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Milk allergen quantification in rice flour: An experimental investigation of sampling and testing procedures

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ABSTRACT

Unintended allergen presence in foods may cause severe allergic reactions, thus posing a health risk for food allergic consumers. Consequently, accurate allergen quantification in foods is relevant for food allergic individuals, the food industry and authorities. However, regulation regarding sampling methods for food allergen analysis is lacking. The aim of the present study was to investigate the influence of the laboratory sample size and test portion size on food allergen analytical determinations by ELISA in an amorphous homogeneous milk contamination scenario.

A sampling plan was designed where seven lots of 1000 g rice flour contaminated with increasing amounts of skimmed milk powder (from 0 to 500 ppm milk protein contamination) were produced, and five 100 g as well as five 200 g laboratory samples were collected. From each laboratory sample, two test portions of 0.5 g and two test portions of 5 g were extracted, and two aliquots from each test portion were analysed for detection of milk allergens using a commercial ELISA kit. To evaluate the sampling plan, the measured concentration variability was estimated using a hierarchical mixed effects model, and the statistical significance of each sampling step was validated using permutation tests.

The experimental data indicated that the variance of the measured concentrations increased as a function of the spiked concentration. While increasing the test portion size from 0.5 to 5 g did not improve test precision, increasing the laboratory sample size from 100 to 200 g improved the test precision by reducing the variance of the laboratory sample by 88% and the total variance by an average of 63%. Thus, when estimating producer's and buyer's risks, increasing laboratory sample size from 100 to 200 g greatly improved the precision compared to increasing sample number from one to two 100 g samples, which reduced the total variance by 50%.

The laboratory sample size was critical for the analytical accuracy of the sampling plan for detection of milk protein contamination in rice flour, which was used in this study as an example of an allergen contamination scenario, and for reducing the risks associated with sample misclassification.

1. Introduction

Food allergy prevalence has been reported worldwide to be as high as 4% and 9% for adults and small children, respectively (Sicherer & Sampson, 2014, 2018; Warren et al., 2020), and the only viable management option available is avoidance of the culprit food. According to EU regulations, all ingredients or processing aids in prepackaged foods must be listed in the list of ingredients. In addition, there are special rules for 14 priority allergenic foods (European Commission, 2003, 2011). However, the unintended allergen presence (UAP), for example, via cross-contact during manufacturing (e.g. shared equipment or production lines, inadequate cleaning of equipment, agricultural practices, etc.) (Codex Alimentarius, 2020), is not regulated. Yet, UAP may be accountable for fatal allergic reactions, representing a health risk for consumers allergic to foods (Blom et al., 2018; Versluis et al., 2015).

To ensure the protection of food allergic consumers and product safety, the food industry is required to know the level of UAP. A range of methods are available for UAP analysis, such as ELISA, polymerase chain reaction (PCR) and mass spectrometry (MS) (Lee et al., 2023; Sena-Torralba et al., 2020), ELISA being the most commonly analytical

Abbreviations: CV, Coefficient of variation; ED, Eliciting dose; ELISA, Enzyme-linked immunosorbent assay; HME, Hierarchical mixed effects; LOD, Limit of detection; LOQ, Limit of quantification; ppm, Parts per million; Q-Q, Quantile-quantile; SD, Standard deviation; UAP, Unintended allergen presence.

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method used for routine analysis for allergenic food. Independently of the method used, the performance evaluation of the chosen method, i.e. specificity, robustness, repeatability and sensitivity, is essential for the correct quantification of allergens in food (Latimer, 2023; Paez et al., 2016; Remington et al., 2022).

There is no EU regulation concerning how and where samples for food allergen analyses should be collected and handled, including their number and sizes. In contrast, guidelines for Genetically Modified Organisms (GMO) (Joint Research Centre, 2014) and regulations regarding methods of sampling and maximum levels of mycotoxins in different foodstuffs (e.g. maize, cereals, nuts, dried fruits, etc.) have been established (European Commission, 2006a, 2006b, 2010). Similarly, the limit of gluten content in gluten-free food is regulated to be 20 parts per million (ppm) (Codex Alimentarius, 2008).

To measure contaminant levels in foods, sampling plans are designed by manufacturers, where parameters such as the number and size of each sample to be tested and the subsequent preparation steps for the analysis are defined. These sampling parameters are critical and contribute to the analytical error (Joint Research Centre, 2014; Sharma et al., 2020, 2021; Tittlemier et al., 2011), thus their optimisation will reduce the analytical variability (Sharma et al., 2020, 2021; Tittlemier et al., 2011). Indeed, several studies have shown that the laboratory sample size was the greatest contributing factor to the total analytical variability of mycotoxins and gluten detection in foods, thus optimisation of this parameter reduced the analytical variability (Johansson et al., 2000; Ozay et al., 2006; Sharma et al., 2020, 2021; Tittlemier et al., 2011; Whitaker et al., 1994).

For food allergen analysis, two recent guides suggested that the number of samples collected should be based on factors such as allergen form (i.e. amorphous vs. particulate), distribution (i.e. homogeneous vs. heterogeneous) (Remington et al., 2022) and level of concern relating to the risk (i.e. increase the number of samples as the risk increases) (Allergen Bureau, 2022; Remington et al., 2022). However, the recommendation for the number of samples to be collected differed between the guides.

To test the performance of a sampling plan, theoretical probability models have been developed depending on the sampling procedure (i.e. steps, sample number and size) and on the acceptance level of contamination (i.e. the maximum level of contamination allowed for a sample to be accepted). The measured and experimental contaminations are compared in relation to the acceptance levels, and the result determines the probability of a sample below the acceptance level being rejected (producer's risk) and the probability of a sample above the acceptance level being accepted (buyer's risk) (Codex Alimentarius, 2004; Miraglia et al., 2005; Sharma et al., 2020, 2021; Whitaker et al., 2007). By altering the sampling parameters (e.g. sample number or size), both the producer's and buyer's risks can be reduced, thus obtaining a robust sampling plan. For instance, in experiments measuring one laboratory sample size and one test portion size, Sharma et al. (2020, 2021) theoretically estimated that the greatest reduction of both risks was accomplished by increasing the laboratory sample size and/or test portion size (Sharma et al., 2020, 2021). Similarly, Whitaker et al. (2007) observed that both risks associated with a sampling plan used to detect aflatoxin in almonds decreased as the sample size increased (Whitaker et al., 2007). To establish an allergen contamination acceptance level, eliciting doses (ED) and food intake, estimated as exposure during a meal, are determined. Recently, new and updated data on doses at which 1% (ED01) and 5% (ED05) of the allergic population is expected to experience objective allergic reactions were established for the 14 priority allergenic foods (Remington et al., 2020).

The aim of the present study was to investigate experimentally, for the first time, the influence of the laboratory sample size and test portion size on food allergen analytical determinations by ELISA in an amorphous homogeneous contamination scenario. An additional aim was to employ a suitable statistical method to determine the producer's and buyer's risks associated with the sampling plan. This scientifically based knowledge will help food authorities and the food industry when designing sampling plans.

2. Materials and methods

2.1. Products

Skimmed milk powder with protein, fat and carbohydrate contents of 34.4%, 0.3% and 55.4%, respectively, and rice flour with protein, fat and carbohydrate contents of 8%, 1% and 81%, respectively, were kindly provided by Nestle (Vevey, Switzerland). The milk ELISA kit (SENSISpec Milk ELISA, HU0030014) for the quantification of casein and β-lactoglobulin was purchased from Eurofins Technologies (Budapest, Hungary).

2.2. Experimental design

2.2.1. Definitions

The term 'sampling' covers several steps before the sample analysis. The following terms are used in this study.

- A lot refers to the experimental lot generated for the experiment.
- A laboratory sample refers to a fraction of the lot that is further mixed.
- A test portion refers to the quantity of material drawn from the laboratory sample that is used for extraction.
- An aliquot refers to the fraction of the extract from the test portion used for testing by ELISA.
- A sampling plan refers to a scheme that defines the number and size of items/samples to be collected.

2.2.2. Experiment 1

Six lots of rice flour, 1000 g each, were spiked with skimmed milk powder to obtain six lots containing 0.5 (lot 2), 5 (lot 3), 20 (lot 4), 50 (lot 5), 250 (lot 6) and 500 (lot 7) ppm milk protein (Fig. 1). Each of the resulting six lots and a non-spiked lot (lot 1, 0 ppm) were individually mixed with a 5L mixer (Varimixer Teddy, Brøndby, Denmark) for 3 min at a speed of 270 rpm. Five 100 g laboratory samples were taken from each lot, therefore 500 g of each lot was not analysed. Each of the 100 g laboratory samples was individually mixed using a blender (Rommelsbacher, Dinkelsbühl, Germany) for 3 × 20 s before taking two 0.5 g test portions and two 5 g test portions. Each test portion was subsequently extracted for measuring milk allergens using an ELISA kit according to the manufacturer's instructions. Two aliquots (100 μl) from each test portion were analysed using the ELISA kit. A total of 280 aliquots were analysed in experiment 1.

2.2.3. Experiment 2

Experiment 2 was performed as experiment 1, except that each lot was divided into five 200 g laboratory samples, therefore the whole lot was analysed, and each laboratory sample was thoroughly mixed for 2 min using a different blender than in experiment 1 (Coop blender, Model no. 82000071, 600W max 1500 ml) due to the bigger sample size.

A total of 280 aliquots were analysed in experiment 2.

2.3. ELISA analysis

As ELISA is the most commonly analytical method used for routine analysis for allergenic food, this was the method of choice for this study. Samples were analysed according to the manufacturer's instructions as follows. Test portions of 0.5 g were extracted for 15 min at 60 °C with 10 ml extraction buffer, and additional 5 g test portions were extracted with 100 ml extraction buffer under the same conditions as for the 0.5 g test portions to investigate the effect of the test portion size. Samples were then, centrifuged for 10 min at 2000 *g*. Two aliquots (100 μl) from each test portion or duplicates from each milk protein standards (0, 0.4, 1, 4

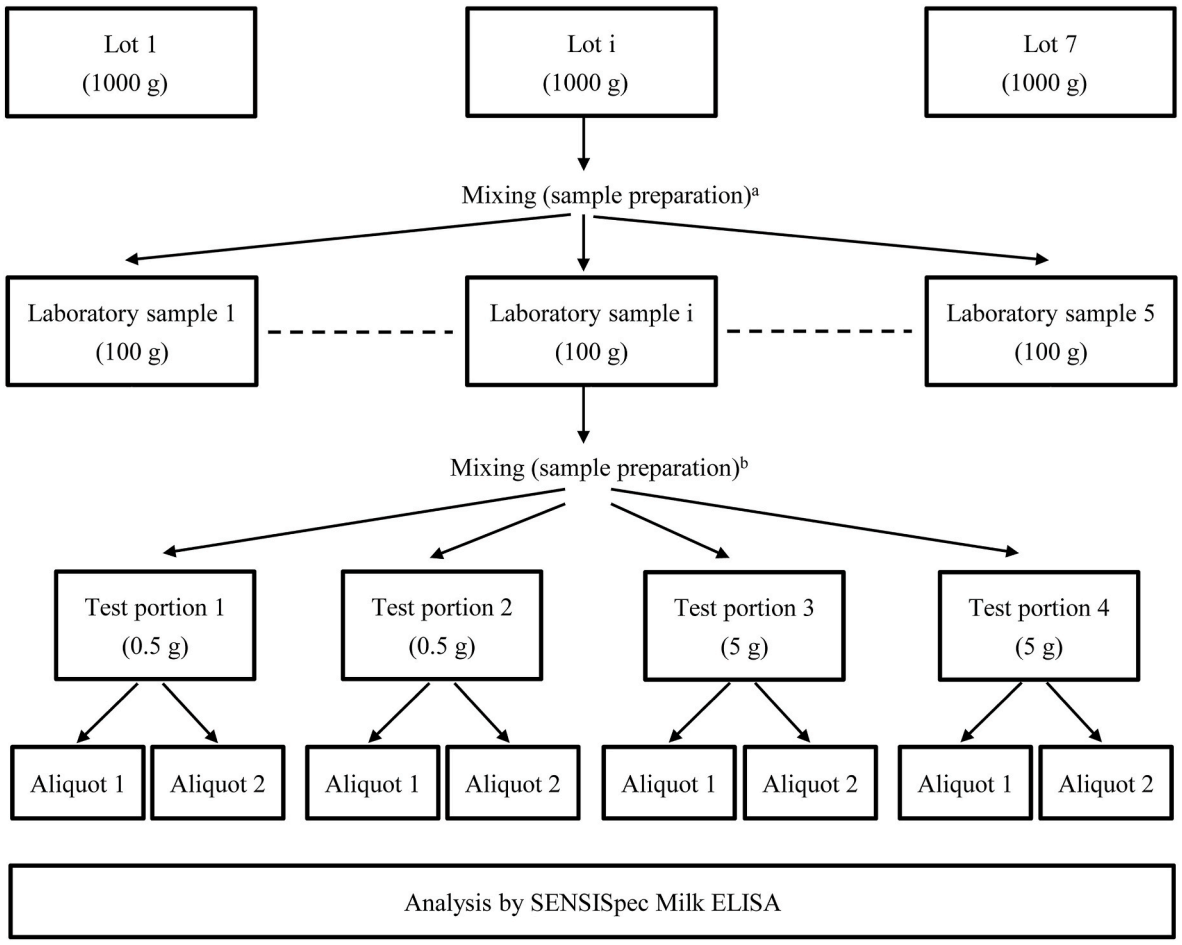

Fig. 1. Flow chart of the design of experiment 1 for the sampling plan to measure analytical variances associated with testing milk protein contamination in rice flour by ELISA. [a] Each individual lot was mixed and subsequently separated into five 100 g laboratory samples. [b] Each individual laboratory sample was mixed and, subsequently, two 0.5 g test portions and two 5 g test portions were taken from each laboratory sample.

and 10 ppm milk protein) were pipetted into a 96-well ELISA plate coated with antibodies specific for detecting casein and β-lactoglobulin and incubated for 20 min at room temperature. After washing the plate three times, 100 μl of peroxidase conjugated anti-milk protein antibody was added and incubated as above. After washing the plate three times, 100 μl of substrate solution (3,3′,5,5′-Tetramethylbenzidine) was added to the plate and incubated as above. The reaction was stopped by adding 100 μl of stop solution (0.5 M H_2SO_4), and the absorbance was measured at 450 nm with a reference wavelength of 630 nm using a microtitre reader (Gen5, BioTek Instruments, Winooski, Vt., US). In each ELISA plate, milk protein standards were included in duplicate, and the average OD of each set was used to calculate the corresponding concentration of milk protein in ppm from the standard curve. The two aliquots from each test portion were not averaged but considered individual samples for statistical purposes to answer the question of whether the aliquots contribute to the measurement variability. A maximum of 43 random test portion samples, corresponding to 86 aliquots, were tested on one ELISA plate together with standards. A total of 560 aliquots were tested on different days.

The analytical sensitivity of the ELISA was assessed by determining the Limit of Detection (LOD) and the Limit of Quantification (LOQ). Ten replicates of the milk protein-free matrix samples (0 ppm) were assayed, and the corresponding concentration of the mean OD +3 Standard Deviations (SD) or +10 SD were defined as LOD and LOQ, respectively, according to manufacturers' specifications (Eurofins Technology).

The intra-assay variation was determined by testing 16 aliquots of each of three test portions, corresponding to three concentrations (0.5, 50 and 250 ppm milk protein), performed on the same assay and ELISA plate. The Coefficient of Variation (CV) and the percentage of recovery were determined. Plate-to-plate variation was determined by testing milk protein standards corresponding to three concentrations (1, 4 and 10 ppm milk protein) in duplicate performed on 10 different assays, ELISA plates and days.

2.4. Statistical analysis

A Hierarchical Mixed Effects (HME) model was chosen to derive maximum likelihood estimates of the variances of each level in the sampling plan. The HME model is a type of linear regression containing both deterministic and random effects (Madsen & Thyregod, 2010), and it is the only regression model that can distinguish variances in the laboratory, test portion, and aliquot sampling levels. Our HME model contained the following terms: A deterministic intercept and random intercepts for each laboratory sample, test portion sample, and a residual error term corresponding to the aliquot samples. Further details can be found in the supplementary material.

The parameters of the HME model were fitted using the package 'nlme' and function 'lme' from the R programming language (Pinheiro & Bates, 2022; R Development Core Team, 2022). Individual HME models were fitted to obtain estimates of the mean and the variances for each combination of spiked concentrations, laboratory sample sizes, and test portion sizes.

The assumption of normally distributed residual error was validated by illustrating the residuals in a Q-Q plot and a histogram. Additionally,

Pearson's chi-squared test was conducted to test the hypothesis of normality using the function 'person.test' in the R programming language (R Development Core Team, 2022).

Permutation tests were used to validate the effect of the spiked concentration, laboratory sample size and test portion size of each sampling level (Good, 2005). Each test was based on 10 000 permutations and a significance level of $\alpha = 0.05$. The insignificantly different estimates were pooled to obtain the final estimates of the mean and variances of each sampling level.

Acceptance probabilities were calculated to assess the producer's and buyer's risks of increasing the spiked concentration and sample sizes. For comparison, the acceptance probability associated with the average of two samples was also validated. $P(x < \rho)$ denoted the acceptance probability of a single new measurement x and a threshold value of ρ. Thus, $P(x < \rho) = \Phi(z)$, where $\Phi(z)$ is the cumulative distribution function of the standard normal distribution (i.e. a normal distribution with a mean of 0 and standard deviation of 1), where z is the standard score. Further details can be found in the supplementary material.

3. Results and discussion

3.1. Variability associated with milk analytical measurements

3.1.1. ELISA parameters

The performance of the commercial ELISA kit in detecting milk proteins in rice flour was evaluated. The standard curve of milk proteins at 0, 0.4, 1, 4 and 10 ppm milk protein, showing the mean of 15 determinations and SDs, is represented in Fig. 2.

The analytical sensitivity is a critical parameter in the suitability of an assay for measuring allergen content in foods, and in this study, LOD and LOQ of the milk ELISA kit corresponded to 0.31 ppm and 0.41 ppm of milk protein in rice flour, respectively.

The intra-assay CV using milk-spiked samples of three concentrations ranged from 5% to 8% depending on the concentration (Table 1), which was below the 10% described by the manufacturers for food matrices other than rice flour (e.g. soy milk, cookies, chocolate etc) (Eurofins Technology). Moreover, the plate-to-plate CV, calculated using standards of three concentrations, ranged from 8% to 14% depending on the concentration (Table 2).

The intra-assay and plate-to-plate variation results indicated that the milk ELISA kit performed better using rice flour than other matrices tested by the manufacturers (Eurofins Technology).

Taking all these results, it can be concluded that this ELISA kit is suitable for the purpose of the present experiment to measure milk protein contamination of rice flour ≥0.5 ppm.

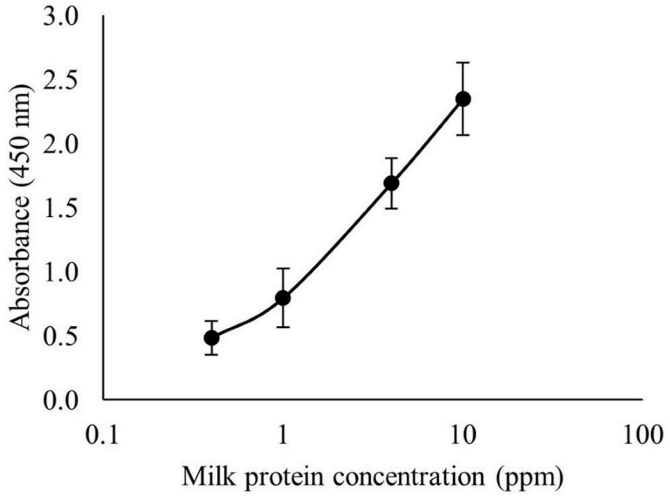

Fig. 2. Standard curve for milk protein standards (0, 0.4, 1, 4 and 10 ppm) obtained by a commercial milk ELISA kit. Each data point represents the mean of 15 determinations ± SD. The x-axis is shown on $\log_{10}$ scale where milk protein concentrations (ppm) are plotted.

Table 1
Intra-assay variation determined by testing milk protein-spiked samples of three concentrations in 16 replicates using a commercial milk ELISA kit.

Spiked concentration (ppm)	Mean ± SD (ppm)	CV (%)	Recovery (%)
0.5	0.67 ± 0.05	7.93	134
50	45.60 ± 2.31	5.07	91.2
250	245.50 ± 14.25	5.80	98.2

ppm: parts per million; SD: standard deviation; CV: coefficient of variation.

Table 2
Plate-to-plate variation determined by testing milk protein standards of three concentrations in 10 different test assays using a commercial milk ELISA kit.

Milk protein concentration (ppm)	Mean ± SD (OD)	CV (%)
1	0.82 ± 0.11	13.95
4	1.77 ± 0.20	11.44
10	2.50 ± 0.19	7.96

ppm: parts per million; SD: standard deviation; CV: coefficient of variation.

3.1.2. Variability and distribution of the measured milk protein concentration

The present study was designed to add science-based knowledge to the different aspects of sampling to help the food industry and food controlling authorities when developing sampling plans. A sampling plan was designed where seven 1000 g lots of rice flour were spiked with different amounts of milk protein (Fig. 1) to cover contamination scenarios where the most sensitive individuals may experience an allergic reaction (ED01 equal to 0.3 mg milk protein). Each lot was divided into five laboratory samples consisting of either 100 g or 200 g, and each laboratory sample was divided into four test portions consisting of 0.5 g or 5 g (Fig. 1). It should be noticed that half of each lot was analysed in experiment 1 (5 laboratory samples of 100 g) while the complete lot (5 laboratory samples of 200 g) was analysed in experiment 2. A total of 560 milk protein concentration measurements were obtained from the two experiments using the commercial milk ELISA kit. The design of the sampling plan was based on guides from the literature since there is no regulation regarding the number and size of samples to be analysed for allergen contamination in foods, and presently, sampling plans for food allergen analyses are based on expert knowledge (Allergen Bureau, 2022; Remington et al., 2022; Sharma et al., 2020, 2021). Indeed, the Allergen Bureau from Australia has developed a guidance concerning agricultural cross-contact that includes suggestions for sampling (Allergen Bureau, 2022). Although the primary target is agricultural cross-contact, the sampling plan may also be used for other situations where testing of products for allergens is needed. The first step in the procedure is to identify the level of UAP risk based on a questionnaire. For example, whereas five samples should be tested if the level of risk is low, a minimum of 15 samples or 10% of consignment (if above 150 units) should be tested if the risk is high. The sample size should be a minimum of 100–200 g. Larger sample sizes (500–1000 g) may be appropriate for bulk commodities (Allergen Bureau, 2022). Another recent guidance from a food industry organisation, ILSI Europe, also suggests strategies for sampling (Remington et al., 2022) and emphasises that because of the many parameters involved, there is no general 'one-fits-all' solution. A minimum sample size of 100 g is recommended, and the sample number is suggested to be based on the level of concern. For example, if the level of concern is low, a single or a small number of samples should be tested, whereas if the concern is medium, 2–6 samples should be tested. If the concern is high, the number of samples would depend on the type of allergen present. For homogeneous allergen

presence, at least six samples or two from each batch should be tested, and for non-regular and heterogeneous presence, incremental sampling could be considered (Remington et al., 2022). Moreover, Sharma et al. (2020) have contributed to the scientific knowledge on sampling by designing a study measuring the gluten content of heterogeneously distributed wheat in oat groats. In the study, 10 lots of 1600 g of oat groats were contaminated with an increasing number of wheat kernels equivalent to gluten contamination ranging from 0 to 164 ppm. Each 1600 g lot was divided into 16 laboratory samples of 100 g, which were then ground. From each of the ground laboratory samples, two test portions of 1 g each were extracted, and from each test portion, two aliquots were tested. The resulting 640 samples were then analysed by a commercial gluten ELISA kit (Sharma et al., 2020).

To determine the accuracy and variability of the measurements in the present study, the HME model was used to calculate the means and variances. The statistical significance of the difference between the estimated means and variances was validated using the permutation tests. For the HME model, the assumption of normally distributed residual errors was confirmed by comparing the results from the theoretical normal distribution histogram (Fig. S1), the quantile-quantile (Q-Q) plot (Fig. S2), and the Pearson's chi-squared test (p-value = 0.231).

Based on the estimates for the means and variances (Table 3) and the statistical significance (p-values) of the permutation tests (Table 4), it was seen that increasing the laboratory sample size from 100 g to 200 g resulted in a reduction of the variance of the laboratory sample by 88% ($p = 0.037$). The total variance is the sum of the variances of each sampling level, thus the proportion of the contribution of the variances of each sampling level to the total variance was determined. For the 100 g laboratory samples, the proportion of the contribution of the laboratory sample variance ranged from 36% to 77%, whereas those of the test portion variance and aliquot variance ranged from 0% to 31% and from 0% to 48%, respectively (Table 3). Thus, when the laboratory sample size was 100 g, the laboratory sample variance was generally the greatest contributor to the total variance among the three variance components. This was in agreement with observations from analysis of gluten-containing wheat contamination in oats and mycotoxins in agricultural commodities (Ozay et al., 2007; Sharma et al., 2020, 2021; Whitaker et al., 2015). For example, Ozay et al. (2007) observed that the laboratory sample size accounted for ~99% of the total variance when a 10 kg sample was taken from a lot with an aflatoxin concentration of 10 ng/g. Thus, Ozay et al. (2007) concluded that optimising the laboratory sample size should be the first step to reduce analytical variability (Ozay et al., 2007). Similarly, when 16 samples of 100 g each were taken from a gluten-containing lot, the laboratory sample variance was the greatest contribution to the total variance, ranging from 47% to 97% depending on gluten concentration, as compared to the test portion variance that ranged from 3% to 53% (Sharma et al., 2020). In our study, when the laboratory sample size was increased to 200 g, the contribution of the laboratory sample was reduced to a range of 19%–52%, generally

Table 4
P-values resulting from the permutation tests. Rows 1–3 contain the p-values related to the main effects, and rows 4–7 the p-values related to the interaction effects. Asterisk (*) indicates significant effects ($p < 0.05$).

Effects	Mean	Variances		
		Laboratory sample	Test portion	Aliquot
Spiked concentration	0.000*	0.000*	0.021*	0.000*
Laboratory sample size	0.626	0.037*	0.890	0.185
Test portion size	0.251	0.783	0.084	0.413
Spiked concentration - Laboratory sample size	0.011*	0.872	0.999	0.595
Spiked concentration - Test portion size	0.4401	0.689	0.928	0.033*
Laboratory sample size - Test portion size	0.023*	0.943	0.906	0.392
Spiked concentration - Laboratory sample size - Test portion size	0.733	0.939	0.978	0.497

Table 3
Mean milk protein concentrations (ppm), variances (ppm^2) and contributions of individual variances to the total variance of the 560 samples analysed by the ELISA kit using 1000 g lot at different spiked concentrations, 100 g and 200 g laboratory samples, 5 g and 0.5 g test portions and 2 aliquots.

Spiked concentration (ppm)	Laboratory sample (g)	Test portion (g)	Mean (ppm)	Variances (ppm^2)				% of total variance		
				Laboratory sample	Test portion	Aliquot	Total	Laboratory sample	Test portion	Aliquot
0	100	0.5	0.16	0.00	0.00	0.00	0.01	52	0	48
0	100	5	0.20	0.00	0.00	0.00	0.01	36	31	33
0	200	0.5	0.16	0.00	0.00	0.00	0.01	52	0	48
0	200	5	0.20	0.00	0.00	0.00	0.01	36	31	33
0.5	100	0.5	0.54	0.09	0.02	0.00	0.11	75	22	3
0.5	100	5	0.42	0.09	0.02	0.00	0.11	75	22	4
0.5	200	0.5	0.85	0.01	0.02	0.00	0.04	27	64	10
0.5	200	5	0.91	0.01	0.02	0.00	0.04	26	63	11
5	100	0.5	4.16	4.18	1.20	0.01	5.39	77	22	0
5	100	5	3.26	4.18	1.20	0.13	5.50	76	22	2
5	200	0.5	4.84	0.50	1.20	0.01	1.72	29	70	1
5	200	5	5.26	0.50	1.20	0.13	1.83	27	66	7
20	100	0.5	25.67	19.80	5.70	4.46	29.96	66	19	15
20	100	5	23.70	19.80	5.70	2.09	27.59	72	21	8
20	200	0.5	23.79	2.38	5.70	4.46	12.54	19	45	36
20	200	5	24.70	2.38	5.70	2.09	10.17	23	56	21
50	100	0.5	63.62	183.39	52.80	39.86	276.05	66	19	14
50	100	5	57.61	183.39	52.80	9.57	245.76	75	21	4
50	200	0.5	45.93	22.01	52.80	39.86	114.67	19	46	35
50	200	5	48.70	22.01	52.80	9.57	84.38	26	63	11
250	100	0.5	326.79	10095.51	2906.82	1360.06	14362.38	70	20	9
250	100	5	282.25	10095.51	2906.82	1690.94	14693.26	69	20	12
250	200	0.5	219.62	1211.36	2906.82	1360.06	5478.24	22	53	25
250	200	5	240.15	1211.36	2906.82	1690.94	5809.12	21	50	29
500	100	0.5	512.61	22765.29	6554.85	4825.05	34145.19	67	19	14
500	100	5	445.72	22765.29	6554.85	4097.41	33417.55	68	20	12
500	200	0.5	667.51	2731.62	6554.85	4825.05	14111.52	19	46	34
500	200	5	698.35	2731.62	6554.85	4097.41	13383.88	20	49	31

ppm: parts per million.

making the test portion size the largest contributor to the total variance. However, increasing the test portion size from 0.5 g to 5 g did not significantly affect the test portion variance ($p = 0.084$), indicating that increasing the sample size by a factor of 10 had a limited effect, which could be due to a gradual demixing of the samples when the test portions were extracted.

Moreover, in general, the mean ($p = 0.000$) and variances of the laboratory sample ($p = 0.000$), test portion ($p = 0.021$), and aliquot ($p = 0.000$) increased as a function of the spiked concentration in the lot (Tables 3 and 4). This was also the case for heterogeneously distributed gluten and mycotoxin analyses (Ozay et al., 2007; Sharma et al., 2020, 2021; Whitaker et al., 2015). For example, Sharma et al. (2020) and Whitaker et al. (2015) observed that the total variance increased as a function of the gluten concentration in oat samples spiked with wheat kernels (Sharma et al., 2020) or as a function of the ochratoxin-A concentration in oats (Whitaker et al., 2015).

In addition, several interactions between the spiked concentration, laboratory sample size, and test portion size were observed (Tables 3 and 4). Interactions between the spiked concentration and laboratory sample size ($p = 0.011$), and the laboratory sample size and test portion size ($p = 0.023$) led to overestimations of the mean concentration of 14% on average for a laboratory sample size of 100 g and 16% for a laboratory sample size of 200 g when the test portion size was 0.5 g. Conversely, when the test portion size was 5 g, the mean was slightly underestimated by −3% for a laboratory sample size of 100 g and overestimated by 23% for a laboratory sample size of 200 g. The spiked concentration also interacted with the test portion size ($p = 0.033$), causing the variance of the aliquot to alternately increase and decrease between the test portion sizes depending on the spiked concentration. Interactions with the laboratory sample size were expected as the 100 g and 200 g sample sizes were obtained under different conditions. The interactions did, however, not affect the variances of the laboratory sample and test portion, which were the focus of the present study.

Since the variance increased as a function of the spiked concentration in the lot, standardisation of the measured values was performed to allow direct comparison across the different spiked concentrations (Fig. 3). The overall means and SDs of each spiked concentration were calculated (Table S1). The blue dots, corresponding to 200 g laboratory samples, were generally less scattered than the red dots, corresponding to 100 g laboratory samples, illustrating that increasing the laboratory sample size from 100 g to 200 g reduces the variance of the laboratory sample, which confirms the findings presented in Tables 3 and 4

The observed dependence between the laboratory sample size and variance of the measured concentration of the laboratory sample can be explained by a study conducted by Lacey (1997). Lacey (1997) performed a mathematical analysis of the concentration variance in homogeneous samples containing arbitrary equally sized particles and noticed that the number of sampled particles followed a binomial distribution (Lacey, 1997). If s denotes the spiked concentration in the lot and n_{total} is the total number of particles in the sample, the distribution of the measured concentration equals the distribution of the sampled proportion for n_{total} binomial trials. In this case, the variance of the measured concentration, $\sigma^2_{measured}$, is:

$$\sigma^2_{measured} = \frac{s(1-s)}{n_{total}} \quad (1)$$

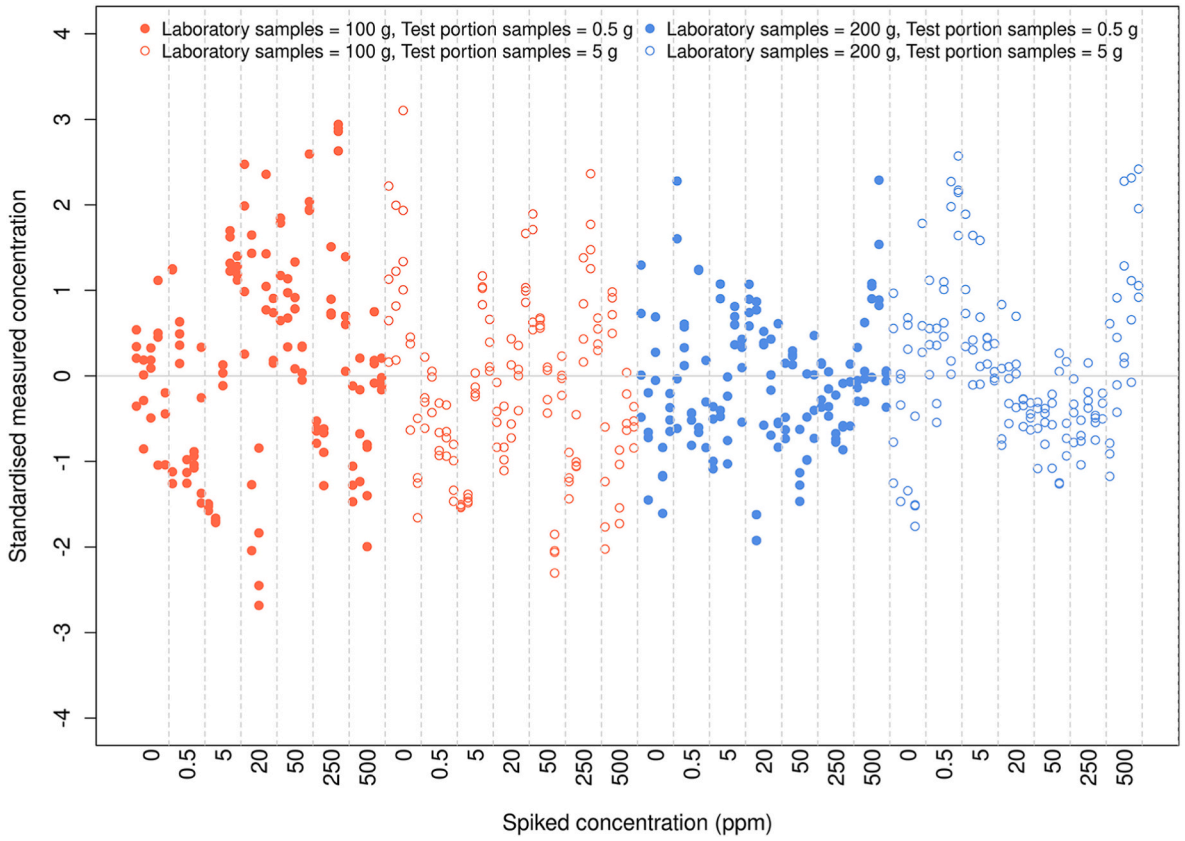

Fig. 3. Standardised measured milk protein concentrations as a function of spiked milk protein concentrations, laboratory sample size and test portion size. The y-axis reflects the number of standard deviations (SDs) from the mean measured concentration (Table S1). The dashed borders encase the 20 measurements of the same laboratory sample and test portion size from a lot of a particular spiked concentration. The 20 measurements contain five laboratory samples, two test portions per laboratory sample, and two aliquots per test portion. Each dot represents a single aliquot. Red symbols indicate a measurement corresponding to a laboratory sample size of 100 g, and blue symbols indicate a laboratory sample size of 200 g. Solid symbols indicate a measurement corresponding to test portions of 0.5 g, and open symbols to test portions of 5 g. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Assuming all particles in a lot have an equal weight of w, the total weight of the sample is $W = n_{total} \cdot w \Leftrightarrow n_{total} = W/w$. Inserting the expression for n_{total} into equation (1) leads to:

$$\sigma^2_{measured} = \frac{ws(1-s)}{W} \quad (2)$$

Equation (2) shows that the variance of the measured concentration is inversely proportional to the total weight of the sample. A similar conclusion was reached by Yalkowsky and Bolton (1990) (Yalkowsky & Bolton, 1990). As a result, larger samples reduce the variance of the measured concentration. In our study, this explains the observed reduction of the laboratory sampling variance when the laboratory sample size was increased from 100 g to 200 g (Fig. 3, Table 3).

It is known that creating homogeneous mixtures is a challenge as particle size, density, and shape, as well as increasing mixing time, may create demixing or separation of the substances, influencing the outcome (Brennan & Grandison, 2011; Fritz et al., 2017; Hogg, 2009). Contamination homogeneity was also investigated by Fritz et al. (2017), who used the R-Biopharm R5 ELISA kit with the recommended extraction of 0.25 g of a minimum of 5 g of homogenised sample to detect gluten in oats (Fritz et al., 2017). A total of 636 pouch servings of gluten free oatmeal were ground and analysed. Among these 636 servings, 10 samples had a gluten content above 20 ppm and 20 samples had a gluten content between 5 and 20 ppm. However, when 10 additional aliquots (0.25 g) from each of the 20 samples were analysed, a high gluten content heterogeneity was observed in the same serving portion with values below the detection level. According to Lacey (1997), the measured concentration of a homogeneous sample will follow the distribution of the sampled proportion from a binomial distribution, which closely resembles the normal distribution (Lacey, 1997). In the study by Fritz et al. (2017), the results followed a log-normal distribution, suggesting a non-homogeneous distribution of gluten particles despite the grounding being performed as recommended. In our study, the distribution of the residual error is not skewed, indicating a homogeneous distribution of milk particles (Fig. S1). This is contrary to the positively skewed data observed for non-uniform gluten or mycotoxin contamination (Sharma et al., 2020, 2021; Whitaker et al., 1972). In addition, the observation that the dependence between the laboratory sample size and the variance of the laboratory sample behaves according to equation (2) is another indication of a homogeneous distribution.

In our study, the HME model was used to derive the variance of each sampling level in the experiment design, whereas Sharma et al. (2020, 2021) used the 'Nested procedure' from the SAS software (Sharma et al., 2020, 2021). According to the SAS documentation, this is equivalent to employing the HME model. While a series of permutation tests to validate the statistical significance of the spiked concentration, laboratory sample size and test portion size on the mean and the variance measured concentration were used in our study, Sharma et al. (2020, 2021) used linear regressions to validate the statistical significance. Additionally, Sharma et al. (2020, 2021) assumed that the variance at each sampling level was inversely proportional to the sample size and used this assumption to extrapolate the results to different laboratory and test portion sizes. Our study proved that, indeed, increasing the laboratory sample size decreased the variance of the laboratory sample.

To determine whether increasing the laboratory sample size was more effective in reducing analytical variability than taking two samples, the total variance for a setting with two laboratory samples, one test portion per laboratory sample, and one aliquot per test portion was calculated (Table 5). The setting uses the average of the two resulting aliquots to estimate the measured concentration. The average total variance was reduced by 63% when the laboratory sample size was increased from 100 g to 200 g, compared to a 50% reduction for two 100 g samples, which indicates that increasing a single laboratory sample size from 100 g to 200 g leads to a lower variance than testing two 100 g samples.

Based on the specific sampling plan designed and used in this study, the overall data indicated that optimisation of the laboratory sample size would be the most efficient step to reduce variability. This is because the laboratory sample has a greater effect on the measurements' variability than the test portion size. This is in line with studies on heterogeneously distributed gluten and mycotoxin contamination (Sharma et al., 2020, 2021; Whitaker et al., 2015).

It should be noted that our conclusions are based on the simplest scenario we could design, a homogeneous amorphous contamination. How our mixing procedure reflects the situation in an industrial setting is unknown. A heterogeneous contamination will add another layer of complexity that our results do not help to clarify. In general levels of food allergen contamination are reported without any information about sampling. Consequently, a first step in adding to knowledge and transparency would be to request a more detailed level of reporting.

3.2. Sample misclassification risk

Prediction of the risks associated with a sampling plan would allow the design of adequate sampling plans to reduce misclassification of lots. In this study, the performance of the sampling plan was tested by determining the probability of accepting an analytical result that surpasses the determined acceptance milk protein contamination levels of 3 ppm and 32 ppm corresponding to ED01 and ED05 values, respectively, and a food intake of 100 g (Table 6). The probability of accepting a non-compliant lot (higher than the milk protein acceptance levels) constitutes the buyer's risk, and the probability of rejecting a compliant

Table 5
Total variances (ppm^2) for three scenarios: A single laboratory sample of 100 g, a single laboratory sample of 200 g and two laboratory samples of 100 g. The estimates were obtained from Table 3. Mean milk protein concentrations (ppm) for two scenarios: A single laboratory sample of 100 g and a single laboratory sample of 200 g.

Spiked concentration (ppm)	Test portion (g)	Total variances (ppm^2)			Mean (ppm)	
		Laboratory size 100 g 1 sample	Laboratory size 200 g 1 sample	Laboratory size 100 g 2 samples	Laboratory size 100 g	Laboratory size 200 g
0.5	0.5	0.11	0.04	0.06	0.54	0.85
0.5	5	0.11	0.04	0.06	0.42	0.91
5	0.5	5.39	1.72	2.7	4.16	4.84
5	5	5.5	1.83	2.75	3.26	5.26
20	0.5	29.96	12.54	14.98	25.67	23.79
20	5	27.59	10.17	13.79	23.7	24.7
50	0.5	276.05	114.67	138.03	63.62	45.93
50	5	245.76	84.38	122.88	57.61	48.7
250	0.5	14362.38	5478.24	7181.19	326.79	219.62
250	5	14693.26	5809.12	7346.63	282.25	240.15
500	0.5	34145.19	14111.52	17072.6	512.61	667.51
500	5	33417.55	13383.88	16708.78	445.72	698.35

ppm: parts per million.

Table 6
Acceptance probabilities for three scenarios: A single laboratory sample of 100 g, a single laboratory sample of 200 g and two laboratory samples of 100 g. The acceptance thresholds corresponded to ED01 (3 ppm) and ED05 (32 ppm).

Spiked concentration (ppm)	Test portion (g)	Laboratory size 100 g 1 sample		Laboratory size 200 g 1 sample		Laboratory size 100 g 2 samples	
		ED01 (3 ppm)	ED05 (32 ppm)	ED01 (3 ppm)	ED05 (32 ppm)	ED01 (3 ppm)	ED05 (32 ppm)
0.5	0.5	1.000	1.000	1.000	1.000	1.000	1.000
0.5	5	1.000	1.000	1.000	1.000	1.000	1.000
5	0.5	0.308	1.000	0.080	1.000	0.239	1.000
5	5	0.456	1.000	0.048	1.000	0.438	1.000
20	0.5	0.000	0.876	0.000	0.990	0.000	0.949
20	5	0.000	0.943	0.000	0.989	0.000	0.987
50	0.5	0.000	0.029	0.000	0.097	0.000	0.004
50	5	0.000	0.051	0.000	0.035	0.000	0.010
250	0.5	0.003	0.007	0.002	0.006	0.000	0.000
250	5	0.011	0.019	0.001	0.003	0.001	0.002
500	0.5	0.003	0.005	0.000	0.000	0.000	0.000
500	5	0.008	0.012	0.000	0.000	0.000	0.001

ppm: parts per million.
ED: Eliciting dose.

lot (lower than the milk protein acceptance levels) constitutes the producer's risk. The acceptance probabilities were calculated using the cumulative standard normal distribution (Section 2.4) and the estimated means and total variances for the measured concentrations (Table 5). As expected, increasing the laboratory sample size led to larger acceptance probabilities below the thresholds and lower acceptance probabilities above the thresholds. For example, the probability of accepting or misclassifying a sample containing 5 ppm milk protein using an acceptance level of 3 ppm was decreased from 30.8% to 8.0% if the sample size increased from 100 g to 200 g, and to 23.9% if two samples of 100 g each were analysed (Table 6). On the other hand, the probability of accepting a sample containing 20 ppm milk protein using an acceptance level of 32 ppm was increased from 87.6% to 99.0% if the sample size increased from 100 g to 200 g, and to 94.9% if two samples of 100 g each were analysed. The only exception was the ED05 threshold, where the milk protein concentration was 50 ppm, and the test portion size was 0.5 g. In this case, the 100 g sample overestimated the mean concentration by 13.6 ppm, leading to a lower acceptance probability (Table 6). The scenario containing two laboratory samples exhibited similar behaviour and only outperformed the 200 g sample due to overestimating the mean concentration. Thus, increasing the laboratory sample size achieved a reduction in the measured concentration variance and a reduction of the producer's and buyer's risks, which confirmed the results and assumptions of other studies (Fritz et al., 2017; Sharma et al., 2020, 2021) and the theoretical analysis by Lacey (1997) (Lacey, 1997). Indeed, a similar behaviour was observed by Fritz et al. (2017), who determined that the probability of accepting a sample being $<$20 ppm was 63.0%, which resulted in the producer's risk of 37.0% of samples rejected (Fritz et al., 2017). The authors concluded that the conventional use of a single 0.25 g test should be treated with caution. No further studies were performed, considering the consequences of the results if the amount extracted was larger. Moreover, Sharma et al. (2020, 2021) assumed that increasing the laboratory size reduced both the producer's and the buyer's risks, thus reducing misclassifying gluten-containing lots (Sharma et al., 2020, 2021). Based on their assumption, the authors concluded that the buyer's risk of a lot with 25 ppm gluten was 59.0% when the laboratory sample size was 100 g and 21.4% when the laboratory sample size was 5000 g. The producer's risk of a lot with 15 ppm gluten concentration was 24.0% and 14.0% when the laboratory sample size was 100 g and 5000 g, respectively (Sharma et al., 2020).

4. Conclusion

In the present study, we experimentally tested the influence of the spiked concentration, laboratory sample and test portion sizes in a model of amorphous homogenous allergen contamination of milk powder in rice flour. The statistical methods used allowed validation of the statistical significance of the different sampling levels on analytical variability of milk protein contamination detection. Based on experimental data, we demonstrated that increasing laboratory sample size was an efficient way to improve measurements' precision and reduce producer's and buyer's risks.

CRediT authorship contribution statement

Anders R. Andersen: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. **Charlotte B. Madsen:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Katrine L. Bøgh:** Writing – review & editing, Funding acquisition. **Ana I. Sancho:** Writing – review & editing, Writing – original draft, Project administration, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2025.111195.

Data availability

Data will be made available on request.

References

Allergen Bureau. (2022). Assessing agricultural cross contact. WWW.allergenbureau.net.
Blom, W. M., Michelsen-Huisman, A. D., van Os-Medendorp, H., van Duijn, G., de Zeeuw-Brouwer, M., Versluis, A., Castenmiller, J. J. M., Noteborn, H. P. J. M., Kruizinga, A. G., Knulst, A. C., & Houben, G. F. (2018). Accidental food allergy

reactions: Products and undeclared ingredients. *Journal of Allergy and Clinical Immunology, 142*(3), 865–875. https://doi.org/10.1016/j.jaci.2018.04.041
Brennan, J. G., & Grandison, A. S. (2011). Mixing, emulsification, and size reduction. *Food Processing Handbook: 2nd Edition, 1*, 363–406. https://doi.org/10.1002/9783527634361.CH11
Codex Alimentarius. (2004). *General guidelines on sampling (CAC/GL 50-2004)*.
Codex Alimentarius. (2008). *Standard for foods for special dietary use for persons intolerant to gluten (CXS 118-1979) GLUTEN*.
Codex Alimentarius. (2020). *Code of practice on food allergen management for food business operators (CXC80-2020)*.
European Commission. (2003). Directive 2003/89/EC of the European Parliament and of the Council of 10 November 2003 amending Directive 2000/13/EC as regards indication of the ingredients present in foodstuffs. *Official Journal of the European Union, L308*, 15–18.
European Commission. (2006a). Commission Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. *Official Journal of the European Union, L70*, 12–34.
European Commission. (2006b). Commission Regulation (EC) No 1881/2006 of 16 December 2006 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Union, L364*, 5–24.
European Commission. (2010). Commission Regulation (EU) No 105/2010 of 5 February 2010 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards ochratoxin A. *Official Journal of the European Union, L35*, 7–8.
European Commission. (2011). Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers. *Official Journal of the European Union, L304*, 18–63.
Fritz, R. D., Chen, Y., & Contreras, V. (2017). Gluten-containing grains skew gluten assessment in oats due to sample grind non-homogeneity. *Food Chemistry, 216*, 170–175. https://doi.org/10.1016/J.FOODCHEM.2016.08.031
Good, P. I. (2005). Permutation, parametric and bootstrap tests of hypotheses. In P. Good (Ed.), *Permutation, parametric and bootstrap tests of hypotheses* (3rd ed.). Springer Science. https://doi.org/10.1007/B138696.
Hogg, R. (2009). Mixing and segregation in powders: Evaluation, mechanisms and processes. *KONA Powder and Particle Journal, 27*(0), 3–17. https://doi.org/10.14356/kona.2009005
Johansson, A. S., Whitaker, T. B., Hagler, W. M., Giesbrecht, F. G., Young, J. H., & Bowman, D. T. (2000). Testing shelled corn for aflatoxin, Part I: Estimation of variance components. *Journal of AOAC International, 83*(5), 1264–1269. https://academic.oup.com/jaoac/article/83/5/1264/5656427.
Joint Research Centre. (2014). Technical Report: Guidelines for sample preparation procedures in GMO analysis. https://doi.org/10.2788/738570.
Lacey, P. M. C. (1997). The mixing of solid particles. *Chemical Engineering Research and Design, 75*, S49–S55. https://doi.org/10.1016/S0263-8762(97)80004-4
Latimer, G. (2023). Appendix M guidance on food allergen immunoassay validation. In G. Latimer (Ed.), *Official methods of analysis of AOAC INTERNATIONAL* (22nd ed.). Oxford University Press.
Lee, N. A., Lopata, A. L., & Colgrave, M. L. (2023). Analytical methods for allergen control in food processing. *Foods, 12*(7), 1439. https://doi.org/10.3390/foods12071439
Madsen, H., & Thyregod, P. (2010). *Introduction to general and generalized linear models*. CRC Press. https://doi.org/10.1201/9781439891148
Miraglia, M., De Santis, B., Minardi, V., Debegnach, F., & Brera, C. (2005). The role of sampling in mycotoxin contamination: An holistic view. *Food Additives & Contaminants, 22*(sup1), 31–36. https://doi.org/10.1080/02652030500389055
Ozay, G., Seyhan, F., Yilmaz, A., Whitaker, T. B., Slate, A. B., & Giesbrecht, F. (2006). Sampling hazelnuts for aflatoxin: Uncertainty associated with sampling, sample preparation, and analysis. *Journal of AOAC International, 89*(4), 1004–1011. https://doi.org/10.1093/JAOAC/89.4.1004
Ozay, G., Seyhan, F., Yilmaz, A., Whitaker, T. B., Slate, A. B., & Giesbrecht, F. G. (2007). Sampling hazelnuts for aflatoxin: Effect of sample size and accept/reject limit on reducing the risk of misclassifying lots. *Journal of AOAC International, 90*(4), 1028–1035. https://doi.org/10.1093/JAOAC/90.4.1028
Paez, V., Barrett, W. B., Deng, X., Diaz-Amigo, C., Fiedler, K., Fuerer, C., Hostetler, G. L., Johnson, P., Joseph, G., Konings, E. J. M., Lacorn, M., Lawry, J., Liu, H., Marceau, E., Mastovska, K., Monteroso, L., Pan, S.-J., Parker, C., Phillips, M. M., … Coates, S. G. (2016). Aoac SMPR® 2016.002. *Journal of AOAC International, 99*(4), 1122–1124. https://doi.org/10.5740/jaoacint.SMPR2016.002
Pinheiro, J., & Bates, D. (2022). nlme: Linear and nonlinear mixed effects models. *R package version, 3*, 1–160. https://CRAN.R-project.org/-package=nlme.
R Development Core Team. (2022). R: A language and environment for statistical computing. https://www.R-project.org/.
Remington, B. C., Baumert, J., Blom, W. M., Bucchini, L., Buck, N., Crevel, R., De Mooij, F., Flanagan, S., Hindley, J., Javed, B., Stavropoulou, D. A., van den Dungen, M. W., van Ravenhorst, M., Wang, S., & Walker, M. (2022). Allergen quantitative risk assessment within food operations: Concepts towards development of practical guidance based on an ILSI Europe workshop. *Food Control, 138*, Article 108917. https://doi.org/10.1016/j.foodcont.2022.108917
Remington, B. C., Westerhout, J., Meima, M. Y., Blom, W. M., Kruizinga, A. G., Wheeler, M. W., Taylor, S. L., Houben, G. F., & Baumert, J. L. (2020). Updated population minimal eliciting dose distributions for use in risk assessment of 14 priority food allergens. *Food and Chemical Toxicology, 139*, Article 111259. https://doi.org/10.1016/j.fct.2020.111259
Sena-Torralba, A., Pallás-Tamarit, Y., Morais, S., & Maquieira, Á. (2020). Recent advances and challenges in food-borne allergen detection. *TrAC Trends in Analytical Chemistry, 132*, Article 116050. https://doi.org/10.1016/j.trac.2020.116050
Sharma, G. M., Pereira, M., Wang, S. S., Chirtel, S. J., Whitaker, T. B., Wehling, P., Arlinghaus, M., Canida, T., Jackson, L. S., & Williams, K. M. (2020). Evaluation of sampling plans for measurement of gluten in oat groats. *Food Control, 114*, Article 107241. https://doi.org/10.1016/J.FOODCONT.2020.107241
Sharma, G. M., Wang, S. S., Pereira, M., Bedford, B., Wehling, P., Arlinghaus, M., Warren, J., Whitaker, T. B., Jackson, L. S., Canida, T., & Chirtel, S. J. (2021). Sampling plan designs for gluten estimation in oat flour by discrete and composite sampling. *Food Control, 129*, Article 107943. https://doi.org/10.1016/J.FOODCONT.2021.107943
Sicherer, S. H., & Sampson, H. A. (2014). Food allergy: Epidemiology, pathogenesis, diagnosis, and treatment. *Journal of Allergy and Clinical Immunology, 133*(2), 291–307.e5. https://doi.org/10.1016/j.jaci.2013.11.020
Sicherer, S. H., & Sampson, H. A. (2018). Food allergy: A review and update on epidemiology, pathogenesis, diagnosis, prevention, and management. *Journal of Allergy and Clinical Immunology, 141*(1), 41–58. https://doi.org/10.1016/j.jaci.2017.11.003
Tittlemier, S. A., Varga, E., Scott, P. M., & Krska, R. (2011). Sampling of cereals and cereal-based foods for the determination of ochratoxin A: An overview. *Food Additives & Contaminants: Part A, 28*(6), 775–785. https://doi.org/10.1080/19440049.2011.559278
Versluis, A., Knulst, A. C., Kruizinga, A. G., Michelsen, A., Houben, G. F., Baumert, J. L., & van Os-Medendorp, H. (2015). Frequency, severity and causes of unexpected allergic reactions to food: A systematic literature review. *Clinical & Experimental Allergy, 45*(2), 347–367. https://doi.org/10.1111/cea.12328
Warren, C. M., Jiang, J., & Gupta, R. S. (2020). Epidemiology and burden of food allergy. *Current Allergy and Asthma Reports, 20*(2), 6. https://doi.org/10.1007/s11882-020-0898-7
Whitaker, T. B., Dickens, J. W., Monroe, R. J., & Wiser, E. H. (1972). Comparison of the observed distribution of aflatoxin in shelled peanuts to the negative binomial distribution. *Journal of the American Oil Chemists' Society, 49*(10), 590–593. https://doi.org/10.1007/BF02609233
Whitaker, T. B., Dowell, F. E., Hagler, W. M., Giesbrecht, F. G., & Wu, J. (1994). Variability associated with sampling, sample preparation, and chemical testing for aflatoxin in farmers' stock peanuts. *Journal of AOAC INTERNATIONAL, 77*(1), 107–116. https://doi.org/10.1093/JAOAC/77.1.107
Whitaker, T. B., Slate, A. B., Hurley, J. M., & Giesbrecht, F. G. (2007). Sampling almonds for aflatoxin, Part II: Estimating risks associated with various sampling plan designs. *Journal of AOAC International, 90*(3), 778–785. https://doi.org/10.1093/JAOAC/90.3.778
Whitaker, T. B., Slate, A. B., Nowicki, T. W., & Giesbrecht, F. G. (2015). Variability and distribution among sample test results when sampling unprocessed oat lots for ochratoxin A. *World Mycotoxin Journal, 8*(4), 511–524. https://doi.org/10.3920/WMJ2014.1858
Yalkowsky, S. H., & Bolton, S. (1990). Particle size and content uniformity. *Pharmaceutical Research: An Official Journal of the American Association of Pharmaceutical Scientists, 7*(9), 962–966. https://doi.org/10.1023/A:1015958209643