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Allergen composition analysis and allergenicity assessment of Chinese peanut cultivars

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ABSTRACT

Peanut (*Arachis hypogaea*) is among the eight major food allergens in the world. Several attempts have been made to decrease or eliminate the allergenicity of peanut. Systemic screening of thousands of peanut cultivars may identify peanut with low allergenicity. In this study, the allergen compositions of 53 Chinese peanut cultivars were characterized, and their allergenicity to sera IgE of Chinese patients and in a mouse model was assessed. Contents of total protein and allergens were quantified by SDS–PAGE and densitometry analysis on gel. Although the contents of allergens broadly varied among cultivars, they were related to one another. The IgE binding capacity of cultivars was tested by ELISA, and their allergenicity was further evaluated in a mouse model by oral sensitization. Results showed that the allergenicity of peanut was affected by allergen composition rather than a single allergen. Peanut cultivars with low allergenicity may contain more Ara h 3/4 (24 kDa), Ara h 2 and less Ara h 3/4 (43, 38, and 36 kDa), Ara h 6. Screening based on allergen composition would facilitate the identification of low-allergenic peanut.

1. Introduction

Peanut (*Arachis hypogaea*) is listed among the eight major food allergens by FAO. Peanut allergy is common, frequently severe, and typically permanent (Sicherer & Sampson, 2010), which makes it an increasingly common life-threatening disorder (Husain & Schwartz, 2012) that has attracted a great deal of interests (Blanc et al., 2011; Bogh, Barkholt, Rigby, Mills, & Madsen, 2012; Delong et al., 2011; Hong et al., 2012). Currently, strict peanut avoidance is still the best therapy choice because oral immunotherapy is still not ready for clinical use (Sampson, 2013; Thyagarajan et al., 2010).

Another strategy is to eliminate the allergenicity of proteins in food. Food processing may change intrinsic allergenicity; hence, several attempts have been made to influence protein allergenicity through food processing. For example, enzymatic cross-linking of peanut proteins increases the bioavailability of major peanut allergen Ara h 2 Radosavljevic et al., 2014); gamma irradiation can reduce Ara h 6 allergenicity (Luo et al., 2013); and boiling can change the formation of aggregates of Ara h 1, resulting in reduced allergenicity (Blanc et al., 2011). Although several processing methods can reduce the allergenicity of allergens, only few are practically used (Somkuti & Smeller, 2013).

Given the huge amount of peanut cultivars, finding a low allergenic peanut variety for food processing is also a good choice (Koppelman et al., 2001). Thousands of peanut cultivars are grown in China, with four major cultivar groups being the most popular, namely, Spanish, Peruvian, Virginia, and Valencia. However, only a few peanut cultivars have been screened on allergenicity in China (Cong et al., 2008).

Given that allergen triggers food allergy, allergen content in peanut can be analyzed to investigate the relationship between allergen content and allergenicity. Specifically, Ara h 1, Ara h 2, and Ara h 3 have been highlighted because of their major contributions to the development of peanut allergic reaction (Cong et al., 2008; Krause et al., 2010; Yusnawan, Marquis, & Lee, 2012).

Currently, thirteen proteins, namely, Ara h 1 to Ara h 13, have been identified as peanut allergens ("UIS Allergen Nomenclature Sub-Committee", 2013-11-07). However, the major allergens

Abbreviations: DPF, defatted peanut flower; sIgG, specific antibodies IgG; sIgG1, specific antibodies IgG1.

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among these proteins may differ in areas. Ara h 1 has been recognized in over 95% of patients from a North American population (Burks et al., 1991), whereas Ara h 2 and Ara h 6 have been reported to account for the majority of the effector activity in a crude peanut extract in westernized countries (Zhuang & Dreskin, 2013). Meanwhile, Ara h 3/4 has been regarded as the major allergen in Asia (Cong et al., 2008; Lin, Wu, Cheng, Huang, & Yeh, 2012). In the Mediterranean area, Ara h 9 is the most important peanut allergen (Krause et al., 2009). However, reducing a single protein may not necessarily cause a reduction in allergenicity (Krause et al., 2010), in which the composition of allergens may play a more important role.

In the present study, allergen compositions in 53 Chinese peanut cultivars were characterized and their allergenicity was assessed. The relationship between the allergen composition and allergenicity of the peanut cultivars in China was discussed.

2. Materials and methods

2.1. Chemicals

Prestained Protein Ladder was purchased from MBI Fermentas (Burlington, Canada). Unless otherwise stated, all other chemicals were obtained from Sigma–Aldrich (St. Louis, MO, USA) and were of analytical grade or superior.

2.2. Peanut samples and protein extraction

Raw peanut seeds were given by Guangxi Academy of Agricultural Sciences, China. The 53 peanut cultivars, which include four peanut types, are listed in Table 1. Defatted peanut flower (DPF) were prepared following the procedure described by Luo et al. (2013). After removing the skin, peanut kernels were finely milled

Table 1
The allergen pattern and IgE binding capacity of peanut.*

Code	Name	Type	Total protein	Ara h 1 (64 kDa)	Ara h 2 (17 kDa)	Ara h 3/4 (43, 38, 36 kDa)	Ara h 3/4 (24 kDa)	Ara h 6 (15 kDa)	IgE binding capacity
1	Rongxianniujiaodou	Peruvian	365.40	102.62	12.66	164.80	84.36	14.55	0.339
2	Ningmingwuqushixing	Peruvian	457.90	102.98	13.48	309.64	133.71	41.61	0.329
3	Yishandazihuasheng	Peruvian	367.60	47.86	28.93	124.94	149.62	34.44	0.335
4	Yaoshangxiaomake	Peruvian	378.50	223.17	79.55	422.19	175.98	32.60	0.287
5	Longanbaowanhuasheng	Peruvian	429.70	69.32	8.71	200.35	100.80	14.79	0.372
6	Lengshuidamake	Peruvian	325.10	94.88	18.01	115.21	47.45	27.12	0.366
7	Pinglezi	Peruvian	377.10	103.91	11.13	135.97	49.69	16.77	0.348
8	Quanzhoumake	Peruvian	380.40	40.00	7.30	118.70	176.85	39.07	0.453
9	Wuxuanmake	Peruvian	425.90	20.92	16.50	249.31	91.54	54.69	0.411
10	Linguimakechangyao	Peruvian	400.20	28.75	11.62	256.64	118.86	32.86	0.384
11	Huningdexianghuasheng	Peruvian	461.30	96.70	9.37	277.84	115.94	9.69	0.366
12	Chongzuoniujiaodou	Virginia	427.50	93.83	13.56	262.19	110.19	34.18	0.397
13	Hexianzhushidou	Virginia	466.60	78.83	15.21	280.68	129.46	24.33	0.340
14	Guipingdahuasheng	Virginia	408.00	87.03	13.88	229.87	87.03	25.32	0.361
15	Ziyuanhuasheng	Virginia	385.10	71.21	20.04	199.26	87.32	38.16	0.363
16	Tengxiandamadou	Virginia	353.80	67.79	10.00	191.72	93.91	27.60	0.386
17	Pingnanzhiyaodou	Virginia	379.30	74.64	11.41	205.72	87.61	11.48	0.315
18	Hexiandahuash	Virginia	425.40	152.67	26.94	170.05	138.16	16.81	0.334
19	Guixianannandou	Virginia	451.50	82.07	10.29	244.85	110.36	20.13	0.392
20	Hexianqingdou	Virginia	427.40	86.75	6.24	260.40	118.40	13.77	0.415
21	Caochunxiyaohuasheng	Virginia	441.00	85.72	13.79	274.68	126.63	23.70	0.345
22	Guihua 17	Virginia	502.50	113.81	18.53	34.87	186.64	26.72	0.371
23	Rongxiandahuasheng	Virginia	412.90	87.87	12.20	251.89	118.06	29.17	0.414
24	Bobaidahuasheng	Virginia	412.30	32.78	10.10	273.89	129.14	30.63	0.400
25	Quanzhoufangkezi	Virginia	352.60	87.39	8.21	204.66	94.30	40.70	0.402
26	Lipufanguidou	Virginia	401.90	82.13	18.65	212.93	89.15	26.88	0.428
27	Puditan	Virginia	370.10	65.06	16.87	186.75	86.94	32.90	0.355
28	Yishanghuasheng	Virginia	413.50	62.14	10.20	122.40	182.34	33.71	0.326
29	Quanxianfanguidou	Virginia	416.40	65.23	14.20	213.63	96.70	20.99	0.380
30	Zuozhoudahuasheng	Virginia	382.90	79.84	17.31	237.23	113.98	23.27	0.402
31	Xinxiandahuasheng	Virginia	398.19	76.38	16.46	119.82	143.82	6.70	0.245
32	7501-11	Virginia	425.22	124.06	19.92	129.86	189.75	7.21	0.263
33	H771	Spanish	375.85	76.54	22.64	103.98	152.77	19.92	0.334
34	Shapohuasheng	Spanish	398.34	43.99	6.42	112.51	183.91	11.06	0.315
35	Luhua 12	Spanish	446.06	104.03	17.71	169.12	210.97	16.59	0.344
36	Minhua 6	Spanish	432.75	118.34	19.56	129.60	194.31	11.63	0.315
37	Guihua 26	Spanish	486.24	88.06	12.69	168.34	226.11	4.32	0.325
38	Quanhua 726	Spanish	451.65	82.53	32.74	103.40	143.66	36.32	0.330
39	Changhua 1	Spanish	397.29	110.09	31.12	116.90	205.41	14.04	0.296
40	8405-3	Spanish	433.90	77.66	29.54	151.78	170.27	1.78	0.295
41	Xinonghua	Spanish	454.10	85.58	25.73	148.98	164.38	9.28	0.330
42	Dalunhuasheng 1	Spanish	396.80	45.88	23.36	142.95	124.77	12.38	0.355
43	Guihua 22	Spanish	445.73	74.76	39.20	145.75	152.21	20.46	0.399
44	0475	Spanish	480.61	113.48	18.12	191.71	252.67	7.16	0.335
45	Shengpinghuasheng	Spanish	430.17	67.30	12.25	196.33	185.72	3.03	0.310
46	Baimahonghuasheng	Spanish	316.51	30.01	96.32	90.43	98.09	12.48	0.331
47	ICGV87281	Spanish	422.73	63.08	6.75	135.19	158.98	1.62	0.312
48	Mangdou	Spanish	451.25	95.02	25.12	158.11	223.76	3.51	0.275
49	Guihua 30	Spanish	487.30	74.05	22.91	210.70	283.97	11.46	0.284
50	Yangdaohonghuasheng	Valencia	404.22	68.61	20.53	142.87	192.39	3.05	0.338
51	Bangjihonghuasheng	Valencia	373.67	46.29	50.23	82.11	201.97	33.05	0.264
52	Ganhua 7	Valencia	500.05	86.91	61.85	217.13	221.82	15.73	0.391
53	Ganhua 92-01	Valencia	507.99	102.55	28.66	246.33	236.43	4.06	0.364

* The unit of protein/allergens listed in the table was mg per gram of defatted peanut flower (DPF).