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Proteomic identification of allergenic seed proteins, napin and cruciferin, from cold-pressed rapeseed oils

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ABSTRACT

In Finland and France atopic children commonly react to seeds of oilseed rape and turnip rape in skin prick tests (SPT) and open food challenges. These seeds are not as such in dietary use and therefore the routes of sensitization are unknown. Possible allergens were extracted from commercial cold-pressed and refined rapeseed oils and identified by gel-based tandem nanoflow liquid chromatography mass spectrometry (LC–MS/MS). Napin (a 2S albumin), earlier identified as a major allergen in the seeds of oilseed rape and turnip rape, and cruciferin (an 11S globulin), a new potential seed allergen, were detected in cold-pressed oils, but not in refined oils. Pooled sera from five children sensitized or allergic to oilseed rape and turnip rape seeds reacted to these proteins from cold-pressed oil preparations and individual sera from five children reacted to these proteins extracted from the seeds when examined with IgE immunoblotting. Hence cold-pressed rapeseed oil might be one possible route of sensitization for these allergens.

1. Introduction

It has been shown that Finnish and French atopic children with clinical allergies to common foods frequently react to seeds of oilseed rape (*Brassica napus* ssp. *oleifera*) and turnip rape (*Brassica rapa* ssp. *oleifera*) in skin prick tests (SPT) and open food challenges (Poikonen et al., 2006, 2009). Major allergens (Bra n 1 and Bra r 1, respectively) were identified as 2S albumins; napins that are seed storage proteins in both of these oil plants (Puumalainen et al., 2006) and represent approximately 20% of the total protein. Napin, consisting of a small and large chain linked by disulphide bonds (Lonnerda & Janson, 1972), is reported to be extremely resistant to pepsin digestion and denaturation caused by heat and low pH (Murtagh et al., 2003). Thus it is possible that napin is not destroyed during conventional food processing. The main seed storage protein in these plants is an 11S globulin, cruciferin, two subunits that are composed of acidic and basic chains linked by a cysteine S–S bridge (Sjodahl, Rodin, & Rask, 1991). Cruciferin is one of the major allergens in white mustard and hazel nut (Beyer, Grishina, Bardina, Grishin, & Sampson, 2002; Palomares et al., 2005).

The route of sensitization in allergy to oilseed rape and turnip rape is unknown since the seeds as such are not in dietary use (Poikonen et al., 2006, 2008, 2009). Napins in oilseed rape, turnip rape, and mustard are highly cross-reactive (Poikonen et al., 2009; Puumalainen et al., 2006). In Finland, however, the consumption of mustard is minimal among lactating mothers or infants and therefore mustard is unlikely to be the first sensitizer. In contrast, in France, mustard is a common food allergen, and its consumption is the highest in Europe (Rance, 2003).

Mixed oil from oilseed rape and turnip rape (i.e. rapeseed oil) is the most widely used vegetable oil in Finnish households and food industry. The clinical relevance of allergy to oilseed rape and turnip rape is disputable, since there is no evidence that rapeseed oil, when ingested as such, causes or worsens symptoms in oilseed rape- and turnip rape-allergic patients (Poikonen et al., 2006). However, some vegetable oils, such as peanut oil, have been reported to contain allergenic proteins (Olszewski et al., 1998). Other vegetable oils have been reported to contain allergenic

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proteins although their role in allergic reactions, especially to refined oils, remains controversial (Crevel, Kerkhoff, & Koning, 2000; Morisset et al., 2003).

During the manufacture of cold-pressed oils, oil is pressed from the oilseeds without heating and the sediment from the seeds is settled down. Thus these kinds of oils may contain traces of seed proteins. To study whether commercial rapeseed oils carry seed allergens, we extracted possible proteins from the oils with acetone and analyzed them by tandem mass spectrometry (LC–MS/MS) from the stained protein gels. The allergenic potential of the oil and seed extracts was assessed by IgE immunoblotting using sera from children sensitized or allergic to the seeds of oilseed rape and turnip rape.

2. Methods

2.1. Acetone extraction of commercial rapeseed oils

Eight commercial rapeseed oils were selected from Finnish grocery stores. Four of them were cold-pressed and four refined (Table 1).

One volume of oil (30 ml) was extracted with four volumes of ice-cold acetone by incubating the mixture at −20 °C for 24–48 h. After centrifugation (4500 g/30 min/+4 °C) the precipitant was washed three times with four volumes of ice-cold acetone and re-suspended in 100 μl of Laemmli buffer (diluted from commercial 10× Tris Glycine SDS Buffer, Bio-Rad Laboratories) overnight at room temperature in a swinging shaker.

2.2. Electrophoresis and immunoblotting of oil protein fractions

The liquid phase from the re-suspended pellet was heated at 93 °C/3 min. We loaded acetone extractions (30 μl) onto 10 to 20% polyacrylamide gradient gels (Criterion™ Precast Gel, Bio-Rad Laboratories). Proteins were separated under reducing SDS–PAGE conditions and silver-stained with LC–MS/MS analysis optimized method (O'Connell & Stults, 1997). For a comparison, extracts from seeds of oilseed rape, turnip rape, and mustard were prepared and analyzed in SDS–PAGE as described earlier (Puumalainen et al., 2006) using PBS (Phosphate Buffered Saline) extraction.

For IgE-immunoblotting, proteins extracted from commercial oils were transferred after gel-electrophoresis to polyvinylidene difluoride (PVDF) membrane (Immobilon Transfer Membrane) and non-specific binding of antibodies was blocked with TBS-Tween®-20 detergent (0.05%), which does not interfere with the protein identification from the immunoblot by LC–MS/MS. Blotted membrane was incubated for 48 h with pooled sera (diluted 1:40) from five children (Patients 6–10 in Table 2, mean age 3.1 *y*, range 0.4–4.9 *y*) who were sensitized to oilseed rape and turnip rape seed proteins. Three of them were allergic to turnip rape, as confirmed by open food challenges. Biotinylated anti-human IgE (diluted 1:1000, Vector Laboratories Inc.) was added, followed by streptavidin-conjugated alkaline phosphatase (diluted 1:24,000, Zymed Laboratories Inc.) and substrate (Alkaline phosphatase conjugate substrate kit, Bio-Rad Laboratories). Dried Western blots were scanned with Image Scanner UMAX GTA 1100 Magic Scan v 4.6 (GE Healthcare). For the immunoblot of seed extracts (oilseed rape, turnip rape, and mustard) we incubated PVDF membrane overnight with individual sera (diluted 1:20) from five children (Patients 1–5 in Table 2, mean age 6.3 *y*, range 2.1–10.7 *y*) who were sensitized to oilseed rape and turnip rape seeds. Two of them were allergic to turnip rape in an open food challenge. The ethical committee of Tampere University Hospital approved the study, and informed written consent was obtained from the children's parents.

Table 1
Proteins detected on silver-stained SDS–PAGE and identified with LC–MS/MS from commercial rapeseed oils.

Oil number	Oil type, country of production	Detection SDS–PAGE + silver staining	Identification Q-TOF-MS	Protein UniProt accession number	Theoretical size kDa[a]	Mascot score[b]	No. matched peptides[c]/ sequence coverage[d]
1	Refined, Finland	<14 kDa	No proteins identified				
2	Refined, flavoured, Finland	<14 kDa	No proteins identified				
3	Refined, Belgium	Weak <14 kDa	No proteins identified				
4	Refined, Finland	Not detected	No proteins identified				
5	Cold-pressed, Finland	Multiple bands	Napin large chain	P27740	9.4	184	3/41%
				P09893	10.1	177	3/41%
			Cruciferin	P33523	30.6	292	6/24%
			Alpha chain	P33522	28.1	277	5/24%
				P33525	32.9	161	2/8%
			Beta chain	P33523	20.8	126	2/21%
				P33522	20.8	102	2/20%
6	Cold-pressed, Finland	Multiple bands	Napin large chain	P27740	9.4	270	5/41%
			Cruciferin	P33522	28.1	315	6/32%
			Alpha chain	P33523	30.6	202	4/20%
				P33525	32.9	163	3/13%
			Beta chain	P33523	20.8	132	2/9%
				P33522	20.8	121	2/9%
7	Cold-pressed, ecological, Finland	<14 kDa	No proteins identified				
8	Cold-pressed, Finland	Not detected	No proteins identified				

[a] Theoretical size of the cleaved subunits after maturation without possible post-translational modifications.
[b] Mascot score, $10 \times \log(P)$, where P is the probability that the observed match is a random event.
[c] No. matched peptides, number of peptides from digested gel spots that have sequence similarity with the identified protein.
[d] Sequence coverage, percentage of matching sequences with the whole protein sequence.