Food Chemistry **39** (1991) 321–327

Active Soybean Lectin in Foods: Isolation and Quantitation

A. M. Calderón de la Barca, L. Vázquez-Moreno, & M. R. Robles-Burgueño

Centro de Investigacion en Alimentación y Desarrollo, AC, Apdo Postal No. 1735, Hermosillo, Sonora 83000, Mexico

(Received 21 July 1989; revised version received and accepted 30 March 1990)

ABSTRACT

An improved method was developed to determine active soybean lectin in processed foods containing soybean. Freeze drying of crude extracts followed by affinity chromatography allowed the authors to purify and quantitate active lectin. After dialysis, concentrated extracts from products were loaded on to a chromatography column of immobilized N*-acetyl-galactosamine. Active lectin content of soybean flours depended on the processing method used. The highest level found was for raw seeds (3600 μg/g), and the lowest for texturized flour (12·9 μg/g). Direct human consumption foods contained various lectin concentrations. While meat substitutes were free of active lectin, milk substitutes, and bakery products had low levels. A cereal type food and a cookie showed the highest lectin concentrations (187·2 and 24·2 μg/g, respectively).*

INTRODUCTION

Feeding of raw soybean (*Glycine max*), a seed of considerable economic importance, results in a poorer than expected growth rate in rats. Poor nutritive value of soybean is usually ascribed to the very high level of trypsin inhibitors. Currently, there is increasing evidence that other antinutritional seed constituents such as lectins, are involved in this problem (Grant *et al.*, 1987).

Food Chemistry 0308-8146/90/$03·50

Although soybean lectin is not generally regarded as toxic for rats, the lectin, along with trypsin inhibitors, might account for the growth-depressing effects. As a matter of fact, a synergistic destabilizing effect of soybean lectin and soybean saponins on rabbit jejunal epithelium has been demonstrated (Pusztai, 1985). Grant *et al.* (1987), have shown that both lectin and trypsin inhibitors can affect pancreatic function, and their combined action may have considerable implications in relation to the use of soybean meal or protein in human and animal nutrition.

Lectins are proteins widely distributed in nature, including food items commonly consumed by man (Nachbar & Oppenheim, 1980; Andersen & Ebbensem, 1986). Knowledge about heat denaturation of these factors is therefore of considerable importance (Jaffé, 1980). Soybean lectin is resistant to inactivation by dry heat (De Muelenaere, 1964), thus a complete detoxification may not always be achieved, especially when soybean flour is used for making baked goods or processed to obtain quick cooking products.

Screening for lectins in common foods has been carried out utilizing several cell-agglutination techniques to measure lectin activity (Nachbar & Oppenheim, 1980; Rea *et al.*, 1985; Klurfeld & Kritchevsky, 1987). These methods are semiquantitative and interference may occur due to the presence of colored substances, gums, and other substances present in foodstuffs.

This paper reports an improved method to determine active soybean lectin in processed foods containing soybean. It utilizes affinity chromatography and the ability of lectins to bind to carbohydrates.

MATERIALS AND METHODS

Raw soybean seeds (*Glycine max*), soybean flours (defatted and texturized), meat substitutes, milk substitutes, bakery products, and breakfast-cereal type foods were obtained from the local markets in Sonora, Mexico. Sugars were purchased from Sigma Chemical Co., St Louis, MO, USA. Agarose activated with divinyl sulphone (Mini-Leak®) was from Kem-En-Tec, Denmark. All other reagents were of analytical grade.

Sample preparation

Finely ground (US mesh No. 20) seeds and products were suspended in a 0·9% NaCl solution (10% w/v). This suspension was stirred for 2 h, the pH adjusted to 4 with 4M acetic acid, and allowed to stand overnight. After

centrifugation, the sediment was re-extracted with a 5% saline solution, and both supernatants were clarified using a glass fiber paper (Whatman GF/A), pooled and concentrated to 20-fold by ultrafiltration using an Amicon System with a PM-20 membrane or freeze dried (Labconco Mod. 4451 F). As a control procedure purified soybean lectin was mixed with three different ground products (100 μg/g) for measuring the recovery efficiency after each step. These samples were then subjected to the extraction, concentration and isolation procedures described above.

For affinity chromatography, dried saline extracts were first suspended in a minimum volume of saline solution, then exhaustively dialyzed and tested for hemagglutination activity with human trypsinized erythrocyte suspension as described previously (Calderón de la Barca & Vázquez-Moreno, 1988). Protein concentration was determined by the Lowry *et al.* (1951) method. Specific activity was defined as hemagglutination titer/protein concentration.

Purification and quantitation of lectin

Two hundred milligrams of *N*-acetylgalactosamine (GalNAc) were coupled to 2 ml of divinyl sulphone activated agarose gel (Mini-Leak®), according to Müller *et al.* (1986). The gel was packed into a 0·7 × 6 cm column and extensively washed with PBS (phosphate buffer saline, 0·02M KH_2PO_4/K_2HPO_4, 0·9% NaCl, 0·03% NaN_3, pH 7·2), and 0·1M GalNAc, and finally reequilibrated with PBS. Samples in batches of 25 ml were run through the column. Unbound proteins were eluted with starting buffer until 0·02 absorbance at 280 nm was obtained. Bound lectin was eluted using 0·1M GalNAc.

The identification of the soybean lectin in each saline extract and its corresponding lectin fraction was done by electrophoresis in a 10% SDS-PAGE gel as described by Laemmli (1970).

RESULTS AND DISCUSSION

Soybean lectin in raw seeds

Soybean lectin has been purified by different methods (Lotan *et al.*, 1974; Allen & Neuberger, 1975) and has been extensively studied. Its agglutination reaction is specifically inhibited by *N*-acetyl-D-galactosamine, and, to a lesser extent, by D-galactose (Allen & Neuberger, 1975). Some problems related to purification by affinity chromatography include time-consuming

preparation of the affinity absorbent and loss of the ligand after a few runs. We have previously found an alternative absorbent which can be prepared by coupling *N*-acetyl-D-galactosamine to divinylsulphone activated agarose (Vázquez-Moreno *et al.*, 1990). This system had a high affinity for soybean lectin and great ligand linkage stability. The absorbed lectin was eluted with a D-galactose solution. From 1 g of raw soybean meal 3·6 mg of lectin were purified, while Allen and Neuberger (1975) obtained 3·2 mg of lectin per gram. In an analysis of 97 lines of *G. max*, the amount of soybean lectin ranged from 2·5 to 12·2 mg per gram of defatted seed meal (Pull *et al.*, 1978).

Soybean lectin in processed foods

Extraction, concentration, and efficiency

Although saline extracts from soybean seed meal can easily be concentrated to 20-fold by ultrafiltration, saline extracts from processed foods, especially texturized products, produce high density solutions that clog ultrafiltration membranes. This prolongs the time required for concentration, and causes partial loss of lectin activity (Table 1). Klurfeld and Kritchevsky (1987), obtained only 10% of recovery efficiency for soybean lectin isolation from soybean oil during extraction and concentration by ultrafiltration apparently due to lectin instability.

On the other hand, saline extracts of all products were easily concentrated up to 20-fold by freeze-drying, with 100% lectin activity recovery. Table 1 also shows that all of the lectin activity was recovered from control samples by the proposed methodology. The percent recovery was based on both protein determinations and hemagglutination activity. Brady *et al.* (1978), used a similar methodology for wheat germ agglutinin quantitation in feces

TABLE 1
Efficiency of Lectin Activity Recovery from Products

Sample[a]	*Lectin activity recovery*[b]		
	Ultrafiltration (%)	*Freeze-drying* (%)	*Affinity chromatography* (%)
Raw seeds	100	100	100
Milk substitute	10	100	100
Textured protein	13	100	100
Breakfast cereal	11	100	100

[a] 100 μg/g of purified soybean lectin were added to each sample.
[b] Lectin activity was measured as specific activity defined in 'Materials and Methods'.

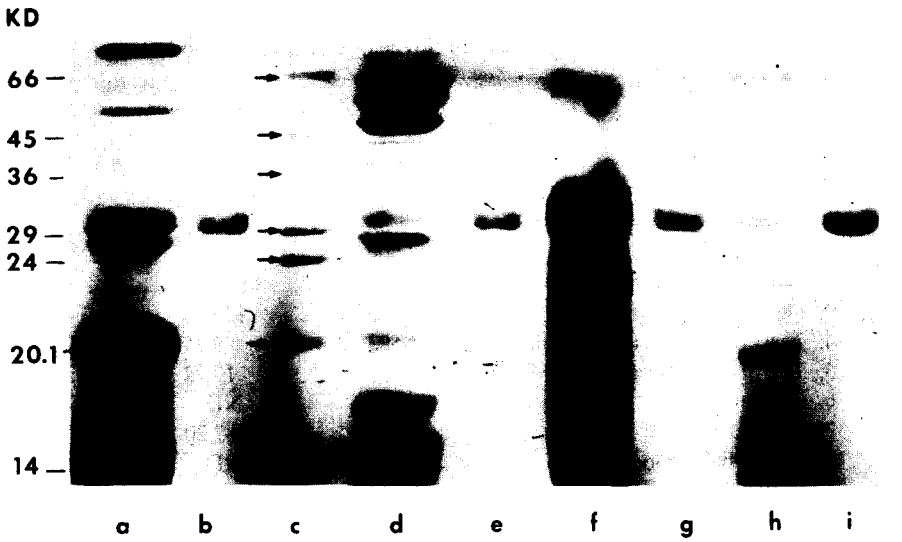

Fig. 1. Electrophoretic patterns of the crude extracts (a, d, f and h) from different products and the corresponding purified fractions (b, e, g and i). (a, b) raw soybeans seeds; (d, e) textured protein product; (f, g) defatted soybean flour; (h, i) cookie; and (c) molecular weight standards.

samples, but recovered only 10% of the added lectin. They attributed the low percentage of recovery to limited efficiency of the extraction procedure rather than to degradation of the protein.

Electrophoretic patterns of isolated lectin from products

The developed method was specifically designed for active soybean lectin isolation from food products. Bound fractions to *N*-acetyl-D-galactosamine were eluted with D-galactose (Allen & Neuberger, 1975), and lectin identification done by SDS-PAGE (Lotan *et al.*, 1974).

Figure 1 shows the electrophoretic patterns for the crude extracts of different products and the corresponding purified fractions with a typical double band at 31 000 D (Lotan *et al.*, 1974), as compared with purified lectin from raw seeds (line b).

Lectin quantitation from products

Table 2 shows the soybean lectin content in processed and unprocessed foods. Active lectin levels in unprocessed foods were estimated from active lectin in the soybean flour content (as indicated by manufacturer).

There was a wide variation of lectin content in unprocessed foods since different products were prepared with various amounts of soybean flour.

Lectin activity levels of soybean flours, as expected, depended on processing (De Muelenaere, 1964). The highest level found was for raw seed (3600 μg/g), followed by whole flour (1692 μg/g), and defatted flour

TABLE 2
Active Lectin Content in Soybean-Containing Foods

Food	*Active lectin content*		
	Unprocessed[a] *item* (μg/g)	*Processed food* (μg/g)	*Residual* (%)
1. Raw soybean seeds	3 600	3 600·00	100·00
2. Whole soybean flour	3 600	1 692·00	47·00
3. Defatted soybean flour	4 583	155·00	3·40
4. Textured protein product 1	4 583	3·75	0·08
5. Textured protein product 2	4 583	12·92	0·28
6. Granola	—	ND	ND
7. Breakfast cereal	180	187·20	104·00
8. Cereal baby food	—	ND	ND
9. Milk substitute 1	1 230	6·91	0·56
10. Milk substitute 2	1 156	16·20	1·40
11. Frankfurter analog product	1 800	ND	ND
12. Bologna	1 800	ND	ND
13. Meat analog product	432	ND	ND
14. Hamburger analog product	1 800	6·19	0·34
15. Baked goods	360	ND	ND
16. Cookie 1	36	2·51	6·97
17. Cookie 2	54	24·20	44·81
18. Whole wheat bread	72	5·68	7·89

ND, Not detectable.
—, Content not provided by manufactures.
[a] As defined in 'Materials and Methods'.

(155 μg/g). The lowest levels found were for textured protein products (3·75 and 12·92 μg/g).

Direct human consumption foods (Table 2, items 6–18) contained different lectin concentrations. Except for the hamburger (6·19 μg/g), most of the meat analogs were free of active lectin. Lectin in milk substitutes ranged from 6·91 to 16·2 μg/g, bakery products from 2·51 to 24·2 μg/g, and cereal-type foods from undetected levels to 187·2 μg/g. According to the manufacturer, the breakfast cereal reported here was processed by extrusion at 140°C for 15 s, and the lectin maintained its activity at 100%. Milk substitute 2 was prepared in a similar way but at 160°C for 90 s, and 98·6% of the lectin activity was lost.

Although it is not known if the lectin levels found in these foods are detrimental to the human diet, there is a theoretical reason to suspect that low levels of lectins are important factors in both allergic and auto-immune diseases (Freed, 1988).

REFERENCES

Allen, A. K. & Neuberger, A. (1975). A simple method for the preparation of an affinity absorbent for soybean agglutinin using galactosamine and CH-sepharose. *FEBS Letters*, **50**(3), 362–4.

Andersen, M. M. & Ebbesen, K. (1986). Screening for lectins in common foods by line-dive immunoelectrophoresis and by haemadsorption lectin test. In *Lectins. Vol. V*, ed. T. C. Bøg-Hansen. Walter de Gruyter & Co., Berlin, Germany, pp. 135–8.

Brady, R. G., Vannier, A. M. & Banwell, J. G. (1978). Identification of the dietary lectin, wheat germ agglutinin, in human intestinal contents. *Gastroenterology*, **75**(2), 236–9.

Calderón de la Barca, A. M. & Vázquez-Moreno, L. (1988). *Amaranthus cruentus* lectin: purification, stability, and some biochemical properties. *J. Food Biochem.*, **12**(2), 117–26.

De Muelenaere, H. J. (1964). Effect of the heat treatment on hemagglutinating activity of legumes. *Nature*, **201**, 1029–30.

Freed, D. L. J. (1988). Lectins in the food—what are they doing to us? In *Lectins. Vol. VI*, ed. D. Freed & T. C. Bøg-Hansen. Walter de Gruyter & Co., Berlin, Germany, pp. 95–108.

Grant, G., Watt, W. B., Stewart, J. C. & Pusztai, A. (1987). Effects of dietary soybean (*Glycine max*) lectin and trypsin inhibitors upon the pancreas of rats. *Med. Sci. Res.*, **15**, 1197–8.

Jaffé, W. G. (1980). Hemagglutinins (Lectins). In *Toxic Constituents of Plants Foodstuffs* (1st edn), ed. I. E. Liener. Academic Press, London, pp. 73–98.

Klurfeld, D. M. & Kritchevsky, D. (1987). Isolation and quantitation of lectin from vegetable oils. *Lipids*, **22**(9), 667–8.

Laemmli, U. K. (1970). Most commonly used discontinuous buffer system for SDS electrophoresis. *Nature*, **227**, 680–5.

Lotan, R., Siegelman, W., Lis, H. & Sharon, N. (1974). Subunit structure of soybean agglutinin. *J. Biol. Chem.*, **249**, 1219–24.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, **193**, 265–75.

Müller, K. G., Schafer, N. & Lihme, A. (1986). Purification of *Ulex europeus* haemagglutinin I by affinity chromatography. In *Lectins. Vol. V*, ed. T. C. Bøg-Hansen. Walter de Gruyter & Co., Berlin, Germany, pp. 135–8.

Nachbar, M. S. & Oppenheim, J. D. (1980). Lectins in the United States diet: a survey of lectins in commonly consumed foods and a review of the literature. *Am. J. Clin. Nutr.*, **33**, 2338–45.

Pull, S. P., Pueppke, S. G., Hymowitz, T. & Orf, J. H. (1978). Soybean lines lacking the 120000-Dalton seed lectin. *Science*, **200**, 1277–9.

Pusztai, A. (1985). Constraints on the nutritional utilization of plant proteins. *Nutrition Abstracts and Reviews* (Series B), Vol. 55(7). Commonwealth Agric. Bureaux, pp. 363–9.

Rea, R. L., Thompson, L. V., David, J. A. & Jenkins, D. M. (1985). Lectins in foods and their relation to starch digestibility. *Nutr. Res.* **5**, 919–29.

Vázquez-Moreno, L., Calderón de la Barca, A. M. & Robles-Burgueño, M. R. (1990). Comparison of divinylsuphone-activated agarose with different matrix materials to purify lectins by affinity chromatography. In *Lectins. Vol. 8*, ed. A. Kallikorm & T. C. Bøg-Hansen. Sigma Chemical Co., St Louis, MO, pp. 256–9.