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Influence of Maturation on the Alteration of Allergenicity of Green Pea (*Pisum sativum* L.)

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The IgE-binding capacity of different maturation levels of green pea seeds (*Pisum sativum* L.) of the variety Maxigolt is examined to determine the influence of maturation on the alteration of allergenicity. Different protein extraction methods to get total protein extracts and the protein fractions glutelin, globulin, and albumin from different maturation levels of green pea seeds are applied to SDS-PAGE/silver staining as well as SDS-PAGE/immunoblotting and EAST inhibition experiments using sera of 15 green pea allergic individuals. The SDS-PAGE/silver-staining experiments show the continuous change of protein pattern during maturation. SDS-PAGE/immunoblot and EAST inhibition demonstrate that all levels of green pea seeds show relevant IgE-binding capacity, as do immature seeds. Total IgE-binding capacity rises with the progress of maturation. Although the main allergenic activity is dependent upon the albumin fraction, the glutelin and globulin fractions are also important. The implication of these results is an obvious allergenic potency of all maturation levels, even immature seeds, whereas an increase of allergenicity during maturation could be notched up. The highest allergenic potency is caused by the albumin fraction, but globulin and glutelin fractions also contribute to the allergenicity of green pea.

KEYWORDS: Food allergy; green pea (*Pisum sativum* L.); SDS-PAGE/immunoblot; EAST inhibition; albumin; maturation

INTRODUCTION

Immediate-type allergic reactions to foodstuffs are usually caused by proteins or glycoproteins as natural components of food with molecular weights between approximately 10 and 70 kDa (*1*). These reactions are mediated by human immunoglobulin E (IgE) antibodies specific to these allergens (*2*). The prevalence of such reactions has been estimated as from 1 to 3% among the adult population and up to 5−8% among the pediatric population (*3*).

The clinical symptoms released through IgE-binding proteins manifest predominantly at the surface of the organism, which means at the skin and mucous membranes of the respiratory and intestinal tracts (*4*). Urticaria, allergic asthma, nausea, vomiting, and diarrhea are common symptoms (see **Table 1**).

Allergic reactions to legumes are widespread in various European countries; 3.3% of affected persons react to beans, 1.5% to peanut, 1.0% to soy, and 0.8% to green pea (*5*). Among adults who suffer from food allergy the prevalence of green pea allergy has been estimated at 1% (*6*). Crespo et al. (*7*) specified the occurrence of food allergy to legumes as 18.9%, referring to the entirety of food allergic persons. Mostly legume allergy is not a group sensitization; rather, reactions to particular kinds of legume are presented. Allergic reactions to soy and peanut dominate (*8*).

The importance of legumes for human nutrition has increased during the past few years due to their oil and protein contents. Many studies exist on allergenicity of soy and peanut but fewer on other legumes, for example, green pea (*9*). Meanwhile, green pea products are used as ingredients for many different foodstuffs. In rare cases even the inhalation of legume flour could cause an allergic reaction (*10*).

The molecular mass of a green pea allergen has been determined as 11 kDa (*11*). Numerous IgE-binding proteins with molecular masses between 14 and 70 kDa were identified by Bernhisel-Broadbent et al. (*12*). Due to their solubility, legume proteins could be divided into three fractions: albumin, globulin, and glutelin. From the quantitatively most important globulin fraction, two main components present in all legumes, vicilin and legumin, have been isolated (*11*). Moreover, it is reported that the major antigenic and allergenic compounds of green pea are associated with the albumin fraction. Bernhisel-Broadbent et al. (*12*) determined specific antigen bindings in all fractions. Studies regarding the chemical composition of ripening green peas indicate that they contain little or no albumin in the very early stages of development and that the rate of albumin synthesis increases slowly during maturation (*13*−*15*). These observations suggest that only the more developed green peas possess any significant allergenic activity.

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Table 1. Characterization of Patients' Sera with IgE Binding to Green Pea Proteins

no.	sex, age	clinical symptoms[a]	specific IgE (units mL^{-1})	EAST class	further sensitivities	IgE-binding proteins in IB
1	female, 24	1, 3, 5	2.67	2	bean, kiwi, banana, carrot, potato	16, 18, 20, 25, 30, 37, 40, 45, 50, 66, 75
2	female, 42	5, 6	0.88	2	kiwi, apple, lychee, birch pollen, apple, carrot	14, 16, 18, 20, 25, 28, 30, 32, 37, 50, 66, 75, 94
3	female, 32	3, 4, 5	1.05	2	apple, carrot, potato, lychee, milk, egg, hazelnut, walnut, fish, soy, bean	<14, 20, 25, 28, 30, 32, 37, 40, 45, 66, 75
4	male, 48	3, 6	19.84	4	green peanut, bean, lentil, soy, mango, lychee, carrot, potato, apple	<14, 14, 16, 18, 20, 22, 25, 28, 32, 37, 45, 50, 66, 75
5	female, 36	1, 3, 4	1.36	2	hazelnut, bean, mango, lychee, carrot, apple	20, 25, 28, 30, 40, 45, 66, 75, 94
6	male, 17	5, 7, 8	1.01	2	soy, bean, mango, lychee, carrot, potato	14, 20, 25, 28, 30, 32, 37, 40, 45, 50, 66, 75, 94
7	male, 60	3, 5	3.02	2	bean	16, 18, 20, 30, 32, 37, 40, 45, 50, 66, 75, 94
8	female, 22	1, 5, 6	1.40	2	soy, bean	16, 18, 20, 30, 32, 37, 40, 50, 66, 75, 94
9	female, 33	5, 6, 7	1.10	2	carrot, potato, tomato, soy, bean, apple, green peanut, lentil	20, 25, 28, 30, 32, 37, 40, 66, 75
10	male, 48	1, 3	5.20	3	soy, green peanut, lentil, bean, carrot	18, 20, 22, 32, 40, 66, 75, 94
11	male, 35	1, 2, 3, 7, 8	3.04	2	kiwi, mango, apple, lychee, carrot, hazelnut, egg, green peanut, soy, bean, lentil	14, 16, 18, 20, 22, 28, 30, 32, 66
12	female, 58	5, 6	2.74	2	bean, carrot, potato, apple	14,18, 20, 25, 32, 40, 50, 66, 75, 94
13	male, 58	1, 2, 3	2.12	2	green peanut, bean, lychee, carrot, potato	<14, 14, 30, 37, 66, 75
14	male, 37	1, 2, 3, 6	0.85	2	apple, lentil, bean, lychee, mango, potato, carrot	20, 25, 28, 30, 32, 37, 45, 50, 66, 75
15	female, 61	5	5.07	3	shellfish, soy, green peanut, bean, lentil, lychee, mango potato, carrot, apple	14, 20, 25, 28, 30, 32, 37, 40, 45, 50, 66, 75, 94

[a] 1, itching and swelling within the area of lips as well as the mouth mucous membrane; 2, problems in swallowing, swelling of uvula and/or in the pharyngeal area; 3, redness, itching, or eczema of skin; 4, nettle rash (urticaria); 5, conjunctivitis, sternutation, sniffles, rhinitis; 6, dyspnea, wheeze, asthma; 7, nausea, vomiting, gastric and/or ventral paroxysms, flatulence, diarrhoea; 8, dizziness, circulatory disturbance, hypotension.

The aim of the present investigation is to verify the influence of maturation on the alteration of allergenicity of green peas. To this end, different maturation levels from the variety Maxigolt of cultivated green pea seeds are analyzed. The IgE-binding capacity of total protein extracts in relation to the maturation status and the incidence of allergenic potency to the individual fractions glutelin, globulin, and albumin are separately investigated by different protein extraction methods, sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE) followed by silver-staining or immunoblotting and EAST inhibition experiments.

MATERIALS AND METHODS

Chemicals. If not otherwise stated, all chemicals were of analytical grade.

Human Sera. A total of 39 sera were collected at the Department of Dermatology and Allergology of the University Hospital Eppendorf, Hamburg, Germany. All sera were tested for specific IgE against green pea protein extract by enzyme allergosorbent test (EAST, Allergopharma, Reinbek, Germany). Twenty-three patients showed EAST classes < 2 (data not shown). A pool serum was composed using equal aliquots of 15 sera with EAST classes ≥ 2. A serum from a nonatopic individual with no history of food hypersensitivity (EAST class $= 0$ to ripe green peas) was used as negative control. The clinical information of the 15 green pea allergic patients is shown in **Table 1**. Many patients showed further sensitivities to different fruits, nuts, and tree pollen.

Plant Material, Growing Conditions, and Harvesting. The variety Maxigolt of commercially obtainable standard green pea seeds suitable for fresh consumption, conservation, drying, and freezing was used for cultivation. Seeds were grown under standard open-land cultivation conditions in Husum, Germany (9° E, 54° N) during one season. The field experimental design allowed separate harvesting at different times.

Harvesting of ~100 g of the cultivated green pea variety was performed after 70, 77, 84, and 91 days, respectively. After the determination of the individual weights of each seed, the mean value was calculated. The subsequent analysis was performed only with seeds showing maximal deviations of 0.05 g from the mean value. The total protein contents and dry weights of four maturation levels in all were determined according to standard methods (see **Table 2**).

Table 2. Characteristics of Harvesting Stages 1–4

harvest stage	growing time (days)	mean value (g)	dry wt (%)	total protein (%)	glutelin (%)	albumin (%)	globulin (%)
1	70	0.21	19.6	5.9	19.8	19.0	61.2
2	77	0.59	27.9	7.6	18.7	22.8	58.5
3	84	0.84	39.4	10.0	15.4	31.5	53.1
4	91	1.11	50.5	10.9	21.5	30.3	48.3

Total Protein Extraction. Selected raw green pea seeds of all maturation levels were ground in acetone at −40 °C immediately after harvesting. The mixture was stored overnight at −80 °C. The precipitates were washed three times with acetone (−20 °C), filtered, and lyophilized.

Protein extracts were obtained by extracting 1.8 g of the lyophilized powder in 20 mL of phosphate-buffered saline [PBS (0.01 M potassium phosphate buffer, pH 7.4, 0.13 M sodium chloride)] for 60 min by ice cooling. The suspensions were centrifuged (4 °C, 10500*g*, 60 min), and the supernatant was filtered. The solution was divided into aliquots of 2 mL, lyophilized, and stored at −20 °C until use.

Fractionation of Green Pea Proteins. The fractionation was performed according to the method of Vioque et al. (*16*). For this purpose lyophilized powders of the four maturation levels were extracted three times with 0.1 M borate buffer, pH 8.3, during 1 h at 4 °C. The pooled suspensions were centrifuged (4 °C, 10500*g*, 10 min), and the solution was removed by suction. The remaining insoluble components contained inter alia the glutelin fraction; the removed solution contained the albumin and globulin fractions.

The residues were lyophilized and the glutelin was extracted by PBS solution. Afterward, the suspensions were centrifuged as described above. The supernatants were separated, and afterward the protein contents were determined according to the method of Bradford (*17*). Finally, the solutions were lyophilized again, and the fractions were used for further investigations.

To separate the albumin and globulin fractions, the solutions were dialyzed against 25 mM sodium citrate buffer, pH 4.6, for 48 h at 4

°C. Afterward, the dialyzed extracts were centrifuged as described above. The resulting supernatants and pellets are the albumin and globulin fractions, respectively.

SDS-PAGE. SDS-PAGE was performed in 10% acrylamide gels (80 mm × 80 mm × 1.5 mm) using the NuPAGE vertical electrophoresis system according to the manufacturer's recommendations (Novex, San Diego, CA). Protein samples dissolved in Tris-HCl/SDS sample buffer (pH 6.8) containing 5% (w/v) 2-mercaptoethanol (*18*) were boiled for 3 min. With the application of ~20 μg of protein per well, electrophoresis was performed at 200 V constant voltage for 0.5 h on the NuPAGE electrophoresis system using an Xcell II power supply (Novex). Gels were silver stained according to the method of Heuckeshoven and Dernick (*19*).

Immunoblot. Proteins were electrotransferred from slab gels to nitrocellulose membrane (0.2 μm, Schleicher & Schuell, Dassel, Germany) at 0.8 mA/cm^2 for 80 min using a NovaBlot electrophoretic transfer kit (Pharmacia LKB, Uppsala, Sweden) according to the method of Kyhse-Andersen (*20*) with a discontinuous buffer system as described by Vieths et al. (*21*). Afterward, the membrane was dried for 30 min and cut into strips of required size.

Immunostaining of IgE was performed according to the method of Vieths et al. (*21*) slightly modified as described previously by Möller et al. (*22*). In brief, dried membrane strips were blocked twice (15 min) in PBS containing 0.3% (v/v) Tween 20 and incubated overnight with pool-serum diluted 1:1 in PBS containing 0.3% (w/v) bovine serum albumin (BSA) and 0.1% (v/v) Tween 20 at room temperature.

Then strips were incubated consecutively with rabbit anti-human−IgE (1:4000, DAKO, Copenhagen, Denmark) and with biotinylated goat anti-rabbit−IgG (1:6000, DAKO) for 1 h each and with streptavidin−horseradish peroxidase (1:20000, Medac, Hamburg, Germany) for 20 min. 3,3′,5,5′-Tetramethylbenzidine and dioctylsodium sulfosuccinate were used as substrates for staining.

To determine the molecular weight of the allergens from each gel, one strip including separated molecular weight (MW) marker (Pharmacia LKB) was brilliant black stained (Bio-Rad, Munich, Germany) according to a modified method of Moeremans et al. (*23*).

Enzyme Allergosorbent Test (EAST). Commercial filter paper disks (d = 6 mm, Schleicher & Schuell) were activated with cyanogen bromide according to the method of Ceska and Lundquist (*24*). Proteins of the extracts from the highest maturation level were coupled to these disks according to a modified procedure described by Möller et al. (*22*).

Specific IgE antibodies against green pea in patients' sera were measured using a commercial test kit [Allergopharma Spezific IgE ELISA TR (RV), Reinbek, Germany] according to the manufacturer's instructions. In this indirect, noncompetitive ELISA IgE binding to allergens was immunodetected by a mouse monoclonal anti-IgE antibody labeled with alkaline phosphatase. The enzymatic activity was monitored by hydrolysis of *p*-nitrophenyl phosphate (PNPP; Bio-Rad, Munich, Germany). After measurement of the absorbances at 405 nm, the results were expressed in EAST classes (0−4) and specific IgE levels [in units mL^{-1} (1 unit = 2.4 ng of IgE)], respectively.

EAST Inhibition. For inhibition experiments the pool-serum was diluted 1:1 in incubation buffer [PBS containing 0.3% (w/v) BSA and 0.1% (v/v) Tween 20]. A 10-fold dilution series of the inhibitor extracts from all maturation levels was prepared in five steps using the same incubation buffer. Nonspecific inhibition was checked with ovalbumin. Fifty microliters of the diluted serum was mixed with 50 μL of the inhibitor solution and an allergen disk. The solutions were incubated overnight at room temperature in the dark. After three washings with 1% Tween 20 in PBS (v/v), 50 μL of anti-human IgE alkaline phosphatase conjugate (Allergopharma, Reinbek, Germany) was added and incubated overnight. The disks were washed again three times, and enzyme activity was stained with PNPP for 60 min at 37 °C. Absorbance was measured at 405 nm (*22*).

RESULTS

Characterization of Patients' Sera. The sera of 39 patients were examined for specific IgE antibodies against green pea. Sera of 23 patients showing EAST classes < 2 were not used for detection (data not shown). The clinical information of the 15 green pea-allergic individuals used for this study is summarized in **Table 1**. Seven patients suffer from oral symptoms to green pea, such as itching in the mouth or throat or swelling of the lips as well as the mouth mucous membrane. Another three patients show problems in swallowing and swelling of the uvula and/or in the pharyngeal area. Nine patients suffer from redness, itching, or eczema of skin and two from urticaria. In addition to this, nine patients show conjunctivitis, sternutation, sniffles, and rhinitis, and six reveal dyspnea, wheeze, and asthma. Three patients suffer from nausea, vomiting, gastric and/or ventral paroxysms, flatulence, and diarrhea, and two further individuals show dizziness, circulatory disturbance, or hypotension after green pea consumption. None of the patients with known anamnesis shows gastrointestinal symptoms or anaphylaxis. As **Table 1** reveals, many patients show further sensitivities to different fruits, nuts, and tree pollen.

The IgE contents and the resulting EAST classes as well as the IgE-binding proteins in immunoblot are also summarized in **Table 1**. The IgE contents vary from 0.85 to >17.5 units mL^{-1} with EAST classes from 2 to 4.

Protein and Dry Weight Determination. The mean values of the individual weights of the seeds, the dry weight contents, and the total protein contents determined according to standard methods of the four harvested stages of green peas are listed in **Table 2**. In addition, the rates of the glutelin, albumin, and globulin fractions are also documented.

As expected, the mean values of the green pea seeds rise during the period of growth. The seeds harvested after a growing time of 70 days show a mean value of only 0.21 g. In the following stages the average value increases steadily from 0.59 to 0.84 g and finally to 1.11 g. The same increase applies to the total protein content. At the outset only 5.9% protein was determined in the small green pea seeds. During the following growing stages the content rises to a value of 10.9%. The dry weight content developed in parallel. Although the early harvested seeds show a content of 19.6% dry weight only, this rises to 50.5% in the fourth growing stage.

The ratios of the individual protein fractions show unsteady tendencies. The protein of the green pea seeds harvested first contains 61.2% globulin, 19.8% glutelin, and 19.0% albumin. In the following stages globulin content falls continuously to a final value of 48.3%. In contrast to this tendency, the albumin content rises during ripening. The second stage shows 22.8%, the third, 31.5%, and the fourth, 30.3%. The glutelin content shows uneven values. Declining from 19.8% in the first stage to 18.7% in the second and to 15.4% in the third, the ripest shows a rise to 21.5%.

SDS-PAGE/Silver Staining. Figure 1 illustrates the silver-stained total protein extracts of all four harvesting stages and the respective three protein fractions glutelin, globulin, and albumin of each stage separated by SDS-PAGE using a 12% acrylamide gel. The molecular weight marker protein bands range from 14 to 94 kDa.

The electrophoretic pattern of the total protein extract of the first harvesting stage (lane 1) shows a multitude of proteins with molecular masses between 20 and 94 kDa; no proteins with low molecular weight are detected. All characteristic bands <67 kDa seen in the first harvesting stage also exist in the following stages (lanes 2−4). Furthermore, some proteins with molecular masses of <20 kDa were detected. In the higher molecular mass range, >67 kDa, some proteins are degraded. New bands or a rise of detection intensity could be assessed for proteins with molecular masses of <14, 16, 18, 20, 25, 28, 30, 40, and 45

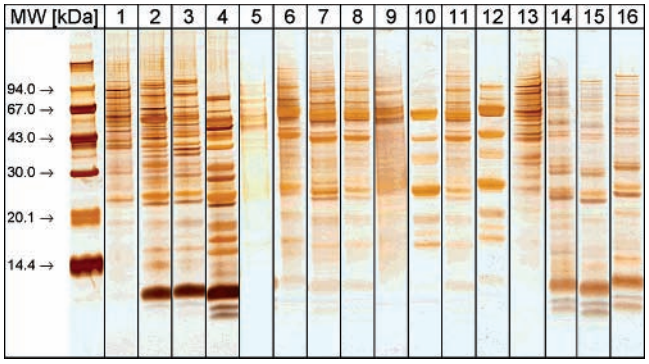

Figure 1. Silver-stained total protein extracts and protein fractions glutelin, globulin, and albumin of harvesting stages 1–4 separated by SDS-PAGE using a 12% acrylamide gel. Approximately 20 μg of protein was applied to each well. Lanes 1–4, total protein extract harvesting stages 1–4; lanes 5–8, glutelin fraction harvesting stages 1–4; lanes 9–12, globulin fraction harvesting stages 1–4; lanes 13–16, albumin fraction harvesting stages 1–4.

kDa. The glutelin fractions of the harvesting stages 1−4 (lanes 5−8) generally show a less distinct protein pattern. In stage 1 (lane 5) only three proteins with MW > 43 kDa are detected. The other glutelin fractions (lanes 6−8) contain many more proteins in the molecular mass range from 14 to 94 kDa. The amount of proteins and the intensity of detection rise, compared to the total protein extracts. Characteristic proteins could be detected at 25, 45, and 66 kDa. Similar proportions, an increase of the number of proteins and the detection intensity, could be observed for the four globulin fractions (lanes 9−12) and the albumin fractions (lanes 13−16). The characteristic bands in the globulin fractions possess molecular masses of 24, 40, 45, and 66 kDa; those in the albumin fractions are <14, 25, and 32 kDa, respectively.

SDS-PAGE/Immunoblot. To characterize the allergen pattern, the investigated extracts are separated by means of SDS-PAGE, electrotransferred to nitrocellulose, and finally detected by immunostaining. The molecular mass marker is stained with brilliant black according to a modified method of Moeremans et al. (*23*). Negative control is incubated with the serum from a nonatopic individual with no history of food hypersensitivity. All other extracts are incubated with a pool-serum of 15 green pea allergic patients. The results of incubated total protein extracts, fractionated proteins, and a negative control are illustrated in **Figure 2**.

The total protein extract of the first maturation level (lane 1) shows seven discrete IgE-binding proteins with molecular masses of 14, 28, 40, 45, 66, 75, and 94 kDa. Furthermore, unspecific staining is visible. In comparison to this, the 66-kDa band is absent in the following total protein extracts (lanes 2−4). All other bands are also detected; mostly the intensity of detection rises. New IgE-binding proteins could be detected at 22 kDa (lane 2−4), <14 kDa (lanes 3 and 4), and 16 and 18 kDa (lane 4). The glutelin fractions of all harvesting stages (lanes 5−8) show stainings at 14, 28, 40, and 45 kDa. Additionally, IgE-binding proteins are detected at 20 and 75 kDa in harvesting stages 2−4 (lanes 6−8) and at 22 kDa (lanes 7 and 8). In contrast to the total protein extracts, the intensity of staining does not rise continuously to the maturation level. Therefore, the total staining of harvesting stage 1 is most intensive. The other levels show comparable total stainings. The globulin fractions (lanes 9−12) indicate the slightest staining of all fractions. IgE-binding proteins are detected at 28, 32, 40, 50, and 75 kDa. The staining intensity develops just as in the case

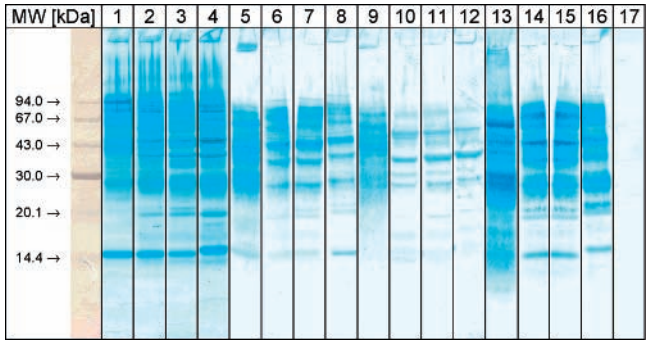

Figure 2. Immunoblot of total protein extracts and protein fractions glutelin, globulin, and albumin of harvesting stages 1–4 separated by SDS-PAGE using a 12% acrylamide gel and transferred to nitrocellulose membrane. Approximately 20 μg protein was applied to each well. Molecular weight marker is brilliant black stained. Negative control was incubated with a serum of a nonallergic individual. All other lanes are incubated with pool-serum from 15 different green pea allergic patients (see **Table 1**). Lanes 1–4, total protein extract harvesting stages 1–4; lanes 5–8, glutelin fraction harvesting stages 1–4; lanes 9–12, globulin fraction harvesting stages 1–4; lanes 13–16, albumin fraction harvesting stages 1–4; lane 17, negative control.

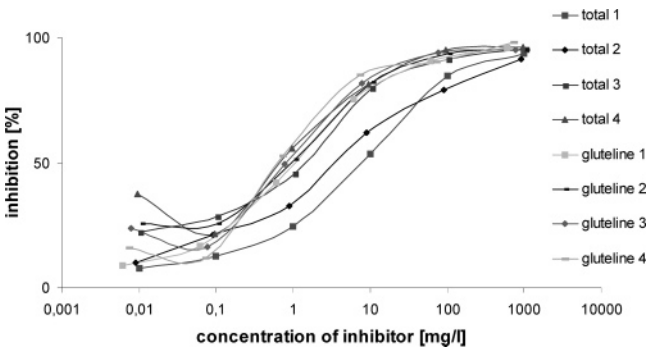

Figure 3. Results of dose-related inhibition of IgE binding with total protein extracts and glutelin fractions of harvesting stages 1–4. Total protein extract from harvesting stage 4 was used as inhibitor. Results are expressed as percent inhibition. The following C_{50} concentrations were obtained with different fractions: total 1, 7.5 mg L^{-1}; total 2, 3.5 mg L^{-1}; total 3, 1.4 mg L^{-1}, total 4 (homologous inhibition), 0.7 mg L^{-1}; glutelin 1, 1.0 mg L^{-1}; glutelin 2, 0.9 mg L^{-1}; glutelin 3, 0.8 mg L^{-1}; glutelin 4, 0.6 mg L^{-1}.

of the glutelin fraction. In contrast to these results, the albumin fractions (lanes 13−16) show the strongest staining. In all extracts, bands at 14, 28, 32, 40, 45, and 66 kDa are detected. Furthermore, albumin fractions 2, 3, and 4 (lanes 14−16) contain IgE-binding proteins with molecular masses of 20, 22, 75, and 94 kDa. Unspecific staining in harvesting stage 1 (lane 13) is discernible.

EAST Inhibition. The EAST inhibition experiments, applied to compare the IgE-binding potency of the different total protein extracts and the respective glutelin, globulin, and albumin fractions, were carried out using the total protein extract from harvesting stage 4 as inhibitor. The inhibition graphs are illustrated in **Figures 3** and **4**. The resulting C_{50} values responsible for a 50% inhibition of the IgE binding, which reflect the extracts' allergenic potencies, are listed in the legends of **Figures 3** and **4**. The results are presented in **Figure 5**.

The decrease of the C_{50} values of the total protein extracts in the course of maturation is justified by the rise in IgE-binding potency. This tendency is also assessed in the case of the glutelin, globulin, and albumin fractions. Only globulin fraction 3 shows a higher C_{50} value than the following harvesting stage.

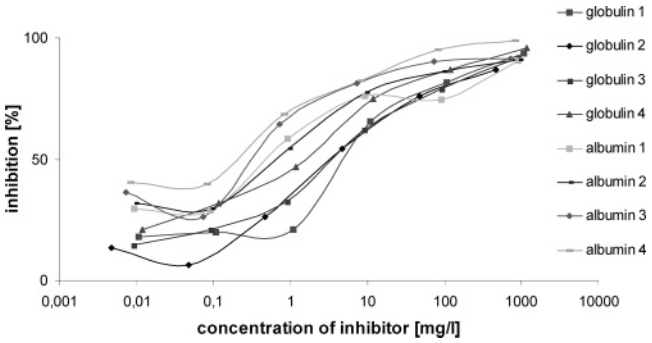

Figure 4. Results of dose-related inhibition of IgE binding with globulin and albumin fractions of harvesting stages 1–4. Total protein extract from harvesting stage 4 was used as inhibitor. Results are expressed as percent inhibition. The C_{50} concentration of the homologous inhibition was 0.7 mg L^{-1} (see **Figure 3**). The following C_{50} concentrations were obtained with different fractions: globulin 1, 4.8 mg L^{-1}; globulin 2, 3.3 mg L^{-1}; globulin 3, 3.5 mg L^{-1}; globulin 4, 2.0 mg L^{-1}; albumin 1, 0.5 mg L^{-1}; albumin 2, 1.0 mg L^{-1}; albumin 3, 0.3 mg L^{-1}; albumin 4, 0.2 mg L^{-1}.

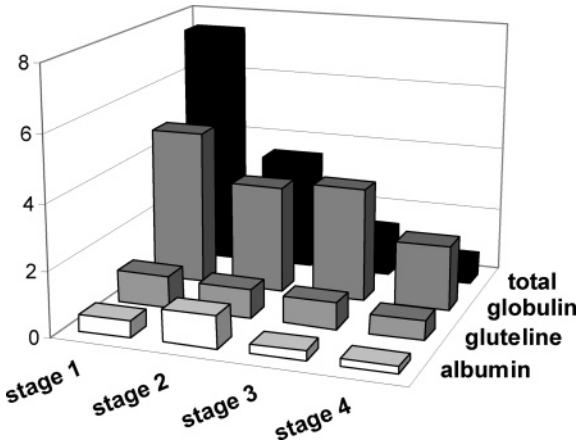

Figure 5. C_{50} concentrations of the total protein extracts and the protein fractions globulin, glutelin, and albumin of harvesting stages 1–4.

DISCUSSION

Although the importance of legumes for human nutrition, due to their oil and protein contents, has increased during the past few years and a serological cross-reactivity between soy, peanut, green pea, chick pea, bean, and lima bean has been verified, studies investigating the allergenicity of green peas are scarce (*9*, *12*, *25*).

In this study 17 different IgE-binding proteins in the range of <14−94 kDa were detected with a pool-serum containing 15 sera of green pea-allergic patients (see **Table 1**). Overall, at least 10 of these IgE-binding proteins in the molecular mass range from 14 to >66 kDa were identified by Bernhisel-Broadbent et al. (*12*), too. Therefore, the results are comparable; differences could be explained with differing patients' sera used for detection and different varieties of green peas.

Despite the generally known variation of the chemical composition of ripening green peas (*13*−*15*) and the assumed differences in allergenic activities of the protein fractions albumin, globulin, and glutelin (*11*, *26*), there are no investigations available concerning the variation of IgE-binding activity depending on maturation level. **Table 2** shows the rise in total protein content as well as the displacement of the concentration in the direction of the albumin fraction. These changes are discovered additionally by SDS-PAGE/silver staining of the total protein extracts and the single protein fractions of the four harvesting stages (see **Figure 1**), showing a differentiation of protein patterns in the course of maturation. The rise in the amount of proteins could be justified in the synthesis of new proteins as well as the disruption of large proteins in fragments during the maturation of green peas. Thereby, albumins as described by Malley et al. (*11*) and globulins as well as glutelins are synthesized. The ability of IgE binding in the total protein extracts as well as in the single fractions in the first harvesting stage is demonstrated (see **Figure 2**). These results are in contrast to the investigations of Malley et al. (*11*), who found that immature green peas showed absolutely no allergenic activity. Probably, the analyzed fractions resulted from an earlier harvesting stage because they contained very little or no albumin. The rise in allergenic capacity in the course of ripening is proved concerning total protein and single fractions of glutelin, globulin, and albumin. These results agree with those of Malley et al. (*11*), who found a close parallel between allergenicity and the albumin content of green peas, whereas other fractions support no allergenic activity. In accordance with the results of the SDS-PAGE/immunoblot studies, the EAST inhibition (see **Figures 3**−**5**) shows a decrease of C_{50} values in the same line. Generally, a rise of allergenic capacity in the progress of maturation is ascertainable. Contrary to further studies (*11*, *26*), the immature green peas as well as the globulin and the glutelin fractions show IgE-binding capacity. The albumin fractions show the slightest C_{50} values, so that these proteins contribute the strongest IgE-binding capacity. This result is comparable to the studies of Malley et al. (*11*).

It has been demonstrated that immature green pea seeds of the variety Maxigolt show a relevant allergenic capacity and that total allergenic capacity rises during the course of maturation. Although the main allergenic activity depends on the albumin fraction, the glutelin and globulin fractions are also decisive for the total allergenic capacity. To generalize these results further, investigations using other green pea varieties are necessary. Besides this, the isolation of the pea allergens and their chemical characterization as described in the studies of López-Torrejón et al. (*27*) have to be done. These SDS-PAGE and immunoblot investigations demonstrate a tendency of the allergenic potency concerning different protein fractions in pea. Definite information about the alteration of the allergenic potency of a certain allergen could not be given. The study is focused on the total allergenic capacity regarding a protein fraction during maturation.

ABBREVIATIONS USED

BSA, bovine serum albumin; EAST, enzyme allergosorbent test; ELISA, enzyme-linked immunosorbent assay; IgE, immunoglobulin E; MW, molecular weight; PNPP, *p*-nitrophenyl phosphate; PBS, phosphate-buffered saline; SDS-PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis.

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