Original:

Investigation of IgE-Binding Patterns and Allergenicity of Mango Fruit Allergens in Different Varieties

Heiko KINDER, Beate SCHARF, Hans STEINHART, Angelika PASCHKE

Institute of Biochemistry and Food Chemistry, University of Hamburg, Germany

SUMMARY

Mango has become one of the most popular tropical fruits. It is cultivated in more than 1000 strains. In the present study the four most frequently consumed varieties (Eden, Ngowe, Osteen and Tommy Atkins) cultivated in different continents were examined. In order to identify IgE binding allergens and determine mutual cross-reactivities of the varieties immunoblot and enzymeallergosorbent test (EAST) inhibition were performed. Seven sera from patients with sensitization to mango fruit were used. All four varieties shared at least five allergens with Mr of approximately 14, 30, 40, 43 and 67 kDa. Results of EAST inhibition elucidated almost the same inhibition curves and 50%-inhibition values for each variety. In conclusion it could be shown that there were no significant differences in allergenic potency among the four mango strains.
(Internet Symposium on Food Allergens 1999, 1(2):43-49)

[Introduction] [Material&Methods] [Results] [Discussion] [References] [Abbreveations]

Correspondence:
Heiko Kinder
Institute of Biochemistry and Food Chemistry
University of Hamburg
Grindelallee 117, D-20146 Hamburg, Germany

Phone: +49-40-42838-4268
Fax: +49-40-42838-4342
E-mail: kinder@lc.chemie.uni-hamburg.de

Heiko Kinder

INTRODUCTION

The spectrum of the food on offer is extending because of the globalisation of world trade. This also applies to tropical fruits, so the consumer is increasingly into contact with them. The annual world production of 14.7 Mio tons makes mango *(Mangifera indica)* one of the most popular tropical fruits. World-wide it is cultivated in approximately 1000 strains. In Europe just a few strains are sold, because most of them taste petroleum-like and are not accepted for that reason. Tommy Atkins, which is cultivated particularly in South America, is the variety most frequently sold. Other well known strains are Osteen (Spain), Eden (Israel) and Ngowe (Kenya) (Franke 1997).
Allergic reactions to mango have become increasingly important. Sensitizations to mango pollen (Vargas Correa et al. 1991), seed (Fernandez et al. 1995) and fruit were described, respectively. In a French study it could be proved that approx. 6 % of the examined persons with clinically manifest food allergy showed a sensitization to mango (Andre et al. 1994). Moreover ingestion of mango fruit caused anaphylactic reactions in several cases (Dang & Bell 1967, Wüthrich & Hofer 1984, Miell et al. 1988, Jansen et al. 1992). Mango fruit allergens are reported to cross-react with latex allergens (Brehler et al. 1997), mugworth allergens (Wüthrich & Hofer 1984), peanut allergens (Fernandez et al. 1995), and birch pollen allergens (Wellhausen et al. 1996).

In some fruits a variety dependence of their allergenic potency is described. For example, apple-allergic patients frequently report that some apple strains usually are highly allergenic (i.e. Granny Smith, Golden Delicious) whereas others (i.e. Jamba, Gloster, Boskop) are tolerated without or with moderate symptoms (Vieths et al. 1993, 1994). With regard to the described phenomena, the aim of the present investigation was to study differences in the IgE-binding patterns and in the allergenicity of fruit allergens in four different mango varieties.

MATERIAL & METHODS

Patients' sera
Seven sera were obtained from patients with RAST classes > 3 for specific IgE to mango by CAP-RAST (Pharmacia, Uppsala, Sweden). All patients were adults (three female and four male), aged between 19 and 59 years. The sera were collected at the University Hospital Eppendorf, Department of Dermatology and Allergy, Hamburg, Germany. The pooled serum contained equal aliquots of the sera.

Mango Extracts
The mango varieties were purchased from Fa. Inter, Großmarkt Hamburg, Germany. All fruits were in a consumable condition and harvested five days before preparing the allergen extracts. They were prepared by a low-temperature extraction method, as described previously but with some minor modifications (Möller et al., 1997a, 1997 b). They were freeze-dried and stored at –20 °C until use. The protein concentration was determined using a Bradford (1976) assay.

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

SDS-PAGE
Discontinuous SDS-PAGE was performed according to the method of Laemmli (1970) with a 5 % (w/v) acrylamide stacking gel and a 13 % (w/v) acrylamide resolving gel. Protein samples were reduced with beta-mercaptoethanol (sample buffer according to Vieths et al. 1992) and loaded onto the gel at a concentration of approx. 10 µg/cm. The gels (250 x 120 x 0.5 mm) were developed in a horizontal slab gel apparatus (Desaphor HF, Desaga, Heidelberg, Germany). Gels were silver stained according to Morissey (1981).

Immunoblotting
For immunoblotting approximately 10 µg protein/cm were loaded onto the gels. Protein transfer was carried out as described previously (Möller et al. 1997a, 1997b). Immunostaining was performed according to Vieths et al. (1992) with minor modifications. The dried nitrocellulose membranes were blocked twice (15 min) with blocking buffer consisting of 5 % skimmed milk powder, and 0.1 % Tween 20 in phosphate buffered saline [PBS (0.01 M potassium phosphate buffer, pH 7.4,

0.13 M sodium chloride)]. Nitrocellulose strips were incubated overnight with 100 µL of patients sera in 1400 µL incubation buffer [0.3 % bovine serum albumin (BSA), 0.1 % Tween 20 in PBS]. After incubation with rabbit anti-human IgE (1:4000, 60 min), biotinylated goat anti-rabbit IgG (1:6000, 60 min; both reagents from Dako, Copenhagen, Denmark) and streptavidin-horseradish peroxidase conjugate (1:20000, 20 min, Medac, Hamburg, Germany), the blots were stained with 3,3',5,5'-tetramethylbenzidine-dioctylsodiumsulphosuccinate (TMB).

One strip was incubated with serum from a non allergic patient to check non-specific binding. To check the efficiency of protein transfer, one strip with separated extract and one strip with low molecular weight marker proteins (Pharmacia) were stained with colloidal gold (Bio Rad).

Immunoblot Inhibition

A total of 100 µL of the pooled serum was incubated with 100 µg of protein of the protein extracts in a total volume of 1.5 mL of incubation buffer for 60 min at room temperature. Afterwards, the preincubated solution was immunostained as previously described (Möller et al. 1997a, 1997 b).

Enzymeallergosorbent test (EAST) Inhibition

Protein extracts were diluted to total protein concentration of 10 mg/L using a sodiumcarbonate-buffer (50 mM $NaCO_3$, pH 9.6). 300 µL were added into each cavity of a Maxisorb-Mikrotiterplate (Nunc, Roskilde, Denmark) and coupled over night at room temperature. Free binding sites were blocked with 5 % skimmed milk powder and 0.1 % Tween 20 in PBS for 1 h. Pooled serum was diluted 1:2 in incubation buffer (0.3 % BSA, 0.1 % Tween 20 in PBS). A dilution series of the inhibitor-extracts was prepared in six steps using the same incubation buffer (undiluted, 1:10, 1:100, 1:500, 1:1000, 1:10000). Ovalbumin was used to check non-specific inhibition. A total of 120 µL of diluted poolserum was preincubated with 120 µL of the inhibitor solution for 1 h. 50 µL of this solution were put into the cavities of the microtiter plate. It was incubated overnight at room temperature in the dark. After three washes with 1 % Tween 20 in PBS, 50 µL of anti-human IgE alkaline phosphatase conjugate (Allergopharma, Reinbek, Germany), diluted 1:200 in incubation buffer, were added and incubated overnight. The plates were washed again and the bound enzyme activity was stained with p-nitrophenylphosphate (PNPP) for 60 min at 37 °C using an alkaline phosphatase staining kit (Allergopharma). Absorbance was measured at 405 nm.
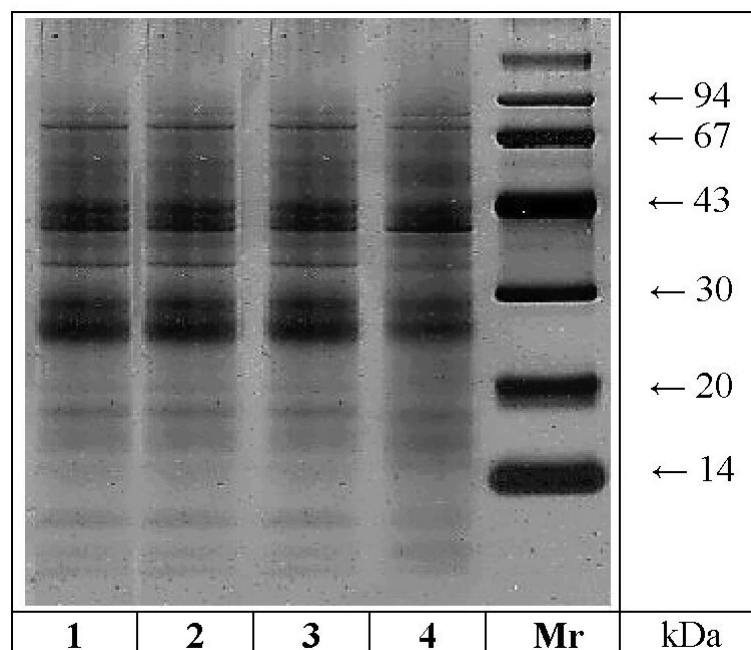
Figure 1: SDS-PAGE / silver staining of protein extracts from different mango varieties. Lane 1: Osteen, lane 2: Eden, lane 3: Tommy Atkins, lane 4: Ngowe (Mr = molecular mass marker proteins).
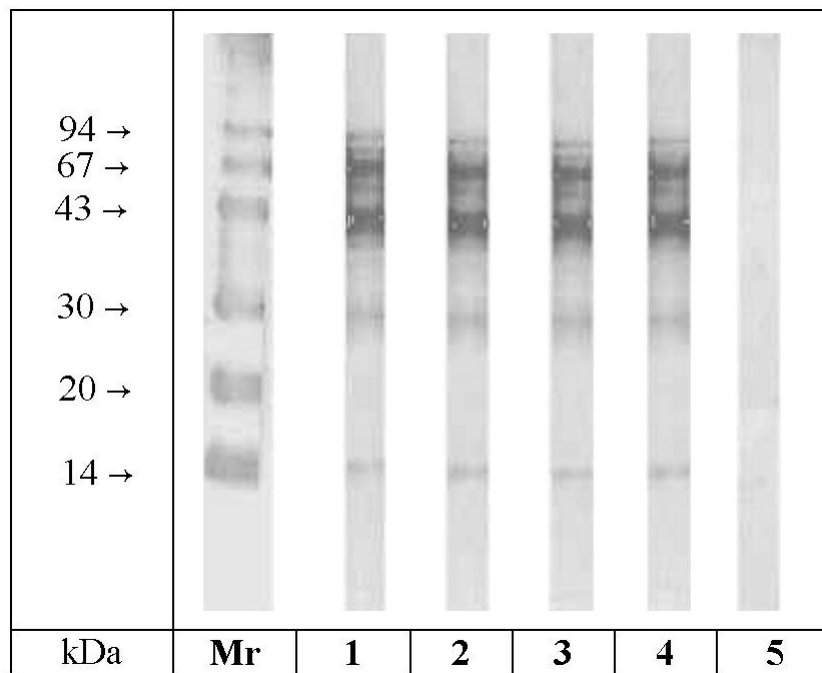
Figure 2: SDS-PAGE / Immunoblotting of different mango varieties. Lane 1: Osteen, lane 2: Eden, lane 3: Tommy Atkins, lane 4: Ngowe, lane 5: Osteen / detection with control serum. Lanes 1-4 were detected with pooled serum from mango- sensitive patients. (Mr = molecular mass marker proteins, colloidal gold staining)

RESULTS

Silver Staining
Extracts of the four mango varieties were investigated by SDS-PAGE / silver staining (Fig. 1). In all varieties proteins with Mr between 14 and 94 kDa were detected. All strains showed three additional protein bands < 14 kDa. Between 14 and 20 kDa three protein bands with Mr of approximately 15, 17 and 19 kDa were detected, at the same time the protein bands with Mr of 15 and 17 kDa were most intense in the strain Ngowe. Very strongly detected proteins became apparent at Mr of approx. 27 and 29 kDa in all four strains. The protein band with Mr of 35 kDa was most intense in the strains Osteen, Eden and Tommy Atkins. At 40 and 43 kDa there were additional strongly detected proteins. Between 43 and 67 kDa there was just a diffuse background of unresolved proteins. At approximately 70 kDa there were two distinct protein bands. In conclusion there were no significant differences in the protein pattern between the four investigated strains.

Immunoblotting
The allergens in extracts of the mango varieties Osteen, Eden, Tommy Atkins and Ngowe were characterized by SDS-PAGE / immunoblotting using seven sera from mango allergic patients.
All sera detected the same allergens in each strain. The detection of the four varieties with pooled serum is shown in Fig. 2. Allergens with Mr of approx. 14, 30, 40, 43 and 67 kDa were visible in each strain (Lane 1-4). Furthermore there was no difference between the varieties in detection intensity of any allergen.

Immunoblot-Inhibition
Cross-reactivity among the different mango varieties was determined by means of immunoblot-inhibition. Fig. 3 shows the separated extract from strain Eden. Preincubation of the pooled serum with mango extracts (incl. Eden itself) resulted in total inhibition of IgE binding by all four strains. No significant inhibition or non-specific binding was observed with ovalbumin and with the control serum, respectively.
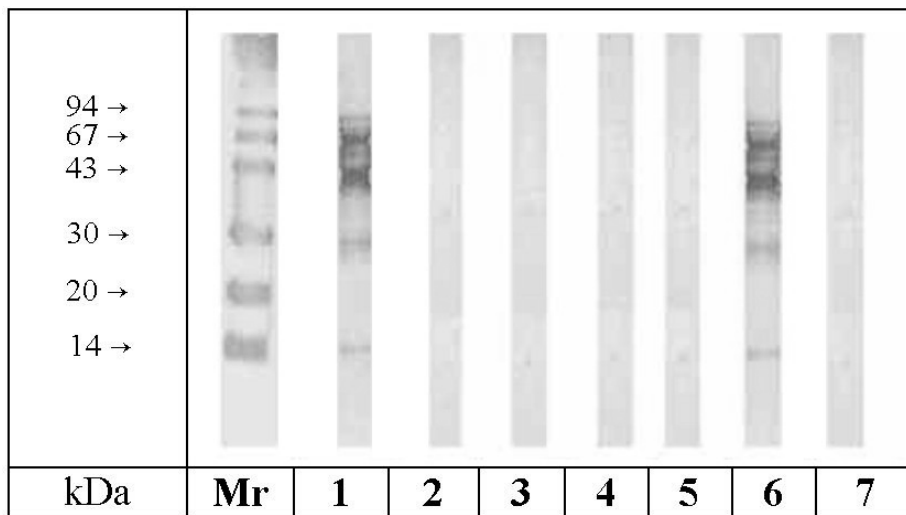
Figure 3: Immunoblot-Inhibition of IgE binding to SDS-PAGE separated mango Eden extract.
Inhibitors (100 µg protein / inhibition): Lane 1: no inhibitor, lane 2: Osteen, lane 3: Eden, lane 4: Tommy Atkins, lane 5: Ngowe, lane 6: non specific inhibition with ovalbumin, lane 7: control serum / no inhibitor. All inhibitors were preincubated with pooled serum from mango-sensitive patients. (Mr = molecular mass marker proteins, colloidal gold staining)
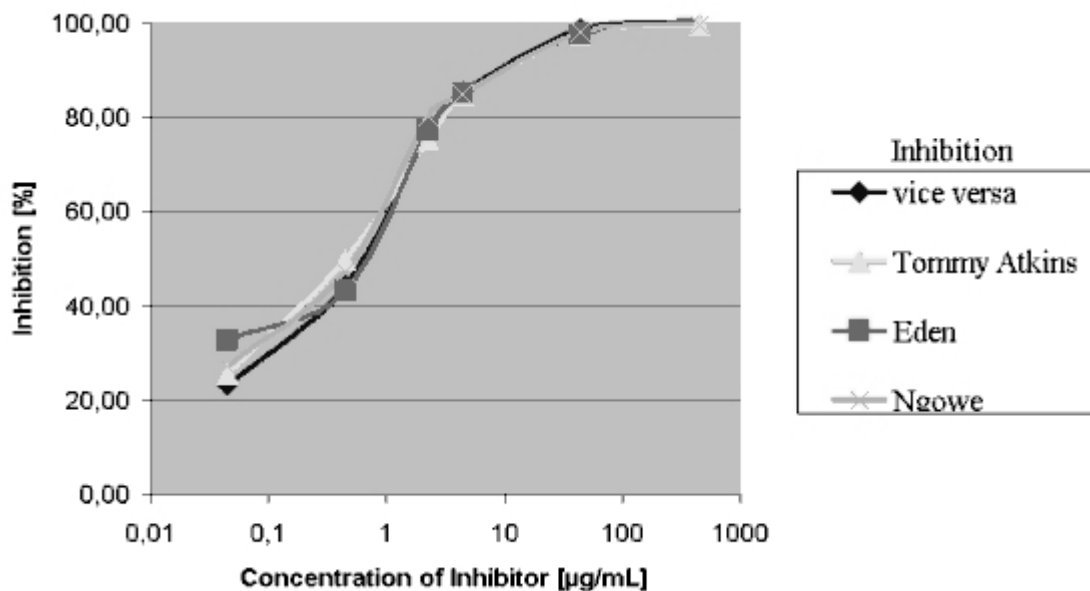
Figure 4: EAST-Inhibition of IgE-binding to mango strain Osteen. Inhibitors: Osteen, Eden, Tommy Atkins, Ngowe.

Table 1: EAST inhibition with different mango extracts.

The protein concentration [µg/mL] for 50 % inhibition is given.

Coupled allergen	Inhibitor			
	Osteen	Eden	Tommy Atkins	Ngowe
Osteen	0.7	0.8	0.5	0.6
Eden	0.9	0.5	0.6	0.8
Tommy Atkins	0.5	0.5	1.0	1.4
Ngowe	1,3	0,7	0,8	0,8

EAST Inhibition

In order to estimate the extent of cross-reactivities among the mango varieties EAST inhibition experiments were performed using the pooled serum. The inhibition of IgE binding to the adsorped protein extract from Mango Osteen is shown in Fig. 4 (Figures of other strains are not shown since they show almost the same results). All four inhibition graphs were nearly identical. The protein concentrations resulting in a 50%-inhibition of IgE binding are given in Table 1. For example IgE binding to Mango Osteen was strongly inhibited by preincubation with all other strains and vice versa. 50%-inhibition values were in the range of 0.5 to 1.4 µg/ml. Using the post-hoc comparison technique there was no difference between the mean values of 50 % inhibition of different strains ($p<0.05$). Total inhibition of IgE binding was achieved by all four varieties at concentrations below 100 µg/mL. No significant inhibition was observed with ovalbumin in all cases.

DISCUSSION

Investigations of different apple strains showed major differences in the allergenic potency of these strains (Vieths et al. 1993, 1994). Because the history of breeding and cultivation of mangos is just as extensive as in the case of apples, the present study was undertaken to prove whether there are variety dependent differences of the allergenic potency of mango fruit or not. For this purpose the four most consumed mango strains, which were cultivated in four different continents (Africa, Asia, Europe and South America) were selected.

The silver staining showed no significant difference between the varieties. These findings were confirmed by immunoblotting. In all strains allergens with Mr of approximately 14, 30, 40, 43 and 67 kDa were detected. Due to the fact that the intensity of the allergen bands seemed to be nearly identical it can be assumed that there are no obvious differences in the IgE binding patterns of mango fruit allergens in the four strains. This result could be confirmed by means of immunoblot inhibition. It was shown that homologous inhibition as well as inhibition with the other strains led to a complete inhibition, indicating the occurrence of identical allergens in all strains.

The results were confirmed by EAST inhibition. It could be shown that all inhibition curves ran almost congruently. Significant differences were not observed. Using statistical examination by means of LSD test it could be stated that the average values of 50 % inhibition do not differ significantly from each other.

Thus it was demonstrated that the allergenic potencies of the four most consumed mango strains in Europe do not deviate from each other.

REFERENCES

● Andre F, Andre C, Colin L, Cacaraci F, Cavagna S (1994) **Role of new allergens and of allergen consumption in the increased incidence of food senitizations in France** *Toxicology 93:77-83*

● Bradford MM (1976) **A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding** *Anal Biochem 72:248-54*

● Brehler R, Theissen U, Mohr C, Luger T (1997) **"Latex-fruit syndrome": frequency of cross-reacting IgE antibodies** *Allergy 52:404-10*

● Dang RW, Bell DB (1967) **Anaphylactic reaction to the ingestion of a mango** *Hawaii Med J 27:149-50*

● Fernandez C, Fiandor A, Martinez-Garate A, Martinez-Quesada J (1995) **Allergy to pistachio: crossreactivity between pistachio nut and other Anacardiaceae** *Clin Exp Allergy 25:1254-59*

● Franke W (1997) **Nutzpflanzenkunde** *6th Edition, Georg Thieme Verlag, Stuttgart*

● Jansen A, de Lijster de Raadt J, van Toorenbergen AW, van Wijk RG (1992) **Allergy to pistachio nuts** *Allergy Proc 5:255-58*

● Laemmli UK (1970) **Cleavage of structural proteins during the assembly of the head of bacteriophage T4** *Nature 227:680-85*

● Miell J, Papouchado M, Marshall AJ (1988) **Anaphylactic reaction after eating a mango** *BMJ 297:1639-40*

● Möller M, Kayma M, Steinhart H, Paschke A (1997a) **Isolation and characterization of a major allergen in kiwi fruits** *Z Lebensm Unters Forsch A 205:364-69*

● Möller M, Paschke A, Vieluf D, Kayma M, Vieths S, Steinhart H (1997b) **Characterisation of allergens in kiwi fruit and detection of cross-reactivity with allergens of birch pollen and related fruit allergens** *Food Agric Immunol 9:107-21*

● Morrisey JH (1981) **Silver stain for proteins in polyacrylamide gels: A modified procedure with enhanced uniform sensitivity** *Anal Biochem 117:307-10*

● Vargas Correa JB, Sanchez Solis L, Farfan Ale JA, Noguchi H, Moguel Banos MT, Vargas de la Pena MI (1991) **Allergological study of pollen of mango (Mangifera indica) and cross reactivity with pollen of piru** *Rev Alerg 38:134-38*

● Vieths S, Schöning B, Baltes W (1992) **Allergy to fruits and vegetables in pollen-sensitive patients: allergen characterization by IgE immunoblotting and peroxidase staining** *Food Agric Immunol 4:181-97*

● Vieths S, Schoening B, Jankiewicz A (1993) **Occurrence of IgE binding allergens during ripening of apple fruits** *Food Agric Immunol 5:93-105*

● Vieths S, Jankiewicz A, Schöning B, Aulepp H (1994) **Apple Allergy: the IgE-binding potency of apple strains is related to the occurrence of the 18-kDa allergen** *Allergy 49:262-71*

● Wellhausen A, Schöning B, Petersen A, Vieths S (1996) **IgE binding to a new cross-reactive structure: a 35 kDa protein in birch pollen, exotic fruit and other plant foods** *Zeitschr Ernährungsw 35:348-55*

● Wüthrich B, Hofer T (1984) **Nahrungsmittelallergie: Das "Sellerie-Beifuß-Gewürz-Syndrom": Assoziation mit einer Mangofrucht-Allergie?** *Dtsch Med Wschr 109:981-86*