Lectin Quantitation in Peanut and Soybean Seeds[1]

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ABSTRACT

A method was developed to determine amounts of lectin in peanut and soybean seeds. Both types of seeds contained active lectins in amounts ranging from 144.7 to 112.2 μg/g defatted meal for peanut and soybean seed, respectively. Lectins were inactivated by heat; moist heat was more effective than dry heat. Roasted peanuts seeds (177 C for 30 min) and boiled peanuts (5% saline solution for 1 hr) were devoid of active lectin.

Key Words: Lectin, peanut seed, soybean seed, RBC agglutination lectin determination, peanut lectin, soybean lectin.

Lectins are a group of chemical substances that are widely distributed in many plants including the edible seeds of common legumes. The term lectin is used interchangeably with the term phytohemagglutinin or hemagglutinin. These terms were proposed due to the ability of these substances to bind to specific sugars present on the surface of red blood cells (RBCs) of humans and different animal species resulting in the agglutination of RBCs. Other biological activities of such substances are amply reviewed (7, 9, 13,15).

Most of the lectins are glycoproteins containing 4-10% carbohydrate (10). Exceptions are jack bean, peanut and wheat germ lectins, which are devoid of carbohydrate; rice and potato lectins contain 25 and 50% carbohydrate, respectively. Most lectins have molecular weights (mw) ranging from 110 KDa to 124 KDa and are composed of 4 subunits, tetramers (8, 10). Lectins from lentil, garden pea and field bean appear to be dimers having MW ranging from 50 KDa to 53 KDa (10).

Chilean legumes and soybean lectins were inactivated by heat with the most reduction in activity occurring with moist heat application at temperatures greater than 100 C (3, 7, 9, 17). The beneficial results of such inactivation were manifested by increased weight gain of rats and higher protein efficiency ratio (PER) values for the diet containing the heated lectins. No reports are available on the quantity of active lectin present in American grown cultivars of peanut seeds or the extent of heat inactivation of lectin in peanut seed.

The objectives of the present study were to investigate and quantify the active lectin in American grown Florunner peanut seeds, peanut products and Bragg soybean seed and the effects of dry and moist heat on the lectin activity.

Materials and Methods

Agglutination mechanism

The human red blood cell (RBC) is enclosed in a membrane consisting

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of a lipid bilayer interspersed with discrete protein aggregates (15). The proteins are of several types, including several glycoproteins and mucoproteins. The proteins contain oligosaccharide side chains with residues of L-fucose, D-galactose, N-acetylgalactosamine and N-acetyl-D-glucosamine. The terminal monosaccharide unit is a negatively charged residue of N-acetyl neuraminic acid (NANA). This acid is a 9-carbon amino sugar derivative consisting of a 6-carbon acetylated amino sugar linked to a 3-carbon sugar acid. Neuraminidase cleaves NANA from the oligosaccharide side chain (Fig. 1). Lectins bind to specific sugars or amino sugars on the side chain of the enzyme treated RBC membrane and bridge two or more RBCs, yielding aggregates. The result of this reaction is the precipitation of the RBCs and the formation of a clear serum. Neuraminidase treatment of the human RBCS is a prerequisite for their agglutination by peanut lectin (13, 14, 16). Sensitization of human RBCs to agglutination by soybean seed lectin could be accomplished by either neuraminidase (this study) or trypsin (6).

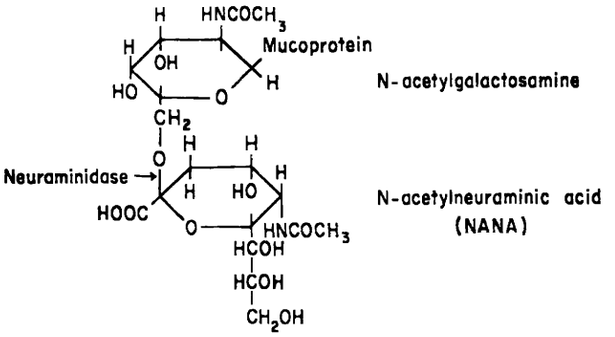

Fig. 1. A general model of glycoprotein with terminal N-acetyl-neuraminic acid (NANA).

Analytical Procedure:

Spin-blanched Florunner peanut seed, peanut seed harvested at R-7 maturity stages, peanut products and dehulled Bragg soybean seed were ground and defatted with petroleum ether in a Soxhlet apparatus. Two g of the well-pulverized fat-free flour were suspended in 100 mL of 0.85% saline phosphate buffered at pH 7.2 (PBS) and stirred magnetically at 4 C for two hr. The mixture was filtered through No. 2 Whatman filter paper and the filtrate was centrifuged for one hour at 16270 x g using RC-5 superspeed refrigerated centrifuge (Sorvall) maintained at 4 C. The supernatant was decanted and passed through a 2 μm Millipore filter. The final filtrate was considered the crude lectin extract.

Agglutination of type A human RBCs was the criterion used to detect the presence of active lectins in peanut and soybean seed extracts. The washed and packed RBCs suspension (50 mL) was incubated with neuraminidase (11.7 mg) for 70 minutes at 37 C. The enzyme treated cells were washed 4 times with 4 X volumes of PBS and made up to a suspension of 1.5% (v/v). The method used was a modification of the methods of Liener (16), Herholzer and Suggs (5), Lis and Sharon (12), and Lotan *et al.* (12). Agglutination of RBCs was followed by absorbance measurments at 620nm using a Coleman Junior Spectrophotometer, at 0 and 150 minutes amd verified after 150 minutes with visual observation of the agglutination of RBCs. The cuvette was filled with the enzyme-treated blood preparation and inserted in the cuvette holder which was masked by black tape in a manner to permit only 1.0 cm^2 aperture located 0.5 cm away from bottom of the cuvette. Thus, all absorbance measurements of the unsedimented RBCs occurred at the same location of the cuvette.

A standard curve of commercially available pure peanut or soybean lectin (Sigma Chemical Co.) was prepared for each run. Lectin concentrations used ranged from 0.062 to 4.0 μg/mL. The blank was a 1:1 of 0.85% PBS and enzyme-treated RBCs. The differences in average absorbance values between 0 time and 150 minutes were plotted against lectin concentration. The higher the difference values, the higher the lectin concentration and consequently the more agglutination of the RBCs.

Twenty sets (each represents a different source of human type A RBCs) of standard curve data were used to calculate a prediction equation to estimate lectin concentration in peanut or soybean extract. The regression coefficients of the first degree and polynomial models were estimated using the Statistical Analysis System program package (1). Comparison of the slopes of the regression lines of the dry and moist heated seeds (T = 5, 10, 15, 20 min) were carried out according to the method outlined by Draper and Smith (4).

Materials

Fresh spin-blanched Florunner peanut seed obtained from Pert Laboratories, Edenton, NC and Bragg soybean seeds obtained from Hickory Farms, AR were exposed to two types of heat treatments:

1 - dry heat: Air heated to 177 C for periods up to 30 minutes.

2 - moist heat: steam at 121 C for periods up to 30 minutes.

Fresh Florunner peanut seed harvested at the R-7 maturity stage (2), were obtained from Sandlin Farms, Williston, FL and boiled in a 5% (w/v) saline solution for one hr.

There were 3 replicates for the heated and unheated seed and peanut products. Results are expressed as μg active lectin/g defatted meal and are presented as means ± standard error of the means.

Results and Discussion

Regression equations to estimate lectin concentration from absorbance values of enzyme treated RBCs are shown in Table 1. Indicators of the goodness of fit of the prediction equation showed that the linear model was the poorest fit while improved goodness of fit was obtained by increasing the number of terms in the equation. Slight differences among the indicators were observed for prediction equation containing polynomials exceeding the cubic level. The regression equation at the cubic level was used to calculate lectin

Table 1. Regression equations to predict lectin concentrations (μ/g) from absorbance values of RBCs suspensions.

Regression equation	Goodness of fit indicators: Mallows CP Stat.	Error MS	R^2
$\hat{Y} = 0.0923 + 0.0727A$	122.36	0.0031	0.7624**
$\hat{Y} = 0.0485 + 0.1924A - 0.0301A^2$	25.98	0.0012	0.9082**
$\hat{Y} = 0.0200 + 0.3483A - 0.1497A^2 + 0.0207A^3$	6.66	0.0008	0.9398**

$\hat{Y}$ = Estimated concentration (μg/g).

A = Difference in absorbance values between 0 time and at 150 minutes.

** = Significant at $\alpha = 0.01$ level.

concentration in this study. The curvilinear relationship between lectin concentration and absorbance values could be due to differences in the number of RBCs in each aggregate of the agglutinated cells or the total number of aggregated RBC.

Heated Florunner peanut and Bragg soybean seed exhibited less lectin activities that the non-heated seed as indicated by the negative slopes of the simple regression equation (Table 2). Active lectin content in heated peanut seeds decreased at a faster degree for the moist heat treatment (slope = -6.75) over times T = 5, 10, 15, 20 min than the dry heat treatment (slope = -1.51). Slope for these two treatments were statistically different. The reverse was true for soybean seed (slopes -5.85 vs -0.29 for the dry and moist heat over times T = 5, 10, 15, 20 min). These slopes were also statistically different. Considerable reduction in active lectin content occurred upon heating soybean seeds for 5 min, with much less additional reduction as heating periods increased to 10, 15 and 20 min (Table 3). This trend was different than the dry heated soybean seed and dry and moist heated peanut seed (Table 3). However, the amounts of active lectin in moist heated soybean were much less that for the dry heated ones. Similary, moist heated peanut seed contained less active lectin than the dry heated seed, especially for the heating periods of 10, 15 and 20 min. Seed were devoid of lectin activity under the analytical procedures used in this study at the heat treatments for 30 and 20 min for peanut and soybean seed, respectively (Table 3). Soybean seed were more susceptible to heat inactivation than peanut seed lectins as shown by the shorter duration of times needs for their inactivation by dry or moist heat. The dry heat treatment of peanut seed at 177 C for 30 min resembled the roasting of shelled peanut seeds in a protable General Electric rotisserie to produce seed possessing a color similar to USDA color standard No. 2 for peanut butter. Thus, it could be stated that roasted peanut seed receiving such heat treatment do not contain active lectin substances. Earlier studies had shown that moist heating of soybean flour at 121 C for 20 min resulted in 96% destruction of the lectin present and increase in the protein efficiency ratio of the rat diet containing this flour of 227% (8, 9, 17). In this study, similar heat treatment of defatted soybean seed meal resulted in 100% destruction of lectin present (Table 3).

Table 2. Comparison of the Slopes[1] of two simple regression (first-degree) lines and R^2 for dry and moist heated[2] peanut and soybean seeds.

	Regression equation			R^2
Peanut	$\hat{Lc}^{3}_{dry}$	=	140.10 - 1.51 T	0.906
	$\hat{Lc}_{moist}$	=	122.00 - 6.75 T	0.873
Soybean	$\hat{Lc}_{dry}$	=	125.25 - 5.85 T	0.910
	$\hat{Lc}_{moist}$	=	5.48 - 0.29 T	0.831

[1] Slopes compared according to Draper and Smith (4).

[2] Heating periods (T) 5, 10, 15, 20 min.

[3] Lc = estimated lectin content, T = times at 5, 10, 15, 20 min.

Table 3. Active lectin content (μg/g defatted meal) of dry and moist heated Florunner peanut and Bragg soybean seeds.

Time (min)	Peanut		Soybean	
	Dry[1]	Moist[2]	Dry[1]	Moist[2]
0	144.7 ± 2.13	144.7 ± 2.13	112.1 ± 2.77	112.1 ± 2.77
5	133.2 ± 0.78	103.3 ± 0.87	85.4 ± 2.05	3.9 ± 0.31
10	122.8 ± 1.18	37.2 ± 1.29	79.7 ± 1.65	3.2 ± 0.57
15	120.7 ± 0.71	9.2 ± 0.60	43.5 ± 2.38	0.6 ± 0.05
20	108.6 ± 1.09	0.4 ± 0.02	0.0	0.0
30	0.0	0.0	0.0	0.0

Data presented as mean ± standard error of the mean (3 replicates)
1 air heated at 177 C
2 steam at 121 C.

Florunner peanut seeds harvested at the R-7 maturity stage contained lesser amounts of active lectin (88.4 ± 3.00 μg/g) as compared to the Florunner seeds harvested at R-8 maturity stage (144.7 ± 2.13 μg/g). This could be due to differences in maturity stages. Boiling in saline solution resulted in complete inactivation of lectin present in peanut seeds harvested at the R-7 maturity stage.

Commercially available peanut seed products showed various amounts of active lectin (Table 4) Differences in active lectin amounts within the same product could be attributed to the degree of roasting prior to product preparation. Differences among the various products could be the result of the differences in type and cultivar used, and the degree of roasting of the seeds.

Table 4: Active lectin content (μg/g defatted meal) of commercially available[1] peanut seed products.

Product	Lectin[2]	
	Brand A	Brand B
Non-roasted Florunner	133.0 ± 5.1	144.7 ± 6.47
Oil roasted spanish	0.5 ± 0.0	5.5 ± 0.17
Dry Roasted	5.6 ± 0.23	8.4 ± 0.46
Roasted in shell	8.4 ± 0.52	6.2 ± 0.29
Solvent defatted flour	21.8 ± 0.92	———
Peanut butter	21.9 ± 0.52	16.9 ± 0.29

[1] Available from various large-chain supermarkets.

[2] Data presented as mean ± standard error of the mean (3 replicates).

Conclusions

Agglutination of human type A RBCs was the criterion used to detect the presence of lectin in peanut and soybean seed. Moist heat was more effective in inactivating lectin than dry heat. However, the amount of heat used in roasting or boiling peanuts in the present study was sufficient for 100% inactivation of peanut lectin. Commercially available peanut products contained lectin in

amounts ranging from 0.5 to 21.9 μg/g defatted peanut meal.

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