IMPORTANCE OF SANITATION AND ALLERGEN PREVENTIVE CONTROLS VALIDATION

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Project Report submitted for the

Virginia Polytechnic Institute and State University

Online Masters of Agricultural and Life Sciences

In

Food Safety and Biosecurity

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May 04, 2020

Keywords: Sanitation, Allergen, Cleaning, Validation

Abstract

The Preventive Controls for Human Food (PC) Rule, published in 2015, expanded on the Hazard Analysis Critical Control Point (HACCP) approach by including sanitation, allergen, and supply chain preventive controls for food manufacturing. In a Preventive Controls food safety plan, the process controls are required to be scientifically validated, but allergen and sanitation controls are not. However, food manufacturers should determine if the sanitation and allergen controls being applied in their facilities are adequate to control identified hazards. This work was conducted to determine the efficacy of cleaning practices to control bacterial contamination and soy allergen in a large bakery that manufactures cracker and wafer products. Cracker and wafer lines were subjected to the typical wet and dry-cleaning procedures used in the facility. Pre-cleaning and post-cleaning surfaces were sampled and tested using the following methods: ATP bioluminescence, microbiological sponge, Aerobic Plate Count (APC), Enterobacteriaceae Count (EC), and soy allergen qualitative and quantitative testing. Wet-cleaning and dry-cleaning methods were generally found to be effective for reducing microbiological contamination and soy allergen presence. However, certain areas of the processing equipment, such as the groove between the roller shaft and wall and the lid lip, were found to have higher APC post-cleaning than pre-cleaning. This work highlights the importance of sanitation validation studies that are specific to the equipment being utilized.

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1. Introduction

Historically, sanitation and allergen programs in food processing facilities were not developed based on a hazard analysis or risk analysis. Hazard analysis, in the context of a HACCP plan, was mainly conducted for processing steps. For many food manufacturing companies, sanitation and allergen control programs were part of the pre-requisite programs (PRPs). In 2011, with the passage of the Food Safety Modernization Act (FSMA), new sanitation and allergen control requirements guided food-manufacturing companies to be more proactive than reactive. The Preventive Controls for Human Food (PC) rule required manufacturers to "conduct a hazard analysis to identify and evaluate based on experience, illness data, scientific reports and other information, known or reasonably foreseeable hazards for each type of food manufactured, processed, packed, or held at your facility to determine whether there are any hazards requiring a preventive control" (FDA, 2019). The adoption of the preventive control rule forced food manufacturers to foresee or predict outcomes of a preventive control program failures. Planning for possible outcomes of a program failure helps food manufacturers develop robust solutions and action plans to undertake in the event of inadvertent situations that have food safety risk.

FDA considers process controls, allergen controls, sanitation controls, supply chain controls and a recall plan as critical preventive interventions to implement when necessary to prevent or control a hazard identified in the hazard analysis. The implementation of these preventive control measures requires a systematic approach defined by the FDA. To be effective, each preventive control requires strict management of its components. The preventive controls required under § 117.135 are subject to the following components: monitoring, corrective actions, and verification (FDA, 2019). Validation is the other critical

component defined by the FDA but not applied to all preventive controls. According to the FDA (2019), "you must validate that the preventive controls identified and implemented in accordance with §117.135 are adequate to control the hazard as appropriate to the nature of the preventive control and its role in the facility's food safety system". However, the FDA does not require validation for food allergen controls, sanitation controls, recall plans, or supply chain programs. This leaves the process control to be the only preventive control that requires validation among the above listed preventive controls. The FDA's position to exclude sanitation and allergen preventive controls from validation is surprising. If validation is about establishing that "preventive controls are adequate to control the hazard as appropriate to the nature of the preventive control" (FDA Reader, 2018), it is unclear why the FDA limits the preventive control validation to the process preventive controls.

This project aims to explain the importance of sanitation and allergen cleaning validation in bakeries and to determine the efficacy of wet cleaning and dry-cleaning protocols to reduce bacterial load and eliminate soy allergens from processing equipment in a bakery.

2. Background

2.1 Sanitation definition and importance

The etymology of the word sanitation is from the Latin word *sanitas*, meaning "health." Applied to the food industry, sanitation is "the creation and maintenance of hygienic and healthful conditions" (Marriott et al., 2006). By this definition, it is clear that sanitation is about the implementation of conditions or systems that will contribute to the production of safe and healthy food for consumption. By "safe," we understand the food to be free of pathogenic microorganisms, toxins, and physical hazards. By "healthy" we understand that the food provides energy and maintains the body.

Sanitation has evolved over quite some time. Originally considered as basic hygiene practices (Marriott et al., 2006), sanitation became complex due to incidents of foodborne illnesses associated with improper sanitation during food manufacturing. Sanitation today involves a systematic approach that is a science rather than an art. Marriott et al., (2006) stated, "sanitation is considered to be an applied science because of its importance to the protection of human health and its relationship with environmental factors that relate to health".

Sanitation contributes to the control of biological, chemical, physical and radiological hazards in food, processing equipment, and the environment. According to the FDA (2019), *"*the sanitation program should implement procedures and monitoring for the following: cleanliness of food-contact surfaces, including food-contact surfaces of utensils, staff wardrobe, and equipment and employees overseeing this program should possess an understanding of the allergen hazard and the principles for control of cross-contact that are required to execute the program". Sanitation applications refer to hygienic practices designed to maintain a clean and wholesome environment for food production, processing, preparation, storage, and transportation. However, sanitation is more than just a visual cleanliness or visual appearance (Marriott, 2003).

It is also clear that sanitation plays a crucial role in maintaining long product shelf life. Ideally, food plants are hygienically designed to allow proper sanitation, "nevertheless, foods can be contaminated with spoilage microorganisms or those that cause foodborne illness if proper sanitary practices are not followed" (Marriott et al., 2006). Sanitation requires sanitary conditions of the processing environment, proper equipment and personnel practices. It is clear that any sanitation failures are the result of a lack of "understanding of the principles of sanitation and the benefits that effective sanitation will provide" (Marriott et al., 2006). Among

these benefits, we can cite the improvement of product quality and shelf life, foodborne illnesses control, preventive control program establishment, environment monitoring programs, and food safety assurance.

Sanitation is the foundation of food safety assurance. In other words, sanitation is the backbone of food safety. When sanitation practices are not strong, they can lead to outbreaks, illnesses, injuries, deaths and recalls. At each step of the food chain, sanitation plays a critical role to assure ingredients and foods meet the necessary criteria to assure good health. However, even with multiple existing sanitation practices, it appears that food manufacturers are far from achieving the goal of consistently producing safe and quality food. Some challenges are still in the way of consistently keep the population safe. In that regard, with the increasing number of recalls, and food outbreaks, the FDA decided to increase the focus on sanitation for current food safety plans.

2.2 Sanitation preventive control

Sanitation is not only an applied science rather it may be used as a predictive or preventive tool that will allow farmers and food manufacturers to foresee and predict the outcomes of sanitation failures and therefore predetermine corrective actions or control measures to act upon in the event these failures occur. Sanitation is among the preventive controls developed by the FDA. The FDA defines preventive controls as "those risk-based, reasonably appropriate procedures, practices, and processes that a person knowledgeable about the safe manufacturing, processing, packing, or holding of food would employ to significantly minimize or prevent the hazards identified by the hazard analysis that is consistent with the current scientific understanding of safe food manufacturing, processing, packing, or holding at the time of the analysis"(FDA, 2019). These controls are called preventive controls for human food

(PCHF). According to the Food Safety Preventive Controls Alliance (2016), "the preventive control for human food regulation requires the implementation of sanitation preventive controls as appropriate to the facility and the food, to significantly minimize or prevent hazards such as environmental pathogens, biological hazards due to employees handling and food allergen hazards".

The implementation of the sanitation preventive controls should effectively remove physical, biological, chemical and radiological hazards from the processing equipment regardless of their complexity or design. This includes the removal of pathogenic bacteria, allergens and preventing biofilms build-ups. Marriott (2006), defined biofilms as "microcolonies of bacteria closely associated with an inert surface attached by a matrix of complex polysaccharide-like material in which other debris, including nutrients and microorganisms, may be trapped". Biofilms when established are hard to remove from processing equipment especially when they are formed in niches and crevices. For sanitation to be effective, it must involve a mechanical action, a systematic cleaning method, an appropriate cleaner or detergent and an effective sanitizer. Sanitation, after all, is a systematic process. There are many sanitation methods and the adoption of one depends on the need, affordability, and effectiveness. Heat treatment, radiation, pulsed light, and electronic pasteurization are some of the available sanitation methods.

Sanitation effectiveness depends on many other factors such as equipment sanitary design, personnel training, the quality of the detergent and sanitizers, and methods. According to Redemann (2005), "the key to good sanitation practices is to provide training to a wide base of plant employees, which may include personnel outside of the sanitation department". If the primary sanitation objective is the removal of food debris, microorganisms, and allergens,

cleaning practices may be specific to the type of soil needing removal. Allergen sanitation may require different practices than general sanitation.

2.3 Allergen sanitation

Sanitation preventive controls aim to prevent allergen cross-contact. In this regard, sanitation for allergen control involves allergen cleaning programs. According to the FDA (2019), "the main purpose of an allergen cleaning program is the removal of the allergens from areas of the processor, including processing and packaging equipment, food-contact surfaces, storage, employee wardrobe, and in the processing and packaging environment". Allergen cleaning may be slightly different from standard cleaning depending on the nature of the food processed, the industry complexity, and cleaning cycles. For example, it may be easier to perform allergen cleaning in a dairy than in a bakery. In a dairy, the majority of the cleaning is a full wet cleaning whereas in the bakery some of the equipment are dry cleaned, and this presents more challenges.

The U.S recognizes eight major allergens commonly called the "Big 8". They are milk, soybean, fish, egg, wheat, shellfish, peanut, and tree nuts. According to Bodendorfer et al., (2004) 2 to 3% of adults and 4 to 8% of infants and young children in the United States are affected by food allergies. Food allergy has become a major health concern for consumers due to an increase in reported cases of food allergy sensitization from a wide variety of foods (Sharma et al., 2017). Undeclared allergens are the number one cause of recalls in the U.S. In 2016, the U.S. Department of Agriculture (USDA) under its jurisdiction reported 34 of the 122 food recalls due to undeclared allergens (FSN, 2017).

The causes of allergen cross-contact are multiple, including improper segregation between non-allergenic and allergenic ingredients, utensils, PPE, formulation error, lack of

equipment sanitary design, incorrect labeling, improper training, and improper cleaning. In some cases, only a very small amount of allergen is necessary to cause a reaction, and that reaction will vary among individuals and the allergen type. According to Roder et al., (2010) "a lack of legal thresholds for adventitious contamination with allergens, so-called 'hidden' allergens, imposes continued challenges for consumers with food allergies".

The best method to prevent food allergies is to avoid foods containing allergens or foods exposed to allergens or foodservice situations where there is a potential risk of allergens cross-contact of meals prepared in a kitchen containing allergenic ingredients with shared utensils. Strict avoidance of the allergy-causing food is currently the only way to completely avoid allergic reactions from foods (Jackson, 2008**).** Proper food labeling can prevent consumption of foods containing an allergenic ingredient (Sharma et al., 2017). However, labeling alone is not adequate to control food allergens. In addition to labelling, other interventions, including sanitation, are required to ensure the safety of foods.

Allergen cleaning can be very challenging based on the type of allergen. However, the cleaning methods or techniques may be similar to other cleaning methods with a slight difference. Effective removal of allergens requires mechanical or physical action followed with appropriate detergent and sanitizer application. Without physical action, it is almost impossible to effectively remove allergens from processing equipment.

Facilities that handle multiple allergens should develop a strong allergen control program to prevent allergen cross-contact. Changeover on lines that produce different allergen containing products must institute an allergen changeover program. According to the FDA (2019), "an effective sanitation program includes procedures, practices, and processes to ensure a facility is maintained in a condition that significantly minimizes or prevents the hazard of allergen cross-

contact". Marriott (2003) stated, "when cleaning operations are not performed between allergen and non-allergen-containing products, a parts-per-million analysis is needed to establish the safety of products that do not list allergens on the label." A visual inspection of a line or equipment will not be sufficient to validate the allergen cleaning.

2.4 Sanitation and allergen cleaning validation

2.4.1 Validation

To understand the effectiveness of sanitation it is imperative to validate the different sanitation or cleaning methods used in food processing plants. While some perceive sanitation and allergen validations as necessary, the FDA has a different approach on sanitation and allergen controls validation. Validation of the methods, techniques, and processes will determine their effectiveness or reliability to guarantee food safety and quality. However, food agencies and industry groups define validation differently. According to the FDA (2019), *"*validation means obtaining and evaluating scientific and technical evidence that a control measure, combination of control measures, or the food safety plan as a whole, when properly implemented, is capable of effectively controlling the identified hazards". According to BRC, "validation is obtaining evidence through the provision of objective evidence that a control or measure, if properly implemented, is capable of delivering the specified outcome" (Belk, 2018). According to SQF, validation is a "system, which identifies, evaluates, and controls hazards, which are significant for food safety. Essentially validation, as applied to control limits, seeks to prove that the intended results was achieved and that it actually worked" (Belk, 2018). *Codex Alimentarius* (2008) defined validation as: "Obtaining evidence that a control measure or combination of control measures, if properly implemented, is capable of controlling the hazard to a specified outcome."

Since the sanitation goal is the assurance of the effective reduction of hazards to an acceptable and safe level, validation will be the proof that the goal is achievable. Validation also answers the question "How do you know it works?" (FDA Reader, 2018). In other words, validation is a confirmation approach that proves that the method, techniques or processes adopted in cleaning is working or is capable of delivering the intended outcome. According to Jackson, et al., (2008) "protocols to validate allergen cleaning efficacy provide the food manufacturer with feedback as to the effectiveness of the cleaning protocol and, very importantly, pinpoint areas of insufficient cleaning".

The FDA Reader (2018) explained the FDA's position on sanitation validation in these words: "sanitation activities, for example, do not need to be validated because most people use a small set of scientifically proven processes (i.e. soap and water, common chemical sanitizers). As a result, there is no need to require each business to prove their sanitation practices work". However, there are numerous instances of failed sanitation regardless of the existence of scientifically proven processes. Factors that contribute to sanitation failure are the following the type of cleaning (wet versus dry), product formulation, the equipment makeup (material), people, methods, chemicals, repeatability, and reliability. Historically, the FDA concept of validation was much more about pharmaceutical industries than food industries. "FDA was more concerned about the contamination of nonpenicillin drug products with penicillin or the cross-contamination of drug products with potent steroids or hormones" (FDA, 2019). The complexity and cost implications of the validation could also be a reason why the FDA did not require the cleaning validation for food industry. It is clear that "FDA does not intend to set acceptance specifications or methods for determining whether a cleaning process is validated" (FDA, 2019). Doing so could legally bind the FDA to any potential cleaning procedure failures. It appears that

the FDA may suggest but leave it to the food industries to establish adequate cleaning procedures.

2.4.2 Sanitation validation

Sanitation validation consists of proving that the cleaning is capable of removing microbiological load to an acceptable limit. Validation consists of directly testing the cleaned surface. There are various methods used for validation. The most common methods are visual inspection of equipment surfaces, the adenosine triphosphate (ATP) bioluminescence test, and microbiological sponge swab test. ATP bioluminescence test is to detect the presence of ATP from biological materials on equipment or cleaned surfaces. Protein detection tests may be used with ATP-type luminometers to detect proteins, some of which may be allergens. This protein test is not a selective test method for allergen testing. Generally, ATP bioluminescence systems remain primarily a hygiene indicator method or hygiene monitoring technique. Testing for indicator organisms allows validation of sanitation effectiveness. Aerobic Plate Count (APC) and Enterobacteriaceae (EB) microbiological plating tests are the commonly used for sanitation effectiveness validation. The presence of these organisms is a sign of lack of hygiene due to improper cleaning and sanitizing.

2.4.3 Allergen cleaning validation

Food industries use ATP-type tests along with ELISA-based (allergen-specific) tests to verify and validate cleaning program effectiveness (Al-Taher, Jackson, & Salter, 2007). Allergen tests are specific to the allergen tested. The validation of a cleaned surface requires the appropriate allergen test. There are kits used to perform a qualitative test, and advanced methods to perform a quantitative testing. According to Jackson et al., (2008) “cleaning validation refers

to the process of assuring that a defined cleaning procedure is able to effectively and reproducibly remove the allergenic food from the specific food processing line or equipment".

3. Material and Methods

3.1 Experimentation

Studies to determine the effectiveness (validation) of cleaning and sanitation practices to control microorganisms and soy allergens were conducted in a bakery located in Virginia, company that produces crackers and wafers. The experiment consisted of performing wet and dry cleaning in the bakery. In the pre-lethality (or pre-oven) area, wet and dry-cleaning methods where used; whereas in the post-lethality (post-oven) area only dry cleaning was performed. During this experiment, cleaning at each step was evaluated by performing ATP, APC, EB and soy allergen tests to monitor, progressively, the effectiveness of the applied methods. All samples were collected before sanitizer application. For this study, Aerobic Plate Count (APC) and Enterobacteriaceae (EB) microbiological plating tests to were used to assess indicators to validate the microbiological cleanliness of each piece equipment during the study.

Five, four and two samples, respectively, were collected for each mixer, machining, and packing table. The samples were collected once for each line and at different cleaning days. The cleaning validation was conducted on three production lines. Line 0, line 1, and line 3. Line 0 is a cracker production line, and lines 1 and line 3 are for various wafers production. Line 0 goes through different allergens changeover and was the only line for allergen testing in this study.

Even though other allergens may be present on that line, soy is the allergen of interest in this study. Each line is comprised of three sections: mixing, machining, and packaging. In the mixer, dry ingredients are mixed with water to produce a dough. At the machining steps (sheeting), the dough is cut into raw cracker or wafer pieces. The raw product travels through an

oven for a specific time depending on the product being baked. After cooling at ambient temperature, the crackers or wafers are packed.

3.2 Processing Equipment

Equipment used includes, mixers, machining (sheeting), and packing belting systems. The study involved three different horizontal mixers, three machining (sheeting) systems and three packing belting systems. Mixer surfaces were stainless steel (304/305). The mixers are composed of a horizontal blade that rotates around a shaft during mixing. Sheeters are a series of metallic gauge rollers and belts. The gauge rollers are stainless steel and belts are laminated. Sheeters are used to reduce dough thickness to obtain a desirable finished product height. The packaging belting systems are a series of laminate belts and stainless steel rollers used to cool down product from the oven. Photographs of the processing equipment and sampling sites are in the Appendix.

3.2.1 Cleaning Compounds and Tools

The cleaning compounds used for this study were Chlorinated Plus (Degreaser; Spartan Chemical Company, Inc.; Maumee, OH) used at a concentration of 3.9 oz./gal, and Sani T-10 (quaternary ammonium sanitizer; Spartan Chemical Company, Inc.; Maumee, OH) used at a concentration of 290 ppm. Cleaning and sanitation compound formulation, as provided by the manufacturer, are shown in Table 1. The water used to make the final concentrations of cleaning and sanitation solutions in the manufacturing facility was sourced from the municipal water of the city located in Virginia where the study was conducted.

Table 1. Formulation of Cleaning and Sanitation Solutions used in this Study*.

Product Name	Component	Percent Weight
Chlorinated Plus	Water	60-100
	Sodium hydroxide	5-10
	Potassium hydroxide	1-5
	Sodium hypochlorite	1-5
	Polycarboxylate, sodium salt	1-5
	Sodium2-ethylhexyl sulfate	1-5
Sani T-10	Water	60-100
	Dialkyl dimethyl ammonium chloride	3-7
	Alkyl dimethyl benzyl ammonium chloride	1-5
	Ethanol	1-5

*Adapted from product package SDS information (Spartan Chemical Company, Inc.; Maumee, OH)

Table 2. Cleaning and Sanitation Tools Used

		
96N Medium Duty Scouring Green Pads (3M; Saint Paul, MN)	White Towel (WY Pall; Kimberly Clark Global; Irving, TX)	Brushes (White; Remco Vikan Products; Zionsville, IN)

Photograph credits: N'Lemahoule Nantob-Bikatui, 2020.

The following chemicals titration kits were used in this study: Chlorinated Plus Kit (8226-01; LaMotte Company; Chestertown, MD) and High Range QAC Kit (3042-01; LaMotte Company; Chestertown, MD)

3.2.2 Surface Testing Supplies

Table 3. Surface Testing Supplies

 Sponges swabs (REF-SSL10NB2G; 3M; Saint Paul, MN)	 Flashlight- E35UE (Fenix; Broken Arrow, OK)	 ATP meter- V.2 Ensure (Hygiena; Camarillo, CA)
 Allergen Kit (902093K Reveal 3-D Soy Test; Neogen; Lansing, MI)		

Photograph credits: N'Lemahoule Nantob-Bikatui, 2020.

Neogen Veratox for Soy Allergen Quantitative Test SOP-SOY -0416- FARRP (Neogen; Lansing, MI; Not Pictured)

3.2.3 Testing Laboratories

Microbiological and allergen samples in this study were tested by the following companies: BioMerieux (Madison, WI) performed the microbiological testing (i.e., APC and EB) from samples taken in the bakery. BioMerieux used the APC Method (AOAC 966.23) and the EB Method (AOAC 2003.01). The Food Allergy Research and Resource Program (FARRP) Analytical Laboratory (Lincoln, NE) tested bakery samples for soy allergen using the Neogen Veratox for Soy Allergen Quantitative Test (SOP-SOY 416; Neogen, Lansing MI). The Internal Laboratory (VA) performed ATP and allergen (Soy Qualitative Test; 3D Reveal; Neogen, Lansing, MI).

3.3 Methods

The study was conducted on mixers, sheeters located in a pre-lethality area (pre-oven), and on packing belts located in a post- lethality area (post oven). Five sites were selected on each mixer, four on each machining line (sheeting) and two on each packing line. Areas that are difficult to clean (e.g., groove, backside of the blade, knife) or areas easily neglected during cleaning (lid lip) were targeted. Each sample site was swabbed for APC, EB, and ATP tests before and after cleaning. ATP test pass value was set at $\leq$ 100 RLU. Accredited laboratories, as indicated above, performed microbiological and allergen tests of sponge swabs for APC, EB and soy allergens. APC value $\leq$ 1000 cfu and EB value $\leq$ 10 cfu were considered to be acceptable. The Almond Board of California recommended the limits are shown in Table 4. Bakery equipment was not tested for pathogens. Also, samples were collected before sanitizer application. Soy tests were considered acceptable when no soy allergen was detected.

Table 4: **Recommended microbiological indicator limits for equipment cleaning before and after application of sanitizer provided by Almond Board of California (**Almond Board of California, 2020).

Quantitative Microbiological Indicator Test	Target/ Acceptable	Post-Heat Treatment Taken Before Sanitizer (cfu/40 in^2)	Post-Heat Treatment – Pre-op Taken After Sanitizer (cfu/40 in^2)
Aerobic Plate Count	Target	< 100	<10
	Acceptable	< 500	<100
Coliforms	Target	<10	<10
	Acceptable	<100	<50
Total Enterobacteriaceae	Target	<10	<10
	Acceptable	<100	<50

3.3.1 Dry cleaning Standard Operation Procedure (SOP)

The dry-cleaning procedure for a pre-lethality and the post- lethality was conducted as follows:

Step1: Equipment Assessment.

Perform the Sanitation Hazard Analysis Work Point (SHAWP) (Carsberg, 2003) to identify the difficult areas to clean and potential points for bacteria growth. Identifying niches, for example, is key to avoid bacterial growth and to request dismantlement of the equipment.

Step 2: Disassemble, dry clean, sweep, and vacuum.

Disassemble or loosen parts of the equipment for cleaning. Scrape the dough thoroughly off the equipment, and belts; vacuum dust or debris and sweep the floor as well.

Step 3: Detergents/cleaners application.

Apply detergent or cleaner on the equipment. Spray the detergent to cover the entire surface to clean.

Step 4: Activation period

Allow 3 to 5 min for the detergent to react and break down soils. Do not allow chemical to dry. This can leave smears on the metallic equipment.

Step 5: Cleaning

Scrub, the equipment. Reach difficult areas to clean.

Step 6: Wet wipe down / Chemical removal

Use a wet towel or cloth (in clean water) to wipe down the equipment to remove the detergent. This step is not a hose down step and is not a wet cleaning.

Step 7: Self-inspection

Inspect the equipment and its surroundings using a flashlight. Clean the work environment as well and arrange tools before the final QA inspection.

Step 8: Formal QA inspection and verification

QA or trained employee will visually inspect the entire equipment to ensure it is visually clean and ready for production. At this step, we collect all post cleaning samples.

Step 9: Sanitizing and Assembly

Sanitize and assemble the equipment. Use clean gloves to handle the equipment parts.

3.3.2 Wet Cleaning Standard Operation Procedure (SOP)

The wet cleaning procedure was conducted as follows:

Step 1: Equipment Assessment.

Perform the Sanitation Hazard Analysis Work Point (SHAWP) to identify the difficult areas to clean and potential points or niches for bacteria growth. Identifying niches, for example, is key to avoid bacterial growth and to request dismantlement of the equipment.

Step 2: Disassemble, sweep, and vacuum.

Disassemble or loosen parts of the equipment for cleaning. Scrape the dough thoroughly off the equipment, and belts; vacuum dust or debris and sweep the floor as well.

Step 3: Pre-rinse

Rinse the equipment *top down* to remove excess gross soil.

Step 4: Detergents/cleaners application.

Apply detergent or cleaner on the equipment. Spray the detergent to cover the entire surface to clean.

Step 5: Activation period

Allow 3 to 5 min for the detergent to react and break down soils.

Do not allow chemical to dry. This can leave smears on the metallic equipment.

Step 6: Cleaning

Scrub the equipment. Reach difficult areas to clean.

Step 7: Rinse / Chemical removal

Rinse the equipment *top down* with clean fresh water to remove the detergent.

Step 8: Self-inspection

Inspect the equipment and its surroundings using a flashlight. Clean the work environment as well and arrange tools before the final QA inspection.

Step 9: Formal QA inspection and verification

QA or trained employee will visually inspect the entire equipment to ensure it is visually clean and ready for production. At this step, we collect all post cleaning samples.

Step 10: Sanitizing and Assembly

Sanitize and assemble the equipment. Use clean gloves to handle the equipment parts.

4. Results

For all three mixers, there was a reduction in ATP values after cleaning. The ATP values were extremely high for line 0 mixer prior to cleaning. However, they were low after cleaning (Table 5). Mixers are the dirtiest piece of equipment during cracker and wafer manufacturing due to the continuous mixing. Low ATP results is an indication of an effective protein residue removal.

The APC swabs results were high for all mixers before cleaning and lower after cleaning. However, the lid lip results were high after cleaning for all three mixers. The groove in the mixer of line 0 result is higher after cleaning as well. For the right sidewall of mixers 1 and 3, the APC results were high after cleaning. The EB results in general were lower than APC results before cleaning for all mixers. The allergen test revealed the presence of soy after processing and before cleaning. FARRP analytical laboratory results confirmed our in- house test results. The results in tables 5, 6, and 7show the soy cleaning effectiveness.

In machining, all ATP results were low prior to cleaning for all lines with the exception of line 3 (Table 10) where the result is higher but not failing for the gauge roller body after cleaning. The machining ATP results were not high before cleaning for lines 1 and 3. However, the ATP was high but not failing for the cracker line 0 (Table 8). APC results were low after cleaning and failing except for the second gauge roller knife (Table 8). The EB results were low

after cleaning (Table 8). The in house allergen tests confirm the absence of soy protein after cleaning. FARRP analytical laboratory validation confirmed the same result as well.

In packaging, for all lines, the ATP results were low prior to cleaning. The results were lower after cleaning. APC and EB results were low before cleaning and lower after cleaning with the exception of the roller in packaging line 1 (Table 12) where the APC was 2600 cfu after cleaning. This may be explained by contamination during cleaning by the operator. The allergen test for line 0 was negative after cleaning. However, the roller did not appear to have soy proteins prior to cleaning (Table 11).

4.1 Pre-Lethality: Wet Cleaning Results

Table 5: Mixer Line 0 ATP, APC, EB, and Allergen Swab Results

Mixer LINE 0	ATP Before Cleaning (RLU)	ATP After Cleaning (RLU)	APC Before Cleaning (CFU)	APC After Cleaning (CFU)	EB Before Cleaning (CFU)	EB After Cleaning (CFU)	Soy Allergen testing before cleaning	Soy Allergen testing after cleaning	Soy Allergen testing before cleaning FARRP	Soy Allergen testing after cleaning FARRP*
Right Side wall	9940	10	>25000	180	110	<10	Fail	Pass	NA	NA
Groove Between Shaft and wall	116	2	18000	41000	60	410	Fail	Pass	Positive	BLQ
Shaft body	7147	3	300000 est.	380	80	<10	Fail	Pass	Positive	BLQ
Back of the left Blade	72	2	>25000	210	90	<10	NA	NA	Na	NA
Lid Lip	9983	64	>25000	26000	120	<10	NA	NA	NA	NA

*BLQ: Below lower Limit of Quantification

Table 6: Mixer Line 1 ATP, APC, and EB Swab Results

Mixer LINE 1	ATP Before Cleaning (RLU)	ATP After Cleaning (RLU)	APC Before Cleaning (CFU)	APC After Cleaning (CFU)	EB Before Cleaning (CFU)	EB After Cleaning (CFU)
Right Side wall	209	1	440	1200	< 10	<10
Groove Between Shaft and wall	155	1	510	320	< 10	<10
Shaft body	440	12	410	170	120	< 10
Back of the left Blade	690	1	410	< 10	< 10	< 10
Lid Lip	336	12	5500	40000	< 10	60

Table 7: Mixer Line 3 ATP, APC, and EB Swab Results

Mixer LINE 3	ATP Before Cleaning (RLU)	ATP After Cleaning (RLU)	APC Before Cleaning (CFU)	APC After Cleaning (CFU)	EB Before Cleaning (CFU)	EB After Cleaning (CFU)
Right Side wall	2	0	230	2700	<10	<10
Groove Between Shaft and wall	0	0	50	<10	10	<10
Shaft body	1	0	3000	90	300	<10
Back of the left Blade	1	0	3800	<10	20	<10
Lid Lip	68	0	3300	78000	110	260

4.2 Pre-lethality - Dry cleaning Results

Table 8. Machining Line 0 ATP, APC, EB, and Allergen Swab Results

Machining Line 0	ATP Before Cleaning (RLU)	ATP After Cleaning (RLU)	APC Before Cleaning (CFU)	APC After Cleaning (CFU)	EB Before Cleaning (CFU)	EB After Cleaning (CFU)	Soy Allergen testing before cleaning	Soy Allergen testing after cleaning	Soy Allergen testing before cleaning FARRP	Soy Allergen testing after cleaning FARRP*
1st Gauge roller body	44	32	>250000	3300	10	<10	Fail	Pass	Positive	BLQ
2nd Gauge roller Side	17	21	27000	3000	10	<10	NA	NA	NA	NA
Belt after 1st gauge Roller	39	9	3500	5900	<10	<10	NA	NA	NA	NA
2nd Gauge Roller Knife	51	5	3300	<10	<10	<10	Fail	Pass	Positive	BLQ

*BLQ: Below lower Limit of Quantification

Table 9. Machining Line 1 ATP, APC, and EB Swab Results

Machining LINE 1	ATP Before Cleaning (RLU)	ATP After Cleaning (RLU)	APC Before Cleaning (CFU)	APC After Cleaning (CFU)	EB Before Cleaning (CFU)	EB After Cleaning (CFU)
1st Gauge roller body	11	0	40	<10	<10	<10
2nd Gauge roller Side	0	0	<10	< 10	<10	< 10
Belt after 1st gauge Roller	7	0	160	<10	<10	<10
Gauge Roller Knife	2	0	860	30	< 10	<10

Table 10. Machining Line 3 ATP, APC, and EB Swab Results

Machining LINE 3	ATP Before Cleaning (RLU)	ATP After Cleaning (RLU)	APC Before Cleaning (CFU)	APC After Cleaning (CFU)	EB Before Cleaning (CFU)	EB After Cleaning (CFU)
1st Gauge roller body	7	17	190	20	<10	<10
2nd Gauge roller Side	6	2	70	< 10	<10	< 10
Belt after 1st gauge Roller	13	1	180	< 10	<10	< 10
2nd Gauge Roller Knife	4	2	100	< 10	<10	< 10

4.3 Post-Lethality - Dry Cleaning Results

Table 11. Packing Line 0 ATP, APC, EB, and Allergen Swab Results

Packaging LINE 0	ATP Before Cleaning (RLU)	ATP After Cleaning (RLU)	APC Before Cleaning (CFU)	APC After Cleaning (CFU)	EB Before Cleaning (CFU)	EB After Cleaning (CFU)	Soy Allergen testing before cleaning	Soy Allergen testing after cleaning	Soy Allergen testing before cleaning FARRP*	Soy Allergen testing after cleaning FARRP*
Belt laminated	22	5	50	<10	< 10	<10	Fail	Pass	Positive	BLQ
Roller	16	5	< 10	<10	< 10	<10	Pass	Pass	BLQ	BLQ

*BLQ: Below lower Limit of Quantification

Table 12. Packing Line 1 ATP, APC, and EB Swab Results

Packaging LINE1	ATP Before Cleaning (RLU)	ATP After Cleaning (RLU)	APC Before Cleaning (CFU)	APC After Cleaning (CFU)	EB Before Cleaning (CFU)	EB After Cleaning (CFU)
Belt laminated	0	0	<10	<10	<10	<10
Roller	5	0	<10	2600	<10	<10

Table 13. Packing Line 3 ATP, APC, and EB Swab Results

Packaging LINE 3	ATP Before Cleaning (RLU)	ATP After Cleaning (RLU)	APC Before Cleaning (CFU)	APC After Cleaning (CFU)	EB Before Cleaning (CFU)	EB After Cleaning (CFU)
Belt laminated	0	0	<10	< 10	<10	< 10
Roller	0	0	30	< 10	<10	< 10

5.0 Discussion

Proper equipment assessment for cleaning and sanitation requires the identification of the material type used to make the equipment for proper chemical selection. The material type and the age of the equipment will provide a clear direction for choices concerning cleaning effectiveness. The development of the sanitation analysis and critical sanitation point should take place during cleaning SOP (standard operation procedure) development.

Disassembling the equipment as much as possible or loosening parts allows proper cleaning of hidden areas. At this step, minimum air hose pressure will help remove (dislodge) dough debris from areas of the equipment where human hands or a vacuum cannot reach. The usage of the air hose needs control to protect adjacent lines from contamination.

Rinsing down the equipment should be done with sufficient clean water and top down to avoid contamination from the floor. The detergent application should be sufficient to break down soil of the cleaning surfaces. The detergent should remain on the equipment for a period of time, as recommended by the manufacturer, to be effective. The operator should avoid allowing the detergent to dry on the equipment. This could leave streaks or smears on the equipment.

During cleaning, the equipment must be brushed (mechanical action) to remove dirt, bacteria, and allergens. Each surface must be covered to avoid failure. An effective scrubbing will guarantee cleaning effectiveness. After cleaning, it is imperative to remove the detergent. Therefore, the equipment should be rinsed top down for wet cleaning. For dry cleaning, a wet or soaked towel can be used to remove the detergent.

Each cleaned piece of equipment must be visually inspected by the operator performing the cleaning. This step shows the diligence of the operator in assuring the cleanliness of the

equipment. The final step of cleaning is a formal QA inspection. The line inspection should be performed by a trained employee, and it is preferred that the inspection not be done by individuals of the same department to avoid conflict of interest.

There are different levels of cleaning which require different validation methods. A visually clean equipment validated by a visual inspection and ATP test, a microbiologically clean equipment validated by a microbiological test, and an allergen clean equipment validated by qualitative and quantitative allergen tests. For a visually clean equipment, a visual inspection is the simple equipment cleaning verification method. Visual inspection of clean equipment verifies the absence of visible food debris on the equipment. According to a study conducted by the FDA (2006), 95% of factories use visual inspection as common method of sanitation verification.

For most food manufacturers the use of the ATP method is sufficient to verify sanitation effectiveness of the equipment. The primary objective of the ATP test is to reveal the absence of organic materials which may serve as a substrate for bacterial growth. However, the ATP tests detect microbial ATP as well as ATP associated with residual foods. Therefore, ATP testing is only effective to verify the effectiveness of wet cleaning procedures (Al-Taher et al. 2007). It is better to perform ATP after a wet cleaning and on a surface free of any chemical residue. This will prevent false results. The visual inspection and the ATP test could be a valuable tool in detecting the presence of allergenic food on food contact surface (Al-Taher et al. 2007). Our study suggests that visual inspection and the ATP test, alone, were not sufficient to validate sanitation on the baking lines tested. Line 0 mixer results after cleaning show that despite the clean appearance of the mixer, some of its sites passed the ATP test and failed the microbiological test.

Microbiological testing is the best method to validate the absence of bacteria on a clean food contact surface. Microbiological cleaning validation requires a sponge swab test to determine bacteria presence. Bacterial cells attached to surfaces in biofilms may be capable of withstanding chemical sanitizers because of the development of extracellular polymers that act as a protective shield against such agents (Gabriel et al., 2017). The bacteria test may consist in pathogen test or bacterial indicators test. Each food facility must determine the appropriate test of their choice based on the level of confidence in product safety.

Allergen cleaning requires allergen testing to validate the removal of allergens from the food contact surfaces. Enzyme-linked immunosorbent assay (ELISA) tests are among the most common tests used for allergen cleaning validation. ELISA tests may be qualitative or quantitative. Qualitative results provide a simple positive or negative result for a sample (Asensio et al., 2008), whereas quantitative results provide numerical values associated when results are above the detectable limit.

Many factors influence the cleaning effectiveness. The identification of difficult areas to clean or areas that are easily neglected is usually associated with the sanitary design of the equipment. The lid lip and the groove on the mixers are difficult areas to clean and present niches or "sandwich area" between the Teflon piece and the stainless steel part of the mixer. These areas fail microbiological test after cleaning simply because during cleaning water will pull contamination out from the groove and the sandwich areas (niches) bringing back accumulated microorganisms that grew behind these areas over time. The Sanitation Hazard Analysis Work Point (SHAWP) should be developed by a food facility. Equipment inspection should identify interior niches that cause contamination since old and new equipment contain hidden areas that harbor microorganisms (Marriott et al., 2006).

During the equipment sanitary design phase, engineers should avoid designing niche areas. The material used to manufacture the equipment plays a role in sanitation effectiveness as well as the age of the equipment. These elements need consideration during cleaning validation. Most equipment used in this study were stainless steel 304/ 305 alloys (SS 304/305). Over time, the stainless steel material develops micro crevices or surface irregularities, often called pits, which are difficult to clean and may become a location of biofilm formation. As the biofilm settles in these pits, they will develop resistance to cleaners. According to Wang et al., (2009), surface topology influences the bacterial attachment to and removal from a surface. "Surface irregularities of polymeric materials or stainless steel have been reported to promote bacterial adhesion and biofilm deposition; whereas ultra-smooth surfaces seemed to reduce the possibility for microbial attachment" (Wang et al., 2009). According to (Mousavi et al., 2009) "the relation between roughness and bacterial adhesion is not always linear and other factors, i.e. the degree of roughness studied, the bacterial strains tested, physicochemical characteristics of the surfaces and the method for detecting bacteria should also be considered".

Operators involved in the cleaning process can greatly influence validation results when they do not properly follow the cleaning SOP. "It is especially important to train sanitation employees in the basics of sanitation because nothing happens in a food establishment until the facility is clean" (Marriott et al., 2006). For rotating equipment, such as belts and rollers, the cleaning operators must carefully ensure the cleaning of each square inch of the entire surface area to avoid validation failure. It is imperative that only well-trained operators should be responsible for cleaning. Additionally, the quality of cleaning compounds is critical during cleaning validation. Chemical concentration validation is necessary to assure the removal of dirt and bacteria on processing equipment.

This study shows that the cleaning methods and cleaning chemicals chosen for use in a bakery environment reduced microbiological contamination and soy allergens significantly. However, the failure to clean some sites is due to the lack of initial sanitation hazards analysis to establish critical sanitation points. This lack of initial work contradicts the FDA approach for not validating crucial sanitation preventive controls. Relying on scientifically proven processes is not sufficient to justify the effectiveness of any cleaning method unless those studies replicate the conditions and equipment design in the particular facility. The purpose of sanitation goes beyond visual inspection, and testing. It should start with a sanitation risk-based hazard analysis. In this regard, we recommend the development of a Sanitation Analysis and Critical Sanitation Point (SACSP) program. SACSP relies on a systematic approach to identify difficult areas to clean and focuses on areas of high risk for hazard presence or development.

6.0 Conclusion

Sanitation failure is a *sine qua non* condition for bacteria proliferation on food processing equipment and in work areas. The validation of sanitation is necessary to guide food processors on the choice of cleaning methods and cleaning compounds. Frequent verification of sanitation effectiveness is reassurance that food establishments are on the right path to assure the production of safe food. The kill step validation alone is insufficient to conclude that the food will be safe after packing. Post-baking or post-lethality contamination could jeopardize the effectiveness of a preventive control. For a preventive control to predict and establish preventive measure for future risks, its validation is necessary to reassure that it will work when these risks emerge.

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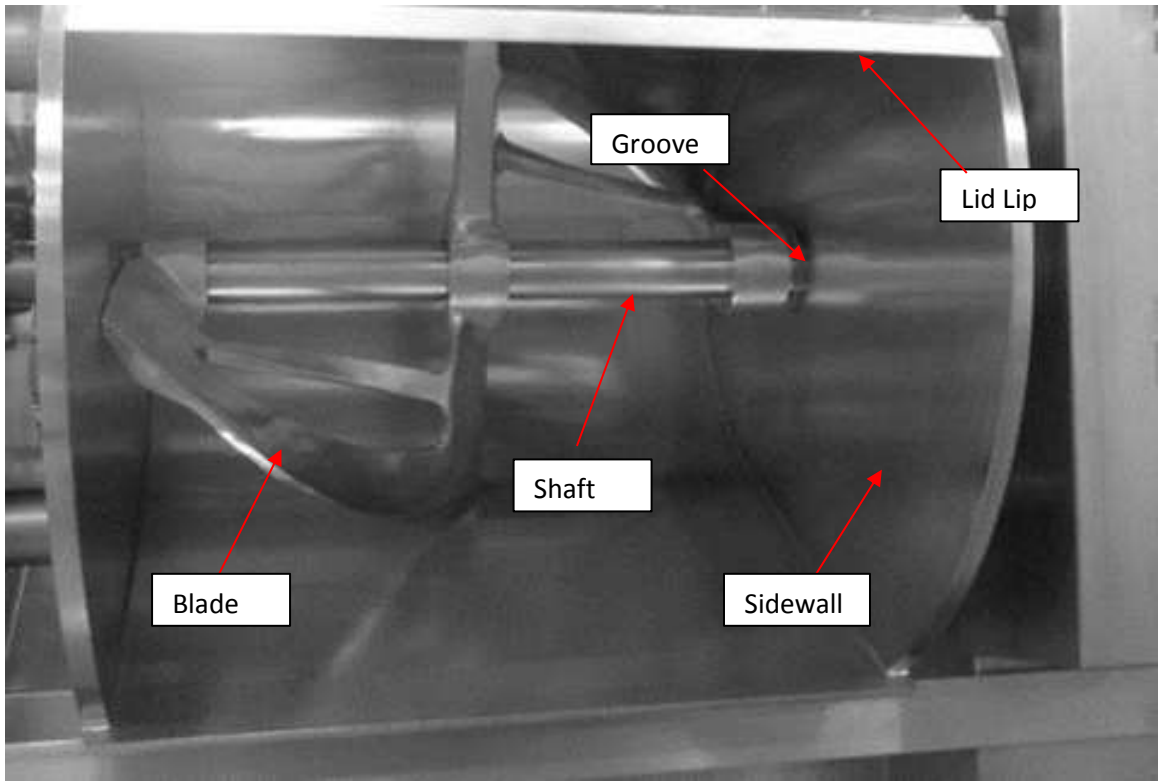
APPENDIX *(Photographs credit: N'lemahoule Nantob-Bikatui 2020)*

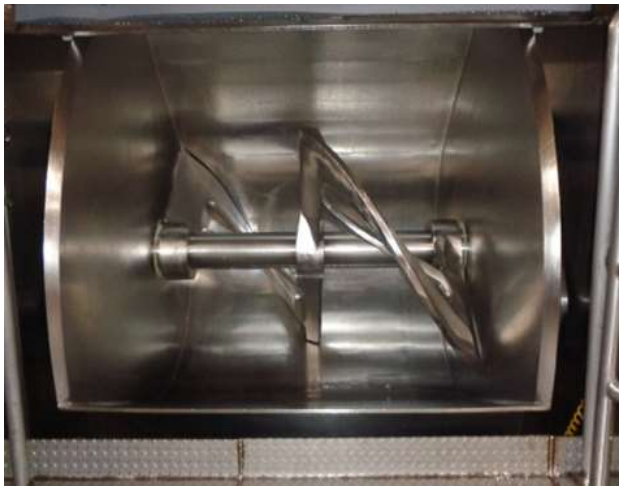


MIXERS

Pic.1 Mixer 0 Before Cleaning

Pic.2 Mixer 0 After Cleaning

Pic.3 Mixer 3 Before Cleaning

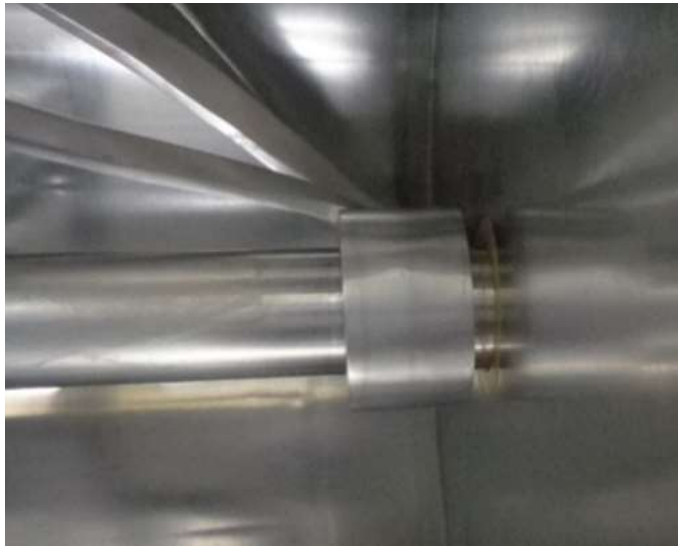


Pic.4 Mixer 3 After Cleaning

Pic.5 Mixer Blade before Cleaning

Pic.6 Mixer Blade After Cleaning

Pic.7 Shaft & Groove Before Cleaning



Pic.8 Shaft & Groove After Cleaning

Pic.9 Mixer Lid Lip Before Cleaning

Pic.10 Mixer Lid Lip After Cleaning

Pic.11 Mixer Lid Lip Sponge swab

Pic.12 Mixer Shaft Sponge swab

Pic.13Mixer sidewall ATP swab

SHEETER

Pic.14 Gauge Roller

Pic.15 Gauge roller belt

Pic.16 Gauge roller knife

PACKAGING

Pic.17 Belt Roller