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COMPARATIVE STUDY ON ALLERGENIC PROPERTIES OF HIGH-OLEIC AND CONVENTIONAL PEANUTS

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Peanuts are one of the most allergenic foods, and peanut proteins are known to cause the allergic reactions. High oleic peanuts have been associated with the greatly enhanced shelf-life, decreased rancidity of roasted peanuts, a preventive effect against hyperlipidemia, and suppressed lung tumorigenesis induced chemically in mice. In this study, 33 raw peanuts of different varieties purchased from various parts of the world were evaluated and compared with two high--oleic peanuts (SunOleic, Florunner 458) in allergenicity. Identification of peanut allergens from defatted extracts was accomplished by means of sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and by using Western immuno-blotting with sera from peanut-allergic patients. The content of oleic acid was also determined and compared to other fatty acids by using gas chromatography (GC) in order to indicate peanut oil quality. SDS-PAGE revealed that the major peanut allergens known as Ara h 1 (64 kDa), Ara h 2 doublet (17 and 19 kDa), and Ara h 3 fragment (14 kDa) were displayed in all protein extracts. In contrast, only low-oleic peanut (LO) varieties contained the protein band at 36 kDa (Ara h 3/Ara h 4) that did not appear in high-oleic and several normal oleic (NO) varieties. The sensitivity to a 14 kDa protein was found to be higher in one patient than another by using immunoblotting. A substantially increased threshold of sensitivity to high oleic peanuts at least 5 times higher than to other peanuts was confirmed by an oral food challenge test, and thus could provide decreased allergenic sensitization.

INTRODUCTION

Allergy to peanut represents a significant health problem because of the severity of the allergic reactions and the widespread use of peanut products. While deaths from food allergies are rare, however, the prevalence of peanut allergy appears to be increasing. Peanuts have a protein complex containing over 30 different proteins [Loza *et al*., 1995] and more than half of these can bind serum IgE from sensitized individuals [Dean, 1998]. Three major peanut allergens, which were recognized by more than 50% of peanut allergic individuals called Ara h 1 (64 kDa vicilin-like seed storage protein), Ara h 2 (17 and 19 kDa conglutin-like), and Ara h 3/Ara h 4 (glycinin-like) have been identified, and their primary structure and major IgE-binding epitopes have been characterized [Burks *et al*., 1998; Shin *et al*., 1998; Rabjohn *et al*., 1999]. In addition, three minor allergens, Ara h 6 (14.5 kDa) and Ara h 7 (15.8 kDa) (both conglutin-like) as well as the plant allergen profilin Ara h 5 (14.0 kDa) were described [Kleber-Janke *et al*., 1999]. The differences in the serology of peanut allergic patients from different parts of the world were reported to may not originate from the differences in the allergen composition [Koppelman *et al*., 2001]. While crude peanut oils can induce allergic reactions, the allergenicity of refined peanut oils is controversial. Allergic reactions to peanut oil proteins are not frequent and may be caused by the oleosins, a family of proteins involved in the formation of oil peanut bodies [Pons *et al*., 2002].

Peanuts high in oleic acid developed at the University of Florida, USA with use of modern breeding methods, have oil containing 80% oleic acid or even more and 2% linoleic acid as compared to 50% oleic and 25% linoleic in oil of conventional peanuts. High oleic peanuts were reported to enhance the shelf-life three times longer [Mugendi *et al*., 1998] and reduce rancidity [Braddock *et al*., 1995]. In addition, new oils have been previously shown to improve substantially the oxidative stability at least 4 times more, but be only moderately better in the frying experiment than conventional peanut oils [Sakurai *et al*., 1999; Parkányiová *et al*., 2000], lower cholesterol levels in hypercholesterolemic women [O'Byrne *et al*., 1993], have a preventive effect against hyperlipidemia, and suppress chemically-induced lung tumorigenesis in mice [Sakurai *et al*., 2000; Yamaki *et al*., 2002].

Moreover, several *in vivo* studies indicated an association between fatty acids and allergy. For instance, Watanabe *et al*. [1994] reported the effects of dietary fatty acids such as linolenic acid and linoleic acid on allergic responses in mice. Diets high in a ratio of linolenic to linoleic acids were shown to suppress allergic responses and anaphylactic shock in mice [Kuwamori *et al*., 1997; Gu *et al*., 1998].

Because high-oleic peanuts are unique in the high ratio of oleic to linoleic acid, it is possible that the allergenicity of high-oleic peanuts may change. In contrast to extensively provided biochemical and immunological knowledges on individual peanut allergens as well as a detailed picture of the

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immune response, little research was done on the differences in the allergen composition and the IgE-binding properties between high-oleic and conventional peanut varieties. The aim of this study was to compare high-oleic and conventional peanuts from different parts of the world in fatty acid composition by means of GC and in allergenicity by using SDS-PAGE, immunoblotting, and in *in vivo* study.

MATERIALS AND METHODS

MATERIALS

All chemicals were analytical reagents of the highest obtainable purity.

Peanut samples. High-oleic and various conventional varieties of peanuts (*Arachis hypogaea*) grown in different countries of the world were generously provided by Denroku Co., Ltd. (Yamagata, Japan) and were stored at -20°C until use. Table 1 summarizes the varieties, and countries of origin.

Blood samples. For detection of IgE binding to different peanuts, immunoblotting was performed with two patient sera supplied by Professor Hideo Takahashi from School of Medicine, Nihon University (Tokyo, Japan). The patients had a positive skin prick test to peanuts and a convincing history of peanut allergy. Three nonallergic subjects were used as controls. Venous blood was drawn from the individuals at the health center of College of Bioresource Sciences, Nihon University (Kanagawa, Japan), mixed 3 times by using a glass rod, allowed to stand for 1 day at 4°C and mixed once again. After centrifugation at 3000 g for 15 min at room temperature (RT), serum was collected and stored at -20°C until use.

Preparation of peanut extracts. Seeds of raw peanuts were dehulled, skin removed, crushed by using a mixer, and then defatted by washing with at least 10 volumes (w/v) of n-hexane (Wako, Osaka, Japan) at RT for 18 h. Defatted powder was obtained after filtration (*) and dried overnight at 4°C.

Protein was extracted from dried defatted powder by mixing with 18 volumes (w/v) of distilled water and the solution was adjusted to pH 8.0 by NaOH, stirred for 1 hour at 4°C, and followed by centrifugation at 15,000 g for 30 min at 4°C. Collected supernatant was taken to 100% ammonium sulfate saturation, stirred for additional 2 h at 4°C, and subsequently centrifuged at 15,000 g for 30 min at 4°C. The obtained precipitate was dissolved in distilled water, and dialyzed against water using a seamless cellulose (Membra-cel) tubing (Viskase Companies Inc., Willowbrook, IL, USA) to remove ammonium sulfate. Protein was collected, lyophilized, and stored at -20 °C until use.

METHODS

Peanut oil preparation and analysis of fatty acids. Filtrate (*) was treated with activated charcoal at room temperature for 1 day. Peanut oil was obtained from filtrate after dehydration by anhydrous sodium sulfate and removing hexane under vacuum in a rotating evaporator. Fatty acid analysis of peanut oils was accomplished by the use of a modification of base-catalysed transesterification method [Christie, 1993].

TABLE 1. Description of peanut samples used.

Sample code	Varieties	Countries of origin
1	Roka-14	China
2	SKS-1	China
3	Hana-37	China
4	8130	China
5	Hanaiku-17	China
6	Roka-10	China
7	79266	China
8	Hanaiku-16	China
9	Umihana-1	China
10	Tachimasari	Japan
11	Runner	Argentina
12	Natal Common	Zimbabwe
13	Flamingo	Zimbabwe
14	Jasper	S. Africa
15	Robbie	S. Africa
16	Anel	S. Africa
17	Kwarts	S. Africa
18	Akwa	S. Africa
19	Harts	S. Africa
20	Sellie	S. Africa
21	ICGV-93415	S. Africa
22	PC 223-K3	S. Africa
23	PC 222-9	S. Africa
24	40/50 Florman	Paraguay
25	38142 Type 1	Paraguay
26	Runner	Paraguay
27	Florunner 96 Medium 45/50	USA
28	Georgia Green Runner Medium 40/50	USA
29	**Florunner 458 Medium 40/50**	USA
30	50/60 “Caiapo” Runner type	Brazil
31	60/70 “Tatu Vermelho” Red skin	Brazil
32	60/70 Not identified variety	Argentina
33	Australian Peanut VKI	Australia
34	Australian Peanut VKJ	Australia
S	**SunOleic**	USA

names in bold indicate high-oleic acid peanut varieties

Preparation of methyl esters. Approximately 10 mg of peanut oil was weighed into a 3 mL vial and 1 mL of hexane containing 3 mg of internal standard (methyl margarate) was added. 1 mL of sodium methoxide (28%) in methanol was added, capped, and heated at 80°C for 20 min. After cooling the sample, 2 mL of hexane, 1 mL of filtered water, and 1 mL of saturated sodium chloride were added, capped, and

shaked. When layers were separated, top layer was pippeted and washed by filtered water. The washed hexane layer was dried with anhydrous $MgSO_4$. The dry solution was then filtered into a vial to get the sample for GC analysis. Triplicate methylations were carried out for each oil sample.

Gas Chromatography. A model Hewlett Packard (HP) 6890 Series GC System gas chromatograph coupled with a FID detector, HP 7683 Series Injector (Wilmington, DE), and HP Chemstation software was used to analyze transesterified methyl esters of fatty acids. A 1-μL portion of the prepared solutions was injected into the GC column under split conditions (50:1). A DB-Wax fused silica capillary column (30 m x 0.25 mm i.d. x 0.25 μm film thickness) from J&W Scientific (Folsom, CA) was used for analysis. The column head pressure was 23.7 psi. The injector temperature was 210°C, and the detector was kept at 250°C. Oven temperature was held at 145°C initially for 5 min, then increased to 210°C at a rate of 4°C.min^{-1}, and finally isothermally maintained at 210°C for 40 min.

Gas Chromatography/Mass Spectrometry (GC/MS). A 2-μL portion of the solutions was injected under splitless conditions into a DB-Wax fused silica column (60 m x 0.25 mm i.d. x 0.25 μm film thickness) (Folsom, CA) installed in a Hewlett-Packard (HP) 5980 Series 2 gas chromatograph (Palo Alto, CA) equipped with an autosampler. The injector and detector temperatures were 210°C and 250°C, respectively. Helium was used as the carried gas at a column head-pressure of 36.6 psi. The mass spectrometer was operated in the electron ionization (EI) mode at 70 eV scanning masses m/z 20-800. The ion source and quadrupole temperatures were 250°C and 100°C, respectively. The oven temperature was programmed from 50°C (5 min isothermal) to 210°C at a rate of 4°C.min^{-1}, and then held for 60 min at final temperature. Compounds were identified by matching of their mass spectra with the mass spectral data of standard compounds in the Wiley PBM MS database and by the comparison of their GC retention times with those of authentic standards.

SDS-PAGE analysis. Proteins were analyzed by using SDS-PAGE, which was performed on the Model AE-8270 PowerStation 500VC (Atto, Tokyo, Japan). Proteins (1 mg) solubilized in 1 mL of an extraction buffer (0.16 mol/L Tris--HCl, 2% SDS, 20% glycerol, 2% 2-mercaptoethanol) were denatured at 100°C for 5 min. Polyacrylamide gradient (Multigel 10-20%) gels (84 x 90 x 1.0 mm width) (Daiichi Pure Chemicals Co. Ltd., Tokyo, Japan) were loaded with 20 μL of the 1 mg/mL peanut protein solution (20 μg protein/well). Electrophoresis was performed at 200 V with a constant current of 18 mA for 200 min in a SDS buffer (0.05 mol/L Tris, 2.88% glycine, 0.1% SDS). Prestained low range molecular weights markers (10 μL) (Bio-Rad Laboratories, Hercules, CA, USA) were run both sides all the samples. The molecular weight markers used were phosphorylase B (113 kDa), bovine serum albumin (93 kDa), ovalbumin (50.3 kDa), carbonic anhydrase (35.5 kDa), soybean trypsin inhibitor (28.8 kDa), and lysozyme (21.4 kDa). Proteins were visualized with Coomassie blue R-250 (Wako, Osaka, Japan).

IgE-immunoblotting. The proteins separated by SDS--PAGE were electrophoretically transferred to a polyvinylidene--difluoride (PVDF) membrane (Atto, Tokyo, Japan) pre-wetted in methanol and a blocking C solution (25 mmol/L Tris, 10% methanol, 0.075% SDS, 0.5% 6-aminohexanoic acid, 0.04% 2-mercaptoethanol) on a semidry device AE-6677 (Atto, Tokyo, Japan) for 1 h at 200 V and 32 mA. Proteins were visualized with Coomassie blue R-250 (Wako, Osaka, Japan).

After soaking at a blockage solution (Yukijirushi, Sapporo, Japan) for 2 h and subsequent 4 washes with PBS-Tween plus (TPBS) (0.06% Tween in a phosphate buffered saline-PBS) for 5 min each, the PVDF membranes were blocked for 10 min at RT in the Avidin and Biotin blocking solutions (Funakoshi, Tokyo, Japan) in TPBS, respectively, washed in TPBS, and incubated with patient and control sera diluted 1:60 (v/v) in TPBS buffer containing 0.3% skim milk for 18 h with gentle agitation at 4°C. After rising with TPBS, bound IgE was detected using Biotin-labelled goat antihuman IgE (Cosmobio, Tokyo, Japan) diluted (1:3000) in TPBS buffer containing 0.3% skim milk, followed by horseradish peroxidase (HRP)-Strept Avidin (Funakoshi, Tokyo, Japan) at RT for 1.5 h and 20 min, respectively, with washes between each step with TPBS, before detection with NADH/POD (Wako, Osaka, Japan) for 20 min at RT in dark. PVDF membranes were air dried.

***In vivo* study design.** Because of the risk of exposing to allergic reactions, only one patient with a positive skin-prick test and reported clinical history of peanut allergy was agreed to be examined. An immediate mild symptom to peanut (throat pruritus) was confirmed after the ingestion of 1 roasted normal peanut. The oral food challenge test with roasted high-oleic acid peanut (SunOleic) was initiated at 1 peanut, and then slowly increased to 5 peanuts, if tolerated.

RESULTS AND DISCUSSION

Fatty acid analysis

Showed in Table 2 is the fatty acid composition of peanut oil from 35 used samples. Palmitic acid (16:0), oleic acid (18:1), and linoleic acid (18:2) are the major fatty acids and the remaining fatty acids, stearic (18:0), arachidic (20:0), eicosenoic (20:1), behenic (22:0), and lignoceric (24:0), belong to the minor ones in peanuts. High-oleic peanut varieties were readily distinguished from the conventional varieties on the basis of the contents of oleic and linoleic acids. High oleic acid and conventional varieties had an oleic acid content of 81.0-81.7% and 41.3-59.1%, respectively. By contrast, the linoleic acid content was 21.8-35.2% for normal oil varieties compared to 2.9-3.6% for high-oleic lines. These findings are in agreement with those of others [Norden *et al*., 1987; Andersen *et al*., 2002]. This resulted in an oleic/linoleic (O/L) ratio of 22.7:1 to 28.4:1 for high-oleic lines, compared to 1.2:1 to 2.7:1 for conventional peanut varieties.

Palmitic acid showed to be up to twice as high for normal oleic acid (8.9-11.1%) compared to high oleic acid (4.8--4.9%) varieties. The negative relationship between palmitic acid and oleic acid most likely represents an increased rate of palmitic acid elongation to stearic acid, with rapid desaturation to oleic acid *via* Δ9 desaturase. The content of eicosenoic acid (2.7-3.1%) in high oleic was about 1.5 to 2-fold

TABLE 2. Fatty acid composition of peanut oil from 35 peanut samples.

Sample code	Fatty acid composition (%)							
	C16:0	C18:0	C18:1	C18:2	C20:0	C20:1	C22:0	C24:0
1	9.46	3.96	54.94	25.68	1.59	0.87	2.23	1.28
2	9.73	3.51	53.76	25.63	1.70	1.18	2.91	1.58
3	9.84	3.42	54.43	25.09	1.67	1.11	2.91	1.52
4	9.81	3.32	53.70	25.70	1.67	1.21	3.04	1.54
5	10.06	3.95	46.80	32.59	1.75	0.87	2.55	1.43
6	10.41	4.35	41.34	35.19	2.03	1.17	3.91	1.59
7	10.48	4.05	53.51	25.74	1.74	0.84	2.47	1.17
8	11.09	4.36	46.70	31.70	1.72	0.83	2.49	1.11
9	12.30	3.93	45.93	31.56	1.71	0.82	2.53	1.21
10	10.56	3.93	51.41	26.95	1.70	1.11	2.72	1.62
11	9.89	2.16	54.24	25.36	1.29	1.76	3.40	1.90
12	11.02	5.30	52.79	22.89	2.11	0.96	3.70	1.24
13	9.96	3.29	54.15	23.13	1.84	1.44	4.52	1.68
14	10.39	2.99	46.75	29.97	1.72	1.62	4.70	1.85
15	9.76	3.06	43.91	32.47	1.85	1.66	5.07	2.22
16	9.64	3.34	43.73	31.88	2.03	1.68	5.50	2.20
17	10.21	3.30	43.96	32.33	1.91	1.49	4.87	1.93
18	10.46	3.08	48.55	29.31	1.70	1.50	3.96	1.44
19	10.67	2.48	49.88	29.01	1.37	1.50	3.55	1.55
20	10.03	3.23	46.41	30.76	1.89	1.48	4.47	1.73
21	10.50	3.76	47.74	29.38	1.89	1.25	3.93	1.55
22	10.33	3.68	48.28	29.48	1.84	1.21	3.78	1.40
23	10.72	3.82	48.48	28.48	1.91	1.27	3.83	1.49
24	9.99	2.72	56.67	23.05	1.47	1.44	2.94	1.72
25	9.67	2.66	59.08	21.78	1.36	1.37	2.63	1.45
26	9.67	2.56	58.72	22.09	1.32	1.53	2.72	1.38
27	8.97	2.19	52.29	27.42	1.36	1.97	3.71	2.09
28	9.10	2.62	54.37	24.20	1.61	1.74	4.07	2.29
Florunner 458	4.79	1.64	81.68	2.88	1.02	3.10	2.90	2.00
30	9.38	3.91	57.12	19.59	2.02	1.43	4.47	2.10
31	11.08	3.68	46.72	28.87	1.88	1.38	4.44	1.96
32	9.88	2.13	54.27	25.08	1.26	2.12	3.43	1.83
33	8.92	3.39	55.71	25.13	1.49	1.36	2.54	1.47
34	9.15	3.20	55.19	25.48	1.51	1.26	2.61	1.59
SunOleic	4.88	2.07	80.96	3.56	1.14	2.65	2.75	1.99

names in bold indicate high-oleic acid peanut varieties

compared to that in other peanuts (0.8-2.1%). Eicosenoic acid was the only fatty acid correlated to oleic acid, because this fatty acid can be synthesized from oleic acid by the addition of an acyl group.

SDS-PAGE analysis of high-oleic *versus* other varieties

Because peanut is used as a protein supplement in a wide variety of processed foods, accidental consumption is almost inevitable. Hypersensitivity to peanuts is a reaction mediated by IgE antibodies in response to several peanut proteins. The identification and characterization of allergens is essential for understanding the relationship between clinical manifestations and the specific IgE mediated immune response to peanut. Moreover, their identification contributes to the improvement of diagnostic tests and treatment of peanut allergy.

The protein composition of high-oleic peanuts was compared with that of other peanut varieties from different parts of the world by means of SDS-PAGE. Typical protein profiles of 33 conventional and two high-oleic peanuts are presented (Figure 1). SDS-PAGE patterns from extracted proteins indicated up to 12 protein bands of differing molecular weight, which were visualized using Coomassie blue. As shown in

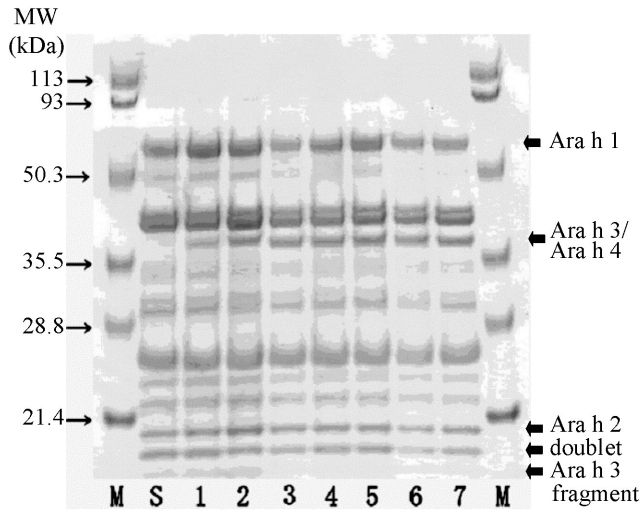
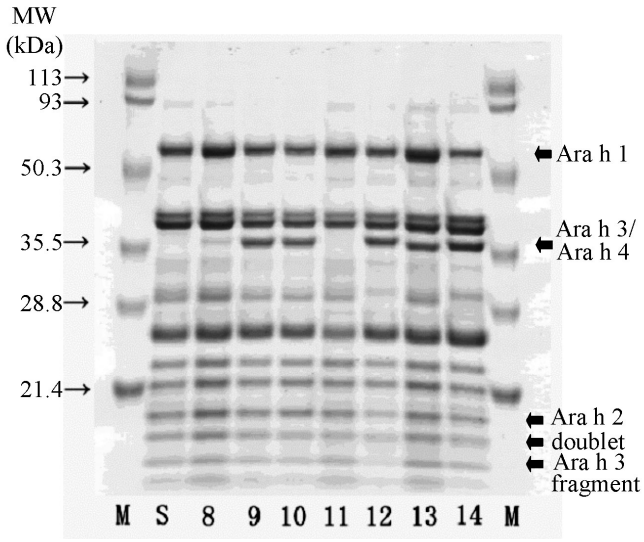
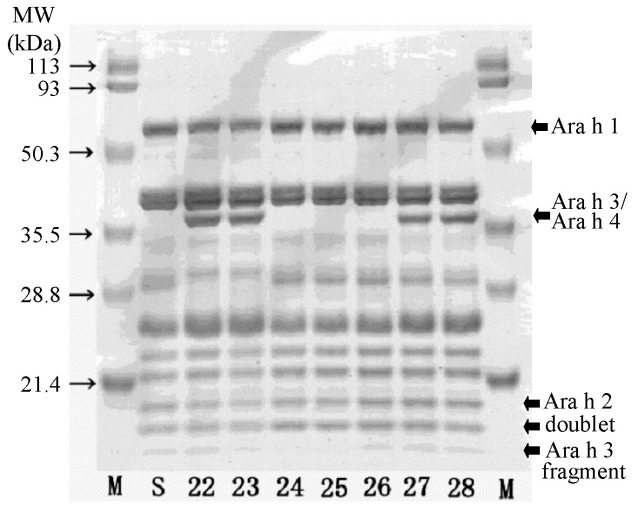
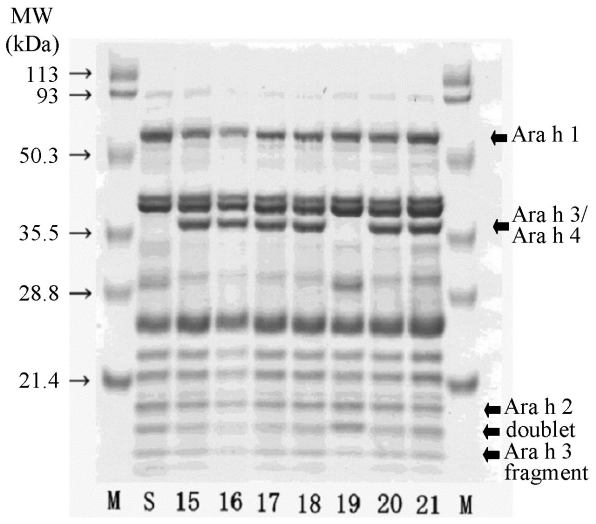


Figure 1, high-oleic peanuts (lanes S and 29) showed little difference from conventional peanuts in the protein composition. All major bands including the monomeric form of Ara h 1 (band with approximately molecular weight of 64 kDa), Ara h 2 doublet (17 and 19 kDa) and Ara h 3 fragment (14 kDa) at lower molecular weights, were visible and appeared to be similar to a great extent in all samples. For conventional peanuts with an oleic content lower than 50% (LO) such as Umihana-1 (no. 9), the protein band with the estimated molecular weight of 36 kDa was markedly detected, whereas its observation found among normal oleic (NO) peanuts with an oleic acid content between 49.9 and 59.1% was striking. This protein band was only present in peanuts no. 1, 2, 3, 4, 7, 10, 12, 13, 27, 28, and 30, but completely undetectable in others no. 11, 19, 24, 25, 26, 32, 33, and 34 like in high-oleic peanuts. It should be noted that this protein may correspond to either a proteolytic product of Ara h 3 (35 kDa) [Rabjohn







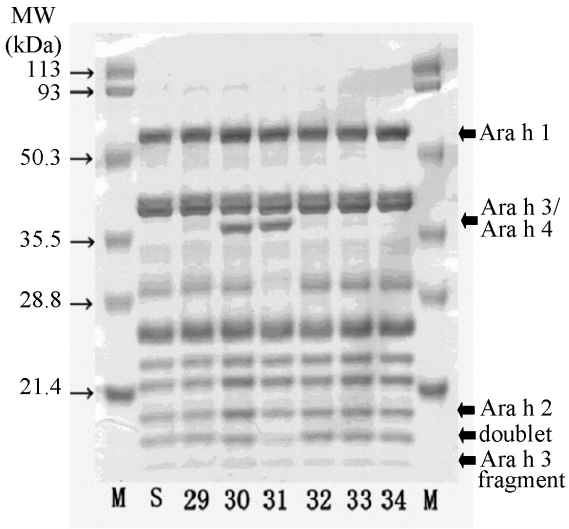


FIGURE 1. SDS-PAGE analysis for 35 different peanut samples stained with Coomassie brilliant blue. Lane M indicates the molecular weights markers MW (kDa). Lane S: SunOleic peanut. Lanes 1-34: peanut samples 1-34 (see Table 1). Arrows indicate the location of the Ara h 1 monomer, the Ara h 2 doublet, the Ara h 3 fragment and Ara h 3/Ara h 4.

et al., 1999] or Ara h 4 (36 kDa) [Kleber-Janke *et al*., 1999], both are members of the glycinin seed storage protein family and share 91.3% amino acid identity. The Ara h 3 product and Ara h 4 were detected by 44% (8/18) and 53% (21/40) of patient selected sera, respectively, and thus indicated a considerable allergic activity of these peanut proteins. The allergenic proteins at 21.6 and 30 kDa may correspond to the previously identified subunit of Peanut I [Sachs *et al*., 1981] and peanut lectin [Koppelman *et al*., 2001], respectively. One band at 27 kDa was a protein with a similar molecular weight as peanut agglutinin, a protein described as a minor legume allergen.

Two recessive genes are responsible for the high-oleic trait [Knauft *et al*., 1993]. The question has been raised as to whether incorporation of these genes into breeding lines has resulted in changes of the protein composition. The apparent loss of Ara h 3/Ara h 4 (at around 36 kDa) in high-oleic peanuts could not be accounted for genetic manipulation effect because this loss was observed also in other conventional peanuts. For instance, for Runner variety, four peanut samples used from Argentina (no. 11), Paraguay (no. 26), USA (no. 28), and Brazil (no. 30) had an oleic content of 54.2, 58.7, 54.4, and 57.1%, respectively. However, the 36 kDa protein band was not displayed in two former peanuts compared with that in two latter ones. Moreover, similar discrepancy was noted also in the samples of Runner and Virginia type peanut cultivars grown in the USA [Koppelman *et al*., 2001]. This indicates that other factors that influence the presence of this allergen such as environmental conditions could be taken into account.

Overall, these results showed that high-oleic peanuts are not different from other peanuts of various varieties and from different countries of the world in the major protein bands, except the 36 kDa one, which is absent in high-oleic and several normal oleic (NO) peanut varieties.

IgE binding of high-oleic *vs*. other varieties

The IgE level and response to the allergens vary with each individual patient. For detection of IgE-binding to the allergens of different peanut varieties, immunoblotting was performed with sera from two patients with peanut allergy. Nonspecific binding to peanut allergens was tested with normal human sera from three nonallergic individuals and used as a negative control. Presented in Figure 2 are typical profiles of the IgE binding pattern for conventional peanuts (Runner and Umihana-1) compared to high-oleic peanut (SunOleic). Compared with patient sera, the lack of IgE reactivity of sera from three nonallergic individuals in the control group was obtained.

As shown in Figure 2, patient serum IgE antibodies recognized not all proteins as demonstrated by SDS-PAGE. Three peanut varieties were similar in IgE binding intensities to Ara h 2 doublet (17 and 19 kDa) and had no IgE binding to Ara h 1 (64 kDa) with both patient sera. The IgE binding activity to Ara h 1 was controversial because this protein was recognized by over 95% of the North American patient population [Burks *et al*., 1991], but by only 35% [de Jong *et al*., 1998], 65% [Kleber-Janke *et al*., 1999] or 70% [Clarke *et al*., 1998] of three European patient populations. The serological differences were found to lesser extent for Ara h 2, the 17 and 19 kDa proteins, which were recognized by IgE antibodies equally well (more than 70%, n=14) [de Jong *et al*., 1998] or by 71% and 37%, respectively, of peanut sensitive patients (n=89) [Clarke et al., 1998]. The 15 kDa protein (most probably Ara h 3) was bound by 54% of patient sera [Clarke *et al*., 1998. Interestingly, with serum of patient B, in parallel with the loss of the 36 kDa protein (Ara h 3/Ara h 4), IgE-binding intensity to Ara h 3 fragment (around 14 kDa) was also completely reduced in high-oleic (SunOleic) and NO (Runner) peanuts compared with that in LO peanut (Umihana-1). These differences in allergenicity were observed, even though the 14 kDa displayed protein band was similar in all 3 peanut samples as shown by SDS-PAGE analysis (Figure 1). In contrary, the Ara h 3 fragment was recognized by the patient's serum A in all peanut samples. The IgE binding to the 15 kDa protein (Ara h 3 fragment) is important because it was shown that there was the relationship between specific IgE sensitivity to the 15 kDa protein and clinical severity [Clarke *et al*., 1998]. Taken together, the absence of IgE sensitivity to this allergen may reduce the patient severe reactions. Other cooking methods than roasting such as boiling or frying peanuts [Beyer *et al*., 2001] or modification of Ara h 3 at critical IgE binding sites by single amino acid changes were reported to reduce also the binding capacity to this allergen [Rabjohn *et al*., 2002].

Moreover, for LO Umihana-1 peanut, although present, IgE antibodies from both tested patient sera have not recognized the 36 kDa allergen. The reason could be due to the amount of serum peanut-specific IgE or no affinity of patient-specific IgE to this allergen. Although not performed, other NO peanuts such as no. 19, 24, 25, 26, 32, 33, and 34 like Runner (no. 11) as well as other LO ones like Umihana-1 (no. 9) in protein composition were thought to give the corresponding similar levels of IgE binding. Results showed that no difference was found in IgE binding of one patient to all three peanut samples. However, with other patient serum, for high-oleic peanuts, in addition to the loss of the 36 kDa allergen, the Ara h 3 fragment showed a similar loss of allergenic pro-

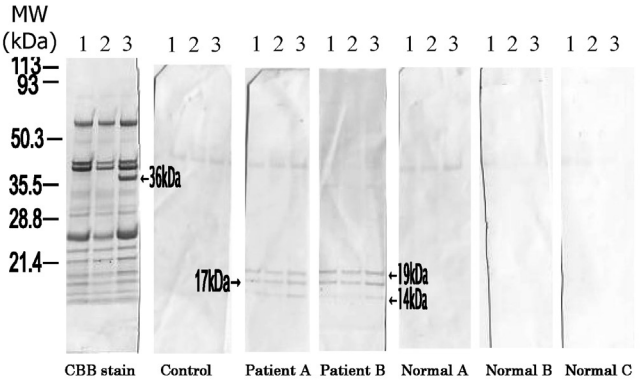


FIGURE 2. IgE-immunoblot of the proteins from 3 peanut varieties with two patient sera (Patient A-B), and three nonallergic patient sera (Normal A-C) served as negative controls; lane 1: SunOleic (S); lane 2: Runner (no. 11); lane 3: Umihana-1 (no. 9). Arrows indicate the location of the Ara h 2 doublet (17 and 19 kDa), and the Ara h 3 fragment (14 kDa).

tein and IgE binding intensities compared with that of LO peanuts, whereas similar loss may be less pronounced in NO peanuts. This finding was probably proportional to the clinical severity of reactions in both patients.

The essential character of high oleic peanuts is, in addition to its high level of oleic acid, their low content of linoleic acid. Although high oleic peanuts appear to help prevent hyperlipidemia, lower cholesterol levels in hypercholesterolemic women [O'Byrne *et al*., 1993], and suppress chemically--induced lung tumorigenesis in mice [Sakurai *et al*., 2000; Yamaki *et al*., 2002], question has been raised as to whether high O/L ratio could indeed result in the lowered specific IgE level and reactivity and thus reduces allergic responses. Dietary linoleic acid is known to associate with allergy. The decrease in the intake of dietary linoleic acid or a diet high in the ratio of linolenic to linoleic acid has been reported to suppress allergic responses in mice [Kuwamori *et al*., 1997; Gu *et al*., 1998].

The chemical modification of Ara h 1 and Ara h 2 and increased levels of potentially allergenic Maillard reaction products formed during roasting at high temperatures were reported to apparently enhance the level of IgE binding or the allergic properties of roasted peanuts compared with those in the raw form [Chung *et al*., 1999, 2001]. However, little difference was found between Korean and American peanuts in allergen components regardless of being raw or roasted [Park *et al*., 2000]. Despite these, the assumption that high-oleic peanuts can reduce allergic sensitization can be made based on the result of *in vivo* study performed at School of Medicine (Tokyo, Japan), which demonstrated that the ingestion of 5 high-oleic peanuts (SunOleic) roasted at 170°C on frying pan for 5 min did not elicit a clinical reaction in mild peanut-allergic patient. This test showed a substantially increased threshold of sensitivity to peanut from a level equal to approximately only 1 normal oleic peanut to at least five high oleic peanuts. The mechanism behind this is probably based on the fact that lower intake of linoleic acid with high-oleic peanuts than with other peanuts results in a lower level of a precursor of prostaglandin E_2 (PGE_2) in allergenic patients, which in turn may decrease IgE production, inhibit IgE binding to mast cells and basophils and thus reduce allergic sensitization.

CONCLUSIONS

With the present study, high-oleic peanuts are shown to be moderately different from conventional peanut varieties in protein composition, and IgE-binding intensity. However, mild peanut-allergic patient reacted negatively in an oral food challenge test to the dose at least 5 times higher, therefore, high oleic peanuts could be less allergenic than other peanuts and may lower a risk of the severity of a reaction.

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