How much bean hemagglutinin is safe for human consumption?

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SUPPORTING INFORMATION

Materials and Methods

Materials and chemicals

Trypsin, sodium hydroxide, sodium phosphate dibasic, sodium phosphate monobasic, hydrogen chloride, and sodium chloride were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used without any purification. Whole rabbit blood in Alsever's solution (R309-0050) was purchased from Rockland Inc. (Limerick, PA 19468). All canned beans were purchased from a local supermarket, including black beans, red kidney beans, white kidney beans, and great northern beans from Bush's, Simply Balanced, Goya, and Market Pantry. Ultrapure water (18.2 MΩ/cm) was used for all experiments, prepared from a Millipore water purification system.

Hemagglutination assay by rabbit red blood cells

The hemagglutination assay was performed according to a method described by Lis and Sharon.[15, 16] To be specific, whole rabbit red blood cells in Alsever's solution were purified by centrifugation and re-dispersed in 0.9% saline solution (w/v). The process was repeated three times to remove interfering plasma. After purification, the red blood cells were suspended at 4% (v/v) in 10 mM phosphate-buffer (PBS) with 0.9% saline (pH 7.4). One part trypsin solution (10%) was added to 9 parts of the blood cell suspension and the mixture was incubated in a 37 ^{o}C water bath for 1 h. After trypsin treatment, the red blood cells were purified three times by centrifugation and re-suspended at a concentration of 4% (v/v) in 10 mM PBS (pH 7.4).

The canned beans were diluted 1.5 times by ultrapure water and blended by a food blender. The blended beans slurry was diluted 10 times by ultrapure water and used directly for the hemagglutination assay. The diluted bean solutions were diluted in serial with 2-fold dilution in 96-well round bottom reading plate. PBS was also added as negative control samples. The trypsinized rabbit red blood cells were added to each well and kept at room temperature for 2 h. The samples in which blood cells precipitated at the bottom of the well and formed in a "teardrop" were considered to be negative. In contrast, when hemagglutinins were present, they coagulated the blood cells by crosslinking, thus the ones that did not form a "tear-drop" were considered to be positive. The last dilution that caused cell agglutination was defined as one hemagglutination unit (HAU). The HAU per gram of solid were thus determined according to the solid concentration of the last well. The bean solids were determined by calculating the weight loss of the bean slurry after overnight incubation in a 105 ^{o}C oven.