



Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Detection of egg and milk residues on working surfaces by ELISA and lateral flow immunoassay tests

Patricia Galan-Malo ^a, María López ^b, Juan-Carlos Ortiz ^c, María D. Pérez ^b,
Lourdes Sánchez ^b, Pedro Razquin ^a, Luis Mata ^{a,*}

^a ZEULAB, Bari 25 Duplicado, 50197, Zaragoza, Spain

^b Departamento de Producción Animal y Ciencia de los Alimentos, Facultad de Veterinaria, University of Zaragoza, Miguel Servet 177, 50013, Zaragoza, Spain

^c Departamento de Sanidad, Distrito Hortaleza, Ayuntamiento de Madrid, Carretera Canillas 2, 28043, Madrid, Spain

ARTICLE INFO

Article history:

Received 30 August 2016

Received in revised form

28 October 2016

Accepted 21 November 2016

Available online xxx

Keywords:

Cross contact

Surfaces control

Swab

Hidden allergens

Beta-lactoglobulin

Ovalbumin

ABSTRACT

In the last few years awareness for allergen management has increased among operators in the food chain. Since a small quantity of allergenic food can trigger an allergic reaction, accurate information in food product labels is essential for people who suffer from food allergy. A key point to reduce the risk of uncontrolled allergen contamination is to implement a strict and validated cleaning process. In this work a sampling method based on swabbing has been optimized with two analytical methods (ELISA and lateral flow tests) for milk and egg analysis on surfaces. Swab material, extraction buffer composition, temperature, time for recovery and the quantity of the allergen residue on the surfaces have been evaluated and a specific procedure was proposed. The new method has demonstrated a good performance to recover egg or milk proteins from two typical working surfaces (stainless steel or Formica). Egg and milk powder were detected by ELISA at a level as low as 0.04 and 0.2 µg, respectively. However, the detection level with lateral flow tests rose to 0.07 µg for egg powder and 0.6 µg for milk, depending on the kind of surface. On average, repeatability of swabbing method ranged from 18 to 32%. Both methods could be applied for validation the cleaning processes and for a routine verification after cleaning.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Food allergy is recognized as an important public health issue. Prevalence of food allergy in Europe has been estimated between 3 and 4% in both children and adults (EFSA Panel on Dietetic Products, Nutrition and Allergies, 2014). According to World Allergy Organization (WAO), between 240 and 550 million people over the world suffer from food allergy (Pawankar, Canonica, Holgate, Lockey, & Blaiss, 2013).

Although more than 160 food ingredients have been documented to cause allergic reactions, the Allergen Labelling and Consumer Protection Act (FALCPA) has identified the most common allergenic foods which account for 90% of food allergic reactions (Hefle, Nordlee, & Taylor, 1996). Individual and environmental factors produce geographical variation in prevalence (EFSA Panel on Dietetic Products, Nutrition and Allergies, 2014). Age is also an

important factor being children more susceptible to suffer allergy against cow milk, egg, peanut, fish and nuts. This distribution can be explained by the early introduction of these foods into the child's diet. In some cases, tolerance can be developed in the adulthood (Fernández-Rivas, 2009). Since very little amounts of an allergen can trigger an allergic reaction, sensitized people must avoid the intake or exposition to the allergens as the most effective caution (Taylor et al., 2014). To protect consumers from the harmful effects of food allergens, the European authorities have established a legislation framework to ensure consumers are appropriately informed as regards the ingredients of the food they consume (Regulation EU 1169/2011). Among other indications, food labels should include information about the presence of food ingredients with allergenic properties. In this context, the inclusion of any of the 14 allergens that are on the regulatory list (Regulation EU 1169/2011) is mandatory.

In order to assure the accurate labelling on allergen content, food industry, distribution and restoration must implement allergen control plans (ACP) inside their Hazard Analysis Critical Control Points system (HACCP). Two out of the six main

* Corresponding author.

E-mail address: lmata@zeulab.com (L. Mata).

components of ACPs are the prevention of cross-contact, also named cross contamination, during processing operations and the use of validated cleaning procedures for equipment (Jackson et al., 2008). Allergen cross-contact is produced mainly by allergenic protein residues that remain on working surfaces after the cleaning process (Jackson et al., 2008; Sakiyama, Sato, Tsuda, Sugiyama, & Hagiwara, 2013). Since these “hidden” allergens may not be controlled through a visual inspection it is very difficult to avoid their presence in final products. Therefore, the effectiveness of the cleaning process should be assessed after each manufactured batch by using appropriate and validated methods.

To protect consumers against the presence of eventual hidden allergens, manufacturers can label their products with the precautionary allergen statement “may contain”. However, indiscriminate use of preventive labelling restrict people choice and could undermine the valid warnings (Schubert-Ullrich et al., 2009). For this reason, food chain operators should consider all the possible countermeasures to prevent cross contamination in order to avoid the use of the precautionary label. Only when the probability of allergen cross contamination is significant, meaning a high risk to the allergic consumer, then the statement “may contain” becomes a useful risk communication tool (Muraro et al., 2014; Reese et al., 2015). In this respect, the U.S. Food and Drug Administration (FDA) has stated that precautionary labelling cannot be used as a substitute for Good Manufacturing Practices (U.S. Food and Drug Administration, 1996). According to EU legislation all the stakeholders must share responsibility in protecting allergic consumers and “may contain” statement should not be applied without a thorough risk allergen management included in their HACCP systems (Muraro et al., 2014; Reese et al., 2015).

Analysis of surfaces is a useful tool to monitor the cleaning effectiveness and to perform mapping studies for cross contamination in food facilities. However, until now the available methods to detect allergens on surfaces have been scarcely characterized. Traditionally, the visual inspection has been the most applied technique for this purpose in food industry. Albeit this approach is a good alarm signal in a first step, negative results should be confirmed by analytical methods (Jackson et al., 2008). Besides visual inspection, samples should be taken with a swab on surfaces after the cleaning to provide an evidence of the protocol effectiveness at the process point. Thus, Schlegel, Yong, and Foo (2007) reported the use of a swabbing technique combined with an unspecific protein analysis based on a colorimetric technique or SDS-PAGE to assess the recovery of peanuts proteins. However, due to their laboriousness, these methods are not suitable for routine control in food manufacturing plants. Alternatively, methods based on ATP detection have been also used as a good hygiene indicator but they do not specifically detect allergenic proteins and their use is limited to wet-cleaned surfaces (Jackson et al., 2008). Moreover, these methods are prone to interferences by ATP from the microflora in water supply and need to establish the ATP background levels at each facility (Shama & Malik, 2013).

Taking into account that allergen reactions are triggered by specific proteins, the analytical methods for detecting those specific proteins are more adequate when applied in allergen risk management (Taylor et al., 2006). Hence, food companies should use quantitative ELISA methods to validate cleaning effectiveness since they achieve a suitable level (milligram per kilogram) of detection (Crevel et al., 2014; Jackson et al., 2008). Once the cleaning process validation is completed, the use of qualitative tests such as lateral flow strips and specific swabs could be employed as the most simple, rapid and cost-effective method for routine monitoring (FoodDrinkEurope, 2013; U.S. Food and Drug Administration, 1996). It seems clear that food industry needs rapid, easy and reliable methods to verify the absence of allergens on the food-contact

surfaces. However, such methods need be validated before they are implemented in the routine control of food industry.

In addition, the analysis of allergens on surfaces should focus on two steps: sample recovery (i.e. by swabbing) and analytical measurement. Both parts have to be performed together and evaluated as a whole. To our knowledge, no completely standardized method of swabbing for specific detection of egg and milk proteins has been described up to date. Some authors has compared different methods for allergen detection on surfaces but they were focused only on the analytical method step and the sample recovery from swabs was not evaluated (Al-Taher, & Jackson, 2007; Jones, Griffith, & Salter, 2006).

The aim of this work has been to optimize an allergen sampling procedure and its subsequent analysis by lateral flow immunoassay (LFIA) and ELISA methods to detect milk and egg allergens. Swabbing was optimized and the recovery and reproducibility of the methods were studied for two different kinds of surfaces.

2. Materials and methods

2.1. Egg and milk samples

Spray-dried whole egg specific for allergen detection (RM 8445) and Non-fat milk powder (SRM 1549) both from NIST (National Institute of Standard and Technology, USA) were used as egg and milk material, respectively. Both were prepared according to manufacture instructions; powder was diluted in distilled water at a concentration of 10 mg/ml. Quantitative ELISA results for egg analysis were expressed as whole egg powder concentration (ppm). Standards used in assay were calibrated against the reference material NIST RM8445 and therefore no conversion was needed. ELISA results for milk analysis were obtained as ppm of β -lactoglobulin. In order to transform results in equivalents of non-fat milk powder reference material, a conversion factor of 45 was empirically calculated. Thus, results for milk analysis in this work were expressed as non-fat milk powder.

2.2. Surface contamination

Different concentrations of allergens (egg or milk powder) were prepared in distilled water from the stock dissolution (10 mg/ml). A volume of 50 μ l from each allergen dilution was spread over two different kinds of surfaces, Formica (HPL according to standard EN-438:2005) or stainless steel (AISI-304). Then, samples were dried at room temperature during at least three hours before performing the swabbing procedures.

2.3. Sampling method

Once the sample was dried, swabbing was performed in a delimited surface of 100 cm² with the help of a hollow square card (10 $\times$ 10 cm). To improve the allergen recovery swab was dipped into 0.5 ml of the corresponding extraction buffer and this pre-wetted swab was rubbed in all possible directions on the surface. Afterwards, the swab was placed again in the extraction buffer and incubated for 30 min at room temperature unless otherwise specified. Four different extraction buffers were assayed: 0.1 M Tris-HCl pH 8.0, 0.15 M NaCl; 0.1 M Tris-HCl pH 8.0, 0.15 M NaCl, 0.02% Tween-20; 0.1 M Borate Buffer pH 9.0, 0.15 M NaCl and 0.1 M Borate Buffer pH 9.0, 0.15 M NaCl, 0.02% Tween-20. Several swab materials were also evaluated: foam (0.05 g of absorbent material) from VWR International Eurolab S.L, Spain; viscose (0.04 g of absorbent material) from Ltd Technical service consultants, UK; Nylon (0.06 g of absorbent material) from COPAN Diagnostics Inc., USA; cotton (0.04 g of absorbent material) and polystyrene (0.05 g of absorbent

material) from Deltalab, S.A., Spain. A surface without spiked allergens was also analysed in each experiment as negative control.

2.4. Analytical methods

Samples were qualitatively analysed by a LFIA test and quantified by ELISA tests based on the determination of ovalbumin or β -lactoglobulin.

LFIA strip tests: PROTEON BLG Express and PROTEON Egg Express (ZEULAB, Spain) were used for milk and egg detection, respectively. Once the sample was extracted as described above the strip test was placed in the sample extraction buffer during 10 min. Then, result was visually interpreted. If only a blue line was displayed the result was negative. When a second red line appeared the test was positive.

ELISA tests: PROTEON Milk and PROTEON Egg (ZEULAB, Spain) were used for milk and egg analysis, respectively. Assays were based on a previously developed method (de Luis, Lavilla, Sánchez, Calvo, & Pérez, 2009; de Luis et al., 2008). A volume of 100 μ l of samples or standards was added to wells of a 96 microtiter well plate coated with specific antibodies depending on each test. The plate was incubated for 30 min at room temperature. Then, wells were washed three times with the corresponding buffer. After that, 100 μ l of conjugate-antibody were added to each well and incubated for 30 min at room temperature. After three washes, 100 μ l of substrate were added and incubated for 30 min at room temperature. Finally, 100 μ l of stop solution were added to each well and then absorbance at 450 nm was measured in a Multiskan microtiter plate reader (Labsystem, Finland).

2.5. Swabbing performance testing

Performance of the swabbing procedure was evaluated by determining the recovery and the intra-laboratory repeatability of the corresponding allergens.

To calculate the recovery efficiency of the swabbing method for each allergen, several areas from the surfaces in study were spread with different amounts of allergen as was previously described. The concentration of allergens in extracted samples was determined by ELISA and the percentage of recovery was calculated using the following formula:

Recovery (%) = μ g of allergen (milk or egg) measured by ELISA/ μ g of allergen placed over the analysed surface $\times$ 100

Repeatability was estimated by analyzing six independent replicates at different levels of allergen (milk or egg) over two different kinds of surfaces using the ELISA test. For each of them, mean and coefficient of variation (CV) were determined. Detection of the allergens in the same samples was also determined by LFIA test.

2.6. Statistical analysis

Statistical analysis was performed with GraphPad 4.0 software by applying ANOVA test with a Bonferroni post-test.

3. Results

3.1. Selection of swabs for allergen recovery from surfaces

Five different swab materials were assayed in order to select those which have the highest ability to capture egg and milk allergens from two different kinds of surfaces: Formica and stainless steel. To test the performance of the swabs, a low amount of

allergens (0.08 μ g and 0.123 μ g of egg and milk powder, respectively) were used because in such conditions differences among materials could be more evidenced. As Fig. 1A shows, swab 3 presented the highest capacity of egg recovery from Formica, while swab 5 was the best to recover egg protein from stainless steel. Viscose and cotton materials showed a very low recovery in both surfaces (swabs 2 and 4). Great differences on egg recovery were evidenced between surfaces when swabs 1 (foam) and 3 (nylon) were used. Therefore, for practical reasons and considering results as a whole, swab 5 (polystyrene) was chosen for analyzing surfaces of stainless steel and Formica during the ongoing assays for egg analysis. Regarding milk (Fig. 1B), swab 5 displayed the highest capacity of recovery from both stainless steel and Formica surfaces. Thus, such material was also selected. In general terms, swabs made of viscose or cotton should be discarded because of their low allergen recovery capacity. However, swabs of foam, nylon and polystyrene could be used depending on the surface properties.

3.2. Selection of the optimal conditions for allergen recovery

Once the allergenic proteins have been adsorbed by the swab, they need to be released in an efficient manner and a suitable structural conformation to allow their detection by ELISA or LIFC tests. In this work, conditions favouring the allergen release from the swabs into an extraction buffer have been studied to achieve the highest recovery. Several conditions were assayed including the incubation time of the swab with the extraction buffer previous to analysis, the effect of shaking the sample during the incubation, the temperature of the incubation and the buffer composition. The effect of such variables on the recovery of milk and egg proteins was determined by ELISA.

Fig. 2 shows the effect of the incubation time of the polystyrene swab after sampling a stainless steel surface with or without shaking. An amount of 5 μ g of egg or 2.5 μ g of milk powder were spread on the surface in study and recovered with a swab by using 0.1 M Tris-HCl pH 8.0, 0.15 M NaCl as extraction buffer. A volume of 100 μ l was removed at different incubation times (10, 20, 30 and 40 min) either with or without previous shaking. Results after 40 min of incubation with shaking were assumed as the reference value (100% of recovery) because it was considered to be the most intensive condition. As Fig. 2 shown, both incubation time and shaking affected allergen recovery. After 30 min of incubation with shaking, recovery of egg and milk increased by 31 and 21% respectively with regards the incubation without shaking. However, extending the incubation step up to 40 min with shaking produced a slight decrease (around 20%) that could indicate protein degradation. When swabs were not shaken, the recovery of allergens increased steadily up to 40 min of incubation. Thus, it may be concluded that shaking during the incubation with the extraction buffers favours the release of allergens from the swab material. The effect was more noticeable for milk analysis in which a similar level of allergen release was reached after 10 min with shaking than after 40 min without it. Regarding egg analysis, shaking did not show any advantage in the range of incubation times evaluated. For practical reasons, inclusion of shaking in the final protocol can be avoided but bearing in mind that incubation times should never be less than 30 min.

Buffer composition could also have an impact on the recovery of allergens from surfaces. Two buffers with different pH or surfactants were tested for egg and milk recovery at room temperature and 40 °C. Buffer 1 was also used in previous assays to select the swab material and the incubation time (Figs. 1 and 2); hence it was referred as the control (100% of recovery). The highest recovery of egg protein was obtained with buffer 2 and 4, around 2 or 3 times more than the control for both Formica and stainless steel

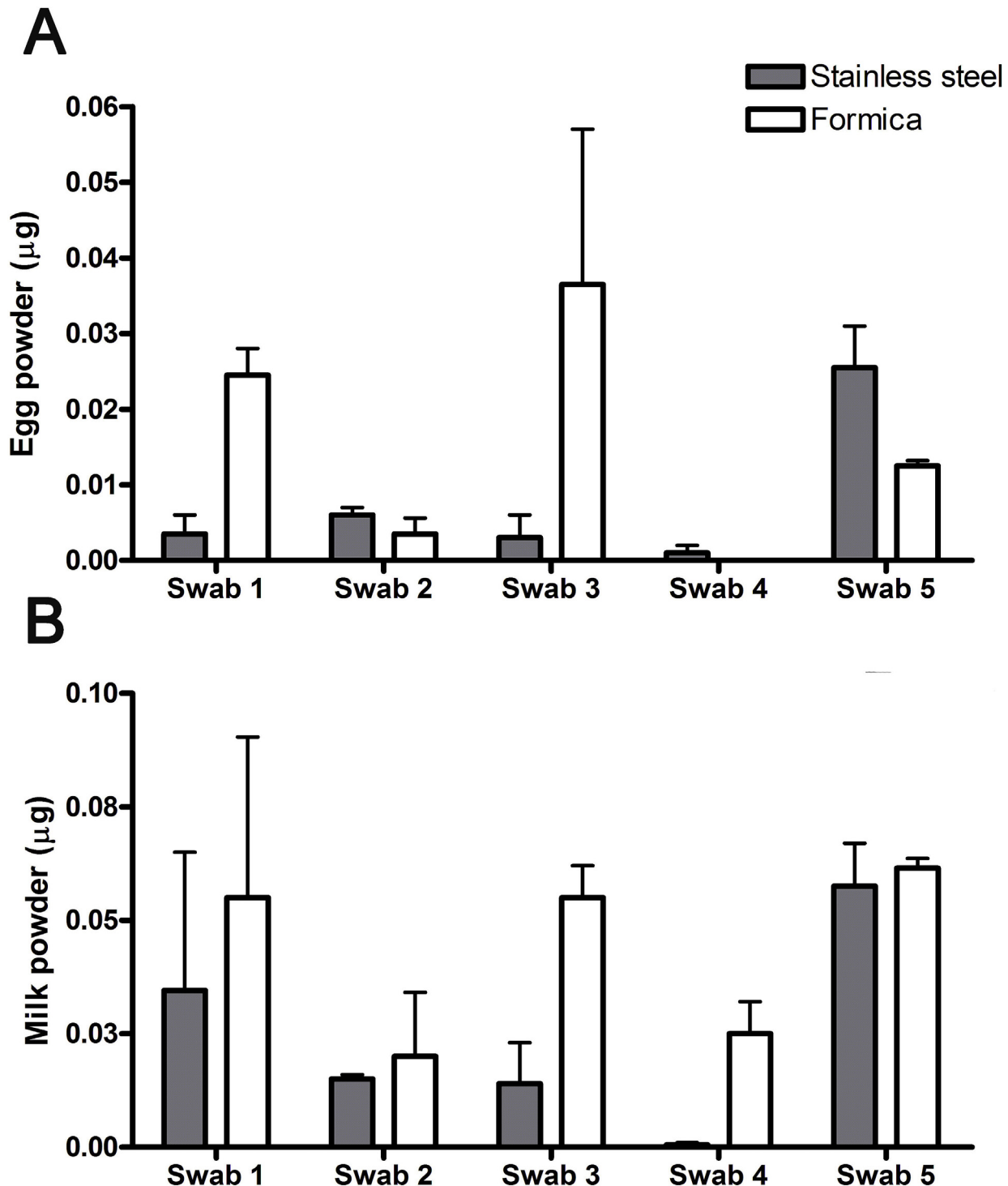


Fig. 1. Recovery of egg (A) and milk powder (B) from stainless steel and Formica surfaces with swabs made of different materials. Swab 1: foam. Swab 2: viscose. Swab 3: nylon. Swab 4: cotton. Swab 5: polystyrene. Results are the mean $\pm$ SD of egg or milk powder recovered (μg) from each surface from four independent experiments. Each surface area to be tested was spread with 0.08 μg of whole egg powder or 0.123 μg of milk powder and then dried for 3 h before applying the swabbing procedure.

respectively (Fig. 3A). For the subsequent assays with this allergen buffer 2 was selected. Similar behaviour was found for both surfaces at room temperature and at 40 °C for any of the buffers evaluated.

Analysis of milk (Fig. 3B) showed that recovery with all buffers was similar, although buffer 2 gave a slight improvement in recovery when incubation was performed at room temperature in stainless steel. Thus, this buffer was selected for the subsequent

assays with this allergen.

All together, results have demonstrated that performing the incubation step at 40 °C did not produce an improvement on allergen protein recovery. Consequently, the following assays were performed only at room temperature. On the other hand, addition of a surfactant (buffer 2 and 4) seems to improve the recovery of egg proteins either in stainless steel or Formica whereas this effect was not observed with milk proteins.

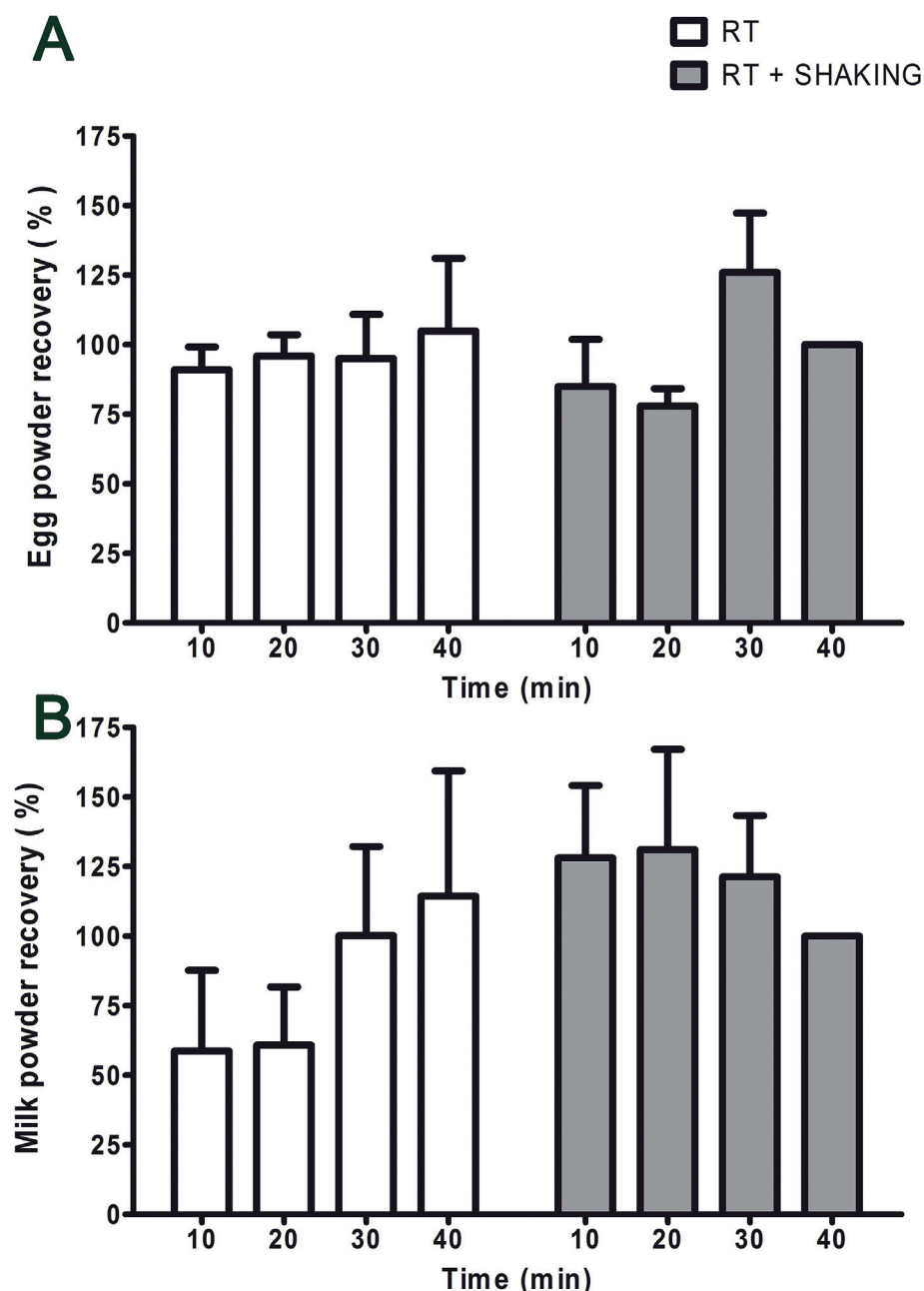


Fig. 2. Effect of the incubation time and shaking after swabbing on the allergen recovery. Stainless steel surfaces spread with egg powder (A) or milk powder (B) were analyzed at different incubation times at room temperature without shaking (bars in white) or with shaking (bars in grey). Results are the mean $\pm$ SD of three independent experiments.

3.3. Effect of consecutive swabbings on the recovery of allergens

Performing consecutive swabbing steps is a way to evaluate the efficacy of the whole swabbing process. To assess the final protocol, a stainless steel surface were spread with 2 μ g of milk powder or 0.8 μ g of egg powder and then, three consecutive independent swabbing samples were taken and analyzed by ELISA (Fig. 4). As expected, the maximum recovery was found with the first swabbing for both allergens. However, recovery was higher for egg (63%) than for milk (20%) proteins. Sum of 3 swabbing allowed detecting around 80% of egg proteins and 40% of milk proteins. The low rate of recovery found for milk proteins could be explained by a saturation of the swab capacity as it was later evidenced in Table 2 in which a very high recovery was found when 0.56 μ g of milk powder were placed on the stainless steel surface but recovery fell down when

levels higher than 1 μ g of milk powder were spread on the surface. However, it could not be excluded that a portion of proteins could remain tightly attached to the surface or it could not be totally released into the extraction buffer after swabbing and then remaining attached to swab material.

3.4. Evaluation of the whole method: swabbing and analytical test

Performance of the swabbing protocol was studied using a qualitative rapid test (LFIA) and a quantitative method (ELISA). Tables 1 and 2 show the efficacy of these methods for detecting egg or milk in stainless steel and Formica. Results are expressed as the percentage of recovery for ELISA tests and the ratio of positive results for LIFC tests ($n = 6$). Variability of the ELISA method is shown as the coefficient of variation (CV).

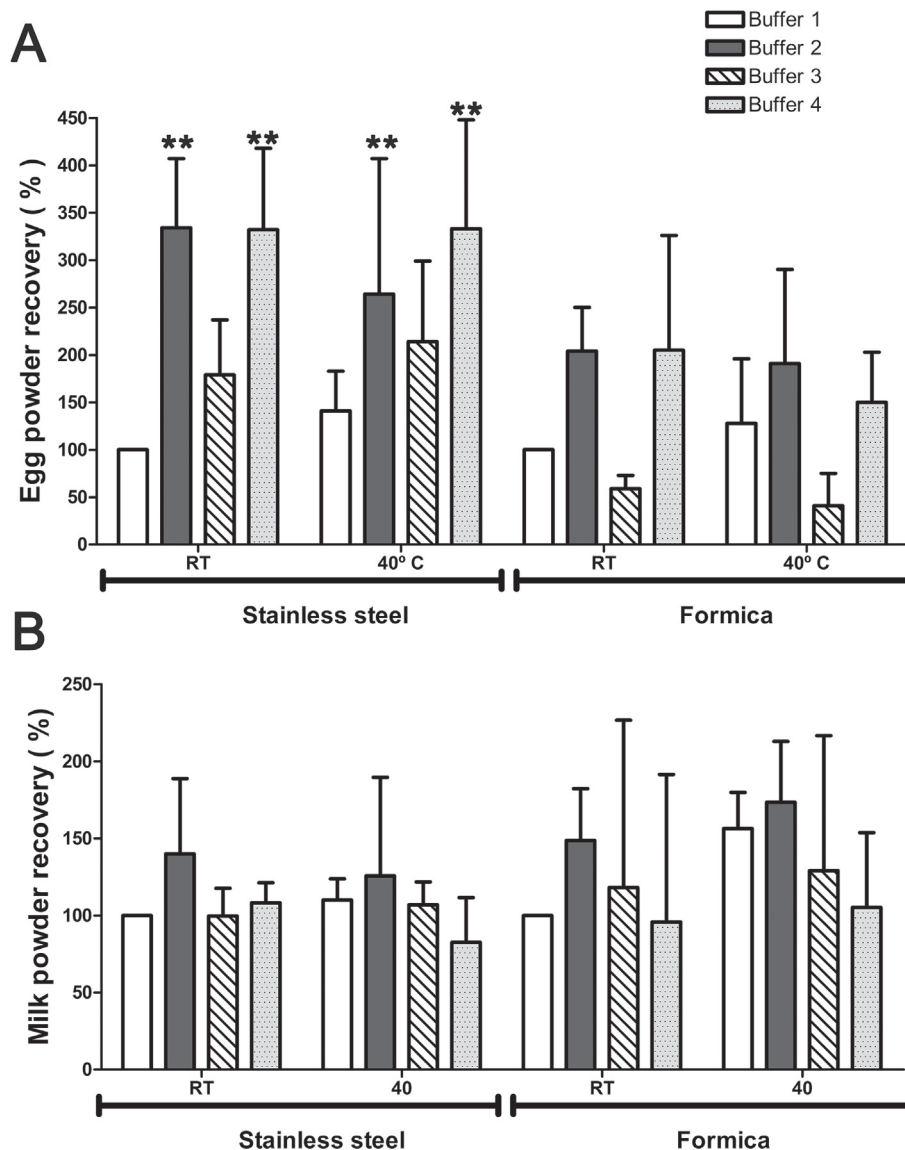


Fig. 3. Effect of the incubation temperature and the buffer composition after swabbing on the allergen recovery. Stainless steel or Formica surfaces spread with egg powder (A) and milk powder (B). Buffer 1: 0.1 M Tris-HCl pH 8.0, 0.1 M NaCl; buffer 2: 0.1 M Tris-HCl pH 8.0, 0.1 M NaCl, 0.05% tween-20; buffer 3: 0.1 M borate buffer pH 9.0, 0.1 M NaCl; buffer 4: 0.1 M Borate buffer pH 9.0, 0.1 M NaCl, 0.05% tween-20. At least three independent assays were performed for each condition including different days and analysts. Samples were analyzed by ELISA. Results are the mean $\pm$ SD. RT: Room temperature. (*) $p > 0.05$, (**) $p > 0.01$.

ELISA test was able to detect at least 0.04 μg of egg protein in both stainless steel and Formica (Table 1) whereas no positive results were obtained by LFIA at such level. At 0.07 μg of egg powder 5 out of 6 samples gave a positive result by LFIA test. These results are the normal consequence of the higher sensitivity of ELISA compared to LFIA. On average, repeatability of swabbing method for egg proteins was similar on stainless steel and Formica (28 vs. 32%) and no level-dependence relationship was observed. Amounts of egg powder up to 0.75 μg were almost totally recovered. However, recovery decreased at higher doses on either Formica or stainless steel.

In general, swabbing process showed good recovery and repeatability at low levels of powder milk (less than 1 μg) on both surfaces but the recovery decreased when the amount of milk was increased at higher levels (Table 2). This could be explained because the capacity of swab to uptake proteins was exceeded and at high levels of allergen no more protein could be adsorbed to the swab material. Thus, it could be inferred that the maximal capacity of

swab to uptake the β -lactoglobulin (the target of the analytical tests) in milk powder was between 0.5 and 1 μg . This level was in the same range which that found for egg proteins and therefore, it could evidence that the maximum capability of swabs to uptake proteins was around 0.5 to 1 μg . ELISA test was able to detect 0.16 μg of milk powder in both stainless steel and Formica. However, LFIC test was not able to detect 0.56 μg of milk powder in stainless steel or 0.16 μg in Formica. As indicated previously it seems a normal consequence of the different sensitivity between the two methods. In the same way that for egg powder, variability of swabbing was similar on stainless steel and Formica (average of 19 vs. 18%) and no clear level-dependency was found.

4. Discussion

The aim of this work was to optimize and evaluate the performance of a method for the detection of egg and milk proteins from surfaces which combines a swabbing procedure with an ELISA or

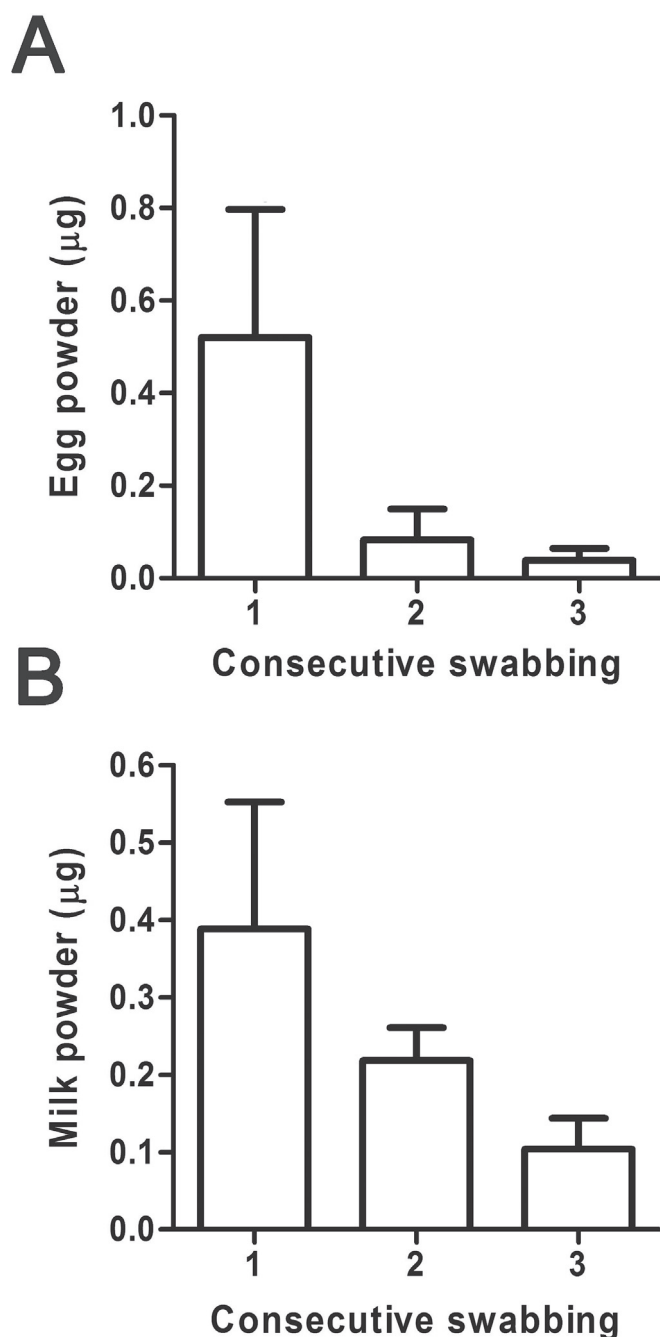


Fig. 4. Effect of three consecutive swabbing on the recovery of egg (A) and milk (B) proteins from a stainless steel surface. Results, expressed as total quantity (μg), are the mean $\pm$ SD of four independent experiments.

LIFC assay. In the last years, food production operators have become aware about allergen cross contamination to assure an accurate food labelling on allergen content (Taylor et al., 2006). To accomplish with this task they need reliable methods to validate and verify the cleaning procedures and the efficiency of anti-cross contact measures. According to several authors, allergen risk management can be approached with classical toxicology principles in a similar way to that followed in pharmaceutical industry (Hattersley, Ward, Baka, & Crevel, 2014; Madsen et al., 2009; Spanjersberg, Knulst, Kruizinga, Van Duijn, & Houben, 2010; Spanjersberg, Kruizinga, Rennen, & Houben, 2007). In this context, the ideal sampling method has to be capable of recover the allergen from a cleaned surface and to detect efficiently the allergenic targets proteins (Yang, Burson, Feder, & Macdonald, 2005).

In the present work, a similar approach to that implemented in pharmaceutical industry to control cross contaminations was followed. An allergen sampling method was developed that combines a swabbing technique with an ELISA or LFIA test to detect egg or milk residues on surfaces. When a swabbing method is developed, two key steps have to be considered. The first is the capacity of swabs to uptake the maximal quantity of the target allergenic proteins from a surface. The second is the ability of the method to release the proteins to a buffer in order to be detected for a selected analytical method. Both of them are complementary and should be considered to obtain the best swabbing performance.

As it was seen in previous sections, recovery of allergens depends on many factors including the nature of allergen, the properties of the surface material, the ability of swab material to uptake specific proteins and to release them in the adequate buffer and the swabbing procedure itself. Therefore, several elements in the swabbing process were optimized before the whole system was evaluated, including the selection of the appropriate material that the swab is manufactured, the specific buffer to release the maximum quantity of egg and milk allergens from swabs and the protocol conditions to get the highest recovery in a suitable structural conformation to allow detection by ELISA or LIFC tests. Our results have shown that swab material from which the swab is manufactured is one of the most important factors in allergen recovery. Swab material should be able to uptake allergens from a surface and then release them into an extraction buffer for the subsequent analysis. Food Allergy Research and Resource Program (FARRP) guidelines about allergen swabbing also warned that all kind of swabs are not effective in allergen testing. Thus, cotton swabs used for cosmetic or personal hygiene can be made of material that could contain allergens and therefore they could give false-positive results during testing (Baumert & Taylor, 2013). Swabs specially designed for clinical uses can be made of different materials to be adapted to different applications, thus, the correct choice depends on a previous evaluation of suitability for allergen recovery. In addition to the swab material selection, the recovery can be also influenced by the interactions among proteins and

Table 1

Recovery and repeatability of the egg analysis from stainless steel and Formica with ELISA and LFIA tests. LFIC test results are shown as percentage of positives ($n = 6$).

Egg (μg)	Stainless steel			Formica		
	ELISA		LFIC	ELISA		LFIC
	Recovery (%)	CV (%)	Positives (%)	Recovery (%)	CV (%)	Positives (%)
0.04	100	19	0	100	31	0
0.07	77	29	83	77	69	83
0.17	112	32	100	101	34	100
0.30	79	33	100	91	26	100
0.75	77	17	100	77	20	100
1.5	34	40	100	60	14	100

Table 2
Recovery and repeatability of the milk analysis from stainless steel and Formica surfaces with ELISA and LFIA tests. LFIC test results are shown as percentage of positives (n = 6).

Milk (μg)	Stainless steel			Formica		
	ELISA		LFIC	ELISA		LFIC
	Recovery (%)	CV (%)	Positives (%)	Recovery (%)	CV (%)	Positives (%)
0.16	63	15	0	130	23	0
0.56	114	7	0	81	3	100
1.01	17	27	100	32	19	100
2.42	16	35	100	33	8	100
3.00	24	19	100	31	15	100
12.48	35	11	100	41	42	100

surfaces themselves. Protein adsorption to surfaces depends on the nature of the binding forces such as hydrophobic or electrostatic (Nakanishi, Sakiyama, & Imamura, 2001; Thammathongchat, Hagiwara, & Sakiyama, 2010). In fact, it has been demonstrated that some products such as citric acid can prevent adsorption of egg white protein to stainless steel (Hagiwara et al., 2015). Food matrix composition can also affect allergen structure and therefore modify its interaction with surfaces (Rabe, Verdes, & Seeger, 2011). Therefore, the recovery of allergens when applying a swabbing method could be compromised by the type of surface and the characteristics of food residues on it. Some authors have reported different interactions between glass, Formica or stainless steel and ovomucoid (Al-Taher, & Jackson, 2007). In our study Formica and stainless steel surfaces were selected because of their wide use in food industry or catering facilities. Surfaces as glass were discarded because others authors have found low allergen protein adsorption (Al-Shabib, 2014) and that material is not widely used in food industry. Urethane, Teflon and Stainless Steel seems to present similar difficulties to be cleaned with wipes (Jackson, & Al-Taher, 2010). For instance, it has been reported that detection of milk and egg residues on urethane and Teflon was performed by ELISA with a similar efficiency as on stainless steel (Al-Taher, & Jackson, 2007; Jackson et al., 2008). As it was mentioned above, food matrices can affect the adsorption of allergenic protein, thus egg and milk powders were preferred as a food matrix instead of specific purified allergens such as β -lactoglobulin or ovalbumin since they are certainly real samples.

The performance of the analysis of allergens from surfaces has to consider not only the effectiveness but also the easy and rapid application by food industry staff. The final method consists of a specific swab material together a specific buffer to capture and release allergenic proteins, and finally a procedure to perform the analysis of allergens. Our results have shown that not all allergens are captured from the surfaces and released at the same level of effectiveness with the same buffer. Thus, specific conditions have to be determined for each allergen and even the specific surface. An assessment of the performance of the swabbing protocol was done and ELISA and LFIA tests were compared with the same sampling method procedure. Milk recovery from Formica was slightly higher than that from stainless steel (58 vs. 45% on average), whereas no difference was observed for egg samples. Other authors have described similar allergen recoveries for egg and milk from urethane, Teflon and stainless steel surfaces (Al-Taher, & Jackson, 2007). The percentage of recovery could also be related to the quantity of food material spread on the surface. Thus, levels up to 0.56 μg of milk powder presented at least 60% of recovery and levels up to 0.75 μg of egg powder at least 70%. A recovery of 68% was found for peanut extracts at a similar level (1.2 μg) from a stainless steel surface (Schlegel et al., 2007). However, when food powder was deposited on the surface at higher levels the recovery was clearly reduced. As the remaining material could be recovered in consecutive analysis, this decrease seems to reveal a saturation

effect of the swab.

Other important issue to be studied in order to determine cross-contact contamination is the minimum quantity of allergenic proteins that can be detected by ELISA or LFIA tests in combination with a suitable swabbing method. According to the technical specifications of the ELISA test, theoretically, up to 0.013 μg of egg powder and 0.09 μg of milk powder can be quantified if the swab could capture all the allergenic proteins spread on the surface. In our work, ELISA method was capable to detect 0.04 μg of egg and 0.16 μg of milk powder whereas around 0.07–0.17 μg of egg and 0.5–1 μg of milk powder could be detected by LFIA depending on the kind of surface. Therefore, factors such as the strength of the binding of proteins to the surfaces or to the swab could compromise the allergen recovery of the system. In any case, detection levels found in this work are lower than those described by other authors (Al-Taher, & Jackson, 2007; Al-Taher, Jackson, & Salter, 2007) and they seem to be adequate to protect consumers from harmful effects of cross-contaminations.

From a practical point of view, LFIA tests could be applied as a routine analysis to check out whether the cleaning procedures have been correctly performed and countermeasures against unintended allergen presence during manufacturing are suitable. However, initial validation of cleaning procedures should be performed by ELISA test due to its higher sensitivity. Periodically or when relevant changes in the cleaning process are introduced an evaluation using the ELISA tests should be performed again. The adequate use of these tools would allow to improve the allergen risk management plans in food industry and to reduce precautionary labelling based on analytical data.

Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

- Al-Shabib, N. A. (2014). Adsorption of ovomucoid as allergenic protein onto surfaces. Presented at the 2014 international conference on food and nutrition technology, Hong Kong, China.
- Al-Taher, F., & Jackson, L. S. (2007). Comparison of visual inspection, an allergen-specific method (ELISA) and nonspecific methods (sensitive ATP and total protein) to detect the presence of allergenic food residues on food contact surfaces. Presented at the IAFP annual meeting, Orlando, Florida.
- Al-Taher, F., Jackson, L., & Salter, S. (2007). Detection of dried egg residues on a stainless steel surface using ELISA and sensitive ATP assays. Presented at the (abstract) annual meeting of the Institute of food technologists, Chicago, Illinois.
- Baumert, J. L., & Taylor, S. L. (2013). *Best practices with allergen swabbing*. Food Safety Magazine. Retrieved from <http://www.foodsafetymagazine.com/magazine-archive1/junejuly-2013/best-practices-with-allergen-swabbing/>.
- Crevel, R. W. R., Baumert, J. L., Baka, A., Houben, G. F., Knulst, A. C., Kruizinga, A. G., et al. (2014). Development and evolution of risk assessment for food allergens. *Food and Chemical Toxicology*, 67, 262–276.
- EFSA Panel on Dietetic Products, Nutrition and Allergies. (2014). Scientific opinion on the evaluation of allergenic foods and food ingredients for labelling purposes. *EFSA Journal*, 12(11), 3894–4180. <http://dx.doi.org/10.2903/efsa>

- jefsa.2014.3894.
- Fernández-Rivas, M. (2009). Food allergy in Alergológica-2005. *Journal of Investigational Allergology & Clinical Immunology*, 19(Suppl 2), 37–44.
- FoodDrinkEurope. (2013). *Guidance on food allergen management for food manufacturers*. http://www.fooddrinkurope.eu/uploads/press-releases_documents/temp_file_FINAL_Allergen_A4_web1.pdf Accessed 31 July 2016.
- Hagiwara, T., Hagiwara, S., Handa, A., Sasagawa, N., Kawashima, R., & Sakiyama, T. (2015). Pretreatment with citric acid or a mixture of nitric acid and citric acid to suppress egg white protein deposit formation on stainless steel surfaces and to ease its removal during cleaning. *Food Control*, 53, 35–40.
- Hattersley, S., Ward, R., Baka, A., & Crevel, R. W. R. (2014). Advances in the risk management of unintended presence of allergenic foods in manufactured food products – An overview. *Food and Chemical Toxicology*, 67, 255–261.
- Hefle, S. L., Nordlee, J. A., & Taylor, S. L. (1996). Allergenic foods. *Critical Reviews in Food Science and Nutrition*, 36(Suppl), S69–S89.
- Jackson, L. S., & Al-Taher, F. (2010). Efficacy of different dry cleaning methods for removing allergenic foods from food-contact surfaces (Vol. CA, pp. 1–47). Presented at the international association for food protection annual meeting, Anaheim, California.
- Jackson, L. S., Al-Taher, F. M., Moorman, M., DeVries, J. W., Tippet, R., Swanson, K. M. J., et al. (2008). Cleaning and other control and validation strategies to prevent allergen cross-contact in food-processing operations. *Journal of Food Protection*, 71(2), 445–458.
- Jones, K., Griffith, C., & Salter, R. (2006). Detection of dried peanut and milk residues form stainless steel coupons using a sensitive ATP test, protein ELISA, and protein lateral flow test. Presented at the 120th AOAC International annual meeting and exposition, Minneapolis.
- de Luis, R., Lavilla, M., Sánchez, L., Calvo, M., & Pérez, M. D. (2009). Development and evaluation of two ELISA formats for the detection of β -lactoglobulin in model processed and commercial foods. *Food Control*, 20(7), 643–647.
- de Luis, R., Mata, L., Estopañán, G., Lavilla, M., Sánchez, L., & Pérez, M. D. (2008). Evaluation of indirect competitive and double antibody sandwich ELISA tests to determine β -lactoglobulin and ovomucoid in model processed foods. *Food and Agricultural Immunology*, 19(4), 339–350.
- Madsen, C. B., Hattersley, S., Buck, J., Gendel, S. M., Houben, G. F., Hourihane, J. O., et al. (2009). Approaches to risk assessment in food allergy: "report from a workshop developing a framework for assessing the risk from allergenic foods". *Food and Chemical Toxicology*, 47(2), 480–489.
- Muraro, A., Hoffmann-Sommergruber, K., Holzhauser, T., Poulsen, L. K., Gowland, M. H., Akdis, C. A., et al. (2014). EAACI food allergy and anaphylaxis guidelines. Protecting consumers with food allergies: Understanding food consumption, meeting regulations and identifying unmet needs. *Allergy*, 69(11), 1464–1472.
- Nakanishi, K., Sakiyama, T., & Imamura, K. (2001). On the adsorption of proteins on solid surfaces, a common but very complicated phenomenon. *Journal of Bioscience and Bioengineering*, 91(3), 233–244.
- Pawankar, R., Canonica, G. W., Holgate, S. T., Lockey, R. F., & Blaiss, M. (2013). *The WAO white book on allergy (update 2013)*. United States.
- Rabe, M., Verdes, D., & Seeger, S. (2011). Understanding protein adsorption phenomena at solid surfaces. *Advances in Colloid and Interface Science*, 162(1–2), 87–106.
- Reese, D. I., Holzhauser, T., Schnadt, S., Dölle, S., Kleine-Tebbe, J., Raithe, M., et al. (2015). Allergen and allergy risk assessment, allergen management, and gaps in the European Food Information Regulation (FIR). *Allergo Journal International*, 24(6), 180–184.
- Sakiyama, T., Sato, K., Tsuda, S., Sugiyama, H., & Hagiwara, T. (2013). Citric acid pretreatment for suppressing adhesion of major egg allergens to a stainless steel surface. *Food Control*, 32(2), 702–706.
- Schlegel, V., Yong, A., & Foo, S. Y. (2007). Development of a direct sampling method for verifying the cleanliness of equipment shared with peanut products. *Food Control*, 18(12), 1494–1500.
- Schubert-Ullrich, P., Rudolf, J., Ansari, P., Galler, B., Führer, M., Molinelli, A., et al. (2009). Commercialized rapid immunoanalytical tests for determination of allergenic food proteins: An overview. *Analytical and Bioanalytical Chemistry*, 395(1), 69–81.
- Shama, G., & Malik, D. J. (2013). The uses and abuses of rapid bioluminescence-based ATP assays. *International Journal of Hygiene and Environmental Health*, 216(2), 115–125.
- Spanjersberg, M. Q. I., Knulst, A. C., Kruizinga, A. G., Van Duijn, G., & Houben, G. F. (2010). Concentrations of undeclared allergens in food products can reach levels that are relevant for public health. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, 27(2), 169–174.
- Spanjersberg, M. Q. I., Kruizinga, A. G., Rennen, M. A. J., & Houben, G. F. (2007). Risk assessment and food allergy: The probabilistic model applied to allergens. *Food and Chemical Toxicology*, 45(1), 49–54.
- Taylor, S. L., Baumert, J. L., Kruizinga, A. G., Remington, B. C., Crevel, R. W. R., Brooke-Taylor, S., et al. (2014). Establishment of reference doses for residues of allergenic foods: Report of the VITAL expert Panel. *Food and Chemical Toxicology*, 63, 9–17.
- Taylor, S. L., Hefle, S. L., Farnum, K., Rizk, S. W., Yeung, J., Barnett, M. E., et al. (2006). Analysis and evaluation of food manufacturing practices used to address allergen concerns. *Comprehensive Reviews in Food Science and Food Safety*, 5(4), 138–157.
- Thammathongchat, S., Hagiwara, T., & Sakiyama, T. (2010). Adsorption of tropomyosin from pink shrimp (*Pandalus eous*) on stainless steel surface. *Food Control*, 21(9), 1250–1253.
- U.S. Food and Drug Administration. (1996). *Label declaration of allergenic substances in foods; notice to manufacturers*. <http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/Allergens/ucm106546.htm> Accessed 31 July 2016.
- Yang, P., Burson, K., Feder, D., & Macdonald, F. (2005). Method development of swab sampling for cleaning validation of a residual active pharmaceutical ingredient. *Pharmaceutical Technology*, 1, 84–94.