

A Research Note
**Selective Inhibition of Lectin Induced Rabbit Erythrocyte
Agglutination by Hydrolysates of Skeletal Beef Protein**

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ABSTRACT

Soybean lectin (SBL) induced agglutination of rabbit red blood cells (RRBC) was selectively inhibited by partial tryptic hydrolysates of beef protein isolate and all-beef frankfurter. These interactions have been utilized to design a noninstrumental agglutination method to detect nonbeef muscle protein in meat products. The presence of nonbeef muscle proteins in cooked sausages is reflected by a reduced level of inhibition established for the all-beef skeletal muscle hydrolysate. The method effectively detected the presence of 10-50% nonbeef proteins (soya, pork, pork rind, chicken, turkey and surimi) substituted in all-beef frankfurter.

INTRODUCTION

SOYBEAN LECTIN (SBL) has an affinity for N-acetyl-D-galactosamine or galactose (Lis et al., 1970). Lectins can selectively agglutinate various types of erythrocytes and SBL was shown to agglutinate rabbit erythrocyte, RRBC, (Liener, 1955, Lis and Sharon, 1972; Sharon and Lis, 1972). These properties suggest suitability of these interactions to detect proteins containing unique glycoproteins in various food sources. Glycoproteins from different species of proteins can have varied binding with soybean lectin (SBL), thus, binding of glycoprotein in the sample with the lectin can lead to inhibition of the lectin-RRBC complex. The objective of this study was to find novel techniques to detect protein additives in all-beef products.

MATERIAL & METHODS

Reagents and equipment

Trypsin (chymotrypsin free), soybean lectin (SBL), rabbit red blood cells (RRBC) were purchased from Sigma (St. Louis, MO); Polytron was from Brinkman Instruments (Westbury, NY); polystyrene cuvettes (1.6 mL), and polypropylene tubes (12 mL and 30 mL) were from Sarstedt (Princeton, NJ); clinical centrifuge was from International Equipment Company (Needham, MA). Soy protein (Supro 620) was a gift from Ralston Purina (St. Louis, MO). Casein and whey powders and other acetone powder isolates were prepared in our laboratory from fresh beef, pork, chicken and fish (*Alaskan pollock*) obtained from local markets. Powdered pork rind was from Grindsted Products (Denmark). Pork muscle, cooked beef tongue, surimi, chicken and turkey frankfurters were purchased from local markets.

Tryptic hydrolysis was carried out in TRIS-CaCl₂, pH 8.1 (0.05M TRIS-0.0086M CaCl₂). Phosphate buffered saline (PBS), pH 7.0, containing 0.041M NaH₂PO₄, 0.061M Na₂HPO₄, 0.31M NaCl and 0.01% thimersol was used as diluent for the lectin, red blood cells and agglutination assay.

Model frankfurters containing approximately 49% lean meat, 25.2% beef fat, 21.2% water, 4.6% spice, sugar and salt, cooked to 71°C internal temperature, were prepared according to industrial standards. Beef protein content was also substituted with soybean protein isolate at 1-5% of the total weight of the emulsion mixture.

Sample extraction and hydrolysis

A 50-g sausage product or other protein sample was macerated in a Waring Blendor. Aliquots of 1g were transferred to 30 mL polypropylene centrifuge tubes. TRIS-CaCl₂ buffer (5 mL) was added to each sample and homogenized 1 min using the Polytron (Power 4). A 100 microliter trypsin solution (10 mg/mL) was added to each tube and samples were mixed in a vortex mixer and incubated in a water bath for 3 hr at 37°C. The samples were transferred to 16 × 100 mm tubes and centrifuged at 2000 rpm (approx. 500 × g, setting #4) for 10 min. Supernatant fluids were decanted and the insoluble peptide fragments were suspended in 5 mL PBS.

Agglutination assay

Tryptic hydrolysates (0.5 mL aliquots) were transferred to cuvettes and 0.2 mL SBL (1 mg/10 mL PBS) was added, covered with parafilm, mixed gently and allowed to stand 1 hr at RT (20-24°C) or 45 min at 37°C. The contents of 1 vial rabbit red blood cells (RRBC) were reconstituted with 10 mL PBS and 0.2 mL gently mixed cell suspension was added to the cuvettes. Cuvettes were covered with parafilm, mixed gently by tilting 4-5 times, placed on a flat surface and agglutination allowed to proceed. The height of the agglutinated cells was measured after 90 min and results were presented as ratio of the sample agglutination to that of all-beef control. Frankfurters fortified with 0-5% soy protein, and other nonskeletal beef proteins were also tested and measured against model all-beef frankfurters.

RESULTS & DISCUSSION

BEEF PROTEIN (SBM) hydrolysates showed a selective inhibition of the lectin-RRBC agglutination while nonskeletal beef proteins (tongue) and other nonbeef proteins showed no inhibition. Optimum conditions were selected from variables presented in a separate publication (Medina and Woychik, 1989). Agglutination heights were recorded after 30, 60, and 90 min following the addition of RRBC to the sample moisture. Results were reported as ratios of height (mm) of test samples over height (mm) of an all-beef (SMB) control after 90 min equilibration. Liener (1955) reported the use of optical density readings of the cell suspension as an end-point measurement; however, that approach was not practical or convenient for the assay we describe here. Measuring the height of the agglutinated cells was simple and adequate for the objectives of our screening assay.

The beef frankfurters containing soy protein isolate had a lower agglutination ratio (Fig. 1) than "SBO" (a standard all-beef sample). Frankfurters containing 0, 1, 2, 3, 4, and 5% soy protein isolate had ratios of 1.0, 0.88, 0.62, 0.69, 0.50 and 0.53, respectively. This figure also shows the inhibition of SBL-RRBC agglutination by all-beef hydrolysates (SBO) in contrast to SBL and RRBC alone (SBL) without any sample added. Agglutination of SBL and RRBC had a mean ratio of 0.13 (±0.02 SD) in 10 separate assays. Furthermore, this agglutination occurs in 5-15 min compared to 90 min equilibration of samples. A minimum of 2% soy protein addition could be detected (ratio of 0.62) and was significantly different from the all-beef control. Deviations from linearity in the soy substituted frankfurters may result from a nonhomogenous mixing of the emulsion by the "chopper" during preparation

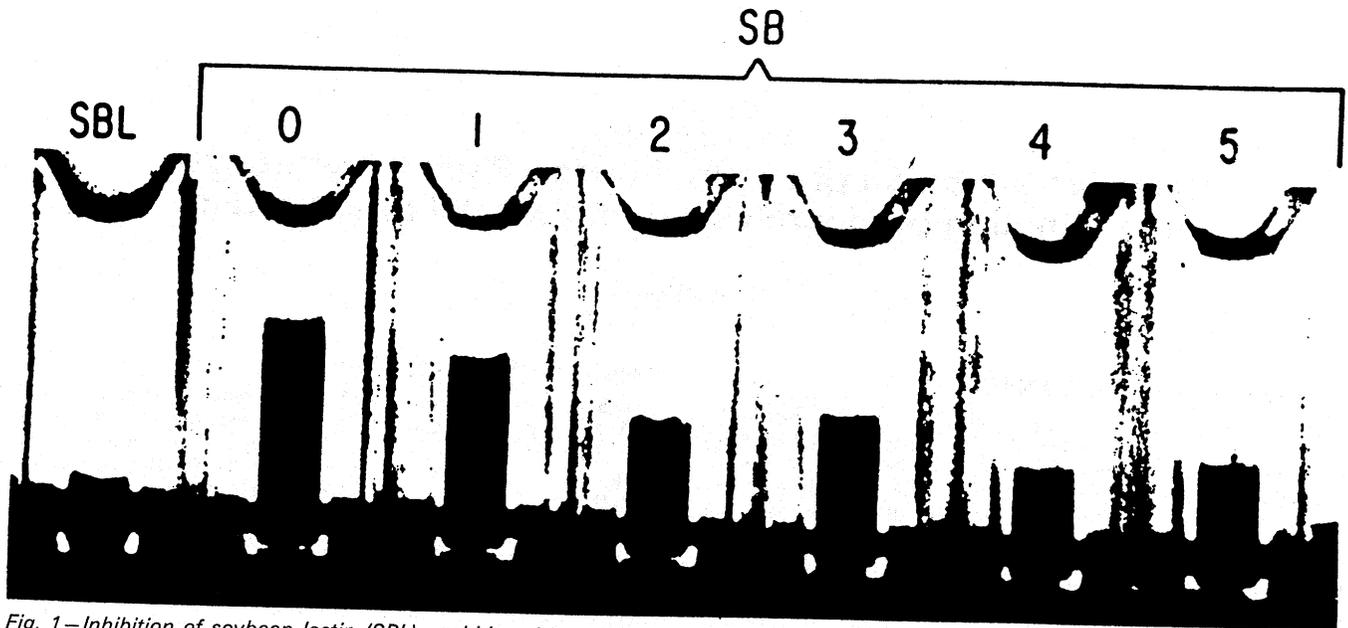


Fig. 1—Inhibition of soybean lectin (SBL) - rabbit red blood cells (RRBC) agglutination by tryptic hydrolysates of soy protein fortified frankfurters. SBL, shows agglutination of lectin and RRBC only. Tryptic hydrolysates containing 0 (all-beef standard), 1, 2, 3, 4, and 5% soy protein isolate are shown in SBO, SB1, SB2, SB3, SB4, and SB5, respectively.

Table 1—Height ratios of agglutinated RRBC's in various mixtures of all-beef frankfurter and nonskeletal beef protein

% Protein substitution	Cooked					
	Pork rind	Pork muscle	Beef tongue	Surimi	Chicken frankfurter	Turkey frankfurter
0	1.00 ± .00 ^a	1.00 ± .00	1.00 ± .00	1.00 ± .0	1.00 ± .00	1.00 ± .00
10	0.80 ± .01 ^b	0.90 ± .04	0.95 ± .03	1.00 ± .01	0.97 ± .01	0.98 ± .02
15	0.68 ± .01	0.50 ± .05	1.00 ± .00	0.87 ± .01	0.97 ± .00	0.95 ± .00
20	—	—	0.82 ± .00	0.91 ± .01	0.95	0.95 ± .00
25	0.56 ± .03	0.58 ± .00	0.57 ± .00	0.66 ± .01	0.97	0.97
30	—	—	—	—	0.79	0.89
50	0.36 ± .03	0.36 ± .03	0.34 ± .03	0.56 ± .04	0.47 ± .00	0.65 ± .03
100	0.16 ± .01	0.33 ± .03	0.25 ± .01	0.30 ± .01	0.33 ± .08	0.26 ± .06

^a Ratios ± standard deviations based on four replicates.

^b Underscored ratios are significantly ($P < 0.05$) different from "0" substituted beef samples.

^c — not analyzed.

of the frankfurters. Non-linear results obtained by the agglutination test agrees with results by ELISA analysis of this same batch of samples (Medina, 1988). ELISA analysis showed that experimentally determined amounts of soy protein in the 3% and 5% were only 85% of the theoretical amounts in the frankfurters. In the agglutination assay, a higher ratio suggests lower amount of non-beef protein additive. The observed ratio in the 3% and 5% soy fortified frankfurter indicates presence of lesser amounts of soy additive compared to the corresponding 2% and 4% samples.

Frankfurters generally contain 12% protein. Protein in an all-beef sausage product was substituted with equivalent amounts of non-meat protein in the range 5–50%. Protein substituted beef samples containing pork rind, pork muscle, chicken, turkey or surimi were mixed and hydrolyzed by trypsin and assayed by RRBC and SBL agglutination procedure. As shown in Table 1, pork rind was detectable in a minimum substitution of 10% with a ratio of 0.68. Substitution with pork muscle, cooked beef tongue (a smooth muscle), surimi, chicken and turkey frankfurters were detectable at 15, 20, 25, 30 and 50% substitution, respectively with ratios of 0.50, 0.82, 0.66, 0.79 and 0.65. These ratios are significantly different from an all-beef control ratio of 1.0. Nonbeef proteins showed minimal interactions between SBL and RRBC.

An all-beef frankfurter containing approximately 12% protein was substituted with 10–50% protein equivalent of the following protein additives: powdered pork rind, pork muscle,

chicken, turkey or surimi. Agglutination assay results showed that pork rind, pork muscle, cooked beef tongue, surimi, chicken and turkey frankfurters were detectable at 10, 15, 20, 25, 30 and 50% substitution, respectively with ratios of 0.80, 0.50, 0.82, 0.66, 0.79 and 0.65. Using Student's t-test, these ratios were significantly ($P < 0.05$) different from an all-beef control ratio of 1.0. Nonskeletal beef proteins (100%) showed minimal interactions between SBL and RRBC as shown by ratios of 0.16 - 0.33. The basis for inhibition by skeletal muscle or myofibrillar proteins may result from carbohydrate ligands which had specific binding to SBL, thus inhibiting SBL induced RRBC agglutination. Such ligands are probably not present in other nonskeletal beef protein additives which had non-inhibitory effect to SBL-RRBC agglutination.

This agglutination assay was applied to 55 different meat products obtained commercially (Medina and Woychik, 1989). Standard deviations of ratios ranged from 0–0.18 when individual samples were analyzed repeatedly (2–10 times). A proposed "cutoff" ratio of 0.70 is also presented wherein an all-beef product may be considered adulterated according to United States legal standards.

CONCLUSION

SBL AND RRBC agglutination was selectively inhibited by hydrolysates of skeletal beef isolate and all-beef frankfurter prepared from lean meat. Presence of nonskeletal beef proteins

SELECTIVE INHIBITION OF LECTIN. . . From page 868

in substituted beef frankfurters diminished this inhibition and were detectable in a range of 10–50% substitution. This study presents a novel method to qualitatively screen presence of protein additives in all-beef products cooked below 100°C. A larger number of protein species used as additives can be detected with this procedure compared to immunochemical assays.

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