

False Positive Detection of Peanut Residue in Liquid Caramel Coloring Using Commercial ELISA Kits

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Abstract: Initial food industry testing in our laboratory using enzyme-linked immunosorbent assay (ELISA) methods indicated that the darkest caramel color (class IV) unexpectedly contained traces of peanut protein, a potential undeclared allergen issue. Caramel production centers on the heating of sugars, often glucose, under controlled heat and chemical processing conditions with other ingredients including ammonia, sulfite, and/or alkali salts. These ingredients should not contain any traces of peanut residue. We sought to determine the reliability of commercially available peanut allergen ELISA methods for detection of apparent peanut residue in caramel coloring. Caramel color samples of classes I, II, III, and IV were obtained from 2 commercial suppliers and tested using 6 commercially available quantitative and qualitative peanut ELISA kits. Five lots of class IV caramel color were spiked with a known concentration of peanut protein from light roasted peanut flour to assess recovery of peanut residue using a spike and recovery protocol with either 15 ppm or 100 ppm peanut protein on a kit-specific basis. A false positive detection of peanut protein was found in class IV caramel colors with a range of 1.2 to 17.6 parts per million recovered in both spiked and unspiked liquid caramel color samples. ELISA kit spike/recovery results indicate that false negative results might also be obtained if peanut contamination were ever to actually exist in class IV caramel color. Manufacturers of peanut-free products often test all ingredients for peanut allergen residues using commercial ELISA kits. ELISA methods are not reliable for the detection of peanut in class IV caramel ingredients and their use is not recommended with this matrix.

Keywords: caramel, coloring, ELISA, food allergy, peanut allergy

Introduction

Food allergies represent a serious public health issue for food manufacturers. Recognizing that 3.5% to 4% of the U.S. population had food allergies (Sicherer and others 2004; Rona and others 2007; Sicherer and others 2010), the U.S. Congress passed the Food Allergen Labeling and Consumer Protection Act (FALCPA) that requires companies to clearly label products containing ingredients derived from 8 commonly allergenic foods or food groups. These major allergenic foods are milk, eggs, fish, crustacean shellfish, tree nuts, peanuts, wheat, and soybeans, and account for approximately 90% of all food-allergic reactions (Bousquet and others 1998). Peanut allergy is among the most prevalent and severe of the food allergies affecting an estimated 0.75% of the U.S. population (Sicherer and others 2010). Peanut allergy also accounts for many severe reactions and is the leading cause of food allergy-related deaths (Bock and others 2007).

In many food-manufacturing operations, cross contact between commonly allergenic ingredients, such as peanut, and other formulations can easily occur through many sources, including inadequate cleaning of shared equipment, inappropriate use of rework, improper storage conditions, improper formulation of ingredients, or use of an incorrect package (Taylor and Baumert 2010). Allergen control programs have widely been adopted by the food industry to guard against such occurrences. Allergen control is

especially important if manufacturers are making a free claim on the product. Food companies perform extensive validation testing on all ingredients and the final food product, especially in these situations, to confirm that no detectable residues of the allergenic food are present.

Reliable detection of peanut residues in foods and ingredients is especially important given the prevalence and severity of peanut allergy. Several commercially available kits are available, which detect peanut protein at low levels (parts per million; ppm) through enzyme-linked immunosorbent assays (ELISAs). In 2005, 3 ELISA-based peanut detection kits, including the Neogen Veratox™ for Peanut, r-Biopharm RIDASCREEN™ FAST Peanut, and Tepnel Biokit™ Assay for Peanut, were performance-tested and validated as successful for the quantitative detection of traces of peanut from 4 different food matrixes (breakfast cereal, cookies, ice cream, and milk chocolate) (Park and others 2005). All of these ELISA methods use polyclonal antisera directed against peanut proteins generally (Neogen Veratox and r-Biopharm) or Ara h 1, a specific peanut allergen (Tepnel). Subsequently, in 2009, Tepnel was acquired by the Neogen Corp. (Lansing, Mich., U.S.A.), but all 3 of these ELISA kits remain in the market.

Caramel colors are incorporated in a wide range of products. Depending on the product being colored, different shades of caramel color are used in the product formulation. The darkest shade, class IV, is primarily found in products such as soft drinks. Class I has the lightest color, while the darkness then progresses through class II to class IV. The standard of identity of caramel coloring permits the presence of basic food-grade carbohydrates including dextrose, invert sugar, lactose, malt syrup, molasses, sucrose, and starch hydrolysates and fractions thereof

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Table 1–Detection of apparent peanut in various lots of class IV caramel color using commercial detection kits.

Sample ID	Neogen Veratox LOQ ^a :2.5 ppm	Neogen Alert LOD ^b :5 ppm	Neogen Reveal LOD:5 ppm	r-Biopharm LOQ:2.5 ppm	Romer Labs LOQ:1 ppm	Neogen/Tepnel Biokit LOQ:1 ppm
Company A Class IV	4.9 ± 1.8 ppm	>5 ppm	>5 ppm	14.6 ± 0.8 ppm	9.8 ± 0.8 ppm	BLQ
Company B Class IV Lot 1	BLQ ^c	BLD ^d	>5 ppm	12.4 ± 1.6 ppm	1.7 ± 0.2 ppm	BLQ
Company B Class IV Lot 2	3.9 ± 1.2 ppm	>5 ppm	>5 ppm	15.1 ± 0.1 ppm	9.8 ± 0.6 ppm	BLQ
Company B Class IV Lot 3	3.7 ppm ^e	BLQ	>5 ppm	12.5 ± 1.2 ppm	2.3 ± 0.1 ppm	BLQ
Company B Class IV Lot 4	BLQ	BLQ	>5 ppm	17.6 ± 0.6 ppm	4.9 ± 0.3 ppm	BLQ

^aLOQ: limit of quantification.
^bLOD: limit of detection.
^cBLQ: below limit of quantification.
^dBLD: below limit of detection.
^eAnalysis of the Company B Class IV Lot 3 sample was replicated 3 times with 1 positive results (3.7 ppm peanut) and the remaining 2 replicates were BLQ.
Note: Analysis of the caramel samples was replicated 2 to 4 times for the quantitative ELISA kits (Neogen Veratox Peanut, r-Biopharm RIDASCREEN FAST Peanut, Romer Labs Agraquant Peanut, and Neogen (Tepnel) Biokit Peanut Assay) and 2 replications were conducted for each of the qualitative ELISA kits (Neogen Alert peanut kit and the Neogen Reveal peanut kit). The average concentration and standard deviation are reported. All concentrations are reported in units of ppm peanut.

Table 2–Detection of apparent peanut in classes I to III caramel color using commercial detection kits.

Sample ID	Neogen Veratox LOQ ^a :2.5 ppm	Neogen Alert LOD ^b :5 ppm	Neogen Reveal LOD:5 ppm	r-Biopharm LOQ:2.5 ppm	Romer Labs LOQ:1 ppm	Neogen/Tepnel Biokit LOQ:1 ppm
Company A Class I	BLQ ^c	BLD ^d	>5 ppm	BLQ	3.3 ± 0.3 ppm	BLQ
Company B Class I	BLQ	BLD	BLD	BLQ	1.2 ± 0.1 ppm	BLQ
Company B Class II	BLQ	BLD	BLD	13.3 ± 0.3 ppm	2.4 ± 0.2 ppm	BLQ
Company A Class III	BLQ	BLD	>5 ppm	BLQ	BLQ	BLQ
Company B Class III	BLQ	BLD	>5 ppm	BLQ	BLQ	BLQ

^aLOQ: limit of quantification.
^bLOD: limit of detection.
^cBLQ: below limit of quantification.
^dBLD: below limit of detection.
Note: Analysis of the caramel samples was replicated 2–3 times for the quantitative ELISA kits (Neogen Veratox Peanut, r-Biopharm RIDASCREEN FAST Peanut, Romer Labs Agraquant Peanut, and Neogen (Tepnel) Biokit Peanut Assay) and 2 replications were conducted for each of the qualitative ELISA kits (Neogen Alert peanut kit and the Neogen Reveal peanut kit). The average concentration and standard deviation are reported. All concentrations are reported in units of ppm peanut.

Table 3–Results of spike-and-recovery experiments with peanut spiked into class IV caramel.

Sample ID	Neogen Veratox Spike:15 ppm	Neogen Alert Spike:15 ppm	Neogen Reveal Spike:15 ppm	r-Biopharm Spike:15 ppm	Neogen/Tepnel Biokit Spike:100 ppm
Company A Class IV	5.9 ± 0.6 ppm	>5 ppm	>5 ppm	14.5 ± 0.6 ppm	BLQ
Company B Class IV Lot 1	3.7 ± 0.5 ppm	>5 ppm	>5 ppm	11.9 ± 1.2 ppm	2.2 ± 0.4 ppm
Company B Class IV Lot 2	5.1 ± 0.01 ppm	>5 ppm	>5 ppm	15.1 ± 0.1 ppm	BLQ
Company B Class IV Lot 3	5.0 ± 1.5 ppm	BLD ^b	>5 ppm	12.9 ± 1.0 ppm	1.6 ± 0.0 ppm
Company B Class IV Lot 4	BLQ ^a	BLD	>5 ppm	17.1 ± 1.7 ppm	BLQ

^aBLQ: below limit of quantification.
^bBLD: below limit of detection.
Note: Spike and recovery analysis of caramel samples was replicated 2 times for each of the quantitative ELISA kits (Neogen Veratox Peanut, r-Biopharm RIDASCREEN FAST Peanut, and Neogen (Tepnel) Biokit Peanut Assay) and qualitative ELISA kits (Neogen Alert peanut kit and the Neogen Reveal peanut kit). The average concentration and standard deviation are reported. All concentrations are reported in units of ppm peanut.

(FDA 2011). The sugars are heated with various acids, alkalis, sulfites, salts, and/or a source of nitrogen as controlled by the Code of Federal Regulations for Caramel. These raw ingredients should not contain any traces of peanut residue. Further, many caramel color manufacturers implement strict allergen-conscious production practices to eliminate the possibility of peanut contamination of the ingredients used in the production of caramel. Unexpectedly, initial testing of class IV caramel in our laboratory using the Neogen Veratox for Peanut kit suggested a possible false positive detection of peanut residue. Thus, we sought to determine the reliability of ELISA-based testing methods on the detection of peanut in various classes of caramel coloring.

Materials and Methods

Liquid caramel color of classes I to IV was obtained from 2 commercial suppliers for analysis of peanut residue using a se-

ries of ELISA allergen detection kits. A total of 10 caramel samples with varying degrees of darkness were collected. The darkest shade, class IV, was the class of most interest due to past experience with consistent low-level positives for peanut residue, so 4 different lots were obtained from 1 supplier and 1 lot of class IV was obtained from the other supplier. Both quantitative (sandwich) and qualitative ELISA kits for the detection of peanut residue were utilized in this study: 4 quantitative kits (Neogen Veratox Peanut, r-Biopharm RIDASCREEN FAST Peanut, Romer Labs AgraquantTM Peanut, and Neogen (Tepnel) Biokit Peanut Assay); and 2 qualitative ELISA kits (Neogen AlertTM peanut kit and the Neogen RevealTM peanut kit). The results of all of the quantitative kits are provided in ppm (μg/g) of peanut.

To assess the ability of the selected kits to reliably recover and detect peanut in the caramel matrix, spike and recovery experiments were performed. For the Neogen Veratox, Alert, and Reveal, and

the r-Biopharm RIDASCREEN FAST peanut ELISAs, 15 ppm of partially defatted, light roasted peanut flour (Golden Peanut Co.), containing approximately 50% protein, was spiked into the class IV caramel matrix. For the Neogen (Tepnel) Biokit peanut ELISA, a 100 ppm spike was used because no peanut residues were detected with a 15 ppm spike. Recovery was assessed using the kits according to manufacturer's instructions.

Results and Discussion

Low levels of presumed peanut were detected in all lots of class IV caramel by 2 or more of the quantitative peanut ELISA methods (Table 1). All of the class IV caramel lots had low levels of presumed peanut when tested by the r-Biopharm RIDASCREEN FAST peanut ELISA and the Romer Labs Agraquant peanut ELISA, while 3 of 5 lots tested positive using the Neogen Veratox peanut ELISA. The Neogen (Tepnel) Biokits peanut ELISA did not test positive for detectable peanut with any of the lots of class IV caramel. Class I, II, and III caramel also yielded low positive results in some of the peanut ELISAs, especially the Romer Labs Agraquant quantitative ELISA and the Neogen Reveal qualitative ELISA (Table 2). Because of the absence of peanut in the ingredients as well as strict allergen conscious manufacturing practices, the detection of peanut in caramel samples of various classes was presumed to be false positives.

When the class IV caramel matrix was spiked with 15 ppm peanut, poor recovery was obtained, and the results were not additive with the unspiked results (Table 3). Moreover, samples spiked with 100 ppm peanut showed little to no recovery with the Neogen (Tepnel) Biokit assay, suggesting false negative results. Qualitative testing using the Neogen Reveal lateral flow test strips was determined ineffective due to color interference of the caramel color masking the test strip indicator line. Interestingly, results obtained from the r-Biopharm RIDASCREEN FAST Peanut kit were very similar in both the spiked (11.9 to 17.1 ppm) or unspiked (12.4 to 17.6 ppm) samples. The spiking results indicate that the peanut ELISA kits would not be able to adequately detect peanut from class IV caramel color even if peanut residues were actually present.

Conclusions

Caramel coloring seems to exert matrix interference when tested for peanut residues using ELISA-based detection methods. This matrix interference is strongest for class IV caramel but occurs with other classes of caramel as well. ELISA-based testing methods for peanut protein are unreliable in detecting peanut residue from class IV caramel coloring (and perhaps other classes as well). Manufacturers who wish to test all ingredients for peanut residues before manufacturing peanut-free products should endeavor to use alternative methods for the testing of caramel ingredients. Admittedly, this matrix interference disappears if the caramel coloring is diluted into a food or beverage product. Thus, this analytical concern only exists if testing the caramel coloring ingredient for peanut residues.

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