

Evaluation of Cleaning Procedures for Allergen Control in a Food Industry Environment

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Abstract: The hygiene of chicken processing surfaces and retention of the wheat protein gliadin and of protein in general on those surfaces were compared in 15 trials after 3 increasingly rigorous cleaning steps. Eleven different chicken products with wheat derivatives as a batter were prepared on 3 processing lines in 15 production runs selected at random over 6 mo (5 runs were thus replicates). Using surface swabs, surface hygiene was monitored by adenosine triphosphate (ATP) bioluminescence, gliadin by immunoassay, and protein by the Coomassie dye method. Gliadin was monitored in 14 trials, protein in 5, and all trials were monitored by ATP bioluminescence. In a typical trial, gliadin values normalized to uncleaned values fell from 100000 arbitrary units, to 6000 after rinsing, to 30 (foam, rinse), to not detected (sanitize, rinse). Parallel ATP bioluminescence values also decreased, but crucially, the relative gliadin value was less than the relative ATP value after foam and rinse in all 14 trials, a result unchanged after sanitize and rinse. In trials comparing ATP and protein, the relative ATP values exceeded the relative protein values in 4 of 5 trials after foaming and after sanitizing. Thus, for these 11 products, ATP bioluminescence was a surrogate indicator of residual gliadin and probably of residual protein. Absolute gliadin concentration on an uncleaned processing line was also the basis of modeling the risk of cross-contamination of gliadin in follow-up product, where the line was hypothetically left uncleaned between production runs. The results show that all follow-up product could be declared “gluten-free” under proposed legislation, and suggest that some industrial cross-contamination risks are currently overestimated.

Keywords: allergen, ATP bioluminescence, cleaning, gliadin

Practical Application: ATP bioluminescence is probably a useful surrogate for rapid validation of allergen-free food processing surfaces.

Introduction

Proteins in 8 common food categories are responsible for over 90% of all food allergic reactions in humans (Hefle and others 1996). For this reason and others, food regulators in many jurisdictions require that the potential allergens must be declared on food product labels. Where processing equipment is used to make multiple products, the cleaning protocols between heterogeneous production runs are intended to remove allergens as well as maintain hygiene. In spite of cleaning, manufacturers often voluntarily include statements to the effect that a particular product was prepared on equipment also used to manufacture another product where an allergen was among the ingredients. These *de facto* disclaimer statements can extend to statements about allergens being present in the same factory.

A recent review of cleaning to prevent allergen contamination (Jackson and others 2008) listed the most common methods used by food companies for allergen cleaning validation. These methods were enzyme-linked immunosorbent assays (ELISA) for specific allergens, and indirect methods such as visual inspection,

adenosine triphosphate (ATP) bioluminescence, and total protein. The basis of the total protein approach is that the major food allergies are caused by proteins. Although sensitive, a total protein test—based on the reduction of cupric ions (Lowry and others 1951; Smith and others 1985) or Coomassie dye color change (Bradford 1976)—typically take about 15 min to perform (Jackson and others 2008), although some kits claim near instant results. The basis of the ATP bioluminescence test for allergen verification is based on the premise that when the concentration of surface-bound ATP from microbes (Selan and others 1992) and food ingredients is lowered to below a defined value, the surface can be declared clean and, by default, also free of allergen. Research on the value of ATP bioluminescence as a predictor of an allergen has been reported for milk and egg proteins in model systems (Salter and others 2005; Al-Taher and Jackson 2007; Al-Taher and others 2007), and show that bioluminescence has the potential to be useful. The motive for using an ATP bioluminescence test is that this test is routinely used as a rapid hygiene index, is fast (as short as 30 s) and can be performed successfully by relatively unskilled personnel. While ELISA tests for specific allergens are uniquely sensitive for the protein of interest, these tests are currently more expensive than ATP bioluminescence, often require relatively skilled personnel and are slower to get results. More positively however, there is much development in rapid ELISA tests (Schubert-Ullrich and others 2009) that may ultimately supersede indirect tests.

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The major part of the present research compares the relative effectiveness of the standard cleaning procedures in a chicken processing facility in lowering the surface concentration of potential allergens. A similar approach was taken by Röder and others (2008) who explored effectiveness of cleaning processing equipment to reduce tree nut allergens in commercial cookies. The processing facility in the present study produces around 140 varieties of chilled, frozen and further processed chicken and turkey products. Most of the products contain ingredients that are variously allergenic in some people. These include wheat, soy, milk, egg, and sesame. The products are produced on processing lines that are reconfigured as required. At the end of each production run the stainless steel equipment is progressively cleaned using a protocol that achieves a satisfactory hygiene criterion as defined by the ATP bioluminescence test from surface swabbing after the final cleaning step. Of the 140 products made in this chicken processing facility, 131 contain wheat-derived ingredients of one form or another. Wheat allergy refers to adverse reactions involving immunoglobulin E antibodies to one or more proteins of wheat. Moreover, a particular class of wheat proteins, the gliadins, is responsible for a digestive syndrome called celiac disease. On the basis of different solubilities, wheat proteins can be classed into groups that include the gliadins (ethanol soluble) and glutenins (Coulter 2002; Akagawa and others 2007), trivially called gliadin and glutenin. Together they comprise gluten, where gliadin constitutes about half the mass of gluten (Metcalf and others 1991).

The particular protein chosen in this study for ELISA testing was gliadin, for which ELISA-based test kits are available. Many wheat proteins including gliadin(s) are potentially allergenic, so a positive result for gliadin will not necessarily predict allergenicity. However, because gliadin is always present in wheat (and similar grains), it is an excellent indicator of the presence of wheat matter. This is important in respect of celiac disease (Anderson and others 1952), which is a cell-mediated immune response rather than an allergy and has a clear hereditary basis (Clot and Babron 2000) unlike an IgE-mediated food allergy that may occur in any individual. Celiac disease occurs in about 1% in the adult U.K. population (van Heel and West 2006), and although low, the level of perceived gluten intolerance is higher in Western countries as demonstrated by the widespread use of "gluten-free" claims even in foods that contain no grain starch.

In addition to monitoring cleaning by ATP bioluminescence and a gliadin test, total protein was chosen as a 3rd analyte to monitor, although this was done in only where sufficient labor was available. Total protein was chosen because the major allergens are all proteins, so low contamination by protein might be regarded as an indicator of low allergenic potential, particularly as not all proteins are allergens. These 3 tests were variously used to monitor progressive cleaning in the hours following 15 production runs over 6 mo, where wheat-based ingredients were used to coat chicken pieces. Within the constraint of wheat-based ingredients, the 15 production runs were chosen at random. The aim was to assess the ability of ATP bioluminescence to be surrogate indicator for gliadin and protein in general. The hypothesis was this: if after cleaning, the relative contamination due to ATP exceeds that due to gliadin or any protein, then the cleaning procedure is even more effective on gliadin or any protein than on microbes and food residues in general, which are both sources of ATP. It follows that by knowing the absolute contamination due to the gliadin or any protein before cleaning, the final contamination can be inferred from the ATP test and judgements can be made about allergenic potential. In addition to these cleaning trials, a computer model

is described that shows the potential for cross-contamination of products with gluten, and other wheat proteins, where a follow-up product purportedly free of gluten is processed on a hypothetically uncleaned processing line.

Materials and Methods

Assay kits

The ATP bioluminescence system from Biotrace Intl., U.K., routinely used for hygiene monitoring in the processing facility, was used in this study. The RIDASCREEN®; Gen-Probe Life Sciences, Manchester, U.K. gliadin test kit (R7001) was obtained from R-Biopharm, Darmstadt, Germany. The test is ELISA-based, and visualized by oxidation of tetramethylbenzidine, ultimately to a yellow pigment measured at 450 nm. The stated limit of detection is 1.5 ppm gliadin, although the concentration of gliadin in the lowest concentration provided is 5 ppm, which is arguably a more realistic limit. Flexi-swab polyurethane foam swabs (Biolab, Australia) were used for the gliadin test. The receiving liquid was 5 mL of 40% ethanol, a concentration to be discussed later.

The Coomassie Plus Kit for total protein was obtained from Pierce, Rockford, Ill., catalog number 23236. The stated limit of detection is 1 ppm. Microbiological cotton swabs on wooden sticks (Livingstone Intl., Sydney, Australia; catalog WAPPSTP) were used to swab surfaces for protein. The protein assay is based on the change in absorption peak from 465 nm to 595 nm when Coomassie dye binds protein in an acid medium. Bovine serum albumin Fraction V (Sigma) was the protein standard. All other reagents were prepared from analytical grade laboratory chemicals.

Swabbing and development of assays

For ATP bioluminescence, the standard swab technique used in the facility was followed and this became the standard for the 3 assays. The conveyers were made of a conventional stainless steel wire mesh supported by cylindrical rollers. For swabbing, a 10 cm by 10 cm swabbing frame was randomly laid on the wire mesh but normal to the conveyer orientation, and for each swab the accessible tops and sides of the conveyer wire were swabbed once in a constant pattern.

Using a fresh calibration curve for each gliadin trial, the kit instructions were followed as recommended, except for one important modification. The gliadin test kit prescribes immersing the loaded swab into the receiving solvent, 60% ethanol, to extract the gliadin from the swab matrix, with a subsequent 1:50 dilution of the solvent by the undescribed diluent that was part of the kit, before addition of the 100- μ L aliquot to the microplate wells. In the present study, the plan was to extract the swab into 5 mL of ethanolic solution, then sampling a 100- μ L aliquot without dilution. The reason for this was because the low levels of gliadin anticipated on cleaned surfaces would require that minimal, if any, dilution would be required to bring samples into the dynamic range of the test. Now, gliadin is soluble in ethanol in the range of 40% to 60% by volume without any significant difference between these concentrations (Wieser and others 1994), but it was not known if 40% ethanol would interfere or not with the ELISA assay. This was tested by the following experiment. Triplicate dilutions of 80 ppm gliadin supplied with the gliadin test kit were prepared in ethanol to yield a final concentration of 32 ppm gliadin in 0%, 0.6%, 6%, 20%, 40%, and 60% ethanol. Absorbances due to the 32 ppm gliadin were compared. Results to be presented later showed that 40% ethanol did not significantly affect the primary immunological reaction, so 5 mL of 40% ethanol was therefore

used as the receiving solvent to extract gliadin from the loaded swabs. The curvilinear response was useful in the dynamic range 0 to 32 ppb gliadin, requiring that the 40% ethanol extracts derived from heavily contaminated swabs often had to be diluted before assay, and this was routinely done with 40% ethanol. Calibration curves were also routinely prepared in 40% ethanol.

In the interests of methods consistency, the Flexi-swab foam swabs used for the gliadin test were trialed for the Coomassie protein assays that were performed in the last 5 of the 15 trials. Blank foam swabs dipped in normal saline turned the brown Coomassie reagent blue in the manner of the protein assay. Enquiry revealed that these swabs contained traces of Tween 20 and peptone, the latter presumably responsible for the reaction with Coomassie. Dry microbiological cotton swabs were used instead. The receiving liquid was 5 mL of 0.9% NaCl. Standard curves at 595 nm were developed for dilute and more concentrated protein samples as specified for the kit.

Trials conducted and standard cleaning procedures

Fifteen trials were conducted over 6 mo on 3 processing lines that generated chicken products destined for food service or retail sale. Within the constraint of wheat-based ingredients, the 15 production runs were chosen at random. In the event, 11 different products were represented, such that there were 5 replicate monitoring trials on a conveyer transporting one particular product. All products were surface coated with various batters that contained wheat flour or wheat starch. This resulted in one conveyer in a processing line being particularly contaminated with raw batter and this was chosen for swabbing after each stage of cleaning. The swabs for gliadin and Coomassie protein were placed in their respective receiving solvent volumes, and those containers were held on ice until analyzed the following day in a remote laboratory. ATP bioluminescence data were generated on site without delay.

Table 1 summarizes the cleaning procedures performed after each production run. In a typical trial, the *Uncleaned* test was done 20 min after the end of the production run that often ended at 2100 h. This 20 min gap allowed gross conveyer contamination to drain from the conveyer wires before swabbing. Swabs were taken from 6 sites of the conveyer surface contaminated with raw batter for each of the ATP bioluminescence, gliadin and Coomassie protein tests carried out. A dedicated cleaning crew then performed the 3 cleaning steps over 5 h and the still-wet rinsed surfaces were swabbed 20 min after each step, again over 6 sites of the conveyer surface. In the case of the *Uncleaned* test, the surfaces swabbed were clearly dirty and for the *Rinse only* tests, the surfaces often showed only minor contamination. However, for the *Foam and rinse* and *Sanitize and rinse* tests, the surfaces were visually clean stainless steel having been scrubbed (*Foam and rinse*) and rinsed clean in both procedures. In swabbing after *Rinse only*, *Foam and rinse*, and

Sanitize and rinse, some of the swab sites may have been previously swabbed, but no attempt was made to mark sites because this was impractical in the production environment where the conveyer was often mobile during cleaning. Although this could mean that contamination values at all cleaning steps beyond *Uncleaned* were underestimates, all 3 assays would on average be similarly affected. Ten trials compared ATP bioluminescence with gliadin, 1 trial compared ATP with Coomassie protein alone, and 4 trials compared ATP, gliadin, and Coomassie protein when more labor was available.

Data analysis

Data from the 6 swab sites on the conveyer in each production run were averaged for each cleaning step: *Uncleaned*, *Rinse only*, *Foam and rinse*, and *Sanitize and rinse*. The results were expressed in ppm of gliadin in the 5 mL of the ethanolic extraction solvent; protein was expressed as ppm of bovine serum albumin equivalents in the 5 mL of saline; ATP was expressed as relative light units (RLU) as defined by the ATP bioluminescence protocol. To compare between the different assays, the means for each *Uncleaned* assay were normalized to 100000 with no regard to units, and the means for *Rinse only*, *Foam and rinse*, and *Sanitize and rinse* were then expressed relative to *Uncleaned*. More detail on how normalized data were compared is given in the discussion of Table 3 and 4.

The data were also summarized in 2 ways. Five of the 15 trials involved one chicken product prepared within 3 mo, so those data were analyzed in a formal statistically based comparison, reported in Table 5. Also, all 15 trials are reported as independent trials, each subject to the question at each cleaning step: was cleaning relatively more effective on reducing the surface contamination of gliadin or protein than of ATP? In all there were 57 possible comparisons that are summarized in Table 6.

For the modeling exercise, summarized in Table 7, the measured concentration of gliadin on a heavily contaminated *Uncleaned* conveyer was the starting point for modeling. The hypothetical modeling proposition was that a particular conveyer was not cleaned at all between productions runs, where chicken pieces in the 2nd run were intended to be gliadin-free. Four operating parameters of a particular processing conveyer (velocity, length, so on) and chicken piece flow (piece/s) were used in a spreadsheet to calculate cross-contamination for each complete cycle of the conveyer. The initial concentration of gliadin on the conveyer was set at 600 mg m⁻², and was calculated by knowing the swab area, the volume of the receiving solvent (5 mL), and the concentration of gliadin in that solvent. This value was rounded up from the highest value found on an *Uncleaned* conveyer in one of the 15 trials. The conveyers were made of wire mesh so contamination could occur only where piece contacted a dirty wire surface. The simplifying assumption was made that the conveyer was a flat surface, an assumption to be discussed later. Two key parameters estimated were the contact area of a chicken piece on the flat surface, estimated and set at 0.001 m², and the gliadin pickup efficiency fraction, ranging between 0 and 1. Three values were chosen: 0.05, 0.10, and 0.50 where the last means that 50% of the available contaminating gliadin would be transferred to a chicken piece on contact. Each cycle of the conveyer involved a calculated loss of gliadin from the surface as the incoming chicken pieces effectively cleaned the conveyer of gliadin contamination. The model adjusted for these losses at each cycle. The potential contamination by gliadin was calculated for a 2 h production run where 225 conveyer cycles generated 4210 packs of 6 chicken pieces of 114 g each. The

Table 1—Progressive cleaning of processing equipment.

Name	Description
<i>Uncleaned</i>	Nothing done
<i>Rinse only</i>	Remove any solid material and wash with water between 40 and 50 °C
<i>Foam and rinse</i>	Apply a 1% enforce foam (Ecolab, New Zealand), comprising NaOH, NaOCl, and surfactant; scrub; wait 20 min; water rinse
<i>Sanitize and rinse</i>	Apply a broad spectrum sanitizer, for example, 1% Sterbac (Ecolab) comprising a range of antimicrobials; wait 20 min; water rinse

parameters chosen for the model were also used by a colleague to develop the model independently. The outcomes of the model presented here were identical to those of the independent check model. The model is also presented as supplementary data.

Results and Discussion

Effect of ethanol concentration on the gliadin antibody-antigen reaction

Table 2 shows the effect of including low to high concentrations of ethanol in the primary incubation reaction of the RIDASCREEN[®] gliadin ELISA test. In the presence of 60% ethanol, the reaction was strongly inhibited (35.2%), but at 40% ethanol the reaction was only slightly inhibited (−3.1%). The concentration of ethanol in the swab receiving solvent was thus set at 40%, so that the receiving solvent did not have to be diluted before addition to the microplate wells.

Limits of detection for gliadin and Coomassie protein

The RIDASCREEN[®] gliadin test kit has a claimed detection limit of 1.5 ppm, based on a 1:50 dilution of the receiving solvent, 60% ethanol. By adopting a lower ethanol concentration, 40%, the receiving solvent could be used where required without dilution, thus increasing the effective sensitivity of the assay markedly. Where a swab yielded no absorbance change compared to the no-gliadin control, the swab was assigned a value of 0 ppm for the purpose of calculation. The same argument was applied to the Coomassie protein assay.

Table 2—Effect of ethanol concentration on the gliadin antibody-antigen reaction.

Ethanol concn. (% v/v)	Mean absorbance at 450 nm ± standard deviation	Percent difference from the 0% ethanol treatment
0	0.968 ± 0.050	
0.6	0.964 ± 0.004	−0.40
6	0.972 ± 0.007	0.48
20	0.976 ± 0.070	0.83
40	0.937 ± 0.026	−3.1
60	0.627 ± 0.052	−35.2

Table 3—Typical effect of progressive cleaning on gliadin concentration (ppm) and on ATP bioluminescence (RLU) (on a conveyer in Trial 1).

Swab	Uncleaned	Rinse only	Foam and rinse	Sanitize and rinse
Swabbing for gliadin after above cleaning step				
1	78.1	0.015	0.08	0
2	29.6	0.018	0	0
3	30.6	0.023	0	0
4	33.0	0.003	0	0
5	34.4	6.0	0	0
6	35.9	8.2	0	0
Mean ± standard deviation	40.3 ± 18.6 ¹	2.37	0.0133	0
Gliadin concn. as a fraction of mean uncleaned set to 100000	100000	5870	33	0
Swabbing for ATP after above cleaning step				
1	138767	48	27	41
2	94679	34	46	32
3	255112	37	47	28
4	201172	63	129	31
5	198258	21654	52	25
6	130680	18392	41	24
Mean ± standard deviation	169778 ± 58666	6705	57 ± 36	30 ± 6
RLU as a fraction of mean uncleaned set to 100000	100000	3950	34	18

¹Data are means of 6 swabs. Standard deviation was not calculated where data were not normally distributed.

Effect of progressive cleaning

Table 3 from Trial 1 of 15 shows typical results of swabbing a processing conveyer for gliadin at the end of a production run. Between-swab variation was often high to the point that standard deviations were not calculated. To compare the relative effect of progressive cleaning on gliadin and ATP, the mean values 40.3, 2.37, 0.0133, and 0 ppm gliadin, respectively, were normalized to become the unitless values 100000, 5870, 33, and 0. Likewise, the RLU values from ATP bioluminescence became the unitless values 100000, 3950, 34, and 18. In comparing the efficacy of cleaning gliadin and ATP from surfaces, 5870 gliadin from *Rinse only* was greater than 3950 ATP, so *Rinse only* was less effective on gliadin than on ATP. After *Foam and rinse* the values were a comparable 33 (gliadin) and 34 (ATP), and after *Sanitize and rinse* 0 and 18, respectively. Thus, after the *Sanitize and rinse* step, cleaning proved to be ultimately more effective on gliadin than ATP.

Table 4 shows equivalent results for protein swabbing. In that Trial 13, the replicate swabs returned normally distributed data, so only means and standard deviations are reported. At all stages of cleaning, the relative value for ATP bioluminescence exceeded that for protein. Thus, in Trial 13, cleaning was more effective at removing conveyer surface protein than conveyer surface ATP.

In 5 of the 15 production runs, the same chicken product was made. The data from these trials could be formally analyzed as replicates (Table 5). Data are means and standard deviations of the means of the 6 replicate swabs at each cleaning step. Thus, each mean represents 30 swabs. It is clear that progressive cleaning is more effective at removing conveyer surface gliadin than conveyer surface ATP.

The relative effectiveness of progressive cleaning in 15 trials is summarized in Table 6 where each production trial is treated independently. In the 14 trials where gliadin contamination was compared with ATP bioluminescence, *Rinse only* was generally more effective at removing ATP than gliadin. However, after the *Foam and rinse* and *Sanitize and rinse* procedures, relative gliadin values were always lower than those of ATP. These 2 wash treatments were thus very effective at removing gliadin. Although the Coomassie protein dataset was limited to 5 trials, the *Foam and rinse* and *Sanitize and rinse* procedures may be marginally

less effective at removing protein in general than in removing gliadin.

It is proposed that in this particular chicken processing facility making its range of products, the ATP bioluminescence test not only offers a quick measure of hygiene but is also a useful surrogate indicator of gliadin contamination and of proteins in general on cleaned processing surfaces. Salter and others (2005) performed similar cleaning experiments on a model surface and in a food industry setting with milk. However, their data cannot be analyzed as was done here because their ELISA test values for milk protein and casein were qualitative. In a study on ground hazelnut contamination on cookie moulding equipment, Röder and others (2008) used only an ELISA test to show the effectiveness of cleaning solid matter off a range of equipment. Because of the nature of the hazelnut cookie process, the cleaning protocol was different and less rigorous than that applied in the present study, but in their cross-contamination trials with follow-up product cleaning

was shown to be particularly effective in reducing contamination to safe concentrations, although that varied with equipment.

Modeling risk of gliadin contamination from processing equipment

Knowing the concentration of an allergen on a conveyer surface has risk assessment value only when the concentration can be translated into contamination of purportedly allergen-free food subsequently prepared on that processing line. This approach was taken by Röder and others (2008) who examined cross-contamination of hazelnut protein in a follow-up product where processing equipment had been left uncleaned or cleaned by defined protocols. A similar experiment could not be performed in this facility so a computer-assisted model was developed to assess cross-contamination when follow-up product purportedly free of gluten was processed on a hypothetically uncleaned processing line. Data are presented as ppm of gliadin in model packs of 6 chicken

Table 4–Effect of progressive cleaning on Coomassie protein concentration (ppm) and on ATP bioluminescence (RLU) on a conveyer in Trial 13.

	Uncleaned	Rinse only	Foam and rinse	Sanitize and rinse
Swabbing for protein after above cleaning step				
Mean ± standard deviation	827 ± 317 ¹	6.25 ± 3.47	1.55 ± 0.73	3.69 ± 1.12
Protein concn. as a fraction of mean uncleaned set to 100000	100000	756	187	446
Swabbing for ATP after above cleaning step				
Mean ± standard deviation	6916 ± 3781	120 ± 36	87 ± 14	74 ± 16
RLU as a fraction of mean uncleaned set to 100000	100000	1735	1256	1068

¹Data are means of 6 swabs.

Table 5–Effect of progressive cleaning on gliadin (ppm) and on ATP bioluminescence (RLU) after 5 replicate production runs.

	Uncleaned	Rinse only	Foam and rinse	Sanitize and rinse
Swabbing for gliadin after above cleaning step				
Mean ± standard deviation	67.3 ± 29.1 ¹	1.00 ± 0.80	0.0024 ± 0.0032	0.0017 ± 0.0035
Gliadin concn. as a fraction of mean uncleaned set to 100000	100000	1,479	4	3
Swabbing for ATP after above cleaning step				
Mean ± standard deviation	190982 ± 35299	1,704 ± 2834	62.8 ± 55.0	27.6 ± 6.0
RLU as a fraction of mean uncleaned set to 100000	100000	892	33	14

¹Data are means ± standard deviation of progressive cleaning outcomes after 5 replicate production runs, and are from Trials 1, 4, 5, 6, and 9.

Table 6–Comparison of the results of the ATP, gliadin, and Coomassie protein assays during progressive cleaning after 15 production trials over 6 mo.

Trial	Compared with Uncleaned, is the relative gliadin value less than the relative ATP value after cleaning?			Compared with Uncleaned, is the relative Coomassie protein value less than the relative ATP value after cleaning?		
	Rinse only	Foam and rinse	Sanitize and rinse	Rinse only	Foam and rinse	Sanitize and rinse
1*	No	Yes	Yes			
2	Yes	Yes	Yes			
3	No	Yes	Yes			
4*	No	Yes	Yes			
5*	No	Yes	Yes			
6*	No	Yes	Yes			
7	No	Yes	Yes			
8	No	Yes	Yes			
9*	Yes	Yes	Yes			
10	Yes	Yes	Yes			
11	No	Yes	Yes	Yes	Yes	Yes
12	Yes	Yes	Yes	Yes	Yes	Yes
13	No	Yes	Yes	Yes	Yes	Yes
14	No	Yes	Yes	Yes	No	No
15				Yes	Yes	Yes
Fraction	4 of 14	14 of 14	14 of 14	5 of 5	4 of 5	4 of 5

*These 5 trials represent replicate production runs and are statistically analyzed in Table 5.

pieces of 114 g each, arising from an initial gliadin contamination of 600 mg m⁻² on an *Uncleaned* surface, which was the highest value recorded in 14 trials and rounded up to the nearest 100. It is acknowledged that 600 mg m⁻² could still be an underestimate because the efficiency of removal of contamination by swabbing from the conveyer wires could not be determined in that industrial environment. However, on uncleaned conveyers that had drained for 20 min after the end of the production run, the contamination was obvious to the eye and, as judged by inspection, could be effectively removed with the swab. Moreover, it is estimated that for a typical batter comprising 30% flour and thus about 1.5% gliadin, a concentration of 600 mg m⁻² would result from a volume of about 40 mL of batter contaminating a square meter the conveyer wire mesh. Thus, 600 mg m⁻² is a plausible value for a worst-case scenario.

An assumption made was that the allergen contamination was uniform on surfaces. This is likely to be valid when the source of contamination is a batter, a relatively homogeneous semifluid, but if the coating formulation were flakes, lumps, or nonaqueous such as peanut butter, the situation would be unacceptably complex for this type of modeling exercise. Another assumption was that the conveyer surface was flat, where it was actually a wire mesh. This assumption is valid because the 2 factors that determine gliadin pickup by follow-up product are the contact area and gliadin pickup efficiency fraction; the exact nature of the contact is unimportant but the values chosen are. The contact area was set at 0.001 m², which was approximately 10% of the chicken piece “footprint.” The value for pickup efficiency fraction is particularly important in the model. If the fraction is high, say 0.50 of the gliadin at the contact area between chicken piece and conveyer, pieces that are produced early in a production run would be much more contaminated than later bags. With a low pickup efficiency fraction, any problem of contamination would linger. The pickup efficiency fractions were arbitrarily set at 0.5, 0.1, and 0.05, while realizing that pickup efficiency is likely to be function of the level of contamination. Intuitively, pickup efficiency fraction will become lower as surfaces became cleaner from prior transfer from surface to chicken piece by follow-up product. This was confirmed by Röder and others (2008) in their hazelnut cookie trials. Replotting of their data shows that the progressive reduction in contamination in follow-up is more complicated than a simple exponential decay curve. There is clear evidence of lower pickup efficiency as surfaces became cleaner, either by dedicated cleaning or by pickup from a follow-up product. In the present study, changes in pickup efficiency fraction as the conveyer surface becomes cleaner can only be guessed, so the 3 pickup efficiency fractions were held constant for the full 225 conveyer cycles.

In Table 7, the gliadin concentrations in 5 equal groups of packs decreased exponentially with time, as was expected, with a 0.50 pickup efficiency yielding the highest initial gliadin contamination but also the lowest gliadin contamination at the end of the production run of 4210 packs. In the worst-case scenario at a 0.50 pickup efficiency fraction, the concentration of gliadin was 2.63 ppm in the first pack off the production line. Gliadin comprises about 50% of gluten by weight (Metcalf and others 1991). Therefore, values for gliadin are doubled to estimate gluten content. Thus, the predicted contamination was no more than say 5.5 ppm gluten and after 100 packs the value had fallen below 5 ppm (details not shown).

In Europe and the United States, food labeling rules or proposals allow for “gluten-free” claims where a food contains no detectable gluten. The current proposal in the United States is that

“gluten-free” be defined as less than 20 ppm gluten (Food and Drug Administration 2007; Codex Alimentarius 2008), although there is pressure from interest groups (for example, Celiac Sprue Assn., Omaha, Nebr., U.S.A.) to lower the threshold to below the claimed limit of detection by conventionally applied current technology, 3 ppm (for example, RIDASCREEN[®]; Gen-Probe Life Sciences). By the current proposal, all the follow-up product in this hypothetical example could be advertised as “gluten-free” under current European and the U.S. legislations. The amount of tolerable gluten varies among people with celiac disease but a review by Akobeng and Thomas (2008) showed that a daily gluten intake of <10 mg is unlikely to cause significant histological abnormalities. Within the limits of the current model, it thus appears unlikely that cross-contamination in follow-up product on an uncleaned conveyer would cause a problem for celiacs (but the same may not be true wheat allergy sufferers to some particular wheat protein). Moreover, because conveyers and associated equipment are always cleaned at least minimally between production runs, cross-contamination by allergens is always likely to be much lower in the real world (Röder and others 2008) than indicated by the present model.

Conclusion

In a commercial processing facility dedicated to value-added chicken products, the hygiene monitoring technique of ATP bioluminescence was a surrogate indicator of gliadin contamination of processing equipment, and in a limited number of trials showed potential in indicating protein contamination. This is important because all major food allergens are proteins. It is emphasized that these outcomes apply only to the chicken product range processed in this facility and are not necessarily able to be extrapolated to other foods and processing protocols. As judged by an ELISA assay for gliadin, the cleaning procedures in this facility were extremely effective at gliadin removal. However, even modest cleaning would be sufficient for gliadin removal in this facility. This was supported by a modeling exercise which suggested that a hypothetical follow-up product, purportedly free of gliadin, prepared on a totally

Table 7–Scenarios of distribution of gliadin in retail packs of nominally gluten-free chicken pieces cross-contaminated from an unclean conveyer.

The pieces were hypothetically prepared on an unclean conveyer contaminated with wheat products from a prior production run with an initial gliadin concentration on the conveyer surface of 600 mg m⁻². In a 2 h, production run of 25259 pieces in 4210 packs, the following distribution was calculated:

Pickup efficiency fraction	Packs	Gliadin concentration on chicken (ppm)	Risk to consumer
0.50	1 to 842	2.63 to 0.38	Worst case
	843 to 1684	0.38 to 0.05	
	1685 to 2526	0.05 to 0.01	
	2527 to 3368	0.01 to 0.00	
	3369 to 4210	0.00 to 0.00	
0.10	1 to 842	0.53 to 0.36	Best case Worst case
	843 to 1684	0.36 to 0.24	
	1685 to 2526	0.24 to 0.17	
	2527 to 3368	0.17 to 0.11	
	3369 to 4210	0.11 to 0.08	
0.05	1 to 842	0.26 to 0.22	Best case Worst case
	843 to 1684	0.22 to 0.18	
	1685 to 2526	0.18 to 0.15	
	2527 to 3368	0.15 to 0.12	
	3369 to 4210	0.12 to 0.10	

uncleaned process line contaminated with gliadin would present no risk for celiacs, as one group displaying an adverse response to a food protein.

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