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Structure and IgE Cross-Reactivity among Cashew, Pistachio, Walnut, and Peanut Vicilin-Buried Peptides

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A.C.Y.F., J.B.N., H.C., E.F.D., S.J.M., B.K.H., and G.A.M. designed experiments. A.C.Y.F., J.B.N., E.F.D., H.C., S.A.Y.G., and G.A.M. performed experiments. A.C.Y.F., S.A.Y.G., and G.A.M. designed and carried out statistical analysis. M.D.K., E.H.K., S.D., and S.M. collected patient sera. A.C.Y.F., J.B.N., S.J.M., and G.A.M. wrote the manuscript. All authors edited and approved the manuscript.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.2c07061.

Microarray peptides from Ara h 1, Ana o 1, Jug r 2, and Pis v 3 (Table S1); patient cohort information (Table S2); constructs used for the structures highlighting the helical regions in gray and the disulfide linkages with red lines (Figure S1); NMR structures of VBP allergens from cashew (AO1.1, AO1.2) and pistachio (PV3.1, PV3.2) (Figure S2); secondary structure determined from NMR (Figure S3); and NMR characterization of the VBP allergens (Figure S4) (PDF)

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Abstract

Peanut and tree-nut allergies are frequently comorbid for reasons not completely understood. Vicilin-buried peptides (VBPs) are an emerging family of food allergens whose conserved structural fold could mediate peanut/tree-nut co-allergy. Peptide microarrays were used to identify immunoglobulin E (IgE) epitopes from the N-terminus of the vicilin allergens Ara h 1, Ana o 1, Jug r 2, and Pis v 3 using serum from three patient diagnosis groups: monoallergic to either peanuts or cashew/pistachio, or dual allergic. IgE binding peptides were highly prevalent in the VBP domains AH1.1, AO1.1, JR2.1, and PV3.1, but not in AO1.2, JR2.2, JR2.3, and PV3.2 nor the unstructured regions. The IgE profiles did not correlate with diagnosis group. The structure of the VBPs from cashew and pistachio was solved using solution-NMR. Comparisons of structural features suggest that the VBP scaffold from peanuts and tree-nuts can support cross-reactivity. This may help understand comorbidity and cross-reactivity despite a distant evolutionary origin.

Keywords

peanut; tree-nut; allergen; vicilin-buried peptide

INTRODUCTION

Peanuts (PN) are among the most common food allergy encountered in Western European societies, with a prevalence rate of 1–2%.[1] Furthermore, peanut allergies are among the leading causes of near-fatal anaphylaxis in the United States, necessitating the use of strict avoidance strategies.[2] This management approach is complicated by the fact that peanut allergy is often comorbid with sensitization or allergy to tree-nuts with walnuts, pecans, cashews, and pistachios being the most common (30%).[3] This comorbidity could be facilitated by the presence of cross-reactive epitopes. Such epitopes would allow for a robust immunoglobulin E (IgE) response against a range of allergen sources, though the

large phylogenic distance between peanuts (legumes) and their tree-nut counterparts makes such an interaction unusual. Given the economic and social burden of peanut allergy,[4,5] quantifying the cross-reactivity potential of peanut/tree-nut proteins and the molecular basis through which this interaction occurs represents a key concern for the scientific community and would provide valuable insight into the design of immunotherapeutic strategies while facilitating allergen avoidance and mitigation approaches.

Vicilins are a family of cysteine-rich seed storage proteins found in most angiosperms, including many common food sources such as peanuts, legumes, fruits, and grains. The vicilin protein is expressed with an N-terminal leader sequence (LS), which contains a variable number of vicilin-buried peptides (VBPs) that are cleaved from the parent vicilin during the maturation process.[6] IgE-reactive VBPs have been identified in peanuts and tree-nuts such as walnuts, cashews, and pistachios.[7–9] The allergen vicilin with the most VBPs is the recently described hazelnut allergen Cor a 16 with a dozen.[10] Previous studies on the peanut LS that contains one VBP domain (AH1.1) revealed that IgE binding levels are uncorrelated to its larger parent vicilin, suggesting that these VBP sequences represent a new allergen family whose immunological properties are independent of their parent vicilins.[11]

VBPs are characterized by a common α-hairpin fold. Unlike other α-hairpin structures, the VBP structure is mediated almost entirely by disulfide bonds between highly conserved CxxxC motifs.[8,12,13] This unique architecture provides a common structural scaffold that can mediate IgE cross-reactivity between evolutionarily distant species. Indeed, previous studies identified the presence of several cross-reactive peanut/walnut epitopes among these VBP sequences.[8,14] Curiously, the patients assessed in these works displayed IgE binding to similar peptides in a microarray analysis regardless of clinical profile (e.g., peanut monoallergic, peanut-walnut dual allergic, etc.). This shared pattern of IgE reactivity suggests that VBPs could represent a common sensitizer with the potential to mediate both initial sensitization and cross-reactivity across different allergen sources.

In addition to peanuts and walnuts, IgE-reactive VBPs have been identified in various other nut species including almonds, cashew, and pistachio.[7,15,16] The latter two are an interesting study: cashews and pistachios generally have different cross-reactivity patterns of IgE binding compared to walnuts and other tree-nuts.[17,18] However, the shared VBP motif could potentially mediate cross-reactivity across these patient pools regardless of initial sensitizing agent. Thus, characterizing the IgE reactivity of the cashew and pistachio VBPs would uncover the role of VBP allergens in cross-reactivity, and the structural/biophysical basis for such an interaction. To this end, we examined the immunological properties of four VBPs from cashews (AO1.1, AO1.2) and pistachios (PV3.1, PV3.2), along with the previously identified VBPs from peanut (AH1.1) and walnut (JR2.1, JR2.2, and JR2.3) counterparts. Using peptide microarrays, we identified numerous pistachio, cashew, and peanut immunodominant IgE epitopes primarily on AH1.1, JR2.1, AO1.1, and PV3.1. To provide further insights into the biophysical basis for these differences, all four domains of cashew and pistachio VBPs were expressed recombinantly, and their structures were solved using solution-NMR to provide insight into the potential for cross-reactivity despite low sequence identity. Taken together, these studies provide valuable insights into the

specific molecular determinants of peanut, cashew/pistachio, and possibly walnut IgE reactivity and cross-reactivity, with implications for both allergic diagnostic testing and immunotherapeutic strategies.

MATERIALS AND METHODS

Microarray Data.

Overlapping 15-mers representing the complete leader sequences from vicilin allergens from peanut, cashew, walnut, and pistachio were printed onto peptide microarrays and assessed for IgE binding using sera from allergic patients. The procedure for measuring IgE binding was identical to that done previously.[8] Table S1 details the specific peptides from Ara h 1, Ana o 1, Jug r 2, and Pis v 3 that were used.

The median signal-to-noise ratio (SNR) for each peptide spot in the microarray was determined. Each SNR value was then converted into modified *z*-scores using the median and median absolute deviation (MAD).[19] Median and MAD were calculated for each patient and leader sequence combination. MAD was calculated in MATLAB using the constant of 1.4826 to approximate standard deviation. After calculating patient and VBP-specific median and MAD, *z*-scores were calculated by subtracting the median from a relevant spot SNR and dividing this value by the MAD. We defined a true IgE binding event as an SNR with a converted *z*-score ≥3.

Patient Cohort.

Serum from peanut and/or cashew/pistachio allergic patients were obtained from multiple clinics to assess IgE binding using microarray. A complete deidentified patient table showing known allergies and conditions is shown in Table S2. All experiments were performed in compliance with the Institutional Review Board at the respective institutions with regard to human donations.

Constructs and Purification.

The sequences for AO1.1, AO1.2, PV3.1, and PV3.2 were identified from the full-length allergen sequences of Ana o 1.0101 and Pis v 3.0101, respectively. According to WHO/IUIS, the allergens studied here should be annotated as Ana o 1.0101 (20–75), Ana o 1.0101 (82–132), Pis v 3.0101 (5–52), Pis v 3.0101 (56–115) (Figure S1). In this article, we abbreviated these as AO1.1, AO1.2, PV3.1, and PV3.2, respectively. The VBP domains AH1.1 from Ara h 1, JR2.1, JR2.2, and JR2.3 from Jug r 2 are similarly defined in Foo et al.[8] Sequences were inserted into the pDest expression system with an N-terminal glutathione *S*-transferase (GST) affinity tag separated from the main sequence by a tobacco etch virus (TEV) protease cleavage site. Plasmids were transformed into BL21 (DE3) *Escherichia coli* cells (Millipore, Burlington, MA), grown to an OD of ~0.8 in 2xYT media at 37 °C, and induced using 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) overnight at 16 °C. Uniformly ^{13}C–^{15}N-labeled samples were grown overnight in 1 L Luria Broth (LB), harvested, and subsequently transferred to M9 media with $^{15}NH_4Cl$ and ^{13}C-glucose as the sole nitrogen and carbon sources respectively. Cells were allowed to acclimatize to the new conditions for 1 h prior to induction.

VBPs were purified using protocols described previously.[8] In brief, GST-VBP was isolated from crude cell lyase using an immobilized glutathione column and eluted with 10 mM reduced glutathione in pH 7.4 phosphate-buffered saline (PBS). The native disulfides were reduced using 2 mM dithiothreitol (DTT) and the GST tag removed via an overnight incubation with TEV protease at 4 °C. The cleaved protein was isolated using a Superdex75 26/600 (Cytivia, Marlborough, MA) sizing column. The resulting protein was then incubated with 1 and 0.5 mM oxidized and reduced glutathione, respectively, to ensure the correct disulfide bonding pattern,[8,20] and exchanged into PBS using the Superdex75 26/600 sizing column to yield the final purified product. Protein concentrations were quantified using a BCA assay kit (Pierce Scientific, Rockford, IL).

NMR Structural Characterization.

NMR spectra were collected on 0.1–1 mM protein samples in PBS using either a 600 or 800 MHz Varian DD2 console equipped with cryogenically cooled probe. Amino acid assignments and nuclear Overhauser enhancement spectroscopy (NOESY) distance restraints were obtained using standard triple resonance techniques employing either the standard VARIAN Biopack or modified BEST-TROSY pulse sequences, with the former using a nonuniform sampling scheme with a 0.25 sampling rate.[21–23] Secondary structure was estimated from the available chemical shifts using the TALOS+ algorithm.[24] NOESY spectra in conjunction with the backbone and side-chain assignments were used to calculate the three-dimensional (3D) structure via the PONDEROSA server.[25–27] Structures of AO1.1, AO1.2, PV3.1, and PV3.2 are deposited in the PDB database as 7UV1, 7UV2, 7UV3, and 7UV4 respectively.

Biophysical Characterization.

Circular dichroism (CD) spectra were collected using a Jasco J-815 CD spectropolarimeter (Jasco, Easton, MD); 2 μM samples in PBS were loaded into a 1 cm pathlength cuvette and read at 25 °C with a scan rate of 20 nm/min and a total of four accumulations. Secondary structure prediction was carried out using the BESTSEL web server.[28,29]

In vitro digestion assays were carried out as described previously.[8] For gastric digestion, samples of VBP (25 μM) were prepared in simulated gastric fluid (30 mM NaCl, pH 2.0) and incubated at 37 °C in the presence of 0.16–3.2 mg/mL pepsin from porcine gastric mucosa (Sigma, St. Louis, MO). To simulate intestinal digestion, 25 μM samples of VBP were prepared in digestion buffer (10 mM Tris pH 7.8, 100 μM sodium azide).[30,31] Digestion was initiated by the addition of porcine trypsin (Sigma, St. Louis, MO) to a final concentration of 1.15 μg/mL. Simulated endosomal degradation was carried out using a 25 μM sample of VBP in endosomal buffer (75 mM citrate pH 5.4, 25 mM NaCl, 1 mM DTT). Digestion was initiated with the addition of 0.25 U cathepsin S (CatS) (Human, recombinant- EMD Millipore, Burlington, MA). In all assays, samples were removed at fixed intervals and quenched using 4× sodium dodecyl sulfate (SDS) buffer containing 0.1 mM NaOH (pH 11) and 2 mM DTT. Loss of the initial VBP substrate was monitored using SDS-polyacrylamide gel electrophoresis (PAGE). The intensity of the SDS-PAGE band corresponding to the initial substrate was quantified using ImageJ,[32] and the resulting data

were fit to an exponential decay function from which a predicted half-life ($t_{1/2}$) of digestion could be obtained.

RESULTS

Microarray Analysis of Peanut and Tree-Nut Leader Sequences.

The leader sequences from peanut, walnut, cashew, and pistachios were assessed for their ability to bind IgE from PN, PN&CP, or CP-allergic patients. The resulting IgE-reactive peptides are depicted in Figure 1A. Numerous IgE-reactive peptides were identified primarily in VBPs AH1.1, AO1.1, JR2.1, and PV3.1 (Figure 1). These peptides appeared to be clustered around specific regions within the VBP, potentially indicating the presence of common IgE epitopes. Conversely, there was very little IgE binding to AO1.2, JR2.2, JR2.3, and PV3.2, nor was there a high prevalence of IgE binding outside the VBP domains. The putative VBP epitopes appear to be broadly similar across all three patient groups (Figure 1B). Likewise, the lack of IgE binding to AO1.2, JR2.2, JR2.3, and PV3.2 was also independent of patient diagnosis group. While minor differences such as IgE binding to the C-terminal region of PV3.1 might occasionally differentiate PN patients from their dual and tree-nut-sensitized counterparts, the overall pattern of VBP peptide recognition between the three patient groups shows more similarities than differences. This observation can be extended to the walnut VBPs: While the walnut-allergic status of the patients employed in this study was not assessed, all patient groups showed similar patterns of IgE reactivity against JR2.1, but not JR2.2 nor JR2.3, further reinforcing the uniformity of the IgE response.

Structural Characterization of Cashew and Pistachio VBPs.

To provide further insight into the physiochemical properties which might mediate cross-reactivity, the structure of all four cashew and pistachio VBPs was solved using solution-NMR. All four VBPs adopted the characteristic α-hairpin fold with two α-helices connected by a turn (Figures S2 and S3). As with their peanut and walnut counterparts, a paucity of long-range distance restraints was observed (Figure S4) relative to other small globular proteins, suggesting that the VBP fold is primarily maintained by disulfide linkages between adjacent CxxxC motifs.[8] Circular dichroism experiments on the VBPs confirm this hypothesis: CD spectra of the intact VBP show minima at 220 and 210 nm indicative of a predominantly α-helical structure (Figure 2A). Reduction of these disulfides with tris(2-carboxyethyl)phosphine (TCEP) altered the shape of the CD spectra, with an increase in the CD spectra at 220/210 nm a new minima emerging at 205 nm indicative of a loss of α-helical content and an increase in random coil. The secondary structure content of the various VBPs obtained from the CD spectra is shown in Figure 2B, illustrating the scale of the α-helix–random coil conversion.

To assess the role of VBP structure in IgE reactivity, the prevalence of peptide binding was used to color the VBP structures to identify regions of common IgE recognition. Figure 3 shows that helix 2 and adjacent residues were commonly recognized in AH1.1, JR2.1, AO1.1, and PV3.1. Helix 1 was commonly recognized primarily in JR2.1 and AO1.1. In contrast, AO1.2 and PV1.2 had a very low prevalence of peptide binding, similar to JR2.2

and JR2.3 (not depicted, see Figure 1B). To assess if these regions were likely to support cross-reactive epitopes, the surfaces of the structures were colored based on a comparison of the physiochemical properties of residues in similar positions (Figure 4). The property distance (PD) metric was utilized instead of the more common Blossum matrix, which is based on evolutionary changes.[33] These colorings show that there are regions of substantial residue similarity that might support cross-reactive epitopes. To provide a comprehensive metric, a surface area similarity (SAS) was calculated (Figure 4).[34,35] These SAS values are substantially higher than one might expect from a comparison with the percent sequence identity suggesting that cross-reactivity is indeed viable;[36] see the Discussion section.

An explanation for the high SAS may be that VBPs come from a surprisingly limited distribution of amino acids. Indeed, seed storage proteins typically contain more nitrogen-containing side chains reflecting their role in germination. Figure 5 plots the normal distribution of amino acids in nonmembrane plant proteins compared to the vicilin domains and leader sequences.[37] If we compare the amino acid content of the vicilin domains of Ara h 1, Ana o 1, Jug r 2, and Pis v 3 with the normal distribution both pie charts display a wide variety of amino acids (Figure 5A,B). There are few changes, perhaps reflecting the mostly β sheet nature of vicilin domains. However, looking at the distribution of amino acids in the leader sequences of the same allergens, there is clearly a narrower distribution of content with 46% of the content dominated by arginine, glutamine, and glutamate compared to 22% in the vicilins, and 16% in nonmembrane plant proteins. Finally, we note that in the VBPs, the large increases in C, E, Q, K, and R are at the expense of the hydrophobic amino acids (L, I, F, W, V, M, A, G, and P), which all show decreases in Figure 5D. These typically reside in the core of “normal” globular proteins providing stabilizing hydrophobic interactions. The lack of these residues in the VBPs is consistent with our observations that the reduction of the disulfide bonds leads to unfolding of the VBPs. This unusual amino acid distribution coupled with the structural confines of the VBP fold restricts the structure space available to these VBP’s, potentially contributing to cross-reactivity despite their low sequence identity.

Proteolytic Resistance of AO and PV VBP Domains.

Previous studies identified resistance to proteolytic cleavage, particularly cathepsin S as a potential determinant of allergenicity among peanut and walnut VBPs.[8] To determine whether the immunogenicity of the cashew and pistachio equivalents follows a similar trend, all four VBPs were subjected to simulated gastric, duodenal, and endosomal digestion (Figure 6). Of these, only AO1.1 displayed significant resilience to all three modes of digestion. It should be noted that the other sequences displayed significantly lower resistance to gastric/duodenal digestion even when compared to their peanut/walnut counterparts ($t_{1/2}$ > 100 minutes), potentially preventing significant exposure to the immune system.[8]

DISCUSSION

In this work, we provide a comprehensive biochemical study of the VBPs in the Anacardiaceae family. The ability of IgE to recognize sequences from both peanut and cashew/pistachio peptides suggests that these VBPs can both act as a potent initial

sensitizing agent in pistachio/cashew-allergic patients and mediate the cross-reactive binding of IgE generated against VBPs from other allergen sources such as peanuts and other tree-nuts. While the walnut-allergic status of the patients employed in this study was not assessed, its high rate of co-allergy with peanuts/other tree-nuts makes it likely that most of the patients tested will have at least some sensitization to walnuts. Indeed, peptides from the walnut VBP JR2.1 were recognized by all three patient groups (Figure 1A,B). Remarkably, the overall pattern of peptide recognition closely mirrors those observed in previous studies on peanut/walnut-allergic patients,[8] despite being derived from a separate patient cohort with different allergic diagnoses. Taken together, these studies reinforce the role of VBPs, particularly the first VBP of walnuts, cashews, and pistachios, in mediating possible cross-reactivity across peanuts and different tree-nuts. The molecular structure of all four VBPs reveals a shared α-hairpin fold with high levels of surface similarity with other previously reported VBP structures.[8,13] The unique disulfide-mediated architecture of these VBP sequences allows them to support a conserved structural motif with cross-reactive epitopes even in the absence of high sequence identity, potentially contributing to their cross-reactivity.

There is considerable literature on the biophysical properties of allergens that may correlate with sensitization.[38,39] One hypothesis is that stability in food allergens confers resistance to gastrointestinal digestion, facilitating the presentation of allergen to the immune system as intact protein with native B-cell epitopes. Stability also confers resistance to endosomal digestion. This reduces the rate of T-cell presentation on major histocompatibility complex (MHC) molecules, which correlates with increased Th2 sensitization.[40,41] Both forms of stability have been shown to play a role in the sensitization process across several allergen families.[38,39] In this work, AO1.1 displayed both the highest resistance to gastric digestion and the highest IgE binding prevalence. In contrast, endosomal digestion did not appear to correlate with IgE binding among the VBPs tested. This contrasts with previous studies on the peanut and walnut VBPs, where the rate of endosomal digestion was a better predictor of IgE binding.[8] While sensitization potential and allergenicity were not directly assessed in this work, these observations along with the stability relationships described elsewhere in the literature suggest that cashew/pistachio VBPs represent an independent sensitizing agent separate from the peanut/walnut counterparts, with different determinants of immunogenicity. This could potentially result in serological differences, which differentiate allergic patients based on their initial sensitizing agent.

Predicting cross-reactivity between proteins is a surprisingly difficult problem. Typically cross-reactivity would be suspected when the sequence identity between proteins is greater than 70% and rare when it is less than 50%.[42] Current World Health Organization guidelines conservatively suggest sequence identity >35% should be tested for cross-reactivity in novel food products. Comparing the VBPs from peanuts and tree-nuts (Figure 4), the sequence identity is clearly below even this threshold. We also compared the VBPs with A-RISC index by Chruszcz et al. that estimates cross-reactivity between allergens in the same protein family using a combination of similarity and identity scores.[43] However, it also did not suggest a high risk of cross-reactivity. Hence, we sought a different metric that might include structural comparisons.

Other attempts to use structure and surface area properties to predict cross-reactivity include the SPADE algorithm[44,45] and the surface area similarity (SAS) metric.[35] In the case of SAS, there are some calibrated comparisons with clinical cross-reactivity. For example, Der p 1 and Der f 1 have numerous reports of cross-reactivity[46,47] and measurements from their structures show SAS scores of 0.86.[35] The cyclophilin allergens Mala s 6 and Cat r 1 were suggested to be cross-reactive,[48] and the SAS score was 0.75. On the other hand, the GST allergen from cockroach (Bla g 5) was not cross-reactive with the GST allergens from mites (Der p 8, and Blo t 8) which had SAS scores of 0.47 and 0.46, respectively.[35] Therefore, the SAS comparisons for the VBP allergens with dominant IgE peptides (AH1.1, AO1.1, JR1.1, PV3.1), which range from 0.57 to 0.61 should be considered as in a gray area where further testing is justified. The fact that the scores are so high is more remarkable when one considers that most of the sequence identity is dominated by the core cysteine residues, which are primarily buried and do not contribute much to the accessible surface. These observations, coupled with the near-universal prevalence of the IgE peptide recognition regardless of patient allergy profile and the ubiquitous nature of VBP sequences across many food sources demonstrate the need for increased testing for VBP reactivity and cross-reactivity when assessing food allergens for both patient diagnosis and allergen avoidance strategies.

The structural and immunochemical findings presented here suggest that VBPs are an important source of both IgE-reactive and cross-reactive epitopes. However, further studies are needed to fully uncover the clinical implications of these findings. For instance, IgE cross-reactivity at the molecular level may not result in clinical symptoms—a phenomenon perhaps best illustrated by the cross-reactive carbohydrate determinants, which confounded plant allergy diagnosis for many years.[49] It is also possible that some patients are simply co-allergic to different allergen sources and competition experiments may help resolve this issue. Finally, IgE binding to allergens frequently does not correlate well with symptom scores in food allergy.[50] Nonetheless, it is becoming increasingly evident that plant VBPs represent an important immunogenic species that can mediate both allergic sensitization and cross-reactive IgE binding across a range of common food sources including peanuts and tree-nuts. Recent works identifying IgE-reactive sequences on VBPs from macadamia nuts, sesame, and almonds[7,10,15,51] further emphasize their immunological potential. The structural and immunological data presented in this work suggests that VBPs from cashews and pistachios could mediate both cashew/pistachio allergy and cross-reactivity with peanut and other tree-nut counterparts via a conserved hairpin motif, with implications for both molecular diagnostic and allergen avoidance strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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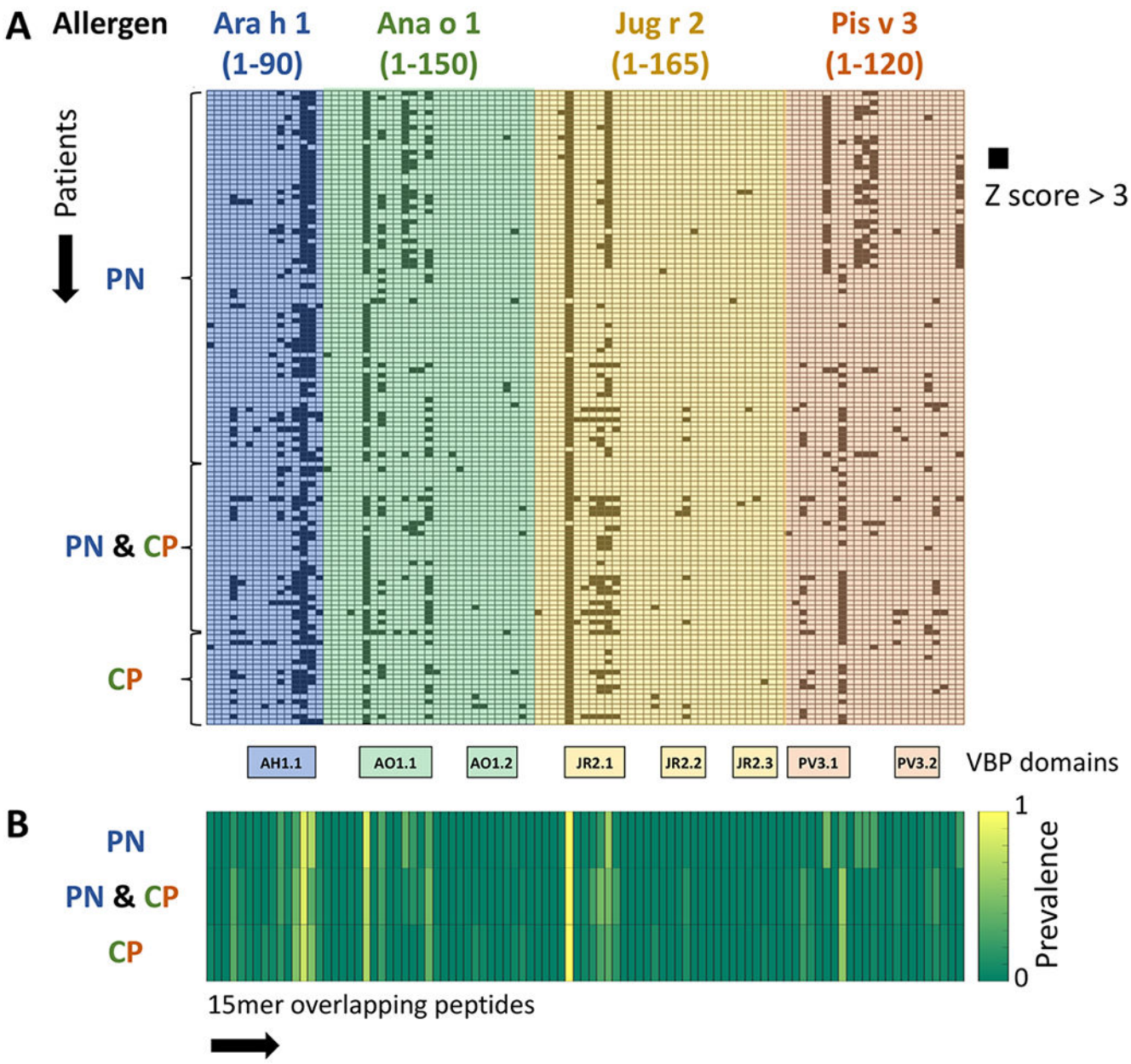

Figure 1.

Microarray analysis of IgE binding to the leader sequence of vicilin allergens Ara h 1, Ana o 1, Jug r 2, and Pis v 3. (A) IgE binding of individual patients against VBP peptides. Squares representing individual peptides examined in this work are arrayed along the *x*-axis. Peptides are color-coded by source organism. The identified VBP domains are represented in the boxes below. A table showing the full sequence of the individual peptides is shown in Table S1. Individual patients are arrayed on the *y*-axis and are grouped according to diagnoses: peanut monoallergic (PN), peanut and either cashew or pistachio (PN&CP), cashew or pistachio (CP). Patient data are in Table S2. Peptides that displayed significant IgE binding, defined as a microarray signal more than 3 standard deviations from the mean

using a modified *z*-score for each patient, are highlighted. (B) Prevalence of IgE binding to each peptide by patient group in a heatmap colored from green (0%) to yellow (100%).

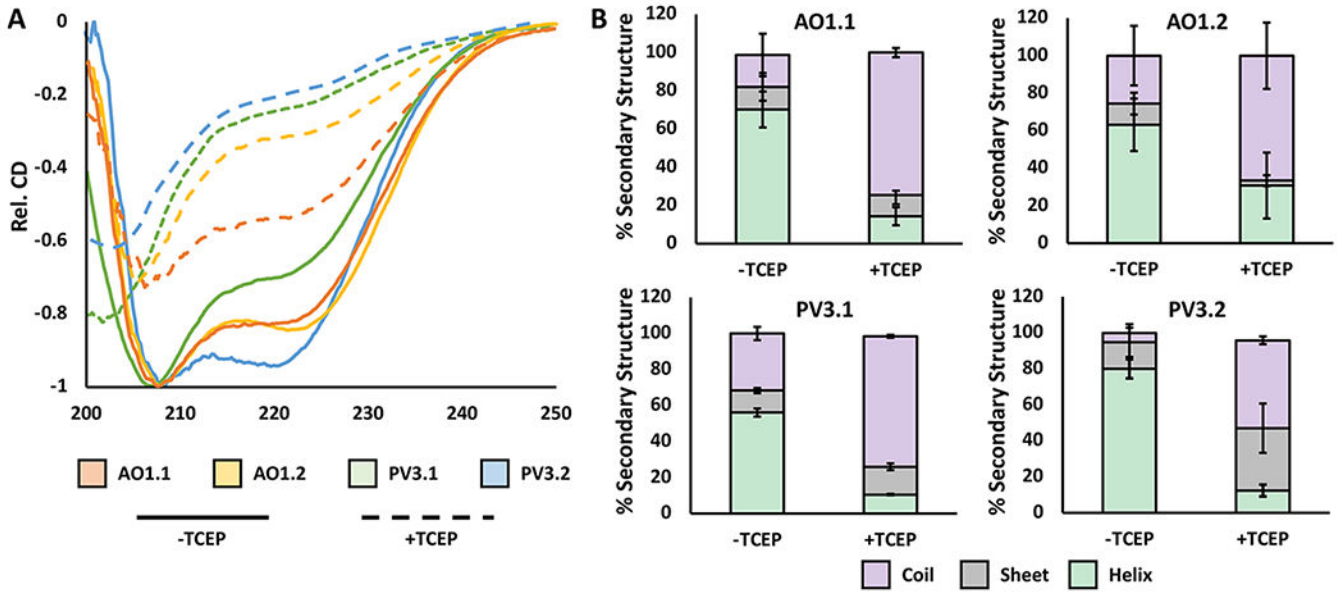

Figure 2.

Secondary structure of VBP's. (A) CD spectra of the VBP domains before and after treatment with TCEP. (B) Secondary structure prediction based on the CD spectra in (A).[28,29]

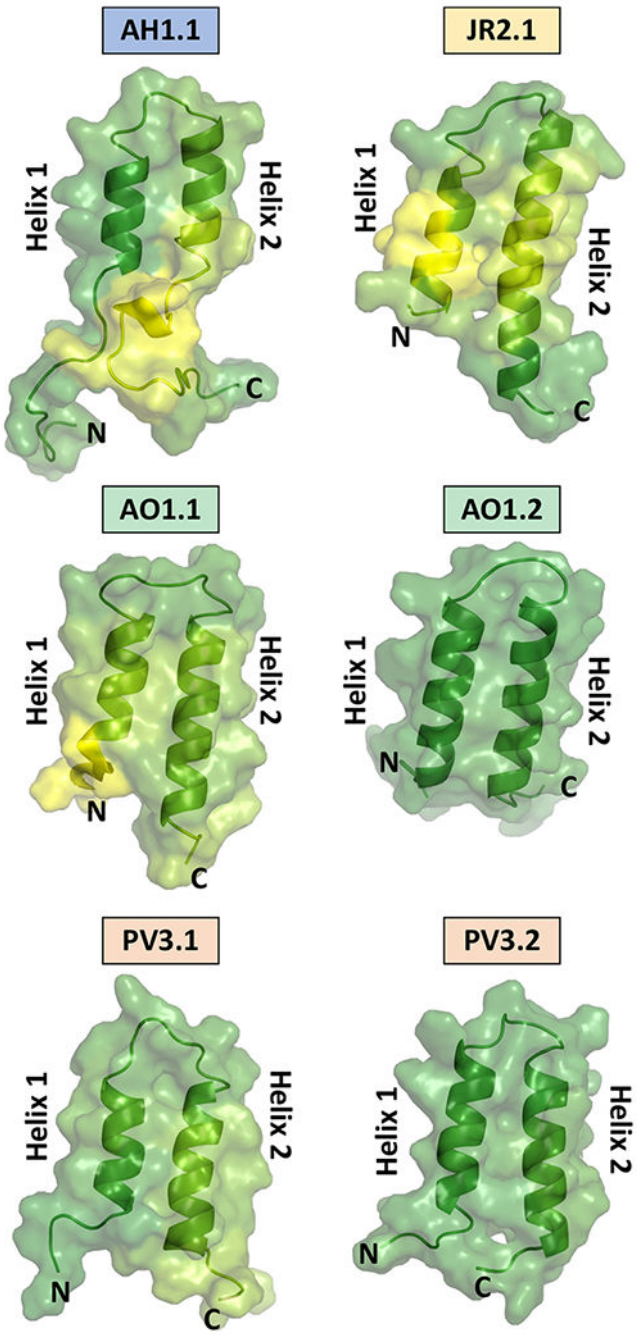

Figure 3.

Prevalence of IgE peptide binding mapped to VBP structures. VBP domains AH1.1 (7LXK), JR2.1 (7LVF), AO1.1 (7UV1), AO1.2 (7UV2), PV3.1 (7UV3), and PV3.2 (7UV4) were colored from 0% (green) to 100% (yellow) prevalence of IgE peptide binding similar to Figure 1B. A color scale was calculated for the central five residues of each 15 mer, weighted by 1/5 for each adjacent 15 mer, and normalized for the whole VBP from 0 to 1. Structures shown are ribbon diagrams with semitransparent surface rendering. N and C termini are annotated.

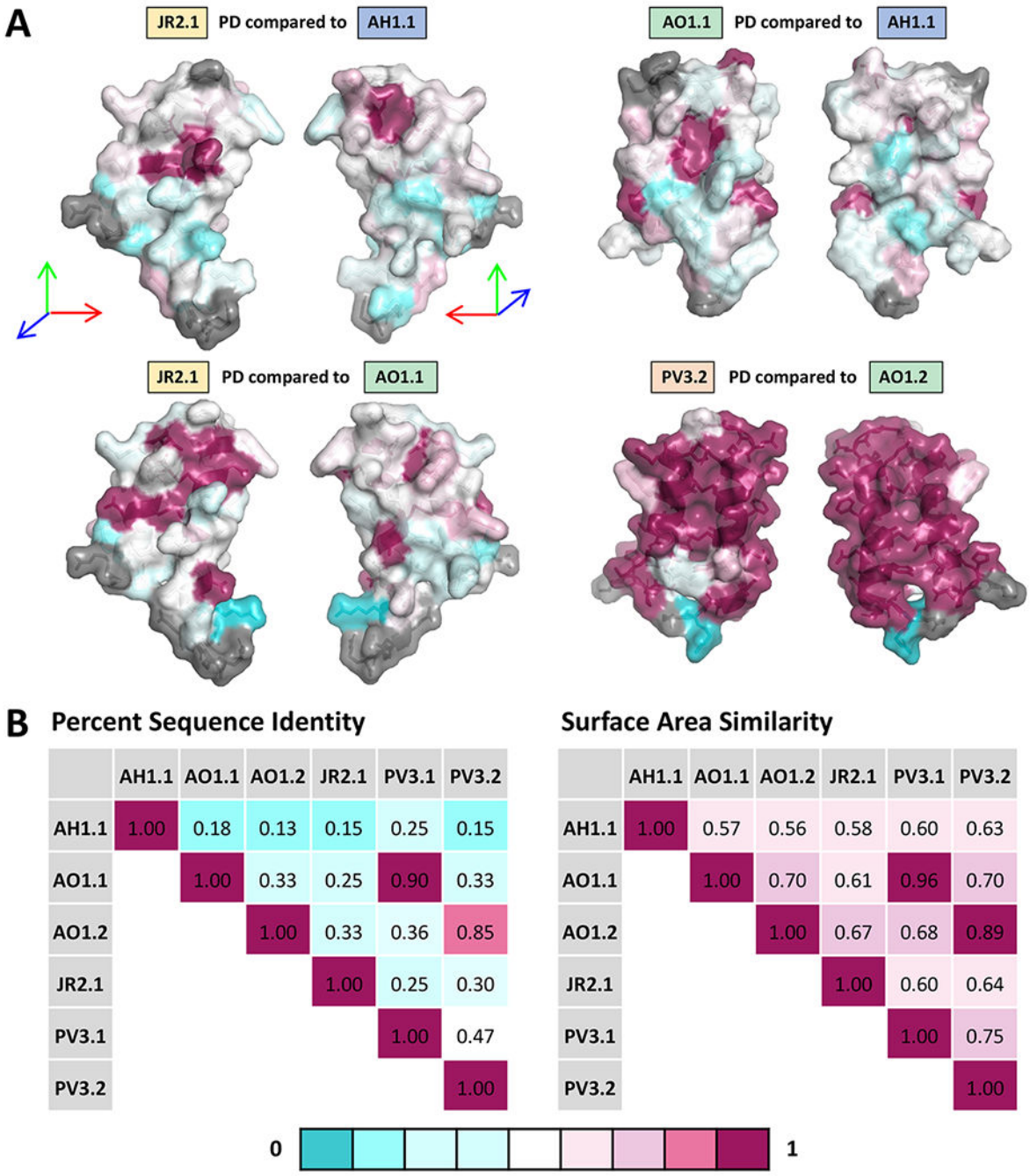

Figure 4.

Surface similarity compared to sequence identity. (A) Surface rendering of various VBPs colored for residue similarity using PD scores to another VBP as indicated in the panel. The color scale is shown at the bottom where 0 is the least similar and 1 is identical. Gray coloring indicates residues that do not align between the two VBPs either due to structural distance or a sequence insertion. (B) Comparison of the sequence identity of six VBPs with the surface area similarity (SAS). SAS is a normalized score from 0 to 1 where the accessible surface area is weighted by the PD comparison of residues in similar positions.

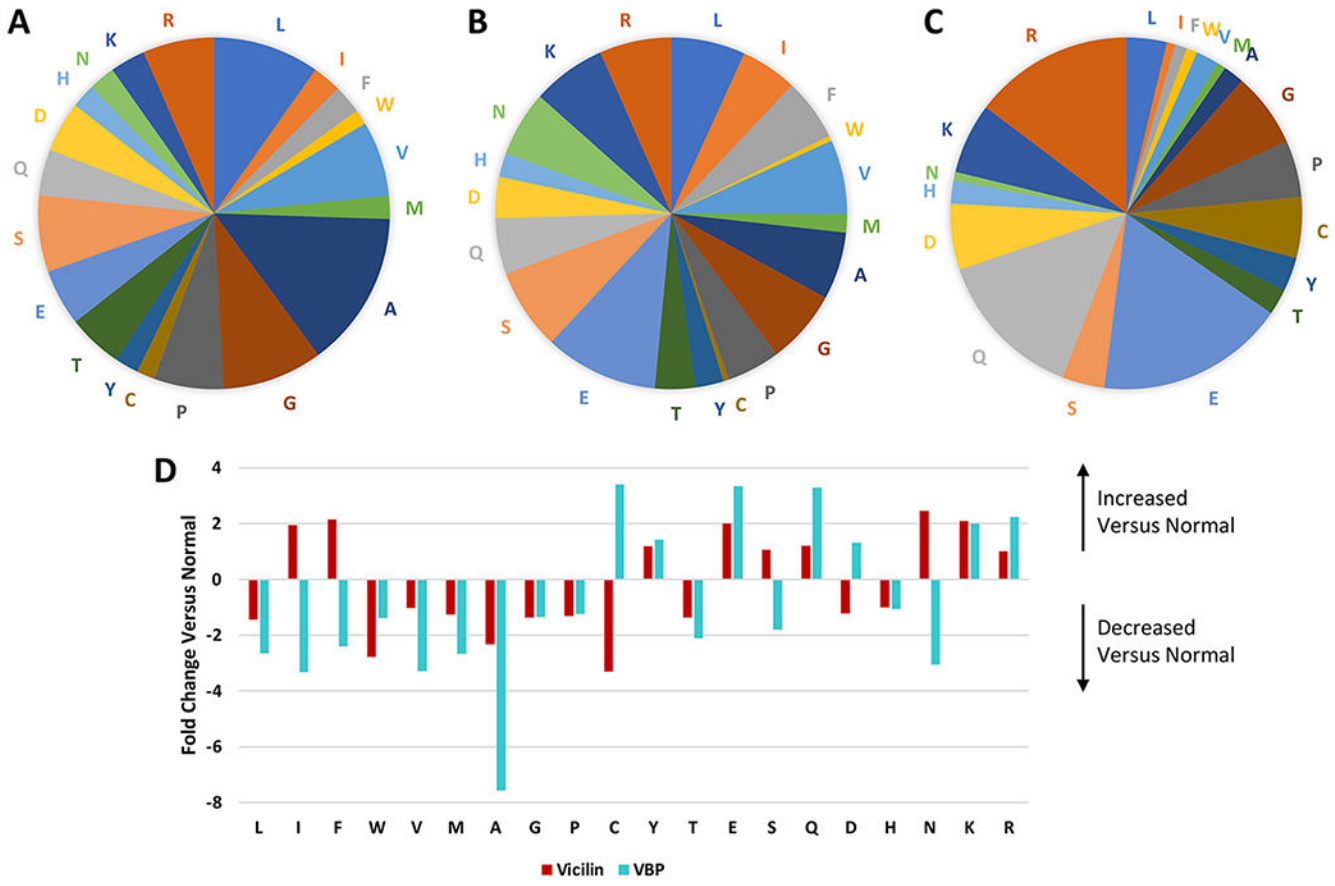

Figure 5.

Relative distributions of amino acids in plants and vicilins. (A) Plants, nonmembrane proteins.[37] (B) Vicilin domains of Ara h 1, Ana o 1, Jug r 2, and Pis v 3. (C) Leader sequences of Ara h 1, Ana o 1, Jug r 2, and Pis v 3. (D) Bar graph showing the fold-change in the individual amino acid levels of the vicilins and leader sequences shown in (B) and (C) relative to the plant nonmembrane proteins (A).

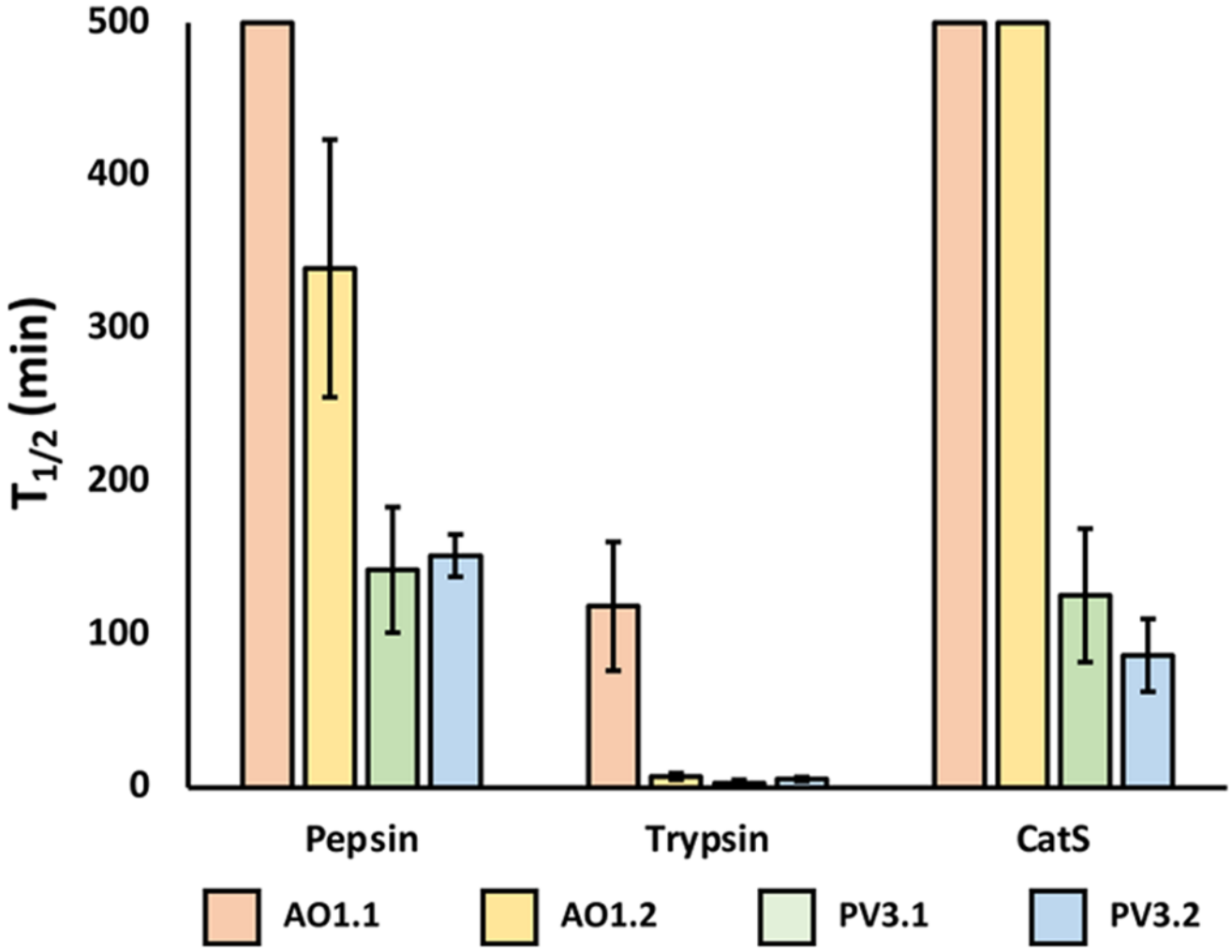

Figure 6.

Simulated endosomal, duodenal, and endosomal digestion of cashew and pistachio VBPs. $T_{1/2}$ in minutes of the simulated digestion using pepsin, trypsin, and cathepsin S (CatS) respectively, as assessed from staining of SDS-PAGE gels for each of the indicated VBPs.