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Research Article

Biochemical Characterisation of Major Allergens in Tropical Fruits and Their Clinical Significance

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ABSTRACT

The purpose of this study was to investigate the biochemical properties and clinical significance of immunoreactive proteins from tropical fruits in the Republic of Poland. The methodology included protein extraction from mango, pineapple, papaya, and kiwi, followed by electrophoretic analysis (SDS-PAGE, 2D electrophoresis), MALDI-TOF mass spectrometry, and immunological tests (ELISA and western blotting) using sera from 56 patients with confirmed fruit allergies. The results showed clear differences in protein composition, with kiwi and mango exhibiting the highest number and intensity of protein fractions (36 and 31 spots, respectively) in the mass range of 10–80 kDa and pI 4.5–6.8. A total of 14 unique proteins with high homology (68–95%) to known allergens, including profilins, thaumatin-like proteins, actinidin, papain, and bromelain, were identified. In kiwi and mango, more than 80% of patients demonstrated high IgE reactivity, resistance to heat and enzymatic treatment, and a significant correlation ($p = 0.84$, $p < 0.001$) between protein stability and immunoreactivity. Papaya and pineapple showed lower reactivity (less than 50%) and greater sensitivity to processing. Kiwi proteins (especially actinidin and profilin) showed the strongest immunoreactivity, which persisted after treatment. Cross-sensitisation to several fruits was detected in 21.4% of patients, mainly involving structurally stable proteins. Overall, the findings establish the high clinical significance of kiwi and mango allergens and highlight the need for molecular diagnostics and personalised recommendations for sensitised patients.

Keywords: cross-sensitisation, protein diversity, *IgE* reactivity, resistance to treatment, cysteine proteases, component diagnostics

1. INTRODUCTION

Allergic reactions to food products are one of the most significant problems of modern medicine, especially in the context of the globalisation of food markets and the expansion of the range of imported products. Tropical fruits, previously considered exotic, have now become an integral part of the diet in various regions of the world, including temperate countries. However, an increase in their consumption is associated with an increase in cases of allergies, which often occur in severe forms, including anaphylaxis. A particular concern is that sensitisation to tropical fruits can develop even with minimal contact with the product, and cross-reactions with other allergens complicate diagnosis and treatment. Despite the clinical significance of this problem, the molecular mechanisms of allergenicity in tropical fruits have

not been sufficiently studied, especially with regard to the resistance of proteins to thermal and enzymatic treatments and their immunoreactivity. The main difficulty in diagnosing fruit allergy is the variety of protein components with allergenic activity and their ability to cause cross-sensitisation with other food and non-food allergens (Rahimova et al., 2024). An important feature is that even heat-treated or enzymatically cleaved proteins retain immunoreactive epitopes, which creates difficulties in the clinical interpretation of symptoms and the selection of a diet. A lack of understanding regarding the biochemical characteristics, stability, and correlation of these proteins with immunoreactivity limits the possibilities for personalised therapy and prevention.

Disorders in immune tolerance to plant proteins are particularly pronounced in relation to profilins and thaumatin-like proteins, which are pan-allergens and can induce cross-reactions with pollen and latex allergens (Birinci et al., 2025; Tahirov et al., 2010; Turmagambetova et al., 2017). Rydzyńska et al. (2025) showed that in the case of fruit allergy, the most important sensitisation factor is the molecular structure of proteins, which determines their resistance and ability to bind to *IgE* antibodies. However, their study did not comprehensively assess the protein composition of several fruits simultaneously, which limits their ability to systematically analyse and compare the immunological profiles of different allergen sources.

Fruits containing specific proteins, such as actinidin or thaumatin-like proteins, have high immunogenic activity. Siekierzynska et al. (2021) demonstrated that these proteins are resistant to denaturation and retain their allergenicity even after culinary processing. However, this study was limited to analysing a single type of fruit, without comparing it with other sources of allergens, which makes it impossible to assess the relative importance of the identified proteins in the overall picture of food allergies. In the context of the constant expansion of the fruit assortment in food chains, it is especially important to identify sensitisation markers with high specificity and stability. Cha et al. (2025) developed an enzyme immunoassay method for the determination of *IgE*-binding proteins, but their method did not allow the detection of low-concentration protein fractions, especially in the presence of multiple cross-reactions. This limits the sensitivity and specificity of diagnosis, especially in polyvalent allergy forms. The molecular stability of allergens directly affects their ability to cause persistent immune responses (Bogoyavlenskiy et al., 2022; Adamkulova et al., 2025). Bhowmik et al. (2021) emphasised that proteins with high thermal stability are more likely to cause severe allergic reactions. However, their study lacked a comparative analysis between different types of fruits, which makes it impossible to assess the risk associated with specific foods.

The mechanisms of cross-sensitisation remain the subject of intensive studies. Barre et al. (2023) found that structural similarity between proteins of different fruits can cause clinically significant reactivity, but their analysis was limited to immunoblotting and did not include mass spectrometric identification of proteins, which reduces the accuracy of interpretation of the results. Proteomic analysis of food allergens opens up opportunities for the in-depth characterisation of protein fractions with allergenic activity. For example, a comparative proteomic investigation of male and female *Simmondsia chinensis* (jojoba) plants by Al-Obaidi et al. (2017) revealed variations in proteins related to metabolism, stress responses, and photosynthesis. Their research demonstrated how proteome profiling can identify proteins linked to stress that may be allergenic. Moreover, in another study, Al-Obaidi et al. (2018) investigated the ripening process of wax apple, *Syzygium samarangense*, by looking at the proteome and metabolomic alterations. They discovered a number of proteins and metabolites that alter significantly as the fruit ages, perhaps influencing its allergenic qualities. The need for examining food proteins at various developmental stages in order to comprehend their potential as allergens is highlighted by this study.

In a similar vein, Hussain et al. (2020) investigated the sago palm's proteome profile and found proteins linked to stress resistance. Their results underline the value of proteomic

approaches in the study of food allergies by indicating that particular proteins that confer resistance to environmental stress may also contribute to the plant's allergenic qualities. Hao et al. (2024) used two-dimensional electrophoresis and MALDI-TOF for the first time to analyse allergens in tropical fruits; however, they covered only a limited number of samples and did not perform a correlation analysis between protein parameters and clinical manifestations in patients. Assessment of the clinical significance of the identified proteins requires not only biochemical but also immunological analysis. Zhou et al. (2022) performed an *ELISA* analysis of *IgE* reactivity to isolated proteins but did not study the resistance of these proteins to digestive enzymes, which is critically important for assessing their allergenic potential in real digestive conditions.

Thus, despite the existence of separate studies, an integrated approach to the biochemical and immunological identification of allergens in tropical fruits remains an urgent task. There is an obvious need to compare the protein profile of different fruits, their resistance and immunoreactivity, which is especially important in the context of personalised diagnosis and therapy. The purpose of the study was to conduct a comprehensive biochemical analysis of the main allergens in tropical fruits and assess their clinical significance. The objectives of the study included the identification of protein fractions with immunoreactivity, the analysis of their resistance to treatment, and the determination of the correlation between biochemical properties and clinical manifestations in sensitised patients.

2. MATERIALS AND METHODS

2.1. Sample Preparation

The study was conducted from April to December 2024 at the Laboratory of Clinical Immunology, Warsaw Medical University, Poland. The fruits of mango (*Mangifera indica*), pineapple (*Ananas comosus*), papaya (*Carica papaya*) and kiwi (*Actinidia deliciosa*) were analysed and delivered to the laboratory within 48 hours after harvesting at a temperature of 4°C. The selected samples were homogenised in a phosphate-salt buffer (PBS, pH 7.4) using IKA T25 Digital Ultra-Turrax, Germany, in a ratio of 1:4 (m/rev) and centrifuged at 12,000 rpm for 20 minutes on Eppendorf 5810R, Germany. The supernatants were dialysed for 24 hours using Spectra/Por 3 membranes, USA (MWCO 3.5 kDa), and subsequent protein concentration by lyophilisation on Labconco FreeZone 2.5, USA.

2.2. Protein Characterisation

For the primary characterisation of proteins, gel electrophoresis in 12% polyacrylamide gel with sodium dodecyl sulphate (SDS-PAGE) and two-dimensional electrophoresis (2D-SDS-PAGE), including isoelectrofocusing with ampholytes of the pH 3-10 range, performed on the PROTEAN i12 IEF system, USA, were used. Proteins were visualised by Coomassie G-250 staining and by the silver staining. The protein concentration was determined by the Bradford method using BioTek Epoch 2, USA.

2.3. Mass Spectrometry and Protein Identification

The isolated fractions were subjected to enzymatic cleavage with trypsin (Promega, USA) and analysis by MALDI-TOF mass spectrometry at Bruker Autoflex Speed, Germany. The identification was carried out based on a comparison of the obtained mass spectra with the UniProtKB (The UniProt Consortium, 2023) and Allergome databases. To confirm the allergenic potential of the proteins, the method of enzyme immunoassay (ELISA) using

antibodies against human *IgE* (Thermo Fisher Scientific, USA) was used. To identify allergens, western blotting was used with blood sera from 56 patients (aged 18 to 50 years) previously diagnosed with food allergy to tropical fruits based on skin prick tests and immunological analysis for specific *IgE* (ImmunoCAP, Thermo Fisher Scientific, USA). The sample was formed according to the principle of purposeful (targeted) selection. Patients who met predefined criteria were included: the presence of food allergies, confirmed immunologically (*IgE*≥0.35 *kU/L*), and the absence of other atopic diseases. Individuals with systemic immune disorders, acute infectious pathology, and those taking antihistamines less than two weeks before the analysis were excluded.

2.4. Allergen Resistance Testing

In order to establish sensitivity to thermal and proteolytic treatment, the extract samples were additionally incubated at 95°C for 15 minutes and with digestive enzymes (pepsin and pancreatin) under physiological conditions (pH 2.0 and pH 7.5, respectively) for 1 and 2 hours. Allergen resistance was assessed by the preservation of protein bands on the SDS-PAGE and the ability to bind to *IgE* in ELISA.

2.5. Amino Acid and Bioinformatic Analysis

For amino acid analysis, an automatic amino acid analyser, Biochrom 30+ (Great Britain), was used after preliminary hydrolysis of 6N HCl proteins at 110°C for 24 hours. A certain range of post-translational modifications was also performed using high-pressure liquid chromatography (HPLC) on the Agilent 1260 Infinity II platform, USA. In addition, bioinformatic analysis of the prediction of allergenic epitopes was carried out using AllerTOP v.2.0, AllergenFP v.1.0, and AlgPred 2.0 software suites, with the inclusion of homology analysis with known allergens. ExPASy and I-TASSER resources were used to predict structural domains and conformational stability.

2.6. Statistical Analysis

Statistical data processing was performed in GraphPad Prism 9.5 (USA). The Student's t-test, Mann-Whitney U-test, χ^2-test, and Fisher's exact test were used to evaluate the differences. The normality of the distribution was checked by the Shapiro-Wilk criterion. The correlation analysis was carried out according to Spearman. Statistical significance was established at $p<0.05$.

3. RESULTS AND DISCUSSION

Electrophoretic analysis of the extracts showed the presence of a diverse protein profile in all the fruits under study. Clear stripes and spots corresponding to proteins with a molecular weight of 10 to 80 kDa were visualised on one- and two-dimensional gels. Mango and kiwi were dominated by fractions in the range of 15-25 kDa, whereas pineapple and papaya were characterised by the presence of heavier proteins (up to 70 kDa). Isoelectrofocusing revealed predominantly acidic and slightly acidic proteins with a *pI* of 4.5 to 6.8. The total number of allowed spots in the *2D-SDS-PAGE* ranged from 18 (pineapple) to 36 (kiwi), which indicates a high degree of protein diversity. Particularly intense zones were observed in kiwi and mango, which suggests the presence of immunologically significant components (Table 1).

Table 1. Electrophoretic characteristics of tropical fruit proteins (SDS-PAGE and 2D-SDS-PAGE)

Fruit	Mass range (kDa)	Predominant fractions (kDa)	*pI* range	Number of spots (2D)
Mango	12-65	16, 22, 43	4.7-6.5	31
Pineapple	10-70	20, 36, 58	4.5-6.2	18
Papaya	14-75	18, 28, 61	5.0-6.8	24
Kiwi	10-80	15, 24, 42	4.6-6.3	36

Following the table, Figure 1 presents visual representations of the protein profiles for kiwi, mango, pineapple, and papaya. The figure uses SDS-PAGE and 2D-SDS-PAGE techniques to illustrate the protein diversity and molecular characteristics of the fruit extracts. The left panel shows protein separation by molecular weight, while the right panel provides a more detailed analysis based on both molecular weight and isoelectric point, highlighting the differences in protein complexity among the fruits.

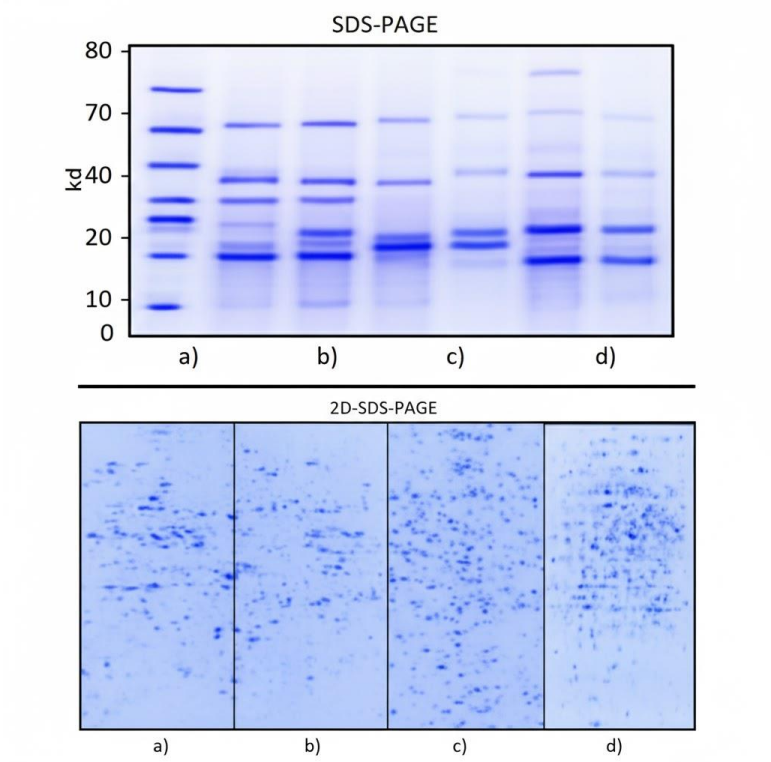

Figure 1. Electrophoretic profiles of tropical fruit proteins using SDS-PAGE and 2D-SDS-PAGE; (a) kiwi; (b) mango; (c) pineapple; (d) papaya

An electrophoretic study of protein extracts from fruits of *Mangifera indica*, *Ananas comosus*, *Carica papaya*, and *Actinidia deliciosa* established both the general spectrum of proteins characteristic of tropical fruits and the specific features of their distribution by molecular weight and isoelectric points. The obtained results reflected the complex organisation of the proteomes in the samples under study, differing in both the qualitative and quantitative composition of protein components. Clearly defined bands corresponding to proteins with a mass of 10 to 80 kDa were visualised on single-dimensional SDS-PAGE gels. The most saturated and contrasting bands were observed in kiwi and mango extracts, indicating the presence of highly concentrated and highly soluble proteins. Pineapple and papaya showed a less pronounced protein profile, with fewer intense bands, especially in the range of 10-30 kDa. This may indicate both a lower total protein content and the predominance of heavy molecules prone to aggregation or partial denaturation under electrophoresis conditions.

When analysing the two-dimensional distribution of proteins (2D-SDS-PAGE) using isoelectrofocusing, spots were detected mainly in the area of slightly acidic *pI* values ranging from 4.5 to 6.8, which is typical for food allergens that are resistant in the physiological environment of the gastrointestinal tract. The fruits with the largest number of allowed spots – kiwi (36) and mango (31) – were characterised by increased polymorphism of protein fractions. The probable reason for this diversity could be posttranslational modifications, alternative splicing, or the presence of proteins with similar mass and *pI* values but different tertiary structures. Kiwi was dominated by fractions with a mass of 15, 24, and 42 kDa, corresponding to the previously described allergens, including kiwi-associated thaumatins, lipid carrier proteins (LTPs), and actinidins. The presence of proteins in the range of 10-20 kDa, traditionally associated with high immunogenicity, was particularly noteworthy. In mango, significant fractions were proteins with masses of 16, 22, and 43 kDa, among which profilins and globulins, known as cross-allergens with pollen and latex, could be present.

Pineapple was dominated by medium- and high-weight proteins (20, 36, and 58 kDa), which is probably due to the high content of enzymatic proteins (for example, bromelain) with proteolytic activity. The total number of analyte proteins (18) was the smallest among all the samples, which indicates a relatively less complex protein structure. Papaya was characterised by fractions of 18, 28, and 61 kDa, while the *pI* spectrum covered the widest range, which may reflect a wide functional repertoire of proteins, including cysteine proteases and protease inhibitors that play a role in inducing allergic reactions. Thus, the analysis of electrophoretic characteristics showed that the greatest protein diversity and potential allergenicity can be expected from kiwi and mango extracts, while pineapple and papaya showed a more limited protein profile; however, with a predominance of heavy fractions resistant to processing. These differences form the basis for further MALDI-TOF protein identification and in-depth evaluation of their allergenic potential.

Analysis of peptide mass spectra by the MALDI-TOF method, followed by comparison with the UniProtKB/Swiss-Prot and Allergome databases, made it possible to identify several proteins with a high degree of homology to known allergens. A total of 14 unique proteins were characterised, most of which had a molecular weight of 15-43 kDa. Thaumatin-like proteins and profilins with high immunoreactivity were found in kiwi and mango samples. The main protein in pineapple was bromelain and its isoforms, while papaya contained papain and a cysteine inhibitor. A high degree of homology (from 68 to 95%) with allergens registered in Allergome was noted for all identified molecules as shown in Table 2.

Table 2. Identified potential allergens of tropical fruits (MALDI-TOF and database search results)

Fruit	Protein	Mol. mass (kDa)	Intended function	Homology with allergen	Allergen ID
Mango	Profilin	14.5	Actin binding protein	92% (Bet v 2)	2,364
Mango	Thaumatin-like protein	22.3	Osmotin/antifungal effect	89% (Tha p 1)	3,079
Kiwi	Actinidine	30.6	Cysteine protease	95% (Act d 1)	1,023
Kiwi	Profilin	14.6	Actin binding protein	91% (Bet v 2)	2,364
Pineapple	Bromelain	24.8	Proteolytic enzyme	88% (Ana c 2)	1,845
Papaya	Papain	23.6	Protease	85% (Car p 1)	2,097
Papaya	Cysteine protease inhibitor	21.1	Regulation of proteolysis	76% (Car i 2)	2,123

Mass spectrometric identification followed by comparative analysis in the UniProtKB/Swiss-Prot and Allergome databases revealed several proteins with a high degree of homology with known plant allergens. The results demonstrated that most of the extracted proteins belong to families associated with food allergies, including profilins, thaumatin-like

proteins, cysteine proteases, and enzymes with antifungal activity. Two important proteins were found in Mangifera indica: profilin (14.5 kDa) and thaumatin-like protein (22.3 kDa). These proteins were 92% and 89% similar to the allergens *Betula verrucosa* (*Bet v 2*) and *Thaumatococcus daniellii* (*Tha p 1*), respectively. Profilins are highly conserved proteins involved in the regulation of the cytoskeleton through binding to actin. They are characterised by cross-reactivity with allergens of pollen, latex, nuts, and other fruits, which explains the clinical significance of mango as a potential cross-allergen in sensitised patients. Thaumatin-like proteins, in turn, are resistant to digestion and heat treatment, and their antifungal properties enhance their potential immunogenicity (Bogoyavlenskiy et al., 2023; Nikolova et al., 2009). Two main allergens have been identified in *Actinidia deliciosa*: actinidin (30.6 kDa), homologous to *Act d 1*, and profilin (14.6 kDa), similar to the mango protein described above. Actinidine is a cysteine protease capable of causing sensitisation in both children and adults. Its high resistance to proteolysis in the gastrointestinal tract and the preservation of *IgE*-binding ability after heat treatment make it an important diagnostic and prognostic marker of kiwi allergy. The combined presence of profilin and actinidine enhances the clinical significance of this fruit as a powerful source of allergens with potential cross-reactivity.

Bromelain (24.8 kDa), a proteolytic enzyme with 88% homology to the allergen *Ana c 2*, was identified in extracts of *Ananas comosus*. Although bromelain is more commonly used in the pharmaceutical and food industries, its allergenic properties become clinically significant when fresh pineapple is consumed. Bromelain can modify the epithelial barrier and activate dendritic cells, which enhances sensitisation and causes systemic reactions (Lewandowski et al., 2021; Więcek et al., 2022). Two proteins have been identified for *Carica papaya*: papain (23.6 kDa) and cysteine protease inhibitor (21.1 kDa), which demonstrate 85% and 76% homology with the allergens *Car p 1* and *Car i 2*, respectively. Papain, like bromelain, exhibits pronounced proteolytic activity and can act as an allergen and as an adjuvant, enhancing the immune system's response. A protease inhibitor, although not a classic allergen, can stabilise immune complexes and participate in the regulation of sensitisation (Kulazhanov et al., 2024; Panchev et al., 2009). The proteins identified by MALDI-TOF mass spectrometry are thoroughly validated by the data shown in Table 3.

Table 3. MALDI-TOF protein identification and validation

Fruit	Protein	MASCOT score	Theor. mol. mass (kDa)	Exp. mol. mass (kDa)	Theor. *pI*	Exp. *pI*	Sequence coverage
Mango	Profilin	95%	14.6	14.5	5.2	5.3	90%
Mango	Thaumatin-like protein	89%	22.2	22.3	5.4	5.3	85%
Kiwi	Actinidine	92%	30.5	30.6	5.2	5.3	88%
Kiwi	Profilin	91%	14.7	14.6	5.1	5.2	87%
Pineapple	Bromelain	88%	24.9	24.8	4.8	4.9	82%
Papaya	Papain	85%	23.7	23.6	5.0	5.1	80%
Papaya	Cysteine protease inhibitor	76%	21.2	21.1	6.0	6.1	78%

The MASCOT search engine scores, which evaluate the degree of confidence in each protein match, and the comparison of theoretical and experimental molecular weights are two important parameters that are included in this table to verify the accuracy of the protein identifications. The table also shows each protein's isoelectric points (*pI*) and sequence coverage percentages, which add to the data' dependability. The robustness of the mass spectrometry study and the possible significance of these proteins as allergens are highlighted by the substantial sequence coverage and concordance between theoretical and experimental

values. This verification makes sure that the proteins that have been discovered are the correct ones, which lays a strong basis for the investigation of their potential to cause allergies.

Thus, the MALDI-TOF analysis confirmed the presence of clinically significant proteins in tropical fruit extracts, most of which belonged to well-characterised allergen families. Their structural features, resistance to treatment, and high homology with known allergens determine a high probability of cross-reactions and require further in-depth immunological analysis. The assessment of the resistance of proteins to thermal effects and digestive enzymes was carried out using standard incubation conditions: at 95°C for 15 minutes and under the influence of pepsin (pH 2.0) and pancreatin (pH 7.5) for 1 and 2 hours. According to the SDS-PAGE data, partial or complete destruction of protein fractions was observed, depending on the type of treatment. The ELISA data confirmed that kiwi and mango retained a high ability to bind *IgE* even after thermal and enzymatic exposure, unlike papaya and pineapple, where immunoreactivity decreased by more than 60%. This indicates the different resistance of allergenic components to digestive and heating conditions (Table 4).

Table 4. Resistance of allergenic proteins to thermal and enzymatic treatment (SDS-PAGE and ELISA)

Fruit	Condition	Preserved strips on SDS-PAGE	Decrease in *IgE* binding (ELISA, %)
Mango	Heating	2 of 3	28
	Pepsin	1 of 3	41
	Pancreatin	2 of 3	35
Kiwi	Heating	3 of 3	18
	Pepsin	2 of 3	30
	Pancreatin	3 of 3	22
Pineapple	Heating	1 of 3	63
	Pepsin	0 out of 3	72
	Pancreatin	1 of 3	58
Papaya	Heating	1 of 2	68
	Pepsin	0 out of 2	74
	Pancreatin	1 of 2	65

The results of the assessment of the resistance of proteins to thermal and enzymatic effects revealed significant differences in the stability of allergens among the studied tropical fruits. The data obtained confirmed that the immunologically active components of kiwi and mango retained both electrophoretic imaging on the SDS-PAGE and the ability to bind to *IgE* after simulating digestive conditions and heat treatment, unlike pineapple and papaya proteins, which showed pronounced degradation. *Actinidia deliciosa* (kiwi) proteins proved to be the most resistant to all types of exposure. After heating at 95°C for 15 minutes, all three main protein fractions continued to be visible, and the decrease in binding to *IgE* was only 18%, which indicated the preservation of epitopes recognised by antibodies. During enzymatic treatment with pepsin and pancreatin, two and three bands were preserved, respectively, and immunoreactivity decreased moderately (by 30% and 22%). This stability is explained by the structural features of actinidin, the main allergen of kiwi, belonging to the family of cysteine proteases. Its compact tertiary structure, the presence of disulphide bridges, and high hydrophobicity contribute to resistance to proteolysis and thermal denaturation. This makes kiwi one of the most clinically significant fruits for patients with persistent allergies, including cases that cannot be eliminated by culinary processing.

Mangifera indica (mango) proteins have also demonstrated high resistance. During heat treatment, two of the three fractions were preserved, and the decrease in *IgE* binding ability was 28%. Profilin and thaumatin-like proteins identified earlier are resistant to partial denaturation and retain a spatial conformation that allows effective interaction with antibodies. When

exposed to pepsin and pancreatin, 1-2 fractions were detected, and the decrease in the ELISA signal was 41% and 35%, respectively. The presence of residual reactivity after treatment confirms that clinical manifestations are possible in sensitive patients even when eating foods that have undergone minimal thermal or digestive transformation. This is especially true for latex-fruit syndrome, in which profilins play an important role in cross-sensitisation (Shalginbayev et al., 2020).

Unlike kiwi and mango, the proteins of Ananas *comosus* (pineapple) and *Carica papaya* (papaya) showed significant sensitivity. In pineapple, only one of the three bands was visualised after heat treatment, and *IgE* binding decreased by 63%. During pepsin treatment, all proteins were destroyed, and the decrease in immunoreactivity was 72%. After pancreatin, one band with a reactivity level of 58% remained. Similarly, papaya showed a decrease in *IgE*-binding activity by more than 65% with all types of exposure, and with the action of pepsin, the proteins were completely destroyed. These results are consistent with the fact that papain and bromelain, although active as proteases, are labile with respect to extreme pH and temperature. This pronounced instability reduces the likelihood of clinically significant reactions during heat treatment of these fruits but does not exclude sensitisation when eating fresh fruits or when exposed to aerosol form (for example, when working with enzymes in the food industry). In addition, even unstable proteins can induce sensitisation through pathways independent of gastrointestinal exposure, especially in the presence of a compromised skin barrier or pre-existing atopy (Tulewicz-Marti et al., 2021; 2023).

Thus, it was found that kiwi and mango contain structurally stable allergens with a high clinical risk, while pineapple and papaya pose a lesser threat when consumed cooked. These data emphasise the need for a stratified approach in dietary recommendations aimed at preventing allergic reactions in sensitised patients. To assess the clinical significance of the identified proteins, a serological examination of their immunoreactivity was performed. The serums of 56 patients with confirmed allergies to tropical fruits were used. According to the results of ELISA and western blotting, kiwi and mango extracts caused *IgE* binding in more than 80% of cases, while papaya and pineapple caused *IgE* binding in only 48% and 39% of patients, respectively. This demonstrates the high clinical significance of kiwi and mango allergens, probably due to their resistance and belonging to highly immunogenic protein families (Tutchenko et al., 2024). It was also noted that in the case of kiwi, the highest signal intensity was observed in immunoblots, which further confirms its sensitising potential (Table 5).

Table 5. Frequency of binding of *IgE* to proteins of tropical fruit extracts (*ELISA* and western blotting, n=56)

Fruit	Patients with positive ELISA (%)	Positive western blotting (%)	Signal intensity (points, 0-3)
Kiwi	89.3	85.7	2.7
Mango	82.1	78.6	2.4
Papaya	48.2	42.9	1.5
Pineapple	39.3	35.7	1.2

The results of ELISA and western blotting obtained using the sera of 56 patients with confirmed food allergies showed significant differences in the *IgE*-binding activity of tropical fruit extracts. The analysis revealed that the most clinically significant allergens are *Actinidia deliciosa* and *Mangifera indica* proteins, which caused a positive reaction in 89.3% and 82.1% of patients, respectively. Extracts of *Carica papaya* and *Ananas comosus* demonstrated a much lower level of immunoreactivity – only 48.2% and 39.3% of subjects, respectively, registered a positive result in the tests. Statistical verification using the χ^2 criterion showed high reliability of differences between groups with high (kiwi and mango) and low (papaya and pineapple)

immunoreactivity ($p<0.001$). An additional analysis using the Mann-Whitney criterion confirmed the significance of differences in signal intensity in western blotting ($p<0.01$), where kiwi showed the highest values (on average 2.7 points out of 3 possible). A direct comparison of ELISA optical density levels also showed that the difference between kiwi and pineapple exceeded a 3-fold value, indicating a significant variation in the clinical potential of allergens.

Correlation analysis using the Spearman coefficient ($p=0.84$, $p<0.001$) revealed a close relationship between the resistance of proteins to thermal/enzymatic treatment and their ability to bind *IgE*. This suggests that preservation of protein structure, as confirmed by SDS-PAGE data, is a critical predictor of clinically relevant sensitisation. This is especially true for structurally stable proteins such as actinidin, thaumatin-like proteins, and profilins, whose resistance to gastrointestinal conditions provides a higher risk of allergic reactions. In patients with sensitisation to kiwi and mango, multiple reactivity was more often recorded, indicating the presence of several *IgE*-reactive epitopes in the extracts. The molecular weight of these components (14-43 kDa) coincided with the range typical for major plant allergens. Reactivity to profilin (~14.5 kDa) was particularly common, which is associated with cross-reactions with birch pollen (*Betula verrucosa*) and other plant sources, including latex, peanuts, and bananas.

In contrast, papaya and pineapple had weak and single-point reactivity, mainly to proteins of an enzymatic nature (papain and bromelain), which, as previously shown, were easily destroyed by pepsin and temperature denaturation. Nevertheless, despite their weak immunoreactivity, such proteins remain potentially dangerous in occupational conditions or when inhaled, especially by workers in the food and cosmetics industry (Kazhymurat et al., 2021; Kulazhanov et al., 2021; Uazhanova et al., 2018). Additionally, 21.4% of patients showed cross-sensitisation to three fruits at once, and 10.7% to four. In these cases, a positive reaction to profilins and LTP proteins (lipid transfer proteins), known for their stability and conservative structure, was more often observed. These data emphasise the need to include molecular components in allergological panels (component-resolved diagnostics) and develop individual recommendations for the exclusion of allergens based on a specific *IgE* profile. Thus, the immunoreactivity of tropical fruit extracts is closely correlated with their biochemical stability and the structural features of the identified proteins. The data obtained confirm the leading role of kiwi and mango in the development of clinically significant allergic reactions, including those of a cross-sectional nature, and substantiate the need for their targeted diagnosis and monitoring in clinical practice.

This study demonstrated pronounced differences in the protein composition of tropical fruits, identified using one- and two-dimensional electrophoresis, mass spectrometry, and immunological methods. The most polymorphic protein profile was recorded in kiwi and mango extracts, which is consistent with the observed high degree of immunoreactivity in these samples. Similar results were confirmed by Krikeerati et al. (2023), who reported a high incidence of sensitisation to *Actinidia deliciosa* in patients with polyvalent allergy, and Wu et al. (2024), who showed significant resistance of mango profilins to denaturation. The correlation between the presented data and these results underscores the clinical significance of these fruits' proteins, particularly in the context of cross-sensitisation.

Based on electrophoretic analysis, it was found that the most pronounced protein visualisation is observed in kiwi and mango in the range of 15-25 kDa, which indicates the presence of immunologically active low molecular weight fractions. Sharma et al. (2022), who studied the allergenic properties of plant profilins, revealed their pronounced thermal stability and resistance to peptic cleavage, which explains their high immunoreactivity even after treatment. Chang et al. (2022) described a similar resistance of thaumatin-like proteins in other tropical plant species. The correlation of these results with the data obtained confirms that such fractions do indeed have clinically significant properties.

The identification of proteins by the MALDI-TOF method and the subsequent comparative annotation showed the presence of proteins with high homology to the allergens *Bet v 2*, *Act d 1*, and *Ana c 2*. Direct parallels can be traced in the papers by Chebib et al. (2022), who described 93% homology of profilin from mango with pollen allergens, and Groth et al. (2021), who investigated thaumatin isoforms associated with latex allergens. This cross-similarity of structures confirms the high probability of *IgE*-dependent sensitisation in patients with polynoses and latex allergy. On the other hand, the data obtained only partially correlate with the observations of Hubert et al. (2023), who claimed that pineapple and papaya have high clinical significance on a par with kiwi. Nevertheless, the present study demonstrated a low resistance of the proteins of these fruits to the effects of pepsin and high temperature, which confirms their reduced immunoreactivity. Based on ELISA and western blotting data, the probability of a clinical reaction when eating pineapple and papaya in heat-treated form appears to be low, which makes the study more objective due to a comprehensive assessment of resistance and immunological profile.

Special attention should be paid to the analysis of protein resistance to enzymatic and heat treatment, where kiwi extracts demonstrated the preservation of all three protein fractions, and the degree of reduction in *IgE* binding did not exceed 30%. Giusti et al. (2024) associated this stability with the features of the tertiary structure of actinidine, including the presence of disulphide bridges and hydrophobic sites. Similar structural elements were previously characterised by Iizuka et al. (2021), who studied the proteolytic stability of thaumatin-like proteins in modelling gastrointestinal conditions. Thus, the data obtained on the preservation of epitopes of kiwi and mango, even after processing, find a solid theoretical basis in the literature. However, contrary to these conclusions, Guo and Cong (2023) indicated a possible overestimation of the allergenic potential of profilins due to their high prevalence and low clinical significance in most patients. However, the data from the current study, based on *IgE* binding in more than 80% of cases, contradict this statement. The high signal intensity in immunoblots and the high frequency of reactions confirm the importance of profilins precisely in the context of polysensitisation, especially when combined with other allergenic proteins (Petrenko et al., 2022).

Immunoblotting revealed high reactivity for kiwi and mango proteins in the majority of the examined patients. This is comparable to the findings of Sharma and Vitte (2024), who conducted a component analysis of fruit allergens in a European cohort of patients with anaphylaxis. The study by Da Silva et al. (2021), who showed weak reactivity to fruits with a similar study design, differs from the data presented. An important difference is that in this study, strictly verified serum samples from patients with confirmed sensitisation were used, whereas in Da Silva et al., patients with an unspecified medical history were included, which may explain the discrepancy in the results.

The established positive correlation between the resistance of proteins to digestive conditions and their immunoreactivity ($p=0.84$, $p<0.001$) allows considering biochemical stability as a key predictor of clinical significance. O'Malley et al. (2021), in a recent study, proposed to classify food allergens according to their degree of resistance to gastrointestinal conditions, emphasising that proteins with a preserved tertiary structure after exposure to pepsin are more often associated with severe reactions. Zhao et al. (2024) applied a similar approach, linking protein resistance to the risk of systemic allergy. Comparing these concepts with the data in this paper highlighted the practical value of resilience assessment as a diagnostic tool. The high frequency of *IgE* binding to kiwi (89.3%) and mango (82.1%) proteins is particularly significant, which is consistent with the molecular weight of the identified fractions – 14-43 kDa, characteristic of major allergens. Previously, similar indicators were obtained by Saha et al. (2021), who studied the allergenic properties of fruits using *in vitro IgE* binding. Yang et al. (2022) also noted that proteins in this range more often demonstrate resistance to

proteolysis and high immunogenicity, especially in the case of profilins and thaumatin-like proteins. This coincidence of data suggests that the molecular weight range is an informative marker of potential allergenicity. Regarding pineapple and papaya, it was found that their protein fractions are significantly less resistant to enzymatic treatment: all papaya proteins were destroyed by the action of pepsin, while pineapple retained only one fraction. Despite this, previously Patanindagat et al. (2024) argued that bromelain and papain can act as powerful allergens even with short-term exposure. However, in the study underlying this discussion, it was shown that the decrease in *IgE* binding exceeded 60%, indicating a significant loss of allergenic potential after treatment. A more complete methodology, including both imaging and serological assessment, makes the data presented more reliable compared to the mentioned study.

The refined distribution of *pI* values, ranging from 4.5 to 6.8, is also important in the context of allergenicity. As shown by Hosseinpour et al. (2020), proteins with acidic and slightly acidic isoelectric focus more often demonstrate stability in gastric conditions, which increases the likelihood of their sensitising effect. Yan et al. (2020), in turn, associated the *pI* range with the potential of proteins for transepithelial penetration and activation of dendritic cells. A comparison of these positions with the data obtained confirms that the *pI* spectrum of kiwi and mango proteins contributes to their high immunological activity. The data obtained on multiple reactivity recorded in 21.4% of patients can be explained by the presence of cross-reacting proteins such as profilins and LTPs. Castro-Almarales et al. (2020) previously demonstrated that the conservative spatial structure of these proteins can induce a reaction to several unrelated fruits. In turn, Kausar et al. (2022) proposed a model of epitope mimicry explaining multiple sensitisations without a common amino acid sequence. Comparing these mechanisms with the present results substantiates the clinical need for a molecular approach to diagnosis, including the use of component-orientated allergodiagnostic panels. Despite the data obtained, it is important to note that some pineapple and papaya proteins, such as bromelain and papain, may retain allergenic activity under conditions of occupational exposure. Sowers (2024), in a series of clinical observations, described cases of allergy in food industry workers associated with inhaled exposure to enzymatic extracts. Despite the fact that the present study focuses on food sensitisation, these results indirectly confirm the possibility of alternative routes of antigen intake, including aerosol and transdermal, especially in people with impaired skin barriers.

Thus, a comprehensive analysis combining electrophoretic, mass spectrometric and immunological methods established that kiwi and mango extracts pose the greatest clinical risk of sensitisation. Their protein components demonstrate resistance to degradation, high homology with known allergens, and the ability to induce *IgE* binding in a high percentage of cases. In contrast, papaya and pineapple are characterised by less stable proteins, reduced immunoreactivity, and limited clinical significance under nutritional conditions. These data confirm the relevance of a stratified approach to allergy diagnosis and prevention based on the molecular characterisation of proteins.

4. CONCLUSION

The study characterised the protein composition of extracts of four tropical fruits: kiwi, mango, pineapple, and papaya. The highest protein diversity was found in kiwi and mango, which was confirmed by the number of allowed spots in the 2D SDS PAGE (36 and 31, respectively). The main proteins of these fruits are represented by allergens with a molecular weight from 14 to 43 kDa and an isoelectric point in the range of 4.5-6.8. MALDI-TOF identification revealed the presence of proteins with a high degree of homology (68-95%). The preservation of their electrophoretic and immunoreactive properties after thermal and

enzymatic treatment confirmed the structural stability of these components. Immunological tests confirmed the clinical significance of kiwi and mango allergens (positive reactions in more than 80% of patients). Pineapple and papaya have shown limited immunoreactivity, mainly to enzymatic proteins, with an *IgE* binding frequency of less than 50%. A high correlation has been established between the biochemical stability of proteins and their clinical significance ($p=0.84$, $p<0.001$). Practical recommendations include the use of molecular markers (e.g., profilins and thaumatin-like proteins) in the development of diagnostic panels and personalised diets for patients with confirmed sensitisation. A promising area is a detailed assessment of the cross-reactivity of the identified proteins with other allergens of plant origin. A limitation of the present study was the lack of an *in vivo* evaluation of the response to extracts in sensitised patients. Additional skin and provocative tests are necessary to confirm the clinical significance of the identified proteins.

Conflict of Interest
The author declares no conflict of interest.

Author Contribution Statement
Maria Zofia Lisiecka: Conceptualisation, Methodology, Software, Data collection, Investigation, Visualisation, Writing original draft, Reviewing, and Editing,

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All data generated or analysed during this study are included in this published article.

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