Processing Environment Monitoring in Low Moisture Foods
Production: Setting Up a Meaningful Program
April 21, 2022

Organized by: ILSI Europe
Moderator: Anett Winkler, Cargill Germany
Sponsored by the IIAFP Foundation
Webinar Housekeeping

• It is important to note that all opinions and statements are those of the individual making the presentation and not necessarily the opinion or view of IAFP.

• All attendees are muted. Questions should be submitted to the presenters during the presentation via the Questions section at the right of the screen. Questions will be answered at the end of the presentations.

• This webinar is being recorded and will be available for access by IAFP members at www.foodprotection.org within one week.
ILSI Europe delivers science-based solutions that improve public health & safeguards the environment.

Microbiological Food Safety Task Force

Cargil
Mondelēz International
Arla Foods
Institut Mérieux
General Mills
Wageningen University
Campden BRI
ILSI Europe delivers science-based solutions that improve public health & safeguards the environment.

- Collaboration
- Common challenges

- Investigate microbial issues in foods that are related to public health risks
- Facilitate the development of harmonised, science-based approaches to predict and prevent microbiological risks
ILSI Europe delivers science-based solutions that improve public health & safeguards the environment.
ILSI Europe delivers science-based solutions that improve public health & safeguards the environment.

- Collaboration
- Common challenges
- Science
- Communicate & disseminate

21ST INTERNATIONAL CONGRESS
ESTLV
SITGES (BARCELONA) SPAIN
21 - 25 NOVEMBER 2022

ILSI Europe's Scientific Session on Food Allergen Quantitative Risk Assessment (QRA)
4-6 May 2022
Munich, Germany
Anett Winkler, Ph.D.
Moderator

Organization: Cargill Germany
Function: EMEA Microbiologist

Work Experience:
- 20 years at Kraft / Mondelez as microbiologist in various roles (regional / global)
- performed numerous validation studies for nut, dairy & cocoa processing
- global expert for thermal processing within Mondelez International
- joined Cargill in October 2017 in her current role
- also active in ILSI Europe (Microbiology Food Safety), and IAFP being the current chair of the Organizing Committee for the IAFP European Symposium
François Bourdichon, Ph. D.

Organization: Università Cattolica Del Sacro Cuore
Function: Research Collaborator

Work Experience:
➢ 15y in the Food Industry: Savencia (FR), Danone (FR), Nestlé (CH), Barry Callebaut (BE)
➢ Since January 2017, Principal Consultant at Food Safety Microbiology and Hygiene
➢ Research Collaborator in DiSTAS, Dipartimento di Scienze e Tecnologie Alimentari per una filiera agro-alimentare Sostenibile, Università Cattolica Del Sacro Cuore, Piacenza, Italy
➢ Member of the IAFP since 2007
Presenter: Marcel Zwietering

Organization: Wageningen University, The Netherlands

Function: Professor Food Microbiology

Work Experiences:

- 19 years professor
- 5 Years Danone Research
- 10 years university
- ICMSF chair
- Active in ILSI Europe (Microbiology Food Safety), and IAFP
Presenter: Séamus Fanning

Organization: University College Dublin, Ireland
Function: Professor of Food Safety & Zoonoses

Work experience:
- appointed to UCD in 2002 and currently is the Director of the UCD-Centre for Food Safety (20-years as a Full Professor)
- more than 30 years research experience, applying molecular methods to food safety challenges
- served as an expert member of several WHO/FAO missions
- a serving member on editorial boards of learned journals including, Journal of Food Protection; Foodborne Pathogens & Disease and Research in Microbiology
- elected as a Fellow of the American Academy of Microbiology (FAAM) in 2019
Processing Environment Monitoring in Low Moisture Foods Production: Setting Up a Meaningful Program

François BOURDICHON
Pathogens in Low Moisture Foods
ILSI Europe: 2010 Dedicated Expert Group

General Interest

Low–Water Activity Foods: Increased Concern as Vehicles of Foodborne Pathogens

LARRY R. BEUCHAT,1,* EVANGELIA KOMITOPoulos,2 HARRY BECKERS,3 ROY P. BETTS,4 FRANÇOIS BOURDICHON,3 SÉAMUS FANNING,6 HAN M. JOOSTEN,5 AND BENNO H. TER KUILE7,8

1Center for Food Safety, University of Georgia, 1109 Experiment Street, Griffin, Georgia 30223-1797, USA; 2Leatherhead Food Research, Randalls Road, Leatherhead, Surrey KT22 7BY, UK; 3Harry Beckers Food Safety Consultant, Achterweg 38, NL-2865 XG Ammerstol, The Netherlands; 4Microbiology Department, Campden BRI, Chipping Campden, Gloucestershire GL55 6LD, UK; 5Nestlé Research Center, Vers-chez-les-blanc, CH-1000 Lausanne 26, Switzerland; 6UCD Centre for Food Safety, School of Public Health, Physiotherapy and Population Science, University College Dublin, Belfield, Dublin 4, Ireland; 7Department of Molecular Biology and Microbial Food Safety, Swammerdam Institute of Life Sciences, University of Amsterdam, Science Park 904, 1098 XH Amsterdam; and 8Office for Risk Assessment and Research, Netherlands Food and Consumer Product Safety Authority, Catharijnestegel 59, 3511 GG Utrecht, The Netherlands

MS 12-211: Received 15 May 2012/Accepted 17 August 2012
Pathogens in Low Moisture Foods
A Code of Hygienic Practice (CXC 75-2015)

CODE OF HYGIENIC PRACTICE FOR LOW-MOISTURE FOODS
CXC 75-2015

Pathogens in Low Moisture Foods
ILSI Europe: a ten year plus initiative

General Interest
Low–Water Activity Foods: Increased Concern as Vehicles of Foodborne Pathogens

LARRY R. BEUCHAT,† EVANGELIA KOMITOPOULOU,‡ HARRY BECKERS,§ ROY P. BETTS,¶ FRANÇOIS BOURDICHO,‖ SéAMUS FANNING,¶† HAN M. JOOSTEN,‡ and BENNO H. TER KUILE,†§

†Center for Food Safety, University of Georgia, 1109 Experiment Street, Griffin, Georgia 30223-1797, USA; ‡Leatherhead Food Research, Randalls Road, Leatherhead, Surrey KT22 7RY, UK; §Harry Beckers Food Safety Consultant, Achterweg 38, NL-2865 XG Ammerstol, The Netherlands; ‖Microbiology Department, Campden BRI, Chipping Campden, Gloucestershire GL55 6LD, UK; ¶Nestlé Research Center, Vers-chez-les-blanc, CH-1000 Lausanne 26, Switzerland; ¶¶UCD Centre for Food Safety, School of Public Health, Physiotherapy and Population Science, University College Dublin, Belfield, Dublin 4, Ireland; †Department of Molecular Biology and Microbial Food Safety, Swammerdam Institute of Life Sciences, University of Amsterdam, Science Park 904, 1098 XH Amsterdam; and †Office for Risk Assessment and Research, Netherlands Food and Consumer Product Safety Authority, Catharijnestraat 59, 3511 GG Utrecht, The Netherlands

MS 12-211: Received 15 May 2012/Accepted 17 August 2012
Pathogens in Low Moisture Foods
ILSI Europe: 2020 – Time for update?

Processing environment monitoring in low moisture food production facilities: Are we looking for the right microorganisms?

François Bourdichon a,b, Roy Betta c, Christophe Dufour d, Séamus Fanning e, Jeffrey Farber f, Peter McClure g, Despoina Angeliki Stavropoulou b, Ellen Wemmenhove h, Marcel H. Zwietering i, Anett Winkler k

a Food Safety, Microbiology, Hygiene, 16 Rue Gaston de Callois, 75015 Paris, France
b Facoltà di Scienze Agrarie, Alimentari Ambientali, Università Cattolica del Sacro Cuore, Piacenza-Cremona, Italy
c Campden BRI, Chipping Campden, Gloucestershire, United Kingdom
d Mérieux NutriSciences, 25 Boulevard de la Paix, 99811 Cergy Pontoise, France
e UCD – Centre for Food Safety, University College Dublin, Belfield, Dublin D04 N27E, Ireland
f Department of Food Science, University of Guelph, Guelph, Ontario, Canada
g Mondelēz International, Bourneville Lane, Birmingham B30 2ET, United Kingdom
h ILSI Europe, Avenue E. Mounier 83, B Box 6, B-1200 Brussels, Belgium
i Arla Foods Ingredients, Sønderøgade 26, Vibybrk, Denmark
j Food Microbiology, Wageningen University, PO Box 17, 6700AA, Wageningen, The Netherlands
k Cargill Germany GmbH, Cornerstr. 2, D-47699 Krefeld, Germany
Pathogens in Low Moisture Foods

2022 related outbreaks:

Abbot, US (Cronobacter spp.)
Ferrero, BE (Salmonella spp.)
Risk-based approach in setting up a meaningful environmental monitoring program

Marcel Zwietering
Presenter: Marcel Zwietering

Organization: Wageningen University, The Netherlands
Function: Professor Food Microbiology

Work Experiences:
- 19 years professor
- 5 Years Danone Research
- 10 years university
- ICMSF chair
- Active in ILSI Europe (Microbiology Food Safety), and IAFP
Review

Processing environment monitoring in low moisture food production facilities: Are we looking for the right microorganisms?

François Bourdichon a,b,⁎, Roy Betts c, Christophe Dufour d, Séamus Fanning e, Jeffrey Farber f, Peter McClure g, Despoina Angeliki Stavropoulou h, Ellen Wemmenhove i, Marcel H. Zwietering j, Anett Winkler k
Low moisture foods

Do not support growth

But survival (in environment and in product)

Low levels can already be an unacceptable risk

Relevant pathogens: *Salmonella*, *Cronobacter*, pathogenic *E. coli*, *B. cereus*

Milk powder, PIF, cereals, dried meats, spices, nuts, chocolate, peanut butter,
Environmental monitoring

Control: If not in your raw materials (or inactivated) and not in your environment

If not in your raw materials (or inactivated) AND not in your environment
FSO: Food Safety Objective: norm set by government

ICMSF

\[ H_0 - \Sigma R + \Sigma G < FSO \]

Sufficient reduction OR prevent growth
FSO: Food Safety Objective: norm set by government

ICMSF

\[ H_0 - \Sigma R + \Sigma G + \Sigma C < FSO \]

Sufficient reduction/prevent growth AND limited recontamination
on log basis

\[ H_0 - \Sigma R + \Sigma G + \Sigma C < FSO \]

\[ H_0 = 2 \log \text{cfu/g} \]
\[ \Sigma R = 6D \text{ reduction} \]
\[ \Sigma G = 2 \text{ logs growth} \]
\[ 2 - 6 + 2 = -2 \log \text{cfu/g} \]
\[ 4 - 8 + 2 = -2 \log \text{cfu/g} \]

\( \Sigma R \) and \( \Sigma G \) not dependant on level
on log basis

\[ H_0 - \Sigma R + \Sigma G + \Sigma C < FSO \]

Contamination is additive on the linear scale!
100 organisms + 1000 recontaminating = 1100
1000 organisms + 1000 recontaminating = 2000

It is not 3 logs + 3 logs = 6 logs!
<table>
<thead>
<tr>
<th>Co (cfu)</th>
<th>C (cfu)</th>
<th>Ho (log cfu)</th>
<th>$H_1$</th>
<th>$\Delta H$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1000</td>
<td>0.00</td>
<td>3.00</td>
<td>3.00</td>
</tr>
<tr>
<td>10</td>
<td>1000</td>
<td>1.00</td>
<td>3.00</td>
<td>2.00</td>
</tr>
<tr>
<td>100</td>
<td>1000</td>
<td>2.00</td>
<td>3.04</td>
<td>1.04</td>
</tr>
<tr>
<td>1000</td>
<td>1000</td>
<td>3.00</td>
<td>3.30</td>
<td>0.30</td>
</tr>
<tr>
<td>10000</td>
<td>1000</td>
<td>4.00</td>
<td>4.04</td>
<td>0.04</td>
</tr>
</tbody>
</table>
Serving of 100 g: FSO<1/100g : -2 log cfu/g

Safe?

If level is -3 log cfu/g, this means 1 organism per 10 bars
1 *Salmonella* has a P illness of 1:400

At FSO=-3 : Pill=1:4000 bars !

Detection probability of C=0.001 cfu/g

<table>
<thead>
<tr>
<th>n</th>
<th>P-</th>
<th>P+</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.88</td>
<td>0.12</td>
</tr>
<tr>
<td>10</td>
<td>0.78</td>
<td>0.22</td>
</tr>
<tr>
<td>60</td>
<td>0.22</td>
<td>0.78</td>
</tr>
</tbody>
</table>
Recontamination difficult to quantify

pathogens: low detection probability
  if not detected not there?
  if detected what will be transfer to product

indicators: higher probability
  how to relate it to the pathogen
  (more Enteros: higher probability of *Salmonella* qualitatively……)

Standardisation difficult:
  where to sample
  how to sample
  how much to sample (quantity and number of samples)
PRP (GMP, GHP, ....)

HACCP

Validated CCPs

Monitor Critical Limits

Verification: testing
Fig. 5.2. Daily count of *Enterobacteriaceae* on surface swabbing in the processing site. From Cordier (2007).
Sampling

Routine
Investigation
Special events
Following a positive sample

Zoning!

<table>
<thead>
<tr>
<th></th>
<th>pathogens</th>
<th>indicators</th>
</tr>
</thead>
<tbody>
<tr>
<td>close to product</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>near production</td>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td>remote areas</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>
Conclusions

LMF outbreaks remain

Environmental monitoring relevant, crucial in low moisture food ..... resident strains and recurrent outbreaks

Combination of control of raw materials, processing, cleaning and disinfecting: ...... and end product and environmental monitoring

Indicators...... pathogens
close to product.... remote areas
routine ..... seek and destroy

Not Black and White: 50 shades of Red
Processing environment monitoring in low moisture foods production—setting up a meaningful programme

Professor Séamus Fanning,
UCD-Centre for Food Safety,
School of Public Health, Physiotherapy & Sports Science,
University College Dublin,
Belfield, Dublin, Ireland
Presenter: Séamus Fanning

Organization: University College Dublin, Ireland
Function: Professor of Food Safety & Zoonoses

Work experience:
- appointed to UCD in 2002 and currently is the Director of the UCD-Centre for Food Safety (20-years as a Full Professor)
- more than 30 years research experience, applying molecular methods to food safety challenges
- served as an expert member of several WHO/FAO missions
- a serving member on editorial boards of learned journals including, *Journal of Food Protection; Foodborne Pathogens & Disease and Research in Microbiology*
- elected as a Fellow of the American Academy of Microbiology (FAAM) in 2019
Preserving food by reducing the moisture content -

- **drying is a traditional method used to preserve food** and low-moisture foods constitute a substantial part of our diet.

- **dried foods have a longer shelf-life** and low- and intermediate-moisture foods have a reduced water activity ($a_w$).

- **low-moisture (LMF) foods include** nuts; cereals; honey along with high-moisture foods such as powdered infant formula (PIF) that have been dried.

- **although erroneously believed to be a low risk,** because these food matrices cannot support microbial growth, nonetheless these foods remain susceptible to microbial contamination and therefore pose a risk to consumers and the brand.

How can the moisture content of a food be reduced & what are the microbiological consequences?

- Freezing
- **Physical removal of water** (such as by spray drying)
- **Addition of humectants** (such as NaCl; sugar or others)

Some of the consequences for the (altered) microbial population:

- metabolism is changed
- spores and vegetative cells **ADAPT** and remain viable for months and years
- cross-contamination of low-moisture foods can arise from exposure to the production environment or
Bacterial adaptation in low-moisture production environments—can sequencing methods identify how they do it?

- **Low-moisture foods are NOT sterile and outbreaks of food-borne diseases** associated with the consumption of low-a_w foods and those formulated in part with low-a_w ingredients have increased in frequency.

- **Little is known about how bacteria behave** in low-a_w food and dry food processing environments.

- Conventional **hygiene protocols** may present a challenge to effective cleaning.

- **Manufacturing practices** used for the production of low-a_w foods must be designed to eliminate pathogens.

- **Pathogens of concern include mainly** *Cronobacter* species [Abbott, USA] and *Salmonella* species [Ferrero, Belgium] that present food safety challenges to low-a_w foods and their production environments.

Precision food safety applied to the processing environment; can protect human health & brand reputation -

Cronobacter species
(formerly known as Enterobacter sakazakii) -

General characteristics:
- member of the Enterobacteriaceae family
- Gram-negative, motile rods
- facultatively anaerobic
- designated as a genus/species in 1980
- taxonomy revised and a new genus recognised (Cronobacter species), now consisting of seven species
- grows readily on laboratory media
- desiccation resistant
- rare opportunistic pathogen & causes nosocomial infections
**Cronobacter** and Powdered Infant Formula Investigation

*Español (Spanish)*

**Updated March 25, 2022**

On February 17, 2022, and February 28, 2022, Abbott Nutrition recalled powdered infant formula produced at its manufacturing facility in Sturgis, Michigan, because of possible *Cronobacter* contamination.

Parents and caregivers of newborns should not feed their baby recalled Similac, Alimentum, or EleCare powdered infant formulas.

**Fast Facts**

- Illnesses: 4
- Deaths: 2
- States: 3 (Minnesota, Ohio, Texas)
- Recall: Yes
- Investigation status: Active

**Why do events like this happen?**
The minimum $a_w$ value required for growth of *Cronobacter* species has been determined to be 0.94

Some *Cronobacter* are relatively resistant to heat

Heat resistance is greatly increased in low-moisture foods along with those with a high fat content

*Cronobacter* can survive for weeks, months and years in low-moisture foods

How does exposure of *Cronobacter* to dry environments confer an adaptation phenotype?
A uniquely adapted *Cronobacter sakazakii* isolate detected in a PIF production environment using PFGE sub-typing

**Note:**
- Similarity: 99%
- Tolerance: 1.5%
- Optimization: 1.5%


Tolerance to desiccation with time -
Uncovering bacterial adaptation to low-moisture environments by detecting gene expression using RNA-seq.

- Cronobacter sakazakii SP291
- Early Stationary Phase
- Desiccation
- Total RNA
- RNA-seq

Gene expression:
- No change in gene expression (n = 2593)
- Highly down-regulated (n = 115)
- Highly up-regulated (n = 361)

Relative expression: Desiccation / Early Stationary Phase (Gene expression)
Expression of stress response genes encoding osmoprotectants during desiccation -

Betaine metabolism

Proline transport

> 4-fold up-regulated

Trehalose metabolism is critical for survival during desiccation.

Do all *Cronobacter sakazakii* respond to low-moisture conditions in the same way?
Comparison between the desiccation curve of 
*C. sakazakii* ATCC™29544ᵀ (clinical) and *C. sakazakii* SP291 (environmental)-

- **Stage I (Desiccation 0-1 h)**
  - no obvious change in viable cell count

- **Stage II (Desiccation 1 to 2 h)**
  - liquid on the coupon evaporated completely
  - a sharp reduction in the viable cell count (~2.5 log₁₀ reduction in 1 h for ATCC™29544)

- **Stage III (Desiccation 2 to 4 h)**
  - bacteria were continuously desiccated on the coupon
  - decrease in viable cell counts at a much slower rate (~1 log₁₀ reduction in 2 h for ATCC™29544)

- **Stage IV (Rehydration 0-30 min)**
  - viable cell count change for *C. sakazakii* ATCC™29544 was larger than that for SP291 during each stage

Expression level fold change

Desiccation phase
Rehydration phase

**C. sakazakii SP291**

- **opuCA**: 260.17
- **otsB**: 250.25
- **proX**: 0.44
- **betI**: 3.39

**C. sakazakii ATCC™29544**

- **opuCA**: 5.59
- **otsB**: 67.89
- **proX**: 1.20
- **betI**: 16.49

**PIF origin**

**Clinical origin**
Datasets

16S rRNA

Insight of microbial communities

Taxonomy profile (bacterial/fungal genus-species resolution)
Diversity and abundance

Shotgun Sequencing

Taxonomy profile (bacterial/fungal/archaea/viruses species-strain resolution)
Metagenomic assembly and binning
Metabolic pathway analysis and function profiling
Selected marker genes profiling

Mining and integration

Integration of the datasets information and experimental results

Overall strategy for sequencing a food/environmental sample containing multiple microorganisms

FIGURE S1. Geographical descriptions of environmental sampling in factory W and M. Numbers in brackets indicate the number of samples collected in each area. Low-care areas are indicated in green, medium-care areas in beige, high-care areas in orange, and doors and other areas in white.
Evaluating risk associated with the microbiota in the food production environment.

- **MC**
  - Water and sludge related bacteria ↑
  - Human related bacteria ↑
  - Weekly

- **LC**
  - General environment related bacteria
  - Human related bacteria ↑
  - Dairy and milk related bacteria ↑
  - Stress endurance related bacteria ↑

- **HC**
  - General environment related bacteria ↓
  - Human related bacteria ↑
  - Dairy and milk related bacteria ↑
  - Stress endurance related bacteria ↑
  - Twice per day

Legend:
- White: Dry environment
- Blue: Wet environment
- Red: Changing room

- ↑ Relative abundance increase
- ↓ Relative abundance decrease

**Outer disturbance**
- Staff
- Non-staff people
- Air
- Raw materials

**Control approaches**
- Routine cleaning
- Changing room segregation
sequencing methods can support food safety control measures in the food production environment [WGS]

understanding the microbial ecology of a food production facility is essential in identifying changes that may signal an increased risk [WGS/16S rRNA/metagenomics]

differentiating persistent from non-persistent isolates recovered is important to refine food safety controls [RNA-seq]

precision food safety measures, including whole genome sequencing of key isolates, linked to their phenotypes, will improve our understanding of how bacteria adapt/behave in these hostile environments and provide novel biomarkers to aid their rapid detection and subsequent risk reduction
Thank you
Contact Information

Anett Winkler                   Anett_Winkler@cargill.com
Francois Bourdichon       francois.bourdichon@gmail.com
Séamus Fanning               sfanning@ucd.ie
Marcel Zwietering           marcel.zwietering@wur.nl
IAFP Upcoming Webinars

April 26  Foundations of Produce Safety in Hydroponic and Aquaponic Operations
May 4   Does Your Food Safety Culture Bridge the Multi-Cultural Challenges?
May 17  Avoiding Premature Water Activity Testing Results When Meeting Safety Regulations
May 26  Making Your Environmental Monitoring Plan Smarter
June 23 7-Steps of Sanitation (Spanish)
Be sure to follow us on social media

InternationalAssociationforFoodProtection

@IAFPFOOD

IAFPFood

international-association-for-food-protection
This webinar is being recorded and will be available for access by IAFP members at www.foodprotection.org within one week.

Not a Member? We encourage you to join today. For more information go to: www.FoodProtection.org/membership/

All IAFP webinars are supported by the IAFP Foundation with no charge to participants.

Please consider making a donation to the IAFP Foundation so we can continue to provide quality information to food safety professionals.