

Claims

What is claimed:

1. A method for determining a signature of peanut allergens in an aqueous medium comprising:

5 digesting peanut allergens present in an aqueous medium to generate allergen digest products;

fragmenting the allergen digest products to generate peptide fragments; and

determining the signature of allergen digest products, wherein the signature comprises Ara h1, Ara h2 and Ara h6 digest products.

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2. The method of any one of claims 1, 8, 13 or 16, wherein the aqueous medium is a dissolution medium, a release medium or an analytical sample.

3. The method of any one of claims 1, 8, 13 or 16, wherein the amount of peanut allergens
15 in the medium is less than 2 µg/ml, less than 1.5 µg/ml, less than 1 µg/ml, or less than 0.5 µg/ml.

4. The method of claim 1 or 8, further comprising the step of comparing the signature to a signature standard.

20 5. The method of claim 1, 8, 13 or 16, wherein the allergen digest products are between 4 amino acids and 50 amino acids in length, between 6 amino acids and 30 amino acids in length, or between 15 amino acids and 20 amino acids in length.

6. The method of claim 1 or 8, wherein the steps of fragmenting the allergen digest products
25 and determining the signature are performed by a method selected from the group consisting of one or any combination of LC-MS, LC-MS-MS, nano-LC-MS-MS, and nanoHPLC-MS-MS.

7. The method of any one of claims 1, 8, 13 or 16, wherein the allergen digest products comprise an amino acid sequence selected from the group consisting of SEQ ID NO:17, SEQ ID NO:70, SEQ ID NO:177, SEQ ID NO:155, SEQ ID NO:93, SEQ ID NO:40, SEQ ID NO:197, SEQ ID NO:198, SEQ ID NO:199, SEQ ID NO:200, SEQ ID NO:201, SEQ ID NO:236 and SEQ ID NO:237.

8. A method for determining a signature of peanut allergens in an aqueous medium comprising:

digesting peanut allergens present in an aqueous medium to generate allergen digest products;

fragmenting the allergen digest products to generate peptide fragments; and

determining the signature of allergen digest products, wherein the signature comprises Ara h1, Ara h2, Ara h3 and Ara h6 digest products.

9. The method of claim 1 or 8, wherein the allergen digest products are present in a majority of isoforms of Ara h1, are present in at least 90% of isoforms of Ara h1, are present in all isoforms of Ara h1, are present in a majority of isoforms of Ara h1, Ara h2 and Ara h6, or are present in a majority of isoforms of Ara h1, Ara h2, Ara h3 and Ara h6.

10. The method of claim 1 or 8, wherein the peanut allergens are digested with one or more proteases selected from the group consisting of trypsin, endoproteinase Lys-C and endoproteinase Arg-C.

11. The method of any one of claims 1, 8, 13 or 16, wherein the aqueous medium further comprises an internal standard that optionally comprises one or more heavy isotopes.

12. The method of any one of claims 1, 8, 13 or 16, wherein the signature comprises allergen digest products that do not contain missed proteolytic cleavage sites.

13. A method for determining an *in vitro* release profile of an aqueous medium comprising peanut allergens comprising:

obtaining a sample from the aqueous medium at each of a plurality of time points;

digesting the peanut allergens present in the samples to generate allergen digest products;

fragmenting the allergen digest products to generate peptide fragments; and

detecting the peptide fragments for at least two of the plurality of time points to identify the allergen digest products, thereby determining the *in vitro* release profile of the peanut allergens, wherein the release profile comprises Ara h1, Ara h2 and Ara h6 digest products.

14. The method of claim 13 or 16, wherein the aqueous medium is a dissolution medium, a release medium or an analytical sample.

15. The method of claim 13 or 16, wherein the steps of fragmenting the allergen digest products and detecting the peptide fragments are performed by a method selected from the group consisting of one or a combination of Liquid Chromatography-tandem Mass Spectroscopy (LC-MS-MS), nanoLC-MS-MS, and nano High Performance Liquid Chromatography-tandem Mass Spectroscopy (nanoHPLC-MS-MS).

16. A method for determining an *in vitro* release profile of an aqueous medium comprising peanut allergens comprising:

obtaining a sample from the aqueous medium at each of a plurality of time points;

digesting the peanut allergens present in the samples to generate allergen digest products;

fragmenting the allergen digest products to generate peptide fragments; and

detecting the peptide fragments for at least two of the plurality of time points to identify the allergen digest products, thereby determining the *in vitro* release profile of the peanut allergens, wherein the release profile comprises Ara h1, Ara h2, Ara h3 and Ara h6 digest products.

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17. The method of claim 13 or 16, wherein the allergen digest products are present in a majority of isoforms of Ara h1, are present in at least 90% of isoforms of Ara h1, are present in all isoforms of Ara h1, or are present in a majority of isoforms of Ara h1, Ara h2 and Ara h6.

10 18. The method of claim 13 or 16, wherein the peanut allergens are digested with one or more proteases selected from the group consisting of trypsin, endoproteinase Lys-C and endoproteinase Arg-C.

15 19. The method of claim 13 or 16, wherein the composition comprises one or both of nanoparticles and microparticles.

20. The method of claim 13 or 16, wherein the release profile is obtained over a three-hour period of time, over a six-hour period of time, over a twelve-hour period of time, or over a twenty-four hour period of time.

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