# REPORT OF ANALYSIS
# MATERIAL PROCESS VALIDATION

Villeneuve de Berg, the 2017/06/01

**Applicant / n° customer**
**FCD SAS / 2307**

N° Customer : 2307
N° File : 000070256
N° Sample : 17-13737

FCD SAS
Monsieur CHEINET
Cantaronne
46230 LABURGADE

***Exam reason : Challenge test TERP***

## REFERENCE - IDENTIFICATION

Foodstuff nature **: Framboises (Raspberries)**
Packaging : PE bag
Taken by the customer, the 2017/05/22 00:00

Date of conditioning : 2017/05/22

Received at the laboratory the : 2017/05/23
Begun analysis the : 2017/05/23

Type of analysis : **challenge testing of Enterococcus faecium NRRL B-2354, according to "guidelines for using E faecium NRRL B-2354 as a surrogate Microorganism in almond process validation" - ABC TERP (Almond Board California Technical Expert Review Panel)**

## RESULTS - CALCULATION

| Analysed samples | Method | before treatment | after treatment | log reduction | Units |
|---|---|---|---|---|---|
| TTI | *ABC Protocol* | 7,36 | ///// | ///// | CFU/g |
| EE1(test) | *ABC Protocol* | 7,36 | **2,84** | **4,52** | CFU/g |
| EE2(test) | *ABC Protocol* | 7,36 | **2,61** | **4,75** | CFU/g |
| EE3(test) | *ABC Protocol* | 7,36 | **2,95** | **4,41** | CFU/g |
| TTI Heat Resistance | *ABC Protocol* | 7,36 | **6,40** | **0,96** | CFU/g |
| TTNI | *ABC Protocol* | 3,04 | ///// | ///// | CFU/g |
| TMIST | *ABC Protocol* | 7,32 | ///// | ///// | CFU/g |
| TMNIST | *ABC Protocol* | 3,20 | ///// | ///// | CFU/g |

CFU : Colony Forming Unit
log CFU = CFU converted in decimal logarithmic unit to base 10

**Criteria of acceptance**
log TTI > 7
log TTI - log TTI heat resistance test <or= 2,5
log TTI min - log EE(x) max >or= 4

**Experimental protocol - see following page**

## GENERAL CONCLUSION OF THE TEST

**All the witnesses and controls samples give satisfactory results, including heat resistance test.**
**Besides, the minimal logarithmic reduction observed after treatment is superior to 4 (4,41), which establishes a satisfactory efficiency of processing treatment according to the criteria defined by the ABC.**

**François DUBOURNAIS**
**Directeur Général**





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*En Région Rhône-Alpes* *En Région Languedoc - Roussillon* *En Région Provence Alpes - Côte - d'Azur*
N°Azur 0 810 810 206 PRIX APPEL LOCAL

*N° de TVA intracommunautaire : FR 47419921580*
*N° Siret 419 921 580 00017*
*S.A.S. ACMARIS au Capital de 7317,54 €*

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## MATERIALS & METHODS

- Method of enumeration : according to ABC protocol
- Microbial strains used : Enterococcus faecium NRRL B-2354 (ATCC8459 purchased from LGC ATCC : batch 63235689 PO No 710787632)

- Culture media : TSA, TSB, pharmacopean phosphate buffer, buffered peptone water
- Petri dish incubation : 35°C for 48 hours

### Inoculation procedure and drying

- Material inoculation with E faecium is performed and validated as follow: To constitute at least 400 g of inoculated material, fractions of matrix were positioned on a sterile stainless tray of about 400 cms ² so as to form a single thickness of raspberries.
The inoculum cellular suspension in peptone buffer water was introduced into a sterile flask with fine spray to vaporize cells on the surface of the matrix, in sterile condition. The quantities of vaporized inoculum were very low so as to deconstruct the least possible the fragile hygroscopic matrix. After every evaporation of surface, the matrix was dried oven in 40°C during 15 minutes, then homogenized. Then the stages of evaporation/drying/homogenization followed one another to deposit the entire biomass of Enterococcus on the surface of the matrix.
Preliminary phases of validation allowed to verify that the cellular integrity of Enterococcus was not questioned by the succession of heating and the potential cellular cutting through the spray was unimportant in front of the total biomass.
This procedure allowed to contaminate the material without damaging it too much.

### Global modus operandi of the test

- in accordance with ABC protocol, inoculated and uninoculated samples was about 50g, stored in PE single-use bags. Transport and conservation was performed à 4+/-1°C. E. faecium was enumerated in samples within 24 hours of treatment. For each test, Enterococcus was characterized as a safety precaution by complementary biochemical tests.

### Samples (tests and controls)

- TTI : Inoculated transport control (This sample has to undergo the same storage conditions and of transport as trial samples. He should not be neither opened nor damaged never)
- TTNI : Uninoculated transport control (This sample has to undergo the same storage conditions and of transport as trial samples. He should not be neither opened nor damaged never)
- TMIST : Inoculated but untreated processing line control (This sample must be kept in the same conditions as the trial samples (storage, transport) and must have passed in the machine WITHOUT the treatment (thermal or other))
- TMNIST : Uninoculated and untreated processing line control (This sample must be kept in the same conditions as the trial samples (storage, transport) and must have passed in the machine WITHOUT the treatment (thermal or other))
- EE : Trial inoculated samples (these samples (triplicate) are going to undergo the treatment)

### Heat resistance, storage and transport of inoculated materials

Heat resistance is performed spreading 25g dried inoculated materials on sterile stainless tray, heating in forced air oven at 138°C for 15 minutes, then enumerating heated sample and control (unheated inoculated material).
Heat resistance is determined by subtracting the lowest log value of survivors (heated sample) from highest log value of non-heated, inoculated control.
Inoculated and raw materials are stored every time dried at 4°C+/-1°C.
Samples (50 g portions) are stored in single-use sterile bags, and transported and handled dried at 4°C+/-1°C.
Samples moisture never exceeds 5,5% (w/w).
Microbial reference strain is enumerated within 24 hours of treatment.

### Data calculation and reporting

Minimum log reduction is calculated by subtracting the highest log of number of survivors in the inoculated treated samples for each process parameter from the lowest log of initial counts in the corresponding untreated inoculated samples (travelling controls).
Please note that the least log reduction values achieved must technically meet the minimum 4-log destruction requirement.

**François DUBOURNAIS**
**Directeur Général**

*This report of analysis concerns only the subdued sample on approval, or the type of subdued product on approval ( R&D).*
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*If you need advice in the interpretation of the results, thank you for consulting us.*



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