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Protein Ingredients Control in Gluten Free Products Using SDS-PAGE, Developed Competitive Enzyme Immunoassays and Commercial ELISA Kits

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Abstract: Some protein ingredients declared in the label of gluten free products are allergenic proteins (milk, soy and egg). The proper identification of these proteins in food products is important for consumers who have food allergies. The aim of this study was to control the presence of protein ingredients declared in the label of gluten free products. Samples were analyzed with sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE), using an extractive solution of total proteins (Tris-ClH buffer 0.0625 M with 3% sodium dodecylsulfate and 2% 2-mercaptoethanol; pH: 6.8) and a selective solvent for the extraction of caseins (isopropanol 55° + 2-mercaptoethanol / ISO + ME). Developed Competitive enzyme immunoassays were used for the detection / quantification of milk, soy and egg proteins in products elaborated with rice flour. Specific polyclonal rabbit antiserums against milk, soy and egg proteins were used as primary antibodies in these competitive enzyme immunoassays. Commercial ELISA kits from Neogen, R-Biopharm and Romer were used to verify the results. In some samples undeclared allergens were detected. Correct allergens labeling is very important for the safety of allergic consumers. In conclusion, it is possible to identify all the proteins ingredients in these gluten-free foods studied, using a combination of electrophoretic methods and immunochemical methods.

Keywords: Protein Ingredients, Allergenic Proteins, Gluten Free Products, SDS-PAGE, Developed Competitive Enzyme Immunoassays, Commercial ELISA Kit

1. Introduction

Food allergies are a growing problem in developing countries but also in emerging countries like Argentina. The main food groups that generate allergic reactions are the "big eight": milk, eggs, soy, wheat, peanuts, nuts, fish and crustaceans [1].

Undeclared allergens in commercial foods are very dangerous for consumers who have food allergies. The clinical symptoms of an allergenic reaction can range from skin and/or gastrointestinal symptoms to anaphylaxis [2]. Food manufacturers must be very carefully in allergen labeling.

There is a need of methodology that enables the detection of allergenic proteins in products. The most common methodology for the analysis of these proteins is ELISA. There are no Latin American companies that produce ELISA kits and in Argentina these kits are very expensive [3].

The aim of this study was to control the presence of protein ingredients in gluten free products. Some of these protein

ingredients are allergenic proteins (milk, soy and egg). The proper identification of these proteins in food products is important for consumers who have food allergies.

2. Materials and Methods

2.1. Gluten Free Products

Ten commercial samples were studied. These samples declared the following protein ingredients: corn, sorghum, soy, rice flour; whole egg powder; ovalbumin; dehydrated whole milk and dehydrated potato, manioc starch and soy lecithin. Two batches of gluten free products were analyzed. The description of each product and the list of ingredients present in each of the samples are shown in Table 1.

2.2. Protein Extraction for SDS-PAGE and Developed Competitive Enzyme Immunoassays

Total protein extraction was used for SDS-PAGE and Developed Competitive enzyme immunoassays. The extraction buffer was: 0.0625 M Tris-HCl (pH: 6.8) containing 3% sodium dodecyl sulfate (SDS) y 2% de 2-mercaptoethanol (2-ME) (Total protein extraction solution). Products (200 mg) were extracted adding 2 mL of total protein extraction solution and heating the mixture in a water bath at 100°C for 5 minutes. Then, the mixture was centrifugated at 2500 rpm for 15 minutes. The supernatants were stored at -20º C until the analysis.

A selective extraction solvent (isopropanol 55° + 2-mercaptoethanol / ISO+ME) was used to evaluate the presence of certain proteins such as caseins with SDS-PAGE. Three hundred and fifty mg of products were extracted with 2 mL of ISO+ME. The mixture was shaken using a VirTis Model 23 homogenizer for 5 minutes at medium speed, then each vial was left for 60 minutes standing and shaken for another 5 minutes. The contents of the extraction vessels were transferred to plastic tubes and centrifuged at 3000 rpm for 15 minutes. The supernatants were transferred to Eppendorf tubes and analyzed the same day of extraction.

2.3. SDS-PAGE

Protein separation by polyacrylamide slab gel electrophoresis with Laemmli system (SDS-PAGE) was used. [4]

The running gel was prepared with 10% acrylamide solution in 1.5 M Tris-HCl containing 0.4% SDS (pH: 8.8). The stacking gel was prepared with 3% solution of acrylamide in 0.5 M Tris-HCl containing 0.4% SDS (pH: 6.8).

An aliquot of the extract of dehydrated whole milk, soy or egg (10 uL) was mixed with 30 uL of a solution of 50 % glycerol in water and 30 uL of a solution of 0.001% bromophenol blue in water. An aliquot of each extract of commercial product (30 uL) was mixed with 15 uL of a solution of 50 % glycerol and 15 uL of a solution of 0.001% bromophenol blue in water. Five uL of each mixture were load in each well.

Electrophoresis was performed using Tetra Mini Protean cell from BioRad at 180 V for 45 minutes.

Gel staining was performed with a solution of 0.1% Coomassie Brilliant Blue R 250 in 40% methanol and 10% acetic acid for 30 minutes. The gels were then decolored with an ethanol: acetic acid (40:10) solution in 2 periods of 20 minutes replacing the decolorizing solution with new one the second period. The decolorizing solutions used were discarded. In all cases the gels were preserved in 7% acetic acid solution with a few drops of dye solution until their analysis, and then dried in a Bio Rad Gelair Dryer.

The densitograms were obtained by transmission with Shimadzu Dual - Wavelength Chromatogram Scanner Model CS - 910 equipment, wavelength of maximum absorption of 550 nm and of 400 nm of minimum absorption. Data acquisition was performed with the Chromatography Station CSW program of DataApex Ltd [5]. All the samples were analyzed in duplicate.

2.4. Immunochemical Methods

Polyclonal antiserum (primary antibodies):

Specific polyclonal rabbit antiserums against milk, soy and egg proteins were prepared by inoculating different NZW rabbits with 100 µg of milk, soy or egg proteins emulsified with complete Freund's adjuvant. A series of four injections (50 µg) of the same antigen in incomplete Freund's adjuvant was administered every 3 weeks. Antibody titres were determined by indirect ELISA.

2.4.1. Developed Competitive Enzyme Immunoassays to Detect Milk, Soy or Egg Proteins in Products Elaborated With Rice Flour

Each extract was analyzed in duplicate. The curve had five points 0; 0.01; 0.03; 0.1 and 0.3 µg soy, milk or egg protein / mL carbonate / bicarbonate buffer, pH: 9.6. For each point of the curve a dilution of the original extract was performed but the SDS and ME concentration remained constant. In this way the components of the extractive solution were diluted (1: 175 (soy), 1:164 (milk) and 1:260 (egg) with carbonate / bicarbonate buffer, pH: 9.6), at all points of the curve. The samples were diluted with carbonate / bicarbonate buffer, pH: 9.6, in the same way as the points of the curve according to the allergen to be detected.

In previous works the optimum dilution for the primary antibody was selected (1:1250 for soy, 1:12500 for milk and 1:60000 for egg with TBS buffer with 0.1% v/v Tween 20 and 3% polyethylene glycol) [6, 7 and not published results]. Seventy five µL of the primary antibody dilution and 75µL of each of the previously prepared curve points or 75µL of the samples extract, were preincubated. In addition, two controls were prepared; a "non-specific control" (NS) containing 200 µL of the buffer used to dilute the primary antibody and a "maximal binding" (M) control containing: 100 µL of the buffer used to dilute the primary antibody and 100 µL of the diluted primary antibody. Preincubates were stored at 4°C in a humid chamber and in darkness for 24 h. Also, an ELISA plate was sensitized by sticking the concentration of antigen (milk, soy and egg) that was previously selected in the optimization test [6, 7 and not published results]. The sensitized plate was

incubated in a humid chamber, in the dark at 4°C for 24 h. The plate was washed 5 times with wash solution (0.9% w/v NaCl and 0.0125% v/v Tween 20 in water). Two hundred µl of blocking solution (1% w/v bovine gelatin and 0.1% v/v Tween 20 in TBS) were seeded into each well and incubated with shacking for one hour in a humid chamber, in the dark at 37°C. The plate was washed 5 times with wash solution.

Subsequently, 100 µL of the preincubates were seeded in the sensitized/blocked plate. Each plate was incubated for one hour in a humid chamber, in the dark at 37°C with shaking. The plate was washed 5 times with wash solution. One hundred µl of Bio-Rad alkaline phosphatase conjugated Anti-IgG secondary antibody (obtained in goats immunized with purified rabbit IgG) was seeded in the wells. The secondary antibody was diluted 1:3000 with TBS buffer with 0.1% v/v Tween 20 and 3% polyethylene glycol. The plate was incubated again for one hour in a humid chamber, in the dark at 37°C with shaking. The plate was washed 5 times with wash solution. Finally, 100 µl of a solution containing 1 mg/mL paranitrophenyl phosphate in a buffer containing 10% v/v diethanolamine and 0.01% magnesium chloride, pH: 9.8 were seeded in each well and the plate was incubated 20 minutes more in the dark at 37°C with shaking. Absorbance was measured on an ELISA microplate reader (ELISA RT-2100C, Rayto, China) at 405nm. The absorbance values were corrected with the average absorbance corresponding to the NS. An absorbance calibration curve was constructed**:** corrected absorbance versus ln µg of milk, soy or egg protein /mL. By interpolation in the calibration curve, the µg of milk, soy or egg protein / mL were obtained. This corresponds to the milk, soy or egg content in the diluted extract analyzed. The amount of milk, soy or egg protein in µg /g of product is calculated according to the following formula:

Amount of protein amount of milk, soy or egg prot.-ug(1)x

$$\text{In the product} = \frac{\text{V} - \text{uL(2) x 1000} - \text{mg (3)}}{\text{6.1 or 5.7or 3.8} - \mu\text{L (4) x P} - \text{mg (5)}}$$

(1) µg of milk, soy or egg protein interpolated in the calibration curve.

(2) Volume of supernatant obtained when extracting the product with extractive solution of total proteins: 1000 µL

(3) 1000 mg: to express the content in 1000 mg of product.

(4) 6.1 or 5.7 or 3.8µL. It is the volume of extract that is taken from the 1000 µl of supernatant and diluted 1:175 (soy), 1:164 (milk) and 1:260 (egg). They are brought to 1000 µL with Buffer carbonate / bicarbonate; PH 9.6.

(5) P: 200 mg. It is the weight of product that is extracted with extractive solution of total proteins.

The detection (LOD) and quantification (LOQ) limits for each developed competitive enzyme immunoassays were: for milk: LOD: 25.0 ppm milk protein, LOQ: 50.0 ppm milk protein. The working range was 13-400 ppm of milk proteins. For soy: LOD: 35.0 ppm soy protein, LOQ: 60.0 ppm soy protein. The working range was 15-420 ppm of soy proteins. For egg: LOD: 6.0 ppm egg protein, LOQ: 16.0 ppm egg protein. The working range was 13-400 ppm of egg proteins.

2.4.2. Commercial ELISA

Milk, soy and egg proteins were detected and quantified with different commercial ELISA kits: Neogen, R-Biopharm and Romer kits. All samples were assayed in duplicate following the protocols of each kit.

The detection (LOD) and quantification (LOQ) limits for each kit were: Ridascreen® Fast milk Protein R-Biopharm LOD: 0.7 ppm milk protein and LOQ: 2.5 ppm milk protein with a quantification range of 2.5 - 67.5 ppm milk protein. Veratox® Allergen Total Milk from Neogen DL: 1 ppm milk protein and LOQ: 2.5 ppm milk protein with a quantification range of 2.5 - 25 ppm milk protein. [8, 9]

Ridascreen® Fast soy Protein R-Biopharm DL: 0.31 ppm soy protein and LOQ: 2.5 ppm soy protein; Romer AgraQuant® Soy Assay LOQ: 16 ppb soy Tripsin Inhibitor and LOQ: 40 ppb soy Tripsin Inhibitor. [10, 11]

Neogen Veratox® for Egg Allergen LOD: 2,5 ppm egg protein with a quantification range of: 2,5-25 ppm egg. [12]

3. Results and Discussion

Table 1 shows the results of milk, soy and egg protein detection in gluten free foods using SDS-PAGE, Competitive enzyme immunoassays and commercial ELISA kits.

In most of the samples that were analyze, the protein ingredients of corn, sorghum, rice, egg, ovalbumin, potato, soy and milk were identified with the SDS-PAGE methodology, using extractive protein solution. In two samples the presence of rice proteins was presumed, although it was not evident (9 y 10). In addition, in six of the samples that presented a complex electrophoretic profile, the presence of milk or soy proteins was not evident (2, 3, 4, 6, 7, 9). In order to confirm the presence of milk, these samples were analyzed by SDS-PAGE using ISO + ME as extraction solvent. The SDS-PAGE methodology with ISO + ME allowed not only to confirm the presence of milk proteins but also the presence of sorghum proteins in the samples that contained them (2, 4, 5, 6, 7 and 8).

As an example figure 1 shows densitograms of samples 1, 2 and 8. In sample 1 soy, corn and sorghum peaks are evident (So, S and C). In sample 2 egg, milk, soy, rice and sorghum peaks can be detected (E, M, So, R and S). Ovalbumin, rice and sorghum can be distinguished in sample 8 (O, R and S).

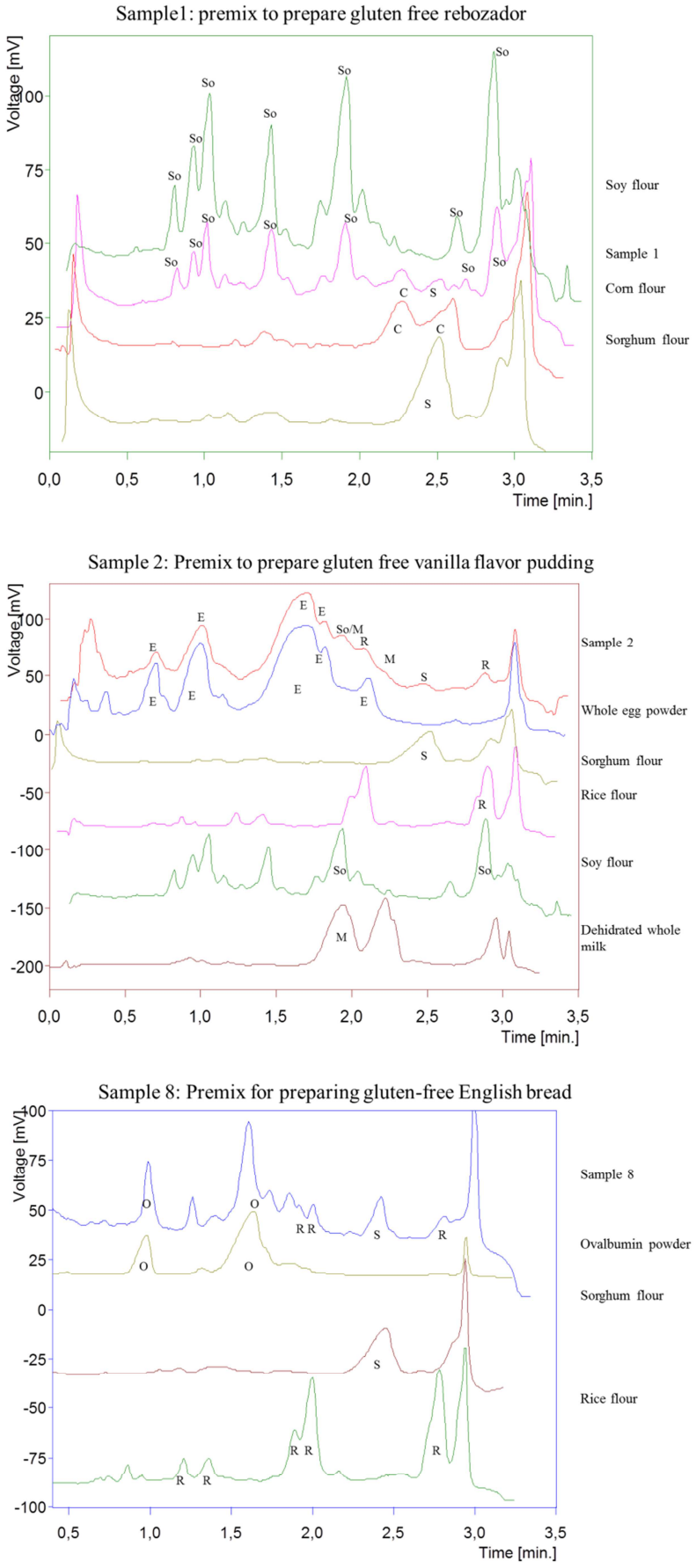

Figure 1. *Densitograms of total proteins in samples 1, 2 y 8 and standards.*

In most of the samples, the presence of milk, soy or egg proteins that were identified using SDS- PAGE, were verified with the developed competitive enzyme immunoassays. Commercial ELISA kits were used to corroborate these results.

In the analyzed samples that declared milk, soy and egg, developed competitive enzyme immunoassays and the commercial ELISA kits detected those proteins (samples 2, 3, 4, 6, 7 and 9). In a sample that did not declare milk or egg, developed competitive enzyme immunoassays detected milk and egg protein. This was corroborated using commercial ELISA kits (sample 1). In two samples that did not declare soy, the developed competitive enzyme immunoassays detected soy protein; this was corroborated using the commercial ELISA kit (samples 8 and 10). In a sample that did not declare milk, the developed competitive enzyme immunoassays did not detect milk proteins, however, they were detected and quantified using the commercial ELISA kits (sample 8). These results are due to the different sensitivity of both methodologies used.

It has been observed in several studies that the results between different ELISAs may be dissimilar. In order to detect the presence of extrinsic proteins an ELISA is often the method of choice because they are considered to have high sensitivity and specificity. Although ELISA method is accepted as standard method for allergen measurement, the results obtained by this methodology seemed to vary from assays to assays. This variation could be due to the lack of standardization of the method, the calibration material used, the extraction solutions used or the antibody specificity [13, 14]. Therefore the quantitative results between developed competitive enzyme immunoassays and commercial ELISA kits cannot be compared.

The significance of the developed competitive enzyme immunoassays that were developed is that their sensibility is higher than the sensibility of the SDS-PAGE and also it allows quantification of the allergens.

SDS-PAGE is the suggested method if it is suspected that allergens proteins were added as an ingredient. The developed competitive enzyme immunoassays that were developed by our work group could be used as a screening method, in cases that it is suspected that allergenic proteins may be present by cross-contact. The cost of the developed competitive enzyme immunoassays was calculated in December 2017 and it was 0.50 U.S dollars per well. At the same time a commercial kit had a market value of 10.30 U.S. dollars per well in Argentina. So the developed competitive enzyme immunoassays has a considerably lower cost than commercial ELISA kits. If a sample shows a positive result with the developed competitive enzyme immunoassays, it is not necessary to use a commercial ELISA kit because the sample contains the allergen. If negative results are obtained with this methodology, it should be confirmed with a commercial ELISA kit of adequate sensitivity, to ensure the absence of allergens proteins.

In different samples that were analyzed and did not declare some proteins, the developed competitive enzyme immunoassays detected those proteins and this was corroborated using the commercial ELISA kits. The presence of these undeclared proteins is a risk for allergic consumers. According to previous studies there are a lot of foods that have a high risk of containing undeclared allergens and may lead to product recalls or to potential hazards. Many studies found higher prevalence of undeclared allergens with or without advisory statement. [14]

4. Conclusions

In conclusion, it is possible to identify all the proteins ingredients in these gluten-free foods studied, using a combination of electrophoretic methods and immunochemical methods.

In some samples undeclared allergens were detected. Correct allergens labeling is very important for the safety of allergic consumers. A correct labeling would be possible if food manufacturers and official food control laboratories had at their disposal methodologies to detect the allergenic proteins.

Table 1. *Detection of milk, soy and egg proteins using SDS-PAGE, developed competitive enzyme immunoassay and different commercial ELISA kits in two batches of gluten free products.*

Sample	Ingredients/allergens declarations	SDS-PAGE Total proteins detected
1 Premix to prepare gluten free breadcrumbs	Corn flour, sorghum flour, soy flour, salt. Contains soy	Soy Corn Sorghum
2 Premix to prepare gluten free vanilla flavor pudding	Sugar, whole egg powder, sorghum flour, corn starch, rice flour. Emulsifiers: mono and diglycerides of lactic acid and propylene glycol. Soy flour, dehydrated whole milk. Chemical leavenings (sodium bicarbonate, sodium acid pyrophosphate and monocalcium phosphate). Vanilla flavoring. Contains egg, milk and soy	Egg Rice Sorghum Milk? Soy?
3 Premix to prepare gluten-free pancakes.	Rice flour, whole egg powder, sorghum flour, corn starch, dehydrated whole milk, soy flour, salt. Contains egg, milk and soy	Egg Rice Sorghum Milk Soy?
4 Premix to prepare gluten-free gnocchi.	Dehydrated potato, rice flour, sorghum flour, corn starch, whole egg powder, dehydrated whole milk, soy flour, salt. Stabilizer: xanthan gum. Contains egg, milk and soy	Egg Potato Rice Sorghum Milk Soy?

Sample	Ingredients/allergens declarations	SDS-PAGE Total proteins detected
5 Premix based on rice flour, sorghum flour and corn starch for gluten-free bakery and pastry	Rice flour, sorghum flour, corn starch, dehydrated whole milk, soy flour, salt. Contains milk and soy	Soy Sorghum Mlik Rice
6 Premix to prepare sweet cookies and free gluten-free chips.	Sugar, rice flour, sorghum flour, corn starch, dehydrated whole milk, soy flour, whole egg powder. Chemical leavenings (sodium bicarbonate, sodium acid pyrophosphate). Vainillina flavoring. Contains egg, milk and soy	Egg Rice Sorghum Milk? Soy
7 Premix to prepare gluten-free vanilla flavor sponge cake.	Sugar, whole egg powder, rice flour, sorghum flour, corn starch. Emulsifiers: mono and diglycerides of lactic acid and propylene glycollester. Dehydrated whole milk, soy flour. Vanilla flavoring. Chemical leavenings (sodium acid pyrophosphate, sodium bicarbonate and monocalcium phosphate). Contains egg, milk and soy	Egg Rice Sorghum Milk? Soy?
8 Premix to prepare gluten-free English bread	Corn starch, sorghum flour, rice flour, sugar, ovalbumin powder, salt, stabilizers (xanthan gum and carboxymethylcellulose), fungal alfa amylase. Contains egg	Ovoalbumin/conalbumin Sorghum Rice
9 Premix to prepare gluten-free egg pasta.	Rice flour, sorghum flour, corn starch, whole egg powder, dehydrated whole milk, soy flour, salt. Stabilizer: xanthan gum. Contains egg, milk and soy	Egg Rice? Sorghum Milk? Soy?
10 Premix to prepare gluten-free pizza.	Corn starch, sorghum flour, rice flour, dextrose, dehydrated whole milk. Emulsifiers: mono and diglycerides of lactic acid and propilenglicolester. Ovalbumin powder, salt. Stabilizers: xanthan gum. Chemical leaners: sodium acid pyrophosphate and sodium bicarbonate. Contains egg and milk	Ovoalbumin/conalbumin Sorghum Milk Rice?

Table 1. *Continued*

Sample	SDS-PAGE ISO + ME proteins detected	Competitive ELISA (CE) Neogen (N) R-Biopharm Milk (R-B)	Competitive ELISA (CE) Romer(R) R-Biopharm Soy (R-B)	Competitive ELISA (CE) Neogen Egg (N)
1 Premix to prepare gluten free breadcrumbs	--	CE: >400 /280 N: >25/10 R-B: >67.5 /17.1	CE: >420/>420 R: >1000/>1000	CE: >400/>400 N: >25/>25
2 Premix to prepare gluten free vanilla flavor pudding	Milk Sorghum	CE: >400 />400 R-B: >67.5 />67.5	CE: >420/>420 R: >1000/>1000	CE: >400/>400 N: >25
3 Premix to prepare gluten-free pancakes.	---	CE: >400 R-B: >67.5 ppm	CE: >420 R: >1000	CE: >400 N: >25
4 Premix to prepare gluten-free gnocchi.	Milk Sorghum	CE: >400 />400 R-B: >67.5/>67.5	CE: >420/>420 R: >1000/>1000 R-B: >20	CE: >400/>400 N: >25
5 Premix based on rice flour, sorghum flour and corn starch for gluten-free bakery and pastry	Milk? Sorghum	CE: >400 R-B: >67.5	---	CE: 305 N: >25
6 Premix to prepare sweet cookies and free gluten-free chips.	Milk Sorghum	CE: >400 R-B: >67.5	---	CE: >400 N: >25
7 Premix to prepare gluten-free vanilla flavor sponge cake.	Milk Sorghum	CE: >400/>400 R-B: >67.5/>67.5	CE: >420/>420 R:>1000/>1000	CE: >400/>400 N:>25
8 Premix to prepare gluten-free English bread	Sorghum	CE: <50 R-B: 36.6 N: >25	CE: 99/75 R: >1000/173 R-B: >20	CE: >400/>400 N: >25/>25
9 Premix to prepare gluten-free egg pasta.	--	CE: >400 R-B: >67.5	CE: >420	CE: >400 N: >25
10 Premix to prepare gluten-free pizza.	--	CE: >400 />400 R-B: >67.5 />67.5	CE: >420/>420 R: 668/761 R-B: >20/>20	CE: >400/>400 N: >25/>25

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References

[1] Ward R. 2014. Chapter 1: Introduction to food aller-gy. In Flanagan S, Handbook of Food Allergen Detection and Control. Woodhead Publishing. Cambridge, UK.

[2] Koeberl M., Clarke D., Allen K. J, Fleming F., Katzer L., Lee N. A., Lopata A. L., Said M., Scheelings p., Shepherd N., Sherlock R., Roberts J. 2018. Food Allergens Management in Australia. Journal of AOAC International. 101(1):60-69(10). DOI: https://doi.org/10.5740/jaoacint.17-0386

[3] López M. C. 2018. Food Allergen Labeling: A Latin American Approach. Journal of AOAC International. 101(1):14-16(3). DOI: https://doi.org/10.5740/jaoacint.17-0382

[4] Laemmli U. 1970. Cleavage of structural protein during the assembly of the head of bacteriophage T4. Nature. 227: 680-685.

[5] López L. B. 2000. Separation, identification and quan-tification of proteins in processed foods. Thesis UBA. Argentina.

[6] Cellerino K. 2016. Control Methodology for the analysis of milk, soy and egg allergens in meat and farinaceous products. Thesis UBA. Argentina.

[7] Cellerino K., Rodríguez V., Docena G., López L. B. 2017. Development of a Competitive Enzyme Immunoassay Technique for the Detection of Soy Traces in Meat Products. Journal of Food and Nutrition Sciences. 5(2): 57-62. Doi: 10.11648/j.jfns.20170502.16

[8] R-Biopharm RIDASCREEN® FAST Milk. 2009. https://food.r-biopharm.com/wp-content/uploads/sites/2/2015/09/R4652-FAST-Milk-15-07-09.pdf. Access: january 2018.

[9] Neogen Veratox® for Total milk allergen. 2016. http://foodsafety.neogen.com/pdf/procedures/8470_pro.pdf Access: january 2018.

[10] R-Biopharm RIDASCREEN® FAST Soya. 2018. https://food.r-biopharm.com/wp-content/uploads/sites/2/2016/10/R7102-FAST-Soya-16-07-18.pdf. Access: january 2018.

[11] Romer AgraQuant®. Soy Assay. 2011. http://shop.romerlabs.com/en/AgraQuant-ELISA/AgraQuant-Allergens/AgraQuant-ELISA-Soy. Access: january 2018.

[12] Neogen Veratox® for Egg Allergen. 2013. http://foodsafety.neogen.com/en/veratox-egg. Access: january 2018.

[13] Diaz Amigo C. 2010. Towards a Comprehensive Validation of ELISA Kits for Food Allergens. Case 2- Milk. Food Analytical Methods. 3: 351-356.

[14] Do A. B, Khuda S. E, Sharma M. G. 2018. Undeclared food allergens and gluten in commercial food products analyzed by ELISA. Journal of AOAC International. 101(1):23-35(13). DOI: https://doi.org/10.5740/jaoacint.17-0384