Shea butter contains no IgE-binding soluble proteins

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To the Editor:

The Food Allergen Labeling and Consumer Protection Act of 2004 requires major allergens to be listed in all packaged goods. The 2006 US Food and Drug Administration guidelines include shea nut among other tree nuts. Shea nut is distantly related to Brazil nut,[1] which cross-reacts with almond, hazelnut, walnut, and peanut.[2] Because of its high content of nonsaponifiable lipids, shea butter is widely used in cosmetic, baby care, food, and confectionary products.[3]

Shea butter is derived from the kernel of the shea nut (Sapotaceae family), which is the seed of the fruit of the karite tree, indigenous to the Savannah region of Africa. Local women manually produce shea butter by shelling the fruit and using the inner nut. The nut is boiled, sun-dried, crushed, and roasted to form a paste. The paste is purified, heated, and mixed with water so that fat rises to the surface, which later hardens to form the butter.[4] It can take 8 hours to produce 1 L butter because of the difficulty in refining the fat and latex within the nuts, which limits solvent extraction.[4] The fatty content of the shea nut kernel varies by region from 29.7% to 53.7%.[3] The protein content is poorly characterized; in one study, 42 mg protein was extracted from 100 g shea nut (0.042%).[5] For comparison, Brazil nut contains 14% protein, cashew and pistachio 21%, and peanut 25%.

In 2003, Lack et al[6] proposed that sensitization to peanut protein may occur through application of skin preparations containing cold-pressed peanut oil to inflamed skin, highlighting the cutaneous route of peanut exposure. There are no reports of ingestion or contact-related reactions to shea butter in individuals with nut allergy. Considering the wide use of skin products containing shea butter, we sought to determine whether there are detectable proteins in shea nut or shea butter extracts and whether such proteins are recognized by subjects with peanut or tree nut allergy.

Extracts were prepared from raw shea nut kernels (Africa Imports, Hackensack, NJ) and white and yellow shea butters from Ghana. Shea nuts were ground and homogenized into a paste. The paste was defatted and extracted by 2 different methods: (1) cold acetone in filter papers and extracted by PBS with protease inhibitor cocktail without EDTA (Roche, Indianapolis, Ind) with or without mercaptoethanol for 4 hours, or (2) petroleum ether in Soxhlet

(Barnstead Electrothermal, Essex, United Kingdom) extracted by the buffered sodium borate method (0.1 mol/L H_3BO_3, 0.025 mol/L $Na_2B_4O_7$, 0.075 mol/L NaCl, pH 8.45 with protease inhibitor) at room temperature for 1 hour.[7] Shea butters were defatted with acetone and extracted by PBS alone or with 0.1 mol/L β-mercaptoethanol with protease inhibitors. Other nut extracts were processed as published.[8] Protein concentration was determined by Coomassie protein assay (Thermo Scientific, Rockford, Ill). Soluble proteins (4 μg/lane) were separated by NuPAGE Novex 4% to 12% Bis-Tris and 3% to 8% Tris-Acetate SDS-PAGE gels (Invitrogen, Carlsbad, Calif) and stained with SimplyBlue SafeStain (Invitrogen). The resolved proteins were transferred to immobilon-P membranes (Millipore, Bedford, Mass). Sera for immunolabeling were obtained from subjects with peanut and peanut/tree nut allergy with a history of convincing IgE-mediated allergic reactions and no known history of allergic reactions to shea nut or shea butter. A nonatopic nut-tolerant individual was used as negative control. Individual and pooled sera were diluted in PBS containing 0.05% Tween 20, 1% BSA, and 10% normal goat serum. Membranes were incubated with Iodine-125–goat antihuman IgE (DiaMed, Windham, Me) and exposed to Kodak bioMax imaging film (Kodak, Rochester, NY) for 1 to 17 days.

ELISA was used to detect small protein fractions, which might not be detected by Western blot. Ninety-six–well plates were coated overnight at 4°C with peanut and shea nut extracts (100 μL/well; protein range, 6.25-200 μg/mL) in carbonate-bicarbonate coating buffer (0.05 mol/L, pH 9.4). Unspecific binding was blocked by 2% BSA, 0.05% Tween 20 in PBS. Peanut/tree nut–allergic pooled sera and a nonatopic control, diluted 1:10 and 1:20 in the blocking buffer, were added and incubated for 2 hours. Allergen-specific IgE was detected with peroxidase-labeled goat antihuman IgE antibody 1:2500 (KPL, Gaithersburg, Md), developed with tetramethylbenzidine (eBiosciences, San Diego, Calif), terminated with stop solution, and read on a microplate reader at 450 nm.

We did not detect any defined soluble protein bands in shea nut or shea butter extracts with SDS-PAGE, even when using gel suitable to detect proteins with molecular weights up to 260 kd (Novex Sharp Protein Standard; Invitrogen). In contrast, multiple well defined protein bands were detected in the peanut, cashew, pistachio, and Brazil nut extracts that corresponded to the known allergens of those nuts. Shea nut and white and yellow shea butter extracts contained 730, 12, and 6 μg/mL water/salt soluble protein by Coomassie assay, respectively. However, this is substantially less compared with cashew extract (25 mg/mL).[8] By Western blot, no IgE binding to shea nut and shea butter was detected, regardless of the method of protein extraction and using sera that strongly bound to the proteins in peanut, cashew, pistachio, and Brazil nut extracts (Fig 1). In ELISA, no IgE binding was detected to shea nut or shea butter, whereas strong binding to peanut proteins was detected (data not shown).

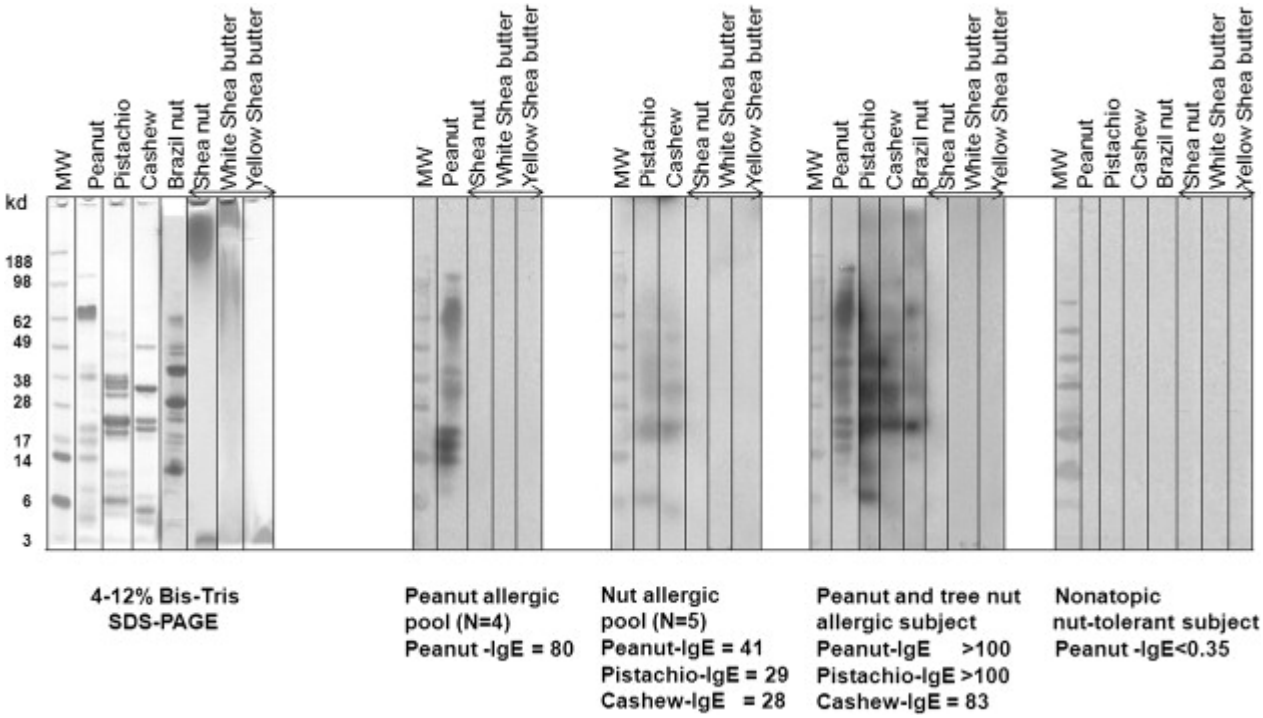

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Fig 1. SDS-PAGE and immune recognition of proteins from shea nut, shea butter, peanut, and tree nut extracts (UniCAP median of specific IgE unit, kilounits of antibody per liter (kU_A/L). We did not detect any defined protein bands in shea nut and shea butter. Nuts were extracted with method 1 (acetone and PBS). *MW*, Molecular weight.

This is the first study examining the potential allergenicity of shea butter. Shea nut and shea butter contain extremely low levels of water/salt soluble protein with undetectable IgE binding by Western blot and ELISA. Protein extraction may be limited by the high fat content of shea nut compared with other tree nuts and peanut and by the presence of latex within the shea nut.[4] These findings are reassuring for individuals with nut allergy who are using shea butter–based products topically. This may explain why no allergic reactions have been reported, despite the popularity of these products. It is unknown whether nipple creams with shea butter used by mothers could predispose to sensitization toward other tree nuts or peanuts in breast-fed infants. In summary, we did not detect any IgE binding to water/salt soluble proteins in shea nut and shea butter extracts with Western blot and ELISA, suggesting minimal availability of protein in commercial shea butter products.

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