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Recovering Gliadin Protein from Guar Gum using Cellulase

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ABSTRACT

Increased awareness of allergens in the food industry has given Neogen the opportunity to test a wide range of ingredients and final products for specific allergens. With greater experience testing different food stuffs, one category of raw ingredients is especially difficult to accurately test for allergens: gums and stabilizers, which only pose a threat when tested as a raw ingredient.

Issues arise with testing gums, stabilizers, and thickeners during the addition of the extraction solution. These products in raw form are hydroscopic in nature and will absorb reagents during extraction. The solution becomes a sponge-like material and any allergenic protein present is difficult to extract. If the said material is positive for an allergen and makes it into a final product, the only way to confirm the presence of the allergen is to test final product, or the product at a point the stabilizer is not concentrated enough to affect the extraction. A potential solution to this problem is increasing the extraction ratio until the ingredient is not absorbing all the liquid, but this poses the threat of diluting out any present allergenic protein to an undetectable level. The other option is to break down the matrix effect by replacing a portion of the extraction solution with cellulase.

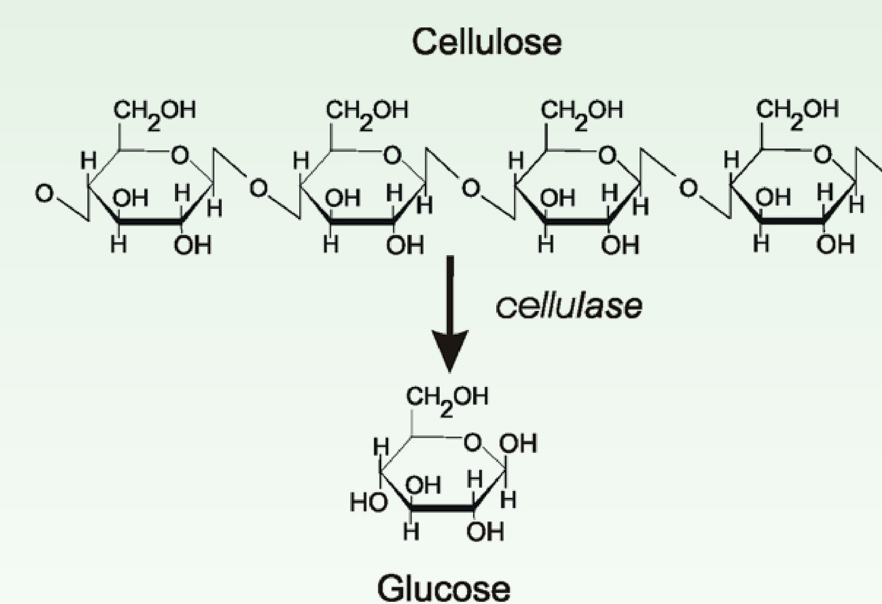
Specifically with gliadin testing, the replacement of a portion of the renaturing solution with a 10% cellulase solution breaks down the absorbance capabilities of the guar gum and allows increased recovery of the gliadin protein. Samples of guar gum either contained gluten naturally or a known level was added to a guar gum negative for gluten. Both types of samples showed an increased gluten recovery when cellulase was added to the extraction.

SPIKE AND RECOVERY TRIALS

Sample	Normal Veratox for Gliadin R5 Procedure (ppm)	20 ppm Gliadin for R5 spike, normal procedure	Average (ppm)	Actual (ppm)	% Recovery	20 ppm Gliadin for R5 spike with cellulase treatment (ppm)	Average (ppm)	Acutal (ppm)	% Recovery (factor in 76%)	% Increase recovery
10% cellulase solution	0.0	15.2 15.3	15.25	15.25	76%					
Guar gum 1 *Derived from guar seeds*	2.6	10.5 10.1	10.30	7.70	39%	18.7 19.1	18.90	16.90	107%	278%
Guar gum 2 *Derived from guar seeds*	11.7	15.2 15.3	15.25	3.55	18%	26.7 26.5	26.60	14.90	98%	550%
Guar gum 3 *Derived from wood/cotton*	0.0	1.0 1.1	1.05	1.05	5%	13.1 12.7	12.90	12.90	85%	1611%
Guar gum 4 *Derived from wood/cotton*	0.0	5.8 6.1	5.95	5.95	30%	14.5 13.4	13.95	13.95	91%	307%
Guar gum 5 *Derived from guar seeds*	0.0	5.0 4.9	4.95	4.95	25%	6.6 6.5	6.55	6.55	43%	174%
Guar gum 6 *Derived from guar seeds*	0.0	2.7 2.4	2.55	2.55	13%	8.3 8.2	8.25	8.25	54%	424%
Guar gum 7 *Derived from guar seeds*	0.0	3.5 3.5	3.50	3.50	18%	3.7 3.4	3.55	3.55	23%	133%
Guar meal	15.8	31.6 32.2	31.9	16.1	81%	41.9 43.9	42.90	27.10	178%	221%
Gum 8 *Derived from guar seeds*	0.0	4.1 3.8	3.95	3.95	20%	7.4 7.3	7.35	7.35	48%	244%

NATURALLY CONTAMINATED

Sample	Naturally contaminated (ppm)	Cellulase treatment (ppm)	% Increased recovery
Guar meal	2.9 2.7	7.6 7.2	264%
Guar gum 1	2.5 2.7	4.0 4.0	154%
Guar gum 2	11.8 11.6	12.1 12.2	103%



OBSERVATIONS

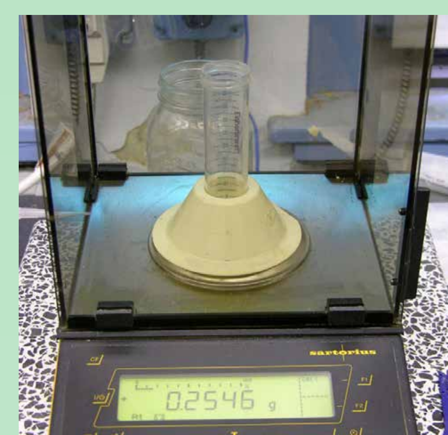
The challenge of testing guar gums, and most other gums, with the normal Veratox for Gliadin R5 extraction procedure is the immediate absorbance of 2.5 mL of Gliadin Renaturing Cocktail Solution. The matrix becomes a thick and spongy during the first incubation, decreasing the efficiency of extracting any gliadin present. This first step of the extraction was the critical point needed to break down the guar gum to increase extraction efficiency.

A 10% cellulase concentration was optimal because all existing procedural steps and equipment were used. A portion of the Gliadin Renaturing Cocktail Solution was replaced with the cellulase to preserve the end dilution factor of the kit. After the 40 minute incubation at 50°C, the solution was liquid enough to pipette, enabling the cocktail solution more access to any gliadin present.

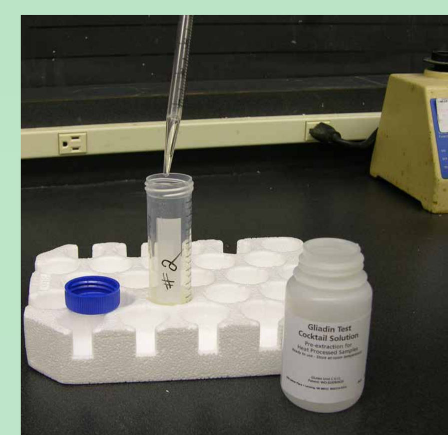
CONCLUSIONS

All samples that were subject to cellulase treatment during the first incubation of the extraction protocol showed increased gliadin recovery. Since there is no analytical method to confirm actual levels of gliadin in naturally-contaminated guar gum, an increased recovery of gliadin was evidence of success of the extraction process. This protocol provides the allergen testing world a tool to combat the difficulties of gums.

VERATOX GLIADIN R5 EXTRACTION PROCEDURE WITH ADDITION OF CELLULASE

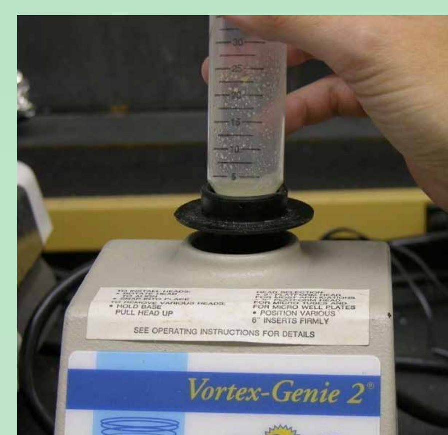


1. Weigh 0.25 g of guar gum powder in 50 cc conical tube.



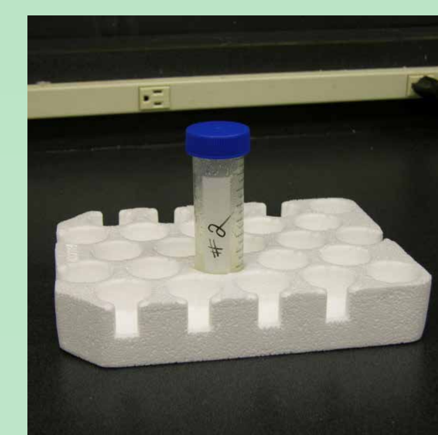
2. Add 2.0 mL of Gliadin Renaturing Cocktail Solution.

3. Add 500ul of 10% Cellulase solution



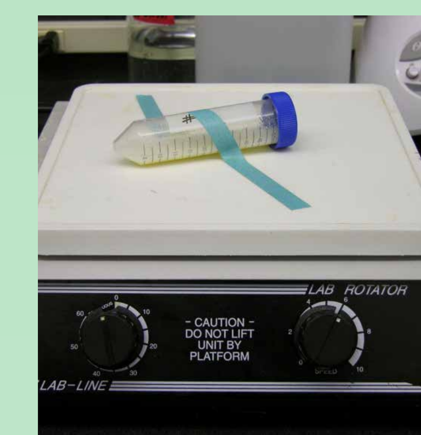
4. Vortex 30 seconds to homogenize cocktail and sample.

5. Incubate 40 minutes at 50°C in water bath or oven.



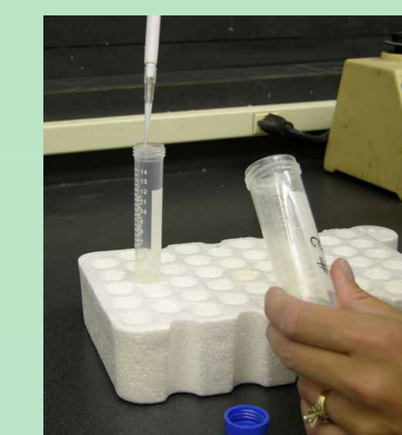
6. Remove and let cool 5–10 minutes.

7. Add 7.5 mL of 80% ethanol and vortex.



8. Shake (150–200 rpm) for 1 hour at room temperature on rotator.

9. Dilute 1:12.5 by adding 2.3 mL Phosphate Buffered Saline (PBS) extract dilution solution to a clean tube.



10. Add 200 uL of the clear sample extract from the top of the sample extract to the dilution tube. Sample is now ready to be tested.