



The Influence of Different Potato (*Solanum tuberosum*) Strains and Technological Processing on Allergenicity

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This study was undertaken to investigate the distinctions between different horticultural strains of potato and the influence of technological treatment on allergenicity. The allergenic potencies of raw and processed fruit extracts were determined by means of electrophoretical and immunological methods. For immunological assays sera of 12 patients with a sensitization to raw potato (EAST-classes ≥ 2) were pooled. The examinations of extracts from six different potato strains showed differences in the pattern of the IgE-binding proteins, but no distinctions could be observed in the allergenic potency by EAST-inhibition experiments. The technological influence on allergenicity was investigated on a model-production of potato-flakes and different heating processes. The data show only a slight decrease of allergenic activity by raising the temperature. In spite of this it proved possible to determine a new protein band in SDS-PAGE with molecular weight of approximately 50 kDa in extracts resulting from heated potatoes, which also showed an IgE binding activity. The application of additives had no influence on allergenic potency.

Keywords: *Potato, food allergy, technological processing, strains*

INTRODUCTION

Potatoes are a common dietary constituent and widely consumed, particularly in Europe. However, reports of adverse reactions to potatoes are rare and described generally in relation to oral allergy syndrome (OAS). Several authors reported that eating and peeling raw potatoes can elicit allergic reactions in both children and adults (Pearson, 1966; Nater & Zwartz, 1967; Castells *et al.*, 1986; Quirce *et al.*, 1989; Wahl *et al.*, 1990; Delgado *et al.*, 1996; Beausoleil *et al.*, 2001). Allergic reactions to raw potatoes have been associated with birch pollen-allergic individuals (Hannuksela & Lathi, 1977; Drebourg & Foucard, 1983). Thereby, protein homologues of the birch (*Betula verrucosa*) pollen allergens Bet v 1 (Breiteneder *et al.*, 1998) and Bet v 2 (Valenta *et al.*, 1991) have been suggested to be

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responsible for adverse reactions to potato. Other Solanaceae, such as tomatoes (Petersen *et al.*, 1996) and bell peppers (Jensen-Jarolim *et al.*, 1998), are also cross-reactive to grass pollen. Furthermore Jensen-Jarolim *et al.* (1998) observed that the protein patterns as well as the IgE binding proteins of different bell pepper strains varied significantly.

In 1967, Nater and Zwartz demonstrated by analysis of heated and unheated potato juice in skin testing that the allergens of potatoes are heat-labile. Hannuksela and Lathi (1977), Larkö *et al.* (1983) and Quirce *et al.* (1989) confirmed these results. In contrast, Seppälä *et al.* (1999) identified a 43 kDa major potato allergen (Sol t 1) as the storage protein patatin, which seems to be heat-stable. In 2001, Seppälä *et al.* also isolated and identified four further potato allergens with molecular masses ranging from 16 to 20 kDa. Investigations on their stability under thermal treatment are lacking. There are only a few cases recorded in the literature of adverse reactions to boiled potatoes. Kästner *et al.* (1984) described an 8-year-old boy who suffered from an asthmatic attack after inhaling the steam of cooking potatoes. Also, Castells *et al.* (1986) reported a child who developed anaphylactic symptoms to cooked potatoes after eating them for the first time at the age of 5 months. Later Hannuksela (1987) related that cooked potatoes were able to elicit symptoms in children under one year of age, whereas older children appeared to be free of symptoms. In all these cases the identification of IgE-binding proteins after thermal treatment is absent.

This study was undertaken to estimate possible differences of allergen pattern and activity of six different horticulture strains. A further major concern of this research was to examine the stability of potato allergens to technological processing, especially the influence of temperature and in particular on profilin, a low molecular protein appearing in nearly every plant which seems to be responsible for the cross-reaction to pollen. Furthermore, this study aimed at the characterization of the proteins causing the allergic symptoms after technological treatment. To this end the protein and allergen patterns are examined, as are the allergen contents of raw fruits, intermediate and final products by means of 12 patients with a history of adverse reaction to potatoes. For immunological assays these sera of patients with a sensitization to potato (EAST-classes ≥ 2) were pooled.

MATERIALS AND METHODS

Allergen Extracts and Technological Processing

For the exploration of the connection between potato strains and allergenicity six different types of fresh potatoes were investigated. The evaluation was made on the basis of two early-maturing varieties 'Karlana' and 'Cilena' and four central early-maturing varieties 'Linda', 'Secura', 'Quarta', and 'Mentor'. The strains 'Karlana', 'Linda', 'Quarta' and 'Mentor', supplied by the company Unilever Bestfoods Deutschland GmbH, are often used in industry, whereas 'Cilena' and 'Secura' are marketed directly.

Potato extracts were prepared from fresh pulps without peel by a previously described low-temperature method with few modifications (Vieths *et al.*, 1992; Möller *et al.*, 1997). For the heating assay in laboratory scale the cultivar 'Linda' was used and autoclaved at 1 bar, 120°C for 10, 20, 30 and 40 min. The potato strain 'Karlana', which is normally utilized for production of potato-flakes, was selected for technological processing. The production of the accordingly defined intermediate and final products was executed in the technical school of the company Unilever Bestfoods Deutschland GmbH in Heilbronn. The main aim of this model-production was to estimate the influence of blanching, the addition of additives, the mechanical cutting as well as the drying temperature and duration on the allergens. Thereby the selection of the process parameters was based on the industrially-used technique. The used additives were monoglyceride for better homogenization and sodium metasilphide as well as ascorbylpalmitate as antioxidation reagents. An overview of the model production process is shown in Figure 1(a) and (b). The utilized intermediate (IP) and final products (FP) are framed. Both intermediate as well as final products were directly analysed

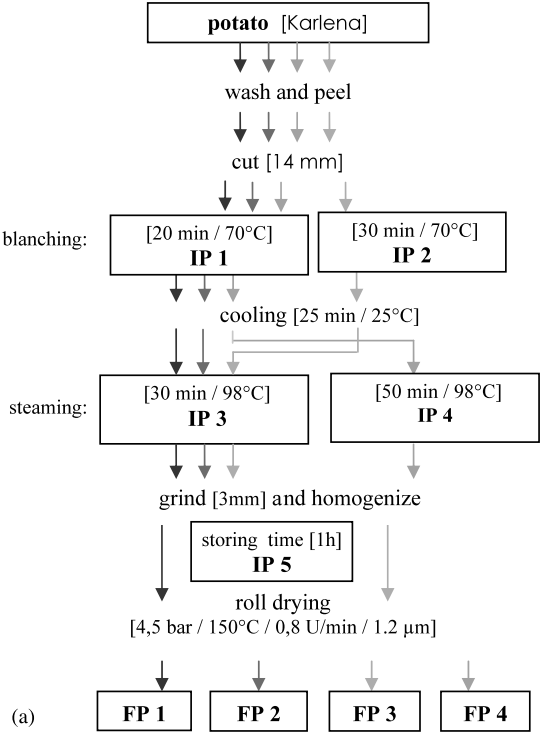


FIG. 1. Flow chart of potato flakes production. (a) Without additives; (b) with additives.

appropriate to the fresh fruits. After lyophilization the extracts were stored at -20°C until use.

Patients and Human Sera

Sera were obtained from 12 patients suffering from adverse reactions to raw potato and collected at the University Hospital Eppendorf, Department of Dermatology and Allergy, Hamburg, Germany. All patients also suffered from pollen allergy and had IgE to birch and mugwort pollen. The relevant clinical information is summarized in Table 1. For immunological investigations all 12 sera were pooled by using equal aliquots. Serum from a non-atopic individual without history of allergic reactions to food served as negative control. Specific IgE was determined by a commercially available enzyme allergosorbent test (EAST) according to the manufacturer's instructions (Allergopharma, Reinbek, Germany). For this, allergenic extracts from fresh potatoes were coupled to cyanogen bromide activated filter paper disks. The results were expressed as U ml^{-1} and divided into EAST-classes.

Electrophoresis, Immunoblotting and Inhibition Experiments

Proteins were separated by SDS-PAGE in 10% Bis-Tris-Gels (Invitrogen, Groningen, The Netherlands) according to the manufacturer's instructions under reducing conditions by use of two different running buffers which contained either MOPS [3-(N-morpholino)propane-sulphonic acid] or MES [2-(N-morpholino)ethanesulphonic acid]. The separated proteins were transferred to Protran nitro-cellulose membranes (Schleicher and Schuell, Dassel, Germany) by semi-dry blotting according to Kyhse-Andersen (1984) with a discontinuous buffer system as described by Vieths *et al.* (1992). The membrane was incubated with 1:15

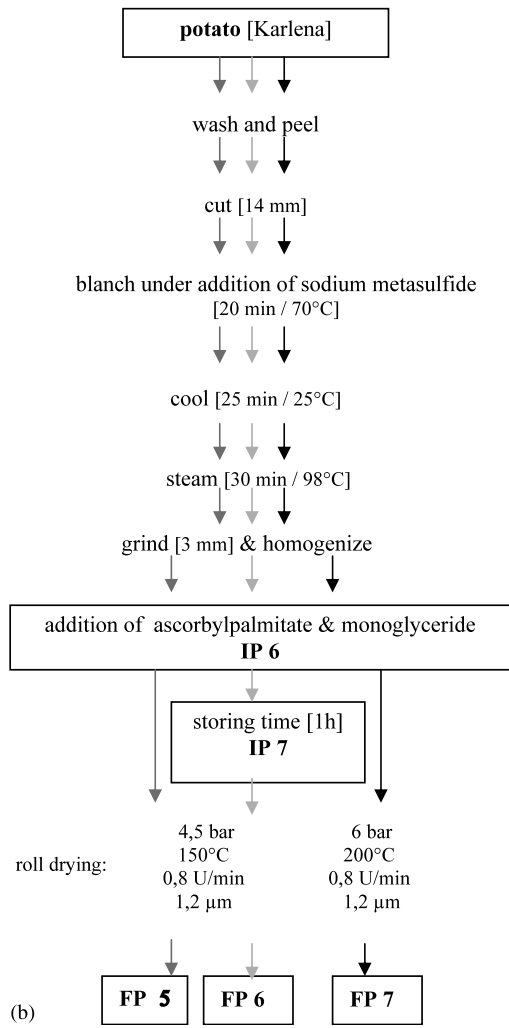


FIG. 1 (Continued)

dilutions of sera, with or without (negative control) specific IgE to potato, respectively, with 1:1000 dilution of a rabbit anti-pear-profileline-antibody. Immunostaining was carried out as described previously (Vieths *et al.*, 1992; Möller *et al.*, 1997). The molecular weights were determined by using low range SDS-PAGE standards (14.4–94.0 kDa). To visualize proteins unspecifically on nitro-cellulose membranes, the Indian ink staining method according to Hancock and Tsang (1983) was used, with some modifications.

For immunoblot-inhibition experiments the preincubation was carried out for 2 h at room temperature with 100 µl of serum and 300 µg of potato fruit extracts (various strains), process-product extracts or ovalbumin as inhibitors in a total volume of 1.5 ml incubation buffer [0.3% (w/v) bovine serum albumin (BSA), 0.1% (v/v) Tween 20 in phosphate-buffered saline (PBS)]. The immunostaining occurred analogous to the standard procedure.

Competitive East-inhibition of IgE binding to coupled potato extract on activated disks took place by preincubation of 1:1 diluted sera in incubation buffer with inhibitor proteins diluted in the same buffer for 60 min. As inhibitors extracts from intermediate and final

TABLE 1. Symptoms of patients and results of specific IgE determination (EAST)

No.	Patient	Sex	East-class spez. IgE (U ml ⁻¹)	Clinical symptoms
1	NB	Female	2–2.436	IM, E, RH
2	RD	Male	2–0.771	SL, IM
3	CG	Female	2–0.787	E, U, RH
4	KDH	Male	3–14.984	RH, D, IM, E
5	RK	Male	2–2.581	DIA
6	SK	Female	4–22.263	E, U
7	KL	Male	2–2.021	RH
8	RF	Male	3–8.900	–
9	LS	Male	2–1.084	IM
10	UW	Female	4–15.729	U, IT
11	JW	Male	2–2.581	IM, E, IT
12	SW	Male	3–4.030	RH, IT

DIA diarrhea; E eczema; IM itching in mouth; IT itching in throat; ND neurodermitis; RH rhinitis; SL swelling of lips; U urticaria.

products as well as different strains in concentrations from 0.08 µg ml⁻¹ to 800 µg ml⁻¹ were used. Non-specific inhibition was checked with ovalbumin.

RESULTS

SDS-PAGE and IgE Immunoblot of Fresh Potato Extracts

The fruits of six different potato strains were extracted and examined by means of SDS-PAGE and MOPS-running buffer. Silver stained gel, depicted in Figure 2(a), showed several protein bands with a molecular weight range of 14–97 kDa. On initial examination all extracts seem to be homologous, but there are slight differences in protein patterns. Besides the distinct marked double band at 43 kDa all extracts showed further clearly visible protein bands in the range of 20–23 kDa. In contrast to all others the extracts of ‘Mentor’ (5) and ‘Linda’ (6) showed an additional band at 45 kDa. Within the lower molecular weight area at approximately 25 kDa an additional protein can be visualized among the extract of ‘Karlena’

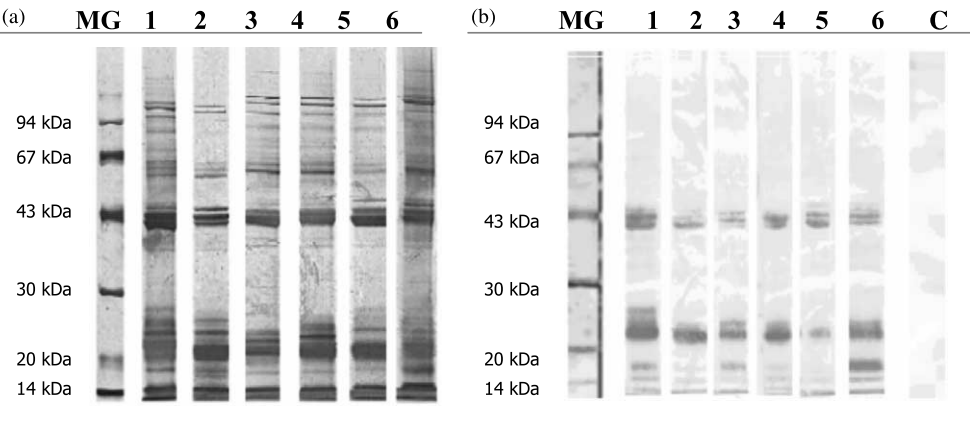


FIG. 2. (a) Silver stained protein extracts of various potato varieties separated by SDS-PAGE. (b) IgE binding patterns of patients’ pooled serum on blotted potato extracts 1–6. MW molecular weight marker; 1 Karlena; 2 Cilena; 3 Secura; 4 Quarta; 5 Mentor; 6 Linda; C negative control.

(1). Immunoblotting revealed in all strains IgE binding to 14, 18, 20–23 and 43 kDa molecules (Figure 2(b)). The differences manifested in silver staining could be confirmed. Extract 1 exhibits an IgE binding protein at 25 kDa and extracts 5 and 6 showed bands at 45 kDa reactive to IgE. In order to estimate whether the 14 kDa allergen is a profilin, immunoblot experiments were performed using rabbit anti-pear-profilin-antibody. The results indicated clearly that the 14 kDa allergen represents a profilin (data not shown).

Inhibition Experiments of Fresh Potato Extracts

By means of immunoblot-inhibition experiments it was examined whether the additional observed 25 kDa IgE binding protein in the extract of ‘Karlana’ respectively the 45 kDa in the extracts of ‘Mentor’ and ‘Linda’ could be inhibited by preincubation of the patients’ sera with extracts of the other potato strains. The IgE depletion experiment with blotted ‘Karlana’ extract, illustrated in Figure 3, shows that IgE binding to the 25 kDa protein can be completely extinguished by preincubation with all other potato strains. The same results were obtained for the experiments wherein the extracts ‘Linda’ and ‘Mentor’ were blotted and preincubated with the extracts of various strains (data not shown). By means of EAST inhibition assays the extent of IgE binding activities was estimated. The results, which are shown in Figure 4 with ‘Karlana’ as coupled allergen extract, do not indicate significant differences. This is discernible by the almost identical inhibition curves as well as by the slightly varying C_{50} -values responsible for a 50% inhibition of the IgE binding, which reflects the extracts’ allergenic potencies. They range from 46 to 130 mg l⁻¹. Graphs of other coupled strains are not shown since they represent the same results. No significant inhibition was observed with ovalbumin in all cases.

SDS-PAGE and IgE Immunoblot of Technical Processed Potatoes

The influence of processing on the allergenic activity of proteins from potato tuber was investigated by a model-production of potato flakes, likewise using different heat treatments in laboratory scale. Figure 5(a) and (b) show the silver staining and immunoblot of autoclaved potatoes for different times. The protein patterns of the extracts from thermally treated potatoes diverge from those of the native potato extract. In addition to the decline of intensity of different protein bands ranged in molecular weight from 14 to 25 kDa and the

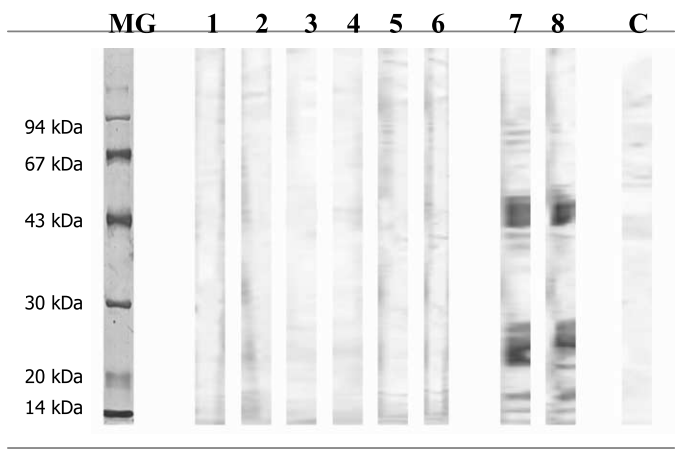


FIG. 3. Inhibition of IgE binding by preincubation of serum with potato strains. Blotted extract: potato cultivar ‘Karlana’. MW molecular weight marker; Inhibitor: 1 Karlana; 2 Cilena; 3 Secura; 4 Quarta; 5 Mentor; 6 Linda; 7 unspecific inhibition with ovalbumin; 8 without inhibitor; C negative control.

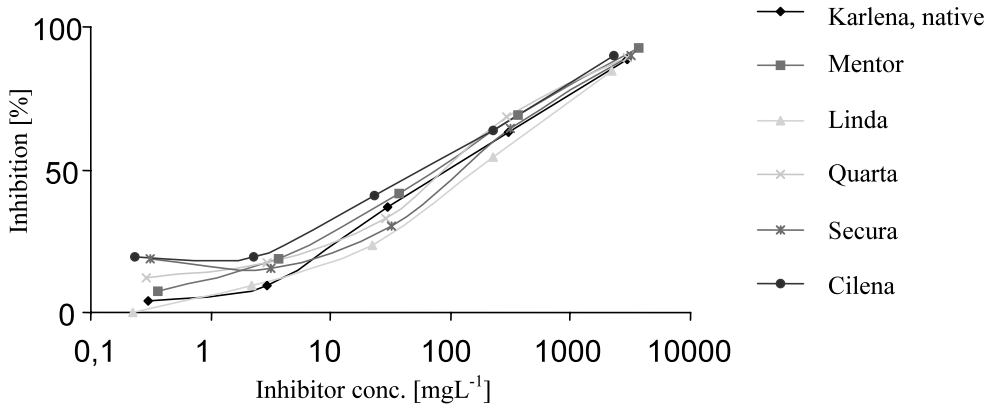


FIG. 4. Dose-related EAST inhibitions with pool serum. Solid phase: potato extract (variety 'Karlena'). Inhibitors: various potato cultivars.

patatin (43 kDa) they also showed new dominant bands at 30 kDa and below 14 kDa. Immunoblotting revealed IgE binding to 23 and 43 kDa proteins with loss in intensity, whereas profilin at 14 kDa is lacking in all heated extracts. Moreover, a new IgE binding protein with approximately 50 kDa could be detected. No differences could be observed between the autoclaved potato extracts. The clearly detected bands in silver staining at 30 kDa and below 14 kDa did not show any IgE binding activity. Figure 6 reveals the inhibition graphs of autoclaved potatoes. In addition the homologous inhibition of the native potato is shown. Compared to the native extract the IgE binding potency was distinctly reduced. No differences could be observed between the extracts of autoclaved potatoes.

The results of SDS-PAGE silver staining and immunoblot of the extracts of the model production are comparable to those of the thermal treatment by autoclaving. In the

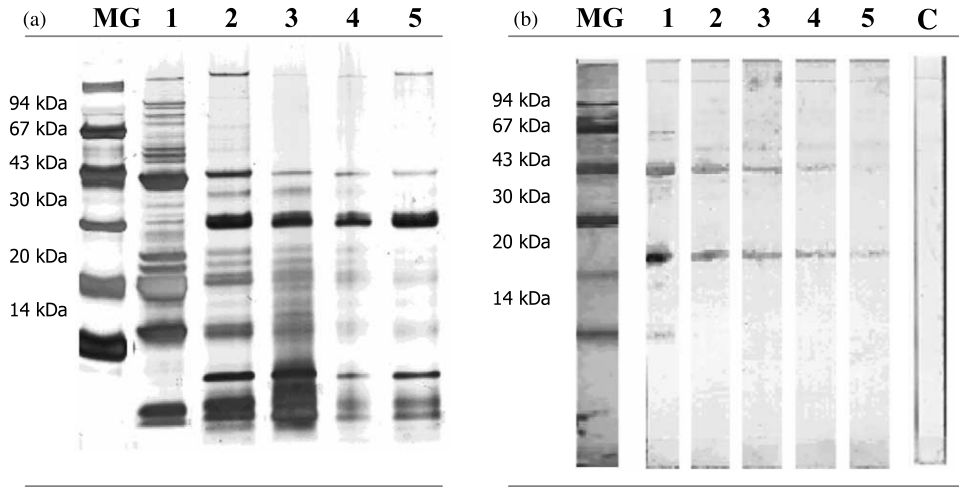


FIG. 5. (a) Silver stained protein extracts of autoclaved potatoes separated by SDS-PAGE. (b) IgE binding patterns of patients' pooled serum on blotted extracts of autoclaved potatoes. MW molecular weight marker; 1 native potato; 2 autoclaved for 10 min; 3 autoclaved for 20 min; 4 autoclaved for 30 min; 5 autoclaved for 40 min; C negative control.

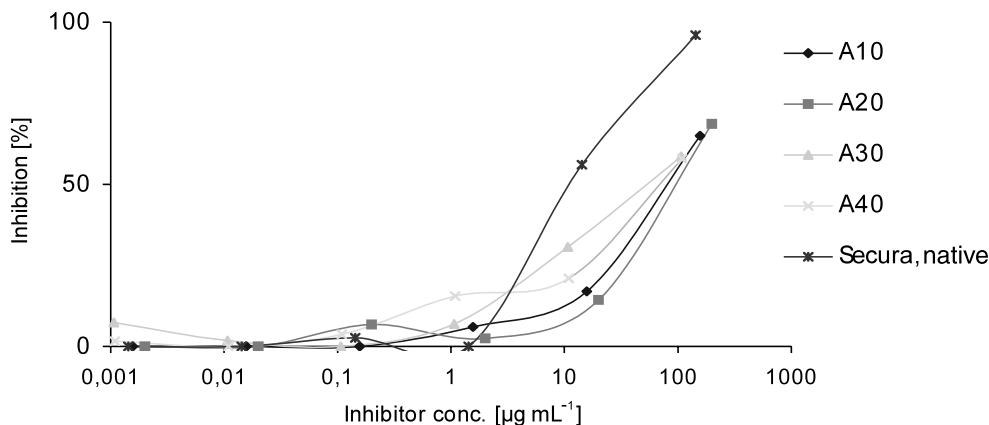


FIG. 6. Dose-related EAST inhibitions with pool serum. Solid phase: potato extract (strain 'Linda'). Inhibitors: extracts of autoclaved potatoes (A 10–A 40).

intermediate products (Figure 7(a)) the pool serum detected the same allergens as in the autoclaved extracts. While these allergens in the extracts of the blanched potatoes (IP 1 and 2) are detectable, the IgE binding diminishes with increasing temperature. In the extracts of IP 3–7 and also of all final products (data not shown) only IgE binding to the new protein with 50 kDa could be clearly observed. No differences in allergenic pattern could be demonstrated by addition of additives or mechanical cutting during the process. This resulted from the electrophoretic patterns of these extracts (IP 6 and 7), which are similar to the corresponding IPs (3–5) without any additives.

Inhibition Experiments of Processed Potatoes

The EAST inhibition experiments, applied to compare the IgE binding potency of the extracts of different treated potatoes, were carried out using the native potato protein extract as a reference on the solid phase and the pool serum. An additional homologous inhibition was performed to check the allergenic activity of the native potato protein extract. Figure 8 illustrates the inhibition graphs. The resulting C_{50} -concentrations of protein extracts are listed in Table 2. The inhibition graphs as well as the C_{50} -values confirm the immunoblot results. The inhibition graphs of the extracts of autoclaved potatoes (Figure 7) showed a weak decrease of allergenic activity with regard to the heating time. The autoclaved products reveal C_{50} -concentrations of approximately $50\text{--}90\text{ mg l}^{-1}$, whereas the homologous inhibition leads to a C_{50} -value of 2 mg l^{-1} . Corresponding to these findings, the C_{50} -concentrations of extracts from IPs and FPs differ from $35\text{ to }130\text{ mg l}^{-1}$ with regard to the increase in temperature. This is also visible in their inhibition graphs (Figure 8, only FP are mapped).

DISCUSSION

The performed investigation of six potato cultivars – the early-maturing 'Karlana' and 'Cilena' as well as the central early-maturing 'Linda', 'Secura', 'Quarta', and 'Mentor' – resulted in an almost identical allergenic potency. Interestingly, the protein and IgE binding pattern in SDS-PAGE varied a little between the cultivars. Extracts from 'Karlana', 'Mentor', and 'Linda' contained an additional IgE-binding protein at approximately 45 and 25 kDa. By means of immunoblot inhibition it was demonstrated that all potato extracts were able to inhibit these additionally manifested allergens. Thereby it would suggest that

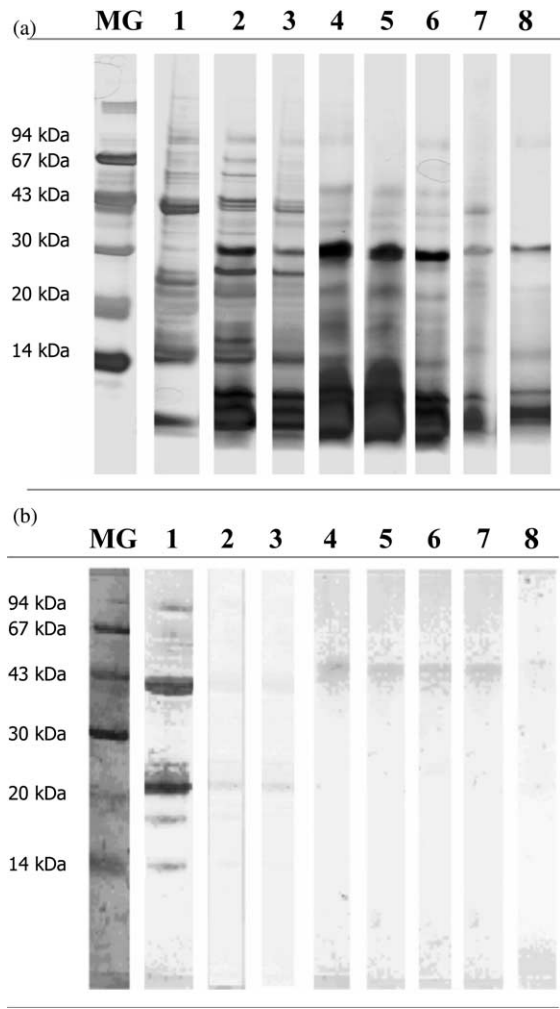


FIG. 7. (a) Silver stained protein extracts of intermediate products (IP) separated by SDS-PAGE. (b) IgE binding patterns of patients' pooled serum on blotted potato extracts of intermediate products. MW molecular weight marker; 1 native potato; 2: IP 1; 3: IP 2; 4: IP 3; 5: IP 4; 6: IP 5; 7: IP 6; 8: IP 7.

one or more allergens, which appeared in every cultivar, carry identical or strongly homologous epitopes to those of the additional ones. For more detailed information about these allergens, further studies which cover the isolation and n-terminal sequencing, are needed. To check which allergens are cross-reactive additional inhibition assays have to be done. However, the EAST-inhibition experiments under use of pool serum showed a comparable allergenic potential of all investigated cultivars. Similar variations of protein pattern in SDS-PAGE were observed by Jensen-Jarolim *et al.* (1998) in different bell peppers, which also belong to the Solanaceae family. According to them, some bell pepper strains are considered to be more allergenic for some individuals than others. For instance Bet v 1 homologues are not expressed in every horticultural strain of bell peppers. Likewise apples were found to have strongly differing allergic potency depending on their cultivar (Vieths *et al.*, 1993; Son *et al.*, 1995). Thus the expression of Mal d 1 seems to be responsible for the distinctions in the allergenicity between different apple strains (Vieths *et al.*, 1993). Wigotzki

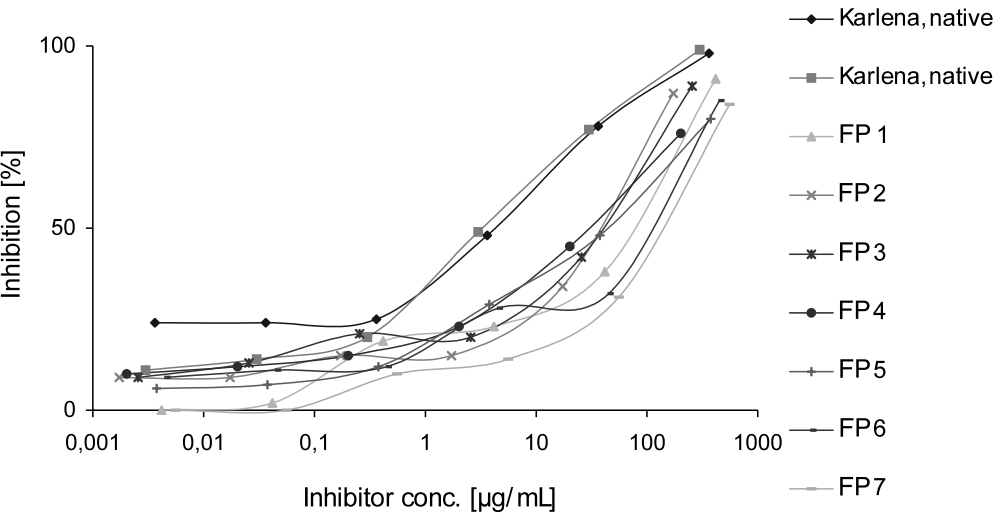


FIG. 8. Dose-related EAST inhibitions with pooled serum. Solid phase: potato extract (strain ‘Karlena’). Inhibitors: extracts of intermediate and final products of potato flake production.

TABLE 2. c_{50} -values and maximum inhibitions of the IPs and FPs of the potato flakes model production

Inhibitor	C_{50} -value (mg l ⁻¹)	Max. inhibition (%)
Karlena, homologous	2	99
IP 1	36	80
IP 2	35	74
IP 3	59	71
IP 4	55	88
IP 5	63	72
IP 6	54	64
IP 7	89	64
FP 1	73	87
FP 2	69	89
FP 3	72	76
FP 4	98	80
FP 5	70	84
FP 6	65	82
FP 7	127	80

et al. (2000), who carried out similar investigations on hazelnut strains, found only slight differences in the IgE-patterns, which were ascertainable by the detection intensity of allergens. In contrast no dependency of allergenic activity on horticultural strains could be observed in several other fruits, such as mangos, lychees, and carrots (Kinder *et al.*, 1999; Zunker, 2001; Wigotzki, 2001).

The investigations on the influence of technological treatment on potato allergens took place in two steps. First heating tests were executed by autoclaving in laboratory scale. Under thermal treatment heat-labile allergens lost their allergenic activity as a result of structural modifications (Besler *et al.*, 2001). Such IgE active molecules often have conformational epitopes, which can be easily destroyed. In contrast, sequential epitopes are most stable to thermal processes. Most of all thermo-stable allergens are found in animal food such as egg

and milk (Taylor *et al.*, 1987). Many food allergens of vegetables contained both heat stable and labile allergens. For example Api g 1 was demonstrated to be heat labile, while celery profilin and especially other allergens (> 30 kDa) were relatively heat stable in SDS-PAGE immunoblot (Jankiewicz *et al.*, 1997, 1998; Vieths *et al.*, 1996).

The silver stained SDS-PAGE revealed a different protein pattern in the thermally processed potato extracts compared to the native potato. In contrast to the native potato extract further proteins with molecular weights of < 14 and 30 kDa without any IgE binding activity were visualized very strongly; and a molecule at 50 kDa was also slightly detectable in all thermally processed potatoes, which showed IgE binding activity in the immunoblot investigations. The 50 kDa IgE binding protein might represent either a neoallergen or a degradation product of a high molecular weight molecule containing IgE binding epitopes, which are also present on the undegraded allergen. Paschke *et al.* (2001) also reported a formation of a 16 kDa neoallergen investigated in soybean lecithins, as did Codina *et al.* (1998). A further example of the appearance of a new allergenic protein is celery, in which such a molecule could be detected after gamma-irradiation (Vieths *et al.*, 1997).

Immunoblot investigations of the thermally treated potato extracts showed only two residual allergenically active proteins, all other allergens of the potato tuber could not be demonstrated, as they seem to be unstable. In relation to the increase in temperature the ability to bind IgE of these two proteins, patatin and the 23 kDa allergen, is reduced. The 14 kDa allergen which could be determined as a profilin seems to be very unstable under heating processes. This finding confirms the results of Wigotzki *et al.* (2000), who described a 14 kDa allergen in hazelnuts which was easily destroyed under heat influence. In contrast the profilins of lychees and mangos turned out to be heat stable (Zunker, 2001).

The EAST inhibition experiments verified the immunoblotting results. The protein extracts of all processed potatoes were able to inhibit the IgE binding activity of the native proteins, whilst the inhibition decreases in connection with the rise in temperature during processing. It was not possible to eliminate the allergenic activity of potatoes, only a slight reduction could be attained.

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