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Bureau of Chemical Safety
Food Directorate

Bureau d'innocuité des produits chimiques
Direction des aliments

R-Biopharm RIDASCREEN® FAST Hazelnut: Performance Evaluation

2009

Our Mission
To ensure chemicals are not present in foods at levels that may cause adverse health effects to Canadians.

Notre Mission
Veiller à ce que les produits chimiques ne soient pas présents dans les aliments à des niveaux pouvant entraîner des effets néfastes sur la santé des canadiennes et des canadiens.

Canada

R-Biopharm RIDASCREEN® FAST Hazelnut: Performance Evaluation

Disclaimer: Inclusion of this method in the compendium does not imply endorsement or approval by Health Canada.

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Introduction

The purpose of this study was to generate performance data on the R-Biopharm RIDASCREEN® FAST Hazelnut test kit for the detection of hazelnut, one of the priority food allergens in Canada, as part of ongoing efforts to evaluate food allergen detection methodologies and further their introduction into the Compendium of Methodologies. A representative roasted hazelnut powder was chosen to be the designated reference material, according to the definition established by the AMC.

This evaluation involved the analysis of selected food matrices, which had been artificially fortified (spiked samples) with the roasted hazelnut powder.

Method Evaluated:

The R-Biopharm RIDASCREEN® FAST Hazelnut. Product Code R6802

Evaluation Level:

Full evaluation under the guidelines developed for the Compendium of Food Allergen Methodologies.

Designated Reference Material:

Roasted hazelnut powder obtained from the Food Allergen Research and Resource Program (FARRP) at the University of Nebraska was used as reference material. This hazelnut powder was a blend of several commercial varieties of hazelnut and had been processed using a freezer mill to a fine powder.

Participating Laboratories:

Health Canada Food Allergen Research Lab
Banting Building,
Ottawa, ON

Health Canada Western Regional Laboratory
Burnaby, BC

CFIA Quebec Regional Laboratory
Longueuil, QC

CFIA Western Regional Laboratory
Burnaby, BC

Spiking Levels:

Samples were spiked at 0, 5.0 and 12.5 ppm of hazelnut powder, which was intended to give a response of 0, 5 and 12.5 ppm on the hazelnut kit. This conforms with the evaluation guidelines of the compendium, which call for spiking levels that give a response from the kit of approximately 0, 2 and 5 times the limit of quantitation (LOQ) when applied to the designated reference material.

Spiking Conditions:

Spiking was done using a 1 mg/g suspension of the hazelnut powder in a CMC solution based on a method by Trucksess et al, *Preparation of Peanut Butter Suspension for Determination of Peanuts Using Enzyme-Linked Immunoassay Kits,* Journal of AOAC International, vol.87, 2, 2004. Samples were left blank, or spiked at one of two levels and given a blind identification code.

Matrices of Interest:

Three different matrices were included in the evaluation: cookies, milk chocolate and dark chocolate. These commodities were included as representatives of some of the matrices, which could likely contain undeclared hazelnut proteins. The potential for cross contamination with hazelnut products at low levels exists when commodities that do not contain hazelnuts as an ingredient are manufactured on the same equipment as commodities that do contain hazelnut.

Materials and Resources

Each participating lab was provided with enough R-Biopharm RIDASCREEN® FAST Hazelnut kits and blind-coded samples (30 of each matrix – 90 samples in total)

Procedure

Spiking Procedure:

The spiking levels were chosen in order to obtain a response of approximately 2 and 5 times the LOQ from the R-Biopharm RIDASCREEN® FAST Hazelnut kit. The R-Biopharm hazelnut kit uses a different standard for its calibration curve than the hazelnut powder used for spiking. However, the two standards behaved very similarly on the kit and therefore no correlation factor was needed. For example, when 5 ppm of the roasted hazelnut powder was spiked in a sample, the sample gave a response of 5 ppm with the R-Biopharm hazelnut kit.

Samples were spiked using a suspension of the roasted hazelnut powder in a solution of CMC (carboxymethylcellulose) with BSA and Thimerosal added. The stock hazelnut powder suspension in CMC was prepared at a concentration of 1 mg/g. The stock hazelnut suspension was diluted in PBS to give a spiking solution with a concentration of 25.3 µg/ml. For the 5 ppm spikes, 197.6 µl of the spiking solution and 300 µl of PBS were added to each sample. For the 12.5 ppm spikes, 494.0 µl of the spiking solution was added to each sample. Blank samples were spiked with 500µl of a solution made by diluting a blank CMC suspension in PBS.

In an effort to confirm the appropriate correlation factor had been used, eight empty bottles were spiked at each of the non-zero spiking levels at the same time as the samples were prepared, using the same spiking solution and spiking volumes as the actual samples. These empty bottles were then treated as samples, extracted and analyzed by each of the laboratories involved in the study.

Preparation of Samples:

The 360 required samples (10 replicates at each of 3 levels in 3 commodities for 4 laboratories) were prepared at the Health Canada's Food Allergen Research Laboratory in Ottawa. Samples (1g) were weighed into 250 ml screw cap bottles (120 samples for each commodity). The samples were separated into groups of 40, then each group spiked at one of the three spiking levels. Each sample was given a code number and then the samples were grouped together for each of the four participating laboratories and shipped by courier.

Sample Extraction and Analysis:

Each laboratory extracted the samples following the procedure outlined in the R-Biopharm RIDASCREEN® FAST Hazelnut kit instruction. The extraction was performed directly in the sample bottles provided. The sample extracts were then analyzed using the R-Biopharm RIDASCREEN® FAST Hazelnut kit, following the instructions provided in the kit insert.

Results / Discussion

The results for the 360 samples in the study showed good inter and intra laboratory consistency. A small number of false positives (4 out of 120 blanks) were reported and three false negatives were reported out of the 120 lower level spiked samples. The positive samples would be confirmed by a second extraction and any false positive results would likely be detected. The three false negative samples were below the LOQ cut-off (2.5ppm), but had OD absorbance values considerably higher than those obtained for the blank sample, which indicates the presence of hazelnut protein, so analysis of these

samples would typically be repeated. These three samples were not included in any further analysis of the data. The remaining data was analyzed for outliers using the Grubb's test at a 95% confidence level resulting in no outliers.

The Z score for an item indicates how far, and in what direction, that item deviates from its distribution's mean, expressed in units of its distribution's standard deviation. A Z-score of 2.0 indicates a result that was two standard deviations above the mean, while a score of -2.0 would indicate a result two standard deviations below the mean. Z-scores were calculated for each lab at each commodity and spiking level. None of the results were above 2.0 and the majority were below 1.0, which shows a good deal of agreement between all the participating labs.

A summary of the results from each of the four labs is presented here.

As mentioned earlier, the roasted hazelnut powder behaved very similar to the hazelnut standard used as a calibrator for this kit in the preliminary evaluation. For this reason no correlation factor was thought to be required. In fact, when the matrix free spikes (empty bottles) were analyzed the average value for the lower level spike sample was 3.8 ppm (instead of 5 ppm) and the average value for the higher level spike sample was 9.8 ppm (instead of 12.5 ppm). Based on these results the expected value was adjusted to 3.8 ppm for the 5.0 ppm spikes and 9.8 ppm for the 12.5 ppm spikes.

Conclusion

The R-Biopharm RIDASCREEN® FAST Hazelnut kit has delivered satisfactory results for the matrices and at the levels tested in this evaluation. The cookie samples gave the highest recoveries with 153% for the low level spiked samples and 108% for the higher spiking level. The chocolate recoveries were quite good, with average milk chocolate recoveries at 97% and average dark chocolate recoveries between 83% and 105% for the upper and lower spiking levels respectively.

The data from this evaluation, based on spiking samples with the roasted hazelnut powder from FARRP, would be complemented by other data based on "naturally incurred" samples which were manufactured using a known amount of hazelnut. Data from any future studies involving these kinds of samples will be added to this report as it becomes available, in order to further document the performance of this commercial test kit.

Table 1 – Summary of Results

Cookie Results

	Cookie 0 ppm*			Cookie 3.8 ppm			Cookie 9.8 ppm		
	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD
Lab 1	0.0-1.7	1.4	0.5	4.9-7.3	6.3	0.8	9.5-14.0	11.4	1.5
Lab 2	1.0-2.2	1.6	0.5	3.0-6.2	4.8	1.0	7.3-12.1	9.2	1.3
Lab 3	0.1-3.4	1.7	1.0	3.9-6.8	5.6	0.9	4.7-10.3	8.6	1.9
Lab 4	1.1-2.1	1.6	0.3	5.9-7.7	6.6	0.6	12.1-13.7	13.0	0.6

Milk Chocolate Results

	Milk Chocolate 0 ppm*			Milk Chocolate 3.8 ppm			Milk Chocolate 9.8 ppm		
	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD
Lab 1	0.0-2.1	0.68	0.89	2.8-3.6	3.1	0.3	8.8-10.5	9.7	0.5
Lab 2	0.0-2.8	1.2	1.1	3.1-6.5	4.3	1.2	6.5-9.9	8.2	1.2
Lab 3	0.0-1.9	1.1	0.5	2.5-5.0	3.3	0.7	6.2-13.7	9.6	3.0
Lab 4	0.0-1.5	0.5	0.6	3.7-5.2	4.3	0.6	9.6-11.0	10.5	0.4

Dark Chocolate Results

	Dark Chocolate 0 ppm*			Dark Chocolate 3.8 ppm			Dark Chocolate 9.8 ppm		
	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD
Lab 1	0.0-1.8	0.2	0.6	3.1-4.3	3.8	0.4	7.6-10.9	8.9	0.9
Lab 2	0.0-1.9	1.0	0.7	2.7-5.4	3.9	0.7	5.9-8.5	7.2	0.8
Lab 3	0.0-1.6	0.8	0.6	3.0-5.1	4.5	0.7	5.9-9.1	7.8	1.0
Lab 4	0.6-1.5	1.0	0.3	3.3-5.4	3.8	0.4	7.4-9.6	8.7	0.7

Results of all Labs

	0 ppm Level*			3.8 ppm Level			9.8 ppm Level		
	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD
Cookie	0.0-3.4	1.6	0.6	3.0-7.7	5.8	1.1	4.7-14.0	10.6	2.2
Milk Chocolate	0.0-2.8	0.9	0.8	2.5-6.5	3.7	0.9	6.2-13.7	9.5	1.8
Dark Chocolate	0.0-1.9	0.7	0.6	2.7-5.4	4.0	0.6	5.9-10.9	8.1	1.1

Z-Scores for the participating labs**

	Cookie			Milk Chocolate			Dark Chocolate		
	1.6 ppm	5.8 ppm	10.6 ppm	0.9 ppm	3.7 ppm	9.5 ppm	0.7 ppm	4.0 ppm	8.1 ppm
Lab 1	-0.3	0.5	0.4	-0.3	-0.6	0.1	-0.8	-0.3	0.7
Lab 2	0.0	-0.9	-0.6	0.4	0.7	-0.7	0.5	-0.2	-0.8
Lab 3	0.2	-0.2	-0.9	0.3	-0.4	0.1	0.2	0.8	-0.3
Lab 4	0.0	0.7	1.1	-0.5	0.7	0.6	0.5	-0.3	0.5

* The levels are referenced to the kit calibrators and have been normalized to the reference material for this study.

** The Z-scores are based on the level of hazelnut protein determined by the labs rather than the theoretical spiking level.