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Preparation and analysis of peanut flour used in oral immunotherapy clinical trials

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Abstract

Background—Oral immunotherapy (OIT) is an investigational therapeutic approach for the treatment of food allergies. Characterization of the drug product used in oral immunotherapy trials for peanut allergy has not been reported.

Objective—To quantify relative amounts of the major peanut allergens and microbial load present in peanut flour used in OIT trials and assess whether these parameters change over a 12 month period. We also anticipate that this report will serve as a guide for investigators seeking to conduct OIT trials under FDA-approved Investigational New Drug applications.

Methods—Densitometric scanning of Ara h 1 and Ara h 2 resolved on SDS-PAGE gels was used to assess allergen content in peanut flour extracts. Microbial testing was conducted on peanut flour under US Pharmacopeia guidelines for the presence of E. coli, Salmonella, yeast, mold, and total aerobic bacteria. Additionally, aflatoxin was quantified in peanut flour. Reported results were obtained from four unique lots of peanut flour.

Results—Relative amounts of the major peanut allergens were similar between different lots of peanut flour and remained stable over a 12 month period. E. coli and Salmonella were absent from all lots of flour. Yeast, mold, total aerobic bacteria, and aflatoxin were within established US Pharmacopeia guidelines on all lots tested and remained within the criteria over a 12 month period.

Conclusions—Peanut flour used as a drug product contains the major peanut allergens and has low levels of potentially harmful microbes. Both of these parameters remain stable over a 12 month period.

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Keywords

Peanut allergy; peanut oral immunotherapy; peanut flour; drug product; stability testing; microbial testing; Ara h 1; Ara h 2

Introduction

Allergy immunotherapy has been practiced for over 100 years and is the only disease-modifying treatment for allergic disease [1]. In the U.S., subcutaneous immunotherapy (SCIT) is used to treat environmental allergies to a variety of allergenic sources including pollens, pet dander, and house dust mite to prevent allergic rhinitis and asthma symptoms [2]. SCIT is also effective for more severe allergic conditions such as anaphylaxis to stinging insect venoms. Recently, the U.S. Food and Drug Administration (FDA) approved sublingual immunotherapy (SLIT) for grass and ragweed pollen allergies [3]. However, there is still no FDA-approved immunotherapy for food allergy.

Patients allergic to foods are instructed to strictly avoid the food they are allergic to and must be prepared to treat accidental ingestions and reactions with emergency medication, including epinephrine [4, 5]. SCIT was studied as a treatment for peanut allergy in the late 1980s, but this approach was abandoned due to a high rate of severe reactions [6, 7]. In the past decade, researchers began to administer food allergens by the oral, sublingual, and epicutaneous routes in an attempt to provide a safe and efficacioustherapy (Reviewed in [8]). Evidence from several studies demonstrated that oral immunotherapy (OIT) for peanut, egg, and milk allergy was often well-tolerated and highly effective in a large portion of subjects [9–15]. OIT appears to modify the immune responses to a greater extent than SLIT leading to desensitization in a higher proportion of subjects [16, 17]. OIT is typically administered in 3 phases: initial escalation, build-up, and maintenance. The initial escalation phase involves several low doses of protein (micrograms to milligrams) given over the course of a few hours. Then subjects undergo the build-up phase by increasing the dose amount approximately every two weeks. After several months, subjects reach the maintenance dose (300 to 4000 mg of protein depending on the study protocol) and ingest it daily for many months or years.

As per FD&C Act (201(g)(1)), a drug is defined as any article that is: *"intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals*." or "*…a substance (other than food) intended to affect the structure or any function of the body*". Used within the context of OIT clinical trials, the peanut product is administered with the intention of providing a treatment to peanut allergic subjects by modulating their immune response to the allergen and therefore meets the definition of a drug. However, as peanut flour is not an FDA approved drug, as per 21 CFR 312.2(b), OIT trials are not eligible for the IND exemption status. In order to comply with the regulatory requirements of conducting OIT clinical studies, an Investigational New Drug (IND) application outlining the characteristics of the peanut flour as well as a detailed description of the manufacturing process is needed. Manufacturing of the peanut flour as an investigational drug product occurs in a Good Manufacturing Practices (GMP)-compliant environment under Standard

Operating Procedures (SOPs) detailing the process. Additionally, the stability of allergenic peanut proteins and microbial growth must be documented.

Within this paper, we present the tests that we have conducted on the peanut flour used in clinical studies of the treatment of peanut allergy in children and adults. Prior to subject administration, the product was subjected to bioburden testing where levels of aerobic/anaerobic bacteria, yeast, and mold were assessed. Due to the specific nature of the peanut flour, the product was also tested for E. coli, Salmonella, and Aflatoxin. Standardizing a drug product for OIT is of paramount importance so that subjects will receive equivalent amounts of allergens even when switching between different lots of the product. Therefore, appropriate testing has to be conducted prior to releasing the product. Here we report our findings related to allergen content, bioburden, and stability of peanut flour used in OIT clinical trials.

Methods

Drug manufacturing process

The overall process of drug manufacturing occurs in several stages: (1) bulk peanut flour is received; (2) the bulk flour is tested for compliance with established US Pharmacopeia guidelines for presence of microbes (See Table 1); (3) the bulk flour is examined for the presence of Ara h 1 and 2 and their consistency in relative quantity to a reference standard and the previous lot of peanut flour; (4) finally, the peanut flour is used to manufacture drug product doses. For the initial treatment under the OIT protocol (referred to as initial escalation phase) the doses of the peanut flour as an investigational drug product are too low weight to be successfully administered with reproducible accuracy as a flour product. Therefore, a peanut liquid drug product is produced by extracting the flour in phosphate-buffered saline (PBS) and used to administer very low doses of allergen in the range of 0.1 mg to 0.8 mg. The peanut extract is filter sterilized, prepared at a concentration of 10 mg/mL, and stored frozen until use. The flour itself is manufactured into drug product doses ranging from 1.5 mg to 4000 mg of peanut protein by weighing out doses on an analytical balance into a vessel. The bulk flour and manufactured drug doses are kept refrigerated to prevent microbial growth and preserve protein integrity; nevertheless, the possibility of protein degradation or microbial growth exists and must be studied.

Peanut flour

Lightly roasted, partially defatted, 12% fat peanut flour was purchased from Golden Peanut Company (Alpharetta, GA) in 50 lb. bags. Upon receipt, the product was broken down into smaller bags (~10 lb. each) and kept refrigerated at 2–8°C. Each lot comes with a Certificate of Analysis (CoA) provided by the Golden Peanut company, including results for physico-chemical properties and microbiological testing. Here we report finding from four unique lots purchased between July 2014 and January 2016.

Extraction of soluble peanut proteins from lightly roasted peanut flour

Peanut flour was mixed with PBS at a 1:4 ratio (weight/volume). The suspension was stirred for 1.5 hours while maintaining a constant pH of 8.5 using 6 M NaOH, then centrifuged at

30,000 x g at 4 °C for 45 minutes. T he supernatant containing extracted proteins was filtered through a 0.2 μm membrane and a bicinchoninic acid (BCA; Pierce) assay was conducted to determine the protein concentration. Finally, the peanut extract was diluted to 10 mg/mL in PBS for SDS-PAGE analysis.

Preparation and storage of a reference standard

The reference standard was generated by extracting protein from peanut flour and storing it frozen at −20°C. Soluble proteins were extracted from the flour as above. The extract was diluted to 10 mg/mL and run on SDS-PAGE to verify the protein bands corresponding to Ara h 1 and 2. The reference standard was split into 500 μL aliquots and stored at −20°C.

SDS-PAGE and densitometric scanning to determine Ara h 1 and Ara h 2 content

There are currently 17 peanut allergens, called Ara h 1–17, recognized by the World Health Organization and the International Union of Immunologic Societies (WHO/IUIS) (allergen.org). Previous biochemical and immunologic studies have demonstrated the major allergens of peanut to be Ara h 1 and Ara h 2 as determined by IgE binding from > 50% of allergic patients' serum [18, 19]. More recently, Ara h 2-specific IgE levels have been shown to have diagnostic importance, confirming Ara h 2 as an important allergen [20, 21]. Accordingly, we chose Ara h 1 and Ara h 2 as allergens to assess for presence and stability in the peanut OIT drug product. SDS-PAGE was conducted with 7.5 μg peanut protein extract per lane. An image of the gel was captured with a camera system and densitometric scanning was performed on the image using ImageJ software (NIH). Duplicate samples were averaged and percent difference in Ara h 1 and 2 from the reference standard was calculated with the following equation: ((Test lot - Reference Standard)/Reference Standard) X 100%. Similarly we calculated percent differences in Ara h 1 and 2 from lot-to-lot comparing the current lot to the previous lot.

Bioburden testing of peanut flour

All microbial testing was conducted at Deibel Laboratories (Lincolnwood, IL) using USP guidelines for defining the acceptance criteria applicable to preparation for a drug used orally. A 150 g sample of peanut flour from each lot was weighed in the GMP environment and shipped for overnight delivery to Deibel Laboratories.

Stability testing of peanut flour

Peanut flour (150 g) was weighed into four separate containers for the microbial testing and 25 g from the same lot of flour was weighed for assessing Ara h 1 and 2. All containers were kept at 2–8°C. The 150 g samples were removed from the refrigerator at approximately 0, 3, 8, and 12 months and shipped overnight to Deibel Laboratories for microbial testing.

Major peanut allergen content was conducted at the University of North Carolina (UNC). 12 months after being placed in the refrigerator at 2–8°C, the 25 g sample of flour was extracted, protein concentration determined by BCA assay, and then diluted to 10 mg/mL to match the concentration of the reference standard. Samples were run on SDS-PAGE gel and subjected to densitometric scanning using ImageJ software (NIH).

Results

Ara h 1 and Ara h 2 are present in lightly roasted peanut flour

As part of the process for the acceptance of the raw material (drug substance, i.e. bulk peanut flour) our group performed analysis of every peanut flour lot prior to being used for the manufacturing of the drug product. Part of the assessment is confirming the identity of the product. Our group and others have demonstrated the presence of Ara h 1 and Ara h 2 in peanut flour [22, 23]. The native proteins have been isolated and their genes cloned [24]. Figure 1 shows an SDS-PAGE gel with purified Ara h 1 and Ara h 2 (purified as in [25]) and an extract from the raw peanut flour used to purify the allergens. A Western blot demonstrating human IgE binding from an allergic patient for both roasted (i.e. the Golden Peanut flour used in OIT) and raw peanut flours is also shown in Figure 1. Ara h 1 has a molecular weight of ~62 kD and is quite abundant in the raw peanut extract with slightly lower abundance in the roasted flour extract. This is thought to be due to the roasting process generating aggregated Ara h 1 via the Maillard reaction which makes the protein less soluble [26]. Ara h 2 consists of two isoforms present at ~17 kD and ~19 kD [27]. As with Ara h 1, Ara h 2 is readily found in both raw and roasted peanut preparations and both bind IgE from peanut allergic patients. These data are important and demonstrate that Ara h 1 and 2 are present in the lightly roasted peanut flour used in OIT.

Total protein and allergen content are consistent between peanut flour lots

The Golden Peanut Company performs several tests to characterize their peanut flour product prior to release to the public. The tests include quantification of biochemical properties and presence of microbes and they are documented on a CoA provided by the company. Table 2 shows results from biochemical testing performed by Golden Peanut for percent protein via the Kjeldahl method (N x 5.1 method) and percent fat, along with microbial testing done by Golden Peanut and by our contracted vendor, Deibel Laboratories. The data demonstrate that the overall protein content is very consistent between lots, with a range of 49% – 51%. Even though total protein content is consistent, it is possible that specific allergens could vary between lots which might lead to unwanted variability of allergen exposure in subjects. To test this we performed extractions on each lot and compared these to both a reference standard as well as to the previous lot. Figure 2 illustrates an example of an SDS-PAGE gel with the Ara h 1 and 2 protein bands gated for densitometric analysis. Densitometry is a semi-quantitative approach that works well to compare relative amounts of proteins present. Data for comparison of four lots to the reference standard are shown in Table 3. There is limited variability of Ara h 2 between lots with a range of 2.04% −6.80%. Ara h 1 had a wider range of variability from 3.12% – 22.59%. Our acceptance criteria were set for 20% variability in Ara h 2 and 30% variability in Ara h 1, thus each lot passed our qualification criteria based on the allergen content. Furthermore, we see that lot-to-lot consistency is evident for both Ara h 1 and 2 when comparing each lot to the previous one (Table 3). These data indicate that Ara h 1 and 2 content are similar between lots and within the ranges we pre-determined to be acceptable to the FDA.

Of note, we established the allowed allergen variability based on a study we conducted using 3 lots of peanut flour that were already used for dosing research subjects with peanut OIT. A retrospective chart review indicated no increase in adverse allergic events related to dosing in any subject following a change from one of these lots to another; the variability of Ara h 1 was <30% when comparing the 3 lots and Ara h 2 was <20% when comparing the 3 lots. The coupling of clinical trial data along with the lab data allowed us to establish the acceptable levels of Ara h 1 and 2 variability.

Microbial and aflatoxin levels are within FDA limits for an orally delivered drug

Microbes are known to exist in food products and their levels must be limited such that consumption will not be harmful to the consumer. Despite regulations, outbreaks of E. coli and salmonella have made headlines in recent years and can cause severe illness when present at high levels. Since peanut flour is being used as an orally delivered drug product, we must monitor microbial levels, including E. coli, salmonella, yeast, mold, and total aerobic bacteria. Additionally, peanut crops can be affected by aflatoxins, so we quantify aflatoxin levels as suggested by the FDA, even though it is not required per USP.

In the four lots of peanut flour reported here, microbial levels were nearly identical when tested by Golden Peanut Company and when tested by our contracted vendor, Deibel Laboratories (Table 2). E.coli and salmonella were absent in all four peanut flour lots. Yeast, mold, and total aerobics were all found to be 10 cfu/g or less for each of the peanut lots. Importantly, the microbial levels were all below the criteria established by the FDA for a non-aqueous drug product being delivered by the oral route (Table 1). Aflatoxin levels were all <3 ppb, well below the 15 ppb suggested by the FDA. These data indicate that microbial burden does not pose a serious risk in the roasted peanut flour used to manufacture peanut OIT drug products.

Peanut flour is stable for at least 12 months when stored refrigerated at 2–8°C

The two main components for testing required by the FDA to use peanut flour as a drug product are allergen content and microbial presence. Since each lot is used to manufacture drug product over a several month period, we determined the stability of the flour over 12 months. Samples (25 g) were kept refrigerated and only brought out of the cold in order to perform extractions to determine allergen content or to be shipped for microbial testing (150 g).

A reference standard was prepared by extracting proteins from a lot purchased in July 2014 and then immediately frozen at −20°C. A sampl e of peanut flour from the same lot was kept refrigerated for 12 months then was brought to room temperature and an extraction was performed. The reference standard and 12 month extracts were run on SDS-PAGE gel and densitometry of the Ara h 1 and 2 bands were performed. Table 4 shows that the differences in Ara h 1 and 2 content were very minimal after 12 months, with the Ara h 1 content varying by less than 5% and the Ara h 2 content varying by less than 1%. These data indicate that the major peanut allergens remain stable in the peanut flour over a 12 month period when kept cold.

Stability of microorganisms was conducted by placing four samples of peanut flour in a refrigerator at 2–8°C on the same day (day 0). One was shipped immediately for the microbial testing. The others were removed from the cold and shipped for testing at 3 months, 8 months, and 12 months. Table 5 shows that over a 12 month period, all microbial levels, as well as that of aflatoxin, remained within the acceptable range required by the FDA (Table 1). It is interesting to note that at 12 months, a small amount of mold growth was detected in the peanut flour, although this level of 40 cfu/g was still below the threshold of 100 cfu/g which would disqualify the product for use as a drug product. Taken together, these data indicate that Ara h 1 and 2 levels remain constant in lightly roasted peanut flour and little to no microbial growth occurs when kept at 2–8°C over a 12 month period.

Discussion

Oral immunotherapy holds great promise as a treatment modality for food allergies, including peanut allergy [28]. OIT is administered in exact quantities using an investigational drug product manufactured from peanut flour. Peanut flour is a fairly crude drug product in that it contains the active ingredients (i.e. peanut allergens) along with presumably inert ingredients including non-allergenic proteins, carbohydrates, lipids, micronutrients, etc. Therefore, the source material needs to be consistent from lot to lot for overall protein content as well as allergen content. Additionally, peanut flour has the potential to allow for microbial growth since it is not a sterile drug product. Both of these factors must be assessed before manufacturing the OIT drug product.

Ara h 1 and Ara h 2 are major peanut allergens and we believe these two proteins are key active ingredients leading to desensitization in peanut allergic subjects. Indeed, we have demonstrated immunologic effects directly against Ara h 1 and 2 following OIT [14, 29]. Ara h 1- and Ara h 2-specific IgE levels decreased during OIT, and IgG4 to both allergens increased. Furthermore, IgE and IgG4 epitope specificity within Ara h 1 and 2 is altered with OIT [29]. Therefore, it is critical that these major allergens be present within peanut flour used in OIT. Here, we demonstrated that Ara h 1 and 2 are present in all lots of peanut flour that we have tested. Furthermore, the relative quantities of Ara h 1 and 2 remained consistent from lot to lot as all testing showed acceptable differences between lots, limited to <25% for Ara h 1 and <10% for Ara h 2. These data provide key evidence that peanut flour is a viable source of active ingredients used in OIT.

The absence of microbes in peanut flour is also critical to its use in OIT. Microbial growth could make the flour harmful if ingested, could alter the composition of protein allergens if microbes use these as a source of nutrients, and could alter the body’s response to the allergens. During the production of the peanut flour doses and prior to its use in OIT, we are required by the FDA to test the product for the presence of specific microbes. In this paper we reported the methods that have been used and results that needed to be obtained to comply with the regulatory requirement of providing the product for OIT clinical trials. Therefore, we have shown that yeast, mold, and total aerobic bacteria are within the USP guidelines for microbial limits. Peanut crops can also be affected by aflatoxins produced by fungi such as Aspergillus flavus and Aspergillus parasiticus. Accordingly, we quantified levels of aflatoxin and these were found to be < 15 ppb in all lots tested, as required by the

FDA. Therefore, when prepared for use in OIT trials, microbial presence in the lightly roasted peanut flour did not pose an issue for use as a drug product.

Finally, the stability of any drug product must demonstrate that under correct storage conditions the product will remain stable in terms of active ingredients and bioburden. We tested the stability of Ara h 1 and 2 and found only slight differences in Ara h 1 and 2 levels compared to the reference standard indicating that these major peanut allergens remained stable in the flour when refrigerated for 12 months. These differences were well within range of our pre-defined acceptable levels of variance for Ara h 1 (30%) and Ara h 2 (20%). Additionally, refrigerated samples from 3, 8, and 12 months were tested for microbial growth. All of the microbes tested, along with aflatoxin, remained within range of the limits established by the FDA for an oral non-aqueous drug product. These data allow us to conclude that peanut flour is stable for at least 12 months when stored at 2–8°C. Therefore, w e only manufacture drug product from the same lot for a maximum of 12 months and any doses that have been prepared are disposed of after the 12 month expiration.

Allergen immunotherapy requires the presence of key allergens to modify an existing IgE-mediated immune response [2]. The peanut flour used in OIT is a crude source of peanut allergens and was used as a starting point for OIT trials. It is a readily available material and is relatively inexpensive, making it attractive for drug development. More advanced approaches using modified, recombinantly produced allergens have shown promise in aeroallergen immunotherapy [30]. However, similar approaches for peanut using mutated allergens with IgE-binding epitopes removed proved unsafe in an initial clinical trial when administered rectally [31]. Other approaches using T cell epitope peptides are also being explored for cat and venom allergies [32] and may be applicable to peanut allergens with known T cell epitopes [33, 34]. As a first-generation therapeutic approach, OIT is relatively safe and highly effective for inducing desensitization. The present report provides evidence that lightly roasted peanut flour is a reliable candidate for development of an OIT drug product. Further studies are needed to examine whether OIT with lightly roasted peanut flour is superior to other potential forms of immunotherapy.

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Highlights

What is already known about this topic?

It is well-established that allergens are the active ingredients in immunotherapy, however, to our knowledge there are no published reports of allergen characterization for investigational peanut oral immunotherapy products.

What does this article add to our knowledge?

Here we report the relative allergen content, microbial load, and stability of these parameters in peanut flour used in oral immunotherapy. The article informs potential investigators of the necessary steps and procedures required by the FDA to use peanut flour for oral immunotherapy under Investigational New Drug applications.

How does this study impact current management guidelines?

The study highlights key parameters that must be addressed when considering implementing clinical trials using a food source to desensitize allergic subjects, as is done in oral immunotherapy.

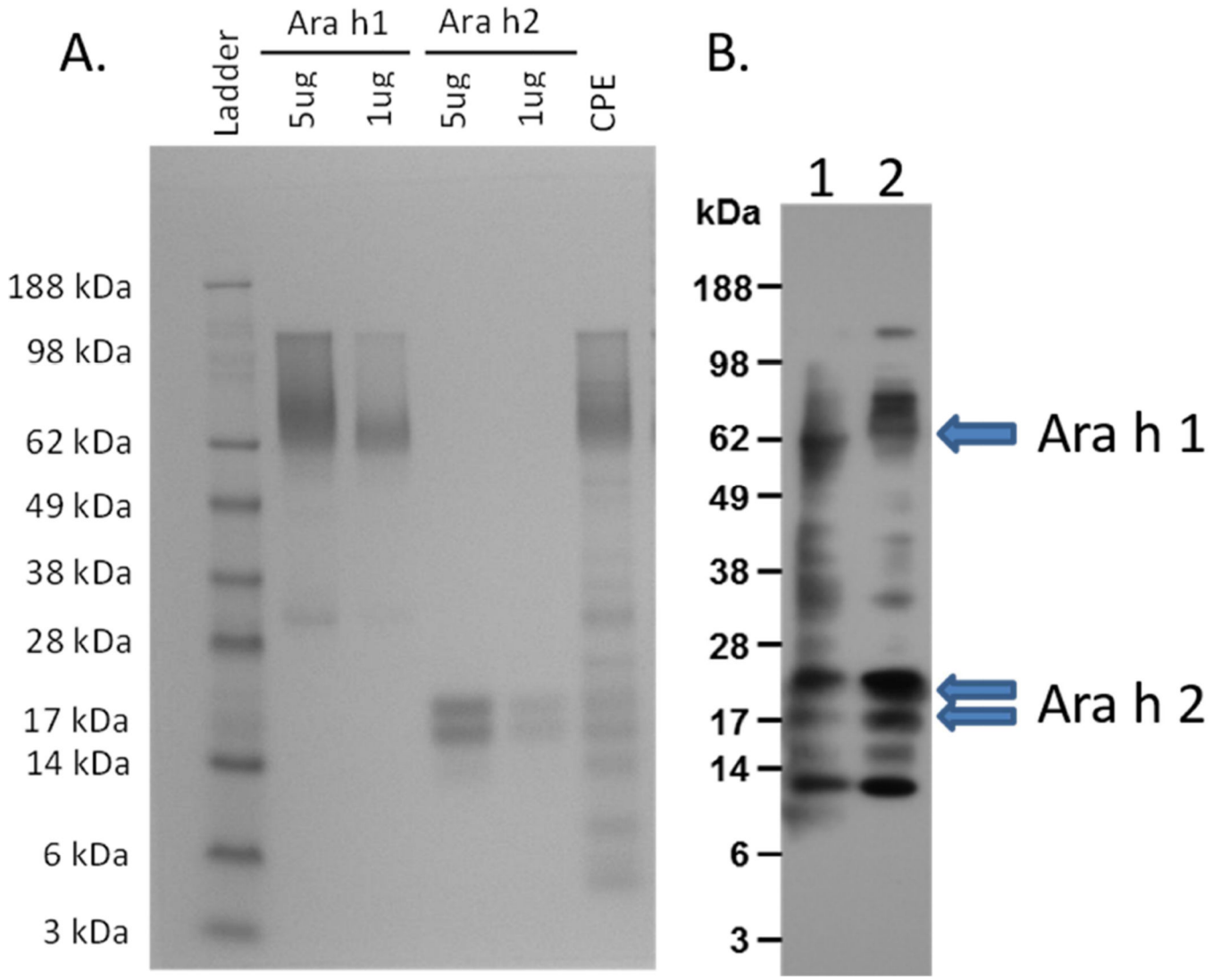
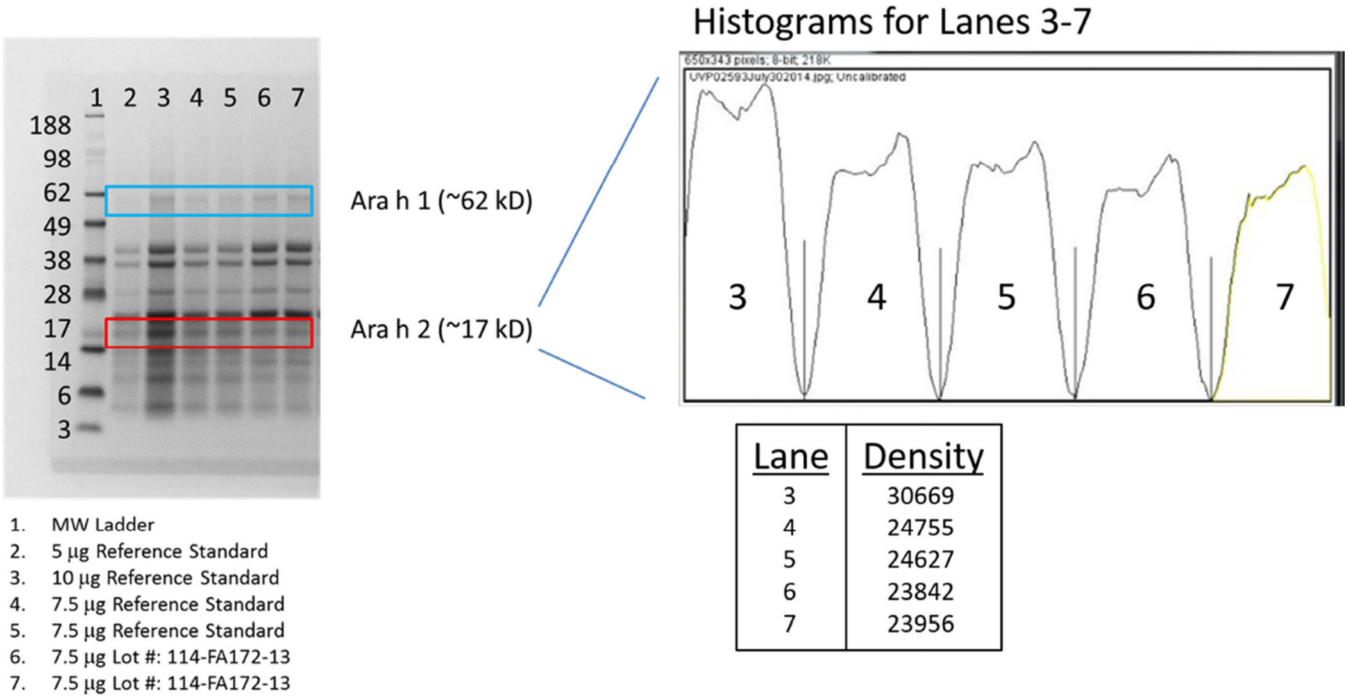


Figure 1.

Ara h 1 and Ara h 2 are present in peanut extracts. A: SDS-PAGE analysis of purified Ara h 1, purified Ara h 2, and a peanut extract (CPE). Intact Ara h 1 monomers appear as a band at ~62 kD. Ara h 2 doublets appear at ~17 kD and ~19 kD. B: Western blot analysis demonstrating human IgE binding to Ara h 1 and Ara h 2 (lane 1: roasted peanut extract; lane 2: raw peanut extract).



Lane	Density
3	30669
4	24755
5	24627
6	23842
7	23956

Figure 2.

Representative example of SDS-PAGE and densitometric scanning for Arah1 and Arah2 present in peanut extracts. SDS-PAGE gel is shown with gating for the individual allergens, along with histogram traces illustrating densities and a table showing final density values.

Table 1

FDA requirements for orally delivered drugs.

	Nonaqueous Preparation	Aqueous Preparation
Escherichia coli (in 1 g or 1 mL)	Absent	Absent
Total Aerobic Microbial Count (cfu/g or cfu/mL)	10^3	10^2
Total Combined Yeasts/Molds Count (cfu/g or cfu/mL)	10^2	10^1
Salmonella (in 1 g or 1 mL)	Absent	Absent
Aflatoxin in peanut products	< 15 ppb	< 15 ppb

Table 2

Characteristics of peanut flour from four consecutive lots.

			E. Coli		Salmonella		Aerobics (cfu/g)		Yeast (cfu/g)		Mold (cfu/g)		Aflatoxin (ppb)	
Lot #	**% Protein**	**% Fat**	**CoA**	**UNC**	**CoA**	**UNC**	**CoA**	**UNC**	**CoA**	**UNC**	**CoA**	**UNC**	**CoA**	**UNC**
1	50	11.38	Negative	Negative	Negative	Negative	10	10	<10	<10	<10	<10	0.	<2
2	49	12.09	Negative	Negative	Negative	Negative	<10	<10	<10	<10	<10	10	0.9	<2
3	49	11.40	Negative	Negative	Negative	Negative	<10	<10	<10	<10	<10	<10	2.4	<2
4	51	11.16	Negative	Negative	Negative	Negative	10	<10	<10	<10	<10	10	1.5	2.05

Microbial testing results are shown from Golden Peanut Company's testing (CoA) and for our testing (UNC) upon receipt.

Table 3

Comparisons of peanut flour lots to the reference standard and to the previous lot.

Lots compared to Reference Standard		
Lot #	**% Difference in Ara h 1**	**% Difference in Ara h 2**
1	3.12%	2.04%
2	7.10%	2.24%
3	22.59%	6.80%
4	9.48%	2.48%
Lots compared to Previous Lot		
Lots Compared	% Difference in Ara h 1	% Difference in Ara h 2
1 to 2	1.32%	2.31%
2 to 3	16.79%	4.73%
3 to 4	1.66%	2.39%

Table 4

Stability of the major peanut allergens, Ara h 1 and Ara h 2, in samples of peanut flour as compared to the reference standard.

Ara h 1		
	Reference Standard Density	Density at 12 months
Sample #1	67580	64807
Sample #2	67184	64231
Average	**67382**	**64519**
Ara h 2		
	Reference Standard Density	Density at 12 months
Sample #1	65445	67250
Sample #2	70998	68427
Average	**68222**	**67839**

The percent difference after 12 months is **-4.25%**

The acceptable limit of difference was defined as 30%

The percent difference after 12 months is **-0.56%**

The acceptable limit of difference was defined as 20%

Table 5

Microbial and aflatoxin test results over 12 months.

Test	Acceptable Limit	Baseline (0 mo)	3 mo	8 mo	12 mo
Total Aerobic Plate Count	< 1000 cfu/g	< 10 cfu/g	< 10 cfu/g	10 cfu/g	10 cfu/g
Yeast	< 100 cfu/g	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g
Mold	< 100 cfu/g	< 10 cfu/g	< 10 cfu/g	10 cfu/g	40 cfu/g
Escherichia coli	Negative	Negative	Negative	Negative	Negative
Salmonella	Negative	Negative	Negative	Negative	Negative
Aflatoxin	< 15 ppb	Not Done	< 2 ppb	< 2 ppb	< 2 ppb