



Special Report: Gluten Contamination of Spices

Gluten Free Watchdog, LLC

Note: This report was originally published for subscribers to Gluten Free Watchdog. It has been modified for public dissemination. Manufacturer names and other identifying information have been removed and are available only to subscribers of Gluten Free Watchdog. If you wish to have access to the full report you must subscribe.

Single ingredient spices have long been considered naturally gluten-free. But a recently released report on gluten in ground spices from the Canadian Food Inspection Agency has called into question whether spices may be contaminated with wheat, barley, and/or rye.

Definition of Spice

The Food and Drug Administration defines spice as “any aromatic vegetable substance in the whole, broken, or ground form...whose significant function in food is seasoning rather than nutritional.” What some people think of as herbs, such as thyme and sage because they come from the leafy portion of the plant are included in the FDA’s definition of spice as are “spices” that come from the other portions of the plant (e.g., roots, flowers, and seeds), such as cumin and clove. _

Canadian Food Inspection Agency Report: Gluten in Ground Spices

The CFIA tested 268 samples of ground spices. 23 samples were domestically processed and 245 were imported. CFIA defines domestically processed spices as including spices being ground and/or packaged in Canada. According to correspondence Gluten Free Watchdog had with CFIA, samples were tested using the Ridascreen Gliadin R7001 assay

and extracted with the cocktail solution R7006 and the addition of milk powder (there will be more information about the use of milk powder later on in this report).

Spices tested by CFIA were allspice, anise, black pepper, cardamon, cayenne, cinnamon, clove, coriander, cumin, fenugreek, ginger, mace, marjoram, mustard, nutmeg, oregano, paprika, sage, thyme, turmeric, and white pepper. 63 (24%) of the 268 samples tested contained quantifiable levels of gluten ranging from 5 parts per million (ppm) to 20,000 ppm. The Ridascreen Gliadin assay has a lower limit of quantification of 5 ppm of gluten. Of the 63 positive samples, 5 were domestically processed and 58 were imported. This means that 22% of the domestic spices tested and 24% of the imported spices tested contained quantifiable gluten.

Of the 5 domestically processed spices containing quantifiable gluten, 3 contained greater than or equal to 20 ppm and would not be considered gluten-free by US standards. They were all coriander. The other two domestic spices containing quantifiable gluten below 20 ppm gluten were coriander and clove.

Of the 63 spices testing positive for gluten, 25 contained \geq to 20 ppm gluten. These spices were clove (4 samples; 24 to 590 ppm gluten), coriander (4 samples; 33 to 260 ppm gluten), cumin (7 samples; 31 to 49 ppm gluten), fenugreek (2 samples; 20 to 39 ppm gluten), mace (2 samples; 83 to 20,000 ppm gluten), sage (1 sample; 21 ppm gluten), thyme (4 samples; 20 to 26 ppm gluten), and white pepper (1 sample; 32 ppm gluten). The other spices testing positive for gluten contained quantifiable gluten below 20 ppm and would be considered gluten-free under US standards.

Note: The imported spice (mace) containing 3,000 to 20,000 ppm gluten almost certainly was cut with wheat flour. If this was the case, the ingredients list should have included wheat flour.

For more information on this report, please see <http://www.inspection.gc.ca/food/chemical-residues-microbiology/chemical-residues/ground-spices/eng/1347987900293/1347988112489>.

Gluten Free Watchdog Testing of Spices

Gluten Free Watchdog tested brands of domestically processed spices that appear to be the most widely used by the gluten-free community based on inquiries made to subscribers of Gluten Free Watchdog.

Gluten Free Watchdog focused primarily on testing the spices that tested at or above 20 ppm in the Canadian survey. These spices (all ground) are clove, coriander, cumin, fenugreek, mace, sage, thyme, and white pepper. Gluten Free Watchdog also tested leaf basil (dried), garlic powder, curry powder, and Italian seasoning containing only leaf spices (dried). In addition we tested pepper packets collected from the lunches served at the International Celiac Disease Symposium. It was the expectation of Gluten Free Watchdog that all domestically processed spices would test gluten-free.

Spices were purchased at retail establishments, including online merchants and sent unopened to the food testing facility Bia Diagnostics in Burlington, Vermont. Spices were tested in duplicate (2 extractions) using the Ridascreen Gliadin R7001 assay and various extraction solutions. The official results used the cocktail extraction R7006. Results comparing a variety of extraction solutions are provided further on in this document beginning on page 9.



Gluten Free Watchdog Report: Gluten Analysis of Spices

Test dates: August 2013 to November 2013

Assay used: R-Biopharm Sandwich R5 ELISA (R7001) with cocktail extraction (Art. No. R7006)

Lower Limit of Quantification for this assay: 5 parts per million (ppm) of gluten

Testing Facility: Bia Diagnostics, LLC, Burlington, Vermont

Spice	Brand	Number of Extractions (ppm gluten)	Labeled Gluten-Free
Cloves Ground	Removed	2 extractions (<5, <5)	No
Coriander Ground	Removed	2 extractions (10, 7)	No
Coriander Ground	Removed	2 extractions (19, 18)	Yes, Certified
Cumin Ground	Removed	2 extractions (<5, <5)	No

Cumin Ground	Removed	2 extractions (<5, <5)	No
Curry Powder	Removed	2 extractions (<5, <5)	No
Fenugreek	Removed	2 extractions (9, 15)	No
Fenugreek	Removed	2 extractions (38, 37)*	Yes, Certified
Garlic Powder	Removed	2 extractions (<5, <5)	No
Italian Seasoning	Removed	2 extractions (<5, <5)	No
Mace Ground	Removed	2 extractions (<5, <5)	No
Pepper**	Removed	1 extraction (<5)	No
Sage	Removed	2 extractions (8, 7)	No
Sweet Basil	Removed	2 extractions (<5, <5)	No
Thyme Ground	Removed	2 extractions (5, 14)	No
White Pepper Ground	Removed	2 extractions (<5, <5)	No

*Original sample tested was highly heterogeneous. Sample was homogenized further and retested.

**Pepper packets were collected from the lunches served at ICDS2013. Unfortunately only enough was collected to test one extraction.

© 2013 Gluten Free Watchdog, LLC. All Rights Reserved.

Test results may be used for personal use only. Results may not be reposted, reprinted, republished, or paraphrased without the express written permission of Gluten Free Watchdog, LLC

Test results provide a snapshot picture of the gluten content of these products at one point in time. There is no way of knowing without testing many more samples whether the results of the samples tested are representative of the gluten content of these products as a whole.

Discussion of Results

For the most part, spices assessed by Gluten Free Watchdog tested gluten-free (defined as less than 20 ppm). The results for one brand of fenugreek and coriander may be concerning to some of you because products from this

company are certified gluten-free. **Nonetheless, it is important to keep in mind that a spice containing 20 ppm gluten is very different from a bread, pasta, or cereal containing 20 ppm gluten.** Each one ounce serving of a product containing 20 ppm gluten contains 0.57 milligrams of gluten. When you eat bread, etc you are likely eating at least one ounce of product at one sitting. When you use a spice you do not use anywhere close to that amount at one sitting. A typical bottle of spice purchased at a grocery store contains 0.5 to 2 ounces of product. Think about how long it takes you to use the entire bottle of spice. Also think about the amount of spice called for in a recipe and how many servings that recipe provides. The bottom line is that the amount of gluten you would ingest if you ate any of the spices tested by Gluten Free Watchdog would be very low. **In general, these results are not cause for undue alarm.**

Recommendations for Buying Spices

1. Purchase spices from well-known domestically located companies.
2. Contact the manufacturer and ask what steps they take to make sure their spices do not come into contact with wheat, barley, or rye in the processing plant and before the spice arrives at the plant.
3. Remember that even if the processing plant does not have gluten-containing grain on the premises this does NOT mean that the spice is free of gluten. The spice may come into the plant already contaminated with wheat, barley, or rye. **The spice may not get any dirtier in the plant but it won't get any cleaner either.**
4. If a manufacturer states that they test their spices for gluten, ask what test they use. Because of the complexities of testing spices (see the section on extraction solutions below), manufacturers should be using a fully validated ELISA assay, preferably the Ridascreen Gliadin R5 ELISA. They should not be using lateral flow devices (dip stick test similar to a pregnancy test).

Extraction Solutions

In addition to testing spices using the cocktail solution, Gluten Free Watchdog also tested spices using two additional extraction solutions. When a food is tested for gluten, gluten first has to be detected and then it has to be extracted so it can be measured. There are several extraction solutions that are used with the various assays that detect gluten in food. These include the Gliadin Extraction Buffer (GEB) typically used with assays that assess the omega-gliadin protein, the Cocktail (Mendez) extraction solution used with the R5 ELISA, various concentrations of ethanol, and various combinations of alcohol and other components, including fish gelatin.

Before proceeding further, it is important to note that the omega-gliadin (Skerritt) ELISA was validated using a 40% ethanol extraction. The R5 ELISA was validated using a cocktail extraction—a mixture of 250 mM 2-mercaptoethanol and 2 M guanidine hydrochloride to which ethanol is eventually added.

From the perspective of a lay person, using these assays and extractions to assess foods for gluten sounds “easy” enough for those who know how to perform these tests but here is the kicker... scientists have known for years that the various constituents in food and combinations of ingredients in these foods can impact how much gluten an extraction solution is able to extract. These effects are termed “matrix effects.”

According to Herbert Weiser writing in the book *Gluten-Free Cereal Products and Beverages*, “Matrix effects caused by different constituents of the sample can affect extraction yield and thus, the results of gluten determination. For example, binding to polyphenols such as those from tea, hops, and cocoa products decreases the yield of prolamins. Addition of casein, urea, or gelatine to the extractant is recommended to avoid underestimation of the prolamin content.”

Jupiter Yeung of the Food Products Association wrote in a powerpoint presentation to the Food and Drug Administration that, “Samples with high polyphenols, tannins and syrups require fish gelatin to improve recovery.”

And finally, a recent article by Terry Koerner et al in the *Journal of AOAC International*, states, “Ideally, methods would be able to analyze all matrixes with equal reliability, but a method that is fit-for-purpose in one, or even several, matrixes may not be applicable in others. Gluten ELISA methods can be susceptible to matrix effects and have diminished performance due to interferences in some matrixes. This could be due to tannins in a sample or a certain degree of hydrolysis in some processes.”

So what we are seeing now from test kit manufacturers are extraction solutions where fish gelatin is being added to ethanol or the Gliadin Extraction Buffer--ethanol with fish gelatin and PVP--are being used, especially with assays that still utilize the omega-gliadin antibody. We are also seeing recommendations from test kit manufacturers to add skim milk powder to the cocktail extraction solution when foods containing polyphenols are tested for gluten.

Specifically, R-biopharm recommends that for tannin and polyphenol containing food samples (e.g., chocolate, coffee, cocoa, chestnut flour, buckwheat, millet and **spices**) skim milk powder be added to the cocktail.

The problem for manufacturers and food testing facilities is that there are many categories of polyphenols and they are found in many foods—the top 100 food sources (obviously far more than listed by R-Biopharm) are listed at http://www.nature.com/ejcn/journal/v64/n3s/fig_tab/ejcn2010221t1.html

All of this information brings several issues and questions to mind:

1. Has the cocktail extraction with the addition of skim milk powder ever been formally validated on foods containing polyphenols? And if so, on which foods?
2. Food may arrive at a lab without an ingredient list so the lab has no idea about the composition of the food they are testing. How are they to make a determination regarding which extraction solution to use?
3. Do we know enough about polyphenols and foods containing polyphenols to know when to use skim milk powder when using the cocktail as recommended by R-Biopharm?
4. The cocktail extraction solution was originally assessed against a 60% ethanol extraction. Has the cocktail ever been formally assessed against other extractions, such as those containing fish gelatin or urea?
5. Is it the case that a variety of extractions should be used with the R5 ELISA depending upon the food matrix?
6. Should labs be testing food matrices to determine which extraction solution works best for any given product?

Now back to spices...

As you just read, spices contain polyphenols. While we first tested spices through Gluten Free Watchdog using the cocktail extraction solution because it is the formally validated method, we also tested most samples using the cocktail plus skim milk powder and the GEB (which includes fish gelatin).

The results of this testing are posted below beginning on page 9. For the most part, spices that tested gluten-free using the R5 ELISA and cocktail extraction also tested gluten-free when the cocktail extraction plus skim milk powder was used. Sometimes the results were a bit higher using skim milk powder and sometimes they were a bit lower. The one exception

was sage—the results were higher using the cocktail plus skim milk powder. You will also note that results were higher on fenugreek when the GEB extraction solution was used.

In my opinion there are no conclusions to be drawn from these results in terms of extraction solutions that should be used to test spices. It seems apparent that a lot more investigation needs to be done to determine the best extraction solutions given the variety of gluten-free foods now available and all the different food matrices. The fact that the amount of gluten extracted from a sample may be decreased due to matrix effects and use of a less than ideal extraction solution suggests that the threshold level for gluten in food should be as low as possible.

Property of
Gluten Free Watchdog



Gluten Free Watchdog Report: Gluten Analysis of Spices Using Multiple Extraction Solutions

Test dates: August 2013 to November 2013

Assay used: R-Biopharm Sandwich R5 ELISA (R7001) with cocktail extraction (Art. No. R7006, official R5 Mendez method) OR cocktail extraction with the addition of skim milk powder (CKTL/Milk), OR GEB extraction

Lower Limit of Quantification for this assay: 5 parts per million (ppm) of gluten

Testing Facility: Bia Diagnostics, LLC, Burlington, Vermont

Spice	Brand	Extraction	Results (2 extractions)	Labeled Gluten-Free?
Cloves Ground	Removed	Cocktail GEB	< 5, < 5 < 5, < 5	No
Coriander Ground*	Removed	Cocktail GEB	10, 7 13, 15	No
		Cocktail CKTL/Milk	8, 12 7, 7	
Coriander Ground*	Removed	Cocktail GEB	19, 18 62, 59	Yes, Certified
		Cocktail CKTL/Milk	23, 27 30, 31	

Spice	Brand	Extraction	Results (2 extractions)	Labeled Gluten-Free?
Cumin Ground	Removed	Cocktail GEB	< 5, < 5 < 5, < 5	No
Cumin Ground	Removed	Cocktail CKTL/Milk GEB	<5, <5 <5, <5 <5, <5	No
Curry Powder	Removed	Cocktail CKTL/Milk GEB	<5, <5 <5, <5 <5, <5	No
Fenugreek Ground	Removed	Cocktail CKTL/Milk GEB	9, 15 11, 8 33, 20	No
Fenugreek Ground**	Removed	Cocktail CKTL/Milk GEB	38, 37 29, 23 57, 44	Yes, Certified
Garlic Powder	Removed	Cocktail GEB	< 5, < 5 < 5, < 5	No

Spice	Brand	Extraction	Results (2 extractions)	Labeled Gluten-Free?
Italian Seasoning	Removed	Cocktail CKTL/Milk GEB	<5, <5 <5, <5 <5, <5	No
Mace Ground	Removed	Cocktail CKTL/Milk GEB	< 5, < 5 < 5, 6 7, 7	No
Pepper***	Removed	Cocktail	<5	No
Sage Ground*	Removed	Cocktail GEB Cocktail CKTL/Milk	8, 7 18, 18 7, < 5 15, 21	No
Sweet Basil	Removed	Cocktail GEB	< 5, < 5 < 5, < 5	No

Spice	Brand	Extraction	Results (2 extractions)	Labeled Gluten-Free?
Thyme Ground	Removed	Cocktail	5, 14	No
		CKTL/Milk	8, 14	
		GEB	8, 11	
White Pepper	Removed	Cocktail	< 5, < 5	No
		GEB	< 5, < 5	

*Two rounds of testing on two different dates using two different homogenized samples were conducted on the coriander and sage products first testing with the cocktail and GEB extraction solutions and then with the cocktail and cocktail plus skim milk powder extraction solutions.

** Results of original testing on the fenugreek were very heterogeneous. The sample was further homogenized and retested. Only the homogenized results are included.

***Pepper packets were collected from the lunches served at ICDS2013. Unfortunately not enough was collected to test two extractions.

© 2013 Gluten Free Watchdog, LLC All Rights Reserved.

Test results may be used for personal use only. Results may not be reposted, reprinted, republished, or paraphrased without the express written permission of Gluten Free Watchdog, LLC

Test results provide a snapshot picture of the gluten content of these products at one point in time. There is no way of knowing without testing many more samples whether the results of the samples tested are representative of the gluten content of these products as a whole.

Acknowledgements

Thank you to Bia Diagnostics, LLC for all of the testing performed on these spices and the due diligence spent to provide homogenized samples for testing. At times, this was not an easy process.

Thank you to my Canadian colleague Shelley Case, RD for alerting me to the testing on spices conducted in Canada by CFIA.

Thank you to all the supporters of Gluten Free Watchdog who encouraged the testing of spices and put up with week after week of spice reports. We could not do this important work without your help.

Sources

Iowa State University. Herbs versus Spices. Available at:
<http://www.ipm.iastate.edu/ipm/hortnews/2003/8-22-2003/herbsnspices.html>

Gluten-Free Cereal Products and Beverages. Edited by: Elke K. Arendt and Fabio Dal Bello. Available at:
<http://www.sciencedirect.com/science/book/9780123737397>

Garcia E, et al. Development of a general procedure for complete extraction of gliadins for heat processed and unheated foods. Eur J Gastroenterol Hepatol. 2005 May;17(5):529-39. Abstract available at:
<http://www.ncbi.nlm.nih.gov/pubmed/15827444>

R-Biopharm News. January 2012. Available at:
<http://www.r-biopharm.com/wp-content/uploads/news/r-biopharm-news/r-biopharmnews-i2012-5-16388/RBN-I-2012-engl.pdf>

Yeung, Jupiter. Commercial Gluten Test Kits/Methods. FDA Gluten-Free Meeting. Powerpoint available at: www.fda.gov/downloads/Food/GuidanceRegulation/UCM205938.ppt

Koerner, et al. Validation Procedures for Quantitative Gluten ELISA Methods: AOAC Allergen Community Guidance and Best Practices. Journal AOAC International. Volume 96, Number 5, September-October 2013, pp. 1033-1040(8).

© 2013 Gluten Free Watchdog, LLC All Rights Reserved. No portion of this report may be reprinted or reposted without the express written permission of Tricia Thompson.

Property of
Gluten Free Watchdog