The assessment of food allergens in food management systems

Sandy Mosia, Nita Sukdeo and Anup Pradhan
Department of Quality and Operations Management
School of Mechanical and Industrial Engineering
University of Johannesburg, Gauteng, South Africa
201248722@student.uj.ac.za; nsukdeo@uj.ac.za; anupp@uj.ac.za

Kemlall Ramsaroop Ramdass
Department of Mechanical and Industrial Engineering
Faculty of Engineering
University of South Africa, Gauteng
ramdakr@unisa.ac.za

Abstract

Food allergens are proteins that are present in the food in significant amounts, they survive food processing environments. The risk caused by food allergens has increased in the last 20 years and it continues to expand. Food intolerances and food allergies are now well-known as a food safety concern, which must be succeeded. In the food industry, consideration of the risk caused by allergenic foods remains unpredictable. Managing this allergic risk would benefit from a better consistency of allergen management, practices and methods. One of the basic human rights, is to have access to safe food and it is of paramount importance that food is accurately labeled in appropriately. Food allergy have recently become a worldwide health concern. Food allergy is a result of an abnormal immune response to certain types of food proteins that may lead to an adverse health reaction and can cause death. In the study, allergen Bradford test was conducted on rinsed water of Diosna Mixers after production. The goal was to determine the organization effectiveness and efficiency of their allergen food management system. The results for products that entailed whew powder and soya as one of the ingredients list, showed traces of protein concentrations. The concentrations were compared with acceptable allergen limits listed on regulations R. 146 and R. 293. It is important that manufacturing industries not only comply with food management regulations but to ensure a robust allergen management plan.

Keywords
Food allergens, food safety, Bradford test,

1. Introduction

Food borne diseases causes severe symptoms such as diarrhea, vomiting, fever and abdominal pains. These symptoms are experienced by people who consumed contaminated foods and every year, millions of people get harmfully affected by the food they consume (Thomas, 2007; Newell, 2010 et al). The major causes of deaths, hospitalizations and illnesses are parasites, viruses, toxins and bacteria. There is a requirement for international and national food safety approaches to mitigate these hazards and to ensure sure that the suitability of food for human consumption traces back to the 1960s (British Retail Consortium, 2013).

Food industries have created significant efforts in putting into operations proper allergen risk management practices. Since these practices are reducing unintentional exposure of allergic consumers to allergens, this has improved the spread of advisory labelling throughout the world. Advisory labelling on likely cross-contact with allergens is

permissible only on the starting point of risk analysis applicable to conscientiously managed operation. In order to manage allergenic conditions, consumers who are affected, must be correctly informed about the compositions and nature of the food they are purchasing. The modifications in food labelling legislation have resulted in significant developments of the allergenic ingredients labeling on food packaging. (Hattersley *et al*, 2014). According to (Stein (2015), the Food Standards Agency's objectives is to improve food safety and providing clear, factual labelling information in order to protect consumers. Based on a study the British Retail Consortium (2013) food allergies are unfavorable reactions to a specific food that comprises the immune system. Allergies are distinguished by their rapid release of chemicals in a human body which causes allergic reactions symptoms. This reaction occurs within minutes after ingestion of allergic food and can last up to an hour. According Vesna *et al* (2017) common food allergens are nuts, egg, peanut, milk, selfish, fish, some sesame and soya.

Food allergies are now considered as a food safety issue, and the management of food allergens is a communal accountability between the government agencies, consumers and food manufactures. Savage *et al* (2010) mentioned that numerous international and national regulations are educating the significance of allergen management and also provide requirements that enforces manufacturing industries to comply with. According to findings reported in Marjanovic-balaban *et al* (2014) report, analysis can assist and provide understanding of allergen management capability and regulator and it should not be used as a sole tool for a sufficient allergen management. Analytical testing purpose can be utilized to validate cross-contamination control competence. Verification of raw materials composition, stipulation of quantitative data for risk assessment purpose, authentication of allergen control measures for an example; segregation and scheduling barriers, monitoring suppliers control capability and confirmation of the significance of any allergen claims are typical applications of analytical testing. The most frequently used allergen analysis is the Enzyme linked immune-sorbent assays (ELISA). This is a protein-based analysis whereby allergens in food are detected by utilizing antibodies that specifically comprehend the food protein of concern (Stein, 2015). Protein assays and Adenosine tri-phosphate (ATP) are onsite essays used on site but are not allergen specific. They are generally used to detect contamination by biological proteins that are not essentially of allergen concerns instead indicates the level of cleaning capability of a factory (Stein, 2015). Bradford assay utilizes a blue die called Coomassie blue that stains water samples that have presence and proteins. It quantifies the results as the blue dye binds with certain primary amino acids such as histidine lysine, and arginine that are present in the protein. The Hydrophobic and Van der Waals forces will interact with the blue dye molecules the overall mass of the protein must be no less than 3,000 Da for quantification. It was highlighted in so and so study that the primary disadvantage of Bradford test is that it is incompatible with surface-active agent at concentrations that are regularly used to penetrate protein membranes.

1.1 Objectives

The main aim of the research is to evaluate the allergen management systems in the food supply chain. To attain more knowledge of how allergens are identified in the factories and how they are analyzed and confirmed safe for consumptions.

The objectives of the study are:

(i) To evaluate d allergen management control in the food sector and allergens legislations.
(ii) To investigate the presence or absence of allergen contaminants.
(iii) To examine the label allergen reporting on labels.

1.2 Problem Statement

What is the control of allergens in the food manufacturing sector? Does manufacturing industries use the risk based approach to the proper use of label statements in order to advise consumers with food allergies due to the risk of accidental allergen cross contamination with food they produce? According to the FSSC 22000, manufacturing industries must implement a plan to control and mitigate the risk of allergens. This must be made aware to the workforce and proper education must be conducted for all employees to be knowledgeable and participate in the procedure. It has been made aware that some labels and unwrapped foods do not have identification of the presence of allergens in the food. Even when ingredients are listed, it should be alerted to the consumer what type of allergen the food contains.

2. Literature Review

Food allergy is described as a hostile health effect that results from a definite immune response that appears reproducibly on contact to a specific food (Benede *et al*, 2016). According to (Soon and Manning, 2017) food allergy is a result of a hostile immune response to proteins in specific food that can be immunoglobulin E (IgE)-mediated, non-immunoglobulin E (non-IgE)-mediated also called food tolerance or grouping of both. Food allergy involves an acute IgE-mediated type-1 hypersensitivity reactions which are wheezing, hives and or vomiting after contact to common allergens such as milk, peanut or egg. Lactose malabsorption is the most familiar food intolerance due to the lack of beta-galactosidase in the adult's small intestines. This is when the enzyme cannot completely hydrolyze the lactose in milk into galactose and glucose. When the incomplete hydrolyzed lactose reaches the colon, it brings about different disturbances such as abdominal pains, diarrhea at times and bloating which is most commonly experienced (Diao, 2017).

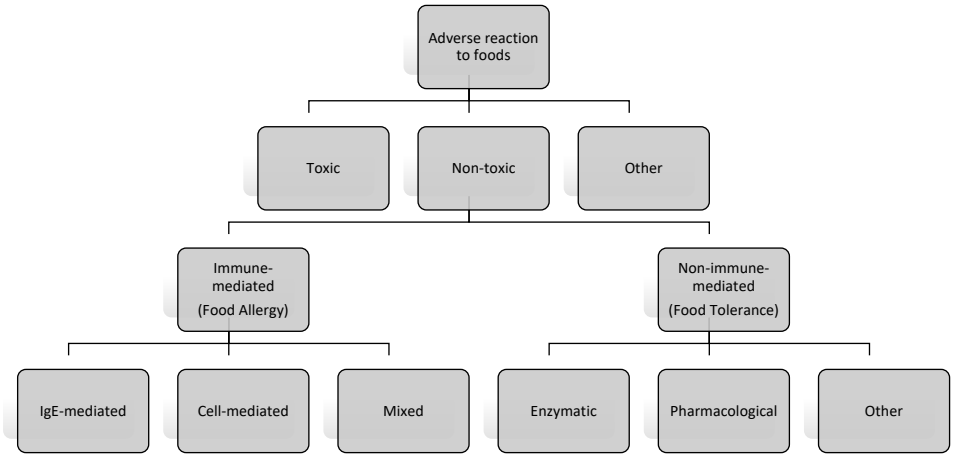

Figure 1. Classification of reactions to food (Diao, 2017).

Choi, (2012) studies classified safe and risky does in relation to managing allergen-free food orders by foodservice workers into three groups: ingredient disclosure, cross-contact prevention, and procedural practices. In their study they discovered that it is very important to have proper practices in place that are in relation to food preparation and service (Gojkoviv *et al*, 2015). This is to avoid food allergy reaction emergencies and also to ensure that all food service workers are informed and trained about their ingredients on the menu. Workers must also be educated on how to appropriately prepare and store allergen materials. Customers are responsible for informing foodservice staff about allergies that they have. Choi, (2012) emphasized the essence of communication between the foodservice staff and customers with food allergies. It is also the responsibility of foodservice staff to provide correct allergen information about the prepared food ready to be served to allergic customers. The responsibility for safe food provision lies between the customer and the foodservice establishment.

Table 1. Eliciting Doses (ED) for customary allergens that causes food intolerance and allergies (Pucholek *et al*, 2018).

Materials or Ingredients that causes food intolerance or allergies	**Level of Protein (MG)**
Eggs	0.03
Peanut	0.02
Milk (Products including lactose)	0.1
Celery	N/A
Sesame seeds	0.2
Cereals that include gluten: Oats, rye, wheat, barley, kamut and spelt	1
Soybeans	1

Crustaceans	1
Nuts: hazelnuts, pecan nuts, pistachio nuts, almonds, walnuts, Brazil nuts, cashews, Queensland nuts, macadamia	0.1
Mustard	0.05
Lupin	4
Sulfates at concentration of greater than 10mg/kg or 10mg/l stated as Sulfur dioxide (SO2)	N/A
Molluscs	N/A

2.1 Food Management Systems

Food safety management system (FSMS) is very significant when it comes to protecting consumer's health. The main goal of European Union's (EU) food safety policy is to provide consumers not only with high quality food but also safe food that has transparent, reliable and accurate information with regards to food products (Pucholek *et al*, 2018). According to (Assuring Food Safety and Quality) guidelines the words food safety and food quality are very unclear; food safety means all chronic or acute hazards that may cause harm to consumers. Food quality refers to all other characteristics that have an impact on any products value to customers. This comprises of negative characteristics such as food contamination, spoilage, off ordours, discoloration, and positive characteristics such as flavor, color, origin, texture and manufacturing process methods of the food. This dissimilarity between food safety and food quality has involvement for public policy and also has an effect on the content and nature of food control systems. These systems are appropriate to meet established national objectives (Assuring Food Safety and Quality). Food control is outlined as an obligatory regulatory action of enforcement by local or national authorities to deliver consumer protection and make sure that all foods in the processing of production to handling, storage to distribution are safe for human consumption. Also to ensure that they conform to quality requirements, safety requirements and are accurately and honestly labelled as stipulated by law (Assuring Food Safety and Quality).

Table 2. Allergen Management requirements provided in the quality and food safety management systems

Standard	**Requirements characteristics**
ISO 22000:2015	Proposed use
	Product groups such as those known to adversely affect consumers that are vulnerable to certain food safety risk e.g. allergens shall be recognized.
ISO/TS 22002-1:2009	Allergen management
	Products that have allergens presence due to cross contamination or product design shall be stated by the manufacture. Unintentional allergen cross-contamination of products shall be protected by proper cleaning and manufacturing line change over.
IFS (Version 6, 2012)	Hazard Analysis and Critical Controlling Points (HACCP) analysis
	Description of product intended use shall be defined, taking into consideration vulnerable consumer groups such as those with allergic reactions.
	Traceability system that consists of allergens and Genetic Modified Organisms (GMOs) shall be in place which aids the documentation of products batches with their connection to raw material batches and packaging material utilized.
	Product contamination risk analysis due to foreign materials from staff facilities shall be assessed and mitigated
	Allergens and precise production conditions
	Raw material specifications classifying allergens that require stating shall be presented. Manufacturing products that may contain traces of allergens and requires declaration shall be executed by ensuring mitigation of cross contamination.
BRC (Issue 7, 2015)	Prerequisite programs

	The production site shall institute and continuously maintain programs that a mandatory to create an environment that is suitable to produce safe and legal products such as creating allergen control program. Recognize intended use Outlining consumer target groups such as the product suitability for vulnerable consumer groups that suffer from allergens Product description Product full description shall be described including allergen information

2.2 Labelling of food allergens

Every food manufacturing industry must make certain that food safety is not compromised. The industries must give accurate information on their food product so that can make an informed decision on what they consume (Pucholek *et al*, 2018). The adoption of regulations on labeling food allergens was due to the cause of the increased scale of problems and possible consequences of allergen reactions for consumer protection. The main information consumers can get about allergies in food is the sufficient product labeling. The law to declare any allergen presence only applies to ingredients that are intentionally added in food products (Assuring Food Safety and Quality).

Figure 2. Typical allergen label (ESR, 2020c)

2.3 Food allergen assessment

Food industries must have HACCP principles in place, implement and continuously sustain the procedure. This procedure includes the identification of hazards that may must be eliminated or mitigated to acceptable levels and create an allergen matrix risk such as figure 2.3 below and allergen risk management figure 2.4. Allergens must be labeled as hazards, products labeled with PAL that exclude HACCP principles have been identified as an incorrect measure with consideration to prevent, eliminate or mitigate allergen risks. The Food Information to Consumer (FIC) labelling does not regulate the word used "may contain traces of…" or additional safety allergen labeling used by business operators in order to alarm consumers of unintended contamination.

SEVERITY	SCORE	LIKELIHOOD: Daily 5	Weekly. 4	Monthly. 3	Every 6 Months. 2	Not at all/ Less than every 6 Months 1
Could it result in the death/ irreversible illness?	5	25	20	15	10	5
Could it result in serious illness/ injury?	4	20	16	12	8	4
Could it result in minor illness/ injury?	3	15	12	9	6	3
Could it result in minor effect on the brand?	2	10	8	6	4	2
Is it unlikely to affect consumer/ brand?	1	5	4	3	2	1

SCORES	CATEGORY
25 TO 17	HIGH RISK
16 TO 7	MEDIUM RISK
7 AND LESS	LOW RISK

Figure 3. Allergen risk matrix (Diao, 2017)

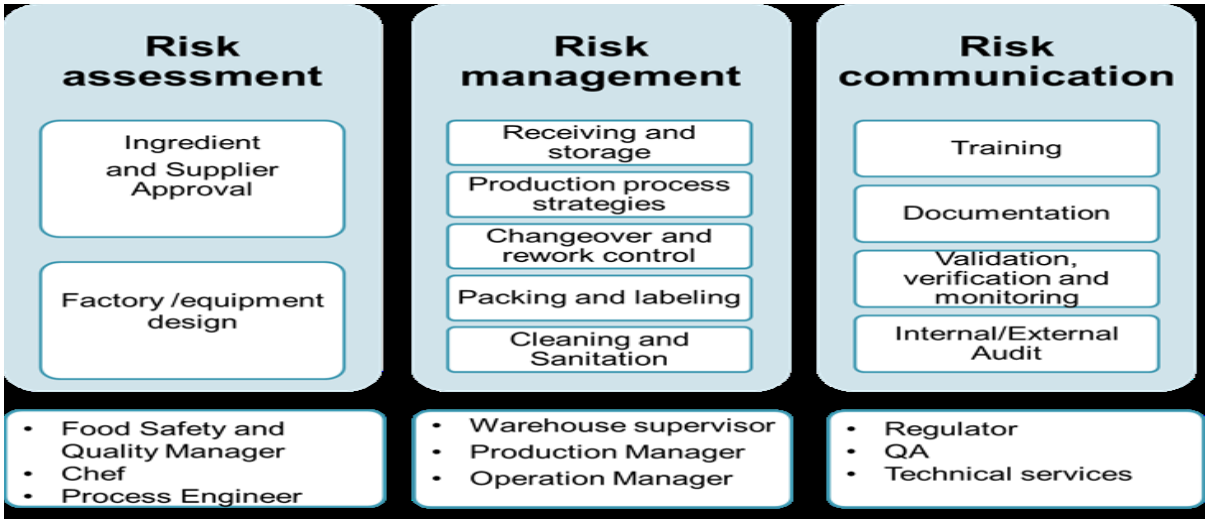

Figure 4. Allergen management risk in food facilities (Diao, 2017)

Food industries often assume that PAL is used to alarm any potential cross contamination for example, in instances whereby different products have shared manufacturing facilities or equipment. It is stated in (Soon and Manning, 2017) article that a lot of countries have been utilizing the PAL method which is not formalized, PAL has statements that are unhelpful to consumers. The employment of PAL is an intended policy to cover manufactures back and mitigate risks.

2.4 Food allergen analysis

ELISA method, Mass Spectrometry (MS), and Polymerase Chain Reaction (PCR) are validation methods performed in laboratories. The methods are expensive and take longer to produce results. They require special resources and skilled technical personnel to conduct the test (Stein, 2015). ELISA is a direct analytical method that detects and measures allergenic residues. This method has been identified to produce accurate results as at is very sensitive and is named the gold standard for food allergens detection unit. The rapid measurement detection device binds to targeted proteins and antibodies and quantifies allergens in finished food products.

Although it is universally recognized, it cannot detect allergen proteins that have undergone hydrolysis or thermal treatments and any other oxidizing agent such as chemicals as they change the protein structure of the allergen. MS aims to test the known allergenic proteins peptide makers as a replacement of testing allergenic proteins in food. An advantage of utilizing MS is that it can detect multiple allergens in a food product in a single test (Stein, 2015).

Results are quantifiable, it is more sensitive than ELISA and it is not affected by cross-reactivity. PCR method targets particular allergen DNA molecules and the DNA that will be detected is an indicative of an allergen presence. This method is best used when reliable ELISA methods have not been established for some allergens. PCR can detect allergen residues and is also sensitive and cross-reactivity does not affect the results.

Coomassie Bradford test is an assay kit that is easy and quick to use for its total protein quantification colorimetric method. Its mode of action is when the protein binds to the Coomassie dye in an acidic environment. During this period there will be an absorption shift that occurs between 465nm and 595nm. This will result in a brown to blue color change, see below illustration on figure 2. 6. The analysis can be conducted in a test tube or microplate which enables the combination of the protein sample and the assay reagent to mixed and incubated before measuring using spectrophotometer absorbance at 595nm (Gojkocic *et al*, 2015).

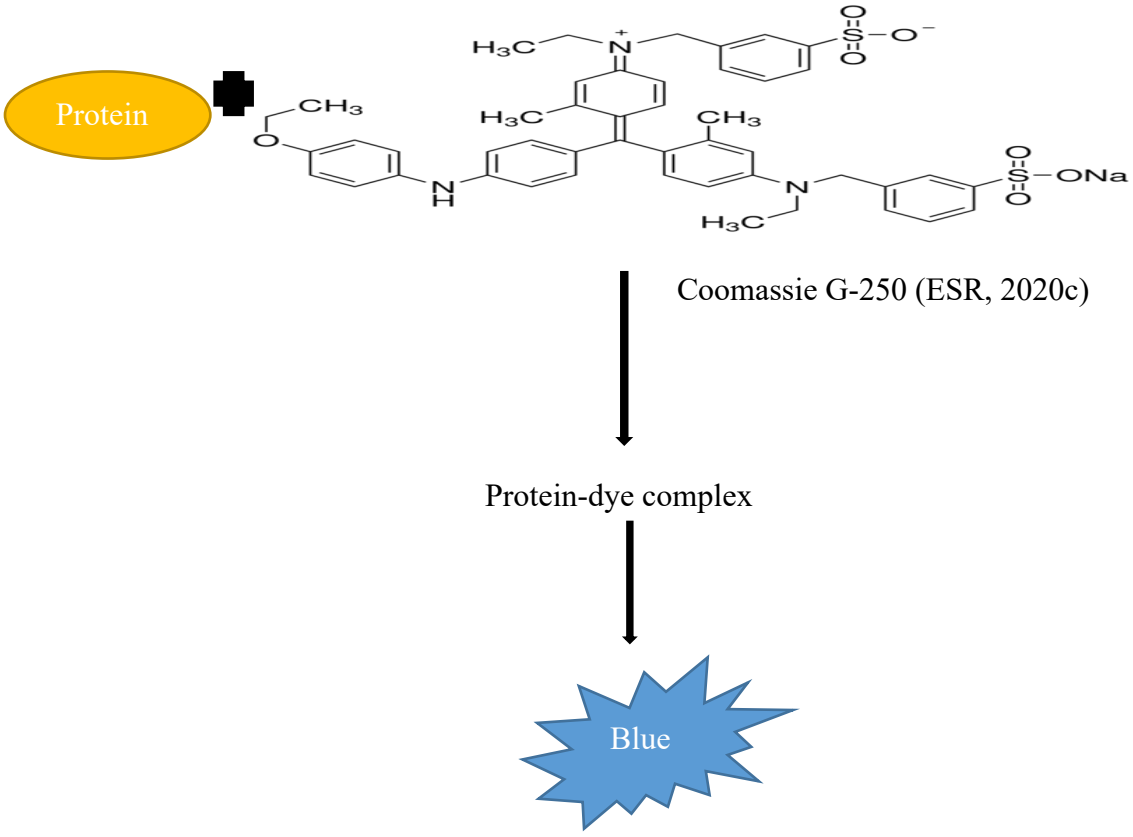

Figure 5. Bradford protein assay reaction with Coomassie dye

Verification methods are rapid test that are commonly used onsite as they cost effective, mobile, and limited skilled technical personnel can conduct the analysis. They are also known as rapid kits and are available for the detection of numerous allergens that are industrially manufactured (Marjanović-Balaban *et al*, 2014).
From left to right test methods represented below on figure 2.7, the first rapid test is also named the semi-quantitative immunochromatographic test that is based on ELISA counterparts (Pacholek *et al*, 2018). Set of colored lines that are reproduced indicates whether a target allergen is present or not in a sample. The allergen concentration detected is directly proportional to the strength of the colored line in the test zone. The second test detects allergen proteins on surfaces, the underlying principle of this method is that if a sample result is found to be protein free (Pacholek *et al*, 2018).
This means that the surface is free from allergens since most allergens are proteins. ATP method does not directly measure allergenic food residues but detects the presence of ATP in rinse water and on surfaces (Gojkocic *et al*, 2015). It is generally used for hygiene monitoring and is it not allergen specific due to lack of sensitivity of an ELISA system. Studies have concluded that ATP is sensitive for validating wet-cleaning processes (Marjanović-Balaban *et al*, 2014).

Figure 6. Three rapid test represented in the food industry for validation methods

3. Methods and Techniques

Two types of allergen test were conducted, the ATP and Bradford assay tests. For ATP test, 4 Diosna mixers were swabbed on hard to reach areas in between two productions after allergen and non-allergen containing ingredients were produced. The swabs were immediately put in the Ensure detection device to obtain results. The rinse water samples were collected after each wash, samples were refrigerated at cool temperatures before Bradford assay. After Bradford test analyses, statistical calculations were conducted. This chapter gives an overview of the research methods that will be used in this study. The study design, study area, study population, sampling, data collection and ethical consideration are discussed. The research methods will enable the researcher to achieve the aim and the objectives of the study.

3.1 Sampling technique

Simple random technique was utilized to produce results that represented an entire population. After each production the four mixers were cleaned with alkaline based soap and rinsed with tap water, 8 x 25mL of rinsed water were sampled from each equipment and labelled accordingly. After the mixers were air dried, 2 X 8 allergen swabs were taken on surfaces that are hard to reach and clean.

Controlled variable: 1mL of Albumin (BSA) Standard.

4. Data Collection

Two allergen data collection method and equipment's were utilized in this study since it is an experiment study. The first method was to collect water samples and analyzing them in the Bradford assay to obtain quantitative data. With the second method, swabs were taken on identified surface areas and inserting into the detection measuring unit to obtain qualitative data.

4.1 Bradford Assay standard preparations

- 5mg/ml of BSA standard was diluted with 0.5g/ml Deionized water.
- Table 3 was used a guide to prepare the diluted standard.
- The standard vials were put into the spectrophotometer and the absorbance was measured at 595nm.

Table 3. BSA standard diluted preparations

Dilution Vial	**Diluent volume (µl)**	**0.5mg/mlBSA volume (µl)**	**BSA final Concentration (µg/ml)**
A	0	300	500
B	60	240	400
C	120	180	300
D	180	120	200
E	240	60	100
F	270	30	50
G	280	12	25
H	300	0	0

4.2 Coomassie blue and rinse water sample preparations

- Coomassie reagent solution was mixed thoroughly by inverting the bottle several times.
- 5µL of rinse water was sampled into the microplate wells, this was prepared in five sets.
- 250µL of Coomassie reagent was added onto the wells and put on a shaker for 30 seconds.
- The plate was incubated for 10 minutes at 37°C to ensure consistent results.
- The plate was put into the spectrophotometer and the absorbance was measured at 595nm
- Protein concentration results of each production rinse water and of the standard were automatically calculated and displayed.

5. Results and Discussion

After all eight production, the equipment were washed with alkaline soap and flushed with tap water. The rinse water that was sampled and analyzed for using Bradford method. The test was quick and easy to perform and due to the automated spectrophotometer, the results were tabulated as follows:

Table 4. Protein concentrations (µg/ml) for standard and rinse water

	1	2	3	4	5	6	7	8	9	10
A	1ppm 0,04128	1ppm 0,03688	1ppm 0,04388	1ppm 0,04558	1ppm 0,04688	P7 0,001680	P7 - 0,001420	P7 - 0,001620	P7 0,01108	P7 0,003080

B	10ppm 0,2228	10ppm 0,2338	10ppm 0,2502	10ppm 0,2417	10ppm 0,2367	P8 0,3860	P8 0,3817	P8 0,3918	P8 0,3576	P8 0,3474
C	P1 0,003480	P1 0,005180	P1 0,005180	P1 0,006780	P1 0,007380					
D	P2 0,003880	P2 0,009980	P2 0,01068	P2 0,01288	P2 0,01288					
E	P3 0,1158	P3 0,1216	P3 0,1278	P3 0,1254	P3 0,1215					
F	P4 - 0,0005200	P4 - 0,0007200	P5 - 0,001620	P6 - 0,0003200	P6 - 0,0005200					
G	P5 - 0,004920	P5 - 0,002320	P5 - 0,003020	P5 - 0,002820	P5 - 0,001820					
H	P6 0,0003800	P6 - 0,004720	P6 - 0,001720	P6 0,002280	P6 - 0,003120					

5.1 ATP Swabbing areas

After the machines were allowed to air dry, ATP swabs were taken on hard to reach and clean surface areas. Figure 4.1 is where the mechanism of stirring parts are dissembled and the first swab was taken. The area appeared to be clean which shows that the cleaners are well informed when it comes too hard to clean areas of the Diaosna mixer.

Figure 7. First hard to reach and clean identified on Four Diosna mixers

The below picture was also identified because most cleaners are used to cleaning the inside of production mixers and are lazy to clean the outside surfaces. Another swab was taken as highlighted on figure 4.2, the swab was visually inspected and was found with no stain or presence of dirt. This can be used as a confirmation that the cleaning process plan is effective.

Figure 8. Second hard to reach and clean identified on Four Diosna mixers

5.2 ATP analysis

Table 5. ATP swab results from the Diosna mixers

	PRODUCTS	1	2	3	4	5	6	7	8
ATP RESULTS	PASS (0 – 10) RLU	0	4		1	2	1	5	
	CAUTION (10 – 15) RLU			11					14
	FAIL (15 >) RLU								

5.3 Concentration of protein allergens

Figures 7 and 8 shows the protein allergens in five water samples of different products namely P1, P2, P3, P4 and P5, P6, P7, P8, respectively. The concentrations found in the macro plate wells, there is an increase in protein concentration for P1 and P2, P3 protein concentration started at a lowest point to highest, the fourth reading went slightly lower followed by the last reading. P3 allergen protein concentration was found higher reading at 0.13 ppm. The two first and last reading points for P4 are precise and the third reading was not agreeing with the rest. P1, P2, and P4 allergen concentration results are all lower than the ED listed on table 2.1.

Concentration points for P5, P6, and P7 are all lower than the ED, however P8 results shows a significant high concentration. The ingredients used in this product entailed both soya and milk allergens and the equipment were not effectively cleaned and rinsed. The product form could have been sticky or the material was not cleaned well leaving behind powder residues. Since this material was the last production for the day, the cleaner's mechanical energy might have been reduced due to close to knock off time. It is human nature especially in production facilities that when the knock off time is approaching, workers rush and skip working procedures. It is advisable for this manufacturing industry to run allergen test at the end of production schedules.

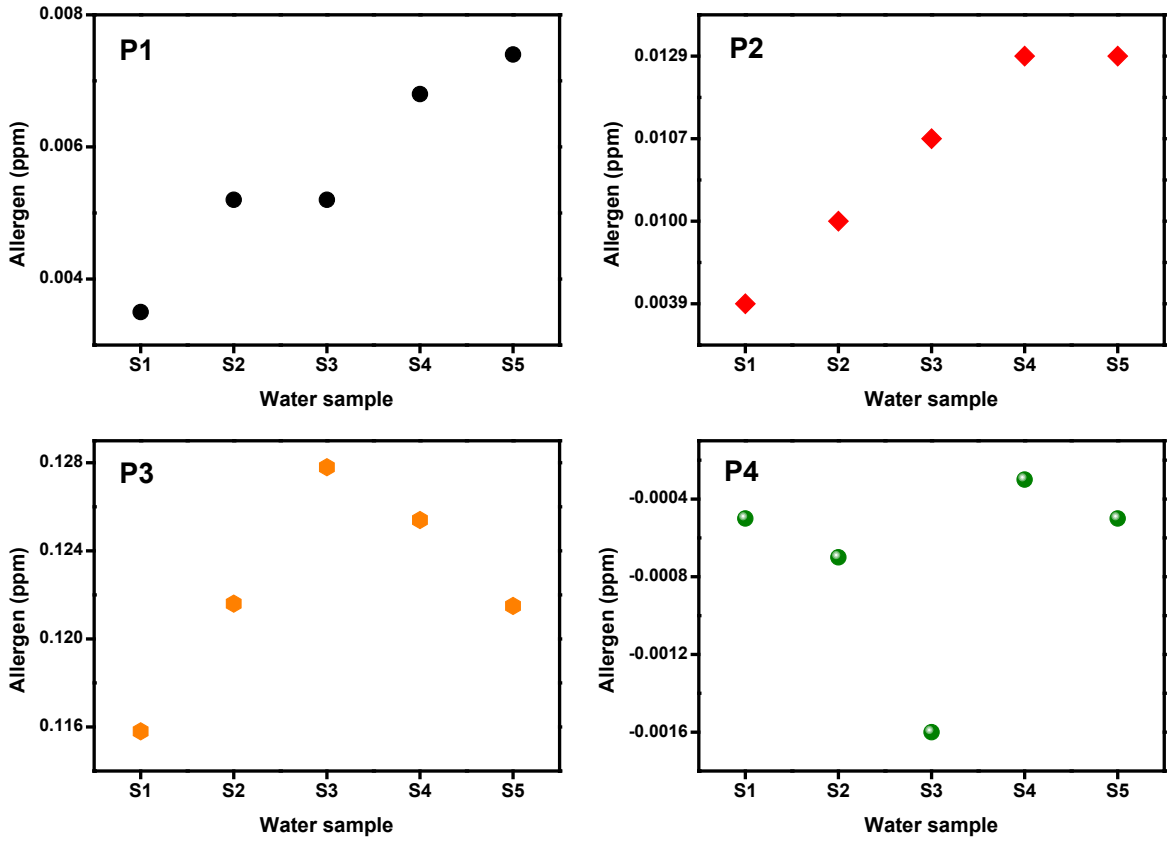

Figure 9. Concentration of protein allergens present in water samples of different products (P1, P2, P3, and P4)

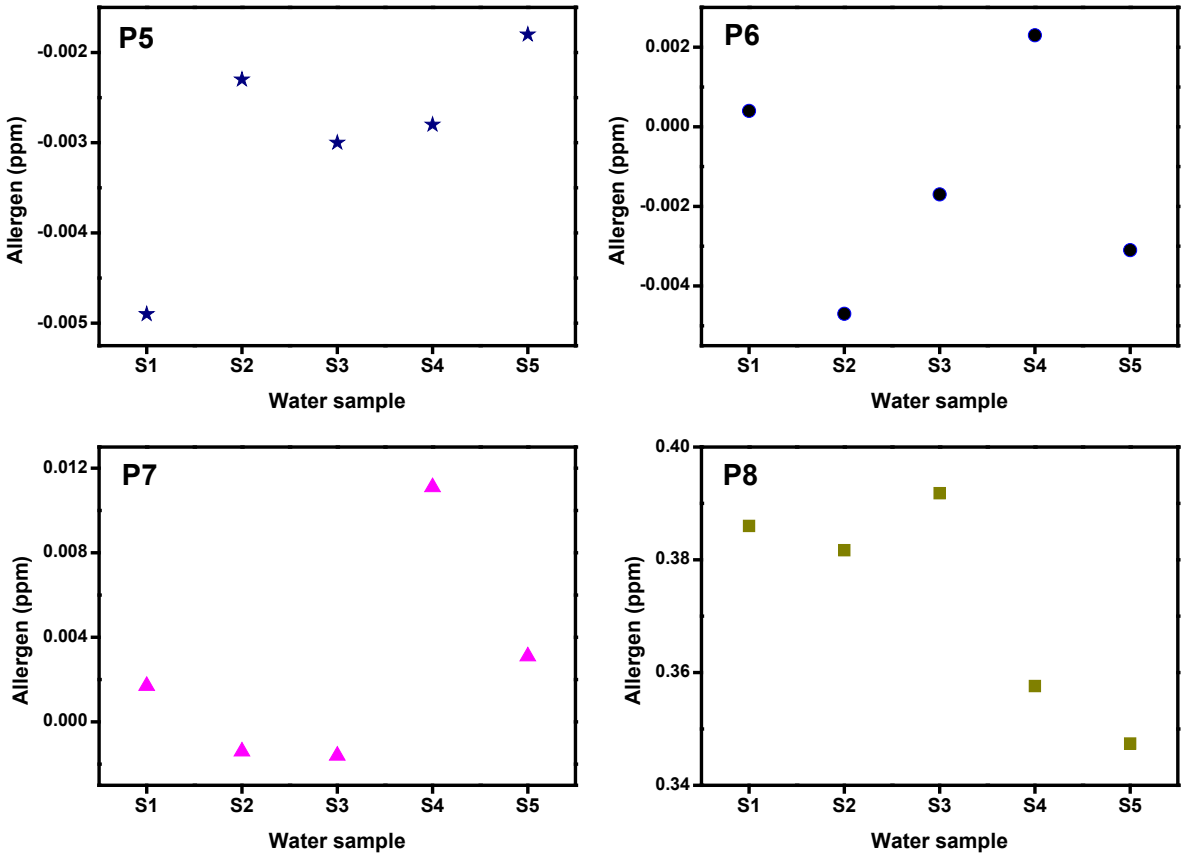

Figure 10. Concentration of protein allergens present in water samples of different products (P5, P6, P7, and P8)

5.4 Comparison of mean concentration of protein allergens

The graphs below illustrates the mean concentration of each product, P3 and P8 shows significant mean values compared to the rest of the products. Figure 4.5b shows the comparison of the standard and all products concentration. It can be deduced that P3 ad P8 entail higher mean values than all other products due to the cleaning procedure and ingredients used in the products.

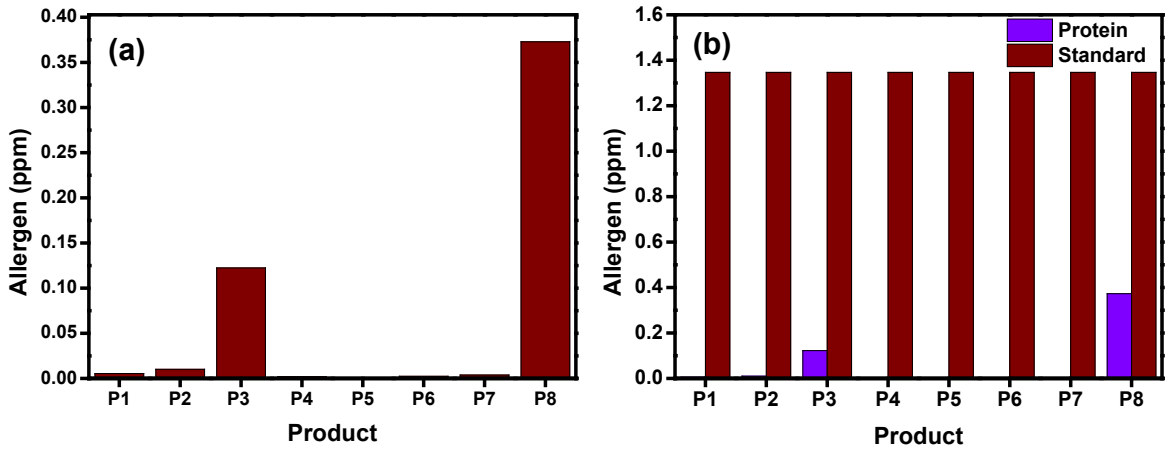

Figure 11. (a) Mean concentration of protein allergens present in different products, (b) Comparison of mean concentration of protein allergens with the world standard value.

5.4 Percentage contribution of protein allergens

Figure 12 illustrates the overall percentage of how much the presence of allergen proteins in the final product has. P4 to P7 only contributes a little followed by P1 and P2. However, P3 and P8 shows a significant contribution to the end product. This means that the products must be rejected or reworked as the soya allergen ED has been reached. This might cause serious health hazrad and might impact the organizations quality standard.

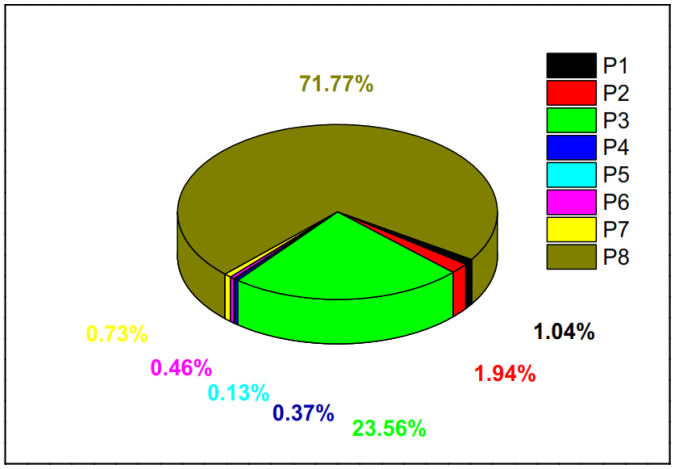

Figure 12. Percentage contribution of protein allergens of different products.

5.5 Pearson's Correlation

Pearson correlation is the linear relationship between two variables (X and Y) with values of +1, or 0, or -1. These values (+1, or 0, or -1) represent positive linear, no linear, and negative linear correlation, respectively. X and Y having the values of +1, or -1 indicate perfect and imperfect relationships, respectively between the variables. A perfect relationship means that Y increases as X increases, while an imperfect relationship exists between the variables when Y increases as X decreases (Oke et al, 2020). The correlation analysis is presented in Table 4.4. From Table 4.4, P1 shows significant positive correlations with P2 (r=0.937), P3 (r=0.500), P5 (r=0.843) and P7 (r=0.513), but poor

positive correlation with P4 (r=0.247), and a negative correlations with P6 (r=-0.057) and P8 (r=-0.869). These types of correlations of P1 with P2, P5 and P8 indicate a strong relationship exists between them. P2 displays significant positive correlations with P3 (r=0.744) and P5 (r=0.895), but poor positive correlation with P7 (r=0.375), and a negative correlation with P4 (r=-0.008), P6 (r=-0.132) and P8 (r=-0.649). The correlations existing among P2, P3, and P5 also indicate a strong relationship. A significant positive correlation exists between P3 and P5 (r=0.534), but negligible positive correlation among P3 and P6 (r=0.35) and P3 and P7 (r=0.127), and a negative correlation among P3 and P4 (r=-0.548), and P3 and P8 (r=-0.041). The correlations between P3 and P5, and P3 and P8 imply a good relationship among the products. The correlations of P4 with P5 and P8 (r=-0.027 and r= -0.643), P6 and P7 (r=0.310 and r= 0.665) implies negative and positive relationship. These correlations of P4 with P5 and P7 indicates a good relationship between the products. P5 also show negligible positive correlation with P7 (r=0.050), good negative correlations with P6 (r=-0.537) and P8 (r=-0.575). The correlation of P7 with P8 indicates the existence of a negative relationship between them. It was also observed that P8 correlated negatively with all studied products.

Table 6. Correlation analysis of examined products

Correlations									
		P1	P2	P3	P4	P5	P6	P7	P8
P1	Pearson Correlation	1	.937**	.500	.247	.843*	-.057	.513	-.869*
	Sig. (2-tailed)		.006	.312	.637	.035	.915	.298	.025
	N	5	35	5	5	5	5	5	5
P2	Pearson Correlation	.937**	1	.744	-.008	.895*	-.132	.375	-.649
	Sig. (2-tailed)	.006		.090	.988	.016	.803	.464	.164
	N	5	5	5	5	5	5	5	5
P3	Pearson Correlation	.500	.744*	1	-.548	.534	.035	.122	-.041
	Sig. (2-tailed)	.312	.090		.260	.275	.948	.818	.939
	N	5	5	5	5	5	5	5	5
P4	Pearson Correlation	.247	-.008	-.548	1	-.027	.310	.665	-.643
	Sig. (2-tailed)	.637	.988	.260		.959	.550	.149	.169
	N	5	5	5	5	5	5	5	5
P5	Pearson Correlation	.843*	.895*	.534	-.027	1	-.537	.050	-.575
	Sig. (2-tailed)	.035	.016	.275	.959		.272	.926	.233
	N	5	5	5	5	5	5	5	5
P6	Pearson Correlation	-.057	-.132	.035	.310	-.537	1	.757*	-.114
	Sig. (2-tailed)	.915	.803	.948	.550	.272		.081	.830
	N	5	5	5	5	5	5	5	5
P7	Pearson Correlation	.513	.375	.122	.665	.050	.757*	1	-.674
	Sig. (2-tailed)	.298	.464	.818	.149	.926	.081		.142
	N	5	5	5	5	5	5	5	5
P8	Pearson Correlation	-.869*	-.649	-.041	-.643	-.575	-.114	-.674	1
	Sig. (2-tailed)	.025	.164	.939	.169	.233	.830	.142	
	N	5	5	5	5	5	5	5	5

**. Correlation is significant at the 0.01 level.
*. Correlation is significant at the 0.05 level.

5.6 Linear relationship

Linear scatted plot shows the relationship and the degree of linear fit (R^2) between products. Matrix scattered plot and histogram of different products were presented in figure 4.7 to confirm the linear relationship between protein allergens. Figure 5 shows that an excellent and good relationship exists among P1 and P2, P2 and P3, P2 and P5, P6 and P7, and P1 and P7, P1, and P3, P3 and P5, P4 and P7, respectively. A poor relationship exists between P1 and P4, P2 and P7, P3 and P6, P3 and P7, P4 and P6, and P5 and P7. Also, a weak relationship occurs among P1 and P6, P2 and P4, P2 and P6, P3 and P4, P5 and P4, and P5 and P6. Furthermore, it was observed that P8 shows a weak relationship with all studied products.

The most significant linear relationship and the degree of linear fit among different products were also displayed in figure 4.8. These significant plots were extracted from figure 4.7 to confirm the Pearson correlation presented in table 4.4. From the same figure, strong linear relationships exist between P1 and P2, P1 and P5, and P2 and P5 with R^2 values of 0.879, 0.711, and 0.805, respectively. A significant good relationship also occurs among P2 and P3, and P6 and P7 with R^2 values of 0.554 and 0.5573, respectively. The most significant weak linear relationship exists between P1 and P8 with R^2 values of 0.754. The linear scatted plots confirm the validity of the Pearson correlation.

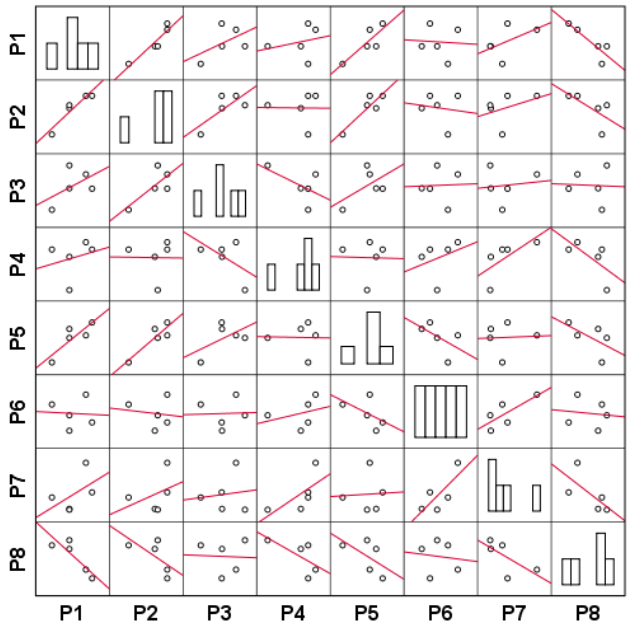

Figure 13. Matrix scattered plot and histogram of protein allergens in different products.

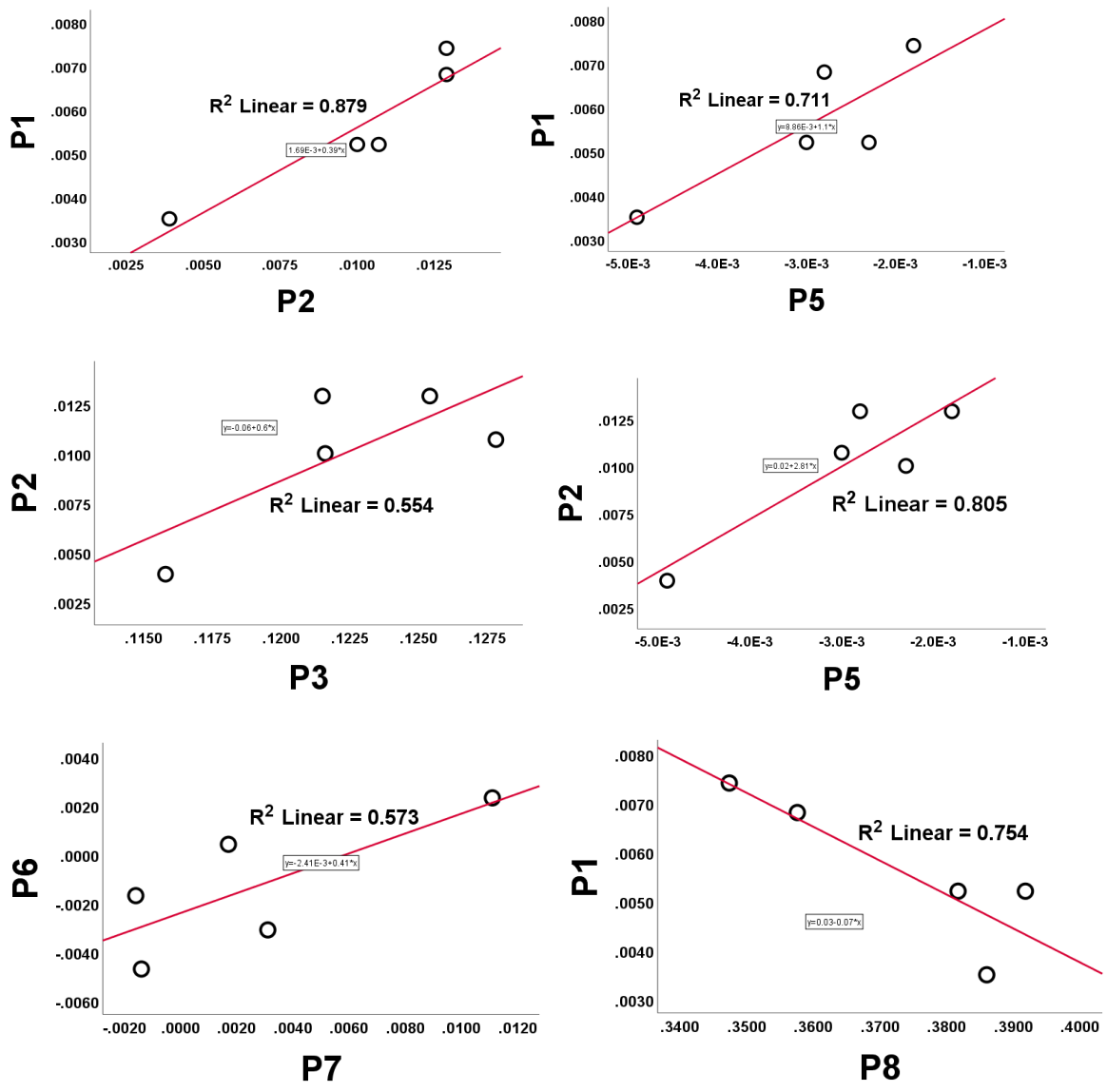

Figure 14. Positive and negative most significant relationship between protein allergens of selected products.

5.7 Cluster analysis

Cluster analysis was performed to understand the hierarchical relationship among objects or data, which is in form of a tree-like diagram. This diagram is also known as a Dendrogram, which comprises the grouping of objects or data like branches attached to a tree. Data are similar when they are grouped as branches attached to a tree. Therefore, trees attached to each other translates to dissimilarity among data. In general, cluster analysis gives information about the similarity and dissimilarity between sets of data (Oke et al, 2020). The number of clusters and the dendrogram representing the hierarchical cluster analysis of studied products is depicted in figure 4.9. From figure 4.9 (a), seven clusters were shown by the figure, but three main clusters were identified from figure 4.9 (b). Figure 4.9 (b) displayed the similarity and dissimilarity among several products, which is an excellent tool to justify the Pearson correlation and linear scattered plot analysis. The clusters were been divided into three main classes. The first cluster shows the similarities between P1, P2, P3, and P5. The second cluster represents the similarities between P4, P6, and P7. The third cluster depicted P8 as a single cluster. The third cluster is indicative of dissimilarities with first and second clusters, while other clusters existing among first and second as depicted in figure 4.9 (a) are less significant. The

cluster analysis was found to be in a relationship and agrees with the Pearson correlation and linear scattered plot analysis.

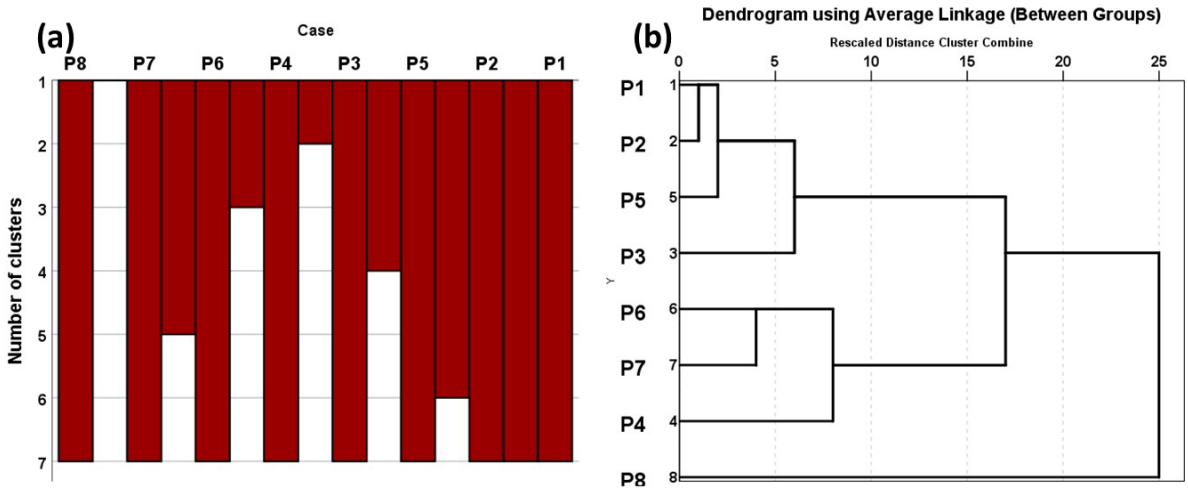

Figure 15. (a) Number of clusters of products, (b) Dendrogram obtained from hierarchical clusters of products

5.8 Validation

According to the results found in the verification and validation methods, it shows that product 3 and 8 significantly differ from other production, this is due to the ingredients used in each production (see appendix). The result obtained from the statistical analysis shows that cluster analysis agrees with Pearson correlation, linear scattered plot analysis. Also, the relationship between studied products indicate that P8 is independent of other products.

The results also conclude that the cleaning method for this facility is poor and needs to be amended based on allergen used in production. This research has also proven that there is a gap between South African regulations, manufactures and consumers. The regulations are amended and communicated; it is the manufactures role to ensure that this information is attained but small food manufactures are clueless on the existence of most food management systems.

6. Conclusion

Allergens are naturally present from food that we eat, in the past years allergens were not known and their health hazards remained unnoticed until consumer eating patterns were traced back. Most children deaths were due to allergic reactions and no one knew how to prevent such a horrific cause. It was later discovered that consumers need to be informed with what they are eating from restaurants to retail stores. Food labelling was the main cause of allergic reactions and food manufactures need to be informed on such cases.

The research was conducted in a flavor industry that supplies raw materials to final manufacturers in the food chain. Allergen samples were taken within the facilities production area after eight successful production and cleaning procedures were complete. The allergen concentrations were measured using the verification and validation methods. Both methods depicted that the third and eighth products had significant presence of allergen proteins. This was due to the high quantities of whey and soya powder used in the ingredients and how the cleaning methods were not effective.

This research also found that the mechanical energy of cleaning workforce significantly decreases near closing business hours. The cleaning protocols must be visited, and sampling time must also be changed. The statistical results show that the last production significantly differed from other products. This was the due to the ingredient whey and soya used in the manufacturing of this product.

References

Assuring Food Safety and Quality: Guidelines for strengthening National Food Control systems, Joint FOA/WHO Publications.

Benede, S., Blazquez, A. B., Chiang, D., Tordesillas, L., and Berin, D. The rise of food allergy: Environmental factors and emerging treatments, *Journal of EBio Medicine*, 7:27-34 2016.

Bolin, Y., and Lindeberg, I. *Undeclared Allergens In Food*. Denmark, Nordic Council of Minister 2016.

British Retail Consortium. Guidance on Allergen Labelling and the Requirements in Regulation 1169/2011. BRC, UK 2013

Choi, J. University foodservice employees' food allergy knowledge, attitude, practices, training and training needs. Masters. Iowa State University 2012.

Diao, X. (2017). *An Update on Food Allergen Management and Global Labeling Regulation*. Masters. University of Minnesota.

ERS. (2020a). GFSI position on mitigating the public health risk of food fraud. Available at https://www.mygfsi.com/files/Technical_Documents/Food_Fraud_Position_Paper.pdf Accessed 22 April 2020

ERS. (2020b). Guidance of Food Allergen Management for Food Manufactures. Available at www.fooddrinkeurope.eu. Accessed 22 April 2020

ERS. (2020c). Coomassie Brilliant Blue G-250. Available at https://media.mpbio.com Accessed 14 November 2020

Gojkocic, V., Marjanovic-Balaban, Z., Vukic, M., Grujic, R., and Novakovic, B. Allergens Management System in the Food Production, *Journal of Hygenic engineering and Design, 6(13): 616-056.3 2015.*

Hattersley S. Ward R., Baka A., Crevel R. W. R. Advances in the risk management of unintended presence of allergenic foods in manufactured food products - An overview. *Journal of Food and Chemical Toxicology,* 67: 255-261 2014.

Marjanović-Balaban Ž. Grujić R., Pećanac B., Jelić D. Methods for Determination of the Presence of Allergens in Foods. *Journal of Quality of Life*, 5, (3-4):93-97 2014.

Newell, D.G., Koopmans, M., Verhoef, L., Duizer, E., Aidara-Kane, A., Sprong, H., Opsteegh, M., Langelaar, M., Threfall, J., Scheutz, F. and van der Giessen, J. Food-borne diseases—the challenges of 20 years ago still persist while new ones continue to emerge. *International journal of food microbiology*, *139*, pp.S3-S1 2010.

Oke, J. A., Faromika, O. P., Aluko, J. O., Oke, S. R. Human health hazard of elemental concentrations in soils of Epe : an implication of gold mining in Nigeria. *International Journal of Environmental Science and Technology*, 17(12):4879-94 2020

Pacholek, B., Sady, S., and Kupinska-Adamczyk, E. Management of Food Allergens in the Food Industry, *Journal of Agribusiness and Rural Development*, 1(47): 73-80 2018.

Padfield, P., Javed, B., Sperrin, M., and Simpaon, A. A protocol for a systematic review to identify allergenic tree nuts and the molecules responsible for their allergenic properties, *Journal of Food and Chemical Toxicology*, 106: 411-416 2017.

Radke, T., J., Brown, L., G., Hoover, R., E., Faw, B., V., Reiman, D., Wong, M., R., Nicholas, D., Barkely, J., and Ripley, D. Food Allergy Knowledge and Attidute of Resturant Managers and Staff: An EHS-Net Study, Journal of Food Protection, 79(9): 1588-1598 2016.

Savage H. J., Kaeding A. J., Matsui E. C., Wood A. R. The natural history of soy allergy. *Journal of Allergy Clinical Immunology*, 125: 683-686 2010.

Stein K. *Effective allergen management practices to reduce allergens in food*. In: Handbook of Food Allergen Detection and Control, Flanagan S. (Ed.), Woodhead Publishing, UK, pp. 103-131 2015.

Shehbaz, M. Evaluation of Current Food Safety Practices at Various Food Establishments in Lahore, *Journal of Food Processing Technology*, 7(3): 2-8 2016.

Soon, J. M., Manning, L. "May Contain" Allergen Statements: Facilitating or Frustrating Consumers? *Journal of Consumer Policy*, 40:447-472 2017.

Thomas K., Herouet-Guicheney C., Ladics G., Bannon G., Cockburn A., Crevel R., Fitzpatrick J., Mills M., Privalle L., Vieths S. *Evaluating the effect of food processing on the potential human allergenicity of novel proteins: International workshop report*. *Journal of Food and Chemical Toxicology*, 45: 1116-1122 2007.

Vesna. G., Zeljka. M.B., Milan. V., Rodaslav. G. and Brane. N. Allergens Management System in the Food Production. Journal of Hygienic Engineering and Design, 613: 76-84 2017

Biography

Prof Nita Sukdeo is currently an Associate Professor and Head of Department in the Department of Quality and Operations Management within the School of Mechanical and Industrial Engineering at the University of Johannesburg, South Africa. She obtained a Masters in Quality from the Durban University of Technology and a PhD in Engineering Management from the University of Johannesburg. She is an active researcher in the field of total quality management and operations management. Her field of expertise also include advanced manufacturing technologies, smart factory, Quality 4.0, quantitative analysis, quality management systems, quality auditing and risk assessment. She is a qualified Lead Auditor, proficient in ISO standards and certification. She is chairperson and director of the Society for Operations Management in Africa (SOMA), a professional member of the South African Society for Quality (SASQ). She is an active participant and session chair of the IEOM Women in Industry and Academia panel session Africa edition.

Dr Anup Pradhan is currently a Senior Lecturer in the Department of Quality and Operations Management, University of Johannesburg, South Africa. He received BSc in Agricultural Engineering from Bangladesh Agricultural University, Bangladesh, ME in Agricultural Engineering from Asian Institute of Technology, Thailand, and PhD in Biological and Agricultural Engineering from University of Idaho, USA. He has held posts at Institute of Engineering and Alternative Energy Promotion Centre in Nepal. His research interests include life cycle assessment, renewable energy, farm mechanization, operations management, smart factory, smart grid management, applied research and optimization, organizational productivity, knowledge management. He is NRF rated researcher in South Africa and a registered engineer with Nepal Engineering Council (NEC). He is a member of American Society of Agricultural and Biological Engineers (ASABE), Engineering Council of South Africa (ECSA), Nepal Engineer's Association (NEA), Gamma Sigma Delta, Golden Key International Honor Society.

Prof Kem Ramdass has worked as a work-study officer, industrial engineer, production/operations manager and skills development facilitator in the clothing, electronics, and textile industries between 1981 and 1999. He joined the academic profession in 1999 as a lecturer with Technikon South Africa. He later moved to UNISA'S Department of Business Management in 2006 lecturing in operations management. He is currently Associate Professor in the Department of Mechanical and Industrial Engineering based as Unisa, Florida Campus. He has a passion for quality and firmly believes that the application of quality management methodologies will highlight deficiencies and instigate the implementation of improvement strategies. He has applied continuous improvement methodologies from an industrial engineering, quality and operations management perspective. He is a process, performance and operations specialist with a driving passion for improving production, quality and competitiveness. He has authored and presented approximately 50 journal and conference papers both nationally and internationally and is a peer reviewer for numerous publications. He has achieved Fellow member status at SAIIE and is a member of PICMET and IEEE. He is registered as Pr Tech Eng at ECSA and is appointed as a member of the Code of Practice Steering at ECSA.