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**Case Report**

# Environmental Monitoring Program in Broccoli (*Brassica oleracea var. Italica*) Packing Plant Guanajuato, Mexico

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## Abstract

The state of Guanajuato in Mexico is known for its large production of vegetables not only for the local and national market, but also for the international market, especially the North American market, to which it exports mainly species such as broccoli (*Brassica oleracea var. Italica*), among other products. In this sense, one of the organizations located in the municipality of Dolores Hidalgo, C. I. N., in the state of Guanajuato, Mexico, has a safety management system in accordance with the PrimusGFS system guidelines, which is recognized by the Global Food Safety Initiative (GFSI) and is therefore recognized worldwide (GFSI, 2022). Within this food safety scheme, a microbiological environmental monitoring program is required as a preventive control to avoid contamination of pathogenic microorganisms to the product from surfaces in direct contact as well as those that are not in direct contact but are part of the production environment. In environmental microbiological assessment, the zone concept is widely used and represents the basis for assessments accepted by international public health and food microbiology bodies such as the International Commission on Microbiological Specifications for Foods, ICMSF.

***Keywords:** Broccoli; microbiological monitoring; risk assessment; pathogens.*

## Introduction

The safety management systems recognized by the Global Food Safety Initiative (GFSI) include among their essential requirements that the organization has a documented and implemented control program for microbiological monitoring of the process environment. It has already been demonstrated that this is an essential tool for strengthening and maintaining the safety of the food manufactured and that the safety system is not only verified but also validated and contamination of the product is prevented, in addition to pinpointing the sites where the presence of microorganisms is potential (SQF, 2018). Any evidence of the presence of pathogenic microorganisms in finished products or process sites are considered as Critical Non-Conformities, which can cost the certification of the company and put the health of consumers at risk, so even the health authorities must be notified to alert the population, initiate the process of investigation and recall of the affected batches.

The PrimusGFS safety system guideline is no exception to the requirement to have a microbiological environmental monitoring plan in place for those companies whose operations include food manufacturing and processing, especially those destined for direct consumption or RTE (Ready to Eat), This is not the case for those organizations that focus solely on primary agricultural activities where there is no processing environment as such and the products are marketed for further treatment or sale with the consumer informed that the product must undergo special treatment during preparation before being consumed. (PrimusGFS, 2022)

According to (Tompkin, 2004) the process environment is the environment in which food is prepared and handled which can have a significant impact on the safety and microbiological quality of the products. The most prevalent incidents of contamination from the process environment to the products are associated with Listeria monocytogenes and Salmonella spp. and result from the lack of effective application of good hygienic practices. The most important application of investigating the presence of pathogens and high levels of hygiene indicators in the environment is the prevention of cross-contamination from the environment to the product, as well as the pinpointing of high-risk areas for action to be taken to eradicate the problem.

Vegetables are among the basic products consumed by people; however, both unprocessed and minimally processed vegetables are a potential source of disease-causing pathogenic microorganisms, as evidenced by the cases of listeriosis associated with broccoli consumption in countries such as Australia, the United Kingdom and Italy. Listeria monocytogenes is a gram negative bacterium that is capable of surviving process conditions and survive for weeks even at freezing temperatures of -18°C, is a pathogen of environmental interest due to the survival and prevalence of development in the inert surfaces of the process environment (Pappelbaum, 2009) hence the constant monitoring on the surfaces of the various hygienic areas contemplates the defined monitoring of this microorganism, This sampling must be adequate from the sampling including a sufficient amount to detect its presence, in essence it is about recovering the most representative amount of sample to locate the presence of the pathogen and prevent it from being transferred to the surfaces in direct contact with the product or to the product itself.

## Method

In environmental microbiological assessment the zone concept is widely used and represents the basis for the assessments accepted by international public health and food microbiology bodies such as the International Commission on Microbiological Specifications for Foods (ICMSF). According to the recommendations and guidelines, the zone concept is divided into four zones based on the degree of risk of contamination to the product, so that zone 1 includes surfaces in direct contact with the product, zone 2 is made up of processing equipment zones and other areas close to the products, zone 3 includes surfaces more distant from the product, but which are within the same processing area and finally zone 4 includes those areas outside the processing area such as cafeterias, lockers, among others.

According to the guidelines of the safety management system implemented in the organization located in Guanajuato, México the microbiological monitoring program of the process environment must comply with a series of essential elements to ensure the absence or timely detection of pathogenic microorganisms or high load of hygiene indicators.

For the design of the sampling points with a zonal approach, it was necessary to know the process flow of the packaging operation, from the reception of raw materials to the output of the finished product. Each of the surfaces in direct contact or that form part of the evaluation to be classified within the classification of hygienic zones according to the criteria established by the ICMSF.

The broccoli packing process begins with the reception of the product taken directly from the field and transferred to white baskets. Once inside the industrial plant, it is temporarily positioned in the input reception area where, once there is enough product, the loading process to the processing line begins. Once on the conveyor belts, the product is selected according to internal quality parameters and placed in waxed boxes that will later be immersed in tubs of ice water with added chlorine concentrations to reduce the microbiological load and increase the shelf life of the product in the market.

At the end of the immersion in ice water, the product goes to the cooling stage. To accelerate the temperature reach, the product is introduced into a tunnel where, by means of forced air, large volumes of cold air are introduced at high pressure to quickly and uniformly extract the heat contained in the product. Once the cooling temperature is reached, the product is transferred to the finished product loading docks for shipment to customers or distributors.

Once the operations involved in the broccoli packaging process had been identified, all the direct contact surfaces were identified, as well as all those that form part of the process environment. Each surface involved in the production process is subjected to a microbiological contamination risk analysis towards the product that takes as a reference the potential development and transfer of pathogenic microorganisms (*Salmonella spp, Listeria monocytogenes*) as well as hygiene indicators (aerobic mesophiles, total coliforms) towards the product, evaluating the probability and severity of cross-contamination towards the product by means of a risk analysis matrix as shown below:

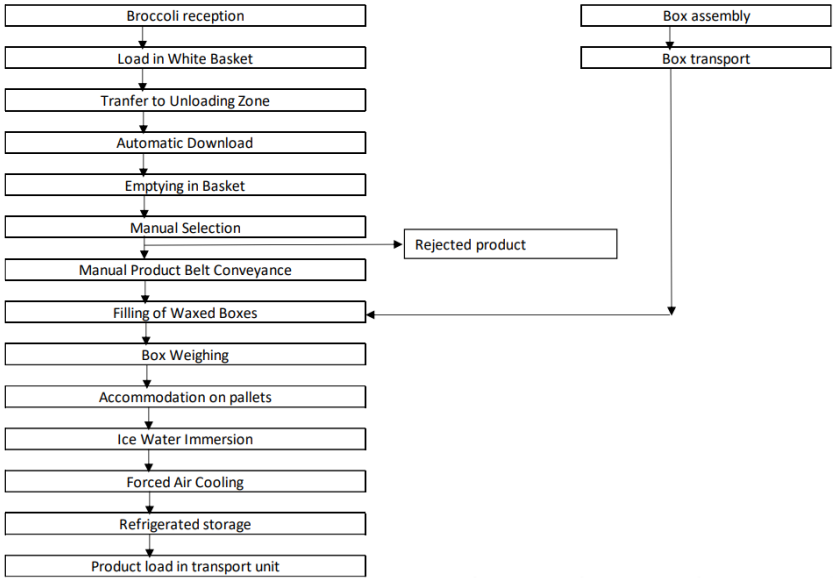


***Fig. 1:*** *Flow chart of operations in broccoli (Brassica oleracea var. Italica) packing process in organization in Guanajuato, México.*

**Table 1:** Risk analysis matrix for classifying process environment surfaces in hygienic zoning for subsequent pathogen sampling definition.

| | | | Probability (Cleaning Difficulty) | | |
|---|---|---|---|---|---|
| | | | Low | Medium | High |
| Hazard (Proximity to food) | High | Zone 1 | | | |
| | Medium | Zone 2 | | | |
| | Low | Zone 3 | | | |

## Results and Discussions

Each surface within the broccoli (*Brassica oleracea var. Italica*) packaging process area in direct or indirect contact with the product must undergo a hazard assessment, depending on the type of microorganism to be evaluated, as well as the frequency of analysis, the surface area to be evaluated and the permissible limits to avoid a risk to the safety of the manufactured products. The risk assessment matrices for all surfaces involved in the process are described in Annex 1.

Under the contamination risk analysis, it is then possible to classify the surfaces in the different hygienic zones to continue with the sampling plan for environmental pathogens (*Listeria monocytogenes, Salmonella spp.)* and indicator microorganisms (aerobic mesophiles, total coliforms), respectively. Considering the above, the zoning of the broccoli packing process at organization is established as follows:

**Table 2:** Classification of surfaces in broccoli (Brassica oleracea var. Italica) packaging process in hygienic zones according to hazard analysis.

| SAMPLING POINT | | |
|---|---|---|
| Process Air in Contact with the Product | ZONE 1 | Basket |
| | | Rubbish band |
| | | Lift |
| | | Iced Water |
| | | Knives |
| | | Cooling Vat Surface |
| | ZONE 2 | Belt rollers |
| | | Box conveyor belts |
| | | Platforms |
| | | Secondary basket emptying conveyor belt |
| | ZONE 3 | Diffusers |
| | | Drainage |
| | | Aerial piping |
| | | Empty basket lowering ramp |
| | ZONE 4 | Sanitary customs pond |
| | | Boot brushes |
| | | Refrigeration area strainers |
| | | Toilets |
| | | Dining room |
| | | Ice drainage pond |

The risk analysis of each of the surfaces makes it possible to establish the type of micro-organisms to be evaluated in each one and to determine the frequency of analysis for each one. Thus, for areas in direct contact with the product (Zone 1), hygiene indicators (aerobic mesophiles and total coliforms) must be evaluated, and in areas that are not in direct contact with the product, the environmental pathogenic microorganisms recommended by the ICMSF for the type of process involved must be evaluated. Salmonella spp. and Listeria monocytogenes are the pathogens best able to persist on surfaces for long periods of time, even years, as well as representing the cause of the highest number of recalls associated with biological contamination associated with the production process (ICMSF, 2018). Within the scope of the Primus GFS safety management system (Primus GFS, 2019), recommendations are also established to determine the frequency of analysis for each sampling point in the hygienic zones, however, this only works as a guide and does not contemplate all the sampling points that according to the zonal analysis that has been determined contemplates a greater scope and therefore contributes to a greater prevention of the contamination of microorganisms towards the packaged product. The frequency established for each of the sampling points distributed in the four hygienic zones according to the level of risk due to contact or proximity to the product in process is shown below.

**Table 3:** Description of frequency of analysis of hygiene indicator microorganisms (aerobic mesophiles, total coliforms) and pathogens associated with process contamination (*Listeria monocytogenes, Salmonella spp.*) in broccoli (*Brassica oleracea var. Italica*) packaging process.

| SAMPLING POINT | | | MICROBIOLOGICAL ANALYSIS | FRECUENCY |
|---|---|---|---|---|
| Process Air in Contact with the Product | ZONE 1 | Basket | CT-MA | WEEKLY* |
| | | Rubbish band | CT-MA | WEEKLY* |
| | | Lift | CT-MA | WEEKLY* |
| | | Iced Water | CT-MA | WEEKLY* |
| | | Knives | CT-MA | WEEKLY* |
| | | Cooling Vat Surface | CT-MA | WEEKLY* |
| | | | CT-MA | WEEKLY* |
| | ZONE 2 | Belt rollers | LM-SS | WEEKLY** |
| | | Box conveyor belts | LM-SS | WEEKLY** |
| | | Platforms | LM-SS | WEEKLY** |
| | | Secondary basket emptying conveyor belt | LM-SS | WEEKLY** |
| | | | CT-MA | WEEKLY* |
| | ZONE 3 | Diffusers | LM-SS | WEEKLY** |
| | | Drainage | LM-SS | WEEKLY** |
| | | Aerial piping | LM-SS | WEEKLY** |
| | | Empty basket lowering ramp | LM-SS | WEEKLY** |
| | | | CT-MA | WEEKLY* |
| | ZONE 4 | Sanitary customs pond | LM-SS | WEEKLY*** |
| | | Boot brushes | LM-SS | WEEKLY*** |
| | | Refrigeration area strainers | LM-SS | WEEKLY*** |
| | | Toilets | LM-SS | WEEKLY*** |
| | | Dining room | LM-SS | WEEKLY*** |
| | | Ice drainage pond | LM-SS | WEEKLY*** |

The sampling plan for the evaluation of indicator micro-organisms corresponds to a three-class plan given the specifications (objective and acceptable) available to evaluate the risk of cross contamination towards the product, being for this plan m=10 CFU/250 cm2 and M=100 CFU/250 cm2 , as these are hygiene indicator micro-organisms the risk is classified as indirect risk, as these groups of micro-organisms indicate the probability of the presence of pathogens when the quantification exceeds the values that have been determined by specification. Likewise, according to the conditions of the process, there are no conditions that modify the associated risk, so the corresponding sampling plan is established with a few samples to be evaluated of 5 (n=5) and a tolerance of 2 of them outside the specification limits (c=2) to rule that the surface is not at potential risk of contaminating the product.

In the case of pathogens, the presence of any quantity of pathogens represents a potential danger to the safety of the products, which is why the analytical tests are of a qualitative nature, independent of the surface area evaluated, with positive results being unacceptable.

**Table 4:** Limits established for evaluation of hygiene indicator micro-organisms (aerobic mesophiles, total coliforms) on food contact surfaces in broccoli (*Brassica oleracea var. Italica*) packing plant.

| Indicator microorganisms | | | | |
|---|---|---|---|---|
| | Before Disinfection (250 $cm^2$) | | After Disinfection (250 $cm^2$) | |
| Quantitative Testing of Microbiological Indicators | Target | Acceptable | Target | Acceptable |
| Total Coliforms (TC) | <10 CFU | <100 CFU | <10 CFU | <50 CFU |
| Aerobic Mesophiles (AM) | <100 CFU | <500 CFU | <10 CFU | <100 CFU |

**Table 5:** Limits established for the evaluation of pathogenic micro-organisms (aerobic mesophiles, total coliforms) on non-food processing surfaces in broccoli (*Brassica oleracea var. Italica*) packing plant.

| Pathogenic microorganisms | | |
|---|---|---|
| Qualitative testing for pathogenic microorganisms | Acceptable | Acceptable |
| Listeria monocytogenes (LM) | Absent | Absent |
| Salmonella Spp (SS) | Absent | Absent |

As this is a qualitative microbiological evaluation in which the result issued can only be the presence or absence of the micro-organism of interest, a two-class sampling plan is established for its evaluation, as in the plan considered for the indicator micro-organisms in accordance with the production process, there are no conditions that modify the risk of microbiological contamination, However, in this case, because of the pathogenic microorganisms, a severe degree of risk is considered, for which a sampling plan is established with a number of samples to be evaluated of 30 (n=30) and zero tolerance to have any sample with a positive result (c=0).

As this is a qualitative microbiological evaluation in which the result issued can only be the presence or absence of the micro-organism of interest, a two-class sampling plan is established for its evaluation, as in the plan considered for the indicator micro-organisms in accordance with the production process, there are no conditions that modify the risk of microbiological contamination, However, in this case, because of the pathogenic microorganisms, a severe degree of risk is considered, for which a sampling plan is established with a number of samples to be evaluated of 30 (n=30) and zero tolerance to have any sample with a positive result (c=0).

**Table 6:** Analytical methods for the detection and enumeration of hygiene indicator microorganisms and environmental pathogens for the environmental microbiological monitoring program.

| Laboratory Tests | Method |
|---|---|
| Suface analysis | ISO 18593 |
| Air sampling | APHA, 2001 |
| *Aerobic Mesophilic* | ISO 4833 |
| *Total Coliforms* | ISO 4833 |
| *Listeria monocytogenes* | ISO 11290 |
| *Salmonella spp* | ISO 6579 |

## Conclusions

Environmental microbiological monitoring program in the organization in Guanajuato, México meets all the requirements of the safety guidelines currently implemented in the PrimusGFS organization in version 3.2, which is approved by the Global Food Safety Initiative (GFSI) and ensures that the food produced by the organization is safe and free of any health hazards to consumers. The program is based on the zonal risk analysis recommended by the International Commission for Microbiological Specifications for Food (ICMSF) of all surfaces in direct and indirect contact with the product as well as the air and process water used. The frequency of analysis is defined for each of the hygiene indicator microorganisms and pathogens for each of the risk zones in which the sampling points are distributed.

## Conflict of Interest

The authors declare no conflict of interest.

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