Cahors, February 9th, 2015

TRIAL REPORT ON DECONTAMINATION OF ALMONDS BY CONTINUOUS STEAM STERILIZATION STERISTEP®

Pilot testing: FCD

Date of the tries: 28th of January 2015

Validity of the tries checked by: Emilie BRARD (05.65.53.45.05), LDA46

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DS : Decontamination System

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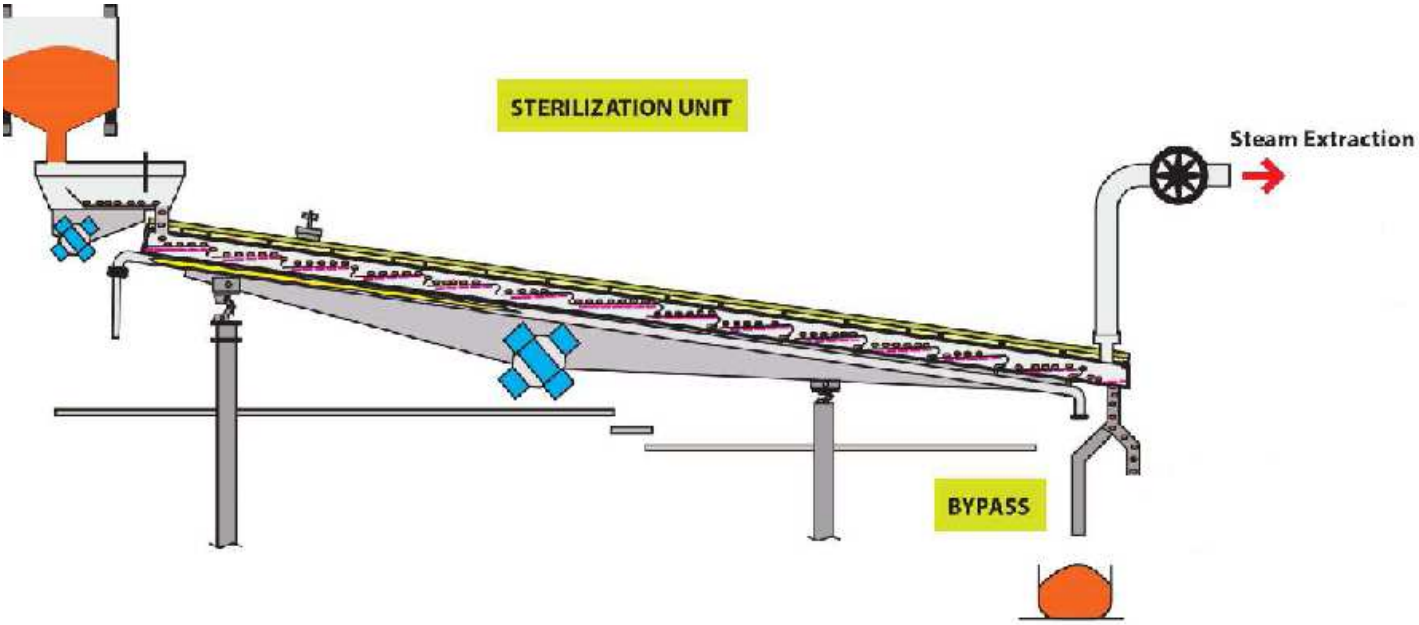

Conveying method

► “Staircase” designs in stainless steel
► 12 steps
► 2 vibrating engines
► Particles move from one step to another – from top to bottom
► Fine powders (2 microns) or whole products (chili pods, breakable herbs) smooth and continuous mixing of the particles regular exposure to steam homogeneous treatment and final product

HEAT and **STEAM:** two sources of energy for more flexibility and effectiveness

Direct heat

► Particles heated by contact with a heated stainless steel surface
► Heat comes from simple electrical resistances
= low energy consumption
► 4 sides heated
= condensation avoided
= high heating surface/m2
= fast rise of product temperature
= short residence time
= preserves the organoleptic properties of the product

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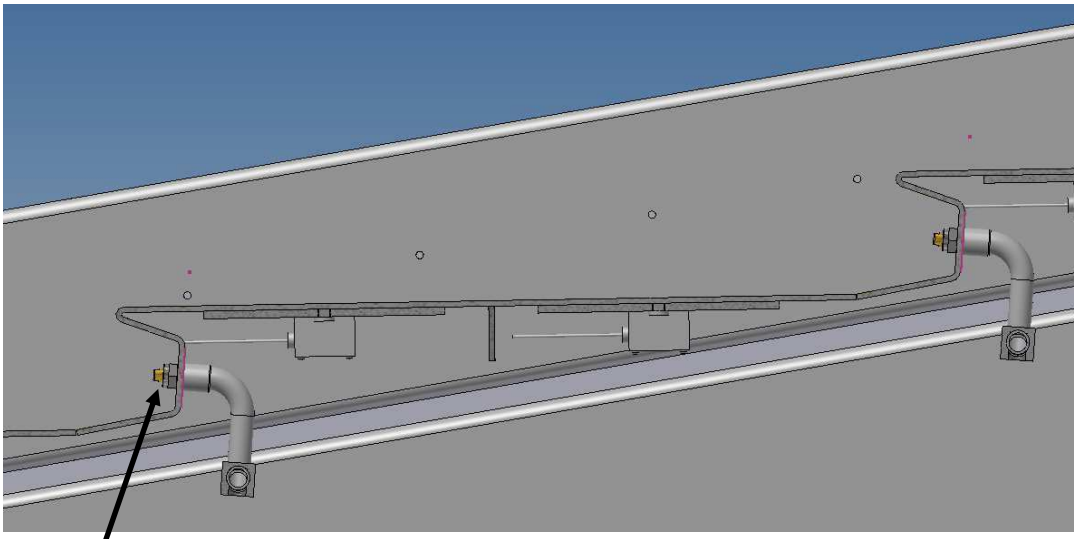
Steam (100 – 120°C): necessary for the decontamination
Injected with nozzles at the bottom of each step:
►To slightly condense on the product
►To increase the heat conductivity
. ►To control the moisture of the product during the treatment



Nozzles

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Guidelines for Process Validation Using *Enterococcus faecium* NRRL B-2354

Inocula Preparation

The fresh culture is streaked onto tryptic soy agar (TSA) and incubated at 35°C for 24+/-2 hours. Isolated typical colonies are picked and transferred into tryptic soy broth (TSB) and incubated at 35°C for 24+/-2 hours. After incubation the culture is transferred into TSB broth and incubated overnight at 35°C. 1-ml aliquots of the overnight (18+/-2 h) culture is spread over each of five TSA plates to produce a bacterial lawn after incubation for 24+/-2 h at 35°C. Following incubation, approximately 5 to 6 ml of 0.1% peptone is added to each large plate. The bacterial lawn is loosened with a sterile spreader and a sterile pipette is used to collect the cells. Prior to inoculating the almonds, the appropriate number of 25-ml preparations (to inoculate 50g portions of almonds) is pooled and thoroughly mixed for a minimum of 1 minute using a magnetic stir bar and stir plate. The inocula are kept on the stir plate until all of the almonds samples have been inoculated.

Preparation of Inoculated almonds

Almonds samples (50 g) should be weighed into plastic polyethylene bags and 1 ml of the pooled inocula added. The bags should be closed and mixed by hand by repeated inversions for 1 minute. Several batches of inoculated almonds may be prepared and pooled together.

Confirmation of Inoculated almonds Batch

The counts of the inoculated almonds should be determined after inoculation. The inoculation level must be greater than 6 logs per gram.

Handling of Inoculated almonds

The inoculated almonds must be used within 14 days.
On the same day that validation trials are being conducted, travelling controls must be handled in the same manner as treated samples, except for the treatment.

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Heat Treatment of Inoculated almonds in Processing Lines

The inoculated almonds should be loosely packed in 50-gram portions in flat thermal-stable netting.

Flat thermal-stable netting

The 50-grams sample bags may be embedded among almonds kernels on the conveyor or mixed in the product flow if the operation does not utilize a conveyor. A sufficient number of sample bags must be used in each validation run to cover representative locations of the conveyor bed.

Almonds in the flat thermal-stable netting

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Enumeration of Treated almonds Samples

After being retrieved from the processing line, the treated samples should be enumerated within 24 hours.

The 50-gram treated samples should be transferred to 100 ml of TSB in a 710-ml Whirl-Pak bag that has been tempered at ambient temperature for 4 hours. The samples bags should be mixed for 2 minutes using a Stomacher Lab Blender, and then held for 3-5 minutes prior to further dilution.

Then, the sample bag should be vigorously shaken by hand 5 times followed by serial dilution in Butterfield's phosphate buffer solution (BPB).

The enumeration procedures described in the FDA Bacteriological Analytical Manual (United States Food and Drug Administration, 1998) may be also followed. Briefly, 50 to 100-g sub-samples were added to 50 to 100 ml of Butterfield's phosphate buffer, respectively. Samples were shaken vigorously 50 times in a 30 cm arc and after standing for 5 minutes were shaken an additional five times before serial dilution and plating.

0.1-ml aliquots (for spread plating) should be plated in duplicate onto TSA plates that have been tempered at ambient temperature for at least 4 hours. The plates may be made the previous afternoon and left at ambient temperature overnight.

Plates should be counted by hand 48h after incubation at 35°C following the procedures outlined for plate counting in the Compendium of Methods for the Microbiological Examination of Foods.

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RESULTS

Enterococcus faecium	***Before Inoculation***	***After Inoculation***
Enumeration of almonds sample	<10 cfu/g	$6{,}8.10^6$ cfu/g

50-gram portions in flat thermal-stable netting.

Plate temperature	Machine speed	Dwell time	Steam fan	Enumeration	Log reduction
120°C / 256°F	17	5'30	13,5	<10 cfu/g	5,3
	18,3	3'20		<10 cfu/g	5 ,3
130°C / 266°F	18,3	2'45		5.10^2 cfu/g	3,6
	20	2'		4.10^3 cfu/g	2,7
	19,4	2'30		$3{,}2.10^5$ cfu/g	0,8
	20	2'15		$1{,}5.10^5$ cfu/g	1,1
	19,8	2'30	30	$1{,}7.10^5$ cfu/g	1,1

Controle sample : 2.10^6 **cfu/g**

CONCLUSIONS

Results show that the conveying method of Steristep® is a basic fact to sterilize products. The conveying method creates a continuous mixing of the particles regular exposure to steam homogeneous treatment. The decontamination system DS associated with a cool unit (flash cooling) provides good results for decontamination (more than 5 logs reduction).

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