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Original Articles

Allergenic potency of kiwi fruit during fruit development

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(Act c 2) change with fruit ripening. These findings should be taken into account during preparation of extracts for allergy diagnosis.

Keywords:

Kiwi fruit | Actinidia deliciosa | food allergy | allergen | actinidin | thaumatin-like protein | development | ripening

Abbreviations

OAS	=	Oral-allergy syndrome
SPT	=	skin prick testing
SDS-PAGE	=	sodium dodecylsulfate-polyacrylamide gel
BSA		
phosph (PBS)		
TLP		
PVPP		
CBB		

SGF	=	simulated gastric fluid
RIE	=	Rocket immunoelectrophoresis

Introduction

Food allergy is an important health problem nowadays. Clinical reactions to food, including cutaneous, gastrointestinal or respiratory disorders or systemic anaphylactic reactions (Sampson 1999) are demonstrated by 8% of children and 2% of adults (Helm & Burks 2000). It is likely that both the incidence and prevalence of food allergies are increasing in line with other forms of allergic diseases (Kimber & Dearman 2001). Food allergy can develop as an isolated reaction to food (fruit, fish, milk) or a secondary reaction after sensitization to pollen (oral-allergy syndrome, OAS) or latex (latex-fruit syndrome) when food allergens cross-react with allergens present in pollen or latex. It is generally believed that food allergens are proteins resistant to digestion because only intact proteins (or their larger fragments) are required for processing by antigen-presenting cells located in gut mucosa. Although stability under digestion may not be the defi tance to digestio esumably by retaining ely accepte y of newly-introduc et al. 2002).

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major allergens of kiwi fruit have been isolated and characterized. Act c 1, a protein of molecular mass about 30 kDa, commonly known as actinidin (Pastorello et al. 1998) is a cysteine protease related to papain (Varughese et al. 1992). Act c 2 is a thaumatin-like protein, the plant defense protein with proven antifungal activity (Gavrovic-Jankulovic et al. 2002b, Wang et al. 2002) that, in kiwi extract, exists in two isoforms differing slightly in pI value (9.4 and 9.5) with molecular mass of about 24 kDa (Gavrovic-Jankulovic et al. 2002b).

Allergenicity of fruit during development depends on the expression of IgE-binding proteins. Vieths et al. (1994) demonstrated that the severity of symptoms in patients allergic to apples was highly correlated with the appearance of 18 kDa apple allergen during storage and suggested that this was caused by ripening. Additionally, Paschke et al. (2001) have correlated the number and intensity of bands in immunoblots with ripening stages of mango fruit and observed no difference caused by ripening.

Allergenic extracts, made by different manufacturers, for the use in diagnosis and therapy of allergic diseases usually show variations in allergenic potency. Especially allergen extracts from fruits, vegetables and other plant foods often lack sufficient biological activity due to the presence of proteolytic enzymes, carbohydrates, and phenol components (Vieths et al. 2001). That is the consequence of applying different extraction methods and source materials. In the last few years several in vivo (such as skin pric ntent of the major al n proposed (Yunging

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Kiwi fruits (Actinidia deliciosa, Monti cultivar) were collected monthly from September to December in the period from 2000 to 2002, from the same tree in Bar, Montenegro. The fruits were stored at −20°C without specific treatment until use. The extracts were made according to previously published protocol (Gavrovic-Jankulovic et al. 2002b). Briefly, the fruits were homogenized 1: 2 (w/v) in 100 mM sodium bicarbonate buffer, pH 9.3 containing 2% polyvinylpoly-pyrrolidone (PVPP) and 0.02% NaN_3 in a blender for 1 min. After extraction and centrifugation, ammonium sulfate was dissolved in the supernatants to achieve 90% saturation. After overnight standing at 4°C the solutions were centrifuged for 20 min at 10000×g. The obtained pellets were dissolved in a minimal volume of a starting buffer and dialyzed extensively against the same buffer. Protein concentration in the extract was determined by the Bradford assay (Bradford 1976).

Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed on 4% stacking gel and 10% or 12% resolving gel according to the method of Laemmli (Laemmli 1970) under reducing and non-reducing conditions. Twelve micrograms of proteins per lane were applied on the gel for Coomassie Brilliant Blue (CBB) staining or semidry transfer ($0.8 mA cm^{-2}$) to a nitrocellulose membrane (Serva) for further examination. The content of major allergen Act c 1 in kiwi fruit extracts

TBS containing 0.05% Tween 20, pH 7.8 for 5 hours. The blots were washed extensively with TBS containing 0.05% Tween 20, pH 7.8 and incubated for 2h with 1: 1000 diluted monoclonal anti-human IgE antibody labeled with alkaline phosphatase (Abcam Ltd., Cambridge Science Park) and, after washing, were visualized using nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate (Sigma Chemical Co., St Louis, MO, USA) as substrates as described (Gavrovic-Jankulovic et al. 2002a). A serum pool of non-allergic persons was used as the negative control.

Digestion with simulated gastric fluid

The digestibility of kiwi proteins in simulated gastric fluid (SGF) was examined according to the method of Yagami et al. (2000). Briefly, 140 µg of kiwi extract proteins was dissolved in 40 µL of prewarmed SGF (US Pharmacopoeia) containing 0.32% w/v of pepsin A (Sigma Chemical Co). Digestion proceeded at 37°C with continuous shaking and an aliquot (8 µL) of the digest was periodically withdrawn (at 8 and 30 minutes). The digestion was stopped with 2.4 µL 0.2 M Na_2CO_3, and samples were mixed with a sample buffer for SDS-PAGE analysis.

Determination of proteolytic activity of actinidin

Proteolytic activity of actinidin in different kiwi fruit samples towards 0.5 mM Nα-Benzoyl- uffer, pH 6.5, cont was evaluate calculated using ex . Relative proteoly tic activity (set up a reparing probes f ead after 1 h of in

A zym ons. Briefly, 12 µg of cusing were applied t analysis. After ele cine, 10 mM Ca^{2+}, 5 staining with CBE

Antibodies against the December kiwi extract were raised in rabbits according to Harboe and Ingild (1983). The animals were injected with 0.5 mL of a 1:1 emulsion of ripe kiwi extract (1 mgmL^{-1}) in complete Freund's adjuvant. Bleeding was performed 50 days after the first immunization and every two weeks thereafter. The presence of antibodies to kiwi proteins was detected by immunodiffusion. The serum was partially purified by ammonium sulfate fractionation (50% saturation).

Rocket immunoelectrophoresis (RIE)

The kiwi extract proteins (5.5 µg) were applied to 1% agarose gel containing 13% rabbit antibodies on a glass plate (12×7 cm). Electrophoresis was carried out in a buffer containing 5 mM barbital, 25 mM Na-barbital, pH 8.6, for 12 h at 2 V cm^{-1}. In order to compare the amount of the major allergen Act c 1 in different kiwi fruit samples, we applied 2.75 µg Act c 1 isolated according to Carne and Moore (1978) without derivatization with S-sulphenyl thiosulphate on the same agarose gel.

Patient sera

Five sera from patients allergic to kiwi fruit were used in this study (1, 2, 3, 4 and 5). All the patients showed OAS and one of them had severe contact dermatitis and anaphylaxis when touching the fruit (case No. 4 in Table I).

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Results

Kiwi fruit extracts

Kiwi fruit extracts (gathered in 2000, 2001 and 2002) were compared using SDS-PAGE, immunoblot and rocket immunoelectrophoresis. The same protein/antigen/allergen patterns were observed in all three years investigated. The concentration of proteins in kiwi fruit extracts increased with fruit development. The results presented here were all obtained using samples from the year 2001.

Protein patterns and actinidin activity in the zymogram

At least 10 protein bands with molecular weights between 67 and 10 kDa were detected by CBB staining in extracts of kiwi fruit from September to December. SDS-PAGE patterns under reducing (see Figure 1a) and non-reducing (Figure 1b) conditions were quite different. The most noticeable difference was in the mobility of the 24 and 30 kDa proteins in reducing conditions (most likely a thaumatin like protein and actinidin, respectively) compared to non-reducing conditions, when they exhibited 20 and 27 kDa molecular weights, respectively. The protein of 29 kDa was constitutively expressed. We also noticed three protein bands of 10, 12 and 14 kDa with similar appearance and in similar amounts from September to December. Several more protein bands of
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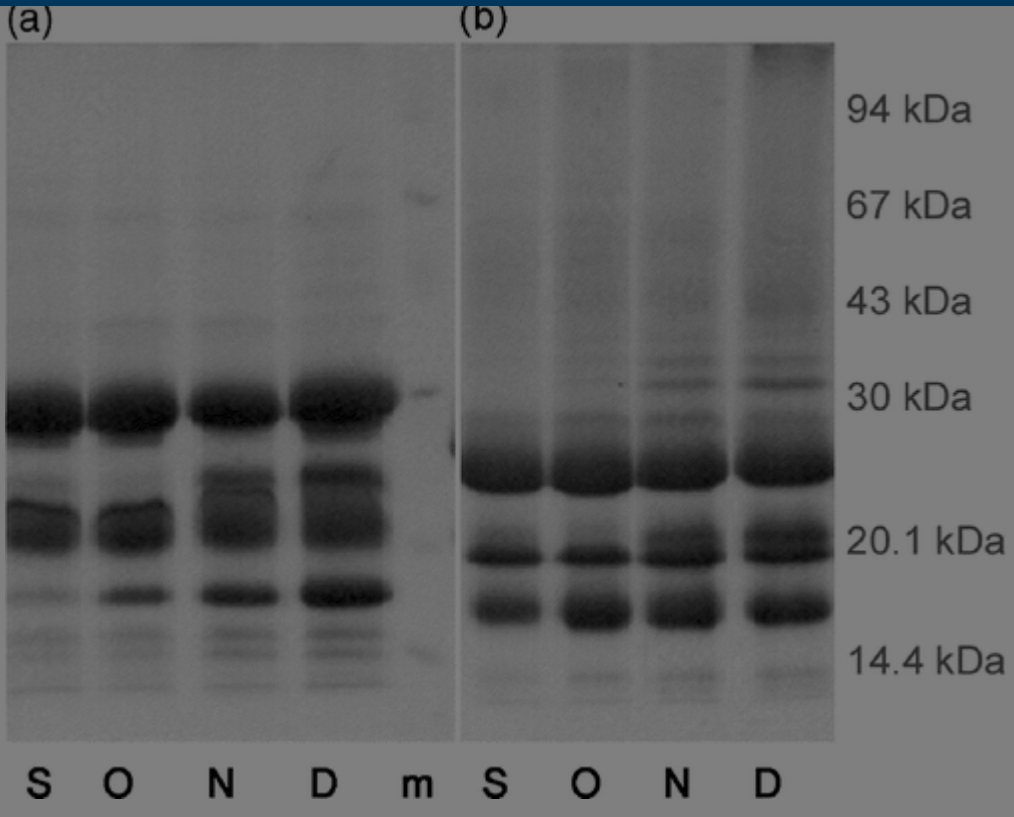

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Two-dimensional PAGE (Figure 2) showed fragmentation of the 30 kDa protein (actinidin), pI 3.6 into a 26 kDa protein with a pI value of 3.2. Both the protein and protein fragment showed proteolytic activity in zymogram (Figure 3). We observed two more proteins of 39 and 17 kDa with pI values of 5.0 and 4.8, respectively.

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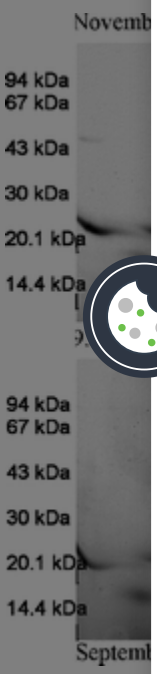

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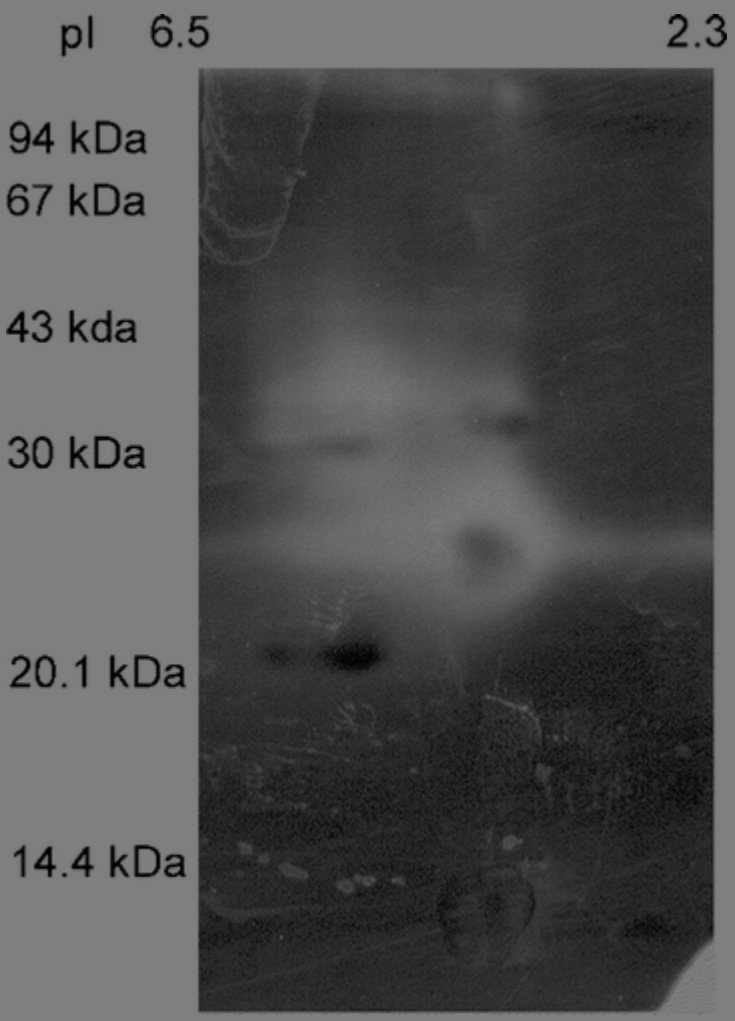
Figure 3. Proteolytic activity of actinidin fragments from the November kiwi fruit extract after 2D PAGE.



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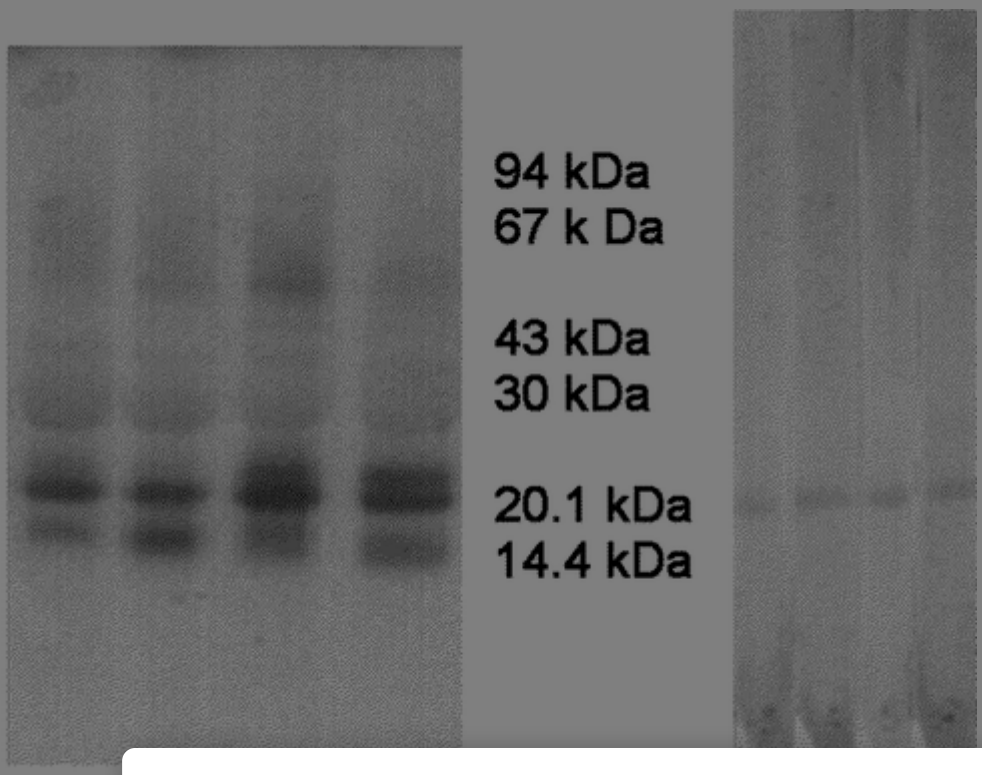
abundant in later months. Allergens of 42 and 68 kDa were present in the October and November samples. The allergen of 22 kDa was most pronounced in the November sample.

Figure 4. Binding patterns of patients' IgE to kiwi fruit extracts after Western blotting. a) immunoblot with serum pool; b) immunoblot with serum from patient 2.

S, September; O, October; N, November and D, December kiwi fruit extract.



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The quantity of actinidin in our samples (see Table II) was measured using three different methods: rocket immunoelectrophoresis, densitometry and determination of proteolytic activity. Rocket immunoelectrophoresis was run according to Hudson and Hay (1989). According to all three methods used in this study, the quantity of Act c 1 was the most pronounced in November kiwi fruit extract (Table II). The most noticeable difference in Act c 1 content between the November kiwi fruit extract and the other three kiwi fruit extract was obtained by determination of proteolytic activity.

Table II. Quantity of major allergen Act c 1 determined by three different methods.

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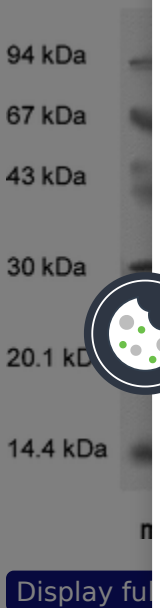
Digestibility of kiwi proteins

All kiwi proteins in September, October, November and December kiwi fruit extracts were decomposed completely within 30 minutes of exposure to simulated gastric fluid (see Figure 5), but the 24 kDa protein (presumably a thaumatin-like protein (TLP), Act c 2) appeared to be more resistant to digestion by gut enzymes in unripe fruit extracts.

Figure 5. Digestibility of kiwi fruit extracts in SGF. Lane m, Molecular weight markers; S, Septemb extract control; sin control.



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The results of SPT and patients' clinical characteristics are shown in Table I. All patients exhibited the most pronounced reaction to the November kiwi fruit extract. The intensity of the reaction to November kiwi fruit extract was approximately twice as strong as the response to the other kiwi fruit extracts (September, October and December).

Discussion

The aim of this study was to correlate the allergen profile and content in kiwi fruit during ripening and to answer the question whether the allergenicity and the digestibility of the fruit depend on its developmental stage or not.

We analysed kiwi fruit extracts from three consecutive years (2000, 2001 and 2002) by SDS-PAGE, 2D-PAGE, immunoblot and rocket immunoelectrophoresis. These extracts exhibited the same allergen and protein profile, ruling out the possibility that our results may depend on specific conditions of the year selected.

The allergenic potential in vivo of the different developmental stages of kiwi fruit (September, October, November and December extracts) investigated by skin prick tests was the highest in extract of fruit collected in November. The amount of Act c 1 (actinidi lergen of kiwi fruit d proteolytic activity. extracts and decrease d by densiton compara ly active in unripe k ing. Acco appropriate metho

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with a presumably higher allergenic potential, appeared to be more prone to digestion in simulated gastric conditions (see Figure 5). Additionally, we noticed that the expression of some until now uncharacterized allergens, started in September and intensified to reach the highest value in November and December (17, 22, 26, 39 and 67 kDa molecular weights). These allergens may also contribute to higher allergenicity of the November extract. Also, it would be of interest to examine the role that these proteins may play in the process of kiwi fruit development.

A previous study by Paschke et al. (2001) showed no difference between allergenic potency of mango fruit during ripening but cannot be directly compared with our results due to the different time-scale of food collection (different fruit developmental stage samples). Kiwi fruits used in our study were harvested in September and October as unripe, while November and December kiwi fruits were ripe and edible. The fruit extracts were made immediately after harvesting. Paschke et al. (2001) performed their experiments only on extracts of consumable and fully developed mango fruit made five to 40 days after harvesting.

According to our results, one of the factors that contribute to the difficulties in proposing well-defined and standardized fruit extracts should also be time (developmental stage) of fruit harvesting. In this particular case, when the kiwi fruit was edib
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Acknow

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Additional information

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References

1. Bradfo … of
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3. Carne … peptides
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PubMed | Web of Science ® | Google Scholar

4. Duffort , OA , Polo , F , Lombardero , M , Diaz-Perales , A , Sanchez-Monge , R , Garcia-Casado , G , Salcedo , G and Barber , D . 2002 . Immunoassay to quantify the major peach allergen Pru p 3 in foodstuffs. Differential allergen release and stability under physiological conditions . Journal of Agricultural and Food Chemistry , 50 : 7738 – 7741 .

PubMed | Web of Science ® | Google Scholar

5. Esch , RE . 1997 . Allergen source materials and quality control of allergenic extracts . Methods: A Companion to Methods in Enzymology , 13 : 2 – 13 .

Web of Science ® | Google Scholar

6. Fu , T-J . 2002 . Digestion stability as a criterion for protein allergenicity assessment . Annals of the New York Academy of Sciences , 964 : 99 – 110 .

PubMed | Web of Science ® | Google Scholar

7. Fu , T-J , Abbott , UR and Hatzos , C . 2002 . Digestibility of food allergens and nonall luid-a compa 4 – 7160 .

8. Garcia 089 . A rare case c gia et Immu

Pu

9. Gavro c , S and Janko en . Journal of Inve

PubMe

10. Gavrovic-Jankulovic , M , Cirkovic , T , Burazer , L , Vuckovic , O and Jankov , RM . 2002a . IgE cross-reactivity between Meadow Fescue pollen and kiwi fruit in patients' sera with sensitivity to both extracts . Journal of Investigational Allergology and Clinical Immunology , 12 : 279 – 286 .

PubMed | Web of Science ® | Google Scholar

11. Gavrovic-Jankulovic , M , Cirkovic , T , Vuckovic , O , Atanaskovic-Markovic , M , Petersen , A , Gojgic , G , Burazer , L and Jankov , RM . 2002b . Isolation and biochemical characterization of a thaumatin-like kiwi allergen . Journal of Allergy and Clinical Immunology , 110 : 805 – 810 .

PubMed | Web of Science ® | Google Scholar

12. Grobe , K , Poppelmann , M , Becker , WM and Petersen , A . 2002 . Properties of group I allergens from grass pollen and their relation to cathepsin B, a member of the C1 family of cysteine proteinases . European Journal of Biochemistry , 269 : 2083 – 2092 .

PubMed | Google Scholar

13. Harbo lins, estima 45 – 351 .

14. Helm ion in Immu

15. Hudso tical Immu fication .

Googl

16. Kimbe oxicology Letter

PubMed | Web of Science ® | Google Scholar

17. Laemmli , UK . 1970 . Cleavage of structural proteins during the assembly of the head of bacteriophage T4 . Nature , 227 : 680 – 685 .

PubMed | Web of Science ® | Google Scholar

18. Malling , HJ . 1993 . “ Methods of skin testing ” . In Allergen standardization and skin test Edited by: Dreborg , S and Frew , A . Vol. Allergy 48(suppl) , 55 – 56 .

Google Scholar

19. Metcalfe , DD , Astwood , JD , Townsend , R , Sampson , HA , Taylor , SL and Fuchs , RL . 1996 . Assessment of the allergenic potential of foods derived from genetically engineered crop plants . Critical Reviews in Food Science and Nutrition , 36(Suppl.) : S165 – S186 .

PubMed | Web of Science ® | Google Scholar

20. Nilsen , BM , Slatten , K , Paulsen , BS , O'Neill , M and van Halbeek , H . 1991 . Structural analysis of the glycoprotein allergen Art v II from the pollen of mugwort (Arten 8 .

Googl

21. Paschl , D and Steinh ening depen y , 13 : 51 – 61 .

22. Pastor , R , Ispano , M , Ir he major allerge 31 – 537 .

23. Pastorello , EA , Pravettoni , V , Ispano , M , Farioli , L , Ansaloni , R , Rotondo , F , Incorvaia , C , Asman , I , Bengtsson , A and Ortolani , C . 1996 . Identification of the allergenic components of kiwi fruit and evaluation of their cross-reactivity with timothy and birch pollens . Journal of Allergy and Clinical Immunology , 98 : 601 – 610 .

PubMed | Web of Science ® | Google Scholar

24. Rudescho , O , Fahlbusch , B , Steurich , F , Schlenvoigt , G and Jager , L . 1998 . Kiwi allergens and their cross-reactivity with birch, rye, timothy and mugwort pollen . Journal of Investigational Allergology and Clinical Immunology , 8 : 78 – 84 .

PubMed | Web of Science ® | Google Scholar

25. Sampson , H . 1999 . Food allergy. Part 1: Immunopathogenesis and clinical disorders . Journal of Allergy and Clinical Immunology , 103 : 717 – 728 .

PubMed | Web of Science ® | Google Scholar

26. Towbin , H , Staehelin , T and Gordon , J . 1979 . Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications . Proce

27. Varug Crystal struct

28. Vie rgy: The IgE-bindin a allergens . Allerg

29. Vieths , M , Haase

PubMed | Web of Science ® | Google Scholar

30. Wang , H and Ng , TB . 2002 . Isolation of an antifungal thaumatin-like protein from kiwi fruit . Phytochemistry , 61 : 1 – 7 .

PubMed | Web of Science ® | Google Scholar

31. Yagami , T , Haishima , Y , Nakamura , A , Osuna , H and Ikezawa , Z . 2000 . Digestibility of allergens extracted from natural rubber latex and vegetable foods . Journal of Allergy and Clinical Immunology , 106 : 752 – 762 .

PubMed | Web of Science ® | Google Scholar

32. Yunginger , JM . 1991 . Standardization of allergenic extracts . Annals of Allergy , 66 : 107 – 112 .

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