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Allergenicity attributes of different peanut market types

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ABSTRACT

Four different market classes of peanut (Runner, Virginia Spanish, and Valencia) are commonly consumed in Western countries, but for some consumers peanuts are a main cause of food-induced anaphylaxis. Limited information is available on the comparative allergenicity of these distinct market classes. The aim of this study was to compare allergenicity attributes of different peanut cultivars.

The protein content and protein profiles were highly comparable for all tested cultivars. All cultivar samples contained the major allergens Ara h 1, Ara h 2, Ara h 3 and Ara h 6, as assessed by SDS-PAGE and RP-HPLC, although some minor differences in major allergen content were found between samples. All samples were reactive in commercial ELISAs for detection and quantification of peanut protein. IgE-binding potency differed between samples with a maximum factor of 2, indicating a highly comparable allergenicity.

Based on our observations, we conclude that peanuts from the main market types consumed in Western countries are highly comparable in their allergenicity attributes, indicating that safety considerations with regard to peanut allergy are not dependent on the peanut cultivar in question.

1. Introduction

Peanut (*Arachis hypogaea* L) is a seed crop legume that is widely used for human food purposes because of its high nutrition value (Oerise et al., 1974) and sensory attributes. The overall annual production of peanut (including Runner, Virginia, Spanish, and Valencia) in the U.S. in 2014 was 2.4 million tons (Anonymous, USDA NASS report, 2015) harvested from 1.4 million acres. The primarily grown species of peanut include two subspecies: *hypogaea* (Virginia market type) and *fastigiata*, the latter divided into two varieties *fastigiata vulgaris* (Spanish market type) and *fastigiata fastigiata* (Valencia market type). The Runner market type is a hybrid of *fastigiata* and *hypogaea* subspecies (Krapovicakas, 1969) and accounts for the majority (78.7%) of the U.S. peanut production. The Virginia market type accounts for approximately 19.9% of the U.S. peanut production. The Runner type is used primarily for the manufacture of peanut butter, and the large-kernelled Virginia type is marketed mainly as snack peanut and in-the-shell peanut products. The Spanish and Valencia market types are commercially less important, representing a combined 1.4% of the overall U.S. peanut production. The Spanish type, with rounder and smaller kernels, is used for snack peanuts, peanut butter and confections while the longer podded Valencia type, containing three to five kernels in each shell, is marketed mostly in the shell for roasting and boiling (American Peanut Council website, 2015).

Peanuts are widely known as potent allergens and count together with tree nuts for the majority of anaphylactic reactions to food (Sicherer and Sampson, 2007). Approximately 0.6% of adults and 1–2% of children/infants in the U.S. are affected by peanut allergy (Dyer et al., 2015; Sicherer et al., 2010). Unfortunately there is

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no treatment currently available to cure peanut allergy and therefore, peanut-allergic patients must avoid consuming peanut. Several experimental immunotherapies such as oral immunotherapy (Anagnostou et al., 2014; Varshney et al., 2011), sublingual immunotherapy (Burks et al., 2015; Fleischer et al., 2013), and epicutaneous immunotherapy (Sampson et al., 2015) show promising results for desensitizing peanut-allergic patients, although complete tolerance to peanut using these approaches appears to occur in only a limited number of patients. A new approach using modified peanut allergens (Bencharitiwong et al., 2015) for subcutaneous immunotherapy is also currently being investigated (ClinicalTrials.gov identifier NCT02163018). The active compound for such therapies is essentially based on peanut proteins that can redirect the immune system. The dosage of peanut protein given in these therapies is controlled for the efficacy and safety of treatment, but it is not known if the source of peanut protein plays a role too.

To help peanut allergic consumers adhere to their peanut avoidance diets, the food industry has invested significant resources to ensure clear labeling of peanut-containing products and has also developed allergen control best practices to prevent peanut cross-contact in food products produced on shared equipment or in shared processing facilities. The validation of the effectiveness of cleaning protocols can be monitored using immunoassays to detecting peanut residue on equipment surfaces and quantifying peanut residue in food samples. Such immunoassays have different sensitivities for specific peanut allergens (Jayasena et al., 2015), i.e. some detect mainly Ara h3, and others detect mainly Ara h2 and Ara h2. Because it is not know if different peanut market types contain different levels of these peanut allergens., it is not known if certain peanut market types are under- or overestimated with such assays.

On occasion, pre-packaged food products have been shown to contain undeclared peanut residue at varying concentrations (Remington et al., 2013). Studies to quantify the risk that undeclared peanut residue poses to peanut allergic consumers who may eat such products rely on population threshold distributions modeled from peanut allergic individuals who have undergone a low-dose peanut challenge using various market types of peanuts (Taylor et al., 2010, 2015). It is not known if different peanut market types have different potencies thereby affecting both individual and population-based thresholds for peanut.

Some studies have investigated differences in the allergenic properties of various peanut types. It was shown that the four main market types (Runner, Virginia, Spanish, and Valencia) had comparable contents of the major allergens, Ara h 1 and Ara h 2 (Koppelman et al., 2001). Although the analytical tools used in that study may have been adequate in that era, quantitation of the major allergens would nowadays require more sophisticated analytical techniques. Also, since the time of that study other major peanut allergens have been identified that should also be taken into account. A more recent study compared different cultivation conditions on the allergen composition of Spanish peanuts (Walczyk et al., 2013) while Kottapalli et al. (Kottapalli et al., 2008) used 2D electrophoresis and proteomics to compare the protein profiles of the four market types commonly grown in the U.S. (Runner, Virginia, Spanish, and Valencia). The authors conclude that Valencia and Runner market types do not contain Ara h 3; however, the 2D gels indicate spots at the approximate position of Ara h 3 that were not identified. Due to complex post-translational processing of Ara h 3 (Piersma et al., 2005), it may migrate in 1D and 2D gel electrophoresis conditions at positions deviating from what is expected, possibly explaining why the authors concluded that Ara h 3 is absent in Valencia and Runner peanuts (Kottapalli et al., 2008). Another proteomics study made a detailed analysis of the proteins present in two different peanut types, i.e. Virginia and an Indonesian type named Kacang Asin or Bali peanut (Schmidt et al., 2009). Over 100 protein spots from 2D electrophoresis were identified, and it was shown that the level of Ara h 1 was substantially lower in the Kacang Asin peanut (Schmidt et al., 2009). Other peanut allergens were present in both peanut types in comparable amounts, but the analytical techniques used were only semi-quantitative, i.e. intensity if mass spectrometry signals (Schmidt et al., 2009).

This study quantitatively compares the allergenicity of the four main peanut market types (Runner, Virginia, Spanish, and Valencia). We have determined the protein content and protein profiles, and have applied different immunoassays to compare antigenic and allergenic potency. Furthermore, we have applied a reversed-phase HPLC method to quantify the allergens Ara h 1, Ara h 2, Ara h 3 and Ara h 6. This knowledge will serve in the development of well-characterized peanut immunotherapy materials for peanut allergy, and will also support risk-assessment and food safety programs for the food industry.

2. Material and methods

2.1. Reference peanut allergens and peanut kernel samples

The purified peanut allergens, Ara h 1, Ara h 2, Ara h 3 and Ara h 6, were obtained from lyophilized stock preparations made as described earlier (de Jong et al., 1998; Koppelman et al., 2003, 2005). Virginia peanuts were obtained from the North Carolina State University Department of Crop Science (Raleigh, NC), Runner and Spanish peanuts from the USDA-ARS National Peanut Research laboratory (Dawson, GA), and Valencia peanut from the New Mexico State University Agricultural Science Center (Clovis, NM). All peanut samples were used raw. Table 1 provides an overview of the peanut cultivar samples. Peanuts were shelled and initially stored according to the guidelines for cold storage of peanuts (American Peanut Council, 2006) for several months. The peanut kernels were later repackaged and stored at −20 °C. The nitrogen content of the intact peanut kernels was determined by the combustion method using a LECO FP-428 nitrogen analyzer at 950 °C combustion temperature (LECO Corp., St. Joseph, MI). Conversion to protein was done by multiplying the nitrogen value with 5.46 (Jones, 1931).

2.2. Preparation of extracts

10 to 15 g of peanut kernels was manually ground with a mortar and pestle until a fine, homogeneous paste was obtained. Three different extracts were prepared. The first series of extracts was prepared by mixing 2 g of ground peanut with 20 mL of extraction buffer (0.01 M Ammonium-bicarbonate, pH 7.9) in a 50 mL Falcon tube. Tubes were vortexed and placed in a rotator device (10 rpm) overnight at 2–8 °C. Tubes were centrifuged at 4500 rpm (2830 × g) for 45 min at 4 °C, and an aliquot of the middle layer was collected and transferred into 15 mL tubes and centrifuged again (4500 rpm; 2830 × g) for 45 min at 4 °C. Again, an aliquot of the middle layer was collected and transferred to several 1.5 mL tubes and centrifuged at 14,000 rpm (10,000 × g) for 20 min at room temperature (RT). Clarified solutions were collected from the middle portion of each microcentrifuge tube; these were pooled per sample, aliquoted in small volumes and stored at −80 °C until further use. Where transportation was required, samples were shipped frozen. The soluble protein concentration in the pooled extracts was determined by Bradford analysis (Sigma–Aldrich, USA) using a bovine serum albumin standard (Sigma–Aldrich) and a UV/Vis spectrophotometer (PerkinElmer, USA). This first series of extracts is referred to as aqueous extracts and was used for protein