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Effect of industrial processing of crackers on the recovery and quantitation of allergens with ELISA kits*

Efecto del procesamiento industrial de galletas en la recuperación y cuantificación de alergenos con kits ELISA

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Abstract

Introduction. Processing foods may generate limitations on the recovery and quantitation of allergens. Factors such as geometry or thermal treatment can influence the veracity of the assay results. **Objective.** To determine the effect of processing on the recovery and quantification of allergens. **Materials and methods.** Study conducted in Costa Rica between 2020 and 2021 in the Compañía de galletas Pozuelo DCR. S.A. The geometry was evaluated with two cracker molds (traditional and XL). The effect of baking was evaluated with the traditional cracker type. For both experiments, samples were taken from four batches, and they were analyzed with three different kits for milk and egg analysis in an independent way. **Results.** The effect of geometry was observed for recovery and quantitation of egg residues was significantly affected by cracker geometry (P= 0.0228) compared to milk (P= 0.4335), regardless of the analytical kit used. The post baking decrease of quantitation effect was presented equally regardless of the kit used (P=0.4245) on egg. Very poor recovery of egg residues (4-5 %) was observed after cracker baking. For milk allergens, there was not a significant difference on the quantitation and recovery after baking among kits (P=0.1682), which is due to the variability of the data among kits. **Conclusions.** Processing reduces the efficacy of kits to detect the real quantity of allergens in foods. The analytical kit must be evaluated with the matrix to be analyzed, to determine how much impact the processing can have on the quantitation of allergens.

Keywords: Food allergies, enzyme immunoassays, detection, labelling, allergenic capacity, food safety.

Resumen

Introducción. El procesamiento de alimentos puede generar limitaciones en la recuperación y cuantificación de alérgenos. Factores como la geometría o el tratamiento térmico pueden influir en la veracidad de los resultados

del análisis de alérgenos en alimentos. **Objetivo.** Determinar el efecto del procesamiento en la recuperación y cuantificación de alérgenos**. Materiales y métodos**. Estudio realizado en Costa Rica entre 2020-2021 en la Compañía de galletas Pozuelo DCR.SA. La geometría se evaluó con dos moldes de galletas soda (tradicional y XL). El efecto de la cocción se evaluó con el tipo de galleta tradicional. Para ambos experimentos, se tomaron muestras de cuatro lotes, y se analizaron con tres kits diferentes para la cuantificación de leche y huevo de forma independiente. **Resultados.** El efecto de la geometría se observó para la recuperación y cuantificación de proteína de huevo (P= 0,0228), pero no para la proteína de leche (P= 0,4335), independientemente del kit analítico utilizado. La disminución del efecto de recuperación y cuantificación después de la cocción se presentó de manera igual independientemente del kit utilizado (P=0.4245) en huevo. Se obtuvo una recuperación pobre (4 y 5%) de proteína de huevo. Para los alérgenos de la leche, no hubo diferencia significativa en la cantidad después de la cocción entre los kits (P=0,1682), lo que se debe a la variabilidad de los datos entre los kits. **Conclusiones.** El procesamiento influyó en la eficacia de los kits para detectar la cantidad real de alérgenos en los alimentos. El kit analítico debe ser evaluado con la matriz de interés, para determinar cuánto impacto puede tener el procesamiento en la cuantificación de alérgenos.

Palabras clave: alergia alimentaria, inmunoensayos enzimáticos, detección, etiquetado, capacidad alergénica, inocuidad alimentaria.

Introduction

Every day it becomes more evident that food allergies represent a global public health issue. Some countries have documented a prevalence greater than 10 % in children (Loh & Tang, 2018) and near 6 % in adults (Sánchez et al., 2019). Multiple mechanisms exists for food allergies and intolerances, but IgE-mediated, immediate hypersensitivities are the basis for the most serious allergic reactions. IgE-mediated food allergies have caused deaths and led to the promulgation of worldwide regulations to improve labeling for the safety of food-allergic consumers. These regulations require processing industries to assume the responsibility of informing consumers with allergies or intolerances about the presence of allergens in each packaged food in a clear and truthful manner. The goal is to protect their health and integrity (Lee et al., 2013; Yue et al., 2023).

The trend regarding allergen management legislation in more advanced countries is clear: precautionary allergen labels (PAL) are permitted only when a company demonstrates that it cannot guarantee the absence of an allergen in a product. This demonstration is typically done through food allergen management systems (Programa de Control de Alimentos de Argentina [PFCA], 2017; Shoji et al., 2018). These allergen management programs include analytical verification of allergenic proteins in both foods and production environments. Worldwide and in Costa Rica, the enzyme-linked immunoassay tests (ELISA) are commonly used to evaluate the presence of allergens in foods (Garber et al., 2020). Declarations of food allergens in Costa Rican foods are established by the Central American Technical Regulation of General Labelling of Prepackaged Foods (Presidencia de la República et al., 2012).

The modifications that can occur in proteins during processing depend on several factors, including the processing conditions, the nature of the protein and composition of the food matrix. Many of the processes applied to foods at industry levels impact both the structure and chemical properties of the proteins. Among the most critical changes are the unfolding and aggregation of proteins, proteolysis, glycosylation, glycation, solubility effects, pH, and network for gel formation, which can increase or decrease its allergenic potential. It cannot be assumed that if an analytical assay is not effective in detecting and quantifying an allergen, the food has lost its potential of causing hypersensitivity (European Food Safety Authority [EFSA], 2014).

It is well established that food production processes such as thermal treatments and extrusion can significantly influence the solubility and extraction capacity of allergenic proteins. Authors should note that solubility can also

be reduced by protein aggregation and note that ELISAs only detect soluble proteins residues. Additionally, these processes can impact the ability of antibody or antibodies used in the ELISA test to recognize allergens due to the loss of conformational epitopes Ig-E (Gomaa & Boye, 2013; Monaci et al., 2011). While it has been demonstrated that the performance of ELISA tests is compromised when extensive food processing techniques, such as baking, are applied, it is important to note that despite the extensive processing, the food still has allergenic potential (Török et al., 2015).

Several factors can influence the results of ELISA tests: (1) interactions with compounds in a food matrix (e.g polyphenols and tannins); (2) reduced solubility and reactivity of denatured proteins due to heat or reactions such as Maillard; and (3) differences in protein profile of a particular food allergen from different species, varieties and geographic origins (Binaghi et al., 2017). Specifically, during food processing (including heating and technological methods), changes in the structure of allergenic proteins can impact antigenic determinants and epitope binding sites. This alteration may compromise the efficient recovery and detection of allergens in ELISA tests (Monaci et al., 2011). Additionally, significant variations exist in the quantitation and detection capabilities of different ELISA kits available on the market (Binaghi et al., 2017).

The geometry characteristics of commercial products are an aspect that has received little attention in the context of allergen quantification during processing. A study that investigated the effect of cookie size on the detection and quantitation of allergens, found that, in general, the recovery of allergens decreases with decreasing size. Interestingly, the impact of baking is more significant than geometry alone. The observed differences related to the cookie size were attributed to the fact that the temperature in the center of the cookie increases as the size decreases (while maintaining the same thickness). These variations in temperature account for the differences observed among cookies of different sizes (Gomaa & Boye, 2013). The aim of this study was to determine the effect of processing on the quantification of allergens in crackers.

Materials and methods

The processing of crackers was conducted in the cookie factory Compañía de galletas Pozuelo DCR. S.A., in San José, Costa Rica between 2020 and 2021. This company has an allergen management program that covers everything from raw material reception to packaging. Additionally, it also has a FSSC 22000 certification, and for these reasons it was selected for this research. The study was conducted in the production processing line for crackers, using the formulation and production process typical of traditional crackers. The base formulation does not include milk and eggs among its ingredients. However, for the experiment, both allergens were intentionally introduced under controlled conditions. Analyzing the raw dough without added milk or egg (non incurred dough), neither milk nor egg were detected or quantified.

For the effect of geometry, the original mold was used and compared with an XL mold which had an area 26 % bigger, resulting in the crackers doubling in weight. Both geometries of crackers were subjected to the same thermal conditions. Direct fire ovens were used with baking times of 2 and 3 minutes at a temperature of 260 and 295 °C. The reference materials used were: NIST 8445 for egg allergen (reference mass fraction value = 48 % +/- 1 %) and for milk MoniQA MQA082016 certified for allergens (17 mg/kg of milk proteins). Given the large quantities produced in the company's production line, the decision was made to introduce the allergen to the crackers after they were molded but before baking. A solution was prepared with the two allergens and deionized water, and the corresponding amount was added to each cracker using a calibrated micropipette.

Initially, a blank sample (referred to as "no incurred dough") was analyzed. Subsequently, raw dough and crackers from both geometries were sampled. To calculate protein content, the weight of each cracker was considered. The doughs were intentionally contaminated with allergens: 200 ppm of protein egg and 138 ppm

of protein milk for batch 1 and 100 ppm of egg protein and 500 ppm of milk protein for batches 2, 3 and 4. The results were reported as the percentage of allergen detected in the cracker relative to the concentration detected in the raw dough. This approach was taken to eliminate other potential sources of variation. The specific level of allergen incurred was determined based on the detection of the reference material at the time of testing. Notably, the formulation excludes both egg and milk as ingredients.

For the incurred samples, a solution of both allergens at established concentrations was elaborated. Molded doughs were incurred using the allergen solution with one micropipette and new tips by applying the solution to the surface of the molded dough in accordance with the weight of the dough The crackers were baked after addition of the allergen solution. These meticulous steps were taken to ensure accurate and consistent results in the analysis.

Four batches of crackers were sampled from different production weeks. These batches included both the traditional geometric shape and the XL mold. To ensure the presence of all added allergens, each incurred batch underwent thorough processing. The dough and crackers were carefully packaged in plastic bags. Subsequently, they were transported to the chemistry laboratory of the National Center of Food Science and Technology (Centro Nacional de Ciencia y Tecnología de Alimentos, CITA) situated on the Rodrigo Facio campus of the University of Costa Rica, San Pedro de Montes de Oca, San José, Costa Rica. It was in this laboratory that the ELISA allergen tests were conducted.

All the collected samples were stored at a temperature of -80 °C until further analysis. To address the challenge posed by the initial water content (ranging from 27 % to 31 % g/100 g) in the doughs, a freeze-drying process was employed. This step ensured a more homogeneous distribution of allergens compared to analyzing the fresh doughs directly. Each sample was analyzed for moisture content with the thermogravimetric analysis (TGA). All tests were conducted in duplicate for each sample. Results are expressed as a percentage of recovery on a dry basis for each allergen. This calculation starts from the analytical quantity determined in the incurred raw doughs. The extraction and ELISA analysis protocols specified in each test kit were strictly followed (Figure 1), despite any unique characteristics they may have had.

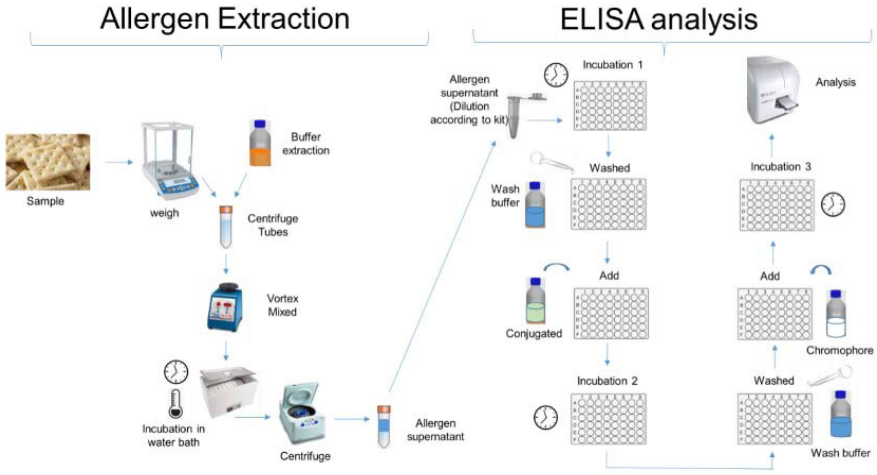

Figure 1. Generic steps to conduct enzyme-linked immunoassay tests in foods. The Figure was elaborated by the authors based on the steps described in the kits for analysis of milk and egg from R-biopharm, Veratox, and 3M, 2021.

Figura 1. Pasos genéricos para realizar las pruebas de inmunoensayo ligado a enzimas en alimentos. La figura fue elaborada por los autores basado en la información descrita en los kits para detectar leche y huevo de R-biopharm, Veratox y 3M, 2021.

All analytical kits used were ELISA sandwich test. Three commercial kits were utilized for detecting milk allergens, and an additional three kits were employed for egg allergens. The kits used correspond to the kits marketed directly in Costa Rica. All kits have different analytical characteristics (Tables 1 and 2). When interpreting results and making comparisons across different kits, factors such as sample quantity, extraction times and temperatures, spinning, incubation and washdown times, and wavelengths for reading must be considered. These variations ensure that the analytical process is tailored to the specific requirements of each allergen detection kit.

Table 1. Main characteristics of analytical kits used for the quantitation of milk allergen, 2021.

Cuadro 1. Principales características de los kits usados para la cuantificación del alergeno leche, 2021.

Allergen	**Milk**		
Kit name	**ELISA RIDASCREEN® Fast Milk***	**Veratox Total Milk Allergen Quantitative 8470****	**Bovine total milk protein ELISA Kit 3M*****
Specificity	casein and β-lactoglobulin	casein and whey proteins	total bovine milk protein
Limits (detection/ quantitation)	detection: 0.7 ppm quantitation: 2.5 ppm	detection: 1 ppm quantitation: 2.5 ppm	detection: 5.8 ppb quantitation: 1 ppm
Result Expression Unit	mg of milk protein/kg (ppm)	ppm of powdered skimmed milk	ppm of total bovine milk protein
Extraction (water bath)	#1: at 100°C/10 min. #2: at 60°C/10 min. Use additive	at 60 °C, 15 min with agitation. Use additive	at 60°, 25 min with agitation
Incubation conditions (minutes)	10 at room temperature.	10 at room temperature	with agitation 30 for incubation 1, 10 for subsequent incubations
Washes (# per incubation)	3	10	3
Reading wavelength (nm)	450	650	450

Sources / Fuentes: * Weiss et al. (2016); **Neogen (2018), ***3M (2017a).

Table 2. Main characteristics of analytical kits used for the quantitation of egg allergen, 2021.

Cuadro 2. Principales características de los kits usados para la cuantificación del alergeno huevo, 2021.

Allergen	**Egg**		
Kit name	**RIDASCREEN®FAST Ei / Egg Protein***	**Egg veratox****	***ELISA kit for egg white protein** 3M
Specificity	egg white protein ovalbumin and ovomucoid	ovomucoid (Gal d1), ovalbumin (Gal d2), Ovotransferrin (Gal d3) and Lysozyme	egg white protein
Limits (detection/ quantitation)	detection: 0.10 ppm quantitation: 0.5 ppm	detection: 1 ppm quantitation: 2.5 ppm	detection: 2.1 ppb quantitation: 0.5 ppm
Result Expression Unit	ppm of whole egg powder	ppm of dried whole egg	ppm of egg white protein
Extraction (water bath)	at 60 °C for 1 min.	at 60 °C for 15 min with agitation. Use additive.	at 60 °C, 25 min with agitation.
Incubation conditions (minutes)	10 at room temperature.	10 at room temperature	with agitation 30 for incubation 1 and 10 for subsequent incubations
Washes (# per incubation)	3	5	3
Reading wavelength (nm)	450	650	450

Sources / Fuentes: *r-biopharm (2022); **Neogen (2018), ***3M (2017b).

The absorbance measurements were incorporated into the software provided by the manufacturers of the ELISA kits. Both the R-biopharm and Veratox-Neogen software allow for the inclusion of applied dilution factors, and the results are reported in ppm after considering these factors. However, there are specific considerations for the kits of 3M whole bovine milk kit and for egg white protein 3M, they indicate that the dilution factor is 100, more than any other dilutions that can be made. These data cannot be included in the software that the manufacturer provides and therefore, calculations must be made separately, and results must be converted from ppb to ppm (equations 1,2,3).

ppb calculated by software from absorbance * 100 = ppb considering dilution kit (1)
ppb considering dilution kit * additional dilution factor = ppb quantified in the sample (2)
ppb obtained / 1000 = ppm quantified in the sample (3)

Regarding the quality of analysis, all tests were conducted with R-biopharm and Veratox-Neogen kits, presented a satisfactory statistical performance Z (less or equal to 2 or -2) with respect to the obtained value in the interlaboratory round FAPAS 27204, 2017 for both allergens. For 3M kits, there were no data of this type, hence, the quality parameter was that the percentage of quantitation of the reference material was in an acceptable range for ELISA tests (50-150 %) (Abbott et al., 2010).

For assessing the geometry effect, a randomized complete block experimental design was employed, where the block was represented by each batch, which in turn coincided with each repetition. A 3x2 factorial arrangement was used, with the following factors: analytical kits (3 different kits), geometry (2 different geometries). The response variable was the percentage of quantitation regarding the concentration of allergen detected in the raw dough. An ANOVA was performed with a significance level of 5 % and the significances of the simple effects of factors and their interactions were evaluated.

For assessing the effect of baking, the experimental design consisted of randomized blocks of a single factor, which corresponds to the kit on three levels (3 different kits) since the difference between raw dough and baked (processing time) was calculated. The response variable was the difference in the percentage of quantitation between the moments of processing (before and after the baking) and an ANOVA was performed with a significance level of 5 %. Both experiments were conducted with four repetitions and each repetition included two replicates. The statistical analysis was conducted using JMP® Pro 9.0.2 software.

Results

Allergen residues were detected by all kits in every case. As shown in Table 3, the recovery of egg residues from baked crackers for the effect of geometry showed a significant difference is observed (P=0.0228), regardless of the kit used. Specifically, less egg allergen was recovered from the XL cracker compared to the traditional one. This effect is consistent across all three kits. However, there was no significant difference in the quantitation of milk allergen based on geometry (P=0.4335), nor was there a difference related to the type of cracker or the kit used for measurement (P=0.4302). The high power obtained for this test (1-β=1.0000) suggests that the lack of difference is not due to data variability.

For the effect of processing time on the quantitation of egg, no significant difference was found among the kits (P=0.4245), and this lack of significance is not due to data variability (power of the test 1-β= 1.0000). It can be affirmed that the effect of the baking is equally presented regardless of the kit used. It is important to note that the quantitation of egg after baking is extremely low (between 4 and 5 %). Regarding the quantitation of milk after baking, a significant difference among the kits (P=0.1682) was not found. However, in this case, when calculating the power of the test (1-β= 0.1079), it is evident that the non-significance is attributable to the variability of the data between and within kits (Table 4).

Table 3. Quantitation percentage of egg and milk allergens with regards to the incurred raw dough for crackers with traditional geometry and XL geometry, Compañía de galletas Pozuelo DCR. S.A, San José, Costa Rica, 2020-2021.

Cuadro 3. Porcentajes de cuantificación de los alergenos huevo y leche con respecto a la masa cruda enriquecida para galletas con geometría tradicional y geometría XL, Compañía de galletas Pozuelo DCR. S.A, San José, Costa Rica, 2020-2021.

Allergen	Kit	R-Biopharm	Veratox	3M
	Geometry	Average[1] [1] ± IC (n=4)		
Egg	Traditional	4 ± 2[a]	4 ± 2[a]	5 ± 2[a]
	XL	3 ± 2[b]	2 ± 1[b]	3 ± 2[b]
Milk	Traditional	50 ± 20[a]	40 ± 20[a]	90 ± 50[a]
	XL	50 ± 40[a]	40 ± 20[a]	50 ± 20[a]

[1]For each allergen, different letters in a same column indicate significant differences ($p<0.05$). / [1]Para cada alérgeno, letras diferentes en una misma columna indican diferencias significativas ($p<0,05$).

Table 4. Difference in the percentage of quantitation between the moments of the process (raw dough and baked crackers) for milk and egg allergens, Compañía de galletas Pozuelo DCR. S.A, San José, Costa Rica 2020-2021.

Cuadro 4. Diferencia en el porcentaje de cuantificación entre los momentos del proceso (masa cruda y galletas horneadas) para los alérgenos de la leche y el huevo, Compañía de galletas Pozuelo DCR. S.A, San José, Costa Rica 2020-2021.

	Kit	R-Biopharm	Veratox	3M
Allergen	Geometry	Average[1] ± IC (n=4)		
Egg	Traditional	96 ± 2[a]	96 ± 2[a]	95 ± 2[a]
Milk	Traditional	49 ± 20[a]	63 ± 20[a]	14 ± 50[a]

[1]For each allergen, different letters in a same row indicate significant differences ($p<0.05$). / [1]Para cada alérgeno, letras diferentes en una misma fila indican diferencias significativas ($p<0,05$).

Discussion

In the present study, the smallest crackers exhibited better quantitation of egg allergens. This observation may be attributed to the fact that under industrial conditions, heat distribution is not entirely uniform. In larger size cookies, the positioning leaves wider gaps in the external areas, resulting in greater exposure to warm air flows compared to crackers with traditional geometry. Importantly, the baking conditions remained consistent in terms of temperature and time across all crackers (Gomaa & Boye, 2013). On the other hand, for the quantitation of the milk allergen, it was found that there is no significant effect of the geometry of the cookie.

In the context of post-baking allergen recovery, no significant difference was found between the kits. However, the low quantification of this post-baking allergen is noticeable across all the kits studied. This low quantitation has been described in several studies (Gomaa & Boye, 2013; Khuda et al., 2012; Török et al., 2014). A specific study investigated the detection and quantitation of eggs using five different ELISA kits, including two commercial brands evaluated in the present study. The study found that, when it came to sweet cookies, none of the kits adequately quantified egg protein in baked cookies in terms of measured mean concentrations and percent recovery. Moreover, the study revealed that detected levels of egg protein dramatically decreased after 30 min of baking time, with kit recoveries ranging from 3.5 to 20.5 % on average (Khuda et al., 2012).

In the present study, more than 95 % of the egg quantitation was lost during baking using the three evaluated kits, which aligns with findings from other studies. For instance, one study reported recoveries ranging from 8 to 48 % (resulting in a loss of 92 and 52 % of quantitation) for one kit, while the other kit exhibited losses of 96 to 100 % recovery, depending on the heat treatment applied (Gomaa & Boye, 2013). The effectiveness of ELISA kits hinges on two critical factors: efficient protein extraction from the matrix and accurate antibody recognition of the allergen (Abbott et al., 2010). The thermal processes significantly impact egg allergens quantitation due to reduced recognition of the native protein modified by the antibodies and/or the decreased protein solubility (EFSA, 2014).

The challenges associated with extracting allergen proteins using extraction reagents from ELISA kits in processed matrices have been thoroughly investigated. This is particularly evident in the case of cookies (Nguyen et al., 2019). It is an issue that warrants consideration in the enhancement of ELISA commercial kits for egg quantitation. Regarding the extraction substance, only one kit explicitly specifies the use of phosphate buffered saline (PBS). However, the other two kits do not provide information about the type of substance used in the insert. Notably, PBS is the most employed extraction medium in ELISA kits at the commercial level (Senyuva et al., 2019). Given the the low percentage of recovering obtained after heat treatment in the present study, it is reasonable to presume that the extraction substance may be a contributing factor to these results.

Regarding the recognition of the allergen proteins, commercial kits use polyclonal antibodies, as is the case with those used in this study. The effect of monoclonal antibodies has also been investigated, finding some advantages such as homogeneity, consistency and high specificity compared to polyclonal antibodies. In one study, recovery percentages for egg allergens in processed products ranged from 61.6–89.3 %, using a kit with monoclonal antibody (Kato et al., 2015), showing better results when using a monoclonal antibody than those found in the present research. A question arises about the use of this antibodies type in commercial ELISA kits versus polyclonal antibodies. The low recovery of allergens in processed matrices and discrepancies between the results from different kits complicate the interpretation of the results (Shoji et al., 2018), therefore studies such as this one serve as a baseline to formalize analytical methods at the regulatory level.

It is crucial to comprehend the factors behind the results obtained in this study, particularly because it was conducted in a real-world industrial setting and reflects the information that will be provided to consumers through food labeling. One of the significant advancements in Japanese food allergen regulation is the establishment of official ELISA methods. These methods are the outcome of extensive research and have led to a revised version of the assay for detecting egg allergens. This updated version incorporates a sample extraction solution that utilizes the detergent sodium dodecyl sulfate (SDS) and the reducing agent 2-mercaptoethanol (2ME). These components enhance the solubilization of food allergen proteins from the food matrix. As a result, the new extraction procedure enables the detection and quantitation of egg allergens even in highly processed foods (Shoji et al., 2018).

Another aspect to highlight is that despite the limited quantification of the egg allergen in the studied crackers, the natural variability observed during, experiments with egg among the 3 kits, was well-controlled compared to the results obtained for milk. When it comes to the impact of baking on milk allergen quantification, it is widely recognized that ELISA kits yield highly variable outcomes. In this specific case, a significant degree of variability was evident in the quantification process. This variability arises from several factors that can interfere with the results, as consistently observed throughout our investigation. The inherent variability of the kits prevents clear differentiation between them. However, it is worth noting that allergen quantification tends to decrease after baking. The mere fact that the acceptable recovery percentage for ELISA kits ranges from 50 to 150 % (Abbott et al., 2010) serves as an indicator of the substantial variability that can be encountered.

It has been noted that commercial ELISA kits used for quantitative assays exhibit variations in extraction substances, calibration procedures, and antibodies quality across different brands and batches. The main limitations associated with these kits include matrix effects, insufficient protein extraction, lack of specificity due to cross-reactions, and inadequate result reproducibility (EFSA, 2014). Similar findings were observed in another study

that evaluated casein recovery after baking sweet cookies using two ELISA kits and one flow cytometry kit. The recovery range for casein was 89 % to 35 % for the Ridascreen kit, 77 % to 21 % for the Veratox kit, and 75 % to 19 % for flow cytometry for casein. These results highlight the significant variability in recovery rates (Gomaa & Boye, 2013).

As evident, variability is not exclusive to ELISA tests; it also occurs with other analytical methods. While both the Veratox kit and flow cytometry yielded lower recoveries compared to the Ridascreen kit, these differences were not statistically significant, except for the small and medium samples baked for 15 min (Gomaa & Boye, 2013). This finding aligns with what was found in the present research regarding the lack of any significant differences between milk kits. This lack of differentiation can be attributed to the inherent variability in the data. Furthermore, another study investigating milk quantitation using five ELISA kits in sweet cookies revealed a wide range of recovery percentages: 2 to 68 % for casein and from 0 to 48 % for β-lactoglobulin. The highest recovery percentages reported by this study correspond to the Morinaga trademark (Khuda et al., 2012). However, Morinaga, was not evaluated in this research because it is not distributed in Costa Rica.

It has been observed that changes in allergenic proteins (chemical and conformational modifications) leading to a decrease in their quantitation occur within the initial minutes of baking. Similarly, as seen with egg, there is a reduction in protein solubility during the process. Food processing is widely recognized to impact the integrity of allergenic proteins, inducing chemical alterations and alterations in their three-dimensional conformation (Monaci et al., 2011). The question arises: Does non-detection or reduced detection of the allergen imply a reduction in its allergenic potency? Unfortunately, it cannot be affirmed that this decrease in detection implies a decrease in the allergenic capacity of the protein (EFSA, 2014).

In certain cases, an improvement in tolerance has been observed among individuals with food allergies when consuming foods that have undergone thermal processing (Liu et al., 2013), particularly baking (Bavaro et al., 2019). However, it is essential to recognize that this phenomenon does not apply universally to all individuals. Given this variability, it becomes crucial to ensure that methods for detecting and quantifying allergens in food are dependable. The results of such analyses significantly impact decisions related to labeling traces, validation of surface cleaning procedures, and overall compliance with national and international regulatory standards.

Interestingly, routine allergen testing within the food industry relies on commercially available test kits. However, vendors often provide minimal details about the characteristics of these kits due to proprietary information (Senyuva et al., 2019). In Costa Rica, the supply of ELISA kits for milk and egg is limited, and most of the kits contain limited analytical information in the protocols provided. For instance, they do not specify whether the antibody used is monoclonal or polyclonal. Out of the six kits studied, only two indicated the type of extraction substance. This limitation hinders informed decisions regarding kit selection based on the specific matrices to be analyzed.

Conclusions

The lack of recovery of protein residues based on cracker geometry was more evident for egg as compared to milk when using the kits employed in this study. The results regarding the effect of baking require attention because, in all cases, a significant decrease in the recovery of allergens was identified after baking. This decrease poses a risk for identifying allergens in food within the food industry, potentially leading to errors in the information provided to consumers about the presence of these allergens in food products. When selecting an enzyme-linked immunoassay tests kit, it is essential to seek technical information to ensure that the kit's characteristics align with the requirements, and its efficacy can be demonstrated. Kit supplier should provide more technical information on the extraction reagents, the antibodies used and the particularities of each kit, to be able to make better decisions when choosing an assay for the detection and quantitation of allergens in foods.

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Interests conflict

The authors of this study do not have any interest conflict.

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