

Allergenicity and antigenicity of fenugreek (*Trigonella foenum-graecum*) proteins in foods

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Background: Fenugreek is an ingredient in Indian-style spiced foods. Reports of adverse reactions reflect a trend toward a more international cuisine. Fenugreek allergy has not been systematically investigated so far.

Objective: Study the allergenicity and antigenicity of fenugreek proteins using patient sera and a newly developed polyclonal antifenugreek antibody.

Methods: Allergenic fenugreek proteins were identified by immunoblotting, using sera from 29 patients with specific IgE to peanut and other legumes. In addition, 2 patients were evaluated by skin prick test and open food challenge with native fenugreek powder. Spiced and flavored food products were analyzed for fenugreek by semiquantitative IgE and IgG immunoblotting.

Results: High levels of specific IgE to both peanut and fenugreek were seen in most sera. Fenugreek sensitization is believed to be a consequence of cross-reactivity in patients with peanut allergy. Primary fenugreek allergy was suspected in only 1 case. The fenugreek dose eliciting objective symptoms was about 2 mg in the open food challenge. Major fenugreek allergens were identified at 50, 52, and 74 kd and peanut proteins at 22, 36, and 40 kd. A specific polyclonal antifenugreek antibody was found suitable for food analysis. In a food survey, about 1/3 of the fenugreek-containing products were labeled correctly.

Conclusion: Fenugreek seed powder, an ingredient in spiced foods, contains several potential allergens. There is evidence for a high rate of cross-reactivity to peanut. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Allergen, allergenicity, antigenicity, cross-reactivity, fenugreek, food, IgE, immunoblotting, legumes, open food challenge, peanut, polyclonal antibody, skin prick tests

Fenugreek (*Trigonella foenum-graecum*) is a member of the genus *Trigonella* belonging to the Fabaceae plant family (legumes). Both the yellowish, rhombic-shaped seeds and fresh leaves are of commercial interest as components in foods, feeds, dyes, and drugs.

Abbreviation used

NorFoodAllergyReg: Norwegian Register and Reporting System for Severe Allergic Reactions to Food

RT: Room temperature

SPT: Skin prick test

Fenugreek has been used for culinary and medical purposes since ancient times, especially in Greece, Egypt, and India, and was introduced to Central Europe in the first millennium as a forage plant.¹ The dried seeds may be used whole or ground to a yellow powder after roasting. The leaves may be consumed fresh in salad or cooked as a vegetable, but fenugreek is most commonly used as a spice in curries, seasoning blends, chutneys, pickles, and teas. Because of its maple aroma and flavor, fenugreek is also used as an ingredient in artificial maple syrup, artificial vanilla aroma, coffee substitute, licorice, rum, cheese, butterscotch, confectionery, and baked goods.²

Consumption of fenugreek has been limited in Western cultures because of its bitter taste and penetrating odor, caused by a high content of alkaloids and volatile oils. However, the recent trend to a more international cuisine has led to an increased popularity of Indian foods. The world production of fenugreek is estimated at 68,000 tons per year, of which more than 50% is consumed in India.¹

Historically, fenugreek is one of the oldest medicinal plants, known for its olfactory, laxative, and galactagogue effects. More recently, immunostimulatory, antidiabetic, antihypertensive, and cholesterol-lowering activities have been demonstrated.³ Fenugreek may therefore be considered beneficial in the daily diet, as a condiment or supplement in bread, for example.⁴ The seeds contain 23% to 26% protein, 6% to 7% fat, and 58% carbohydrates, of which 25% is dietary fiber.⁵ The protein fraction is lysine-rich and comparable in quality to that of soybean.

Fenugreek is generally considered safe for human consumption.⁶ However, after the identification of curry spices mixture as the cause of immediate allergy reactions, the components in different curries have been studied separately,^{7,8} and fenugreek has been identified as potentially allergenic.⁹ More recently, a total of 7 cases of fenugreek allergy have been described,¹⁰⁻¹² including anaphylaxis and occupational asthma elicited by ingestion, inhalation, or external application.

Only 2% to 6% of adults with food allergy in Western countries are allergic to spices,^{13,14} often as a result of pollinosis, and the associated risk of secondary spice allergy as seen in the mugwort-celery-spice syndrome.¹⁵ Fenugreek may demonstrate cross-reactions with other plants from the legume family such as peanut, soy, lupin, lentil, pea, bean, and chickpea.

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TABLE I. Patients registered by the NorFoodAllergyReg with specific IgE against legumes*

Patient ID	Age (y)	Sex	IgE class				
			Peanut	Soy	Pea	Lupin	Fenugreek
135	24	F	3	0	0	NM	3
231	18	M	6	2	2	3	NM
282	7	M	3	2	2	2	2
310	1	F	0	0	1	0	0
320	53	F	3	0	0	0	0
323	11	F	4	2	2	2	3
340†	18	F	5	2	0	0	3
341†	13	M	2	2	2	0	1
343†	9	M	6	3	1	0	1
376	24	M	4	2	3	3	2
389	19	F	2	6	6	3	NM
402†	25	M	3	1	0	1	3
405†	1	F	3	1	2	0	0
426†	20	F	2	2	3	3	2
427	17	F	3	2	0	2	3
429	19	F	2	1	0	0	NM
469†	37	M	3	1	1	0	1
482†	11	M	6	0	2	2	3
484	15	F	0	2	2	0	2
485†	24	M	4	3	2	0	3
490†	21	F	4	1	0	0	2
498†	30	F	5	2	3	3	3
502	17	F	2	0	1	0	2
508	44	M	1	1	1	1	1
509	13	M	2	2	2	2	2
513	14	M	2	2	2	2	2
536	13	M	1	0	0	0	3
583	2	M	6	3	2	2	3
594†	17	M	2	1	0	2	2

F, Female; M, male; NM, not measured.

*IgE classes were determined by ImmunoCap according to IgE levels (kU/L; 0: <0.35; class 1: 0.35-0.7; class 2: 0.7-3.5; class 3: 3.5-17.5; class 4: 17.5-50; class 5: 50-100; class 6: >100).

†The patient was additionally skin prick tested and food-challenged with fenugreek seed extract.

‡Fenugreek-containing foods are suspected to have caused these incidents.

Recently, the Norwegian Register and Reporting System for Severe Allergic Reactions to Food¹⁶ (NorFoodAllergyReg) has received a number of reports of patients with peanut allergy who have experienced allergic reactions after consuming curry-containing meals. Peanut is a major food allergen with a high frequency of fatal allergic incidents.¹⁷ It was therefore the aim of this study to evaluate the allergenicity and antigenicity of fenugreek proteins.

METHODS

Patients

Sera were obtained from 29 patients, registered by the NorFoodAllergyReg with specific IgE against the legumes peanut, soy, pea, lupin, and fenugreek (Table I). Registration is voluntary and based on a form submitted by the physician, a blood sample, and in some cases, food samples. Case histories suggested that 12 of the allergic incidents were caused by curry-containing food products. Serum IgE specific for peanut (f13), soy (f14), pea (f12), lupin (Rf335), and fenugreek (Rf305) was determined by using ImmunoCap (Phadia, Uppsala, Sweden).

Two patients with histories of anaphylaxis after intake of Indian food were referred to the Voksenterpen Children's Center for Asthma and Allergy

(Rikshospitalet University Hospital, Oslo, Norway) for skin prick testing (SPT), serum specific IgE analyses, and open food challenges. Fenugreek antigen for SPT was prepared by mixing native fenugreek powder (*T. foenum-graecum* semen 0.7 mm; Norsk Medisinaldepot ASA, Oslo, Norway) with the negative control diluent (Allergopharma, Reinbek, Germany) directly on the skin on the volar side of the underarm. Testing was performed by pricking through the material. Further SPTs were performed for lupin (*Lupinus angustifolius* seed flour, Lupipan; Soja Austria, Vienna, Austria), peanut (5000 protein nitrogen units/mL), pea (5000 protein nitrogen units/mL; Allergopharma), and soy 1:20 wt/vol (Solu-prick; ALK-Abelló Laboratories, Copenhagen, Denmark). A positive SPT result was defined as a wheal with a diameter (mean of longest and the midpoint orthogonal diameter) ≥ 3 mm larger than the negative control. Histamine dihydrochloride (10 mg/mL; ALK-Abelló Laboratories) served as a positive control. Open food challenges were performed with 2 mg fenugreek powder (Norsk Medisinaldepot ASA) placed directly inside the lower lip. Informed consent was obtained from all patients. The studies were approved by the Regional Committee for Medical Research Ethics, Southern Norway.

Fenugreek extraction

Fenugreek powder was suspended (wt/vol 1:10) in TRIS/glycine buffer, pH 8.7. After overnight extraction at 45°C in a shaking water bath (OLS 200; Grant, Cambridge, United Kingdom) and centrifugation for 25 minutes at 4°C at 18,000g (J2-MC; Beckman Instruments, Palo Alto, Calif), the supernatant was diluted with distilled water (1:10) and filtered through glass wool. Protein was precipitated in a glass beaker by adding 90% ammonium sulfate (Riedel-de-Haen, Seelze, Germany), at room temperature (RT) under continuous stirring. After further centrifugation (15 minutes, 4°C, 9000g), the pellet was resuspended in distilled water, extensively dialyzed against water in a dialysis tube with 6 to 8 kd pore size (Spectra/Por; Spectrum Medical Industries, Los Angeles, Calif) at RT and overnight at 4°C, and freeze-dried (Heto, Allerød, Denmark). The protein was resuspended in PBS, pH 7.4. The total protein content was determined with the Lowry Protein Assay (BioRad Laboratories, Hercules, Calif), and adjusted to 2 mg/mL.

Production of polyclonal antifenugreek antibody

A polyclonal antiserum was raised against fenugreek by injecting a male chinchilla rabbit subcutaneously with 1.0 mL 200 μ g/mL fenugreek extract, diluted 1:1 with Freund complete adjuvant. After reimmunization at the same dose level (diluted in Freund incomplete adjuvant, weeks 4 and 8), the rabbit was exsanguinated in week 12. Serum was obtained by centrifugation and stored in aliquots at -80°C until use.

Samples for specificity testing and food survey

Peanut (*Arachis hypogaea*), soy (*Glycine max*), common pea (*Pisum sativum*), lupin (*Lupinus albus*), white bean (*Phaseolus vulgaris*), lentil (*Lens culinaris*), chickpea (*Cicer arietinum*), and food samples obtained from local retail stores were extracted with TRIS/glycine buffer, pH 8.7 (wt/vol 1:5), directly or, if necessary, after homogenization in a mechanical blender (Retsch GmbH & Co, Haan, Germany) as described. Total protein contents were measured by the Lowry method; samples were stored in aliquots at -20°C .

Gel electrophoresis and immunoblot

The NuPAGE Gel System (Invitrogen, Carlsbad, Calif) was used for electrophoretic separation of protein samples in accordance with the manufacturer's instructions. Protein extracts were diluted to 2 mg/mL and prepared with lithium dodecyl sulfate sample buffer and dithiothreitol (0.05 mol/L) reducing agent (all from Invitrogen) for gel analysis. The samples were loaded (3 μ g for legume extracts, 10 μ g for food extracts), and separation was performed under reducing conditions for 40 minutes at 200 V in 2-(N-morpholino) ethane sulfonic acid SDS running buffer, using 4% to 12% Bis-Tris gels and Mark12 unstained standard or SeeBluePlus2 (Invitrogen)

TABLE II. Specific IgE, SPT, and food challenge results from 2 patients diagnosed at the Voksentoppen Children's Center for Asthma and Allergy*

	Patient	
	A	B
Total IgE (kU/L)	409	2100
Specific IgE (kU/L)		
Fenugreek	15.9	36.4
Peanut	>100	>100
Lupin	4.2	2.9
Pea	5.3	11.3
Soy	<0.35	3.2
Hazelnut	1.4	41.2
Almond	2.5	3.9
SPT		
Fenugreek	19 mm	4.5 mm
Peanut	7.5 mm	8 mm
Lupin	8 mm	Neg
Soy	4 mm	3 mm
Pea	NM	3 mm
Hazelnut	Neg	8 mm
Birch	Neg	8.5 mm
Timothy grass	Neg	6 mm
Open food challenge		
Fenugreek	Pos†	Pos†
Lupin	Pos	NM

Neg, Negative SPT (<3 mm wheal diameter); NM, not measured; Pos, positive open food challenge.

*Patient A (identical with ID482 in Table I): 11-year-old boy with anaphylaxis after intake of a meal with curry mix; patient B: 12-year-old boy with anaphylaxis after eating prepacked Indian food.

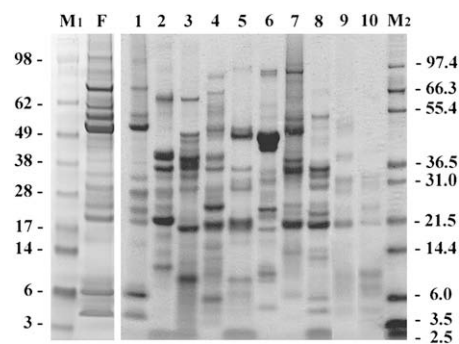
†Both patients reacted with urticaria when they were challenged with about 2 mg fenugreek seed powder.

pre-stained reference standard. The proteins were either stained with SimplyBlue Safe Stain (Invitrogen) or electrophoretically transferred onto nitrocellulose membrane (Bio-Rad, Hercules, Calif) for 60 minutes at 30 V by using transfer buffer in an XCell II Blot Module (Invitrogen).

TRIS-buffered saline containing 0.1% Tween 20, pH 7.6, was used as washing buffer, and with 3% BSA as blocking and assay buffer for the immunoblots. After blocking for 30 to 60 minutes, blots were incubated under gentle shaking overnight at 4°C with patient sera, diluted 1:20. All further steps were performed at RT. The blots were washed (3 × 15 minutes) and incubated successively with 2 antibodies, first for 1 hour with rabbit antihuman IgE (1:1000; Dako, Glostrup, Denmark), and after an intermediate wash, for 1 hour with horseradish peroxidase-conjugated goat antirabbit antibody (1:5000; Zymed, San Francisco, Calif). After a final wash, the membrane was developed with TMB substrate solution (Invitrogen) until bands of satisfactory intensity appeared (10 minutes). In addition, blots were developed by using polyclonal antifenugreek antibody diluted 1:105 or 1:106, followed by incubation with horseradish peroxidase-conjugated goat antirabbit secondary antibody diluted 1:5000.

GelPro Analyzer Image Analysis

Immunoblots were scanned and processed using GelPro Analyzer Image Analysis (MediaCybernetics, Bethesda, Md). Protein band intensities were determined by applying Standard Optical Density Fitting correlating (second-order polynomial) the number of pixels measured and the OD. For blot-to-blot comparison, the measured relative maximum OD values were normalized by using the 38-kd band in the protein reference standard on each blot. In some experiments, the total OD elicited by a sample was calculated for sample comparison by summing up the individual band intensities in the respective immunoblot lane.

**FIG 1.** Coomassie-stained SDS-PAGE of legume extracts. *M*₁, SeeBluePlus2 prestained molecular weight marker; *F*, fenugreek; 1, fenugreek; 2, peanut; 3, lupin; 4, pea; 5, soy; 6, bean; 7, lentil; 8, chickpea; 9, curry; 10, tikka masala; *M*₂, Mark12 molecular weight marker. Protein sizes (kd) are indicated on the sides of the gel.

RESULTS

Patients with potential fenugreek hypersensitivity

The NorFoodAllergyReg has collected data on suspected fenugreek allergy incidents since 2006. Sera from the respective patients were analyzed for IgE specific to a number of legumes. The majority of the patients had a known peanut allergy, and were additionally sensitized to soy, pea, lupin, or fenugreek (Table I). However, 2 patients included in the current study (ID310, ID484) had no detectable anti-peanut IgE, and 1 patient (ID320) was sensitized to peanut only. IgE levels of classes 1 to 3 were measured for fenugreek. High antifenugreek IgE titers were associated with equally high or higher anti-peanut IgE titer, with the exception of ID536.

Food challenge with fenugreek

Two confirmed patients with peanut allergy were clinically examined by using SPT and open food challenge (Table II). Both had anti-peanut IgE class 6 and were sensitized to other legumes, nuts, and pollen. Patient A had antifenugreek IgE class 3 and developed a 19-mm wheal on SPT with the same allergen, whereas patient B had IgE class 4 and developed a 4.5-mm wheal. Both patients had a history of anaphylaxis after eating curry-containing foods. On open food challenge, patient A developed urticaria on the face and shoulders approximately 45 minutes after ingestion of about 2 mg native fenugreek. Patient B experienced throat tingling and urticaria after 5 minutes. The symptoms resolved after some hours in both cases. The study was discontinued because of the low eliciting dose.

Protein patterns in extracts of fenugreek and other legumes

The band pattern seen in SDS-gel analysis of fenugreek proteins differed slightly from those of other legumes (Fig 1). Fenugreek showed a characteristic quintet of proteins at 50 kd, 52 kd, 58 kd, 62 kd, and 74 kd. A group of 4 proteins was detected at 22 kd, 24 kd, 30 kd, and 34 kd, and 2 small proteins were found at 4.5 kd and 6 kd. When a higher concentration was applied, several more protein bands became visible in the range from 10kd to 100 kd. In the extracts of the other legumes tested (peanut, lupin, pea, soy, bean, lentil, chickpea) and the 2 spice blends curry and

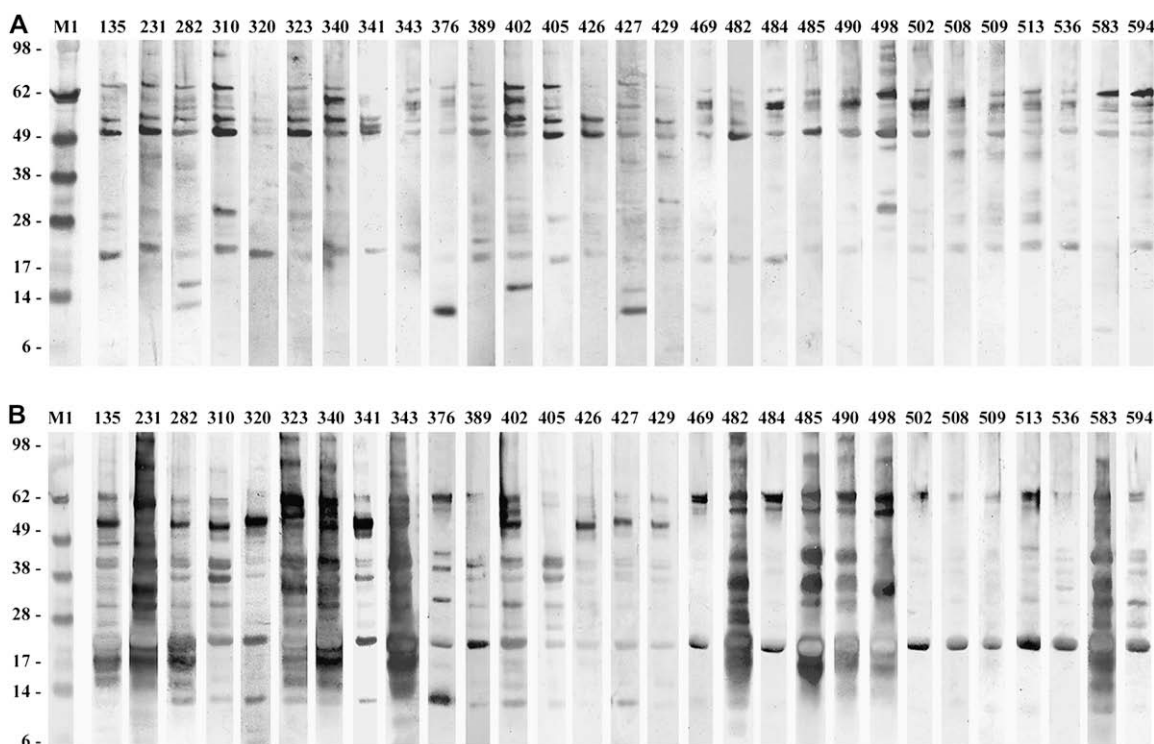


FIG 2. Immunoblot using sera from fenugreek-sensitized patients (Table I). **A**, Fenugreek. **B**, Peanut. *M1*, Marker (kd).

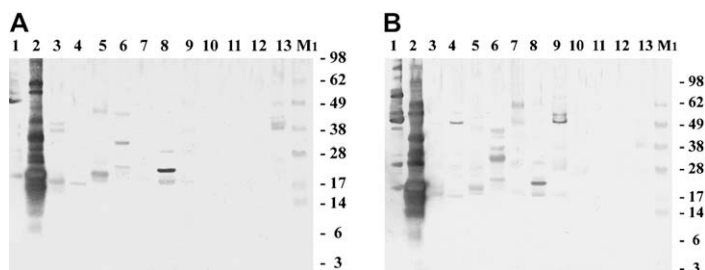


FIG 3. Immunoblot using sera from patients with fenugreek allergy (Table II). **A**, Patient A. **B**, Patient B. 1, Fenugreek; 2, peanut; 3, lupin; 4, pea; 5, soy; 6, bean; 7, lentil; 8, chickpea; 9, curry; 10, tandoori masala; 11, cinnamon; 12, Indian tikka masala; 13, naan authentic; *M1*, Marker (kd).

tikka masala, the most dominant protein bands were detected at 22 kd, 30 kd, 36 kd, and 40 kd.

Allergenicity of fenugreek and peanut proteins

Sera from patients with potential fenugreek allergy (Table I) were used in immunoblot experiments to investigate the allergenicity of the 8 different legume extracts. Fenugreek and peanut were studied in more detail by comparing the respective lanes from each blot. The fenugreek proteins at 50 kd, 52 kd, and 74 kd bound to IgE in almost all patient sera (Fig 2, A), and the protein at 22 kd was probably also highly allergenic. Proteins at 14 kd, 17 kd, and 30 kd were detected only by 1 or a few patient sera.

Peanut proteins showed very different IgE-binding patterns for the individual patients (Fig 2, B). The proteins at 22 kd, 36 kd, and 40 kd produced strong signals. In addition, several minor allergens were detected, ranging from 14 kd to 90 kd.

IgE-profiling of the food-challenged patients

The sera of 2 fenugreek-challenged patients were analyzed with immunoblot to elucidate IgE binding to different legume extracts, fenugreek-containing foods, and control samples (Fig 3, A [patient A] and B [patient B]). Both patients demonstrated a strong affinity to the 22-kd peanut protein. The characteristic fenugreek quintet of protein bands and the 22-kd protein were also recognized. In addition, the serum of patient B bound to proteins at 28 kd and 45 kd.

Reactivity to other legumes was weaker. Serum from patient A bound with low intensity to the 22-kd protein in lupin, pea, soy, chickpea, and curry, and recognized higher molecular weight bands in lupin, soy, bean, chickpea, and spiced bread. This patient did not have IgE against lentil. The binding pattern for patient B was slightly different because a number of larger proteins in addition to the 22-kd band were recognized. This patient had IgE against all legumes tested, the spice blends, and spiced bread.

TABLE III. Food products purchased in Norwegian retail stores*

	Food product	Labeled with fenugreek	Protein content (mg/L)	Western blot Polyclonal antibody†	Western blot Patient sera†
1	Mixed pickles	✓	2.6	±	+
2	Chili pickles	✓	5.9	+++	++
3	Lime pickles	✓	5.4	±	±
4	Tikka masala (1)	—	4.6	+	—
5	Tikka masala (2)	—	4.2	±	±
6	Indian tikka masala	—	3.1	±	±
7	Tandoori masala	✓	10.6	+++	++
8	Garam masala	✓	35.2	+	+
9	Curry powder	✓	22.0	+++	++
10	Curry sauce	—	3.4	±	±
11	Spice mix	—	2.8	+++	+
12	Indian wok spice mix	—	14.8	+++	++
13	Indian tandoori rice	—	8.5	+	++
14	Indian tandoori sauce	—	16.4	+	++
15	Wok sauce	—	4.0	±	±
16	Oyster and shrimp sauce	—	2.7	—	—
17	Minced meat sauce	—	11.1	+	+
18	Paneng	—	19.9	++	±
19	Papadam	—	32.6	++	++
20	Papadums	—	98.1	++	++
21	Naan authentic	—	4.7	—	++
22	Naan bread	—	3.5	++	++
23	Spiced cake dough	—	66.8	—	++
24	Spiced cake sauce	—	6.4	—	—
25	Cappuccino spice (1)	—	59.6	—	—
26	Cappuccino spice (2)	—	21.7	—	—
27	Cappuccino instant	—	48.2	—	+
28	Cinnamon powder	—	96.3	—	—
29	Chewing gum	—	1.6	—	—
30	Vanilla sauce	—	19.6	±	±
31	Rum essence	—	1.5	—	—
32	Halva	—	10.0	++	+++

*Results of immunoblot analyses (10 µg each sample) developed with polyclonal antifenugreek rabbit serum or patient sera (median of 5).

†Relative ODmax values (GelPro analyzer) derived from Figs 4 and 5 (not using patient ID320): <0.01: none (—), 0.01–0.2: weak (±); 0.2–0.8: medium (+); 0.8–4: strong (++); >4: very strong (+++).

Specificity of antifenugreek antibody

The specificity of the polyclonal rabbit antifenugreek antibody was tested by immunoblot using different legume and spice extracts (data not shown). Diluted 1:10⁶, the antibody bound to only 3 of the protein bands in the fenugreek quintet pattern, and also to an 88-kd band. No cross-reactivity to other legume proteins was observed. At higher antibody concentrations (1:0.5 * 10⁵ and 1:10⁵), the binding to fenugreek increased, with all bands except the 2 low-molecular proteins at 4.5 kd and 6 kd recognized. However, cross-reactivity to proteins at 22 kd in lupin, 30 kd in bean, 46 kd in pea, and 24 kd in chickpea were observed.

Fenugreek-related antigenicity of foods

The polyclonal antifenugreek antibody was used to trace fenugreek in 32 spiced prepacked foods from the Norwegian market. Six foods were labeled with fenugreek (Table III), although the presence of fenugreek protein was detectable in 21 of these products (Fig 4). Fenugreek proteins detected in the foods included the quintet, with the strongest signals at 50 kd and 74 kd, as well as other bands with molecular weights of 14 kd, 22 kd, 43 kd, and 88 kd. The signal intensities in each blot lane were combined and arranged into categories ranging from weak to very strong (Table III).

Fenugreek-related allergenicity of foods

IgE binding to the same 32 foods was tested by immunoblot, using 6 patient sera selected according to their IgE profiles (Table I; Fig 2). They included the 2 positively fenugreek-challenged patients A and B, patient ID376 showing strong reactivity to low-molecular proteins, ID484 without anti peanut IgE, ID536 containing more antifenugreek than anti peanut IgE, and ID320 sensitized to peanut only. The individual sera showed different binding patterns to the respective food products (Fig 5), predominantly recognizing bands from the fenugreek quintet and also proteins ranging from 22 to 43 kd. The peanut monoallergic subject ID320 differed from the other 5, showing no reaction to potentially fenugreek-containing food products, with the exception of the confectionery halva. Total IgE binding to the foods was obtained by combining the signal intensities in each lane and determining the median values for the 5 fenugreek-sensitized patients, which ranged from weak to very strong for 24 foods (Table III).

The semiquantitative results from the antigenicity and allergenicity experiments correlated relatively well.

DISCUSSION

The presence of fenugreek is often disguised by the generic term “spices” on food labels. Spices are generally added in small



FIG 4. Fenugreek in food products. Immunoblot using polyclonal rabbit antifenugreek antibody. 1-32, Food products in the same order as in Table III. M, M₁ Marker (kd).

amounts to food products as seasoning and may be overlooked in cases of food allergy. However, fenugreek was not included in a large study on spice allergenicity, analyzing 40 different species from a total of 17 plant families.¹³ Spice allergenicity results mainly from cross-reactivity with pollen allergens (celery-birch-mugwort-spice syndrome) and is a consequence of homology between the major birch pollen allergens Bet v 1 and Bet v 2 with the respective spice allergens.¹⁸

There have, however, been reports of patients without birch allergy who have reacted to the mixed-spice blend curry.^{19,20} Indian-style meals often contain the legume fenugreek, which may cross-react with major allergens such as peanut, soy, and lupin.

The clinical relevance of *in vitro* cross-reactivity is uncertain. Whereas a study of 9 different legumes in Spanish children suggested a good correlation between ELISA inhibition and food challenge results,²¹ this was not found to be the case in an American patient cohort.²² It has been further suggested that dietary habits may influence sensitization patterns.²³

Various techniques have been used to diagnose spice allergy, such as specific IgE-analysis, SPT, RAST, and scratch testing, as well as inhalation and food challenges. Specific IgE analyses and food challenges appear to be positively correlated,^{24,25} although the results may depend on the assay system used.²⁶ Reproducibility of SPT was better using native spices than commercially available extracts.²⁷ Wheal size diameters have been successfully used to predict the outcome of food challenge studies.²⁸ RAST appeared to be less reliable, negative in more than 50% of the diagnosed cases.²⁹ Spice allergy should be confirmed by using double-blind placebo-controlled food challenge.

In the current study, sera from 29 patients with reported anaphylaxis to legume-containing foods have been analyzed for specific IgE to a number of legumes. In most cases, the anti-peanut IgE levels were higher or equal to the antifenugreek IgE-levels, indicating a secondary fenugreek allergy as a consequence of a previously existing peanut allergy. Primary fenugreek allergy was suspected in only 1 case (ID536), indicated by a stronger IgE-specific reaction to fenugreek (class 3) compared with peanut (class 1). The majority of the patients were additionally sensitized to soy, pea, and lupin. However, this appeared to be of less relevance because allergic reactions caused by these foods were rarely reported.

IgE-mediated allergy to fenugreek was confirmed by open food challenge in 2 patients with peanut allergy by using small doses of native fenugreek seed powder. Remarkably, patient A, with specific antifenugreek IgE class 3, developed a 19-mm diameter wheal in the SPT, whereas patient B, with IgE class 4,

produced a wheal of only 4.5 mm diameter. In immunoblot, however, signal intensities were stronger for patient B. The discrepancies probably resulted from the different test materials used in the respective procedures. Whereas native fenugreek was applied in the SPT, specific IgE was determined by using ImmunoCap preparations, and gel electrophoresis was performed with extracts under denaturing and reducing conditions. It might therefore be concluded that patients A and B were sensitized to fenugreek proteins with differing stability and characteristics.

Fenugreek produced a typical protein pattern on SDS gel, with multiple bands, ranging from 4.5 to 100 kd. In 3 case reports of fenugreek allergy,¹⁰⁻¹² diagnosing a total of 7 patients, IgE binding was demonstrated to fenugreek proteins from 14 kd to 80 kd. A combined analysis of allergenicity experiments performed in the current study was used to confirm these findings. IgE binding was strongest to the characteristic fenugreek protein quintet from 50 kd to 74 kd. In contrast, the major allergens in peanut and other legumes were observed at around 22 kd. The molecular basis for cross-allergy is similarity between epitopes; therefore, homology between fenugreek proteins and major legume proteins such as Ara h 1, Ara h 2, and Ara h 3 from peanut³⁰ might be assumed. We will attempt to identify and characterize fenugreek allergens by proteomic analysis in a follow-up study.

A specific polyclonal rabbit antifenugreek antibody was produced, using extracted fenugreek seeds, and bound mainly to the larger fenugreek proteins. The antibody was used in a survey of 32 spiced food products, and showed that about 2/3 of the foods contained traces of fenugreek, although only 6 were labeled with fenugreek. In several typical Indian foods like tandoori masala and curry powder, the fenugreek content appeared to be relatively high.

When the allergenicity of the same food products was compared, the outcome was similar. Not unexpectedly, the IgE binding varied between sera from patients who were sensitized to different legumes, and in some cases other foods. Complex food products were associated with a risk of nonfenugreek-related reactions. Patients ID376 and ID536 had, for example, specific antiwheat IgE class 3 and 6, respectively, which resulted in intensive signals for the wheat flour-containing foods naan authentic, naan, and spiced cake dough. However, selecting the median value for the result evaluation lessened the influence of outliers, and moreover, a good correlation between the antigenicity and allergenicity studies could be demonstrated.

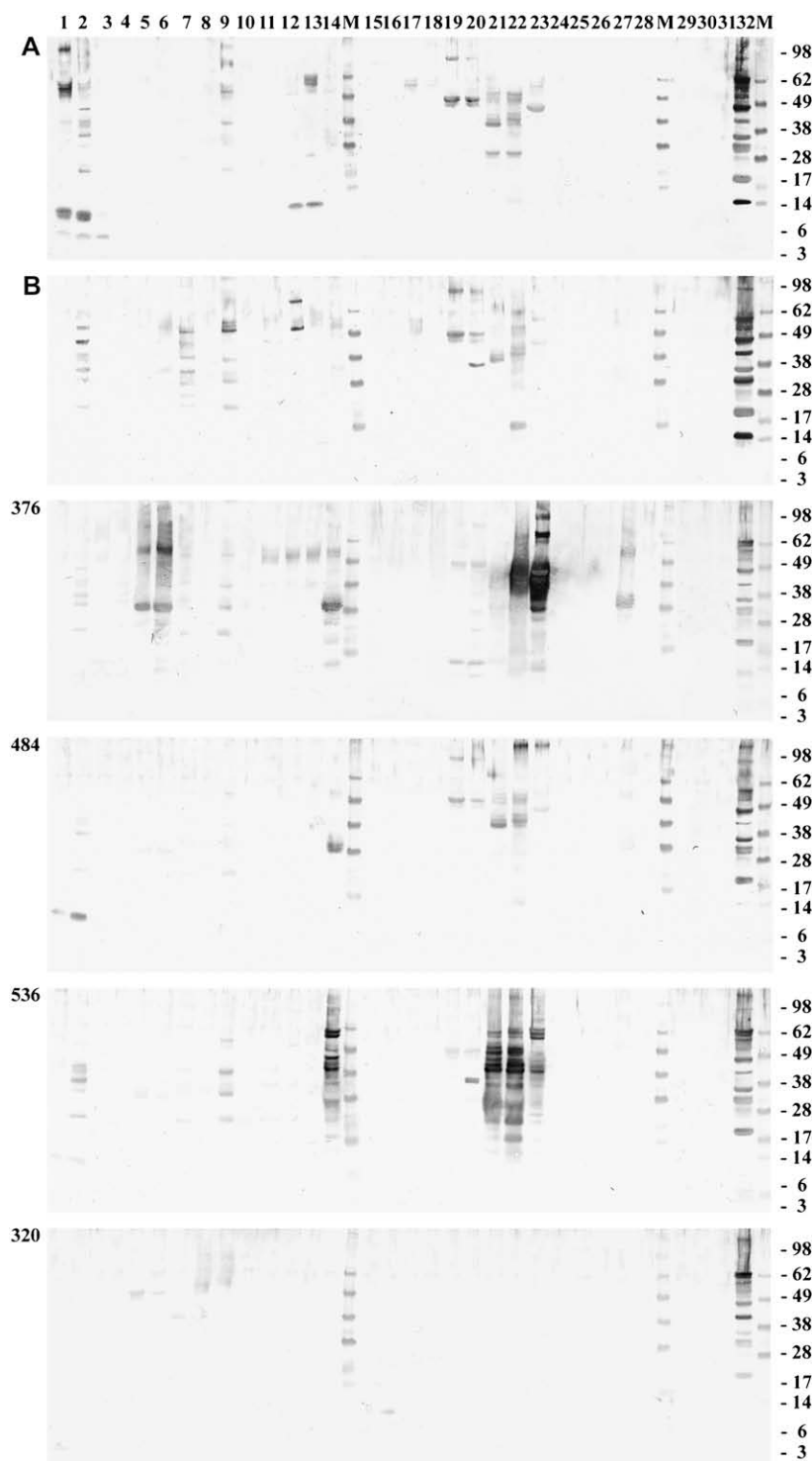


FIG 5. Allergenicity of fenugreek-containing foods. Immunoblot using sera from patients differently sensitized to peanut and fenugreek. 1-32, Food products in the same order as in Table III. M, M₁ marker (kd).

Considering the relatively low levels of spices that are added to foods, the thresholds for sensitization and provocation of allergic incidents are probably low.²⁰ In the case of fenugreek, the potential cross-reactivity with peanut, responsible for most of the food-related fatal allergic incidents in the United States, should be

taken into account.³¹ Physicians might include fenugreek in the diagnostic program for patients with peanut allergy and advise them to avoid Indian-style food. Food manufacturers should consider adding fenugreek to lists of ingredients even if it has not yet been included in food safety regulations.

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Clinical implications: Fenugreek seed powder is allergenic and potentially cross-reactive with peanut allergens. The consumption of fenugreek-containing foods represents a risk for persons with peanut allergy.

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