-reactions. Flaxseed allergy is rare and could be detected by PIP to heated extruded flaxseed. Increasing consumption calls for monitoring of clinical risk.

## Introduction

Food allergy is a public health issue and any new food appearing on the market should be carefully monitored. Amongst health foods, omega-3 polyunsaturated fatty acids (PUFA) have a preventive effect on cardiovascular disease. Flaxseed contains PUFA. PUFA exert a protective role in atopic dermatitis and reduce the prevalence of

atopy (1-6). Flaxseed given to horses with *Culicoides* species sensitivity significantly reduced the size of prick test wheal areas (7).

The recommended ratio of omega 6/omega 3 is around 1/5, but this is not obtained and other sources of supplementation are investigated, either of animal origin: fish (particularly sardines and tuna), or of vegetable origin (rapeseed oil, olive oil, blackcurrant seed oil, etc.) (8). A

**Abbreviations:** PUFA (Polyunsaturated Fatty Acids); PIP (Prick-in-prick) = prick-prick using the fresh seed; FTIR spectroscopy (Fourier Transform Infra Red spectroscopy); CCD (Cross-reactive Carbohydrate Determinants); DBPDFC (Double Blind Placebo Controlled Food Challenge); MW (Molecular Weight)

current strategy is to add varieties of flaxseed (*Linum usitatissimum*) rich in omega 3 into cereals. Detoxified flaxseed flour is now used in human food, but there are few data concerning the risk of food allergy. Only a few cases of food allergy with flaxseed or to flaxseed oil used as a laxative have been published (9-12).

It would therefore seem useful to envisage allergic risk related to novel food, such as that reported for the Micronesian nut Nangai (*Canarium indicum*) (13). The potential allergenicity of a new food has to take into account possible modifications of the natural food by food technologies (14). The primary objective of this study was to evaluate the prevalence of sensitization and allergy to natural flaxseed flour in 1,317 patients consulting for diverse allergic disorders. The secondary objectives were to study cross reactivity of flaxseed with other seeds (soybean, lupine, rape) and to compare sensitization to natural flaxseed with flaxseed found in food after transformation by two industrial methods: heating (heated flaxseed) then extrusion (extruded flaxseed).

## Material and methods

### Studied population

The prospective study was conducted over 18 months in 1,317 patients attending the allergology department (Internal Medicine, Clinical Immunology and Allergology, Nancy University Hospital Center). All subjects were routinely interviewed to detect consumption and possible food allergy to flaxseed and underwent prick tests to 12 standard airborne allergens. When food allergy was suspected, the routine diagnosis relied on PIP to 25 standard food allergens, extending to other allergens when required (9).

These patients were classified in two groups:

The first group comprised 603 non-atopic patients without any anamnesis of atopic disease, and negative prick tests to 12 airborne allergens. These patients consulted for allergy to Hymenoptera, drug-related adverse reactions, non-allergic rhinitis.

The second group comprised 713 atopic patients with atopic dermatitis, food allergy, allergic rhinitis or allergic asthma, confirmed by prick tests with at least one reference airborne or standard food allergen.

### Material

Airborne allergens were Allerbio extracts (Varennnes en Argonne)

The natural flaxseeds were obtained from a health food shop that guaranteed the absence of pesticides and other

products used in traditional agriculture. These products are commonly found in human food. They were stored at -80°C. They were ground to powder and mixed into a saline solution for prick tests.

Flaxseed flour was supplied by Valorex (35210 Combournillé, France). It contained eight varieties of *Linum usitatissimum*. Seeds were steamed at a temperature not exceeding 100°C. Extruded flaxseed was obtained from heated flaxseed that had been processed in an extruder (patented manufacturing technique). These products are used by the food industry.

### Prick tests

Basal skin reactivity was assessed by prick tests to 9% codeine and saline solution (negative control).

The 12 standard airborne allergens were: *D. pteronyssinus*, cockroach, *Alternaria alternata*, cat and dog epithelia, latex, grass pollen, birch pollen, *Artemisia pollen*, plantain pollen, ash pollen, rape pollen (extracts from Allerbio, Varennes en Argonne, France).

24 standard food allergens were tested by PIP (prick-prick using the fresh seed) (15). They were: milk, egg, fish, wheat flour, rye, celery, carrot, kiwi, peanut, hazelnut, walnut, Brazil nut, almond, sesame, buckwheat, crab, green peas, lentils, chicken, pork, garlic, onion, mustard, potato. Other allergens often tested were: various seeds (natural flaxseed, poppy seed etc.), tomato, Prunoideae, maize, soy, baker's yeast, spices, molluscs, beef and other meats.

Six patients consented to undergo PIP to Valorex products: natural, heated and extruded flaxseed flour.

Criteria for positivity was 3 mm on the condition that codein control be positive and saline solution be negative.

### Specific IgE

Specific IgE were assayed using Cap System RIA (Pharmacia, Uppsala, Sweden). Tests were performed on sera remaining from the blood samples from 26 patients with positive prick tests to flaxseed.

### DBPCFC

They were performed according to the procedure conducted in the department since 1990. Briefly, flaxseed flour from Valorex was mixed into stewed apple. Six doses were given at 20 minutes intervals: 5 mg – 10 mg – 50 mg – 150 mg – 285 mg – 465 mg. A further dose increase rose to 7,110 mg. Pulse, blood pressure peak flow rate, aspect of the skin and oral mucosa were evaluated.

#### *Preparation of protein extract*

Seed mixtures were prepared using one gram of seeds ground by Ultraturrax and one ml of buffer at pH 8 (0.1 M Tris, 0.14 M NaCl, 2 mM EDTA, 0.05% Triton, 0.05% Deoxycholate, 0.1% Nonidet P40). Bromelain extract was prepared by dissolving one gram of bromelain (Sigma, France) in 3 ml of PBS. After incubation overnight at 4°C while shaking, each mixture was centrifuged at 3,500 rpm for 15 min. The protein concentration of the supernatant, measured using the pyrogallol red method, was 10 mg/ml (natural flaxseed), 6 mg/ml (heated flaxseed), 3 mg/ml (extruded flaxseed), 11 mg/ml (soybean), 5 mg/ml (peanut), 49 mg/ml (lupine), 3 mg/ml (rapeseed), 4 mg/ml (wheat), 39 mg/ml (bromelain). Rapeseed pollen extract was supplied by Allerbio (Varennes en Argonne, France). After preparation, the extracts were immediately aliquoted and stored at -20°C.

#### *RAST inhibition assay*

RAST inhibition of flaxseed was conducted using the serum of the 14 patients with flaxseed-specific IgE higher than 1 kU/L. It was performed at a dose of 1.50 mg/mL of protein with each of the following extracts: peanut, soybean, wheat, rapeseed, rapeseed pollen, lupine, bromelain, natural flaxseed, heated flaxseed and extruded flaxseed.

Inhibition mixtures, containing 25 µL of the patient's serum and 25 µL of the test allergen extract, were first incubated with continuous stirring for 30 minutes at room temperature and subsequently incubated with the CAP allergen support before addition of the detecting reagents and measurement of the resulting radioactivity. A mixture of patient's serum and PBS served as the control mixture. All the experiments were carried out using the Pharmacia CAP System. The inhibition percentage was calculated as follows:  $1 - (\text{cpm bound to inhibiting mixture} / \text{cpm bound to control mixture})$ . The limit of positivity is currently 30% of inhibition. Inhibition tests could not be done in duplicate nor at several dilutions due to lack of serum. Because a single concentration of inhibitor was tested, the limit of positivity was raised to 40% of inhibition.

#### *Fourier Transform InfraRed (FTIR) spectroscopy*

Each type of seed (i.e. natural flaxseed, heated flaxseed, extruded flaxseed) was ground with KBr. Each KBr pellet was analysed using FTIR (Mattson, Unicam). The protein structure was studied by second derivative spectrophotometry between the wave-numbers 1,600-1,720

cm<sup>-1</sup>, 1,500-1,600 cm<sup>-1</sup> and 1,200-1,350 cm<sup>-1</sup>, corresponding to the absorption domains of the primary, secondary and third amide function of proteins.

#### *SDS-PAGE and immunoblotting*

Two sera were selected because of the high level of specific IgEs (8.5 and 11 kU/L): serum from patient 6 (sensitized patient) and serum from one allergic patient (case 2), respectively. Negative control immunoblots were tested using either an aqueous solution containing 1% BSA or the sera from 2 non-allergic patients.

Electrophoresis was conducted as previously described (14). Briefly, 4 µg of each protein extract (natural flaxseed, heated flaxseed, extruded flaxseed) were denatured and loaded on an 8-25% polyacrylamide gradient gel (Phast-System Pharmacia).

A molecular weight standard consisting of biotinylated proteins (Covalab, Dako) was also run on the gels.

After migration, the gel was either stained with Coomassie brilliant blue or transferred to a cellulose membrane with 0.45 µm pores. The Western blot was conducted as previously described (Franck 2002). Briefly, the membrane was incubated at room temperature for 1 hour in 5% bovine serum albumin, washed, incubated overnight with the serum, washed and incubated for 1 hour in a 1:10,000 dilution of peroxidase-conjugated human anti-IgE antibody (Dako) in 1% BSA, 0.05% Tween in PBS. After washing, the membrane was immersed in a solution of luminol in the presence of H<sub>2</sub>O<sub>2</sub> (Covalab, Dako) directly on a Kodak Digital Science 1D image analyzer. An image was acquired after an exposure time of 6.6 minutes (20 captures). In order to visualize the molecular weight standards, the membrane was incubated for 15 minutes in the presence of peroxidase-conjugated avidin (diluted 1:2,000) and revealed as described above.

#### *Immunoblot inhibition*

As a sufficient quantity of serum was required, immunoblot inhibition was conducted using only one serum, that of patient 6.

A volume of 500 µl of serum was pre-incubated overnight at 4°C in the presence of 500 µl (2.5 mg of protein) of the peanut extract. As described above, an immunoblot was carried out with this pre-blocked serum. A mixture of the patient's serum and PBS served as the control mixture. A positive control was conducted using flaxseed extract as an inhibitor.

## Results

### Clinical data

PIP tests to flaxseed were performed in 714 patients with food allergy or respiratory allergy (360 children aged under 15 years, 354 adults) and in 603 non-atopic patients (213 children, 390 adults). There was 12.2% prevalence of sensitization in atopic children and 8.2% in atopic adults. No sensitization was detected in 213 non-atopic children and the prevalence of 1% of sensitization characterized 390 non-atopic adults (Tab. 1).

Among the 77 patients sensitized to flaxseed, 73 were atopic. Of 44 children, 39 had food allergy and 31 were sensitive to airborne allergens, including 30 to pollens. Of 33 adults, 10 had food allergy, 21 had respiratory allergy or atopic dermatitis and were sensitive to airborne allergens

(including pollens). The study of associated sensitizations in atopic children and adults sensitized to flaxseed proteins, showed polysensitization to airborne allergens and food allergens in nearly all cases (Tab. 2). Standard airborne allergens were predominant: grass pollen, birch pollen, ash pollen, plantain pollen, *Artemisia* pollen and dog and cat epithelia.

Two cases of food allergy to flaxseed were diagnosed. Case 1 was a 41-year old female with atopic dermatitis and asthma, allergic to birch, grass, ash, pollens, cat epithelia, latex, polysensitized to avocado, banana, sweet pepper, buckwheat. She experienced laryngeal angio-edema after eating 13 g of flaxseed. The prick test was positive (10.5 mm) and flaxseed RAST was at 0.51 kU/L. The DBPCFC was positive at the cumulative dose of 265 mg, eliciting generalized urticaria.

**Table 1** – Sensitization to flaxseed assessed by PIP to natural flaxseed

|          | Non-atopic    | Evolutionary atopic diseases | Total           |
|----------|---------------|------------------------------|-----------------|
| Children | 0/213 (0%)    | 44/360 (12.20%)              | 44/573 (7.68%)  |
| Adults   | 4/390 (1.02%) | 29/354 (8.2%)                | 33/744 (4.44%)  |
| Total    | 4/603 (0.66%) | 73/714 (10.2%)               | 77/1317 (5.84%) |

**Table 2** – Sensitizations associated to flaxseed sensitization in atopic children and adults

|                             | Airborne allergens (12) |                  |                    | Food allergens (24) |                  |
|-----------------------------|-------------------------|------------------|--------------------|---------------------|------------------|
|                             | Atopic <15 years        | Atopic >15 years |                    | Atopic <15 years    | Atopic >15 years |
| Grass                       | 55%                     | 100%             | Pulses*            | 51%                 | 16%              |
| <i>Artemisia</i>            | 26%                     | 35%              | Nuts*              | 76%                 | 32%              |
| Rape                        | 16%                     | 23%              | Cereals*           | 16%                 | 40%              |
| Plantain                    | 29%                     | 29%              | Egg (white, yolk)  | 38%                 | 12%              |
| Birch                       | 42%                     | 59%              | Sesame             | 16%                 | 20%              |
| Ash                         | 42%                     | 53%              | Fish               | 17%                 | 8%               |
| <i>Alternaria alternata</i> | 19%                     | 12%              | Kiwi               | 12%                 | 12%              |
| Dog                         | 32%                     | 53%              | Milk               | 12%                 | 0                |
| Cat                         | 45%                     | 47%              | Potato             | 0                   | 12%              |
| Cockroach                   | 6%                      | 6%               | Mustard            | 4%                  | 12%              |
| <i>D. pteronyssinus</i>     | 32%                     | 76%              | Garlic             | 2%                  | 12%              |
| Latex                       | 0 %                     | 18%              | Apiaceae*          | 0                   | 12%              |
|                             |                         |                  | Buckwheat          | 0                   | 8%               |
|                             |                         |                  | Meats*             | 2%                  | 8%               |
|                             |                         |                  | Crustaceans (crab) | 2%                  | 0                |

Explanatory note: Pulses = green peas, lentils; Meats = chicken, pork; Apiaceae = carrot, celery; Cereals = wheat, rye; Nuts =hazelnut, walnut, peanut, Brazil nut, almond

**Table 3** - Compared allergenicity of natural flaxseed, heated flaxseed and extruded flaxseed in a flaxseed-allergic patient (FA) and five sensitized patients (S)

| Clinical status | Sex | IgE Flaxseed<br>kU/L | Codeine<br>(mm) | Flaxseed<br>(mm) | PIP wheal diameter (mm)        |                         |                           |
|-----------------|-----|----------------------|-----------------|------------------|--------------------------------|-------------------------|---------------------------|
|                 |     |                      |                 |                  | Natural flaxseed<br>flour (mm) | Heated flaxseed<br>(mm) | Extruded flaxseed<br>(mm) |
| FA              | F   | 0.51                 | 3               | 10               | 10.5                           | 15.5                    | 13.5                      |
| S               | M   | 8.8                  | 3               | 5                | 6                              | 0.5                     | 0                         |
| S               | M   | 3.7                  | 2.5             | 4                | 3                              | 1                       | 1                         |
| S               | F   | <0.35                | 5               | 3                | 6                              | 0                       | 0                         |
| S               | M   | <0.35                | 5               | 4                | 5                              | 2                       | 0                         |
| S               | M   | <0.35                | 1.5             | 5.5              | 5                              | 0                       | 0                         |

Case 2 was a 40-year old female with past history of asthma, presenting recurrent systemic reactions (generalized urticaria, gastric pain and conjunctivitis after eating bread containing flaxseed. The prick test was positive (10.5 mm) and elicited conjunctivitis. Flaxseed RAST was at 11 kU/L. An oral challenge was not performed because of the syndromic reaction to prick test.

Globally 63% of patients sensitized to flaxseed had some kind of food allergy, 67% were sensitized to pollens and 0.15% had food allergy to flaxseed. It should be noted that case 2 had positive PIP to heated and extruded flaxseed, whereas five sensitized patients had negative results (Tab. 3).

Flaxseed-specific IgE were determined for 26 cases of sensitization detected by PIP. They were present in 16 cases. Amongst these, 14 sera with flaxseed-specific IgE ranging from 1 to 11 kU/L were analysed by RAST inhibition assays with extracts of soybean, peanut, lupine, wheat and rape. Inhibition reached significant levels up to 78%, and was homogenous for the five seeds tested and for rape pollen (Tab. 4). The comparison of the rate of rapeseed inhibition and rape pollen inhibition may indicate common allergens between seeds and pollens. Out of the 14 sera, 11 had specific IgEs to bromelain. Bromelain consistently and impressively inhibited flaxseed RAST. However, testing several concentrations of inhibitor would have improved the quality of the results.

A study was carried out in 6 subjects comparing natural, heated and extruded flaxseed flour reactivity by PIP. The flaxseed allergic patient had positive heated and extruded flaxseed PIP, while sensitized patients had negative PIP (Tab. 3). RAST inhibition of flaxseed using natural, heated and extruded flaxseed showed significant inhibition with 12/14 sera, i.e. not for patients 5 and 9 who showed inhibition percentages ranging between 14 and 20% (Tab.

5). Patient 9 had no food allergy and was sensitized only to pollens (birch and grass). The fact that we observe a positive result to flaxseed and a negative inhibition of this Cap by flaxseed might indicate a very low affinity of IgE to flaxseed. A technical bias cannot be excluded. We postulate that there was cross reactivity between flaxseed and pollens and the high rate inhibition by bromelain may indicate the presence of anti CCD IgEs. Besides, in Table 4, no inhibition at all is observed by any food. Case 5 had cashew allergy and inhibition of flaxseed RAST was obtained by all other seeds than flaxseeds (Tab. 4). The meaning of this discrepancy is not clear.

#### SDS-PAGE

A similar electrophoresis pattern was observed with the extracts from natural, heated and extracted flaxseed (Fig. 1). For each sample, three protein fractions with molecular weights of 14, 25 and 35 kDa were stained.

#### Western blot

The immunoblot conducted using the serum of patient 6 (flaxseed sensitized patient, RAST= 8.5 kU/L) showed several bands from 5 to 40 kDa, present in natural and processed flaxseed (heating and extrusion). In extruded flaxseed flour, only one band of 30-35 kDa appeared (Fig. 2). The immunoblot from case 2 (flaxseed-allergic patient, RAST= 11 kU/L) showed two bands at 25 and 38 kDa (natural flaxseed) and one band (38 kDa) with heated and extruded flaxseed. Control tests performed using a BSA solution or sera from two non-allergic patients were negative.

#### Blot inhibition

Incubation of serum from patient 6 with peanut extract showed complete inhibition of the blot. The same result

**Table 4** – RAST inhibition of flaxseed using different extracts in the 14 sera of sensitized patients with RAST values exceeding 1 kU/L

| Patient | Clinical status             | Flaxseed PIP (mm) | Sex | Age (years) | Flaxseed RAST (kU/L) | Peanut inhibition | Soybean inhibition | Wheat inhibition | Rape seed inhibition | Rape pollen inhibition | Lupine inhibition | Bromelain RAST (kU/L) | Bromelain inhibition |
|---------|-----------------------------|-------------------|-----|-------------|----------------------|-------------------|--------------------|------------------|----------------------|------------------------|-------------------|-----------------------|----------------------|
| 1       | Peanut allergy              | 13.5              | F   | 10          | 10.9                 | 69%               | 56%                | 70%              | 30%                  | 21%                    | 60%               | 2.02                  | 90%                  |
| 2       | Wheat flour allergy         | 4                 | M   | 47          | 2.9                  | 62%               | 60%                | 70%              | 35%                  | 40%                    | 60%               | 7.87                  | 85%                  |
| 3       | Peanut allergy              | 4                 | M   | 11          | 10.9                 | 54%               | 30%                | 34%              | 57%                  | 48%                    | 42%               | 0.67                  | 89%                  |
| 4       | Peanut allergy              | 4                 | M   | 11          | 2.22                 | 62%               | 35%                | 65%              | 49%                  | 47%                    | 48%               | 1.88                  | 90%                  |
| 5       | Cashew allergy              | 4.5               | M   | 11          | 2.86                 | 53%               | 54%                | 33%              | 56%                  | 57%                    | 50%               | 1.11                  | 92%                  |
| 6       | Peanut allergy              | 2.5               | M   | 11          | 8.54                 | 71%               | 50%                | 74%              | 52%                  | 32%                    | 55%               | 1.07                  | 92%                  |
| 7       | Peanut allergy              | 7                 | M   | 6           | 10                   | 60%               | 82%                | 70%              | 73%                  | 55%                    | 85%               | 1.5                   | 85%                  |
| 8       | Atopic dermatitis           | 4                 | M   | 3           | 2.68                 | 57%               | 63%                | 51%              | 60%                  | 40%                    | 60%               | < 0.35                | 80%                  |
| 9       | Atopic dermatitis           | 5                 | M   | 58          | 7.78                 | 5%                | 3%                 | 2%               | 0%                   | 0%                     | 5%                | < 0.35                | 93%                  |
| 10      | Peanut allergy              | 5                 | M   | 11          | 1.14                 | 70%               | 40%                | 78%              | 53%                  | 45%                    | 50%               | 1.98                  | 47%                  |
| 11      | Wheat flour allergy (cured) | 5                 | M   | 12          | 3.24                 | 62%               | 45%                | 32%              | 57%                  | 40%                    | 55%               | 7.54                  | 76%                  |
| 12      | Peanut allergy              | 3                 | F   | 6           | 4.46                 | 68%               | 56%                | 61%              | 55%                  | 41%                    | 62%               | < 0.35                | 91%                  |
| 13      | Peanut allergy              | 4                 | M   | 9           | 1.4                  | 55%               | 50%                | 45%              | 55%                  | 39%                    | 53%               | 1.9                   | 75%                  |
| 14      | Peanut allergy              | 3                 | M   | 7           | 8.06                 | 62%               | 55%                | 55%              | 50%                  | 35%                    | 60%               | 1.2                   | 92%                  |

was obtained using a protein extract of natural flaxseed, tested as a positive control (Fig. 3).

#### FTIR

Analysis by Fourier Transform Infrared (FTIR) spectrometry showed a modification in the spectrum at the 1,317 cm<sup>-1</sup> and 1,650 cm<sup>-1</sup> regions (alpha structure) and at the 1,242 cm<sup>-1</sup>, 1,620 cm<sup>-1</sup> and 1,640 cm<sup>-1</sup> regions (beta structures), after heating. Major changes between 1,560 and 1,570 cm<sup>-1</sup> were observed after heating (Fig. 4).

#### Discussion

Flaxseed (*Linum usitatissimum*) belongs to the Dicotyledone family. Although the risk of sensitization has already been

reported (16), clinical case reports are rare, and related to other seeds (in bread) or to flaxseed oil used as a laxative (11, 12). The presence of specific IgE has been demonstrated by RAST confirming positive prick tests (10). Cases of occupational asthma have been reported (17, 18).

Flaxseeds are rich in protein: 20% to 49% of the seed (Viking cultivar). 40% are albumins, 40% are globulins, 6.6% are prolamins and 13.5% are glutelins. The storage proteins are essentially of the legumin family (19).

Allergenicity to flaxseed has been studied in two cases of allergy to flaxseed, corresponding to reactive bands at 38, 35, 30, 22 and 20 kDa (11). Under non-reducing conditions, proteins of 150–175 kDa bind intensively to IgE, showing that there are many potentially allergenic subunits (10, 12). A 56 kDa allergen was identified in one

**Table 5** - Cross-reactivity evaluated by RAST inhibition of flaxseed by extracts of natural, heated and extruded flaxseed in the 14 sera with flaxseed RAST higher than 1 kU/L

| Patients | Flaxseed RAST (kU/L) | Natural flaxseed inhibition (%) | Heated flaxseed inhibition (%) | Extruded flaxseed inhibition (%) |
|----------|----------------------|---------------------------------|--------------------------------|----------------------------------|
| 1        | 10.9                 | 63                              | 69                             | 72                               |
| 2        | 2.9                  | 60                              | 62                             | 70                               |
| 3        | 10.9                 | 41                              | 37                             | 40                               |
| 4        | 2.22                 | 62                              | 66                             | 55                               |
| 5        | 2.86                 | 14                              | 21                             | 32                               |
| 6        | 8.54                 | 60                              | 75                             | 77                               |
| 7        | 10                   | 79                              | 90                             | 89                               |
| 8        | 2.68                 | 48                              | 49                             | 61                               |
| 9        | 7.78                 | 20                              | 30                             | 28                               |
| 10       | 1.14                 | 65                              | 74                             | 66                               |
| 11       | 3.24                 | 52                              | 59                             | 63                               |
| 12       | 4.46                 | 61                              | 65                             | 67                               |
| 13       | 1.4                  | 55                              | 59                             | 63                               |
| 14       | 8.06                 | 60                              | 63                             | 70                               |

case and could be a dimer corresponding to the 28 kDa subunit. A malate dehydrogenase would be one candidate (12). It is possible that the band we observed at 25 kDa corresponded to this protein.

In this population of 1,317 patients consulting in the allergology department, 5.8% were sensitized to natural flaxseed. 54% of the 1,317 patients presented with atopic disease (food or respiratory allergy). Extrapolation to the French population, on the basis of an overall rate of 5% to 10% for atopic disease (respiratory allergies, atopic dermatitis, food allergies), produces an estimated prevalence of flaxseed sensitiza-

tion in the French population ranging from 0.54% to 1.08%. However, allergy to flaxseed is extremely rare (2 cases out of 1,317), corresponding to an estimated 0.01 to 0.02% of the population (1 allergic patient for 6,000 inhabitants). The frequency of positive prick tests to multiple airborne and/or food allergens indicates possible cross-reactions that could explain positive prick tests to flaxseed. There was marked positivity of RAST inhibition assays with different seeds. There was complete immunoblot inhibition to flaxseed by a peanut extract. This could indicate that part of the cross-reaction is related to the storage proteins that represent a large percentage of the total proteins. The protein composition of flaxseed, because of shared homologous proteins with the other seeds (lupine soy, rape seed wheat), explains the inhibition of flaxseed

**Figure 1** - SDS-PAGE (8-25%) of different protein extracts stained with Coomassie brilliant blue

Band 1: standard MWs  
Band 2: natural flaxseed  
Band 3: heated flaxseed  
Band 4: extruded flaxseed

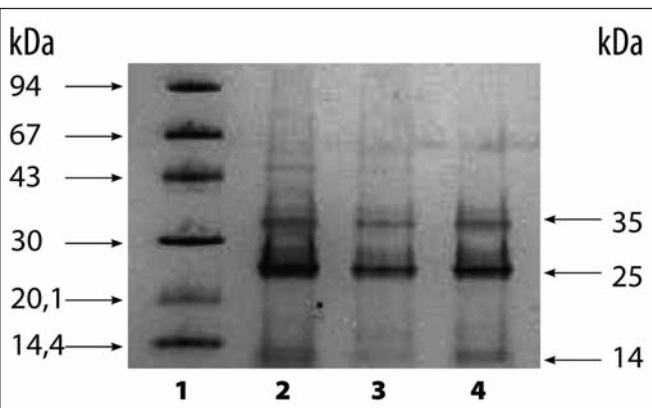


**Figure 2** - Immunoblot carried out with one serum from a sensitized patient (patient 6) and one serum from a flaxseed-allergic patient (case 2)

Band 1: natural flaxseed  
Band 2: heated flaxseed  
Band 3: extruded flaxseed

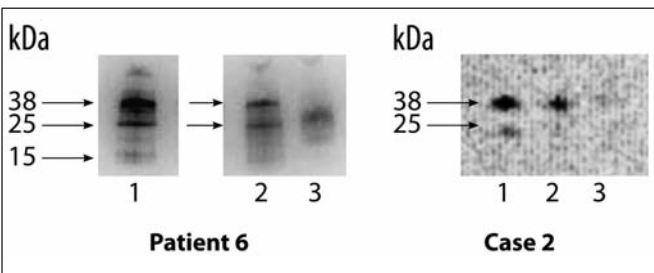
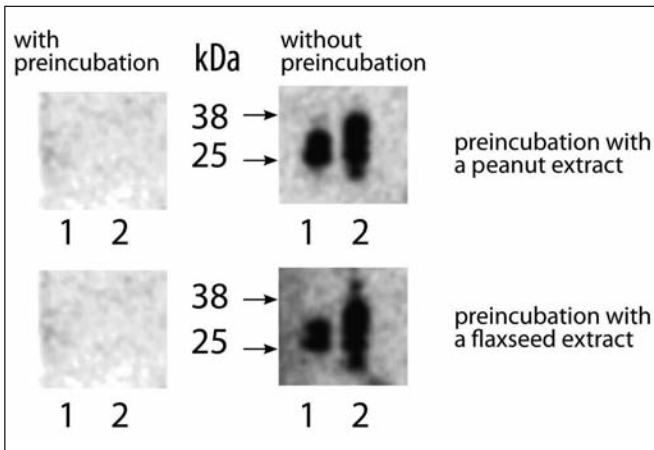


**Figure 3** - Inhibition of immunoblots conducted with the serum of a sensitized patient (Patient 6) with or without pre-incubation of serum with a peanut extract, or with a natural flaxseed extract.

Band 1: extruded flaxseed

Band 2: natural flaxseed



RAST by these seeds. However, further details cannot be discussed because of the use of a single dose inhibition. The frequency of pollen polysensitization and the constant RAST inhibition of flaxseed by bromelain suggest that cross-reactive carbohydrate determinants (CCD) are involved in these cross-reactions. In three cases, a discrepancy between the positive inhibition by bromelain and the negative RAST to bromelain might point to poor availability of CCD on the RAST matrix.

Anti-CCD antibodies are relatively frequent, particularly in patients sensitized to pollen and hymenoptera venom (20-22). The clinical relevance of anti-CCD IgE is much discussed (23, 24). In general, these antibodies do not seem to be involved in food allergy (25). Besides, prick tests to vegetable glycosylated proteins are negative when the sensitization is exclusively directed towards CCD. In patients having positive PIP to natural flaxseed, negative PIP with heated and extruded products are observed. Indeed, FTIR spectrometry confirmed the profound modification of alpha and beta structures after heating and extrusion. These data support the hypothesis of sensitization to conformational epitopes.

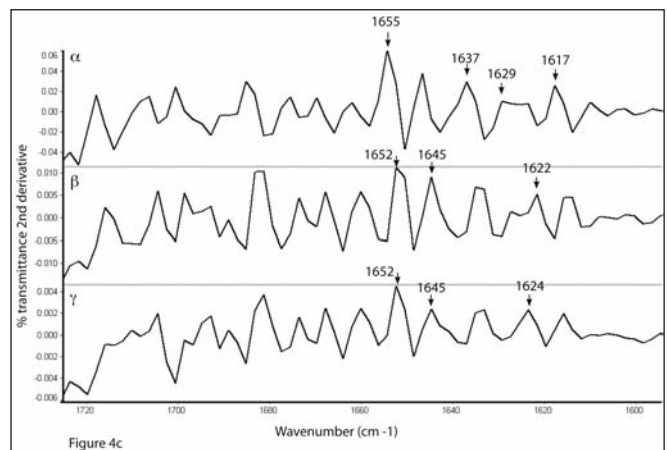
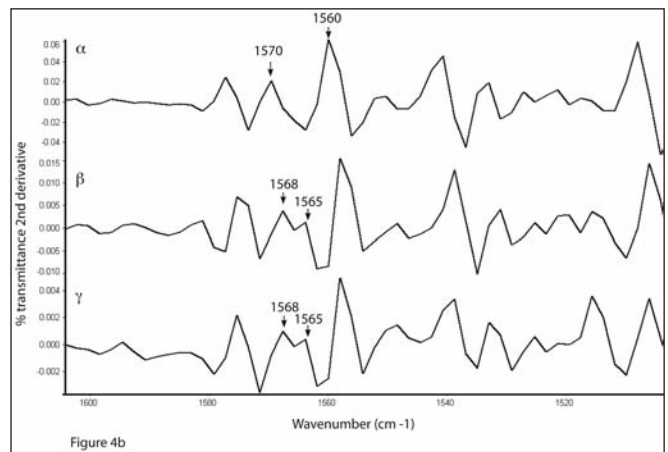
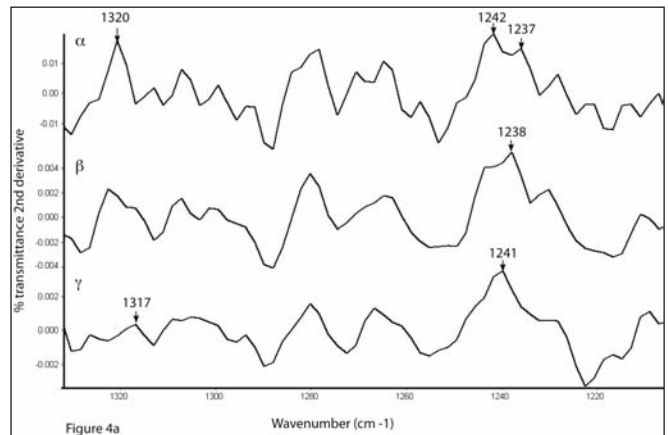
The patient allergic to flaxseed (case 2) had positive PIP tests with heated and extruded flaxseed. Immunoblot testing confirmed IgE binding to a 38 kDa band present in both heated and extruded flaxseed. Hence the basis of allergenicity could be related to thermostable peptides that

**Figure 4** - Fourier Transformed InfraRed (FTIR) spectroscopy. Comparison of transmittance second-derivative spectra in the amide I (1,600-1,720 cm<sup>-1</sup>), amide II (1,500-1,600 cm<sup>-1</sup>), and amide III (1,200-1,350 cm<sup>-1</sup>) regions of natural, heated and extruded flaxseed.

$\alpha$ : Natural flaxseed

$\beta$ : Extruded flaxseed

$\gamma$ : Heated flaxseed



resist extrusion. This justifies the hypothesis of clinically relevant linear epitopes.

To conclude, the relative frequency of skin sensitization, demonstrated by PIP to natural flaxseed, seems to mainly reflect cross-reactivity, and corresponds to allergens that are not clinically relevant. These may be related to CCD in vegetable proteins, and/or to homologies between storage proteins in seeds. The latter would have to be studied. Only two out of 77 patients with positive PIP to natural flaxseed were allergic to flaxseed, documenting the assertion that PIP to natural flaxseed are of no use for diagnosis of flaxseed allergy. If flaxseed allergy is suspected, PIP should be carried out with heated flaxseed or extruded flaxseed obtained from industry.

Given the nutritional benefits of flaxseed, it is becoming increasingly popular in food preparations, indicating that structures like the Allergy Vigilance Network will have to monitor the potential clinical risk. No other case has been declared out of the 812 anaphylactic food reactions reported over the last 7 years (26). Studies of allergenicity of new foods have to include these natural foods but moreover must take into account the modifications of allergenicity induced by various food technologies.

## References

1. Yu G, Björkstén B: Polyunsaturated fatty acids in school children in relation to allergy and serum IgE levels. *Pediatr Allergy Immunol* 1998; 9: 133-8.
2. Kankaanpää P, Sütas Y, Salminen S, Lichtenstein A, Isolauri E: Dietary fatty acids and allergy. *Ann Med* 1999; 31: 282-7.
3. Beck M, Zelczak G, Lentze MJ: Abnormal fatty acid composition in umbilical cord blood of infants at high risk of atopic disease. *Acta Paediatr* 2000; 89: 279-84.
4. Duchon K, Casas R, Fageras-Böttcher M, Yu G, Björkstén B: Human milk polyunsaturated long-chain fatty acids and secretory immunoglobulin A antibodies and early childhood allergy. *Pediatr Allergy Immunol* 2000; 11: 29-39.
5. Field C, Clandinin M, Van Aerde J: Polyunsaturated fatty acids and T-cell function: implications for the neonate. *Lipids* 2001; 36: 1025-32.
6. Oomah BD: Flaxseed as a functional food source. *J. Sci. Food Agric* 2001; 81: 889-94.
7. O'Neill W, McKee S, Clarke AF: Flaxseed (*Linum usitatissimum*) supplementation associated with reduced skin test lesional area in horses with *Culicoides* hypersensitivity. *Can J Vet Res* 2002; 66: 272-7.
8. Harel Z, Riggs S, Vaz R, White L, Menzies G: Omega-3 polyunsaturated fatty acids in adolescents: knowledge and consumption. *J Adolesc Health*. 2001; 28: 10-5.
9. Stricker WE, Anorve-Lopez E, Reed CE: Food skin testing in patients with idiopathic anaphylaxis. *J Allergy Clin Immunol* 1986; 77: 516-9.
10. Lezaun A, Fraj J, Colás C, et al: Anaphylaxis from linseed. *Allergy* 1998; 53: 105-6.
11. Alonso L, Marcos ML, Blanco JG, et al: Anaphylaxis caused by linseed (flaxseed) intake. *J Allergy Clin Immunol* 1996; 98: 469-70.
12. León F, Rodríguez M, Cuevas M: The major allergen of linseed. *Allergy* 2002; 57: 968.
13. Sten E, Stahl Skov P, Andersen SB, et al: Allergenic components of a novel food, Micronesian nut Nangai (*Canarium indicum*), shows IgE cross-reactivity in pollen allergic patients. *Allergy* 2002; 57: 398-404.
14. Franck P, Moneret Vautrin DA, Dousset B, et al: The allergenicity of soybean-based products is modified by food technologies. *Int Arch Allergy Immunol* 2002; 128: 212-9.
15. Dreborg S: Skin tests in the diagnosis of food allergy. *Pediatr Allergy Immunol* 1995; 6 (suppl 8): 38-43.
16. Grant LR: A report of six cases of flaxseed sensitization with review of the literature. *J Allergy* 1931; 3: 469-77.
17. Charpin J, Zafropoulos A, Luccioni R: Occupational asthma in the fat bodies industry. *Poumon Coeur* 1966; 22: 513-21.
18. Vandenplas O, D'Alpaos V, César M, Collet S, Tafforeau M, Thimpoint J: Occupational asthma caused by linseed oilcake. *Allergy* 2008; 63: 1250-1.
19. Sammour RH: Proteins of linseed (*Linum usitatissimum* L.), extraction and characterization by electrophoresis. *Bot Bull Acad Sin* 1999; 40: 121-6.
20. Mari A: IgE to cross-reactive carbohydrate determinants: analysis of the distribution and appraisal of the in vivo and in vitro reactivity. *Int Arch Allergy Immunol* 2002; 129: 286-95.
21. Malandain H: IgE-reactive carbohydrate epitopes--classification, cross-reactivity, and clinical impact. *Allerg Immunol* 2005; 37: 122-8.
22. Jappe U, Raulf-Heimsoth M, Hoffmann M, Burow G, Hübsch-Müller C, Enk A: In vitro hymenoptera venom allergy diagnosis: improved by screening for cross-reactive carbohydrate determinants and reciprocal inhibition. *Allergy* 2006; 61: 1220-9.
23. Fötisch K, Altmann F, Hausteiner D, Vieths S: Involvement of carbohydrate epitopes in the IgE response of celery-allergic patients. *Int Arch Allergy Immunol* 1999; 120: 30-42.
24. Vieths S, Scheurer S, Ballmer-Weber B: Current understanding of cross-reactivity of food allergens and pollen. *Ann N Y Acad Sci* 2002; 964: 47-68.
25. van der Veen MJ, van Ree R, Aalberse RC, et al: Poor biologic activity of cross-reactive IgE directed to carbohydrate determinants of glycoproteins. *J Allergy Clin Immunol* 1997; 100: 327-34.
26. Moneret-Vautrin DA, Morisset M, Flabbee J, Beaudouin E, Kanny G: Epidemiology of life-threatening and lethal anaphylaxis: a review. *Allergy* 2005; 60: 443-51.