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Validation of an Aseptic Packaging System of Liquid Foods Processed by UHT Sterilization

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The Ultra High Temperature sterilization process (UHT) followed by aseptic filling, requires validation of the operational conditions, including air quality control, equipment operation and good manufacturing practices to avoid recontamination of processed food. The objective of this study was to validate an aseptic filling system applied to sterile liquid foods through microbiological study of air quality and processing practices. It was used a Merck MAS-100 Eco to collect air samples inside the aseptic filling and process surrounding area, being inoculated in PCA (bacteria) and PDA (yeasts and molds) medium. In addition, in the aseptic filler were collected air samples by petri plate opened during two hours in the filling process and four hours after processing, to the evaluation of the microbiological filter integrity. This evaluation were made in 13 process repetitions, and the results were compared to the ISO 14644 and Fung scale parameters, where the aseptic filler counts resulted in an absence with the use of the air sampler, and for the plates exposed for 2 h during process, a critical condition, showed a 100 % absence for aerobic mesophilic bacteria and 71.4 % for fungi. The 4 h exposition counts showed a 100 % absence for bacteria and fungi, concluding for the filter integrity. In the surrounding process area, the results were below the allowed limit of 300 cfu.m^{-3} air. Personnel (operators) received training in the operational procedures related to the good manufacturing practices and were evaluated with the use of a checklist, also determining the critical control points of process steps. This training results in failure control and improvements in microbiological results. The checklist showed 95 % compliance and in each process was made training operational improvements, and the critical points (UHT sterilization, filling and packaging closing) were controlled. The results validate the aseptic filler system, highlighting the importance of the staff training and process monitoring.

1. Introduction

Developments in the study of food science contributed to several changes in the habits of the population, and among them is the consumption of ready-to-eat food (Amorim, 2008), whose products require no preparation or additional treatment (Yam-fung et al., 2013). The ultra high temperature sterilization technology (UHT) innovated the food market, in the production of safe food, allowing storage at room temperature and long shelf life (Baglinière et al., 2012).

UHT heat treatment involves temperatures between 130 and 150 °C for 3 to 5 s, in continuous flow, reaching lethal effect on microorganisms and bacteria spores (Jaeger et al., 2010), and immediately cooled to temperatures below 32 °C and filled aseptically in sterile sealed packaging (Pereira et al., 2013). Monitoring of aseptic filling must be constant throughout the process, since the steps of filling and sealing are critical in terms of risk of contamination control after UHT treatment (Pujol et al., 2013). Petrus et al. (2009) also reported the activities post thermal treatment related to packaging system as the greatest impact on the stability of the product, influencing the shelf life. Usually, microbiological analysis is used to evaluate the

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hygiene conditions of the environment, equipment, utensils, and employees after cleaning (Oliveira et al., 2008).

The air serves as a carrier of microorganisms adhered to the circulating dust particles, liquid droplets or as individual particles, until they fall and are deposited on surfaces (Curiel et al., 2000), and thus as primary packaging, important points of analysis and control, directed to good manufacturing practices (GMP) (Jesus et al., 2007). Clean rooms are environments that offer the necessary conditions for procedures that require operational sterility (Sbarai, 2007). The air supply, filtering, construction materials and operational procedures aim to control particles in the air, given the appropriate levels of cleanliness of current technical standards (Blois, 2002), and the ISO 14644 (ABNT, 2005) is one of the standards for clean rooms, free from contamination.

This study aimed to validate an aseptic filling system of liquid food treated by UHT sterilization process in a pilot plant, through microbiological study of environmental air quality in the processing unit.

2. Materials and methods

The evaluation of the system was performed in a UHT processing unit for coconut water, followed by filling in equipment and aseptic conditions, which aimed to validate. Microbiological analyzes were performed in air samples to assess the filling environment, staff training and discussion about factors considered critical points and that should be controlled in accordance with the principles of HACCP (Hazard analysis and critical control points).

2.1 Green coconut water, UHT processing and aseptic filling

The green coconuts suitable for coconut water extraction, were washed and sanitized by immersion during 15 minutes in free chlorine solution 50 $mg.kg^{-1}$, rinsed and side cut for extraction and filtration of the water. The packaging (212 mL clear glass bottles and polypropylene screw caps) were sterilized by soaking for 15 minutes in a 1 % peracetic acid solution, and rinsed with sterile water coming from the UHT equipment. The fresh coconut water followed the steps of UHT processing and aseptic filling. The processing flowchart is shown in Figure 1.

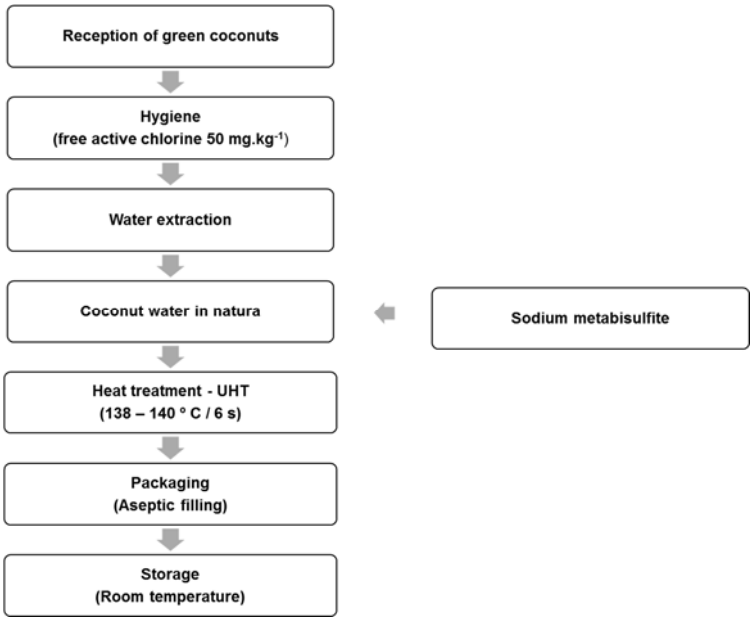

Figure 1 – Flowchart – coconut water UHT processing

The UHT treatment was performed in a Armfield FT74 UHT sterilizer, connected to a aseptic filler FT83, a chamber with laminar air flow, with HEPA filter, clean room specification - Class 5, according ISO 14644-1 standard (ABNT, 2005). Before processing, the aseptic filler chapel and the metal surfaces were sanitized with an alcohol solution and plastic surfaces with benzalkonium chloride 500 $mg.L^{-1}$. The pipes were sterilized by circulating superheated water at 120 °C and keeping the tubes at temperatures above 120 °C for 20 min. After sterilization, to have the process conditions (steady state), a water cooling system Armfield FT63 was connected. UHT process was in temperature from 138 to 140 °C and 6 s retention time, followed by cooling

below 30 °C in continuous mode, flow rate of 10.2 $L.h^{-1}$, and pressure of 4 to 5 bar. In the aseptic area the bottles were filled with coconut water, closed by hand, encoded and stored at room temperature. The air velocity in the aseptic chamber was monitored with an anemometer THA-30 Northeast Sammar model.

2.2 Microbiological analysis

Air samples from seven environments were analyzed (aseptic filler, two process areas and four cooling chambers), with mesophilic aerobic using plate count agar (PCA) medium and yeasts and molds with potato dextrose agar (PDA). The Merck MAS-100 Eco air sample collector was used inside the aseptic filling (1 m^3 air samples) and process surrounding area (200 L samples). In addition, in the aseptic filler were collected air samples by simple sedimentation in open petri plates (APHA, 2001). The sedimentation time was two hours opened during the filling process and four hours after processing, to the evaluation of the microbiological filter integrity. These microbiological evaluations were made in 13 process repetitions, totaling 324 samples collected in triplicate. The plates were incubated at 35 ± 1 °C / 48 h for mesophilic bacteria and at 25 ± 1 °C / 2 to 5 days for yeasts and molds. It was performed visually counting the colony forming units (cfu) and the results were expressed as mean in cfu / m³, cfu / 2 h and cfu / 4h.

2.3 Evaluation of the hygienic and sanitary conditions

For the evaluation of GMP, it was prepared a checklist adapted from Brazilian regulation 368/97 of the Ministry of Agriculture (MAPA) (Brazil, 1997a), with 20 items evaluation, divided into hygiene of workers, cleaning equipment and utensils, layout, cleaning, disinfection and storage / conservation. Another regulation, No. 40/97 (MAPA) (Brazil, 1997b) was used for the hazard analysis and critical control points (HACCP).

3. Results and discussion

3.1 Aseptic filler results

The results of the microbiological analyzes for the aseptic filler chamber are shown in Table 1, expressed as a percentage of analyzes that showed plates with no colonies (satisfactory) or counts (unsatisfactory), according to the microbiological parameter established by the ISO 14644 cleanroom -1 - Class 5. In the sedimentation test, with medium exposure for 4 h without any activity except airflow, the results were the absence of microorganisms in 100 % of cases, showing the microbiological integrity of the filter and appropriate to the process of filtering the air. The results of the air quality during filling process, showed 92 % and 75 % compliance for mesophilic aerobic and molds, using the sampler, and 100 % and 72 %, using the method of exposition of the open plates for two hours during the process.

Table 1. Microbiological evaluation of the aseptic filler conformity.

	Conformity (%)*	
Evaluation method	Mesophilic aerobic	Yeasts and moulds
MAS-100 Eco®	92	75
Open plates during processing (2 hours)	100	72
Open plates after processing (4 hours)	100	100

(*) In a total of 13 process evaluation. Conformity means the absence of colony former units.

The air flow speed circulating inside the aseptic filler showed an average of 0.45 m / s, from the microbiological filter towards the outlet opening, according to the manufacturer specifications, and preventing airflow from outside to inside, carrying the microorganisms present in the outside air to the filling chamber.

The results shown in Table 1, compared to those discussed by Ponce et al. (2011), which indicated no contamination in air samples in cleanroom areas, indicating non-compliance with respect to the absence of plates with colony-forming units. The first process showed a non-compliance, addressed to workers fails, and corrective actions were taken, related to the training of operators and some changes regarding the way to carry out the work and the followed process showed no contamination during aseptic filling. According to Nazir et al. (2013), a well-trained operator has the responsibility to ensure the smooth operation of the plant, and is required an accurate and comprehensive knowledge of the process and its operating conditions.

In food processing environments, factors such as activity of employees, floor drains, ventilation systems, communication between sectors, spilled food and equipment surfaces are examples of aerosol sources (Burfoot et al., 2007; Byrne et al., 2008). In addition to the evaluation of microbiological counts and monitoring of the airflow, were also evaluated the operational conditions associated with the work of operators and good manufacturing practices. As the initial processing had unsatisfactory results, it was considered as caused by

operational failure, with an indication of the need for better staff training. The results after corrective actions, however, presented no microbiological contamination, confirming that the training was effective. About the filter integrity, as all sampling has absence of microbiological counts during a 4-hour period, it also proved to be effective action of the filter and the airflow as a barrier to microorganisms from outside to inside.

3.2 Assessment of air quality of processing areas and refrigeration unit

Table 2 shows the results of microbiological evaluation of the environment, classifying as "good" and "unsatisfactory", according to the microbiological air quality parameters (Fung, 2002). In the "process area 1", the results for aerobic mesophilic counts were satisfactory in 92 % of the tests, and to molds and yeasts, 75 % satisfactory. In the "second process area" adjacent to the aseptic filler environment, all the mesophilic aerobic tests were satisfactory, and yeasts and molds had 85 % of the results as satisfactory. The two process areas had mold and yeast count above the aerobic mesophilic. There were more satisfactory results in the evaluations than unsatisfactory, but even so, it was considered as warning and observed greater care to environmental hygiene in sequential processes. In cooling chambers, as there are no parameters defining limits of microbiological counts, we use the parameters recommended in Fung (2002) although very restrictive, were applied as a way to establishing decision limit, and take corrective action related to the frequency of cleaning operations. The yeasts and molds counts presented lower satisfactory results comparing to mesophilic aerobic.

Table 2. Microbiological evaluation of the processing area and refrigeration units.

	Conformity (%)*	
Place:	Mesophilic aerobic	Yeasts and moulds
Processing area 1	92	75
Processing area 2	100	85
Refrigeration unit 2	80	60
Refrigeration unit 3	92	58
Refrigeration unit 4	82	58

(*) In a total of 13 process evaluation. Conformity means less than 300 ufc.m^{-3}.

Studies point to a significant occurrence of mesophilic in food processing areas. Salustiano (2002), evaluating the quality of the air in a dairy industry, obtained averaged counts greater than 90 cfu.m^{-3} in all environments. Research from cheese buns industries showed a high percentage (85.7 %) samples with counts above the proposed limit (Tomich et al., 2005). In this study, however, found lower values of unsatisfactory results in all environments, which highlights sanitary conditions conducive to the realization of this type of processing.

Table 3 shows an example of one of the 13 tests, with the microbiological count performed, considering the aseptic filling and surrounding processing areas. The results showed a non-conformity level in three environments (counts > 300 cfu.m^{-3}), where the worker´s activities affects the contamination of ambient air through airborne particles (Austin, 1965).

Table 3. Microbiological evaluation of the processing surrounding area and refrigeration units.

Environment	Mesophilic aerobic (cfu.m^{-3})	Yeasts and molds (cfu.m^{-3})
Aseptic filler	0	0
Processing area 1	75	120
Processing area 2	35	560
Refrigeration unit 2	395	170
Refrigeration unit 3	50	190
Refrigeration unit 4	65	210
Refrigeration unit 5	25	370

3.3 Hygienic and sanitary evaluation

The checklist presented 95 % of conformity and, considering the average of unsatisfactory count in aseptic filling chapel, in the initial tests, it was necessary to strengthen the operator's team training and establish operational adjustments in order to reduce the risk of contamination during the processes. The training involved the GMP practices and microbiological assurance, including the use of gloves and utensils disinfection early in the process and each new setting in the machine; proper handling material and packaging

within the aseptic filler, avoiding contact with non-sterile material; proper cleaning of the internal surfaces of equipment, packaging and materials and minimizes contact during filling operations. The discussion about cleaning frequency in the environments and equipment indicated the existence of a satisfactory cleaning plan, which enables a safe use of the equipment involved.

About HACCP, Critical Control Point (CCP) is defined as any point, step or procedure, in which there are preventative measures to keep an identified hazard under control in order to eliminate, prevent or reduce health risks consumer (Ribeiro-Furtini and Abreu, 2006). In the Figure 1 (flowchart), according to the methodology proposed by the Codex Alimentarius via decision tree (Afonso, 2006), were identified four Critical Control Points: PCC1 - UHT sterilization, PCC2 - sterilization of packaging, PCC3 - aseptic filling and PCC4 - closing packaging. These are considered, therefore, as the steps that represent risk of microbiological contamination and require strict control by the operator.

According to Scott (2005), validation is proof that the critical limits adopted are able to control the hazards identified at critical points. The list of identified CCP helped, however, in getting processing improvements and operational control, indicating the importance of monitoring the hazards and critical limits for the implementation of corrective actions related to UHT sterilization and aseptic filling process.

4. Conclusions

The aseptic filling system was validated as an enabling environment for the aseptic filling of processed foods UHT sterilization, capable of commercial sterility of the product during the process steps. Most of the results obtained in the microbiological analyzes of the environments are in agreement with the standards, indicating satisfactory air quality in the analyzed environments.

The training provided improvements in microbiological results and performance of operators. Thus demonstrating the importance of continuous staff training, along the quality and frequency of cleaning and sanitization of process environments, microbiological monitoring and equipment maintenance, as preventive quality control measures for the final product.

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