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Effect of Technological Processing on the Allergenicity of Mangoes (*Mangifera indica* L.)

MARK DUBE,‡ KATY ZUNKER,† SYBILLE NEIDHART,‡ REINHOLD CARLE,‡ HANS STEINHART,† AND ANGELIKA PASCHKE*,†

Institute of Biochemistry and Food Chemistry, Section of Food Chemistry, University of Hamburg, Grindelallee 117, D-20146 Hamburg, Germany, and Institute of Food Technology, Section Plant Foodstuff Technology, Hohenheim University, August-von-Hartmann-Strasse 3, D-70599 Stuttgart, Germany

In parallel with the rising popularity of exotic fruits in Europe, allergy against mango is of increasing importance. Because mangoes are also consumed as processed products such as chutneys or beverages, the influences of different process conditions on their allergenicity were investigated. Mango purees and nectars were manufactured at small pilot-plant scale, and the allergenic potencies of the resulting intermediate and final products were determined by means of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), immunoblotting and inhibitive enzyme allergosorbent tests (EAST-inhibition), using a pool serum of 9 individuals with manifest mango allergy. The mango allergens were shown to be very stable during technological processing. Irrespective of enzymatic matrix decomposition, mechanical tissue disintegration and heating during peeling, mash treatment, and pasteurization, significant loss of allergenicity could not be observed in the extracts of mango purees and nectars derived thereof. These results were confirmed by analogous investigation of commercial mango drinks and nectars. Hence, conventional mango processing into pulp-containing products typical for this species obviously does not allow complete elimination of the allergenic potency.

KEYWORDS: Food allergy; mango pulp; *Mangifera indica* L.; juice; immunoblotting

INTRODUCTION

Numerous epidemiological studies have proven the increasing frequency of allergic diseases during the past years, concurrent with the significant rise in food allergies (*1*−*2*). The affected portion of the Central European population is estimated at 0.3−2.4% for adults and at the higher rate of 1.3−8% for children (*3*−*6*). Apart from settling the pathogenetic reaction, a set of food allergens of animal and plant origin was discovered and has been partly characterized. Detailed studies were performed on apple (*7*, *8*), celery (*9*, *10*), carrot (*10*), and kiwi (*11*, *12*). Sensitivity of the allergens from celery and peanut to heat was thoroughly examined (*13*−*15*). Attempts to reduce the allergenicity by means of specific processing were described for rice by Hayakawa et al. (*16*) and for peach by Brenna et al. (*17*).

In contrast, technological effects on the allergenic potency, especially of exotic fruits, have been scarcely considered. Due to the rising popularity of exotic fruits and their meanwhile nonsaisonal availability on international food markets, allergy against mango increasingly moves into the focus of public interest in the consumer countries, particularly regarding the postulated birch and mugwort pollen association (*18*−*19*).

With the annual world production of approximately 25.8×10^6 Mt (*20*), mango (*Mangifera indica* L.) is the most frequently cultivated tropical fruit besides banana. Mangoes are consumed as fresh fruits and after processing into pickles, chutneys, canned or dried goods, juices, or nectars (*21*−*23*).

The objective of this study was to investigate the influence of various process parameters on the allergenicity of the mango fruit, aiming at strategies to eliminate its allergenic potency by means of processing. Whereas usual case records on food allergies do not consider the exact botanical determination of the plant material and the modalities of its industrial processing into food, the present study is directed to the production of well-defined purees and nectars, which were manufactured at the small pilot-plant scale by analogy to industrial practice, and their immunological characterization, determining the allergenic potencies of the intermediate and final products by means of established methods. Because mangoes usually generate viscous, pulp-containing juices, enzymatic pectin degradation is commonly used to adjust product viscosity (*24*). Therefore focus was layed on the effects of technological heating measures and enzymatic treatments on mango allergenicity. In addition to the identification of individual allergens, a relative quantification of the allergenicity was attempted by means of EAST-inhibition experiments. To verify the results obtained on the experimentally

* To whom correspondence should be addressed. Tel.: 49 40 42838 4353. Fax: 49 40 42838 4342. E-mail: angelika.paschke@uni-hamburg.de.
† University of Hamburg.
‡ Hohenheim University.

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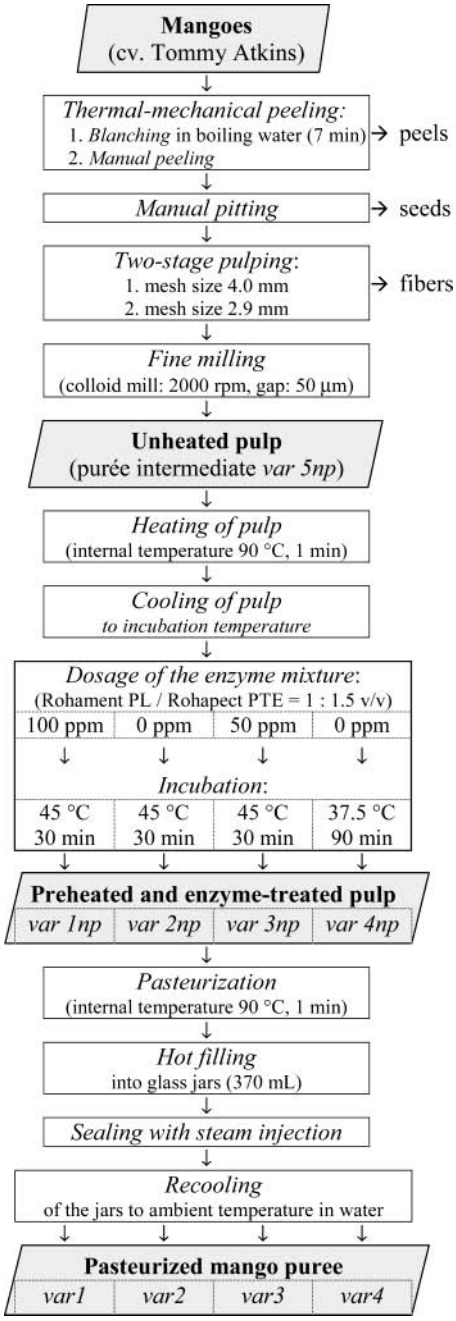

Figure 1. Experimental production of mango puree for immunological investigations. Raw material, intermediate, and final products used for stepwise process control and immunological analyses are presented in gray rhombic fields.

manufactured products, commercial products of the local market were comparatively considered.

MATERIALS AND METHODS

Small-Scale Production of Mango Purees and Nectars. Ripe mango fruits (cv. Tommy Atkins), originating from Costa Rica, were purchased from a local wholesaler (Staiger GmbH, Stuttgart, Germany). Four variants of purees (*var1* − *var4*) were produced, processing the fruits in batches of 4 kg, according to the procedure shown in **Figure 1**. For the two-stage pulping process with decreasing mesh size, a pulper type UMA (Alexanderwerk AG, Remscheid, Germany) was applied. Instead of conventional finishing of the pulp in a pulper at mesh sizes below 0.5 mm, which was not available, fine milling of the pulp was performed in a colloid mill, type K60/O/RDV (Probst and Class, Rastatt, Germany). To inactivate detrimental endogenous enzymes, which otherwise cause, for example, browning, the pulp was heated under stirring (75 rpm) prior to enzymation in a steam-heated kettle, type EL3 (Esco-Labor AG, Riehen, Switzerland) equipped with an anchor stirrer. At permanent stirring of the good (75 rpm), the same kettle was subsequently used for cooling and incubation of the preheated pulp and the final puree pasteurization, cooling or thermostating the kettle with water and heating it with steam. Sealing of the filled glass jars (370 mL) by means of a semiautomatic steam-vacuum-sealing machine (SLW 100 Vaporette, Schmalbach-Lubeca AG, Hannover) was associated with steam injection into the free bottleneck to remove excessive air and to sterilize the uncovered jar surface. For all enzymatic treatments of mango pulp, commercial preparations of a polygalacturonase (Rohament PL) and a pectin lyase (Rohapect PTE; both AB Enzymes GmbH, Darmstadt, Germany) were blended at the constant ratio of 1:1.5 (v/v) according to the manufacturer's recommendation, resulting in degradation of the pectin in the pulp. Thus, efficiency of enzymatic pulp decomposition was monitored by measuring the induced decrease in puree viscosity. To ensure that the rheological properties of the finally produced variant (*var1*) were significantly modified by enzymatic pulp degradation when compared to the control (*var2*), incubation parameters were preliminarily optimized, using a statistical experimental design for 3 factors (enzyme dosage, incubation time, and temperature; results not shown).

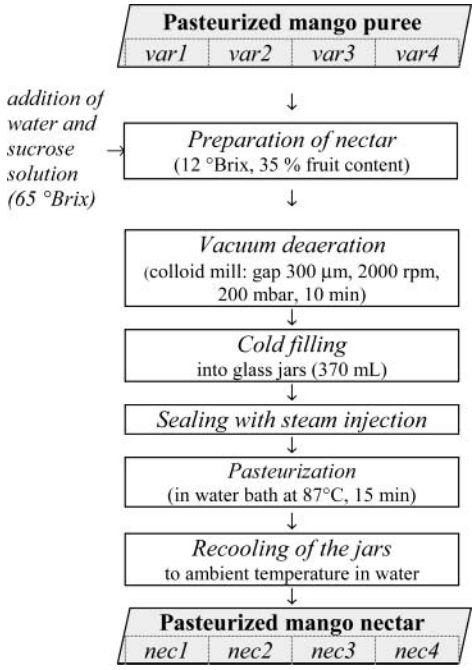

Figure 2. Experimental production of mango nectar for immunological investigations. Raw material and final products used for stepwise process control and immunological analyses are presented in gray rhombic fields.

In addition to the preheated pulp intermediates *var 1np* − *var 4np* (**Figure 1**), the intermediate *var 5np* was collected immediately after pulping. Thus, sample *var 5np* underwent neither an enzymatic treatment nor the necessary preheating. To mimic the usual nectar production from purees imported from producer countries, the pasteurized purees (*var1*−*var4* in batches of 1 kg) were processed into nectars (**Figure 2**), blending the puree with water and sucrose solution. Vacuum deaeration of the unpasteurized nectar was achieved by circulating it in the colloid mill mentioned above under vacuum (200 mbar) for 10 min to minimize the risk of oxidation. In contrast to the hot-filled purees, the nectars were cold-filled due to the small batch sizes, using the sealing machine mentioned above, and subsequently pasteurized, heating the jars in a water bath (Haake Mess-Technik GmbH & Co, Karlsruhe, Germany) at 87 °C for 15 min.

Process control including the determination of total soluble solids and titratable acidity was performed according to IFU (*25*). For comparison, one industrially produced mango nectar (35% mango pulp content) bottled in Germany (sample *cn1*) and two nectar-like mango drinks, orginating from Southeast Asia, with 35% pulp content (*cn2*)

and unspecified pulp content (*cn3*), respectively, were obtained from local markets in Stuttgart and Hamburg (Germany).

Rheological Measurements. Characterizing the flow behavior of the pasteurized mango purees and nectars, their viscosities were measured at 25 °C as a function of a preset shear rate ramp with a controlled-strain rheometer (ARES FV4, TA Instruments, New Castle, DE). Parallel plates of 50-mm diameter were used at a gap of 1 mm for rheological analyses of the purees. To measure the viscosities of the nectars, a Couette system, consisting of a fixed bob with 48-mm diameter and a rotating cup of 50-mm diameter, was employed because of their lower viscosity range. To prevent evaporation of water, the sample surface was covered with low-viscous liquid paraffin (VWR International, Darmstadt, Germany).

Patients' Sera. Sera were obtained from nine patients (5 male and 4 female) suffering from adverse reactions to raw mango. Most of them also reported symptoms after ingestion of fresh apples and carrots. Apart from anamnesis, allergy diagnosis was based on a positive skin prick test and the determination of specific serum IgE antibodies against mango by an enzyme allergosorbent test (EAST classes 1−4). A serum from one nonallergic person (male) served as negative control. The sera were collected at the University Hospital Eppendorf, Department of Dermatology and Allergy, Hamburg, Germany.

Mango Protein Extracts. From the fresh endocarp and the purees (*var 1np*−*var 5np*, *var1*−*var4*), extracts in the form of lyophilized acetone powders were prepared according to the low-temperature extraction method previously described by Vieths et al. (*26*), with slight modifications. A 2-g sample of the acetone powder was extracted with 30 mL of phosphate-buffered saline (PBS), consisting of 0.01 M potassium phosphate buffer (pH 7.4) and 0.13 M sodium chloride, for 60 min under stirring and cooling with ice. The suspension was centrifuged (10 500*g*, 60 min, 4 °C) and the supernatant was filtered. The clear solution was divided into aliquots of 2 mL, lyophilized (Beta 1−16, Christ, Osterode, Germany) and stored at −20 °C until use. From the trial nectars and the commercial samples, the protein extracts were obtained by extraction of the freeze-dried samples with PBS as described above, without the initial low-temperature extraction generating acetone powders. Total protein concentration of the extracts was determined by Bradford (*27*), with bovine serum albumin (BSA) as standard.

SDS-PAGE. For electrophoresis, the protein samples were diluted and reduced in β-mercaptoethanol buffer according to Vieths et al. (*28*) prior to heating at 90 °C for 3 min. SDS-PAGE was performed in 10% polyacrylamide gels, using the NuPAGE vertical electrophoresis system according to the manufacturer's recommendations (Novex, San Diego, CA). Electrophoresis was carried out for 40 min at a constant voltage of 200 V (EI-9001-Xcell II Mini Cell, Novex, San Diego, CA). For silver staining, the method of Heuckeshoven and Dernick (*29*) was applied.

Immunoblotting. Protein samples were separated by SDS-PAGE as described above and transferred on a nitrocellulose membrane (0.2 μm, Schleicher and Schüll, Dassel, Germany) by the method of Towbin and Gordon (*30*). After blotting, one strip with separated extract and one strip with molecular-mass-marker proteins were briefly stained with colloidal gold (Bio Rad, Herkules, CA) to visualize the transfer of proteins. Western blots were performed according to Vieths et al. (*26*), with slight modifications. To prevent unspecific binding, the dried membranes were treated twice with 5% skimmed milk powder and 0.1% Tween 20 in PBS. Subsequently nitrocellulose strips were incubated with pooled patients' sera diluted 1:14 with incubation buffer (0.3% BSA, 0.1% Tween 20 in PBS). The strips were then incubated with rabbit antihuman IgE (1:4000, 60 min; Dako GmbH, Hamburg, Germany), biotinylated goat antirabbit IgE (1:6000, 60 min; Dako GmbH, Hamburg, Germany), and streptavidin-horseradish peroxidase conjugate (1:20000; 20 min; Medac, Hamburg, Germany), respectively, and stained with 3,3′,5,5′-tetramethylbenzidine (TMB) and dioctylsodiumsulfosuccinate (DONS).

For immunoblot-inhibition experiments, a preincubation of the pooled sera with any sample extract as inhibitor was carried out for 1 h at ambient temperature, using 100 μL of the pool-serum and 200 μL of extract from either the native mango, a trial nectar, one of the commercial products or ovalbumin as inhibitors in a total volume of 1.5 mL incubation buffer (0.3% BSA, 0.1% Tween 20 in PBS). Subsequently, immunoblotting of the allergens from the native mango extract, which were fixed on the nitrocellulose membrane, was performed as described above for each of the preincubated sera containing dissolved inhibitor allergens.

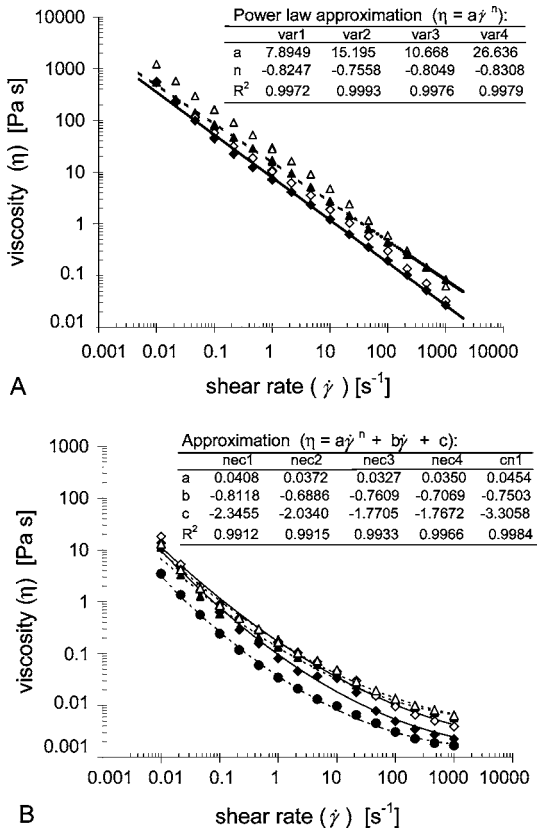

Figure 3. Effect of enzymatic pulp treatments on the flow behavior of (**A**) mango purees and (**B**) the derived mango nectars at 25 °C. Legend **A**: (◆), *var1*; (▲), *var2*; (◇), *var3*; (△), *var4*; — regression curve for *var1*, - - - regression curve for *var2*. Legend **B**: (◆), *nec1*; (▲), *nec2*; (◇), *nec3*; (△), *nec4*; ●, commercial nectar *cn1* (reference, 35% mango pulp content); — regression curves of samples produced by pulp incubation with enzymes (*nec*1*, nec3*); - - - regression curves of samples produced by pulp incubation without enzymes (*nec2, nec 4*); – • – regression curve for sample *cn1*.

EAST-Inhibition Assays. According to the method of Möller et al. (*11*), the extracts of the native mango fruit (cv. Tommy Atkins; protein concentration 236 μg/mL) were coupled to bromocyan-activated cellulose paper disks. Pooled patients' sera were diluted 1:2 in incubation buffer (0.3% BSA, 0.1% Tween 20, 0.05% NaN_3 in PBS). A 6-fold dilution series of the inhibitor extracts was prepared in the same buffer. The EAST was performed in 96-well microtiter plates (Nunc, Roskilde, Denmark). The wells were coated with 50 μL of inhibitor extracts and 50 μL of the pooled sera and incubated overnight at ambient temperature. The plates were washed thrice with 1% Tween 20 in PBS. Finally 50 μL of anti-human IgE alkaline phosphatase conjugate (1:2 in incubation buffer) was added into the cavities of the microtiter plate, with subsequent incubation overnight. Bound IgE antibodies were detected by color reaction with *p*-nitrophenyl phosphate (PNPP) for 60 min at 37 °C, using an alkaline phosphatase staining kit (Allergopharma, Reinbek, Germany). Absorbance was measured at 405 nm.

RESULTS

Effect of Enzymatic Pulp Degradation. Irrespective of the process technology, all mango purees displayed the typical flow behavior of a shear-thinning fluid (**Figure 3A**). A 10^4-fold

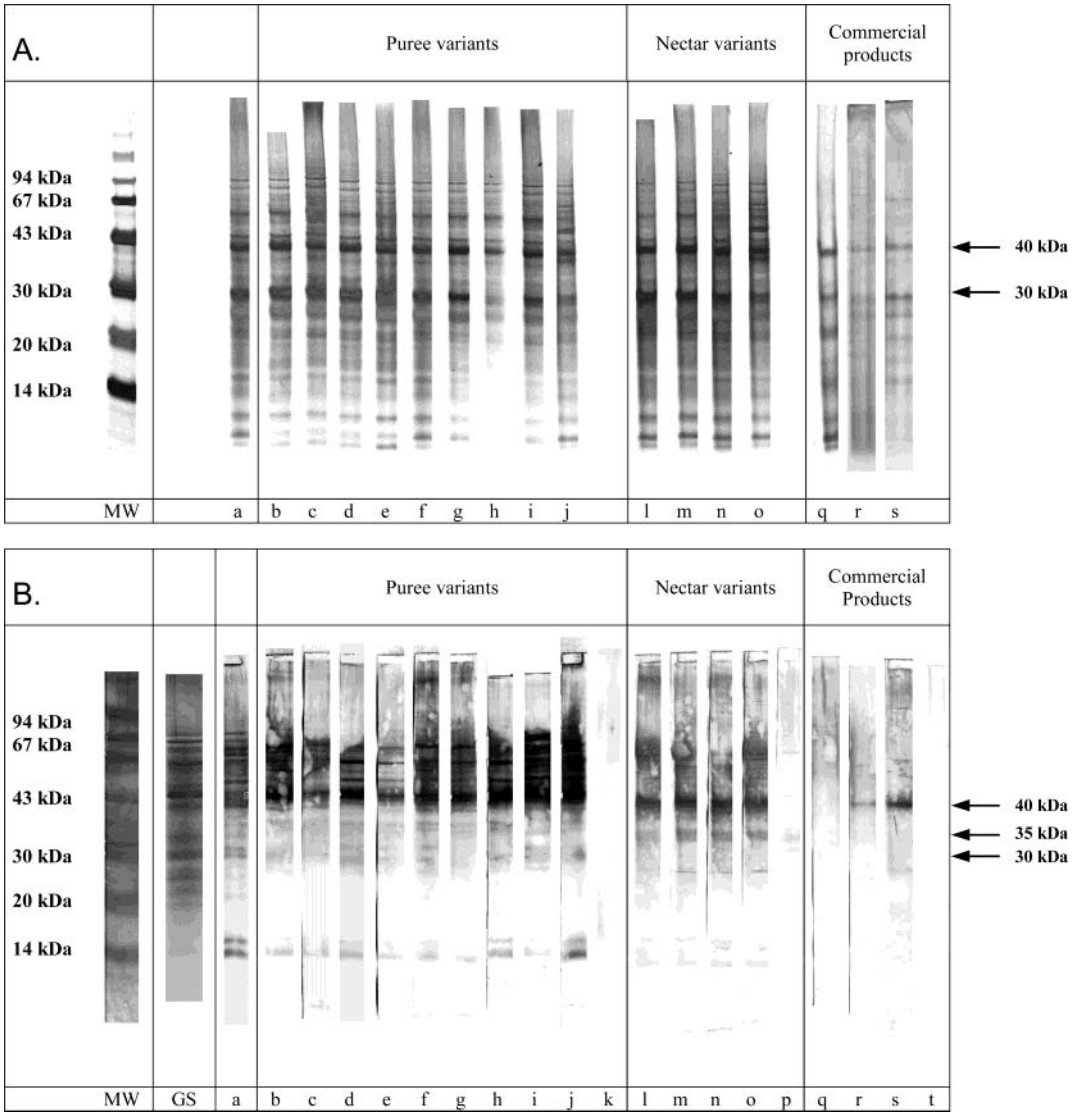

Figure 4. SDS-PAGE (**A**) and Immunoblot (**B**) of the trial mango purees, the derived nectars thereof, and commercial products. Lanes (protein concentration of the extracts in parentheses): MW, molecular mass marker; GS, native mango extract (gold-stained); a, native mango extract (436 mg/L). Puree intermediates: b, *var 1np* (327 mg/L); c, *var 2np* (311 mg/L); d, *var 3np* (290 mg/L); e, *var 4np* (207 mg/L); f, *var 5np* (325 mg/L). Pasteurized purees: g, *var1* (275 mg/L); h, *var2* (291 mg/L); i, *var3* (293 mg/L); j, *var4* (227 mg/L); k, *var3* with control serum. Nectar samples: l, *nec1* (316 mg/L); m, *nec2* (296 mg/L); n, *nec3* (344 mg/L); o, *nec4* (287 mg/L); p, *nec3* with control serum. Commercial products: q, *cn1* (128 mg/L); r, *cn2* (158 mg/L); s, *cn3* (111 mg/L); t, *cn3* with control serum.

decrease in viscosity was observed when the shear rate rose from 0.01 to 1000 s^{-1}, and the relation between viscosity (η) and shear rate ($\dot{\gamma}$) could be described by power laws $\eta = a\dot{\gamma}^n$. Compared to the control (puree variant *var2*: preheated pulp, incubated at 45 °C for 30 min without addition of enzyme), the enzymatic pectin degradation at 45 °C for 30 min of the variants *var1* (100 ppm enzyme mixture) and *var3* (50 ppm enzyme mixture) resulted in 56 and 32% reduction of viscosity, respectively, at a shear rate of 100 s^{-1}. To estimate the average viscosity reduction for the whole considered shear rate range, the reduction of the power law coefficients *a* were calculated. Thus, average viscosity of the purees *var1* and *var3* was reduced by 48% and 30%, respectively.

Enzymatic pulp degradation also significantly contributed to viscosity reduction of the respective nectars, which still showed shear-thinning behavior (**Figure 3B**). However, the nectar *nec1*, which resulted from pulp incubated at maximum enzyme dosage (*var1*), still displayed markedly higher viscosities at each shear rate than did the commercial nectar *cn1*, despite their identical pulp content (35%). Nevertheless, the trial nectars and the commercial sample were quite analogous in their shear-thinning flow behavior.

SDS-PAGE. In the extracts of native mango (lane a), the puree intermediates (lanes b−f) and the pasteurized purees (lanes g−j), at least 14 protein bands with molecular masses ranging from 14 kDa to 94 kDa were detected (**Figure 4A**). All lanes showed three additional protein bands below 14 kDa. Those of greater intensity corresponded to molecular masses of 30 kDa and 40 kDa, known as the major allergens (*31*). The protein patterns of the native mango extract and those of the puree variants were quite similar.

Additionally, the protein patterns of the mango nectar extracts are shown in **Figure 4A**. As described for the puree extracts, corresponding SDS-PAGE behavior of the native mango extract (lane a) and mango nectar extracts (lanes l−o) was found. Moreover, protein bands of the native mango extract (lane a) were similar to those of extracts from the commercial mango

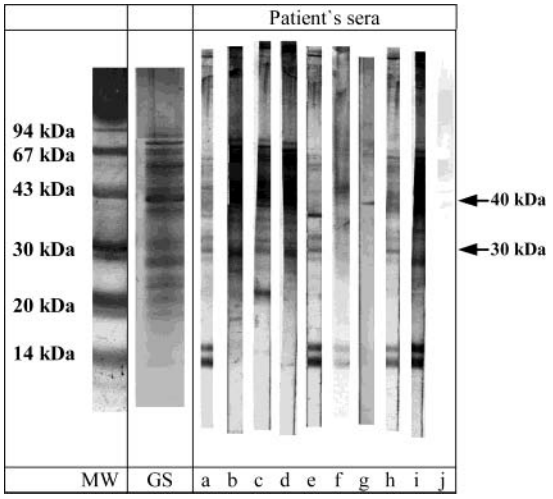

Figure 5. Immunoblot of the patients' sera. Lanes: MW, molecular mass marker; GS, native mango extract; a, RD; b, KDH; c, SK; d, FR; e, LS; f, DS; g, GS; h, SW; i, UW; j, control serum.

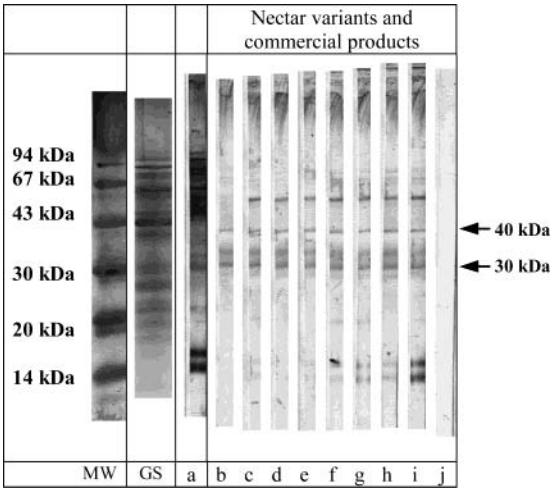

Figure 6. Immunoblot inhibition of the trial mango nectars and the commercial products. Lanes (protein concentration of the extracts in parentheses): MG, molecular mass marker; GS, native mango extract (gold-stained); a, native mango extract (no inhibitor; 436 mg/L). Fixed allergens from the native mango extract with inhibitors from b, native mango extract (237 mg/L); c, *nec1* (222 mg/L); d, *nec2* (146 mg/L); e, *nec3* (229 mg/L); f, *nec4* (175 mg/L); g, *cn1* (100 mg/L); h, *cn2* (154 mg/L); i, *cn3* (85 mg/L); j, control.

nectar (*cn1*, lane q) and the imported nectar-like mango drinks (*cn2* and *cn3*; lanes r−s).

Immunoblotting. Among the 9 sera from patients with mango allergy that were tested, 7 had IgE antibodies capable of binding to the major allergen with a molecular mass of 30 kDa (**Figure 5**, lanes a−i). IgE antibodies of 6 sera bound to the 40 kDa major allergen. Specific binding was also observed for allergens with molecular masses of 67, 60, 43, 37, 20, 18, 16, 14 kDa, and above 67 kDa.

A pool-serum of the 9 sera described above was employed to investigate IgE-reactivity to extracts of the mango purees and nectars as well as the commercial products. In all strains, the strongest binding was observed at 40 kDa (**Figure 4B**). In the extracts of the native mango (lane a) and the puree intermediates (lanes b−f), the IgE binding band with a molecular mass of 30 kDa was merely detectable, whereas all strains of extracts from the pasteurized purees (lanes g−j) were devoid of the band representing the molecular mass of 30 kDa. No binding was detected when the control serum was applied instead of the pool-serum (lane k, exemplarily for *var3*, control strains of other puree extracts not shown).

Immunoblot studies on the mango nectar variants (**Figure 4B**, lanes l−o) revealed that specific IgE was very strongly bound to the 40 kDa allergen. Additionally, an allergen with a molecular mass of 35 kDa was detected in the nectar extracts. On the other hand, the mango-specific protein at 30 kDa was not identified by adding the pool-serum to the nectar extracts. The band representing the 40 kDa major allergen, which was characteristic of the native mango extract, was visible in the immunoblots of the extracts of two commercial products (*cn2* and *cn3*, lanes r−s in **Figure 4B**). It should be noted that allergens were not detectable in the commercial nectar sample *cn1* (lane q). As expected, no binding was observed with the control serum instead of the pool-serum (lane p and t, exemplarily for *nec3* and *cn1*, respectively; control strains of other nectar and commercial extracts not shown).

To verify the results of immunoblot investigations, immunoblot-inhibition experiments were performed. Preincubation of pool-serum with the extracts of native mango, commercial products, and nectar variants, respectively, acting as inhibitors, resulted in a significant reduction of IgE binding to fixed allergens of the native mango with molecular masses of 14, 16, 20, 30, 40, 55, and 67 kDa (**Figure 6**). The great number of inhibited protein bands indicated the residual allergenic potency of the mango nectars and the commercial products under investigation.

EAST-Inhibition. The aim of this assay was to determine semiquantitatively the allergenic potency of the mango products. Allergen disks prepared from the native mango fruit (cv., Tommy Atkins) were incubated with pool-serum, while the puree extracts were used as inhibitors. The resulting inhibition graphs are presented in **Figure 7A**. The principal run of the curves was nearly identical for all puree variants. Irrespective of originating from an intermediate or a final product, mango puree extracts caused an almost complete inhibition of IgE binding to mango allergen disks.

Similar inhibition was achieved with the extracts from the trial mango nectars and the commercial mango products, respectively (**Figure 7B**). Even though the nectar variants (*nec1*−*nec4*) were inhibited by 50% at concentrations (C_{50}-values), which were 3−5 times higher than the C_{50}-value of the extract from the native mango (4.92 mg/L), these results still reflected high allergenic activities. This increase in the C_{50}-values is in agreement with the 2.86-fold dilution of pulp during the production of nectars with 35% pulp content.

DISCUSSION

Initially, the mango allergens were characterized by means of SDS-PAGE-immunoblot, using sera of 9 patients with manifest sensibilization to mango. Two allergens displaying molecular masses of 40 and 30 kDa were detected by 67 and 78% of the assigned sera, respectively. These allergens were previously characterized as the major allergens *Man i 1* and *Man i 2* by Paschke et al. (*31*).

The influence of technological processing on the allergenic potency of mango was examined on two levels. First, various purees were produced from the fresh fruit. Here, focus was on the enzymatic treatment of the pulp and on heating steps associated with peeling, thermal inactivation of native enzymes detrimental to product quality, and puree pasteurization. Second, mango nectars were manufactured by dilution of the purees with

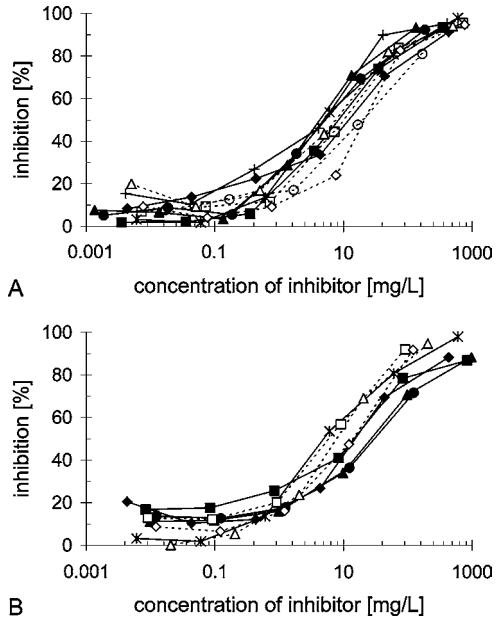

Figure 7. EAST-inhibition of IgE-binding to mango allergen disks by (**A**) mango puree extracts and (**B**) extracts from mango nectar and commercial nectar-like products. Results are expressed in percent inhibition. Native mango extract was used as positive control. Legend (C_{50}-values in parentheses): (∗), native endocarp (4.92 mg/L); puree intermediates (**A**) (■), *var 1np* (8.41 mg/L), (▲), *var 2np* (4.28 mg/L), (◆), *var 3np* (12.09 mg/L), (●), *var 4np* (5.25 mg/L), (+), *var 5np* (4.30 mg/L); pasteurized purees (**A**) (□), *var1* (10.01 mg/L), (△), *var2* (7.47 mg/L), (◇), *var3* (21.27 mg/L), (○), *var4* (19.77 mg/L); nectar samples (**B**) (■), *nec1* (14.58 mg/L); (▲), *nec2* (26.77 mg/L); (◆), *nec3* (25.27 mg/L); (●), *nec4* (30.31 mg/L); commercial products (**B**) (□), *cn1* (5.98 mg/L); (△), *cn2* (7.47 mg/L); (◇), *cn3* (13.86 mg/L).

water and sucrose solution. Additional heating was applied during final pasteurization of the nectar. Including the blanching of the fruit during hot water peeling, the mango tissue was thus subjected to four heating steps at 87−96 °C during processing of the fruit into nectar. After cold filling of the nectar, pasteurization at 87 °C of the bottled product in hot water required a 15-min holding time to ensure sufficient product stability. Owing to the small batch size in experimental nectar production, milder treatments such as much shorter continuous pasteurization combined with aseptic or hot filling, usual in industrial practice, were not applicable. Moreover, the mango tissue was exposed to severe mechanical stress during pulping of the fruit and fine-milling of the pulp. Also, vacuum-degassing of the nectars resulted in additional tissue disintegration. The impact of shear stress caused by mechanical operations may implicate surface denaturation or aggregations of proteins (*32*). On the other hand, allergenic proteins may be liberated by disintegration of cell compartments and subjected to inactivating reactions, e.g. with genuine enzymes or tannins. In a commercial mango puree concentrate, phenolic acids, mangiferin, flavonol glycosides and a gallotannin consisting of glucose and four gallic acid units were identified (*33*). However, the total amount of polyphenols in the pulp was reported to be much lower than that in mango peel (*33*), and knowledge on the polyphenol pattern in mango peel (*34*) and pulp is still lacking to judge the significance of the tanning effects of mango polyphenols. Nevertheless, numerous process parameters may influence the allergenic potency.

Concerning puree production, enzymatic treatments of mashed endocarp after destoning of the fruit and prior to pulping was recommended by Johann (*35*), to increase the puree yield and to facilitate puree concentration owing to reduced viscosity. In the experimental production of mango puree, enzymation was applied after pulping to reduce product viscosity, improving sensory properties. Initially, incubation time, temperature, and enzyme dosage were optimized with respect to viscosity reduction. The use of a statistical experimental design elucidated the complex interactions among the incubation parameters and enabled the purposive production of the reported puree variants for the allergological investigation. Thus, these variants (*var1*−*var4*) demonstrated the wide range of potential puree viscosities $\eta(\dot{\gamma})$ from this raw material, reflecting different degrees of enzymatic tissue degradation. In the purees *var1* and *var3*, genuine pectin was more or less degraded. On the other hand, the different viscosities of samples *var2* and *var4*, incubated without addition of enzymes for 30 and 90 min, respectively, may suggest that pectin molecules and tissue particles swelled, or that pectin initially bound to the tissue was partly dissolved during long pulp incubation, enhancing final product viscosity especially of sample *var4* (**Figure 3A** in accordance with the results of the preliminary technological optimization of mango pulp enzymation). The negatively charged pectin molecules may act as protecting colloids by forming hydrate hulls around tissue particles, and more specifically, by ionic interactions with proteins, which carry positive surface charges in the acid foodstuff (*36*).

In the immunoblot, the extracts of the differently produced intermediates *var 1np*−*var 5np*, especially that of the unheated pulp (*var 5np*), showed an allergen pattern which was analogous to that of the native fruit, where the major allergen with a molecular mass of 40 kDa as well as the low-weight proteins with 14 and 16 kDa were easily detected. Irrespective of the varying process parameters, the allergen activity determined by EAST-inhibition remained nearly unchanged. Neither the total enzyme dosage nor incubation time or temperature affected the allergenic potency during the enzymatic treatment (*var 1np*−*5np*). Conversely, enzymation could induce structural changes of food proteins (*32*), which may be ascribed to minor protease activities of technical mash enzyme preparations or to protein modifications affected by texturization with transglutaminase (*37*). The assigned enzyme preparations were likewise examined for their allergenic potency by means of EAST-inhibition, using them as inhibitors, which competed with the fixed allergens of the native mango extract for the pool-serum. Applying the extract of native mango as inhibitor in the positive control of this experiment, none of the enzyme preparations showed an allergenic activity (data not shown).

Due to structural changes caused by heat treatments, thermally unstable allergens frequently lose their allergenic activity (*38*). Such IgE-binding proteins often possess conformational epitopes, which are destroyed by the deconvolution of the peptid chains and breaking of the disulfide bonds. Some food allergens, such as those in kiwi fruit (*39*), are known to have conformational epitopes. Allergens with sequence epitopes are not affected by thermal treatments (*40*). Thermally stable allergens mainly comprise animal proteins (e.g., in egg and milk) but they are also found in peanut (*41*) and soy (*42*). Even at the end of the puree production process, pasteurization at 90 °C for 1 min (*var1*−*var4*) prior to hot filling did not reduce mango allergen activity. Comparing the immunoblots, none of the pasteurized variants presented the band at 30 kDa. Nevertheless, the allergen with a molecular mass of 40 kDa as well as the low-weight proteins with 14 and 16 kDa were clearly visualized. As indicated by EAST-inhibition experiments, the allergenic potency of all pasteurized purees was maintained. The C_{50}-values

of the pasteurized purees *var1* to v*ar3* only increased 1.2 to 1.8-fold, when compared with the respective nonpasteurized variants. Only the C_{50}-value of the pasteurized puree *var4* rose by the factor 3.8. Nevertheless, the concentration at maximum inhibition was quite similar among all four extracts and analogous to that of the native fruit.

From these results, it can be deduced that the activity of the mango allergens is hardly affected by the different process parameters during puree processing. According to Gall et al. (*39*), heating of kiwi fruit at 40 °C up to 90 °C resulted in reduction of allergenic potency with increasing temperature. Although Helbing et al. (*43*) gave evidence of various proteins in extracts from carrots cooked at 100 °C for 10 min, only the major allergen could be detected in the immunoblot. Also Gomez et al. (*44*) and Quirce et al. (*45*) observed significant reduction of the allergenic potency of carrots caused by heating. By analogy, heating of apples for 30 min at 100 and 175 °C resulted in dismantling of the IgE-binding proteins (*46*, *47*). In contrast, high thermal stability of peanut allergens has been reported (*41*).

Nectar production from the pasteurized purees required dilution of all mango compounds and additional pasteurization. Despite these treatments, the 40 kDa major allergen was intensively visualized. Although the 30 kDa allergen was not detectable, EAST-inhibition experiments only revealed minor reduction of allergenic activity. This was possibly due to the observed binding to a 35 kDa allergen, which was described as a minor allergen in birch pollen, cross-reactive with proteins of comparable molecular size from mango, banana and apple (18). Thus, the stability of mango allergens during technological processing was evident. Irrespective of heating, mechanical or enzymatic tissue disintegration, allergenicity of mango puree and nectar was maintained. Brenna et al. (*17*) found pronounced thermal stability of peach allergens during juice processing of the respective fruits, even during heating at 121 °C for 10 and 30 min. The production of a hypoallergenic, clear peach juice was only achieved after final ultrafiltration, but not by variation of the usual process parameters. However, typical characteristics of peach juice products, such as cloudiness and mouth-feeling were thereby lost. Because studies considering the effect of mango processing on the allergenicity of the products are lacking, the results gained by experimental mango processing were verified by investigating three different commercial products. Particularly, potential effects of down-scaling of mango processing could be excluded. All commercial samples displayed residual allergenicity in the EAST-inhibition experiment, although allergenic proteins of one commercial nectar (*cn1*) were obviously not detectable by immunoblotting using the pool-serum. In contrast, the 40 kDa allergen was evident in the extracts of the other commercial samples (*cn2* and *cn3*). However, using single sera (EAST-classes 2 to 4) instead of the pooled sera, evidence of residual allergenic potency was provided for all three commercial products (results not presented). Moreover, results of immunoblot-inhibition experiments approved residual allergenic potency of all trial mango nectars, commercial mango nectars or nectar-like mango beverages under study. As confirmed by the EAST-inhibition experiments, the residual allergenic potency of the commercial products was comparable to that of the trial nectars. Therefore, conventional mango processing into pulp-containing products typical for this species obviously does not allow complete elimination of the allergenic potency.

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LITERATURE CITED

(1) André, F.; André, C.; Colin, L.; Cacaraci, F.; Cavagna, S. Role of new allergens and of allergen consumption in the increased incidence of food sensitizations in France. *Toxicology* **1994**, *93*, 77−83.

(2) Moneret-Vautrin, D. A.; Kanny, G. Food-induced anaphylaxis. A new french multicenter survey. *Ann. Gastroenterol. Hepatol.* **1995**, *31*, 256−263.

(3) Castillo, R.; Delgado, J.; Quiralte, J.; Blanco, C.; Carilli, T. Food hypersensitivity among adult patients: epidemiological and clinical aspects. *Allergol. Immunopathol*ogy **1996**, *24*, 93−97.

(4) Chandra, R. K. Food hypersensitivity and allergic disease. *Am. J. Clin. Nutr.* **1997**, *66*, 526S−529S.

(5) Hill, D. J.; Hosking, C. S.; Zhie, C. Y.; Leung, R.; Baratwidjaja, K.; Iikura, Y. The frequency of food allergy in Australia and Asia. *Environ. Toxicol. Pharmacol.* **1997**, *4*, 101−110.

(6) Sampson, H. A. Food allergy. *J. Am. Med. Assoc.* **1997**, *278*, 1888−1894.

(7) Vieths, S.; Janck, K.; Aulepp, H.; Petersen, A. Isolation and characterization of the 18 kDa major apple allergen and comparison with the major birch pollen allergen (Bet v 1). *Allergy* **1995**, *50*, 421−430.

(8) Hsieh, L. S.; Moos, M.; Lin, Y. Characterization of apple 18 and 31 kDa allergens by microsequencing and evaluation of their content during storage and ripening. *J. Allergy Clin. Immunol.* **1995**, *96*, 960−970.

(9) Breiteneder, H.; Hoffmann-Sommergruber, K.; O'Riordain, G.; Susani, M.; Ahorn, H.; Ebner, C.; Kraft, D.; Scheiner, O. Molecular characterization of Api g 1, the major allergen of celery (*Apium graveolens*), and its immunological and structural relationships to a group of 17 kDa tree pollen allergens. *Eur. J. Biochem.* **1995**, *233*, 484−489.

(10) Hoffmann-Sommergruber, K.; O'Riordain, G.; Ahorn, H.; Ebner, C.; Laima Da Camara Machado, M.; Pühringer, H.; Scheiner, O.; Breiteneder, H. Molecular characterization of Dau c 1, the Bet v 1 homologous protein from carrot and its cross reactivity with Bet v 1 and Api g 1. *Clin. Exp. Allergy* **1999**, *29*, 840−847.

(11) Möller, M.; Paschke, A.; Vieluf, D.; Kayma, M.; Vieths, S.; Steinhart, H. Characterization of allergens in kiwi fruit and detection of cross-reactivity with allergens of birch pollen and related fruit allergens. *Food Agric. Immunol.* **1997**, *9*, 107−121.

(12) Pastorello, E. A.; Conti, A.; Pravettoni, V.; Farioli, L.; Rivolta, F.; Ansaloni, R.; Ispano, M.; Incorvaia, C.; Giuffrida, M. G.; Ortolani, C. Identification of actinidin as the major allergen of kiwi fruit. *J. Allergy Clin. Immunol.* **1998**, *101*, 531−537.

(13) Jankiewicz, A.; Baltes, W.; Bögl, K. W.; Dehne, L. I.; Jamin, A.; Hoffmann, A.; Haustein, D.; Vieths, S. Influence of food processing on the immunochemical stability of celery allergens. *J. Sci. Food Agric.* **1997**, *75*, 359−370.

(14) Lüttkopf, D.; Ballmer-Weber, B. K.; Wüthrich, B.; Vieths, S. Celery allergens in patients with positive double-blind placebo-controlled food challenge. *J. Allergy Clin. Immunol.* **2000**, *106*, 390−399.

(15) Koppelmann, S. J.; Bruijnzeel-Koomen, C. A.; Hessing, M.; De Jongh, H. H. Heat induced conformational changes of Ara h 1, a major peanut allergen, do not affect its allergenic properties. *J. Biol. Chem.* **1999**, *274*, 4770−4777.

(16) Hayakawa, K.; Linko, Y. Y.; Linko, P. Mechanism and control of food allergy. *Lebensm. Wiss. Technol.* **1999**, *32*, 1−11.

(17) Brenna, O.; Pompei, C.; Ortolani, C.; Pravettoni, V.; Farioli, L.; Pastorello, E. Technological processes to decrease the allergenicity of peach juice and nectar. *J. Agric. Food Chem.* **2000**, *48*, 493−497.

(18) Wellhausen, A.; Schöning, B.; Petersen, A.; Vieths, S. IgE-binding to a new cross-reactive structure: a 35 kDa protein in birch pollen, exotic fruit, and other plant foods. *Zeitschr. Ernährungswiss.* **1996**, *35*, 348−355.
(19) Paschke, A.; Kinder, H.; Zunker, K.; Wigotzki, M.; Wessbecher, R.; Vieluf, I.; Steinhart, H. Characterization of cross-reacting allergens in mango fruit. *Allergy* **2001**, *56*, 237−242.
(20) Food and Agriculture Organization of the United Nations (FAO). FAOSTAT-Agriculture. In *Statistical Databases of the Food and Agriculture Organization of the United Nations*, Internet-edition, FAO, Economic and Social Department, Statistics Division: Rome, Italy, 2003, http://www.fao.org.
(21) Ramteke, R. S.; Vijayalakshmi, M. R.; Eipeson, W. E. Processing and value addition to mangoes. *Indian Food Ind.* **1999**, *18*, 155−163.
(22) Pott, I.; Marx, M.; Neidhart, S.; Mühlbauer, W.; Carle, R. Quantitative determination of β-carotene stereoisomers in fresh, dried, and solar-dried mangoes (*Mangifera indica* L.). *J. Agric. Food Chem.* **2003**, *51*, 4527−4531.
(23) Singh, N. G. I.; Dhuique-Mayer, C.; Lozano, Y. Physicochemical changes during enzymatic liquefaction of mango pulp. *J. Food Process. Preserv.* **2000**, *24*, 73−85.
(24) Janser, E. Enzyme applications for tropical fruits and citrus. *Fruit Process.* **1997**, *7*, 388−393.
(25) Methods of analysis. International Federation of Fruit Juice Producers (IFU), Loose-leaf book, Swiss Fruit Association, Zug, Switzerland.
(26) Vieths, S.; Schöning, B.; Brockmann, S.; Aulepp, H. Untersuchungen zur Allergie gegen Lebensmittel pflanzlicher Herkunft: Herstellung und Charakterisierung von Obst- und Gemüseextrakten für serologische Untersuchungen. *Dtsch. Lebensm. Rundsch.* **1992**, *88*, 239−243, 273−278.
(27) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye-binding. *Anal. Biochem.* **1976**, *72*, 248−254.
(28) Vieths, S.; Brockmann, S.; Schöning, B. Nahrungsmittelallergie gegen Obst und Gemüse: Eine aktuelle serologische Untersuchung zur Sensibilisierung von Pollenallergikern. *Allergologie* **1992**, *15*, 367−379.
(29) Heukeshoven, J.; Dernick, R. Neue Ergebnisse zum Mechanismus der Silberfärbung. In *Elektrophoreseforum*; Radola, B. J., Ed.; TU München, Germany, 1986; pp 22−87.
(30) Towbin, H.; Gordon, J. Immunoblotting and dot blotting: current status and outlook. *J. Immunol. M.* **1984**, *72*, 313−340.
(31) Paschke, A.; Kinder, H.; Zunker, K.; Wigotzki, M.; Wessbecher, R.; Vieluf, I.; Steinhart, H. Characterization of allergens in mango fruit and ripening dependence on the allergenic potency. *Food Agric. Immunol.* **2001**, *13*, 51−61.
(32) Vieths, S.; Fischer, K.; Dehne, L. I.; Bögl, K. W. Allergenes Potential von verarbeiteten Lebensmitteln. *Ernaehr. Umsch.* **1994**, *41*, 140−143.
(33) Schieber, A.; Ullrich, W.; Carle, R. Characterization of polyphenols in mango puree concentrate by HPLC with diode array and mass spectrometric detection. *Inn. Food Sci. Emerg. Technol.* **2000**, *1*, 161−166.
(34) Schieber, A.; Berardini, N.; Carle, R. Identification of flavonol and xanthone glycosides from mango (*Mangifera indica* L. cv. "Tommy Atkins") peels by high-performance liquid chromatography-electrospray ionization mass spectrometry. *J. Agric. Food Chem.* **2003**, *51*, 5006−5011.
(35) Johann, P. Besonderheiten bei der Verarbeitung von tropischen Früchten. *Flüss. Obst.* **1987**, *54*, 430−434.
(36) Mensah-Wilson, M.; Reiter, M.; Bail, R.; Neidhart, S.; Carle, R. Cloud stabilizing potential of pectin on pulp-containing fruit beverages. *Fruit Process.* **2000**, *10*, 47−54.
(37) Motoki, M.; Kumazawa, Y. Recent research trends in transglutaminase technology for food processing. *Food Sci. Technol. Res.* **2000**, *6*, 151−160.
(38) Besler, M.; Steinhart, H.; Paschke, A. Stability of food allergens and allergenicity of processed foods. *J. Chromatogr. B.* **2001**, *756*, 207−228.
(39) Gall, H.; Kalveram, K. J.; Forck, G.; Sterry, W. Kiwi: a new birch pollen-associated food allergen. *Dermatol. Monatsschr.* **1993**, *179*, 99−104.
(40) Kreft, D.; Bauer, R.; Görlich, R. Nahrungsmittelallergene-Charakteristika und Wirkungsweise. de Gruyter: Berlin, New York, 1995; p 269.
(41) Burks, A. W.; Williams, L. W.; Thresher, W. Allergenicity of peanut and soybean extracts altered by chemical or thermal denaturation in patients with atopic dermatitis and positive food challenge. *J. Allergy Clin. Immunol.* **1992**, *90*, 889−897.
(42) Taylor, S. L.; Lemanske, R. F.; Bush, R. K.; Busse, W. W. Food allergens: structure and immunologic properties. *Ann. Allergy* **1987**, *59*, 93−99.
(43) Helbing, A.; López, M.; Schwarz, H. J.; Lehrer, S. B. Reactivity of carrot-specific IgE antibodies with celery, apiaceous spices, and birch pollen. *Ann. Allergy* **1993**, *70*, 495−499.
(44) Gomez, M.; Curiel, G.; Mendez, J.; Rodriguez, M.; Moneo, I. Hypersensitivity to carrot associated with specific IgE to grass and tree pollens. *Allergy* **1996**, *51*, 425−429.
(45) Quirce, S.; Blanco, R.; Diez-Gomez, M. L.; Cuevas, M.; Eiras, P.; Losada, E. Carrot-induced asthma: immunodetection of allergens. *J. Allergy Clin. Immunol.* **1997**, *99*, 718−719.
(46) Drebourg, S.; Foucard, T. Allergy to apple, carrot, and potato in children with birch pollen allergy. *Allergy* **1983**, *38*, 167−172.
(47) Vieths, S.; Hoffmann, A.; Holzhauser, T.; Müller, U.; Reindl, J.; Haustein, D. Factors influencing the quality of food extracts for in vitro and in vivo diagnosis. *Allergy* **1998**, *53*, 65−71.

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