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Supporting Information

Influence of peanut matrix on stability of allergens in gastric-simulated digesta: 2S albumins are main contributors to the IgE-reactivity of short digestion resistant peptides

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Running Title: Gastric digesta of peanut reveals the highest IgE reactivity to 2S albumin peptides.

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Abbreviations

1D – one dimensional

2D – two dimensional

CD – circular dichroism

CPS – control peanut sample

cCBB – colloidal Coomassie Brilliant Blue

CHAPS – 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate

DPS – digested peanut sample

DTT – dithiothreitol

ELISA – enzyme-linked immunosorbent assay

FDR – false discovery rate

IAA – iodoacetamide

IPG – immobilised pH gradient

nLC-MS/MS – nano-liquid chromatography coupled to tandem mass spectrometry

PBS – phosphate buffered saline

SDRPs – short digestion resistant peptides (<10 kDa)

Tris – tris(Hydroxymethyl)aminomethane

Methods

Materials

α-Amylase from human saliva (EC 3.2.1.1; A0521-500 UN; Type IX-A, lyophilized powder 1000–3000 U/mg protein) and porcine pepsin from gastric mucosa (EC 3.4.23.1; P6887-1G, lyophilized powder 3200–4500 U/mg protein) were purchased from Sigma–Aldrich (St. Louis, MO, USA). The enzyme activities were measured according to the assays detailed by Minekus et al. [1]. Chemicals for gel electrophoresis as Tris(Hydroxymethyl)aminomethane (Tris), glycine, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), urea, thiourea, dithiotritol (DTT), dimethylformamide, acrylamide, bis-acrylamide, trichloroacetic acid (TCA), Coomassie Brilliant Blue R-250 (CBB), and iodoacetamide (IAA), sequencing grade trypsin, formic acid, and acetonitrile of HPLC grade were also purchased from Sigma-Aldrich. Ampholytes and immobilised pH gradient (IPG) strips were supplied by GE Healthcare (Uppsala, Sweden). All other chemicals were of the analytical reagent grade, and Milli-Q water (18 MΩ cm at 25 °C) was used (Millipore, Bedford, MA, USA) in all the experiments.

Simulated oral and gastric in vitro digestion conditions

Oral phase: Solid milled peanut (0.4 g) was mixed with 320 μL SSF stock solution. Human salivary α-amylase (40 μL, 1500 U/mL in water) was added to achieve a final concentration of 75 U/mL in the digestion mixture, followed by addition of $CaCl_2$ (40 μL, 15 mM) to achieve final concentration of 0.75 mM. The reaction mixture was incubated for 2 minutes at 37 °C with agitation. All reagents were previously pre-warmed at 37 °C for 5 minutes. Controls without peanut (solid peanut replaced by sand) and controls without amylase (amylase replaced by water) were also included.

Gastric phase: Complete oral phase material was mixed with 400 μL of SGF stock solution and 8 μL of $CaCl_2$ (15 mM) to achieve a final concentration of 75 μM in the digestion mixture. Porcine pepsin (320 μL; 10,000 U/mL 10 mM HCl) was added, to achieve a final concentration of 2000 U/mL in the digestion mixture. The mixture was adjusted to pH 3 with 1 M HCl, then water was added, such that the final volume of reaction mixture was 1600 μL. The reaction mixture was incubated for 120 minutes at 37 °C with intense agitation (600 rpm). Control samples were run in parallel: pepsin control (oral bolus without amylase with addition of 160 μL 10 mM HCl instead of pepsin solution) at 0` (P0) and 120` (P120), and

peanut control (with 0.4 mL of SSF stock solution and 0.4 g of sand instead of oral bolus) at 120` (C120). Digestion was stopped by addition of 200 μL 1 M NaHCO3 to change the pH of the final reaction mixture to 8. The samples were centrifuged at 10,000 *g* for 20 minutes; the liquid phase was separated from solid material and immediately frozen at −20 °C. Protein concentration was determined using BCA assay (Thermo Fisher Scientific Inc., Bremen, Germany) after diluting the liquid phase of digestion mixtures 20 times in phosphate buffered saline (PBS).

Identification of digested peanut proteins

Identification of peanut proteins was performed by PEAKS Studio 8.5 (Bioinformatics Solutions Inc., Canada). Signature MS/MS spectra were searched using PEAKS DB algorithm against a hybrid database consisting of a UniprotKB/Swiss-Prot (reviewed only) peanut (*Arachis hypogaea*) database (downloaded on 14/08/2017 from http://www.uniprot.org/) and cRAP (The common Repository of Adventitious Proteins) database (downloaded on 18/01/2017 from http://www.thegpm.org/crap/). The following modifications were taken into account as variables: oxidation (Met), deamidation (Gln, Asn), and hydroxylation (Pro), while carbamidomethylation (Cys) was set as fixed. Up to 2 missed cleavages with non-specific cleavage at both ends of a peptide were allowed. Mass tolerances were set to ±10 ppm for parent ions and ±0.5 Da for fragment ions. Protein filters were as follows set to a one unique peptide and -10logP of value 20. Peptide filters were as follows: input of -10logP for Peptide-Spectrum Matches (PSM) was the lowest values securing less than 0.5% of resulting peptide sequence FDR and 0% FDR at protein level and de novo ALC Score ≥ 80%.

1D SDS-PAGE was performed on a 14% gels according to Laemmli method [2], stained with CBB. Dried TCA/acetone protein pellets from liquid portion of gastric-simulated digesta were re-suspended in Laemmli sample buffer (reducing and non-reducing conditions). Isoelectrofocusing and 2D SDS-PAGE were done as per method of Apostolovic et al. [3]. Briefly, dried TCA/acetone pellets were re-suspended in isoelectrofocusing rehydration buffer (8 M Urea, 2% CHAPS, 0.5% IPG buffer 3-10NL, 50 mM DTT, and 0.002% bromophenol blue). Protein samples (250 μg) were applied on 13 cm; pH 3–10, nonlinear IPG strips (GE Healthcare, Uppsala, Sweden). Isoelectrofocusing was done with Ettan IPGphor system (GE Healthcare) and strips were reduced with DTT, and alkylated with IAA according to the method of Apostolovic et al. [3]. The second dimension was carried out on 14% gels, and protein spots were visualized with colloidal CBB staining. The 2D gels were

scanned with Typhoon FLA 7000 (GE Healthcare) and spots were quantified and matched with Image Master 2D Platinum software v7.0 (GE Healthcare).

Separation of SDRPs obtained after gastric-simulated digestion and their analyses with Orbitrap shotgun peptidomics identification

Ethanol (2.4 mL) was added to 800 µL of liquid phase separated from the digestion mixture and incubated at 4 °C for 20 hours. After centrifugation at 4 °C and 12,000 *g* for 10 minutes, the supernatant containing the released SDRPs was separated and dried in a vacuum concentrator in low binding tubes. The dried peptides were dissolved in 500 µL of 10 mM HCl and subjected to size-exclusion chromatography. The Sephadex G25 column (0.8 × 30 cm) was equilibrated, and the separation was carried out with 10 mM HCl at a flow rate of 5 mL/h at room temperature. Fractions of 500 µL were collected, and the separation was monitored by ultraviolet absorption at 214 nm, 280 nm, and 220 nm (Figure S1). To minimize low molecular mass species other than peptides (such as polyphenols), fractions with highest absorbance values at 214 nm and lowest absorbance values at 280 and 340 nm (fractions 8–20 Figure S1) were pooled, and were analysed by electrophoresis and immunoblotting with Ara h 2 antibodies to confirm the absence of intact allergens. They were then divided into two parts. One part was concentrated 4 times on SpeedVac (Eppendorf, Hamburg, Germany) and used for the ImmunoCAP inhibition assay. The second part was evaporated, and then subjected to nLC-MS/MS analysis as intact or pre-treated by reduction, alkylation, and trypsin digestion according to the method of Johnson et al. [4], where reduction time was prolonged to 1.5 hours at 80 °C. The peptides obtained were analysed according to the method reported by Apostolovic et al. [3, 5] using LTQ Orbitrap XL mass spectrometer with an EASY- nano liquid chromatography (nLC) II system (Thermo Fisher Scientific Inc., Bremen, Germany), with change in the Orbitrap resolution from 30000 to 60000. Identification of peanut peptides was performed using PEAKS Studio 8.5 (Bioinformatics Solutions Inc., Canada). Signature MS/MS spectra were searched using PEAKS DB algorithm against a hybrid database consisting of a UniprotKB/Swiss-Prot (reviewed only) peanut (*Arachis hypogaea*) database (downloaded on 14/08/2017 from http://www.uniprot.org/) and cRAP (The common Repository of Adventitious Proteins) database (downloaded on 18/01/2017 from http://www.thegpm.org/crap/). The following modifications were taken into account as variables: oxidation (Met), deamidation (Gln, Asn), and hydroxylation (Pro), while carbamidomethylation (Cys) was set as fixed. Up to 2 missed cleavages with non-specific cleavage at both ends of a peptide were allowed. Mass tolerances

were set to ±10 ppm for parent ions and ±0.8 Da for fragment ions. Protein filters were as follows set to a one unique peptide and -10logP of value 20. Peptide filters were as follows: input of -10logP for Peptide-Spectrum Matches PSM was the lowest values securing less than 0.5% of resulting peptide sequence FDR and 0% FDR at protein level and de novo ALC Score ≥ 80%. Identified peptides were searched in the IEDB database (Immuno Epitope Database and Analysis, http://www.iedb.org) in order to find sequences overlapping with characterized epitopes. The following IEDB search parameters were applied: linear sequence for epitope structure, substring for BLAST option, and human as host.

IgE-binding properties of peanut digests

ELISA inhibition. The IgE-binding properties of the liquid phase from the digestion mixtures, as well as standard defatted peanut extracts were analysed using an inhibition ELISA. Standard defatted raw peanut extract was prepared according to the method reported by Radosavljevic et al. [6]. Half-area microtiter plates (96 wells, Greiner bio-one, Frickenhausen, Germany) were coated with 50 µL per well of 10 µg/mL with defatted peanut extract, and incubated overnight at 4 °C in coating buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$ pH 9.6). The remaining binding sites were blocked with 1% BSA in TPBS (20 mM phosphate buffer with 0.9% NaCl pH 7.4 containing 0.05% of Tween 20 (w/v)), for 1 hour at 37 °C. Serum pooled from 10 peanut sensitised patients (patient #1-10, Table S3) was prepared by following the EMEA Note for Guidance on Allergen Products (EMEA/CHMP/BWP/304831/2007). Samples (defatted raw peanut extract, defatted liquid phase of control and digested peanut) were diluted 2-fold with 1% BSA in tPBS (concentration range 10–0.04 µg/mL). Samples were pre-incubated 1:1 with the serum pool (final dilution of serum pool was 30-fold in blocking buffer) for 1 hour at 37 °C before their addition on the plate for incubation of 1 hour at 37 °C. Detection of bound IgE was performed with 50 µL mouse-anti-human IgE monoclonal antibody (2000 times diluted in TPBS containing 1% BSA; Abcam, Cambridge, UK) conjugated to horseradish peroxidase. Finally, staining was performed by enzymatic conversion of 3, 3′, 5, 5′-tetramethylbenzidine (Biolegend, San Diego, CA, USA). Inhibition of IgE-binding was calculated as $[(OD_{\text{no inhibitor}} - OD_{\text{inhibitor}})/OD_{\text{no inhibitor}}] \times 100$, and the concentration needed to inhibit 50% of this signal was calculated (IC_{50}). The results were analysed using GraphPad Prism6 (La Jolla, CA, USA).

ImmunoCAP inhibition. IgE-binding of the SDRPs fraction of digested peanut was determined using ImmunoCAP inhibition (ImmunoCAP System, Phadia/Thermo Fisher Scientific, Uppsala, Sweden). Seven undiluted individual sera (200 µL; patients #1–7 Table S2) were pre-incubated with 200 µL peptides prior to the measurement for allergen-specific IgE to: peanut (f13), Ara h 1 (f422), Ara h 2 (f423) and Ara h 3 (f424). Applied peptides are released from about 3.3 mg of milled peanut e.g. released from about 800 µg of peanut proteins extracted to liquid phase during digestion. The inhibition of IgE-binding was expressed as percentage based on non-inhibited serum, using the following formula: % IgE inhibition = 100 – (IgE binding to the solid surface in the presence of the inhibitor/IgE binding to the solid surface) × 100).

Immunoblotting. After TCA precipitation, samples were resuspended in 2% SDS. 1D electrophoresis was carried out on a 14% gel. The samples (25 µg) were loaded in the well. Proteins were separated on 1-DE and transferred onto nitrocellulose membranes with 0.2 µm pore size (Bio-Rad, Solna, Sweden). Ponceau S staining was used to verify success of the transfer. The membranes were blocked with 2% BSA in PBS pH 7.4 containing 0.05% Tween 20 (TPBS) for 1 hour at room temperature (RT). Subsequently, membrane was incubated overnight at 4 °C with 1:10 diluted serum pool from patients with proven peanut allergy. The serum pool consisted of sera of seven peanut sensitised patients (#4-10 Table S3; range and mean of total peanut-specific IgE: 11- 415 kU/L and 146 kU/L, respectively; range and mean of Ara h 2-specific IgE: 5–192 kUA/L and 61 kUA/L, respectively). The secondary antibody, anti-human IgE produced in rabbit (Miab, Uppsala, Sweden), was diluted 1:2000 and incubated for 1 hour at RT. Tertiary antibody, AP-labelled goat anti-rabbit IgG (Jackson ImmunoResearch, West Grove, PA, USA), diluted 1:2000, was added to the strips and incubated for 1 hour at RT. The binding patterns were visualized with a substrate solution consisting of 1.5 mg BCIP and 3 mg NBT in 10 mL of 100 mM Tris, containing 150 mM NaCl, and 5 mM $MgCl_2$, pH 9.6.

Circular Dichroism (CD) Spectroscopy

CD spectroscopy was performed on control and digested samples after re-solubilization of TCA/acetone pellet in 2% SDS. Samples were diluted in 10 mM sodium phosphate buffer (pH 7.4) to achieve final concentrations of 1 mg/mL for far-UV CD (SDS concentration was < 0.2 %). Far UV CD spectra were recorded using a Jasco J-815 spectrophotometer (Japan Spectroscopic Co. Ltd., Tokyo, Japan) at RT.

De novo modelling and molecular graphics

The sequences of Ara h 1, Ara h 2, Ara h 3 and Ara h 6 were obtained from UniProt (www.uniprot.org, identifiers P43238, Q6PSU2-2, B5TYU1 and A5Z1R0, respectively). For Ara h 6 structure PDB code 1W2Q, model #1 was used. The missing regions in the Ara h 1, Ara h 2.01 and Ara h 3 partial crystal structures (PDB code 3SMH, 3OB4 and 3C3V, respectively) [7] were built using Rosetta all-atom *de-novo* loop modelling. After clustering of 10,000 modelled structures (per protein) by structural similarity, the lowest energy models of the most populated cluster were chosen (Figures 2 and 5). Molecular graphics of Ara h 1, Ara h 2, Ara h 3 and Ara h 6 3D modelled structures were created using BIOVIA Discovery Studio Visualizer (Dassault Systems BIOVIA, Discovery Studio Modelling Environment, Release 2017, S. Diego; http://accelrys.com/products/discovery-studio/).

Supporting Tables

Table S1. Summary of published data on major peanut allergens digestibility by *in vitro* simulated gastric digestion.

Allergen	Size (kDa)	Pepsin : allergen ratio (w/w) (in final reaction mixture)	Enzyme activity unit/mg allergen (in final digestion mixture)	pH	Peanut extract/ purified protein	Digestion time [min.]	Protein stability [min.]	Peptide fragment (kDa)	Ref.
Ara h 1,Cupin (Vicilin type,7S globulin)	64	nd	170	2.5	PP	120	< 10	<4	[8]
		0.025	80	2.1	PP		1	<20	[9]
		0.05	162	2.5	PP	120	1	5.5	[10]
		12.8	nd	1.2	PP	120	5	nd	[11]
		3.04	10,000	1.2	PP	60	0.5	nd	[12]
		0.3	1,000	1.2	PP	60	0.5	nd	[12]
		0.03	100	1.2	PP	60	0.5	nd	[12]
		3.04	10,000	1.2	PE	60	0.5	nd	[12]
		0.63	2540	1.2	PE	60	1	nd	[13]
		0.0001	nd	2	PE	1200	30	<35	[14]
Ara h 2, Conglutin (2S albumin)	17	3	10,000	1.2	PP	60	0-2	10	[15]
		3	10,000	2	PP	60	0-30	10	[15]
		19	nd	1.2	PP	60	/	/	[16]
		12.8	nd	1.2	PP	120	0.5	nd	[11]
		3.04	10,000	1.2	PP	60	16	10	[12]
		0.3	1,000	1.2	PP	60	/	/	[12]
		0.03	100	1.2	PP	60	/	/	[12]
		3.04	10,000	1.2	PE	60	16	/	[12]
		0.63	2540	1.2	PE	60	15	nd	[13]
Ara h 3, Cupin (Legumin-type, 11S globulin, Glycinin)	60	3.04	10,000	1.2	PP	60	0.25	nd	[12]
		0.3	1,000	1.2	PP	60	0.25	nd	[12]
		0.03	100	1.2	PP	60	0.25	nd	[12]
		0.002	nd	2	PP	120	<2	<14	[17]
		3.04	10,000	1.2	PE	60	0.25	nd	[12]
		0.63	2540	1.2	PE	60	1	nd	[13]
Ara h 6 Conglutin (2S albumin)	15	3.04	10,000	1.2	PP	60	4	10	[12]
		0.3	1,000	1.2	PP	60	16	10	[12]
		0.03	100	1.2	PP	60	/	/	[12]
		3.04	10,000	1.2	PE	60	60	10	[12]
		0.63	2540	1.2	PE	60	15	nd	[13]

PP, peanut protein; PE, peanut extract; nd, not described;

Table S2. Stock solutions preparation for simulated digestive fluids.

Constituent	Concentration in SSF stock solution	Final concentration in oral phase reaction mixture	Concentration in SGF stock solution	Final concentration in gastric phase reaction mixture
KCl	15.1 mM	6.04 mM	6.9 mM	6.67 mM
KH_2PO_4	3.7 mM	1.48 mM	0.9 mM	1.19 mM
$NaHCO_3$	13.6 mM	5.44 mM	25 mM	15.22 mM
NaCl	-	-	47.2 mM	23.6 mM
$MgCl_2(H_2O)_6$	0.15 mM	0.06 mM	0.1 mM	0.08 mM
$(NH_4)CO_3$	0.06 mM	0.024 mM	0.5 mM	0.263 mM
HCl	1.1 mM	0.44 mM	240 mM	122.45 mM
pH	7.05	6.68±0.12	3.00	2.91±0.18

Table S3. IgE levels of peanut sensitized patients determined by ImmunoCAP

Patient's ID	Whole peanut extract	rAra h 1	rAra h 2	rAra h 3
		kU_A/L		
1	415	96	192	52
2	11	<0.10	5	<0.10
3	65	12.40	36	6.40
4	48	14	20	2.60
5	34	2.60	24	0.66
6	152	2.40	78	<0.20
7	218	92	68	34
8	225	66	63	3.90
9	23	0.19	0.24	0.58
10	11	3.20	0.14	<0.10

Table S4 is provided separately as pdf file. It contains identification results of proteins and their fragments from spots and bands of standard peanut extract (SPE), control peanut (CPS) and digested peanut samples (DPS) from Figs. 1, 3 and 2S achieved by tandem bottom up proteomics on Orbitrap LTQ hybrid and PEAKS Suite 8.5 softwares

Table S5. Sequences of intact SDRPs from Ara h 3 (18) and Ara h 1 (27), found after in vitro oral-gastric digestion of whole kernels peanut, matching with Ara h 3 and Ara h 1 epitopes reported in IEDB. The SDRPs fraction was analyzed by mass spectrometry as intact. Epitopes found in identified peptides are bolded and reported with their ID.

Peptide No.	Peptide sequence	Allergen accession no	Epitope ID IEDB	Epitope sequence IEDB	Reference
1	**LKNNNPFKF**	Ara h 3 (A1DZF0, Q6IWG5, Q0GM57)	106026	QARQ**LKNNNPFKF**FV	[18]
1	**LKNNNPFKF**		106042	Q**LKNNNPFKF**FVPPS	[18]
2	**RQLKNNNPFKF**	Ara h 3 (A1DZF0, Q6IWG5, Q0GM57)	106026	QA**RQLKNNNPFKF**FV	[18]
3	**SYGLPRE**	Ara h 3 (A1DZF0)	105678	AN**SYGLPRE**QARQLK	[18]
4	**IAVPTGVAF**	Ara h 3 (A1DZF0, Q6IWG5, Q0GM57)	99266	GDL**IAVPTGVAF**WLY	[19]
4	**IAVPTGVAF**		99325	**IAVPTGVAF**WLYNDH	[19]
5	**IAVPTGVA**	Ara h 3 (A1DZF0, Q6IWG5, Q0GM57)	53687	RFDEGDL**IAVPTGVA**	[6]
5	**IAVPTGVA**		99266	GDL**IAVPTGVA**FWLY	[19]
5	**IAVPTGVA**		99325	**IAVPTGVA**FWLYNDH	[19]
6	**RILSPDRK**	Ara h 3 (A1DZF0)	71559	VTVRGGL**RILSPDRK**	[20]; [6]
6	**RILSPDRK**		99738	TVRGGL**RILSPDRK**R	[18]; [19]
6	**RILSPDRK**		70725	VRGGL**RILSPDRK**RR	[6]
6	**RILSPDRK**		99277	GGL**RILSPDRK**RRAD	[19]
6	**RILSPDRK**		105826	GGL**RILSPDRK**RRQQ	[18]
6	**RILSPDRK**		106076	**RILSPDRK**RRQQYER	[18]
7	**KKNIGRNRSPDIYNP**QAG	Ara h 3 (A1DZF0)	31642	**KKNIGRNRSPDIYNP**	[18]; [19]; [6]
7	KKN**IGRNRSPDIYNPQAG**		99331	**IGRNRSPDIYNPQAG**	[18]; [19]; [6]
8	**RSPDIYNPQAGSL**	Ara h 3 (A1DZF0)	99513	N**RSPDIYNPQAGSL**K	[18]; [19]
9	**SPDIYNPQAGSL**	Ara h 3 (A1DZF0)	99513	NR**SPDIYNPQAGSL**K	[18]; [19]
10	**LRGRAHVQVVD**	Ara h 3 (A1DZF0)	99363	IYR**LRGRAHVQVVD**S	[19]
10	**LRGRAHVQVVD**		99447	**LRGRAHVQVVD**SNGN	[19]
11	**LRGRAHVQVVDSNG**	Ara h 3 (A1DZF0)	99447	**LRGRAHVQVVDSNGN**	[19]
12	**ARQLKNNNPFKF**	Ara h 3 (Q6IWG5)	106026	QAR**QLKNNNPFKF**FV	[18]
13	NG**RAHVQVVDSNGNRVY** DF	Ara h 3 (Q6IWG5	99597	**RAHVQVVDSNGNRVY**	[19]
14	NG**RAHVQVVDSNGNRVY** D	Ara h 3 (Q6IWG5	99597	**RAHVQVVDSNGNRVY**	[19]
15	**RAHVQVVDSNG**	Ara h 3 (A1DZF0	99597	**RAHVQVVDSNG**NRVY	[19]
15	**RAHVQVVDSNG**	Ara h 3 (A1DZF0	99447	LRG**RAHVQVVDSNG**N	[19]
16	**LQEGHVL**	Ara h 3 (A1DZF0, Q6IWG5,	99141	DEE**LQEGHVL**VVPQN	[19]
16	**LQEGHVL**		99440	**LQEGHVL**VVPQNFAV	[19]
17	**GHVLVVPQNF**	Ara h 3 (A1DZF0, Q6IWG5,	99280	**GHVLVVPQNF**AVAGK	[19]
17	**GHVLVVPQNF**		99440	LQE**GHVLVVPQNF**AV	[19]
18	**HVLVVPQNF**	Ara h 3 (A1DZF0, Q6IWG5,	99280	G**HVLVVPQNF**AVAGK	[19]
18	**HVLVVPQNF**		99440	LQEG**HVLVVPQNF**AV	[19]
19	**VLPKHADADNIL**	Ara h 1	100389	PNTLVL**PKHADADNIL**VIQQ	[21]

19	**VLPKHADADNIL**	(P43238, N1NG13	190791	IEAKPNTLVLPKHADADNIL	[22]
20	**VLPKHADADNI**	Ara h 1 (P43238, N1NG13,	100389	PNTLVL**PKHADADNIL**VIQQ	[21]
20	**VLPKHADADNI**		190791	IEAKPNTLVLPKHADADNIL	[22]
21	**VLPKHADADN**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237	99393	KPNTL**VLPKHADADN**	[19]
21	**VLPKHADADN**		100389	PNTL**VLPKHADADN**ILVIQQ	[21]
21	**VLPKHADADN**		190791	IEAKPNTL**VLPKHADADN**IL	[22]
22	**VLPKHADAD**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237	99393	KPNTL**VLPKHADADN**	[19]
22	**VLPKHADAD**		100389	PNTL**VLPKHADADN**ILVIQQ	[21]
22	**VLPKHADAD**		190791	IEAKPNTL**VLPKHADADN**IL	[22]
23	**PKHADADNIL**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237, B3IXL2)	100389	PNTLVL**PKHADADNIL**VIQQ	[21]
23	**PKHADADNIL**		190791	IEAKPNTLVL**PKHADADNIL**	[22]
23	**PKHADADNIL**		190849	L**PKHADADNIL**VIQQGQATV	[22]
23	**PKHADADNIL**		523624	**PKHADADNIL**VIQQGQATVTVANG	[23]
24	**PKHADADNILVI**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237	190849	L**PKHADADNILVI**QQGQATV	[22]
24	**PKHADADNILVI**		523624	**PKHADADNILVI**QQGQATVTVANG	[23]
24	**PKHADADNILVI**		100389	PNTLVL**PKHADADNILVI**QQ	[21]
25	**SFNLDEGHA**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237	99616	RKSFNLDEGHALRIP	[19]; [24]
25	**SFNLDEGHA**		190952	RKSFNLDEGHALRIPSGFIS	[22]
25	**SFNLDEGHA**		191006	TVTVANGNNRKSFNLDEGHA	[22]
26	**LRIPSGF**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237, B3IXL2)	99142	DEGHA**LRIPSGF**ISY	[19]
26	**LRIPSGF**		99312	HA**LRIPSGF**ISYILN	[19]
26	**LRIPSGF**		100063	GHA**LRIPSGF**ISYILNRHDN	[21]
26	**LRIPSGF**		190781	HA**LRIPSGF**ISYILNRHDNQ	[22]
26	**LRIPSGF**		190952	RKSFNLDEGHA**LRIPSGF**IS	[22]
27	**LRIPSGFI**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237, B3IXL2)	99142	DEGHA**LRIPSGF**ISY	[19]
27	**LRIPSGFI**		99312	HA**LRIPSGF**ISYILN	[19]
27	**LRIPSGFI**		100063	GHA**LRIPSGF**ISYILNRHDN	[21]
27	**LRIPSGFI**		190781	HA**LRIPSGF**ISYILNRHDNQ	[22]
27	**LRIPSGFI**		190952	RKSFNLDEGHA**LRIPSGF**IS	[22]
28	**ILNRHDNQNL**	Ara h 1 (P43238, N1NG13,	100169	ISY**ILNRHDNQNL**RVAKISM	[22]; [21]
28	**ILNRHDNQNL**		99355	ISY**ILNRHDNQNL**RV	[19]; [24]
29	**RVAKISM**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237, B3IXL2)	100169	ISY**I**LNRHDNQNL**RVAKISM**	[22]; [21]
29	**RVAKISM**		99511	NQNL**RVAKISM**PVN	[19];[24]; [25]
29	**RVAKISM**		100433	QNL**RVAKISM**PVNTPGQFED	[21]
29	**RVAKISM**		190882	NQNL**RVAKISM**PVNTPGQFE	[22]
30	**AKISMPVNTPGQF**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237, B3IXL2)	100433	QNLRV**AKISMPVNTPGQF**ED	[21]
30	**AKISMPVNTPGQF**		190882	NQNLRV**AKISMPVNTPGQF**E	[22]
30	**AKISMPVNTPGQF**		434773	V**AKISMPVNTPGQF**EDFFPASSR + MGM(K2)	[26]
30	**AKISMPVNTPGQF**			V**AKISMPVNTPGQF**EDFFPASSR	[26]
31	**VVVNKGTGNLE**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237, B3IXL2)	98841	KAMVI**VVVNKGTGNLE**LVAV	[27]; [21]; [28]
31	**VVVNKGTGNLE**		148699	NSKAMVI**VVVNKGTGNLE**LV	[29]
31	**VVVNKGTGNLE**		190708	AM**VIVVVNKGTGNLE**LVAV	[22]
31	**VVVNKGTGNLE**		523259	NSKAMVI**VVVNKGTGNLE**LVAVRK	[23]
32	**VKVSKEHVEE**	Ara h 1	98910	NEGVI**VKVSKEHVEE**	[19]; [30]; [24]

32	**VKVSKEHVEE**	(P43238, N1NG13)	99757	VI**VKVSKEHVEE**LTK	[19]
32	**VKVSKEHVEE**		100323	NEGVI**VKVSKEHVEE**LTKHA	[21]; [22]; [31]
32	**VKVSKEHVEE**		106968	**VKVSKEHVEE**LTKHAKSVSK	[31]
33	**SEEEGDITNPINL**	Ara h 1 (Q6PSU3, P43237,	99657	**SEEEGDITNPINL**RE	[19]
33	**SEEEGDITNPINL**		190971	SEEEGDITNPINLREGEPDL	[22]
34	**LAGDKDNVIDQI**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237	100137	IF**LAGDKDNVIDQI**EKQAKD	[22]; [21]
34	**LAGDKDNVIDQI**		434746	IF**LAGDKDNVIDQI**EK + MCM(K7)	[26]
34	**LAGDKDNVIDQI**		434747	IF**LAGDKDNVIDQI**EK	[26]
35	**LAGDKDNVIDQ**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237	100137	IF**LAGDKDNVIDQ**IEKQAKD	[22]; [21]
35	**LAGDKDNVIDQ**		434746	IF**LAGDKDNVIDQ**IEK + MCM(K7)	[26]
35	**LAGDKDNVIDQ**		434747	IF**LAGDKDNVIDQ**IEK	[26]
36	**IVVVNKGTGNLEL**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237, B3IXL2)	98841	KAMV**IVVVNKGTGNLEL**VAV	[27]; [21]; [28]
36	**IVVVNKGTGNLEL**		148699	NSKAMV**IVVVNKGTGNLE**LV	[29]
36	**IVVVNKGTGNLEL**		190708	AMV**IVVVNKGTGNLEL**VAV	[22]
36	**IVVVNKGTGNLEL**		523259	NSKAMV**IVVVNKGTGNLEL**VAVRK	[23]
37	**IVVVNKGTGNL**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237, B3IXL2)	98841	KAMV**IVVVNKGTGNL**ELVAV	[27]; [21]; [28]
37	**IVVVNKGTGNL**		148699	NSKAMV**IVVVNKGTGNL**ELV	[29]
37	**IVVVNKGTGNL**		190708	AMV**IVVVNKGTGNL**ELVAV	[22]
37	**IVVVNKGTGNL**		523259	NSKAMV**IVVVNKGTGNL**ELVAVRK	[23]
37	**IVVVNKGTGNL**		99364	KAMV**IVVVNKGTGNL**	[24]; [19]
38	**IVKVSKE**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237, B3IXL2)	98910	NEGV**IVKVSKE**HVEE	[19]; [30]; [24]
38	**IVKVSKE**		100323	NEGV**IVKVSKE**HVEELTKHA	[21]; [22]; [31]
38	**IVKVSKE**		99757	V**IVKVSKE**HVEELTK	[19]
38	**IVKVSKE**		190967	RWSTRSSENNEGV**IVKVSKE**	[22]
38	**IVKVSKE**		191030	WSTRSSENNEGV**IVKVSKE**	[22]
39	**IMPAAHPVAINA**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237	148649	KEGDVF**IMPAAHPVAINA**SS	[22]; [29]
39	**IMPAAHPVAINA**		99167	DVF**IMPAAHPVAINA**	[19]
39	**IMPAAHPVAINA**		190764	GDVF**IMPAAHPVAINA**SS	[22]
40	**IMPAAHPVAIN**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237	148649	KEGDVF**IMPAAHPVAIN**ASS	[22]; [29]
40	**IMPAAHPVAIN**		99167	DVF**IMPAAHPVAIN**A	[19]
40	**IMPAAHPVAIN**		190764	GDVF**IMPAAHPVAIN**ASS	[22]
41	**IMPAAHPVA**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237, B3IXL2)	148649	KEGDVF**IMPAAHPVA**INASS	[22]; [29]
41	**IMPAAHPVA**		99167	DVF**IMPAAHPVA**INA	[19]
41	**IMPAAHPVA**		190764	GDVF**IMPAAHPVA**INASS	[22]
41	**IMPAAHPVA**		98843	KEGDVF**IMPAAHPVA**	[19]; [30]
41	**IMPAAHPVA**		540385	EGDVF**IMPAAHPVA**I	[24]
42	**EVKPDKKNPQL**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237, B3IXL2)	99242	F**EVKPDKKNPQL**QDL	[19]
42	**EVKPDKKNPQL**		99283	GKLF**EVKPDKKNPQL**	[19]
42	**EVKPDKKNPQL**		148695	NNFGKLF**EVKPDKKNPQL**QD	[29]
42	**EVKPDKKNPQL**		190745	**EVKPDKKNPQL**Q	[32]; [22]
42	**EVKPDKKNPQL**		190750	F**EVKPDKKNPQL**QDLDMMLT	[22]
42	**EVKPDKKNPQL**		190877	NNFGKLF**EVKPDKKNPQL**Q	[22]
42	**EVKPDKKNPQL**		523002	NNFGRLF**EVKPDKKNPQL**QDLDMM	[23]
42	**EVKPDKKNPQL**		540393	**EVKPDKKNPQL**QDLD	[24]

43	**EVKPDKKNPQ**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237, B3IXL2)	99242	F**EVKPDKKNPQ**LQDL	[19]
43	**EVKPDKKNPQ**		99283	GKLF**EVKPDKKNPQ**L	[19]
43	**EVKPDKKNPQ**		148695	NNFGKLF**EVKPDKKNPQ**LQD	[29]
43	**EVKPDKKNPQ**		190745	**EVKPDKKNPQ**LQ	[32]; [22]
43	**EVKPDKKNPQ**		190750	F**EVKPDKKNPQL**QDLDMMLT	[22]
43	**EVKPDKKNPQ**		190877	NNFGKLF**EVKPDKKNPQ**LQ	[22]
43	**EVKPDKKNPQ**		523002	NNFGRLF**EVKPDKKNPQ**LQDLDMM	[23]
43	**EVKPDKKNPQ**		540393	**EVKPDKKNPQ**LQDLD	[24]
43	**EVKPDKKNPQ**		190729	DLSNNFGKLF**EVKPDKKNPQ**	[22]
43	**EVKPDKKNPQ**		190876	NNFGKLF**EVKPDKKNPQ**	[22]
44	**EEGDITNPINL**	Ara h 1 (P43238, N1NG13)	99657	SE**EEGDITNPINL**RE	[19]
44	**EEGDITNPINL**		190971	SE**EEGDITNPINL**REGEPDL	[22]
45	**DITNPINL**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237, B3IXL2)	98731	**DITNPINL**RE	[30]
45	**DITNPINL**		98732	DITNPINLREGEPDL	[30]
45	**DITNPINL**		99196	EG**DITNPINL**REGEP	[19]
45	**DITNPINL**		99657	SEEEG**DITNPINL**RE	[19]
45	**DITNPINL**		190971	SEEEG**DITNPINL**REGEPDL	[22]

Table S6. Sequences of SDRPs from Ara h 3 (30), Ara h 1(28) and Ara h 2 (2), found after in vitro oral-gastric digestion of grained peanut, matching with Ara h 3 and Ara h 1 epitopes reported in IEDB. The SDRPs fraction was subjected to reduction, alkylation and trypsin digestion before mass spectrometry analysis. Epitopes found in identified peptides are bolded and reported with their ID.

Peptide No.	Peptide sequence	Allergen source	Epitope ID IEDB	Epitope sequence IEDB	Reference
1	**AHVQVVDSNG**	Ara h 3 (Q647H4, Q8LKN1, Q6T2T4, A1DZF0, Q6IWG5, Q0GM57, E5G077, Q9SQH7)	99447	LRGR**AHVQVVDSNG**N	[19]
1	**AHVQVVDSNG**		99597	R**AHVQVVDSNG**NRVY	[19]
2	**ALRRPFYSNAPQE**	Ara h 3 (Q647H4, Q8LKN1, Q6T2T4, Q9SQH7, A1DZF0)	99484	N**ALRRPFYSNAPQE**I	[18]; [19]
3	**IETWNPNNQE**	Ara h 3 (Q647H4, Q8LKN1, Q6T2T4, A1DZF0, Q6IWG5, Q0GM57, E5G077, Q9SQH7)	25997	**IETWNPNNQE**FECAG	[6]
4	**IQQGRGYFG**	Ara h 3 (Q647H4, Q8LKN1, A1DZF0, Q9SQH7)	16280	F**IQQGRGYFG**LIFPG	[18]; [19]; [6]
4	**IQQGRGYFG**		99561	QEIF**IQQGRGYFG**LI	[18]; [19]
5	**LKNNNPFKF**	Ara h 3 (Q647H4, Q8LKN1, Q6T2T4, A1DZF0, Q6IWG5, Q0GM57, E5G077, Q9SQH7)	106026	QARQ**LKNNNPFKF**FV	[18]
5	**LKNNNPFKF**		106042	Q**LKNNNPFKF**FVPPS	[18]
6	**LQEGHVLVVPQN**		99440	**LQEGHVLVVPQN**FAV	[19]
6	**LQEGHVLVVPQN**		99141	DEE**LQEGHVLVVPQN**	[19]
7	**LQEGHVLVVPQNF**	Ara h 3 (Q647H4, Q8LKN1, Q6T2T4, A1DZF0, Q6IWG5, Q0GM57, E5G077, Q9SQH7)	99440	**LQEGHVLVVPQNF**AV	[19]
8	**LRILSPDR**	Ara h 3 (Q647H4, Q8LKN1, Q6T2T4, A1DZF0	71559	VTVRGG**LRILSPDR**K	[20]; [6]
8	**LRILSPDR**		99738	TVRGG**LRILSPDR**KR	[18]; [19]
8	**LRILSPDR**		70725	VRGG**LRILSPDR**KRR	[6]
8	**LRILSPDR**		99277	GG**LRILSPDR**KRRAD	[19]
8	**LRILSPDR**		105826	GG**LRILSPDR**KRRQQ	[18]
9	**NGRAHVQVVDSNGNR**		99597	**RAHVQVVDSNGNRVY**	[19]
10	**NIGRNRSPDIYNPQAG**	Ara h 3 (Q647H4, Q8LKN1,	99331	IGR**NRSPDIYNPQAG**	[18]; [19]; [6]
11	**NNNPFKF**	Ara h 3 (Q647H4, Q8LKN1, Q6T2T4, A1DZF0, Q6IWG5, Q0GM57, E5G077, Q9SQH7)	105988	**NNNPFKF**FVPPSEQS	[18]
11	**NNNPFKF**		106026	QARQLK**NNNPFKF**FV	[18]
11	**NNNPFKF**		106042	QLK**NNNPFKF**FVPPS	[18]
12	**NRSPDIYNPQAG**	Ara h 3 (Q647H4, Q8LKN1, Q6T2T4, A1DZF0)	99331	IGR**NRSPDIYNPQAG**	[18]; [19]; [6]
12	**NRSPDIYNPQAG**		99513	**NRSPDIYNPQAG**SLK	[18]; [19]
13	**NRSPDIYNPQAGS**	Ara h 3 (Q647H4, Q8LKN1,	99513	**NRSPDIYNPQAGS**LK	[18]; [19]
14	**NRSPDIYNPQAGSL**	Ara h 3 (Q8LKN1, Q6T2T4,	99513	**NRSPDIYNPQAGSL**K	[18]; [19]
15	**NSYGLPR**	Ara h 3 (Q647H4, Q8LKN1,	105678	A**NSYGLPR**EQARQLK	[18]
16	**PDIYNPQAGSL**	Ara h 3 (Q647H4, Q8LKN1,	99513	NRS**PDIYNPQAGSL**K	[18]; [19]
17	**QEGHVLVVPQNF**	Ara h 3 (Q647H4, Q8LKN1,	99440	L**QEGHVLVVPQNF**AV	[19]
18	**QLKNNNPFKF**	Ara h 3 (Q6IWG5, Q0GM57, E5G077, Q9SQH7, Q647H4, Q8LKN1, Q6T2T4, A1DZF0)	106026	QAR**QLKNNNPFKF**FV	[18]
18	**QLKNNNPFKF**		106042	**QLKNNNPFKF**FVPPS	[18]
19	**RAHVQVVDSNGNRVY**	Ara h 3 (A1DZF0, Q6IWG5,	99597	**RAHVQVVDSNGNRVY**	[19]
20	**RPFYSNAPQE**	Ara h 3 (Q647H4, Q8LKN1,	99484	N**ALRRPFYSNAPQE**I	[18]; [19]
21	**RPFYSNAPQEI**	Ara h 3 (Q647H4, Q8LKN1,	99484	N**ALRRPFYSNAPQEI**	[18]; [19]
22	**RSPDIYNPQAGSL**	Ara h 3 (Q647H4, Q8LKN1,	99513	**NRSPDIYNPQAGSL**K	[18]; [19]
23	**SLPYSPYSPQ**	Ara h 3 (Q647H4, Q8LKN1,	106093	R**SLPYSPYSPQTQPK**	[18]

23	**SLPYSPYSPQ**	Q6T2T4)	106122	SRRR**SLPYSPYSPQT**	[18]
24	**SLPYSPYSPQTQPK**	Ara h 3 (Q8LKN1, Q6T2T4)	106093	R**SLPYSPYSPQTQPK**	[18]
25	**SPDIYNPQAG**	Ara h 3 (Q647H4, Q8LKN1, Q6T2T4, A1DZF0)	99331	IGRNR**SPDIYNPQAG**	[18]; [19]; [6]
25	**SPDIYNPQAG**		99513	NR**SPDIYNPQAG**SLK	[18]; [19]
26	**SPDIYNPQAGSL**	Ara h 3 (Q647H4, Q8LKN1)	99513	NR**SPDIYNPQAGSL**K	[18]; [19]
27	**SYGLPR**	Ara h 3 (Q647H4, Q8LKN1,	105678	AN**SYGLPR**EQARQLK	[18]
28	**YEEPAQQGR**	Ara h 3 (Q9SQH7, Q8LKN1, Q6T2T4, A1DZF0, Q9SQH7)	105700	CPST**YEEPAQQGR**RH	[18]
28	**YEEPAQQGR**		106150	T**YEEPAQQGR**RHQSQ	[18]
29	**YEEPAQQGRR**	Ara h 3 (Q647H4, Q8LKN1, Q6T2T4, A1DZF0)	105700	CPST**YEEPAQQGR**RH	[18]
29	**YEEPAQQGRR**		106150	T**YEEPAQQGR**RHQSQ	[18]
30	**YGLPR**	Ara h 3 (Q647H4, Q8LKN1, Q6T2T4, A1DZF0, Q6IWG5, Q0GM57, E5G077, Q9SQH7)	105678	ANS**YGLPR**EQARQLK	[18]
30	**YGLPR**		106196	**YGLPR**EQARQLKNNN	[18]
31	**CLQSCQQEPDDLKQK**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237,B3IXL2)	98978	R**CLQSCQQEPDDLKQK**ACES	[21]; [28]
31	**CLQSCQQEPDDLKQK**		190885	PCAQR**CLQSCQQEPDDLKQK**	[22]
32	**VVVNKGTGNLE**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237,B3IXL2)	99053	**VVNKGTGNLE**LVAVR	[19]; [30]
32	**VVVNKGTGNLE**		148699	NSKAMVI**VVVNKGTGNLE**LV	[29]
32	**VVVNKGTGNLE**		190708	AMVI**VVVNKGTGNLE**LVAV	[22]
32	**VVVNKGTGNLE**		523259	NSKAMVI**VVVNKGTGNLE**LVA	[23]
33	**VVVNKGTGNL**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237,B3IXL2)	98841	KAMV**IVVVNKGTGNLE**LVAV	[27]; [21]; [28]
33	**VVVNKGTGNL**		99364	KAMV**IVVVNKGTGNL**	[24]; [19]
33	**VVVNKGTGNL**		148699	NSKAMV**IVVVNKGTGNLE**LV	[29]
33	**VVVNKGTGNL**		190708	AMV**IVVVNKGTGNLE**LVAV	[22]
33	**VVVNKGTGNL**		523259	NSKAMV**IVVVNKGTGNLE**LVA	[23]
34	**VVNKGTGNL**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237,B3IXL2)	98841	KAMVIV**VVNKGTGNL**ELVAV	[27]; [21];
34	**VVNKGTGNL**		99364	KAMVIV**VVNKGTGNL**	[24]; [19]
34	**VVNKGTGNL**		99053	**VVNKGTGNLE**LVAVR	[19]; [30]
34	**VVNKGTGNL**		148699	NSKAMVIV**VVNKGTGNL**ELV	[29]
34	**VVNKGTGNL**		148985	**VVNKGTGNL**ELVAVRKEQQQ	[29]
34	**VVNKGTGNL**		190708	AMVIVVVNKGTGNLELVAV	[27]
34	**VVNKGTGNL**		523259	NSKAMVIV**VVNKGTGNL**ELVA	[23]
35	**SFNLDEGHA**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237,B3IXL2)	99616	RK**SFNLDEGHA**LRIP	[24]; [19]
35	**SFNLDEGHA**		190952	RK**SFNLDEGHA**LRIPSGFIS	[22]
35	**SFNLDEGHA**		191006	TVTVANGNNRK**SFNLDEGHA**	[22]
36	**SEEEGDITNPINL**	Ara h 1 (P43238, N1NG13)	99657	**SEEEGDITNPINL**RE	[19]
36	**SEEEGDITNPINL**		190971	**SEEEGDITNPINL**REGEPDL	[22]
37	**REGEPDLSNNFGKL**	Ara h 1 (P43238, N1NG13)	98979	**REGEPDLSNNFGKL**F	[30]
37	**REGEPDLSNNFGKL**		190893	PINL**REGEPDLSNNFGKL**FE	[22]
38	**PKHADADNIL**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237,B3IXL2)	100389	PNTLVL**PKHADADNIL**VIQQ	[21]
38	**PKHADADNIL**		190791	IEAKPNTLVL**PKHADADNIL**	[22]
38	**PKHADADNIL**		190849	L**PKHADADNIL**VIQQGQATV	[22]
38	**PKHADADNIL**		523624	**PKHADADNIL**VIQQGQATVTV	[23]
39	**NNPFYFPSR**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237,B3IXL2)	99031	TSR**NNPFYFPSR**RFS	[19]; [30]
39	**NNPFYFPSR**		99031	TSR**NNPFYFPSR**RFS	[19]; [30]
39	**NNPFYFPSR**		99607	REETSR**NNPFYFPSR**	[19]
39	**NNPFYFPSR**		100478	R**NNPFYFPSR**RFSTRYGNQN	[21]
39	**NNPFYFPSR**		148966	TSR**NNPFYFPSR**RFSTRYGN	[29]
39	**NNPFYFPSR**		190878	**NNPFYFPSR**RFSTRYGNQNG	[22]

39	**NNPFYFPSR**		190973	SHVREETSR**NNPFYFPSR**RF	[22]
39	**NNPFYFPSR**		540582	R**NNPFYFPSR**RFSTR	[24]
40	**LAGDKDNVIDQ**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237,B3IXL2)	100137	IF**LAGDKDNVIDQ**IEKQAKD	[22]; [21]
40	**LAGDKDNVIDQ**		434746	IF**LAGDKDNVIDQ**IEK +	[26]
40	**LAGDKDNVIDQ**		434747	IF**LAGDKDNVIDQ**IEK	[26]
41	**LAFPGSGEQVEKL**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237)	98859	**LAFPGSGEQVEKL**IK	[30]
41	**LAFPGSGEQVEKL**		98859	**LAFPGSGEQVEKL**IK	[30]
41	**LAFPGSGEQVEKL**		190804	KD**LAFPGSGEQVEKL**IKNQK	[22]
42	**KGSEEEGDITNPIN**	Ara h 1 (P43238, N1NG13)	98850	K**KGSEEEGDITNPIN**	[19]; [30]
43	**IVVVNKGTGNLE**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237,B3IXL2)	98841	KAMV**IVVVNKGTGNLE**LVAV	[27]; [21];
43	**IVVVNKGTGNLE**		523259	NSKAMV**IVVVNKGTGNLE**LVA	[23]
43	**IVVVNKGTGNLE**		190708	AMV**IVVVNKGTGNLE**LVAV	[22]
43	**IVVVNKGTGNLE**		148699	NSKAMV**IVVVNKGTGNLE**LV	[29]
44	**ISMPVNTPGQF**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237,B3IXL2)	100433	QNLRVAK**ISMPVNTPGQF**ED	[21]
44	**ISMPVNTPGQF**		190882	NQNLRVAK**ISMPVNTPGQF**E	[22]
44	**ISMPVNTPGQF**		434773	VAK**ISMPVNTPGQF**EDFFPASS	[26]
44	**ISMPVNTPGQF**		434774	VAK**ISMPVNTPGQF**EDFFPASS	[26]
45	**IMPAAHPVAINAS**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237,B3IXL2)	190764	GDVF**IMPAAHPVAINAS**S	[22]
45	**IMPAAHPVAINAS**		148649	KEGDVF**IMPAAHPVAINAS**S	[22]; [29]
46	**IMPAAHPVAINA**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237,B3IXL2)	190764	GDVF**IMPAAHPVAINAS**S	[22]
46	**IMPAAHPVAINA**		148649	KEGDVF**IMPAAHPVA**INASS	[22]; [29]
46	**IMPAAHPVAINA**		99167	DVF**IMPAAHPVAINA**	[19]
47	**IFLAGDKDNVIDQ**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237,B3IXL2)	100137	**IFLAGDKDNVIDQ**IEKQAKD	[22]; [21]
47	**IFLAGDKDNVIDQ**		434746	IF**LAGDKDNVIDQ**IEK +	[26]
47	**IFLAGDKDNVIDQ**		434747	IF**LAGDKDNVIDQI**EK	[26]
48	**FQNLQNHR**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237,B3IXL2)	100445	QRSRQ**FQNLQNHR**IVQIEAK	[29]; [21]
48	**FQNLQNHR**		99646	RSRQ**FQNLQNHR**IVQ	[19]
48	**FQNLQNHR**		98971	QRSRQ**FQNLQNHR**IV	[30]; [24]
48	**FQNLQNHR**		99239	FDQRSRQ**FQNLQNHR**	[19]
48	**FQNLQNHR**		190748	FDQRSRQ**FQNLQNHR**IVQIE	[22]
48	**FQNLQNHR**		190757	**FQNLQNHR**I	[22]
48	**FQNLQNHR**		190758	**FQNLQNHR**IVQIEAKPNTLV	[22]
48	**FQNLQNHR**		40406	**FQNLQNHR**IVQIEAK	[24]
49	**FIMPAAHPVAINA**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237,B3IXL2)	148649	KEGDV**FIMPAAHPVAINA**SS	[22]; [29]
49	**FIMPAAHPVAINA**		99167	DV**FIMPAAHPVAINA**	[19]
49	**FIMPAAHPVAINA**		190764	GDVF**IMPAAHPVAIN**ASS	[22]
50	**EDFFPASSRDQSSYLQG**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237,B3IXL2)	99243	**FFPASSRDQSSYLQG**	[19]
50	**EDFFPASSRDQSSYLQG**		190749	F**EDFFPASSRDQSSYLQG**FS	[22]
50	**EDFFPASSRDQSSYLQG**		524091	QF**EDFFPASSRDQSSYLQG**FSR	[23]
51	**EDFFPASSRDQSSY**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237,B3IXL2)	190749	F**EDFFPASSRDQSSYLQG**FS	[22]
51	**EDFFPASSRDQSSY**		524091	QF**EDFFPASSRDQSSYLQG**FSR	[23]
51	**EDFFPASSRDQSSY**		99241	F**EDFFPASSRDQSSY**	[19]
52	**EDFFPASSRDQSS**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237,B3IXL2)	190749	F**EDFFPASSRDQSS**YLQGFS	[22]
52	**EDFFPASSRDQSS**		524091	QF**EDFFPASSRDQSS**YLQGFSR	[23]
52	**EDFFPASSRDQSS**		99241	F**EDFFPASSRDQSS**Y	[19]
52	**EDFFPASSRDQSS**		98955	QF**EDFFPASSRDQSS**	[30]
53	**EDFFPASSR**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237,B3IXL2)	99241	F**EDFFPASSR**DQSSY	[19]
53	**EDFFPASSR**		98955	QF**EDFFPASSR**DQSS	[30]
53	**EDFFPASSR**		190749	F**EDFFPASSR**DQSSYLQGFS	[22]
53	**EDFFPASSR**		524091	QF**EDFFPASSR**DQSSYLQGFSR	[23]

53	**EDFFPASSR**		434773	VAKISMPVNTPGQF**EDFFPASS**	[26]
53	**EDFFPASSR**		434774	VAKISMPVNTPGQF**EDFFPASS**	[26]
53	**EDFFPASSR**		99530	PGQF**EDFFPASSR**DQ	[19]
53	**EDFFPASSR**		100400	PVNTPGQF**EDFFPASSR**DQS	[21]
53	**EDFFPASSR**		19983	SMPVNTPGQF**EDFFPASSR**D	[22]
53	**EDFFPASSR**		421060	GQF**EDFFPASSR**DQS	[24]; [25]
54	**DLAFPGSGEQVEKL**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237,B3IXL2)	99368	K**DLAFPGSGEQVEK**L	[25]; [19]
54	**DLAFPGSGEQVEKL**		190804	K**DLAFPGSGEQVEKL**IKNQK	[22]
55	**DLAFPGSGEQVEK**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237,B3IXL2)	99368	K**DLAFPGSGEQVEK**L	[25]; [19]
55	**DLAFPGSGEQVEK**		190804	K**DLAFPGSGEQVEKL**IKNQK	[22]
56	**CVYDPR**	Ara h 1 (P43238, N1NG13)	99385	KLEYDPR**CVYDPR**GH	[19]
56	**CVYDPR**		99782	YDPR**CVYDPR**GHTGT	[19]
56	**CVYDPR**		99919	**CVYDPR**GHTGTTNQRSPPGE	[21]
56	**CVYDPR**		100455	RCTKLEYDPR**CVYDPR**GHTG	[21]
56	**CVYDPR**		190820	KLEYDPR**CVYDPR**GHTGTTN	[22]
57	**AENNHRIF**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237,B3IXL2)	100037	FGIN**AENNHRIF**LAGDKDNV	[21]
57	**AENNHRIF**		190771	GIN**AENNHRIF**LAGDKDNVI	[22]
57	**AENNHRIF**		190988	SSELHLLGFGIN**AENNHRIF**	[22]
57	**AENNHRIF**		420973	FGIN**AENNHRIF**LAG	[24]; [25]
57	**AENNHRIF**		521205	LHLLGFGIN**AENNHRIF**LAGDK	[23]
58	CLQSCQ**QEPDDLKQKA**	Ara h 1 (P43238, N1NG13, Q6PSU3, P43237,B3IXL2)	98953	**QEPDDLKQKA**	[30]
58	CLQS**CQQEPDDLKQKA**		99129	**CQQEPDDLKQKACES**	[19]
58	C**LQSCQQEPDDLKQKA**		99443	**LQSCQQEPDDLKQKA**	[19]
59	**CMCEALQQIMENQ**	Ara h 2 (Q6PSU2, Q6PSU2-2, Q6PSU2-3, Q6PSU2-4)	53291	R**CMCEALQQIMENQ**SDRLQG	[33]; [22]; [34]
59	**CMCEALQQIMENQ**		15608	FENNQR**CMCEALQQIMENQ**	[35]
59	**CMCEALQQIMENQ**		53290	R**CMCEALQQIMENQ**SDRLQ	[35]
59	**CMCEALQQIMENQ**		178803	FENNQR**CMCEALQQIMENQ**S	[33]
60	**NLPQQCGLRAPQR**	Ara h 2 (Q6PSU2, Q6PSU2-2, Q6PSU2-3, Q6PSU2-4)	33124	KRELR**NLPQQCGLRAPQR**CD	[22]; [34]
60	**NLPQQCGLRAPQR**		39150	LR**NLPQQCGLRAPQR**CDLD	[35]
60	**NLPQQCGLRAPQR**		99448	LR**NLPQQCGLRAPQR**	[19, 36]
60	N**LPQQCGLRAPQR**		105306	**LPQQCGLRAPQR**	[37]
60	**NLPQQCGLRAPQR**		179200	LR**NLPQQCGLRAPQR**CDLDV	[33]
60	**NLPQQCGLRAPQR**		179375	QFKRELR**NLPQQCGLRAPQR**	[33]
60	**NLPQQCGLRAPQR**		514924	ELR**NLPQQCGLRAPQR**CDLEV	[23]
60	**NLPQQCGLRAPQR**		515929	FKRELR**NLPQQCGLRAPQR**CD	[23]

Supporting Figures

Fig. S1

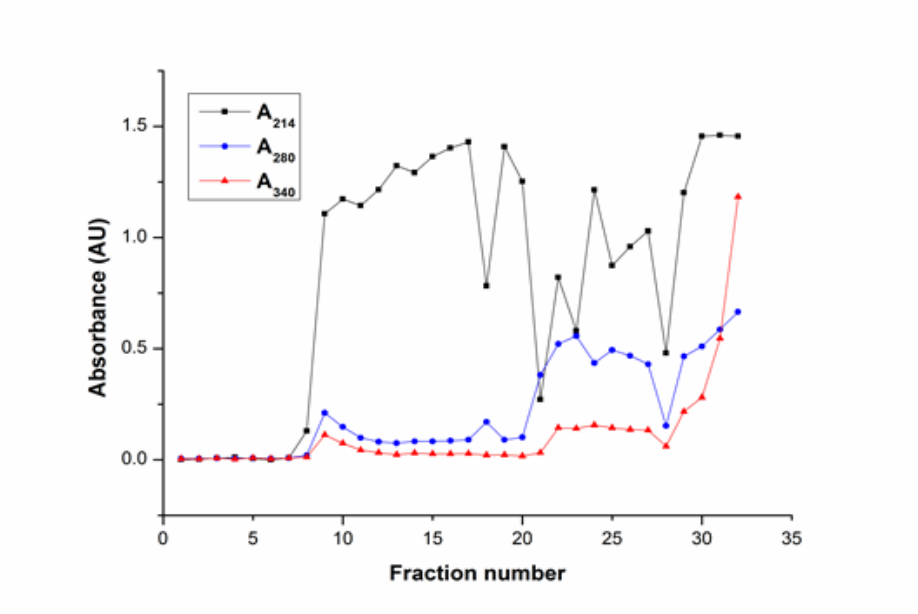

Fig. S1. Gel filtration of SDRPs obtained after in vitro oral-gastric phase of digestion of whole kernels peanut. After digestion liquid phase of digestion mixture was precipitated by ethanol and non-precipitated solution was applied to Sephadex G-25 column (20 ml of matrix; column size 0.8x40cm).

Fig. S2

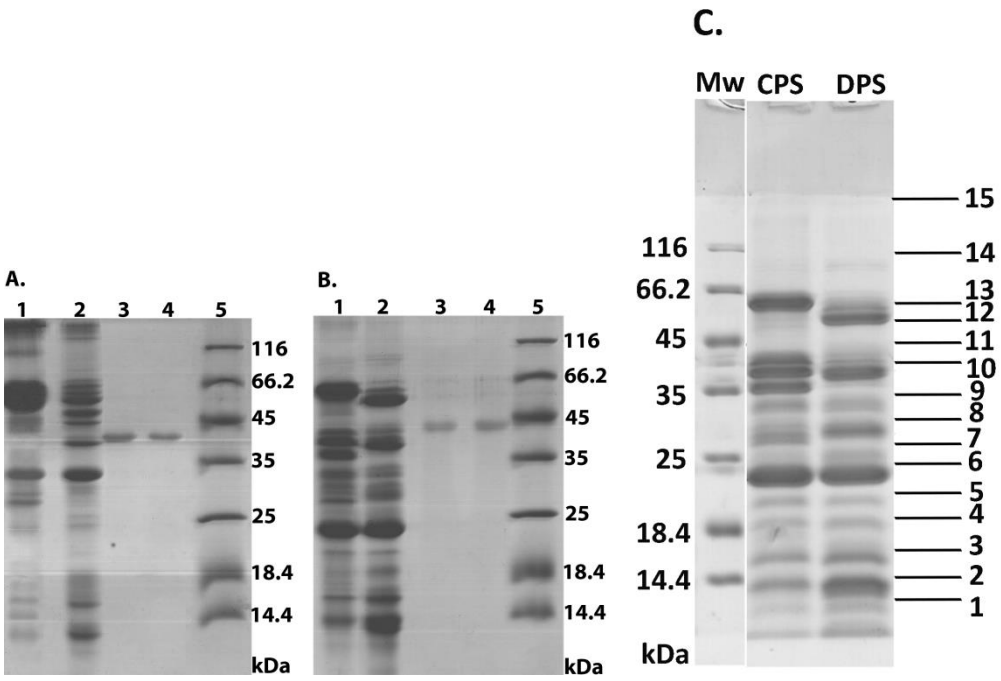

Figure S2. SDS PAGE profiles of digested and control peanut samples**.** A) non-reducing conditions; B) reducing conditions. Lane 1- control sample (without amylase and pepsin), lane 2 – digested sample, lane 3 – pepsin control at 0`, lane 4 – pepsin control at 120`, M-molecular weight markers. 23 µg of peanut proteins and 6 µg of pepsin were applied per lane. (C) 1D SDS PAGE profiles of peanut control sample (CPS) and digested peanut sample (DPS) analyzed by nLC-MS/MS spectrometry; identification results shown in Table S4.

Digestibility of peanut proteins from the whole grain was analyzed by non-reducing and reducing SDS-PAGE after simulated *in vitro* oral and gastric digestion (FigS2. A and B). Proteins from separated liquid phase of digestion mixture were precipitated by TCA and analyzed by SDS PAGE. We have analyzed TCA precipitated protein fraction in order to get insight into pepsin resistant protein fraction. TCA was able to precipitate about 30 % of protein extracted from peanut during digestion e.g about 10 % of whole peanut grain proteins.

Under non-reducing conditions (Fig S2.A), at the top of separating gel, high molecular mass aggregates of Ara h 1 could be observed in control sample, while they are much less intense in digested sample. It was reported that Ara h 1 when transferred from acidic (pH 2) to basic (pH 8) environment forms disulfide cross-linked aggregates with mass of about 250 kDa, and pepsin digestion destroys ability of Ara h 1 to form these aggregates [38]. In undigested

sample there is intensive band in region 55-65 kDa, containing Ara h 1 and disulfide linked acidic and basic Ara h 3 subunits [39], while in digested sample intensive series of discrete bands in the range 45-65 kDa, originating from proteolysis of Ara h 1 and Ara h 3, could be observed instead. Also, bands with Mr of approximately 30, 15 and 12 kDa are more intensive in digested sample. Under reducing conditions (Fig S2. B), it is obvious that almost all Ara h 1 was proteolyzed mainly to its 50 kDa form, and probably to forms with mass about 12 and 16 kDa. Proteolysis of Ara h 3 acidic forms (region 35-45 kDa) is also visible under reducing conditions, in contrast to basic forms which looks almost intact. These results ambiguously implies that both Ara h 1 and Ara h 3 were partly proteolyzed.

Fig. S3

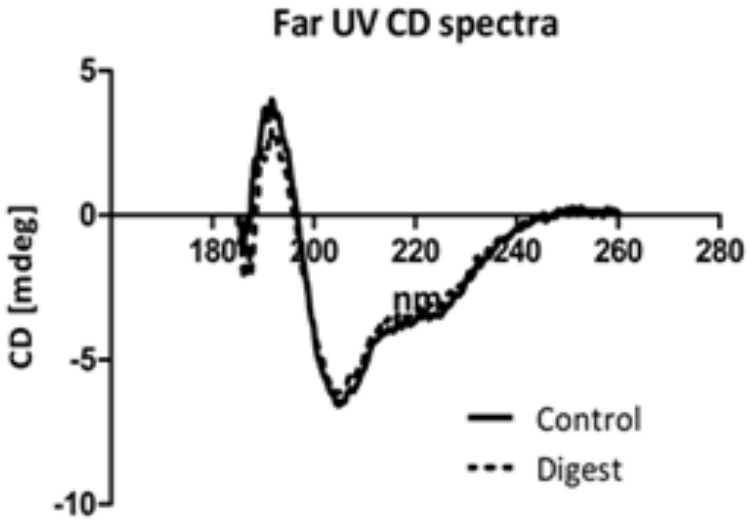

Figure S3. CD spectra of control and digested peanut.

Fig. S4

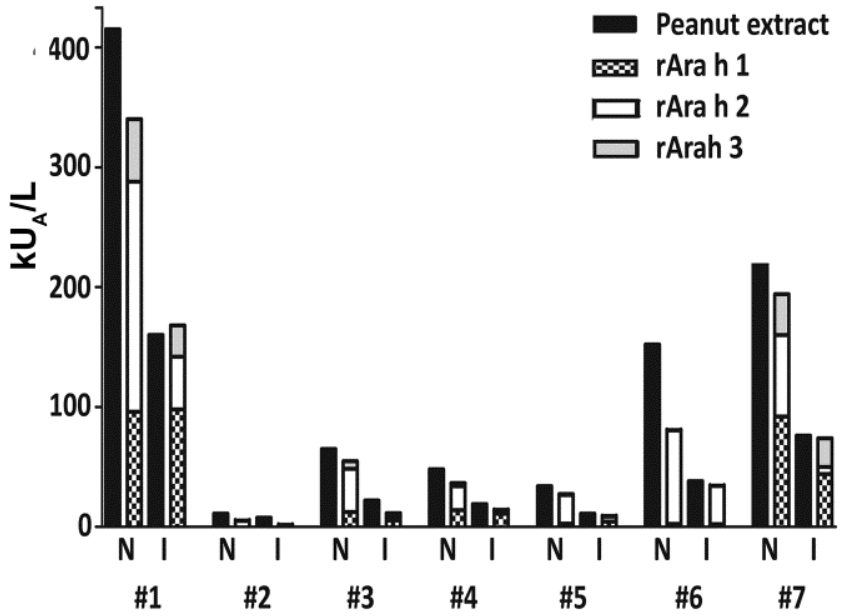

Fig. S4 ImmunoCAP absolute values of IgE binding for whole peanut extract, rArah 1, rAra h 2, and rAra h 3 inhibited by short digestion resistant peptides (SDRPs) fraction of peptides released during peanut gastric digestion. N – noninhibited; I –inhibited. X axis' numbers denote patients in Table S3.

Fig. S5

Ara h 1

a) KSSPYQKKTENPCAQRCLQSCQQEPDDLKQKACESRCTKLEYDPRCVYDPRGHTGTTNQRSP
PGERTRGRQPGDYDDDRRQPRREEGGRWGPAGPREREREEDWRQPREDWRRPSHQQPRKIRP
EGREGEQEWGTPGSHVREETSRNNPFYFPSRRFSTRYGNQNGRIRVLQRFDQRSRQFQNLQNH
RIVQIEAKPNTLVLPKHADADNILVIQQGQATVTVANGNNRKSFNLDEGHALRIPSGFISYILNR
HDNQNLRVAKISMPVNTPGQFEDFFPASSRDQSSYLQGFSRNTLEAAFNAEFNEIRRVLLEENA
GGEQEERGQRRWSTRSSENNEGVIVKVSKEHVEELTKHAKSVSKKGSEEEGDITNPINLREGEP
DLSNNFGKLFEVKPDKKNPQLQDLDMMLTCVEIKEGALMLPHFNSKAMVIVVVNKGTGNLE
LVAVRKEQQQRGRREEEEDEDEEEEGSNREVRRYTARLKEGDVFIMPAAHPVAINASSELHLL
GFGINAENNHRIFLAGDKDNVIDQIEKQAKDLAFPGSGEQVEKLIKNQKESHFVSARPQSQSQS
PSSPEKESPEKEDQEEENQGGKGPLLSILKAFN

b) KSSPYQKKTENPCAQRCLQSCQQEPDDLKQKACESRCTKLEYDPRCVYDPRGHTGTTNQRSP
PGERTRGRQPGDYDDDRRQPRREEGGRWGPAGPREREREEDWRQPREDWRRPSHQQPRKIRP
EGREGEQEWGTPGSHVREETSRNNPFYFPSRRFSTRYGNQNGRIRVLQRFDQRSRQFQNLQNH
RIVQIEAKPNTLVLPKHADADNILVIQQGQATVTVANGNNRKSFNLDEGHALRIPSGFISYILNR
HDNQNLRVAKISMPVNTPGQFEDFFPASSRDQSSYLQGFSRNTLEAAFNAEFNEIRRVLLEENA
GGEQEERGQRRWSTRSSENNEGVIVKVSKEHVEELTKHAKSVSKKGSEEEGDITNPINLREGEP
DLSNNFGKLFEVKPDKKNPQLQDLDMMLTCVEIKEGALMLPHFNSKAMVIVVVNKGTGNLE
LVAVRKEQQQRGRREEEEDEDEEEEGSNREVRRYTARLKEGDVFIMPAAHPVAINASSELHLL
GFGINAENNHRIFLAGDKDNVIDQIEKQAKDLAFPGSGEQVEKLIKNQKESHFVSARPQSQSQS
PSSPEKESPEKEDQEEENQGGKGPLLSILKAFN

Ara h 3

c) VTFRQGGEENECQFQRLNAQRPDNRIESEGGYIETWNPNNQEFQCAGVALSRTVLRRNALRRP
FYSNAPLEIYVQQGSGYFGLIFPGCPSTYEEPAQEGRRYQSQKPSRRFQVGQDDPSQQQQDSH
QKVHRFDEGDLIAVPTGVAFWMYNDEDTDVVTVTLSDTSSIHNQLDQFPRRFYLAGNQEQEF
LRYQQQQGSRPHYRQISPRVRGDEQENEGSNIFSGFAQEFLQHAFQVDRQTVENLRGENEREE
QGAIVTVKGGLRILSPDEEDESSRSPPSRREEFDEDRSRPQQRGKYDENRRGYKNGIEETICSAS
VKKNLGRSSNPDIYNPQAGSLRSVNELDLPILGWLGLSAQHGTIYRNAMFVPHYTLNAHTIVV
ALNGRAHVQVVDSNGNRVYDEELQEGHVLVVPQNFAVAAKAQSENYEYLAFKTSRPSIANL
AGENSIIDNLPEEVVANSYRLPREQARQLKNNNPFKFFVPPFDHQSMREVA

d) VTFRQGGEENECQFQRLNAQRPDNRIESEGGYIETWNPNNQEFQCAGVALSRTVLRRNALRRP
FYSNAPLEIYVQQGSGYFGLIFPGCPSTYEEPAQEGRRYQSQKPSRRFQVGQDDPSQQQQDSH
QKVHRFDEGDLIAVPTGVAFWMYNDEDTDVVTVTLSDTSSIHNQLDQFPRRFYLAGNQEQEF
LRYQQQQGSRPHYRQISPRVRGDEQENEGSNIFSGFAQEFLQHAFQVDRQTVENLRGENEREE
QGAIVTVKGGLRILSPDEEDESSRSPPSRREEFDEDRSRPQQRGKYDENRRGYKNGIEETICSAS
VKKNLGRSSNPDIYNPQAGSLRSVNELDLPILGWLGLSAQHGTIYRNAMFVPHYTLNAHTIVV
ALNGRAHVQVVDSNGNRVYDEELQEGHVLVVPQNFAVAAKAQSENYEYLAFKTSRPSIANL
AGENSIIDNLPEEVVANSYRLPREQARQLKNNNPFKFFVPPFDHQSMREVA

Ara h 2

e) RQQWELQGDRRCQSQLERANLRPCEQHLMQKIQRDEDSYGRDPYSPSQDPYSPSQDPDRRDP
YSPSPYDRRGAGSSQHQERCCNELNEFENNQRCMCEALQQIMENQSDRLQGRQQEQQFKREL
RNLPQQCGLRAPQRCDLEVESGGRDRY

Ara h 6

f) MRRERGRQGDSSSCERQVDRVNLKPCEQHIMQRIMGEQEQYDSYDIRSTRSSDQQQRCDELN
EMENTQRCMCEALQQIMENQCDRLQDRQMVQQFKRELMNLPQQCNFRAPQRCDLDVSGGR
C

Figure S5. The regions with peptides of Ara h 1 (**a, b**), Ara h 3 (**c, d**) and Ara h 2 (**e**) and Ara h 6 (**f**) found in short digestion resistant peptide (SDRP) fraction of peanut digested by pepsin; (**a, c**) intact peptides, (**b, d, e, f**) peptides found after reduction, alkylation and trypsin digestion of low molecular mass fraction of peanut digested by pepsin. [a] Continuous epitopes are underlined, [b] discontinuous epitopes are highlighted in green, and identified short digestion resistant peptides (SDRPs) of gastric digesta are in red letters. [a] Continuous epitopes found by Otsu et al. [40] for Ara h 2 and Ara h 6, Burks et al. [41] for Ara h 1, and Rouge et al. [42] for Ara h 3. [b] Motifs/consensus found in the mimotopes found by Chen et al. [43] for Ara h 2 and Ara h 6, Bogh et al. [44] for Ara h 1.

Fig. S6

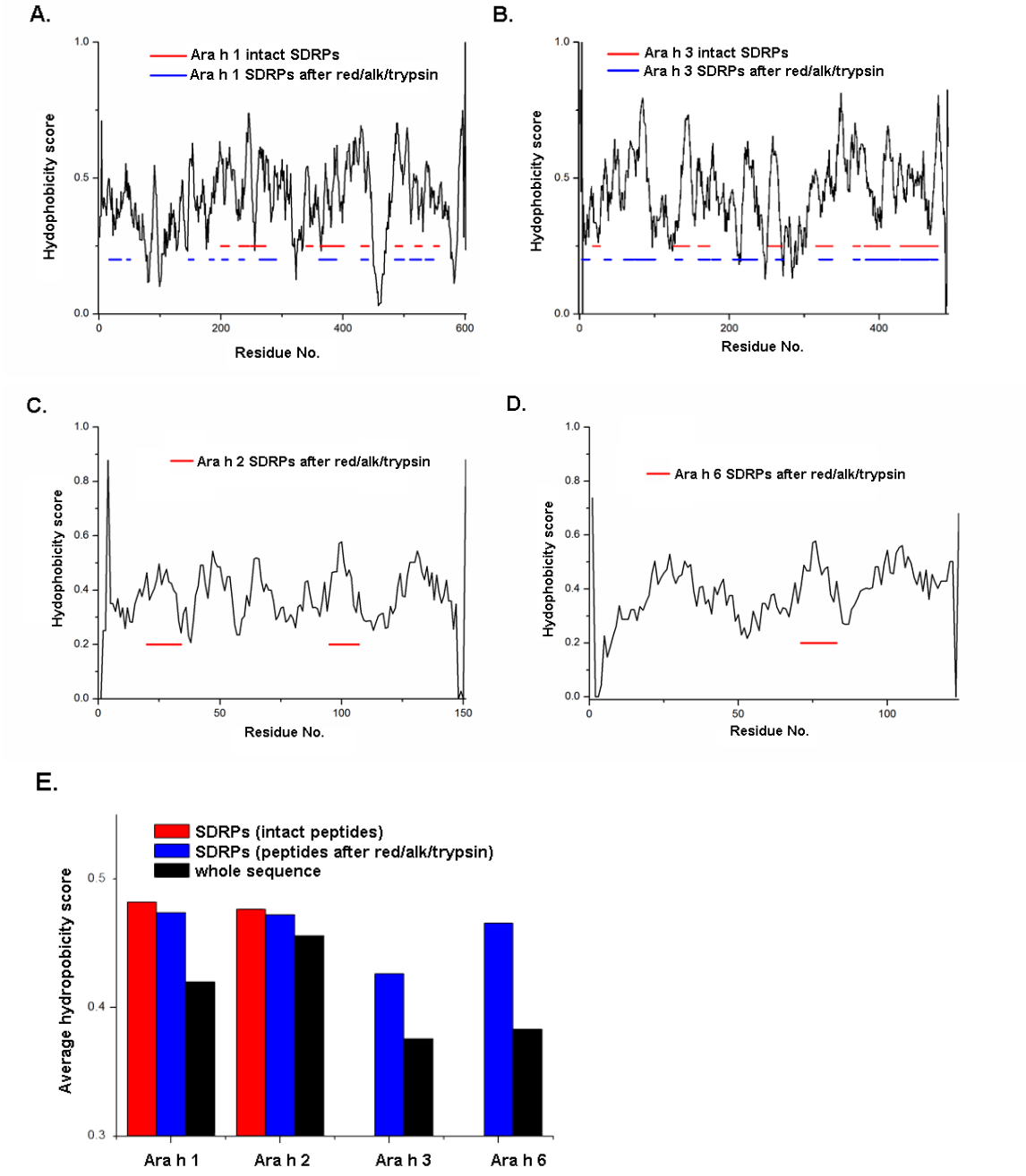

Fig. S6 Hydrophobicity curves of Ara h 1 (A) Ara h 2 (B), Ara h 3 (C) and Ara h 6 (D) with underlined regions of peptides found in SDRPs fraction of digested peanut. E) Average hydrophobicity scores of Ara h 1, Ara h 2, Ara h 3 and Ara h 6 regions of peptides found in SDRPs fraction and whole protein sequence. Hydropathy curves were made by ExPASy - ProtScale (web.expasy.org/protscale/), according to Black et al. [45] amino acid scale and using UniProtKB/Swiss-Prot accession number P43238 for Ara h 1 and Q6IWG5 for Ara h 3.

Fig. S7

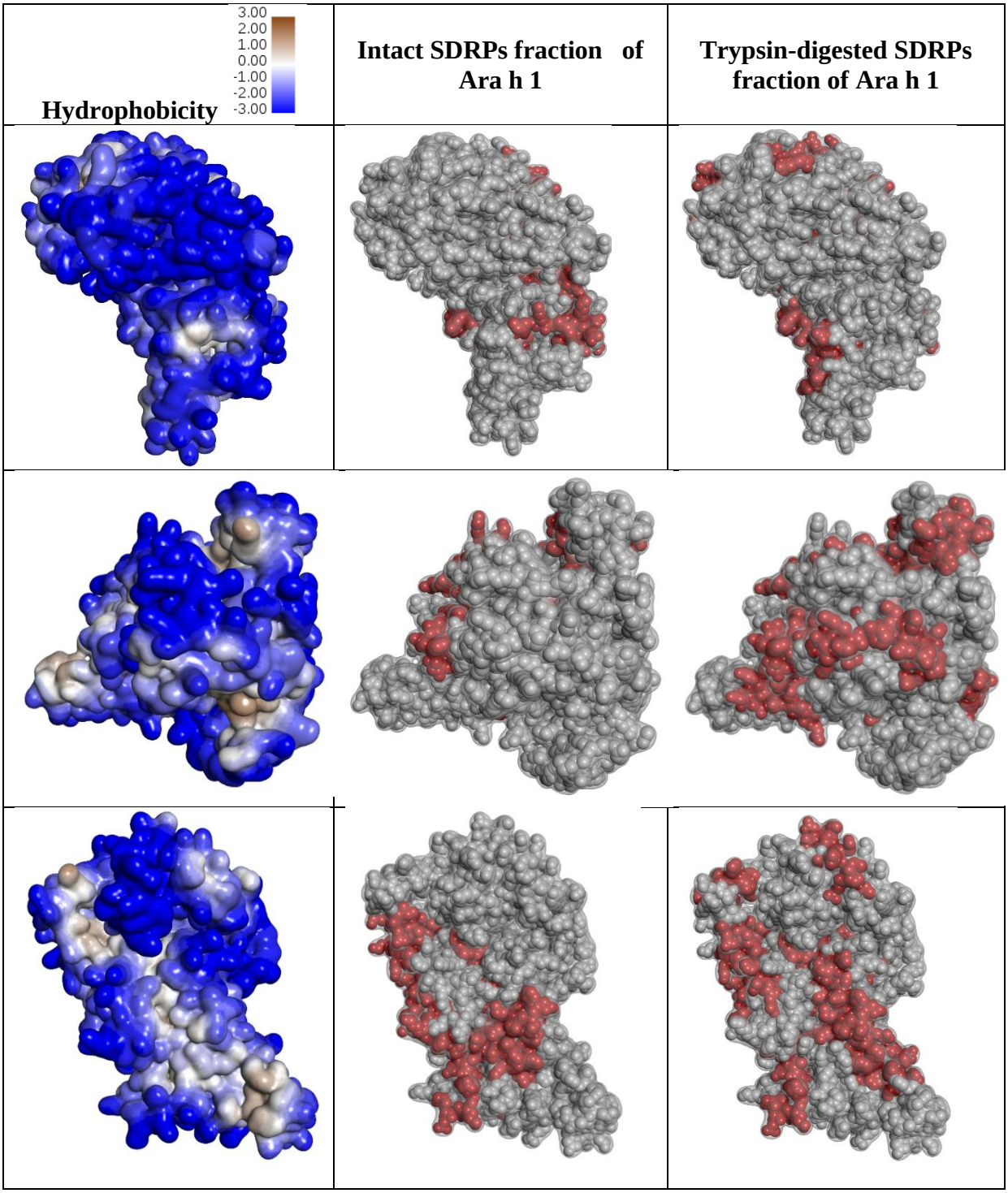

Fig. S7. Solvent accessible surface of Ara h 1 from three different angles with labelled gradual hydrophobicity level (from deep blue for the least hydrophobic to brown for the most hydrophobic area). The regions with identified peptides of Ara h 1 found in the short digestion resistant peptides (SDRPs) of peanut digested by pepsin are in red.

Fig. S8

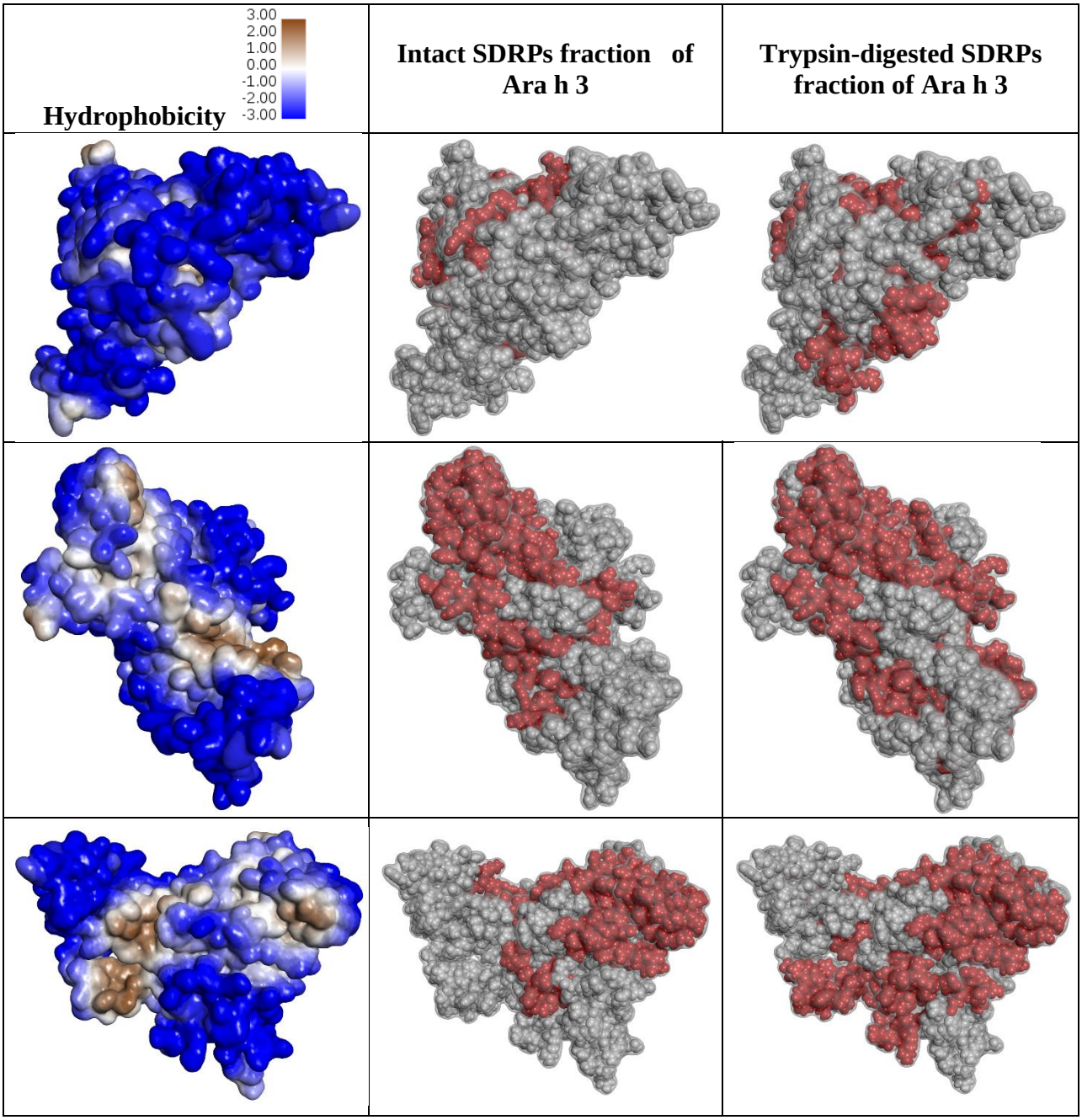

Fig. S8. Solvent accessible surface of Ara h 3 from three different angles with labelled gradual hydrophobicity level (from deep blue for the least hydrophobic to brown for the most hydrophobic area). The regions with identified peptides of Ara h 3 found in the short digestion resistant peptides (SDRPs) of peanut digested by pepsin are in red.

Fig. S9

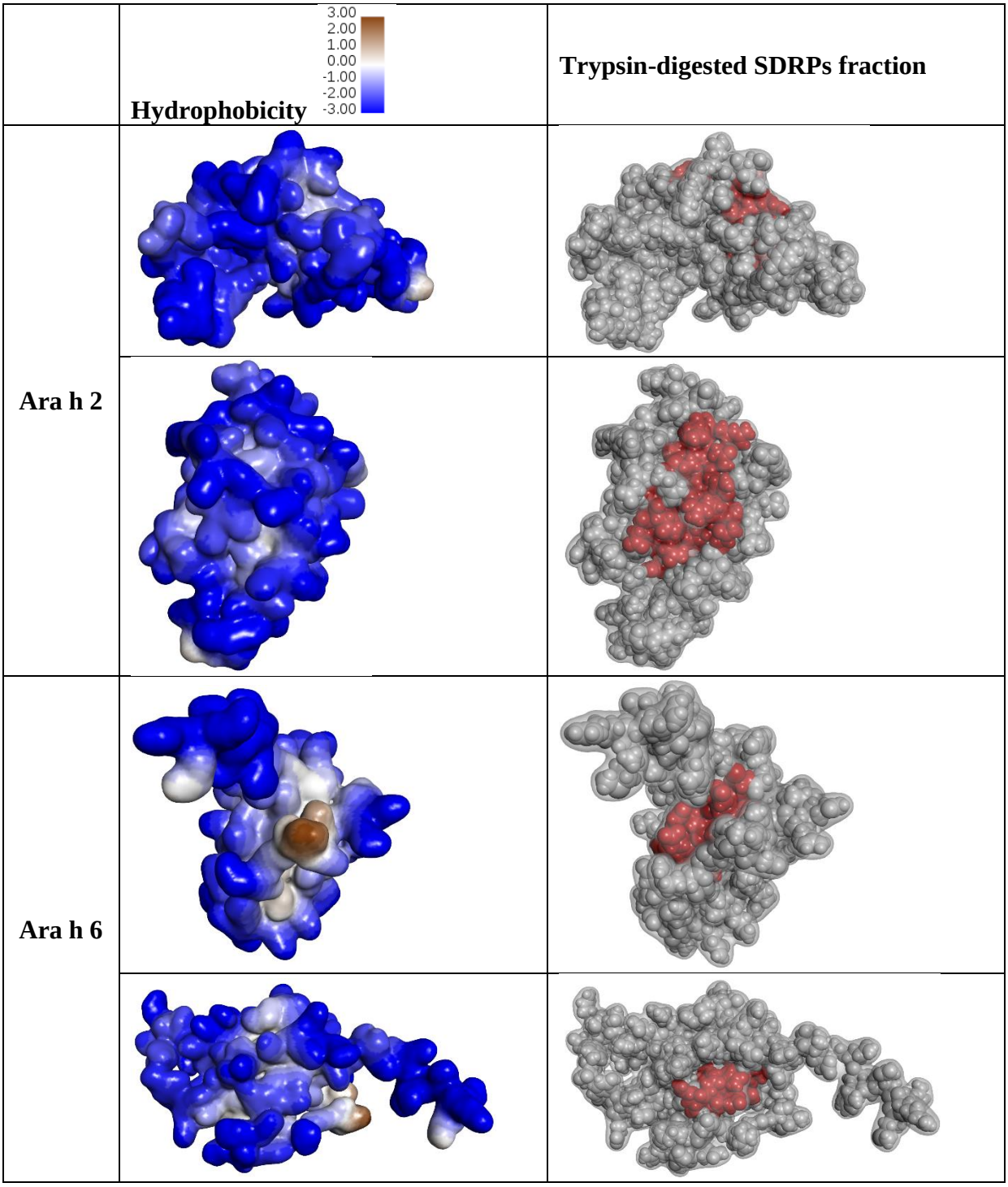

Fig. S9. Solvent accessible surface of Ara h 2 and Ara h 6 from two different angles with labelled gradual hydrophobicity level (from deep blue for the least hydrophobic to brown for the most hydrophobic area). The regions with identified peptides of Ara h 2 and Ara h 6 found in the short digestion resistant peptides (SDRPs) of peanut digested by pepsin are in red.

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