

Allergen Sanitation in the Food Industry: A Systematic Industrial Scale Approach To Reduce Hazelnut Cross-Contamination of Cookies

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ABSTRACT

Recently, we investigated the impact of shared equipment on cross-contamination of cookies at a pilot plant scale. Based on those findings, this study investigated the extent and subsequent sanitation of hazelnut cross-contamination (HNCC) of cookies at the industrial scale. Similarly, a product change from cookies with hazelnut ingredient to cookies without hazelnut was performed on standard equipment. HNCC in the hazelnut-free follow-up product was quantified by enzyme-linked immunosorbent assay for each production device and the applied cleaning procedure. All experiments were repeated in duplicate. The highest HNCC was found in concordance with previous studies after mere mechanical scraping: more than 1,000 mg of hazelnut protein per kg was quantified in the follow-up product after processing by a cookie machine. Additional cleaning with hot water decreased the HNCC irrespective of the processing device to levels at or below 1 mg of hazelnut protein per kg. Furthermore, raw materials for cookie production were monitored over a period of 24 months for unwanted preloads of hazelnut and peanut: hazelnut was quantified in 16% of the investigated raw materials as being between 0.26 and 90 mg/kg. Further critical control points at the industrial scale, where cross-contamination might occur, were identified but did not display noteworthy sources of cross-contamination. In conclusion, the quantitative monitoring of the cleaning efficiency at the industrial scale confirmed the procedure of manual scraping plus wet cleaning as a qualified sanitation procedure to effectively reduce the hazelnut protein cross-contamination down to a level at which severe hazelnut-related allergic reactions are unlikely to occur.

Food allergy is an increasingly reported health problem. Currently, it is estimated that food allergies affect approximately 2 to 3% of the adult population and 4 to 8% of children (15, 16, 22). Allergies to hazelnut (*Corylus avellana*) and peanut (*Arachis hypogaea*) have each been listed among the top five serious food allergies (1, 11). Even minute amounts of hazelnut or peanut protein, at or below the low milligram level, can cause adverse reactions in highly sensitized individuals. At higher levels both can cause life-threatening symptoms and fatalities (27–29). In contrast to the United States, in Europe hazelnut is much more commonly used in the food industry than peanut. Hazelnut is used as a basic compound of nut fillings in various cakes and in a wide variety of confectionary products (8, 13, 27).

Although some studies show promising results for a successful induction of oral tolerance for certain allergens (4, 20), a general causative immunotherapy for food allergy is currently not available (19). As a consequence the treatment of food allergies requires strict avoidance of the

offending food allergens (5, 17). Therefore, reliable labeling of allergenic constituents is of paramount importance; but it is not yet fully available.

The labeling of most common allergenic foods that are added as ingredients in prepackaged foods has improved under regulatory amendments, such as the European Union Labeling Directive 2007/68/EC and the U.S. Food Allergen Labeling and Consumer Protection Act (6, 24). A lack of legal thresholds for adventitious contamination with allergens, so-called “hidden” allergens, imposes continued challenges for consumers with food allergies. For food manufacturers these hidden allergens represent a problem that is difficult to control. Fairly little is known about the extent and frequency of cross-contamination, and the producer is challenged to decide on the use of precautionary labeling. To limit advisory labeling to products where it is necessary, food manufacturers require sound knowledge about the frequency and the extent of allergen cross-contamination during manufacture of ingredients and retail packaged goods.

Using the example of shortbread cookie production, we recently demonstrated that hazelnut cross-contamination due to shared production equipment can be a serious

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TABLE 1. *Cleaning protocols between product changes*

Protocol	Description
I	Manual scraping only
II	Manual scraping plus cleaning with 52°C hot water
III	Manual scraping plus cleaning with 52°C hot water and excessive final rinse with hot water

problem in pilot plant equipment (14). Depending on the application of cleaning between product changes and the availability of analytical testing to verify cleaning efficiency, qualified allergen sanitation procedures may be established and precautionary labeling thus avoided. This study describes the investigation of the extent of allergenic hazelnut cross-contamination due to shared equipment and the efficiency of various cleaning procedures in two industrial enterprises. In addition, we investigated the relevance of airborne contamination of the processing environment due to the production of hazelnut cookies. Lastly, we report results of a 2-year ingredient-monitoring program that examined ingredients and raw materials for the presence of unwanted hazelnut and peanut from different suppliers.

MATERIALS AND METHODS

Production plants for cookie manufacture and investigated machines. The plants for cookie manufacture were located at Würselen, Germany (Wilhelm Kinkartz GmbH & Co. KG), and at Krefeld, Germany (Wilhelm Gruyters GmbH & Co. KG). In each plant one dough mixer and one cookie machine were investigated. Experiments were performed with the following machines: spiral kneader, z-kneader, pastry-forming machine (rotary moulder), and dosing machine for the production of piped cookies with wire-cut (wire cutting machine). The kneading capacities of the spiral and the z-kneader were approximately 220 and 300 kg, respectively.

Production of shortbread cookies. The recipes were different for the rotary moulder (moulded cookies) and the wire cutting machine (piped cookies) and are not published to preserve corporate secrets. Hazelnut as an ingredient consisted of finely ground hazelnut flour for both recipes. All manufactured cookies consisted of “typical cookie ingredients.” Samples of all ingredients were analyzed prior to the study for hazelnut protein (HNP). The moulded hazelnut cookie consisted of 7% hazelnut and was produced with a spiral kneader and a rotary moulder. The piped hazelnut cookie consisted of 10% hazelnut and was manufactured with a z-kneader and a wire cutting machine. The hazelnut-free dough that was used for the investigations at the cookie machines (rotary moulder and wire cutting machine) was produced in precleaned z-kneader or spiral kneader to exclude any hazelnut contamination. The absence of hazelnut contamination of this dough was verified analytically.

Cleaning methods. After processing and removal of the dough that contained either 7 or 10% hazelnut, the manufacturing device under investigation was cleaned from adherent dough according to various protocols that are summarized in Table 1. For manual scraping of the equipment, common plastic scrapers were used. The wet-cleaning procedures were performed with hot tap water (52°C) from a hose and typical household brushes and sponges. Cleaning protocol I consisted of only manual scraping of

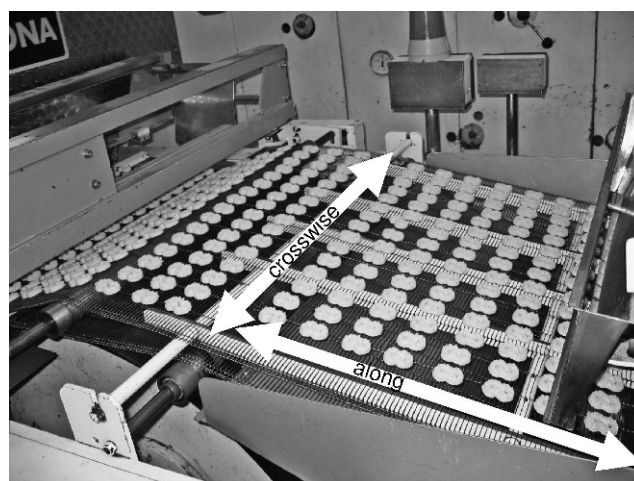


FIGURE 1. Sampling strategy at the cookie machines, with the wire cutting machine as an example. Dough samples were taken crosswise and along from the conveyor belt, depending on rotations of the feeding or moulding roller.

the devices. Cleaning protocol II, “manual scraping plus cleaning with 52°C hot water,” consisted of manual scraping and the use of hot tap water from a hose. Hot water from the hose was applied until no water turbidity was visible at the effluent water from the equipment. The additional excessive rinse step in cleaning protocol III, “manual scraping plus cleaning with 52°C hot water and excessive final rinse with hot water,” consisted of hot water cleaning that was applied for one-half of the time that was needed to perform cleaning method II.

Study design and sample drawing for shared production equipment. The homogeneous dough that contained finely ground hazelnut at either 7 or 10% was processed by one of the devices under study. After cleaning of the device by one selected cleaning protocol (Table 1), dough without hazelnut was processed. All samples were drawn directly after dough processing at the device for subsequent quantification of HNP cross-contamination caused by the individual device. All experiments were performed in duplicate. For the cookie machines only one of the two wet-cleaning methods was investigated, because wet cleaning caused downtimes of several hours. Cleaning method III was selected because it yielded lower cross-contamination in the previous study. Three different cleaning protocols were performed for the mixers. After kneading, samples of approximately 120 g were drawn from the spiral kneader 5 times and from the z-kneader 10 times from different areas of the kneader bowls. Sampling was performed at the rotary moulder and the wire cutting machine as the equipment was operating. In detail, cookie dough samples were taken separately crosswise and along from the conveyor belt next to the cookie machines, depending on rotations of moulding and/or feeding roller (Fig. 1). Each sample at the rotary moulder and the wire cutting machine consisted of approximately 30 cookies and represented an average of ~200 g. Samples were immediately stored at –21°C for quantification by enzyme-linked immunosorbent assay (ELISA). The impact of heat treatment during the baking process on the detectability of hazelnut was determined by investigating dough samples before and after baking.

Monitoring of common ingredients and raw materials used in the production of fine bakery wares. Over a period of 24 months, 148 single batches of ingredients and raw materials used for cookie production from three cookie manufacturers

(differing in company size) were analyzed for unwanted HNP and peanut protein, respectively. The following raw materials (with number of investigated batches in parentheses) were analyzed: chocolate coatings (37), different spices (18), baking agents and flavors (15), flour (13), marzipan (12), hazelnut (10), sugar (12), fat (10), skim milk powders (7), fruit fillings (5), soy lecithin (3), persipan (3), and chopped almonds (3).

Investigation of airborne hazelnut cross-contamination. In both cookie plants petri dishes were positioned at contamination-susceptible areas of cookie production, adjacent to mixers, cookie machines, balances, and cookie-packaging machines. Dust was collected in petri dishes during a 24-h time course of production of a cookie having 10% hazelnut ingredient. After dust sampling, sealed petri dishes were stored at -20°C until analysis by ELISA.

Sample homogenization and extraction protocol. Each sample was homogenized, and the presence of HNP was analyzed and quantified by ELISA as described elsewhere (14). Briefly, 10 g of homogenized dough was adjusted to 200 ml with extraction buffer and suspended with a homogenizer. Samples were then extracted at 37°C for 1 h. Extracts were clarified by centrifugation, and the supernatant was analyzed by ELISA.

Quantification of hazelnut or peanut cross-contamination by specific ELISA. The hazelnut-specific sandwich ELISA and peanut ELISA were performed as described previously (10, 21). The limits of detection (LOD) and quantification were determined to be 0.06 ± 0.05 and 0.11 ± 0.09 mg of HNP per kg, respectively, for the hazelnut-specific ELISA (10). For the peanut-specific ELISA, the LOD and the limit of quantification were determined to be 0.07 ± 0.04 and 0.15 ± 0.12 mg of peanut protein per kg, respectively (21). The ELISA results were only accepted for triplicates with a coefficient of variation of less than 15%.

RESULTS

Results for industrial investigations. A summary of the results of all investigations performed with shared production equipment is shown in Table 2. All ingredients were free from HNP.

Kneaders: spiral kneader and z-kneader. After cleaning the z-kneader from 10% hazelnut-containing dough according to protocol I, the hazelnut-free follow-up dough contained an average of 17 mg of HNP per kg in both tests. Additional hot-water cleaning after hazelnut dough removal (protocol II) reduced the HNP in the “hazelnut-free” follow-up dough to an average level between 0.2 and 0.7 mg/kg in the follow-up products of the two tests. The application of cleaning protocol III did not reduce the HNP any further than did cleaning protocol II. The results are shown in Figure 2A.

The application of manual scraping (protocol I) at the spiral kneader between the product changes from dough that contained 7% hazelnut to hazelnut-free dough resulted, on average, in about 1.5 to 9.2 mg of HNP per kg in the hazelnut-free follow-up dough per test. After additional cleaning with 52°C hot water (protocol II), the HNP was reduced to a level below 1 mg/kg in the follow-up product. After further excessive rinsing with hot water in accordance with protocol III, the HNP was not decreased further in

TABLE 2. Range of cross-contamination of the follow-up products with HNP depending on the cleaning protocol and the investigated device

Device	Range of product contamination by HNP (mg/kg) after cleaning protocol:		
	I	II	III
Spiral kneader	1.5–9.2	0.4–0.5	<LOD–0.3
z-Kneader	16.6–17.3	0.2–0.7	0.3
Rotary moulder	3.9–140	ND ^a	<LOD–1.0
Wire cutting machine	1.7–>1,000	ND	<LOD–0.9

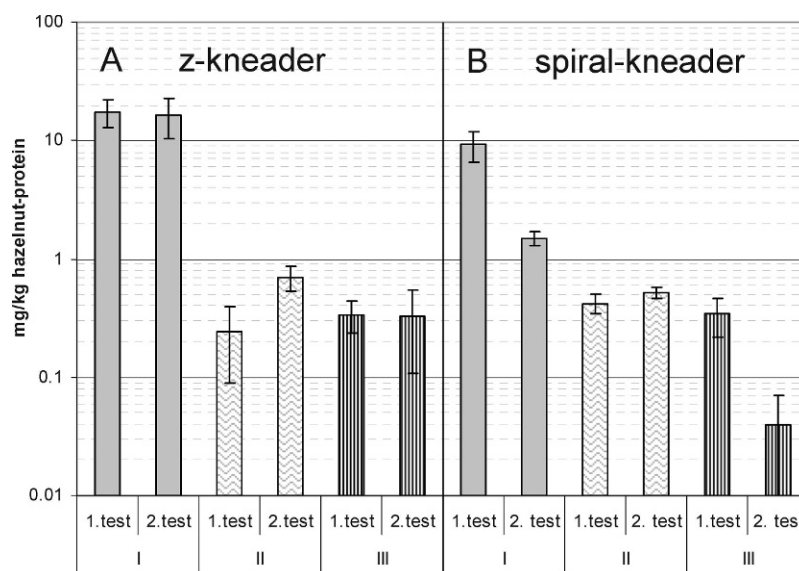
^a ND, not determined.

comparison to cleaning protocol II. The detailed results are shown in Figure 2B.

Cookie machines: wire cutting machine. Samples were analyzed during the dough processing. The running production was reflected by increasing rotations of the feeding roller. Therefore, samples were analyzed from rotation 1 to 11 after each second rotation, and thereafter, from rotation 15 to 50 after each fifth rotation. From rotations 50 to 70, samples were analyzed after each 10th rotation. No noteworthy differences were seen between samples analyzed crosswise and along the wire cutting machine. By applying cleaning protocol I after processing of the 10% hazelnut-containing dough, cross-contamination of up to 1,000 mg of HNP per kg in the follow-up dough samples was determined. At the beginning of production, HNP cross-contamination increased and decreased during the ongoing production process (Fig. 3). But still more than 10 mg/kg could be detected in samples drawn from the last investigated rotation. Hot-water cleaning in accordance with protocol III decreased the detectable HNP in the “HN-free” follow-up product to below 1 mg/kg (Fig. 3) and irrespective of the time course of dough processing.

Cookie machines: rotary moulder. The running production was reflected by increasing rotations of the moulder roller, and samples were analyzed from rotation 1 to 11 after each second rotation, and thereafter, from rotation 15 to 50 after each fifth rotation. From rotation 50 to 100, samples were analyzed after each 10th rotation. No noteworthy differences were seen between samples analyzed crosswise and along the rotary moulder. After processing of the dough with 7% hazelnut content and scraping of the feeding and the moulding roller of the rotary moulder (cleaning protocol I), the follow-up product was cross-contaminated with up to some 100 mg of HNP per kg (Fig. 4). Even after 100 rotations up to 10 mg of HNP per kg was detectable. The results demonstrate that a general reduction of HNP occurred through the time course of production but occasionally resulted in a random detection of elevated levels of HNP. After cleaning with hot tap water and final rinsing in accordance with cleaning protocol III, the HNP cross-contamination in the drawn samples was reduced to ≤ 1 mg/kg during the complete running operation (Fig. 4).

FIGURE 2. HNP in the follow-up product of the z-kneader (A) and the spiral kneader (B) after application of the different cleaning protocols (I through III). Each experiment was done twice (test 1 and test 2). One bar represents the average of quantified HNP from 5 samples, except for cleaning method I applied at the z-kneader, which is the average of 10 samples. The standard deviation is illustrated by error bars.



Twenty-four-month monitoring of common raw materials used in the production of fine bakery wares. Peanut was not detected in any of the 148 analyzed batches. The hazelnut-specific ELISA was not performed with those batches containing chopped or ground hazelnut; therefore, only 138 batches were screened for hazelnut cross-contamination. Wheat flour was analyzed at a portion of 100% but led to an unspecific signal equivalent to ≤ 0.25 mg of HNP per kg. This observation has already been reported and discussed in our previous study (14). Therefore, the LOD of the hazelnut-specific ELISA was set at 0.25 mg of HNP per kg to avoid false-positive results. Twenty-one of the 138 investigated batches contained hazelnut above the LOD. Most frequently batches of marzipan, persipan, and almond were cross-contaminated, containing HNP concentrations of between 0.26 and 90 mg/kg.

Three further batches were identified as being cross-contaminated with HNP: in one batch each of coriander, fruit filling, and shortening, HNP was detectable at concentrations between 0.3 and 0.4 mg/kg. In total, the frequency of HNP cross-contamination in all ingredients and raw material tested was 15%, and the concentration ranged from 0.26 to 90 mg/kg.

Investigation of airborne hazelnut cross-contamination. At manufacturer A, 11 of 12 sampled and investigated petri dishes contained less than $1 \mu\text{g}$ of HNP per cm^2 after a holding time of 24 h. One petri dish with HNP at a level of $72.7 \mu\text{g}/\text{cm}^2$ contained not only dust but also a small dough lump. Therefore, the dust experiments were repeated. This time all petri dishes contained only dust and less than $1 \mu\text{g}$ of HNP per cm^2 . Results of airborne contaminations for

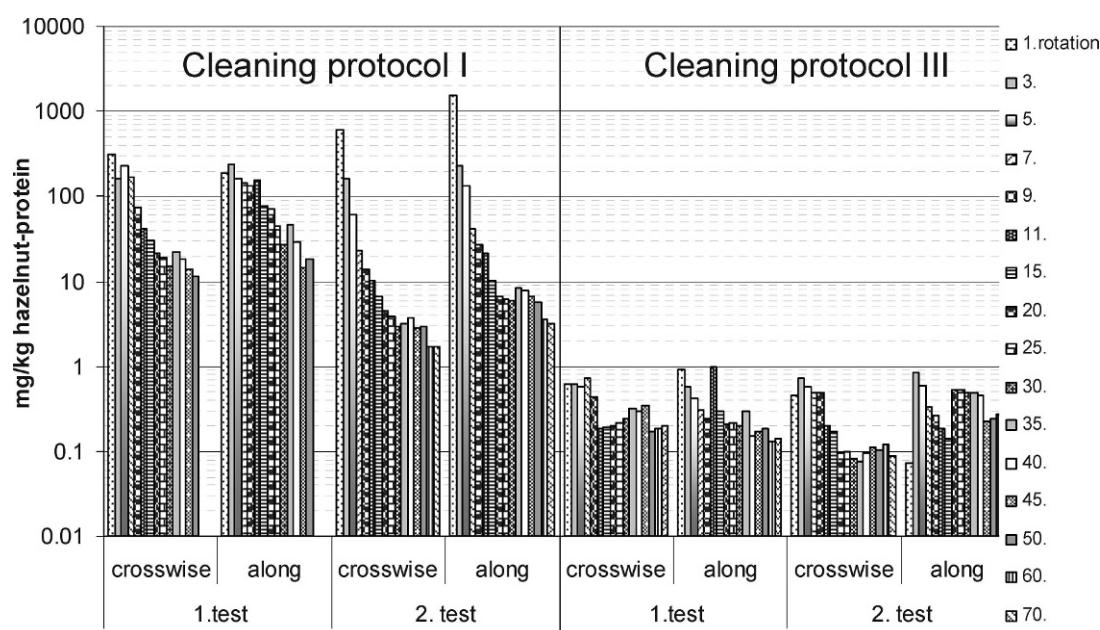


FIGURE 3. HNP in the follow-up product of the wire cutting machine after application of cleaning protocols I and III. The bars represent the level of HNP determined in the follow-up product. The chronological sequence of cookie production is reflected by rotation numbers of the feeding rollers that are given in the legend.

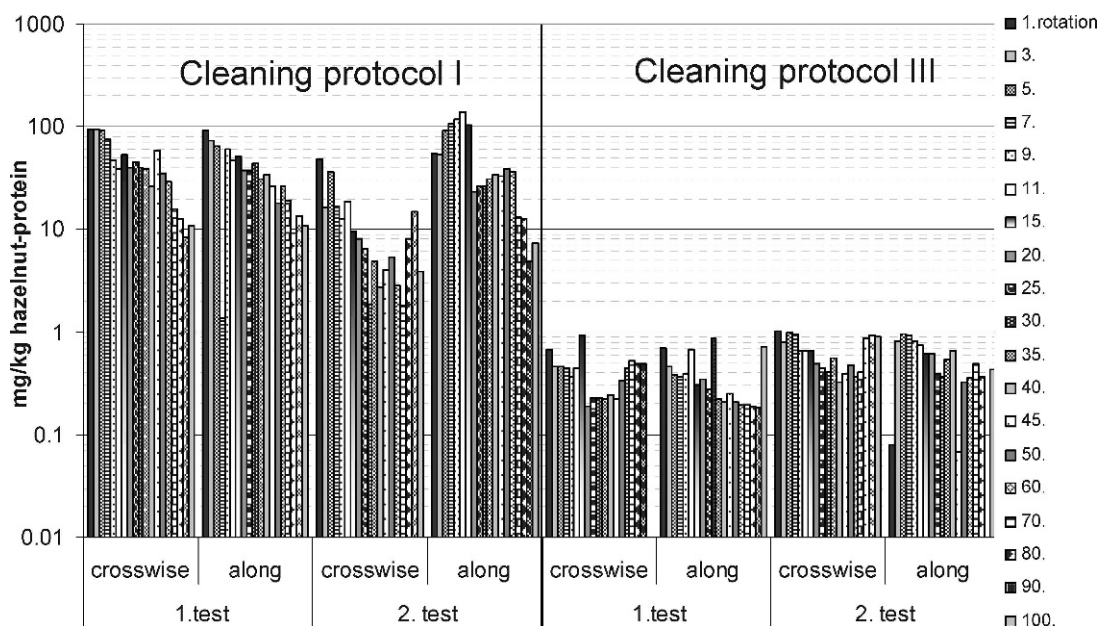


FIGURE 4. HNP in the follow-up product of the rotary moulder after application of cleaning protocols I and III. The bars represent the level of HNP determined in the follow-up product. The chronological sequence of cookie production is reflected by rotation numbers of the moulding roller that are given in the legend.

manufacturer B were comparable. None of the 16 petri dishes contained more than $1 \mu\text{g}$ of HNP per cm^2 .

Baking test. The heat stability of HNP was investigated in each of the two doughs that contained 7 or 10% hazelnut, respectively. Test samples of 20 cookies were drawn before and after baking. Water evaporation of approximately 15% during baking was taken into account. Almost no change in detectability was found in cookies that contained 7% hazelnut. However, in the dough containing 10% hazelnut, detectability of HNP decreased by approximately 20% after baking (Fig. 5).

DISCUSSION

From the manufacturers' point of view, applying precautionary labeling means that the clientele of consumers allergic to certain foods avoids such products. Not applying precautionary labeling might have more serious implications like food product recalls (25) or claim damages from affected allergic individuals, if they suffer from unexpected severe allergic reactions. Despite following good manufacturing practices, the manufacturers' concern about potentially hidden allergens increasingly leads to advisory labeling such as "shared equipment," "shared facility," "may contain," or "produced in a factory that also produces . . ." (2, 9). A study investigating the effect of precautionary labeling concludes that consumers with food allergy are overextended with this kind of information, because they are becoming less avoidant of products with advisory labels and assume incorrectly that the different mentioned terms indicate different levels of risk (9). Despite the increase in precautionary labeling, accidental consumption of "hidden allergens" in unlabeled food has been frequently reported (3, 7, 18, 30).

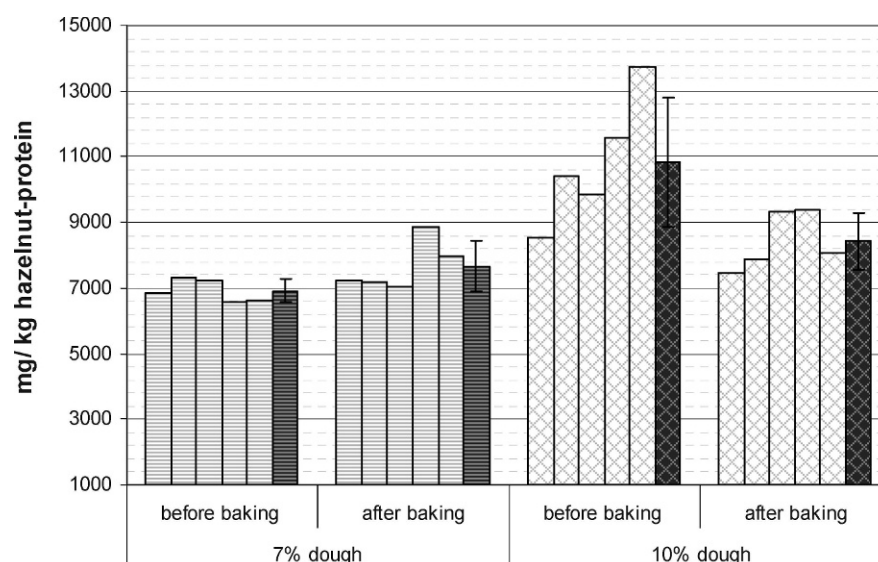
Thus, from the allergic individuals' point of view, the current use of precautionary allergen labeling is unsatisfying. This statement is confirmed by a study conducted in European countries by Pele et al. (13), which found that no relationship could be observed between the use of precautionary labeling and the analytical results of hazelnut and peanut traces in prepackaged cookies and chocolates.

To reduce precautionary labeling to relevant prepackaged products that inevitably might have become cross-contaminated with allergens during production, manufacturers need knowledge about the crucial steps where cross-contamination could occur.

Two years before the writing of this article, our group published the results of "pilot plant investigations on cleaning efficiencies to reduce hazelnut cross-contamination in industrial manufacture of cookies." This first study revealed critical points of cross-contamination during cookie manufacture on shared equipment (14). Therein we concluded that shared production equipment can be a serious health risk. We further concluded that the application of an appropriate cleaning procedure in combination with quantitative monitoring of the cleaning efficiency can effectively reduce the HNP cross-contamination to a level of approximately 1 mg/kg per device in pilot plant equipment, thus effectively reducing the risk of severe allergic reactions. However, these findings were to be verified for the industrial scale and environment.

Now, this study has verified the results on an industrial scale. In collaboration with two German cookie manufacturers we investigated the potential of wet-cleaning procedures between product changes with special regard to the reduction of hazelnut cross-contamination. In our previous study chopped hazelnut was demonstrated to be distributed randomly; therefore, a reproducible analytical

FIGURE 5. Differences in the detectability of hazelnut protein before and after baking of raw dough samples. Light gray bars, five individual analyses of a 20-cookie sample drawing; dark gray bars, mean of five individual analyses; error bars, standard deviations.



monitoring was not possible. Based on these findings, we eliminated the investigation of chopped hazelnut cross-contamination but systematically undertook studies with finely ground hazelnut that equally distributes in the cookie dough.

Moulded cookies were produced by manufacturer A, whereas piped cookies were produced by manufacturer B. The study design was developed to be consistent with our aforementioned study (14). Therefore, after processing of a hazelnut-containing dough, a cleaning method was applied and afterward a hazelnut-free dough was processed. The hazelnut-free dough was analyzed for HNP by ELISA. Since the use of detergent did not improve cleaning efficiency in the pilot plant study, it was not applied in the current investigations. This time, three different cleaning methods were applied at the kneaders whereas method III differed from method II by only one additional rinsing step with hot water. The cookie manufacturers involved in this study considered the wet-cleaning procedures at the industrial scale very time-consuming. Accordingly, the basic studies were done on pilot plant equipment to identify general starting points and crucial conditions to successfully undertake limited but well-adapted experiments at an industrial scale that could be run in the industrial environment, where production time-outs are generally a critical issue. At the industrial scale two cleaning methods were performed in replicate for both cookie machines: manual scraping (I) and manual scraping with hot water cleaning and additional rinsing (III). In contrast to pilot plant studies, sample drawing for both cookie machines was divided into samples taken crosswise from the production zone and samples taken from the border of the production zone (Fig. 1). This additional distinction was performed because in pilot plant studies it appeared that the HNP cross-contamination was higher in the border areas of feeding and moulding rollers.

Experiments were first done in plant A. The results for all cleaning methods performed at the spiral kneader correlated at the industrial and the pilot plant scales. Depending on the manual scraping efforts of cleaning

method I, a very subjective method, the detectable HNP of the virtually hazelnut-free dough produced in the spiral kneader was determined to be on average 1.5 and 9.5 mg/kg per independent test. The two experimental approaches with the wet-cleaning method (II) reduced the HNP below 1 mg/kg, as it did in pilot plant scale, indicating that the additional rinse in cleaning method III is not necessary.

The ELISA results obtained from experiments with the industrial rotary moulder were also in good concordance with findings from pilot plant equipment. Manual scraping of the equipment yielded a high cross-contamination level of more than 100 mg/kg at the beginning of the cookie production of the follow-up product and was still clearly detectable with a level of up to 11 mg/kg after 100 rotations of the feeding roller. This is equivalent to more than 2,000 cookies or 40% of the dough batch of one production. As seen in pilot plant studies, the contamination level does not lower gradually during the run. The wet-cleaning procedure was very time-consuming, lasting approximately 2.5 h because the equipment was not designed and built for cleaning in place. A maintenance employee was needed to coordinate the disassembly of the feeding roller, the moulding roller, and the conveyor band and after the cleaning procedure for the reassembly of the machine. The processing of hazelnut-free dough at the wet-cleaned rotary moulder resulted in both independent tests in up to 100-fold-reduced HNP levels of less than 1 mg/kg. These results have been very comparable to the pilot plant scale.

In plant B, the tested devices were the z-kneader and the wire cutting machine. Due to the assumption that the z-kneader might distribute hazelnut less equally than the spiral kneader, 10 randomly drawn samples were drawn at each test. The analysis of all 10 samples after cleaning method I (Fig. 2A) revealed that this assumption did not apply to the z-kneader. Therefore, only the first 5 of 10 samples were analyzed from each of the two wet-cleaning methods. The newly obtained results of the z-kneader again matched nicely those from the pilot plant scale. Cleaning method I led in the follow-up product to a cross-contamination between 10 and 20 mg of HNP per kg in single drawn

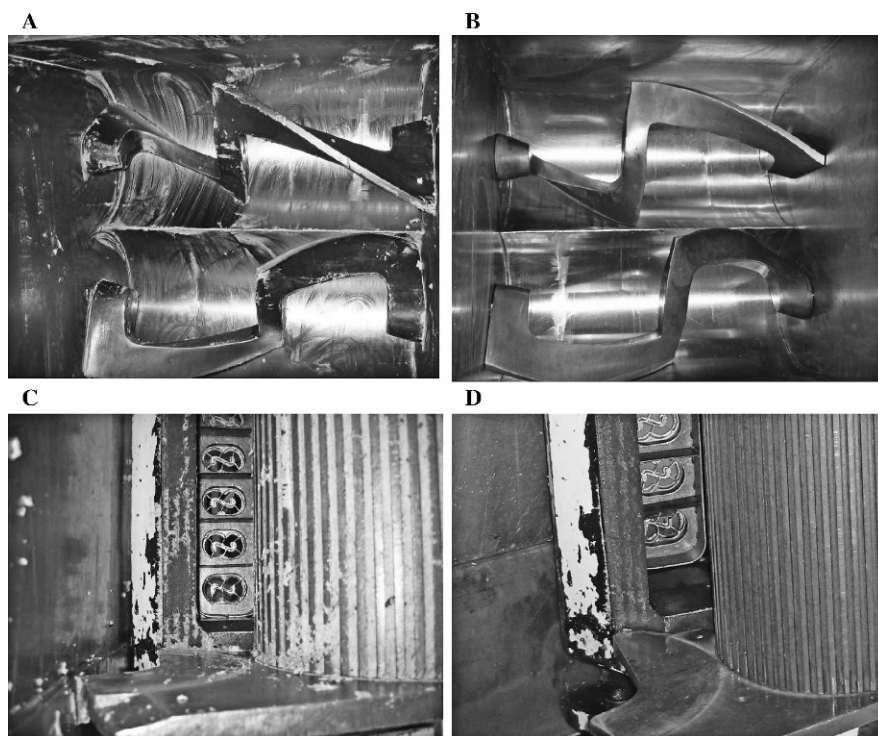


FIGURE 6. Z-kneader and a detail of one of the two feeding rollers from the wire cutting machine after the application of cleaning protocol I (A and C) and after application of cleaning protocol III (B and D).

samples. Both hot-water cleaning methods reduced HNP contamination below 1 mg/kg independent of an additional rinse.

The results for the wire cutting machine differed from those obtained with pilot plant equipment. Using manual scraping as cleaning method, the HNP cross-contamination is 100-fold higher for industrial scale than for pilot plant scale. This was not astonishing since the cleaning of industrial equipment was very rudimentary. In contrast to pilot plant equipment, the flutes of the feeding roller were still completely filled with hazelnut-containing dough. The minimal cleaning efforts between products were typical for products having similar colors. As a consequence the contamination level of the industrial-scale follow-up product was more similar to that obtained in the test performed at pilot plant scale, where the wire cutting machine was not cleaned at all after product change. There, similar HNP amounts were detected, ranging from >1,000 mg/kg at the beginning to ~20 mg/kg after 70 rotations of the feeding roller. Again the HNP concentration detected in dough samples that were processed after the hot-water cleaning method matched the results obtained at pilot plant scale. While the samples tested below 1 mg/kg, the method required an extensive amount of time to disassemble the equipment. The z-kneader and a detail of the wire cutting machine after the cleaning protocols I and III are shown in Figure 6.

The baking experiment results regarding antigenicity that are reflected by the heat stability of HNP confirmed findings from the previous pilot plant study. Only a slight reduction of 20% was observed for the HNP content in one of the baking experiments. We have no conclusive explanation for this finding, but perhaps the hazelnut used for the 7% hazelnut-containing dough had been roasted under harsher conditions than the hazelnut ingredient in the

10% hazelnut dough, and therefore no reduction could be seen.

We analyzed typical ingredients from three cookie manufacturers that are used in the production for cookies and snacks for the presence of hazelnut and peanut over a period of 24 months. No peanut protein was detected in 148 samples. However, 21 (15%) of 138 ingredient samples contained HNP above the LOD, and HNP was frequently detected in almond samples. It is likely that the suppliers of marzipan ingredients also produced hazelnut ingredients like chopped hazelnuts or nougat cream, and shared equipment might have been the cause of cross-contaminated raw materials. Summarizing the results for investigated raw materials, the overall contamination rate was fairly low. Based upon prior industry experience, one would have expected chocolates to have been contaminated more frequently. The study of Pele et al. (13) on peanut and hazelnut traces in cookies and chocolates revealed that in several countries up to 80% of chocolate products tested positive for hazelnut. This suggests that cross-contaminations might occur more frequently at the point of manufacture of prepackaged products than at the point of supply of raw materials.

Airborne dust has been discussed as one source of allergen cross-contamination. Hence, we collected dust in petri dishes along the production line of a hazelnut-containing cookie. After 24 h, the petri dishes were visibly covered with dust. Except for 1 of 40 petri dishes, the ELISA did not detect relevant HNP contaminations. Most probably due to the high fat content of approximately 58% (23), ground hazelnut shows a tendency to adhere to surfaces, where it can form sticky coverings, and might therefore not be distributed via the air like wheat flour. The petri dish that tested positive had a clearly visible dough piece. After consultation of the lead worker, the visible dough piece might have been

propelled into the petri dish due to the use of compressed air for equipment cleaning. It has to be highlighted that compressed air, if strong enough, could vector a small clog not only to a petri dish but also to parallel lines. Thus, the control of compressed air during equipment cleaning is highly critical to prevent cross-contamination.

In summary, the results of our study corroborate the findings of the previous pilot plant study and demonstrate that investigations performed with pilot plant equipment are a suitable alternative for industrial investigations. Hidden allergens resulting from shared equipment can be a serious health risk; when manual scraping methods were applied as the single cleaning method between product changes from allergen-containing food to allergen-free food, HNP cross-contaminations of more than 1,000 mg/kg occurred. Wensing et al. (28) calculated that 50% of individuals allergic to hazelnut would react to dried HNP amounts of 2 to 11 mg. Even if only extremely small serving sizes of 12 g (theoretically containing 12 mg of HNP, based on a 1,000-mg/kg level) were to be consumed (approximately two cookies), the majority of allergic consumers could suffer from allergic reactions. To achieve the same level of HNP uptake after the application of a hot-water cleaning method, more than 12,000 g (12 kg) of cookies would have to be consumed. In concordance with the results at pilot plant equipment, only the hot-water cleaning method reproducibly reduced the allergen content to a level at or below 1 mg of HNP per kg. At this level the majority of allergic individuals are thought to be protected from severe allergic reactions (26). Thus, wet cleaning appears to be an advisable measure to effectively reduce hidden allergens in subsequently manufactured products that need to be allergen free.

Irrespective of our findings, this technique might not be the method of choice in all cases. Especially in enterprises where the production equipment has not been designed for easily accessible cleaning, such as cleaning in place, wet cleaning will cause expensive downtime. In cases where stringent time schedules with several changes of products containing allergenic ingredients are required in a day, a product change connected with downtimes of several hours and the availability of trained maintenance personnel might be an economic challenge. Finally, it has been taken into account that wet cleaning may cause microbial contamination if product equipment is not completely dried before use for a subsequent product. Nonetheless, measures need to be taken to effectively reduce hidden allergens in follow-up products to ensure the safety of food for the consumer with food allergies.

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