ALLERGENS IN FOOD PROCESSING: INDUSTRY CASE STUDIES

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by

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ALLERGENS IN FOOD PROCESSING: INDUSTRY CASE STUDIES

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CONTENTS

SUMMARY **1**

1 INTRODUCTION **3**

1.1 The Regulatory Environment 3
1.2 Food Industry Initiatives 3
1.3 Current project 4

2 DAIRY CASE STUDY **5**

2.1 Process Description 5
2.2 Allergen Analyses 6
2.3 Stage One Sampling 6
2.3.1 Milk and juice packaging 6
2.3.2 Soy and milk processing 6
2.4 Stage One Results 7
2.4.1 Milk to Juice Changeover 7
2.4.2 Soy to Milk Changeover 8
2.5 Stage Two Sampling 8
2.5.1 Milk and juice packaging 8
2.5.2 Soy and milk processing 8
2.6 Stage Two Results 8
2.6.1 Milk to juice changeover 8
2.6.2 Soy to milk changeover 8
2.7 Conclusions – Dairy Case Study 8
2.7.1 Assessment under the VITAL risk assessment framework 9
2.7.2 Level of risk associated with dairy process 9

3 BAKERY CASE STUDY **10**

3.1 Process Description 10
3.2 Allergen Analyses 10
3.3 Stage One Sampling 11
3.4 Stage One Results 12
3.4.1 Adhered Material Scrapings 12
3.4.2 Surface Swabs 12
3.4.3 Other Environmental Samples 13
3.4.4 Bread and Dough Analyses 13
3.5 Stage Two Sampling 14
3.6 Stage Two Results 15
3.6.1 Following low sesame batch 15
3.6.2 Following high sesame batch 15
3.7 Conclusions – Bakery Case Study 17
3.7.1 Assessment under the VITAL risk assessment framework 17
3.7.2 Level of risk associated with bakery process 18

4 COMMENTS ON THE APPLICATION OF THE VITAL FRAMEWORK **19**

4.1 Form of Allergenic Material – Particulate or Readily Dispersible Form 19
4.2 Comparison of Product Allergen Levels to VITAL Action Levels 19

5 REFERENCES 20

APPENDIX 1 VITAL DECISION TREE FOR ALLERGEN LABELLING..... 21

LIST OF TABLES

Table 1: VITAL (Voluntary Incidental Trace Allergen Labelling) action levels 4
Table 2: Concentration of casein protein (ppm) in samples from the dairy plant for the changeover between liquid milk and fruit juice 7
Table 3: Concentration of sesame (ppm) in adhering dough samples from the bakery plant for the changeover between sesame containing and non-sesame containing bread 12
Table 4: Level of sesame in swabs from contact surfaces from the bakery plant for the changeover between sesame containing and non-sesame containing bread 13
Table 5: Concentration of sesame (ppm) in doughs and related bread from the bakery plant for the changeover between sesame containing and non-sesame containing bread 14

LIST OF FIGURES

Figure 1: Schematic of case study dairy process 5
Figure 2: Schematic of bakery case study process 10
Figure 3: Apparent sesame content (ppm sesame seed) of bread from batches following low and high sesame batches 16

SUMMARY

The Voluntary Incidental Trace Allergen Labelling (VITAL) industry initiative has been developed to provide guidance on appropriate labelling of foods that have the potential to cross-contact allergenic source materials.

Two case studies were conducted, with two rounds of sampling carried out for each case study. The first sampling (stage one) aimed to define the potential for cross-contact, while the second (stage two) aimed to determine the impact on final product of the cross-contact.

Dairy Case Study

Two processes were examined at a diary processing facility in Christchurch:

- Changeover from liquid milk to juice processing
- Changeover from soy to milk processing

Samples analysed during stage one included flush water, surface swabs from cleaned processing sites, product post-clean used to flush system, and finished product post changeover. Samples analysed in stage two were all finished product, with the exception of some clean-in-place (CIP) flush water from the soy to milk changeover. Samples were analysed for casein (milk to juice process) or soy (soy to milk process) using enzyme-linked immunosorbent assay (ELISA) test kits.

No test results were found to be positive for soy in the soy to milk process changeover, in stage one or stage two. In the stage one sampling of the milk to juice process changeover, 1/15 water samples from the mix tank demonstrated a positive result for casein, while 1/15 finished products contained casein at the limit of detection of the analytical method (2 ppm). No samples were positive in the stage two sampling.

Using the VITAL decisionmaking framework neither of these processes would be considered to require any allergen labelling due to cross-contact.

Bakery Case Study

One process was examined at a bakery facility in Christchurch:

- Changeover from sesame-containing bread to non-sesame-containing bread

Samples analysed during stage one included bread samples, dough samples, surface swabs and scrapings of adhering material on process surfaces post changeover. Only dough and bread samples were analysed during stage two sampling. Samples were analysed for sesame using enzyme-linked immunosorbent assay (ELISA) test kits.

During stage one, a high proportion of scrapings and surface swabs were found to be positive for sesame, confirming the potential for sesame to be carried over into subsequent processing batches. Doughs following low-sesame (0.5%) batches did not contain detectable sesame residues, while doughs following high-sesame (6%) batches were found to contain sesame at concentration in the range 14-130 ppm sesame (approximately 2.8-26 ppm sesame protein). Breads following high-sesame batches were found to contain sesame. However, quantitative sesame results from heat-treated samples can be unreliable.

During stage two sampling, no sesame residues were detected in doughs following a low sesame batch and were rarely detected (2/29 samples) in doughs following a high sesame batch. Sesame was detected in bread samples following both low and high sesame batches, but at very low concentrations (0.6-2.6 ppm sesame protein).

Using the VITAL decisionmaking framework and data from stage two dough analyses, the VITAL approach suggests that there is no need to label non-sesame containing product for the incidental presence of sesame. Application of the VITAL framework to results from stage two bread analyses are equivocal. However, results from breads should be viewed with caution due to the known influence of heating on the performance of the sesame analytical method.

1 INTRODUCTION

Allergenic source material may inadvertently be present in processed foods due to cross-contact in the processing environment. A key area with potential for cross-contact is in the changeover from production of a product containing allergenic material to a product not containing that material.

1.1 The Regulatory Environment

Standard 1.2.3 of the joint Australia New Zealand Food Standards Code requires mandatory labelling of food products where specified allergenic source materials are present as an ingredient, a food additive or a processing aid or a component of any of these. Allergenic source materials covered by the standard are:

- Cereals containing gluten and their products
- Crustacea and their products
- Egg and egg products
- Fish and fish products
- Milk and milk products
- Peanuts and soybeans and their products
- Added sulphites in concentrations of 10 mg/kg or more
- Tree nuts and sesame seeds and their products

Allergen cross-contact situations are not explicitly covered by Standard 1.2.3.

1.2 Food Industry Initiatives

A series of projects were conducted under the auspices of the Australian Food and Grocery Council (AFGC) to establish best practice with respect to allergen management. One of these projects considered risk assessment for cross-contact of allergenic source material between allergen containing and non-allergen containing foods. This project produced an assessment system and decision framework called Voluntary Incidental Trace Allergen Labelling (VITAL; see http://www.allergenbureau.net/allergen-guide/vital/ for details).

The VITAL tool used published dose-response information and an exposure algorithm to define three allergen action levels, on the basis of allergen protein concentration in a processed food. The VITAL action level values are reproduced in Table 1.

Table 1: VITAL (Voluntary Incidental Trace Allergen Labelling) action levels

Allergenic Source	Action Level (ppm allergenic source protein) in final product		
	Level 1	**Level 2**	**Level 3**
Milk	<5	5-50	>50
Egg	<2	2-20	>20
Soy	<10	10-100	>100
Fish	<20	20-200	>200
Peanut	<2	2-20	>20
Tree nuts	<2	2-20	>20
Sesame Seeds	<2	2-20	>20
Crustacea	<2	2-20	>20
Gluten	<20	20-100	>100
Sulphites	<10		>10
Labelling Action	**No labelling**	**Precautionary labelling**	**Ingredient labelling**

ppm parts per million = mg/kg = mg/L

The level of allergen in the finished product is used to inform a decision on what form of labelling is required or whether any labelling is required. The VITAL decisionmaking framework is shown in Appendix 1.

Two points are worth noting with respect to the action levels in Table 1:

- The action levels are expressed in terms of 'ppm allergenic source **protein'**. Some testing kits express their results in the same protein format, while others express their results as 'ppm allergenic source material'. Examples of this are the Tepnel peanut and sesame kits. In these cases, an assumption must be made to convert analytical results to a protein basis for comparison with the VITAL action levels. The usual assumption is that the allergenic source material contains an average or typical level of protein for that food. For example, 20 ppm sesame would equate to 5 ppm sesame protein, assuming a typical protein content for sesame seeds of 20%.
- The action levels and most analytical results are expressed in terms of total protein from the allergenic source material, not allergenic protein. This is a practical approach, as different individuals have different spectrums of response to proteins from allergenic materials. Studies on provoking doses, the basis for the action levels, also express doses in terms of total protein from the allergenic material.

1.3 Current project

The current project was a collaboration with two industry partners, to assess cross contact at the point of change over from allergen-containing product to non-allergen-containing product. Each case study was run in two stages; an initial assessment of the degree of cross contact at the changeover point and the impact of this cross contact on the allergen status of final product, and a second stage which focused more on final products. The second stage was designed to test the validity of the labelling decisions made by the industry partners, in the context of the VITAL framework.

2 DAIRY CASE STUDY

The case study was conducted with a dairy processor and packager based in Christchurch. Two processes were selected for evaluation:

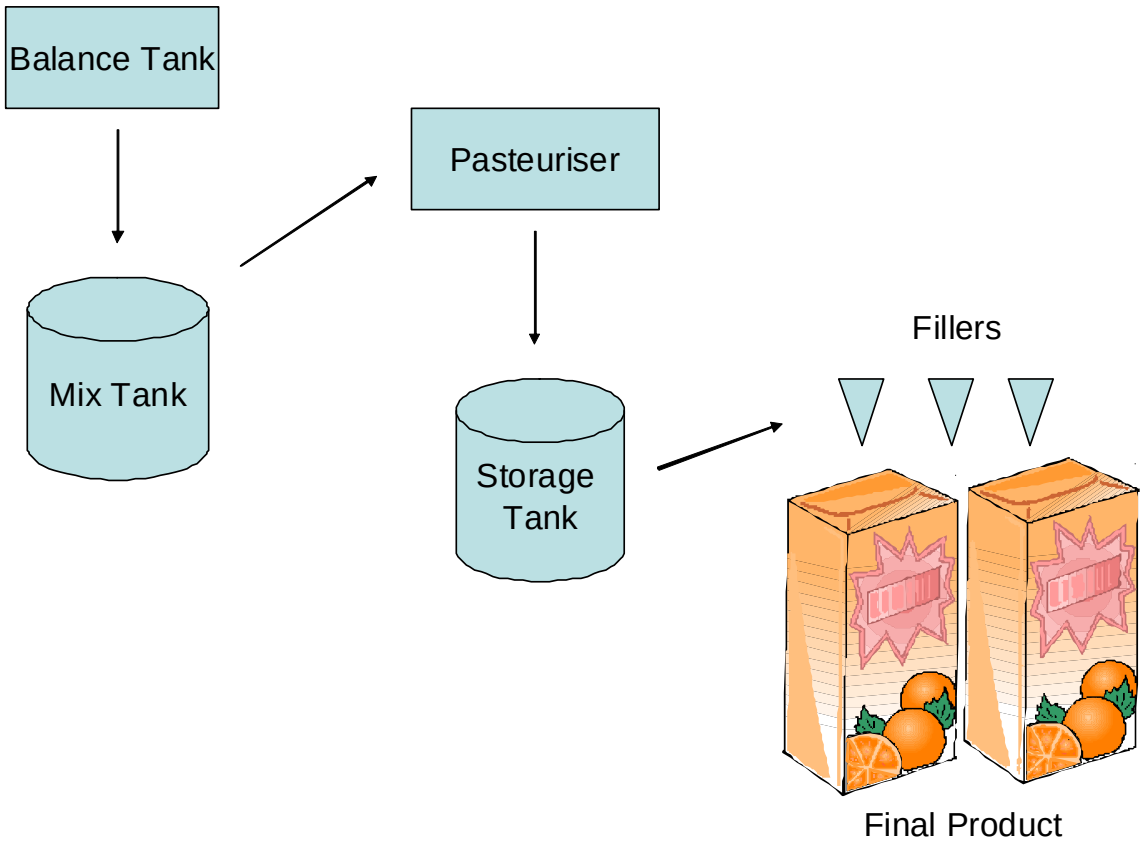
- Changeover from packaging of milk to packaging of fruit juice
- Changeover from processing of soy-based drink product to processing of milk products

The first of these was selected as the primary case study, although samples were also collected and analysed from the second process.

2.1 Process Description

All ingredients are combined in a **balance tank.** Ingredients are blended then proceed to a **mix tank** where mixing is completed. The mixed liquid is then **pasteurised** before proceeding to a **storage tank** and on to **fillers**. The fillers dispense the liquid final product into either cardboard or plastic retail containers. A schematic of the process is shown in Figure 1.

Figure 1: Schematic of case study dairy process



2.2 Allergen Analyses

Samples from the juice/milk process were analysed for casein (the major milk protein, accounting for 80% of milk protein) using Biokits Casein Assay Kit (Tepnel Biosystems). The method is an indirect competitive enzyme-linked immunosorbent assay (C-ELISA).

Samples from the soy/milk process were analysed for soy protein using Elisa Systems soy residue kit (Elisa Systems). The method is a sandwich ELISA (S-ELISA).

Both methods have previously been evaluated in our laboratories to determine sensitivity, specificity, matrix effects and thermal stability of the method (Cressey and Jones, 2005). Both methods were found to be suitable for their current usage.

2.3 Stage One Sampling

2.3.1 Milk and juice packaging

Five sample types were taken from the process:

- Flush water from the balance tank
- Surface swabs from the clean storage tank
- Water for making up juice sampled from the mix tank, prior to addition of other juice ingredients
- Juice run-off to waste at beginning of run
- Finished juice product

Each sampling point was sampled on three occasions with five samples taken at each point on each occasion. Samples were taken at approximately 1-3 minute intervals. All samples were analysed individually.

2.3.2 Soy and milk processing

This process is largely the same as that for milk and juice.

Samples taken were:

- Swabs from the balance tank following the clean-in-place (CIP) process following soy processing
- Swabs from the mix tank following the clean-in-place (CIP) process following soy processing
- Swabs from the UHT holding tank (equivalent to storage tank) following the clean-in-place (CIP) process following soy processing
- Line flush water post-CIP
- Flush water from pasteuriser
- Milk flush samples from pasteurizer to UHT holding/storage tank
- Flush water from UHT holding/storage tank
- Finished milk product after soy

Each sampling point was sampled on two occasions with five samples taken at each point on each occasion. Samples were taken at approximately 1-3 minute intervals. The project was originally designed to consider only a single process under each case study, and lacked

sufficient resource to analyse all individual samples from this process. However, the additional information from this process was considered to be of value and to accommodate the additional analyses samples from each point/occasion were composited.

2.4 Stage One Results

2.4.1 Milk to Juice Changeover

Table 2 summarises the results from analyses of samples taken during the changeover from milk to juice processing.

Table 2: Concentration of casein protein (ppm) in samples from the dairy plant for the changeover between liquid milk and fruit juice

Date	Sample point	Sample				
		1	2	3	4	5
13-Nov-07	Flush water (balance tank)	ND	ND	ND	ND	ND
13-Nov-07	Swabs (Clean finished product tank)*	ND	ND	ND	ND	ND
13-Nov-07	Make up water (mix tank)	ND	ND	ND	ND	ND
14-Nov-07	Juice run-off before pack	ND	ND	ND	ND	ND
14-Nov-07	Juice finished product	ND	ND	ND	ND	ND
16-Nov-07	Flush water (balance tank)	ND	ND	ND	ND	ND
16-Nov-07	Swabs (Clean finished product tank)*	ND	ND	ND	ND	ND
16-Nov-07	Make up water (mix tank)	ND	11	ND	ND	ND
17-Nov-07	Juice run-off before pack	ND	ND	ND	ND	ND
17-Nov-07	Juice finished product	ND	ND	ND	2	ND
24-Dec-07	Flush water (balance tank)	ND	ND	ND	ND	ND
NS	Swabs (Clean finished product tank)*	ND	ND	ND	ND	ND
NS	Make up water (mix tank)	ND	ND	ND	ND	ND
NS	Juice run-off before pack	ND	ND	ND	ND	ND
NS	Juice finished product	ND	ND	ND	ND	ND

NS Not stated
ND Not detected at a limit of detection of 2 ppm
* As swab samples are on an area basis, rather than a volume basis results should be considered as either positive or negative, rather than quantified

These results generally indicate a process under good control, with no casein protein detected in any sample for the first and third sampling occasions. On the second sampling occasion one of five water samples taken from the mix tank, prior to the addition of juice ingredients, exhibited a strong positive result for casein protein. One marginal positive result for casein in finished product was also observed.

2.4.2 Soy to Milk Changeover

None of the samples from the soy to milk changeover were positive for the presence of soy protein at a detection limit of 1 ppm. The need to composite samples on a sampling point/sampling occasion basis may have lead to single low level positive samples being missed, however, the high sensitivity of this method suggests that any soy contamination in these samples would have been well below the VITAL action level one limit of 10 ppm.

On the basis of these samples, it appears that the cross-contact between the soy and milk processes is negligible. Under the VITAL decisionmaking framework, no specific allergen labelling of product from this process would be required.

2.5 Stage Two Sampling

2.5.1 Milk and juice packaging

Samples of packaged juice were taken on each of nine processing days, with five samples taken on each sampling day, with the intervals between successive samples varying in the range 3-40 minutes.

2.5.2 Soy and milk processing

Three samples of final clean-in-place (CIP) rinse water and three samples of first product were taken, one each on three successive days.

2.6 Stage Two Results

2.6.1 Milk to juice changeover

Casein protein was not detected in any of 45 samples of juice taken over nine processing days. The detection limit for the analytical method was 2 ppm of casein protein.

2.6.2 Soy to milk changeover

Soy protein was not detected in any samples of rinse water or first product. The detection limit for the analytical method was 1 ppm soy protein.

2.7 Conclusions – Dairy Case Study

The samples analysed from the dairy plant suggest that current clean-in-place procedures are effective in preventing significant allergen cross-contact between processing batches containing allergenic source material and following batches that are not intended to contain the allergenic source material.

On the one occasion where casein protein was detected in fruit juice (first sampling), the analytical result was at the limit of detection of the analytical method and below the VITAL action level one concentration limit for this allergen.

2.7.1 Assessment under the VITAL risk assessment framework

The evidence collected during the course of this case study suggests that the current practice of applying no precautionary labelling to fruit juice packaged at the dairy plant is consistent with the interpretation of the available analytical data via the VITAL risk assessment framework.

2.7.2 Level of risk associated with dairy process

An allergen risk model has been developed to determine the probability of adverse allergic events resulting from defined cross-contact scenarios (Cressey, 2007). Using data from the current case study (1/60 juice samples containing 2 ppm of milk protein), the model estimates a probability of an adverse allergic outcome of 1.2×10^{-7} or approximately one in every ten million servings consumed.

3 BAKERY CASE STUDY

The case study was conducted with a bakery which is primarily a bread baker and pre-mix formulator based in Christchurch. One process was selected for evaluation:

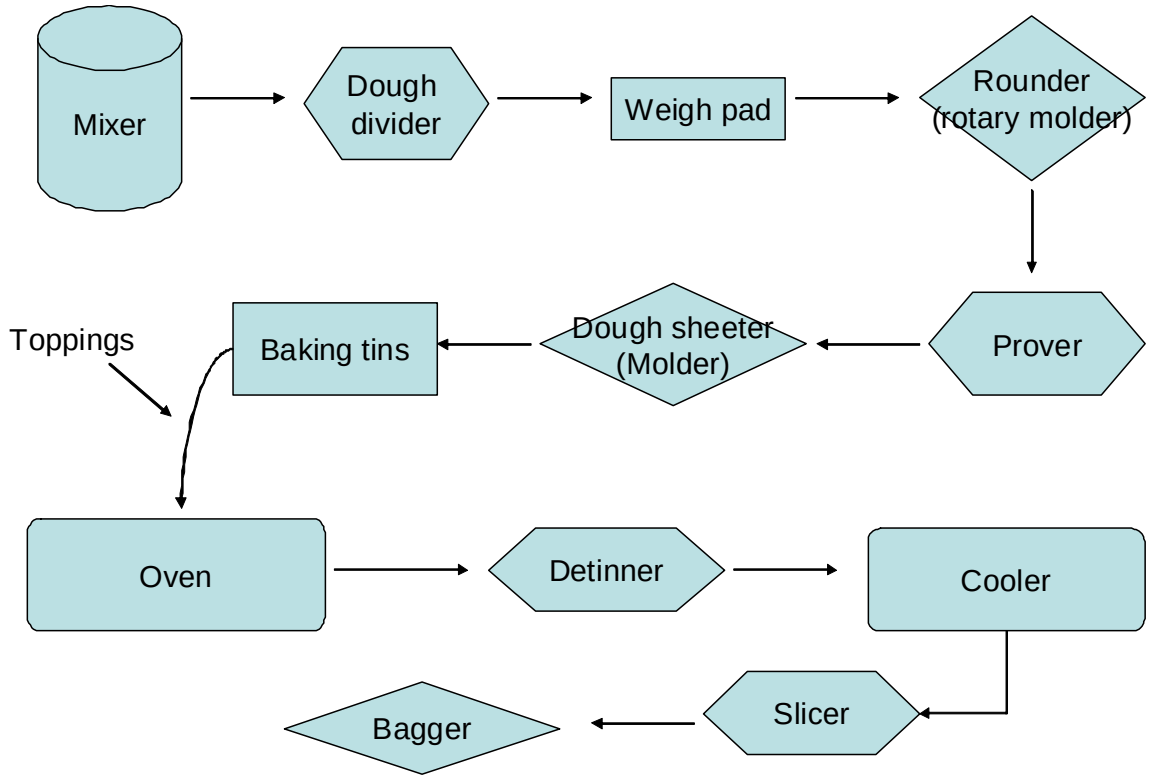
- Changeover from sesame containing product to non-sesame containing product

Two sesame containing products were considered; a low sesame product (0.5% sesame) and a high sesame product (6% sesame).

3.1 Process Description

All ingredients are combined in a **dough mixer.** After mixing, the dough passes through a **dough divider**, across a **weigh pad** and through a **rounder**, to shape the dough pieces. Dough pieces then go through a **prover**, before final dough shaping by a **dough sheeter**. Dough pieces are placed in tins before **topping, baking, detinning, cooling, slicing** and **bagging.** A schematic of this process is shown in Figure 2.

Figure 2: Schematic of bakery case study process



3.2 Allergen Analyses

Samples were analysed for sesame seed protein using Biokits Sesame Assay Kit (Tepnel Biosystems). The method is a direct sandwich enzyme-linked immunosorbent assay (S-ELISA). Results are expressed in terms of 'ppm sesame'.

The method has previously been evaluated in our laboratories to determine sensitivity, specificity, matrix effects and thermal stability of the method (Cressey and Jones, 2006). The method is suitably sensitive and specific for the current project and shows acceptable performance in cereal matrices. However, the sesame test kit shows some sensitivity to heating of the matrix. Frying of sesame seeds resulted in the sesame protein becoming undetectable, while heating in an oven resulted in an apparent increase in sesame protein (over-recovery), possibly due to partial denaturation of the protein exposing additional epitopes[1]. Available information does not allow any judgment to be made as to whether the test epitopes are also allergenic epitopes and, therefore, it is uncertain whether the postulated partial denaturation would change the allergenicity of the sesame seed.

These effects mean that quantitative results from heat-treated samples should be viewed with some caution and results from analysis of breads should be viewed as indicative only.

To establish how relevant thermal effects seen in the laboratory were to the thermal processes in a bakery, analyses were carried out on a bread known to contain sesame, to determine if the sesame protein was still detectable following the baking process. Samples of crust and crumb were analysed separately, as the crust will experience higher temperatures than the crumb. Sesame was detected in both crust and crumb samples, at similar concentrations (60,000 and 50,000 ppm respectively). The concentrations of sesame determined were reasonably consistent with the known composition of the bread (6% sesame = 60,000 ppm). It was concluded that, while the baking process may have some impact on the performance of the sesame test kit, sesame protein remains detectable following exposure to baking temperatures and quantitative results are of an expected order of magnitude.

3.3 Stage One Sampling

The sampling was carried out on two occasions, one following processing of a bread mix containing 0.5% sesame seed (20 December 2007) and one following a bread mix containing 6% sesame seeds (9 April 2008).

Four sample types were taken from the process:

- Scraping samples of material adhering to plant surfaces between batches (and able to contaminate subsequent batches), taken from mixer, divider and sheeter
- Surface swabs, taken from mixer, divider, rounder, prover, sheeter, tins and slicer
- Dough pieces, ex-rounder and ex-sheeter
- Baked bread, ex-bagging

Wherever possible at least three separate samples were taken of each sample type from each sampling point over the course of the two sampling days.

[1] An epitope is a sequence of usually 5-7 amino acids. This sequence is 'recognised' by the bodies immune system, in the case of an allergic reaction, or by antibodies bound to the test well, in the case of the ELISA method. Proteins and their associated epitopes used for ELISA methods for allergenic source materials are not necessarily the same as those causing allergic reactions, but are chosen as good markers of the allergenic source material.

3.4 Stage One Results

3.4.1 Adhered Material Scrapings

Table 3 summarises the results from analyses of samples of adhering dough material taken during the changeover from sesame to non-sesame containing bread.

Table 3: Concentration of sesame (ppm) in adhering dough samples from the bakery plant for the changeover between sesame containing and non-sesame containing bread

Sample point	Sample		
	1*	2#	3#
Scraping - Mixer	ND	ND	ND
Scraping - Divider	3	>100	63
Scraping – Dough sheeter	ND	ND	ND

ND Not detected at a limit of detection of 3 ppm
* 20 December 2007, following dough containing 0.5% sesame
\# 9 April 2008, following dough containing 6% sesame

While it is tempting to assume that material adhering to production surfaces will have originated from the immediately previous production batch, the inconsistency of these results suggests that dough material adhering to production equipment may relate to the immediately previous sesame containing production run (e.g. positive results in scraping from the divider) or may relate to even earlier non-sesame containing production runs (e.g. negative results in samples from the mixer and the dough sheeter). This suggests that there is potential for material to persist on contact surfaces through several subsequent batches and this material may cross contact a number of production processes and not just the process immediately following.

3.4.2 Surface Swabs

Table 4 summarises the results of analyses of swabs taken from surfaces that would be subsequently contacted by dough pieces (‘contact surfaces’). It should be noted that swabs are measuring sesame on an area basis, rather than a weight basis and are not directly comparable to other results given in this report. To simplify interpretation, results from analysis of swabs have been presented as negative (-), low positive (+), medium positive (++) or high positive (+++). This approach allows assessment of relative contamination of various surfaces.

Table 4: Level of sesame in swabs from contact surfaces from the bakery plant for the changeover between sesame containing and non-sesame containing bread

Sample point	Sample		
	1	2	3
Swab – Mixer	+*	-*	
Swab – Mixer	+++#	+++#	+++#
Swab – Dough divider	-*	++#	+#
Swab – Rounder	-*	+++#	+++#
Swab – Prover	+++*	+++#	++#
Swab – Dough sheeter	-*	+++#	+++#
Swab – Baking tin	-*	-*	-*
Swab – Slicer blades	+++#	+++#	+++#

- Not detected at a limit of detection of 3 ppm
+ Detected at an apparent concentration <10 ppm
++ Detected at an apparent concentration of 10-100 ppm
+++ Detected at an apparent concentration of >100 ppm
* 20 December 2007, following dough containing 0.5% sesame
# 9 April 2008, following dough containing 6% sesame

The results in Table 4 indicate significant retention of sesame protein on production contact surfaces following processing of doughs containing sesame. While this phenomenon was more pronounced following processing of a high-sesame recipe, it was also apparent following doughs containing lower levels of sesame. It should be noted that intact sesame seeds were not apparent on any of these surfaces.

3.4.3 Other Environmental Samples

A small number of samples were taken from non-contact areas of the plant, to determine if these may act as reservoirs of contamination for the plant. Breadcrumb material taken below the conveyor belt after the detinner and from below the slicer both contained sesame in excess of 100 ppm. This indicates a high level of general contamination of the processing environment with sesame.

3.4.4 Bread and Dough Analyses

Results from dough and bread analyses are summarised in Table 5.

Table 5: Concentration of sesame (ppm) in doughs and related bread from the bakery plant for the changeover between sesame containing and non-sesame containing bread

Sample type	Concentration of sesame (ppm) in sample number (Sample position*)		
	1	2	3
Dough, following low sesame batch#	ND (1)	ND (2)	ND (3)
Dough, following high sesame batch§	18 (1)	130 (3)	14 (6)
Bread, following high sesame batch§	320 (1)	130 (3)	210 (5)

* The sample position refers to the sequence of doughs or bread loaves passing the sampling point i.e. sample position 1 refers to the first dough or bread of the batch to exit the sheeting molder or the bagger, respectively

20 December 2007, following dough containing 0.5% sesame

§ 9 April 2008, following dough containing 6% sesame

ND Not detected at a detection limit of 3 ppm sesame

Dough samples (x3), of a non-sesame containing recipe, taken after exit from the rounder, following a dough containing 0.5% sesame, did not contain detectable sesame at a limit of detection of 3 ppm sesame.

Dough samples of a non-sesame containing recipe, taken after exit from the dough sheeter (the final step before tinning), following a dough containing 6% sesame, were found to all contain detectable sesame.

Finished bread samples from the same production run were found to contain sesame at apparent concentrations of 320, 130 and 210 ppm. The difference in measured sesame content between the doughs and breads in this production run appears unlikely, based on observation of the process, and it is possible that heating during the baking process has resulted in 'over-recovery' of sesame protein in the assay. However, these results do confirm the presence of sesame material in finished breads from production runs following processing of sesame-containing runs.

3.5 Stage Two Sampling

The first sampling sought to determine the extent of environmental contamination within the bakery environment, due to sesame material, and to establish that there was carryover from one batch to the next. The second sampling sought to establish the persistence of the carryover into the following batch. Samples were taken following a low-sesame batch (0.5%) and a high-sesame batch (6%). Samples collected were:

Following low-sesame batch:

- 4-8 dough samples, spread throughout the following batch
- 3 bread samples, spread throughout the following batch

Following high-sesame batch:

- 9-10 dough samples, spread throughout the following batch
- 3 bread sample, spread throughout the following batch

Each of these samplings was repeated on three different days.

3.6 Stage Two Results

The bakery incorporated some process scheduling changes between the stage one and stage two samplings. These changes allowed for inclusion of a mixer wash step following the processing of high sesame doughs.

3.6.1 Following low sesame batch

Sesame protein was not detected in any of 17 dough samples taken from batches immediately following the processing of a low sesame batch. The limit of detection of the analytical method was 3 ppm of sesame seed (approximately 0.6 ppm of sesame protein, based on a typical 20% protein content of sesame seed). These results are consistent with findings from stage one of the cases study, where sesame protein was not detected in doughs from batches immediately following low sesame batches.

In contrast, sesame was detected in three of nine baked loaves from the batch immediately following a low sesame batch. Observed concentrations were in the range 6-13 ppm sesame seed or approximately 1.2-2.6 ppm sesame protein.

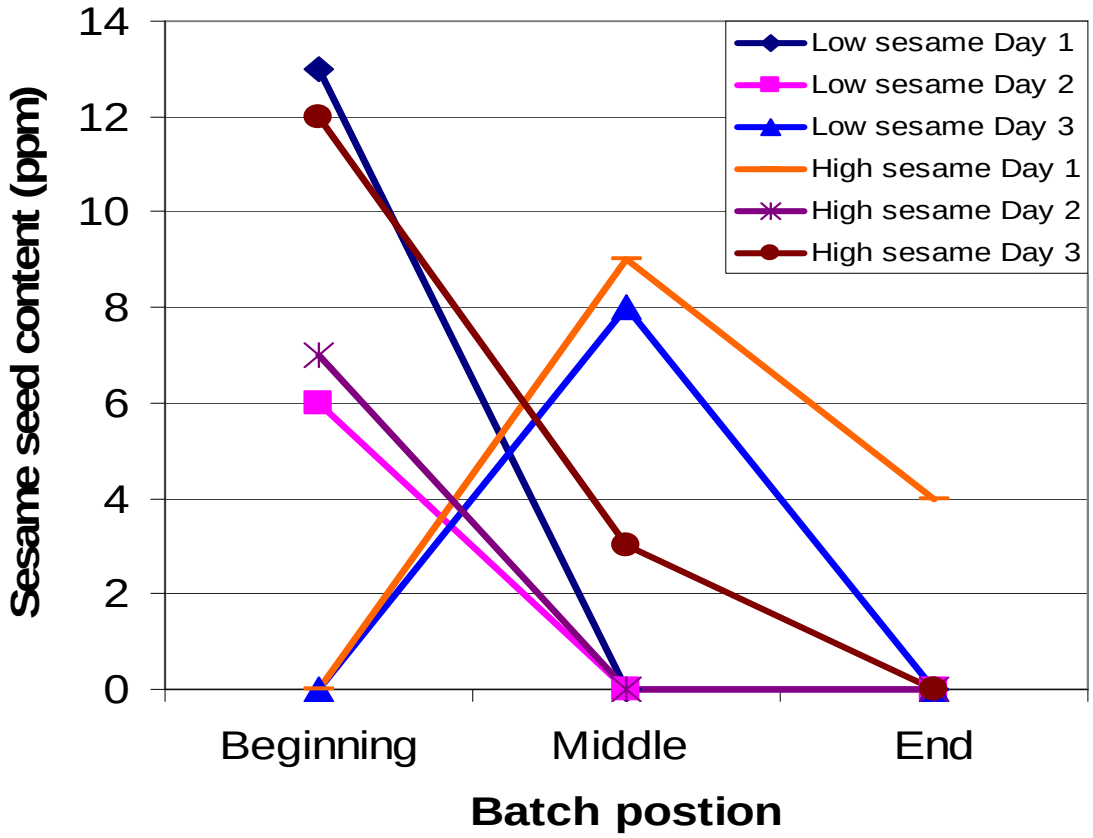
3.6.2 Following high sesame batch

Sesame was detected in two of 29 dough samples taken from batches immediately following the processing of a high sesame batch. Concentrations of sesame seed were in the range 3-4 ppm (0.6-0.8 ppm sesame protein). The frequency of detection and the concentrations of detected sesame were significantly lower in doughs following high sesame doughs for the stage two sampling than for stage one sampling.

The two doughs that contained detectable sesame protein were the second and ninth doughs taken in one day's sampling. This observation supports the sporadic nature of allergen cross contact within the bakery environment.

Sesame was detected in five of nine bread samples from batches following a high sesame batch. Apparent concentrations of sesame seed in breads were in the range 3-12 ppm (approximately 0.6-2.4 ppm sesame protein). Results for sesame in bread following both low and high sesame batches are shown graphically in Figure 3.

Figure 3: Apparent sesame content (ppm sesame seed) of bread from batches following low and high sesame batches



In the majority of cases, the pattern of occurrence of apparent sesame residues in non-sesame containing breads is largely as would be expected, with cross contact more likely to impact loaves near the beginning of a batch following a sesame containing batch (Figure 3). Mean sesame concentrations across all days show a steady decline from the beginning to the end of the production batch. However, there was considerable day to day variability and on two occasions the peak sesame concentration observed was for the middle sample, rather than the first sample.

3.7 Conclusions – Bakery Case Study

The baking process provides a challenging environment with respect to minimising allergen cross-contact, due to the large number of surfaces that the product contacts and the minimal opportunities for surface decontamination between processing runs.

Stage one of the current study assessed some of the available surfaces for non-visible surface contamination and carryover of visible contamination (adhering dough pieces). Sesame residues were found in a number of samples, particularly following processing of doughs with a high sesame content. These results confirm that opportunity exists for doughs formulated without sesame to acquire sesame material from contamination of contact surfaces and carryover of dough material. The frequent detection of sesame protein on surfaces which were visually free of contamination suggests that, although sesame enters the process in a particulate form (seeds), the sesame protein quickly becomes dispersed (non-particulate).

No sesame residues were detected in doughs following a low-sesame dough (0.5%) for samples from both stage one and stage two. This suggests that any sesame protein carried over from the low-sesame batch is insufficient to result in detectable levels of sesame in the following batch.

In the stage one sampling, all doughs analysed following processing of a high-sesame dough (6%) were found to contain sesame residues. However, the stage two sampling, following some modifications to the process, only resulted in detection of sesame protein at low levels in only a small number of doughs (2/29) from batches following the high sesame batches.

Bread samples from both stages of the project and from batches following low or high sesame batches were found to contain sesame protein more often and at levels higher than those seen in the related dough samples. It is uncertain whether this is due to additional contamination acquired during the baking, cooling, detinning and slicing processes or due to the impact of heat on the performance of the sesame analytical method. These observations should be viewed with caution.

Given these methodological uncertainties, the levels of sesame protein found in bread samples from stage two of the project were significantly lower (0.6-2.6 ppm sesame protein) than those found in stage one of the project (26-64 ppm sesame protein). It is possible that the reduction in apparent cross-contact between the stage one and stage two samplings was due to changes made to the process.

3.7.1 Assessment under the VITAL risk assessment framework

All dough analyses from stage two of the project were below the VITAL action level one value of 2 ppm sesame protein. Strict interpretation of these results, in the context of the VITAL framework, would suggest that no specific labelling of non-sesame products with respect to sesame residues was necessary.

Of 18 bread samples analysed during stage two of the study, two contained apparent sesame residues at levels above the VITAL action level one of 2 ppm sesame protein, but below the VITAL action level two of 20 ppm sesame protein. Interpretation of results from analyses on

breads, in the context of the VITAL framework, is slightly more problematic and must consider:

- The proximity of the highest analytical values to the VITAL action level 1 (2.6 ppm compared to 2 ppm);
- Uncertainties related to the analysis of sesame in heat-treated products (e.g. bread);
- Uncertainties related to the derivation of action levels for sesame. In the absence of clinical threshold data for sesame, VITAL action levels for peanut were used as a surrogate for sesame. This assumes that sensitive sesame allergy sufferers will react to similarly low amounts of protein as sensitive peanut allergy sufferers. The very limited available evidence does not support this assumption (Morisset *et al.*, 2003) and the assumption should be viewed as quite conservative; and
- Customer complaints received by the manufacturer.

3.7.2 Level of risk associated with bakery process

An allergen risk model has been developed to determine the probability of adverse allergic events resulting from defined cross-contact scenarios (Cressey, 2007). However, it was concluded that there was only currently sufficient information to establish the risk model for four allergens: peanut, milk, egg and soy.

Relatively little information has been published on sesame allergy. In a study of 798 6–year old children on the Isle of Wight, five were reported to suffer adverse reactions to sesame (0.6%; 95% Confidence interval 0.3-1.5%) (Venter *et al.*, 2006). Of 700 children who consented to skin prick tests, three gave positive reactions (0.4%; 95% CI 0.2-1.2%). Two of the sensitised children received an open food challenge, with one demonstrating objective symptoms. An Israeli study diagnosed sesame allergy by a positive history and sensitisation (positive skin prick test) for 16 of 9070 children (0.2%; 95% CI 0.1-0.3%) (Dalal *et al.*, 2002). The prevalence of sensitisation to sesame in Australian children was estimated to be 0.42%, although no attempt was made to determine the proportion of sensitised individuals who were actually allergic (Hill *et al.*, 1997).

A lowest reactive dose for sesame of 30 mg sesame protein has been reported, compared to lowest reactive doses of 2 mg, 5 mg and 0.1 ml for egg, peanut and milk respectively (Morisset *et al.*, 2003).

When the allergen risk model was set up to simulate the conditions observed during stage two sampling (44% of bread samples containing sesame, average concentration 1.6 ppm sesame protein, range 0.6-2.6 ppm sesame protein), the highest exposure calculated from 10,000 model iterations was 0.75 mg. This is well below the lowest reactive dose for sesame, reported as 30 mg (Morisset *et al.*, 2003). Even if a ten fold safety factor is applied to the lowest reactive dose (30/10 = 3 mg), the highest expected dose of sesame protein result from the observed situation is still a factor of four lower.

4 COMMENTS ON THE APPLICATION OF THE VITAL FRAMEWORK

The VITAL framework represents a useful initiative for the assessment of risks associated with allergen cross contact in food processing. Several aspects of the VITAL framework and process may require further consideration.

4.1 Form of Allergenic Material – Particulate or Readily Dispersible Form

Studies on sesame cross-contact suggest that the distinction between particulate and readily dispersible forms of an allergen may not be clear-cut and the distinction may be of limited utility. In the current study, sesame protein was often detected on surfaces where there was clearly no particulate sesame seeds. Sesame was included in the process in the form of sesame seeds.

Physical processing may reduce a particulate allergenic material to a readily dispersible form, while mixing with other food ingredients may result in solubilisation of allergen proteins outside of the particulate matrix. Therefore, ensuring that there is no carryover of particulate allergenic material is not equivalent to ensuring no carryover of allergenic protein.

4.2 Comparison of Product Allergen Levels to VITAL Action Levels

The VITAL framework allows evaluation of potential allergen contributions from raw material, raw material cross contact and processing cross contact. This approach considers total amounts of allergenic protein that may be in a production batch and averages the amount over the production batch of interest. Risk modelling carried out at ESR indicates that this approach is satisfactory for determining the level of risk associated with a particular batch of product (Cressey, 2007).

However, the VITAL framework offers little guidance to food producers who base labelling decisions on analysis of multiple end product samples. In particular, if the results of these analyses vary widely, it is unclear whether the labelling decision should be based on comparison of **average** or **maximum** end product allergen concentrations with VITAL action levels. Further guidance on how to use surface swab testing results and within process test results to formulate VITAL decisions would add to the usefulness of the VITAL framework.

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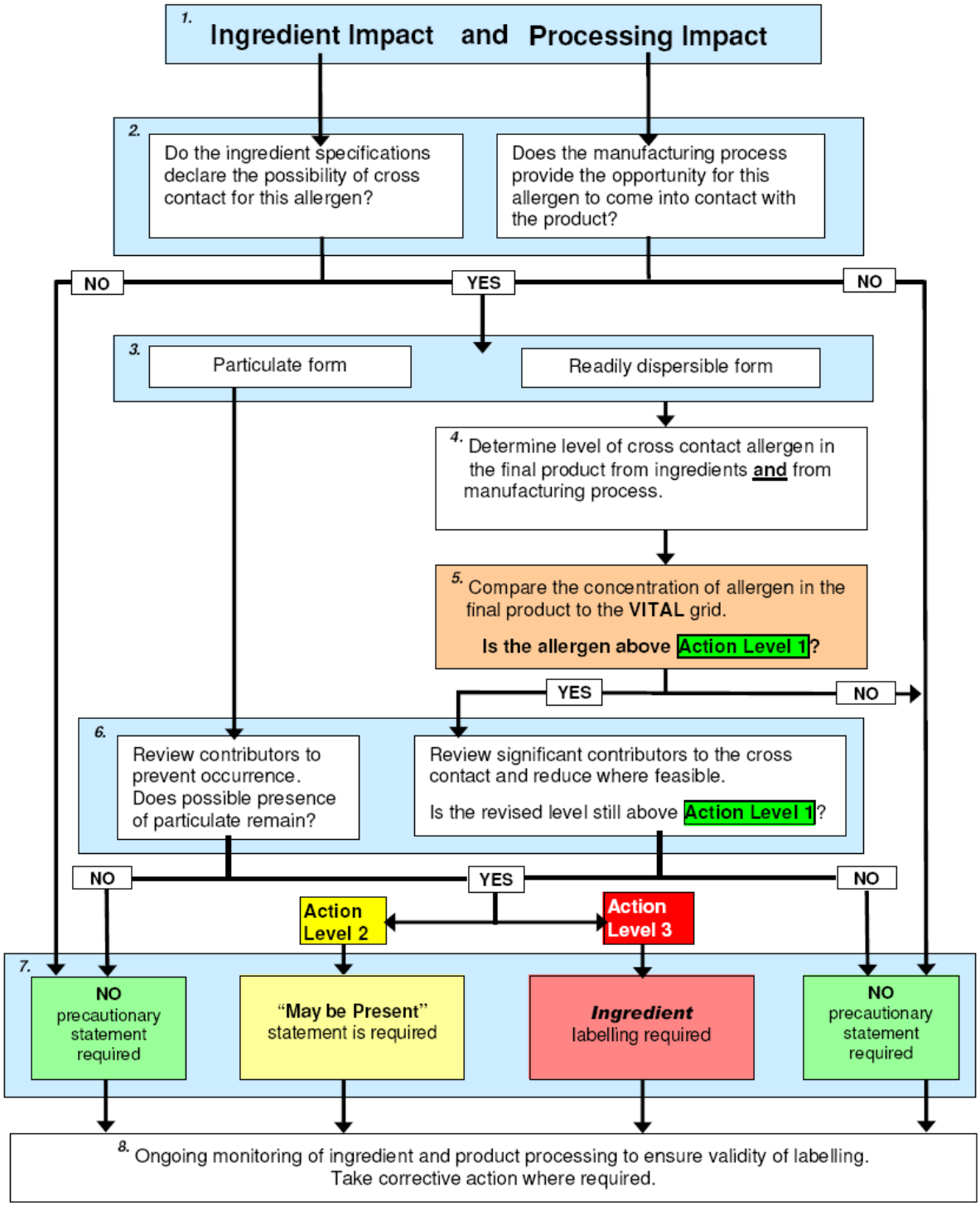
APPENDIX 1 VITAL DECISION TREE FOR ALLERGEN LABELLING



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