



Final Report for:

## **Analysis of Psyllium Fractions**

Submitted to:

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## OBJECTIVE

The objective of this study was to analyze several psyllium fractions by SDS-PAGE and immunoblot analysis.

## METHODS

### Source of Materials

The Procter & Gamble Company provided approximately 11 g of each of the following materials for testing:

Material
1. Standard Psyllium
2. Fraction B
3. Fraction B/C

The following samples of Metamucil were purchased locally either for this study or for earlier psyllium studies:

Purchased	Texture	Flavor	Lot	Exp. Date
09/94	Original	Regular	2014XB05N	01/95
03/95	Original	Orange	4343XB09	11/96
01/02	Original	Regular	(L)1131XA05	03/03
01/02	Original	Orange	(L)1164XA04	05/03
01/02	Smooth	Orange	(L)1261XA10	08/04
01/02	Smooth	Orange, Sugar-free	(L)1277XA00	09/03

Wright State University provided the following materials from stocks previously prepared for P&G:

Material	Identification
1. Psyllium standard	#000914, H <sub>2</sub> O extract stored at -80C
2. Rabbit anti-needle/kernel	#930325 prep; #000306 aliquot
3. Rabbit anti-psyllium	#000815 Pool B (rabbits #331-339)
4. Human anti-psyllium	#931001 Pool from 5 P&G employees

Aqueous extracts of all psyllium and Metamucil samples were prepared by suspending 25 mg powder in 1.00 ml glass-distilled water. Samples were also extracted with "SDS Extraction Buffer" (125 mM Tris, pH 6.8, 4% sodium dodecyl sulfate) at the same concentration. Samples were allowed to extract overnight (16 - 20 hrs) and soluble proteins were collected in the supernatant following centrifugation for 10 min at 14,000 x g. Protein contents of aqueous

samples were determined by the Coomassie Blue dye method of Bradford using a protein assay kit from Bio-Rad. Bovine serum albumin served as the protein standard.

### **Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Immunoblotting.**

Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting were performed under reducing conditions using Bio-Rad mini-gels and our standard protocol. All lanes were loaded with the same volume of extract. Samples and molecular weight markers were first separated on a polyacrylamide gel, using a discontinuous (Laemmli) buffer system at room temperature. Each experiment had 4 identical gels. One gel from each set was stained with silver nitrate to visualize all proteins.

The PAGE-resolved proteins on the other 3 gels of each set were electrophoretically transferred from the separating gels to PVDF membranes and the membranes (blots) were blocked. Two blots from each set were incubated for 5 hours with rabbit antiserum at a dilution of 1:1000 (20  $\mu$ l antibody/20 ml buffer). Antibody binding was detected using peroxidase-labeled anti-rabbit Ig (at 1:1000) for 1 hour followed by chromogenic detection.

The fourth blot in each set was incubated overnight with human serum. The serum was a pool of 7 samples from 5 persons. Previous commercial analysis reported that the serum pool had a total IgE concentration of 528 U/ml and was RAST 3+ to ispaghula (psyllium). Human IgE binding was detected by overnight incubation in  $^{125}$ I-labeled goat-anti-human IgE at 0.8  $\mu$ Ci per blot followed by autoradiography for 1, 4 or 7 days.

Molecular weight marker proteins were run on each gel and blot. Sample protein molecular weights (MWs) were calculated by comparison to those of the markers using Kodak 1-D software. Consensus molecular weights were determined for the allergen proteins and are reported in Table 1. The allergen content in the test samples was also determined using this software.

## RESULTS

### Protein concentrations of the aqueous extracts.

The protein concentrations of the aqueous extracts of the various samples were as follows:

Sample	Protein Conc. ( $\mu\text{g/ml}$ )
Standard psyllium	56
Fraction B	2
Fraction B/C	6
1994 Metamucil, Original texture, Regular flavor	14
2002 Metamucil, Original texture, Regular flavor	26
1995 Metamucil, Original texture, Orange flavor	4
2002 Metamucil, Original texture, Orange flavor	4
2002 Metamucil, Smooth texture, Orange flavor	2
2002 Metamucil, Smooth texture, Orange flavor, Sugar-free	18

It must be noted that the protein concentration of the sugar-free Metamucil is unreliable since this product contains Aspartame, which is a peptide that interferes with this protein assay. Only the protein concentrations of aqueous extracts are reported since SDS also interferes with this assay.

Aqueous extraction released substantially less protein from Fractions B (96% less) and B/C (89% less) than from the Standard Psyllium samples used as a reference.

### Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblot analysis using rabbit antiserum.

A preliminary analysis was performed using 12% acrylamide gels to optimize conditions. These gels were run using psyllium samples extracted in water and in SDS Extraction Buffer to determine if one extractant released more protein from the psyllium matrix. On the silver stained gel (Fig. 1, upper panel), more bands were visible in the lanes containing samples extracted in SDS (lanes 6-9) than were present in lanes with samples extracted in water (lanes 2-5). The same pattern was observed on the immunoblots probed with rabbit antisera (Fig. 1, middle and lower panels).

Since the preliminary experiment showed more protein was extracted with SDS than with water alone, the subsequent experiments were performed using SDS extracts. Additionally, subsequent experiments used gels with smaller pores (acrylamide concentrations of 15% and 8 – 16% gradient) since most of the proteins on the 12% gels were observed on the lower half of the gels with molecular weights < 40 kD.

Samples analyzed on 15% and 8-16% gradient gels were better resolved than those on the 12% gels (Figs. 2, 3). As before, the silver stained gels (upper panels) showed less protein in Fractions B and B/C than in the Standard Psyllium sample. The various lots of Metamucil tested also showed more protein than did the test Fractions. Immunoblots probed with rabbit antiserum

also indicated that less antigenic protein was present in the Fractions (Figs. 2 and 3, middle and lower panels).

**Immunoblot analysis of SDS-PAGE resolved proteins using human serum to identify IgE-binding allergens.**

One blot from each set (12, 15 and 8-16% gels) was incubated in the human serum pool to identify IgE-binding allergens present in the samples (Figs. 4 – 6). All blots were exposed to film for 1, 4 and 7 days to allow the grading of the intensity of IgE binding as strong (first visible at 1 day), moderate (4 days), or weak (7 days). A detailed analysis of these data is presented in Table 1.

On all blots at all times, the Standard Psyllium sample was the darkest lane indicating that this sample contained the largest number and highest amount of allergenic protein (Figs. 4 – 6). Conversely, the Fraction B and B/C lanes showed no IgE binding at 1 day exposure and binding that was clearly less than all other samples at 4 and 7 days exposure indicating that the allergenicity of these samples was significantly less than that of the Standard Psyllium. All samples of Metamucil showed IgE binding that was less than for Standard Psyllium but greater than for the Fractions.

The amount of allergen present in the Fractions was compared to that present in the Standard Psyllium using a computer analysis of the intensity of radiostaining (IgE binding) on the blots. These data indicate that Fraction B contains  $\leq 3.5\%$  of the allergenic protein extractable from an identical sample of Standard Psyllium while Fraction B/C contains  $\leq 4.9\%$  as much allergen.

## CONCLUSIONS

**Fraction B** contains **> 96%** less allergenic protein than does the Standard Psyllium used as a reference. **Fraction B/C** contains **> 95%** less allergenic protein than does Standard Psyllium.