



ArtiSaneFood - Innovative bio-interventions and risk modelling approaches for ensuring microbial safety and quality of Mediterranean artisanal fermented foods

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Food









Content

- Background of the ArtiSaneFood project
 - Objectives, partners, food products
- Pillars: Biointerventions, fate studies, dynamic modelling, process control, process risk models
- The ArtiSaneFood free tool
 - Database
 - Process risk models

Background: The ArtiSaneFood project



- Duration: June 2019 May 2023
- Objective: to develop efficient bio-intervention strategies, enhanced process criteria, and an easy-to-use food safety decision support IT tool, aiming to the reduction and control of food-borne pathogens in artisanal fermented foods of meat or dairy origin produced in the Mediterranean.

First PRIMA project led by a Portuguese institution



- Website:
 - http://www.ipb.pt/artisanefood/
- The decision-support tool
 - https://arti-sane-food-frontend.vercel.app/
- **Repository:**
 - https://zenodo.org/communities/artisanefood

Partners

Portugal, Morocco, Tunisia,
 Spain, France, Italy, Greece

Artisanal foods







1. Biointerventions

Functional starter cultures

- Development of functional starters
 - Assessment and ID of potential LAB
 - Apart from technological properties, antimicrobial properties were tested and quantified in situ



Natural extracts with antimicrobia properties





In-situ evaluation of the growth or survival of selected foodborne pathogens (*Salmonella*, *L. mono*, *S. aureus*) artificially inoculated in the food to assess the effects of (bio-)interventions

Treatments

Control
Improved process criteria
Added lactic acid bacterium/cultures
Added extracts or EOs

Stage of monitoring (CCP?)
 Fermentation
 Maturation or curing
 Storage (shelf life)







Nonetheless our fate studies are characterised for having no fixed conditions

Product: Alheira
Stage: Maturation
Changing property: pH
Treatment: Control



Product: Merguez
Stage: Fermentation and drying
Changing properties: aw and temperature
Treatment: Control



Consider that some bio-interventions further affect the evolution of the changing properties (!)

Product: Raw milk cheese
Stage: Maturation
Changing property: pH
Treatment: Addition of 1% spearmint extract

Product: Fermented milk
Stage: Cold storage
Changing properties: pH and acidity
Treatment: Addition of lemon extract



Modelling is challenging, because of dynamic data and interactions:



Dynamic data:
 Fermented food products

 Interactions:
 Kinetic parameters <-> Bio-interventions



Predictive microbiology: controlling factors in foods and responses of pathogenic and spoilage microorganisms are quantified and modelled by mathematical equations

- The main problems are:
 - The kinetic parameters that depend on environmental parameters cannot be treated as constant, then resort to derivatives

$$\int_{N_0}^{N_t} \frac{dN}{N} = \int_0^t \mu dt$$

Lactic acid bacteria may (or not) retard the development of foodborne pathogens





•*L. monocytogenes* was inactivated by added LAB culture in fermented sausage at two different storage temperatures



17

Jameson-effect competition model with gamma interaction parameter

$$\frac{1}{N_{LM}} \frac{dN_{LM}}{dt} = \mu_{LM} \left(1 - \frac{N_{LM} + \boldsymbol{\gamma} N_{LAB}}{N_{\max tot}} \right)$$
$$\frac{1}{N_{LAB}} \frac{dN_{LAB}}{dt} = \mu_{LAB} \left(1 - \frac{N_{LAB}}{N_{\max tot}} \right)$$

•If $\mu_{LM} > 0$: • $\gamma < 1$: LM still grows after LAB reaches a maximum

 $\gamma > 1$: LM stops growing after LAB reaches a maximum

• If $\mu_{LM} < 0$:

 $\gamma >1$: when LAB reaches maximum growth, LM population can become less sensitive to the inhibitory effect of LAB



•*L. monocytogenes* was inactivated by added LAB culture in fermented sausage at two different storage temperatures



Log cfu/g



•*L. monocytogenes* grows in *salchichón* sausage during storage, but its growth is delayed by added LAB culture



Lotka-Volterra competition model with alpha interaction parameter

$$\frac{1}{LM}\frac{dLM}{dt} = \mu_{LM} \left(1 - \frac{LM + \alpha_{LM-LAB} \times LAB}{LM_{max}}\right)$$
$$\frac{1}{LAB}\frac{dLAB}{dt} = \mu_{LAB} \left(1 - \frac{LAB}{LAB_{max}}\right)$$

•If $\alpha_{LM-LAB} = 0$:

No effect of LAB, each bacterial group grows independently

A good way to test if there is really inhibition effect

•*L. monocytogenes* grows in *salchichón* sausage during storage, but its growth is delayed by added LAB culture



S. aureus in raw milk cheese is inactivated during maturation, and it is further inactivated by adding spearmint extract in goat's milk cheese,
pH changes during maturation





Geeraerd inactivation model coupled with Bigelow model for pH

$$\frac{dN}{dt} = -\frac{kN}{\left(\frac{1}{1+C_c}\right)}\left(1-\frac{N_{res}}{N}\right)$$

$$\frac{dC_c}{dt} = -\mathbf{k}C_c$$

$$D = \frac{\log (10)}{k}$$
$$\log D = \log D^* - \left(\frac{pH - pH^*}{Z_{pH}}\right)^2 \quad \text{if }$$

S. aureus in goat's milk cheese is inactivated during maturation, and it is further inactivated by adding spearmint extract in goat's milk cheese,
pH changes during maturation



. *monocytogenes* in soft cheese grows during curing in both, without added starter culture and with added starter culture



100

200

Time (h)

300

400



100

200

Time (h)

300

400





Huang growth model coupled with cardinal parameter model for pH and aw

L. monocytogenes in soft cheese grows during curing in both, without added starter culture and with added starter culture *pH* profiles are different









4. Process control Food safety control system Variability between lots assessed, since it must be small in comparison to I. Knowledge of factors necessary for control I. Knowledge of extent of variability and factors that influence variability

3. Establishing criteria for the factors that must be controlled

4. Establishing monitoring procedures

5. Organising and interpreting data

Detect process deviations (when it has gone out-ofcontrol)

variability within a

lot

6. Using the data to improve control and measure change

7. Investigating and learning from unforeseen events

4. Process control

Verification testing

Can rely on **statistical** process control to make informed decisions about the capability of a process to produce safe food



Standards Quality monitoring tools

Regulatory verification: use of a microbiological criterion to demonstrate that the upstream process is under control

chain

Processing of fermented sausages Reception of raw materials **Process control** Mixing verification can ♣ \ be applied at any Stuffing point in the food **Fermentation** Smoking **Maturation** End product



 A process risk model is an exposure assessment model that describes the pathways of a foodborne pathogen along processing

Process risk model

Process

variables

Prevalence (P) and concentration (C) in end product Stage 4 Stage 3

Stage 2

✓ Surveys
 ✓ Stan
 in
 ✓ Bio factories
 inter

Initial P

and C

Standards ✓ Biointerventio ✓ ns

 ✓ Dynamic models
 ✓ Effect of bio-

Microbial

kinetics

interventions

Stage 1





- Generic model for cheesemaking
 - Customisable for any cheese and fermented milk
 - *L. monocytogenes* only

•Time (min, mode, max), Temp (min, mode, max), N_{LAB} (min, mode, max), a_w (min, mode, max), pH (min, mode, max), [LAC] (min, mode, max), $\mu_{maxref Path}$ (mean, sd), $\mu_{maxref LAB}$ (mean, sd), MPD_{Path}, MPD_{LAB}

Reduction (min, mode, max) if extracts added

•W_{cheese}, rho_{Pressed}, Yield, Dispersion

•Vol_{milk}, C(mean, sd), P_{lot}, f_{entrapped}



- Generic model for sausage
 - Customisable for any fermented sausage
 - L. monocytogenes, Salmonella or S. aureus

Time (min, mode, max), Temp (min, mode, max), EGR_{5°C} (mean, sd), MPD_{Path}, T_{min}, Lnq0 (mean, sd)

Reduction (min, mode, max) if extracts added

Casings (P_{lot}, N), Dispersion

Meat (P, C, prop), Fat (P, C, prop), Spices (P, C, prop)

• The PRM enabled the assessment of the safety of the actual manufacturing processes, and those with potential interventions.

Lben fermented milk

Baseline (Prev=0.05)



L. monocytogenes counts, CFU/ml

Lben fermented milk

Added with citrus peel extract (Prev=0.05)



Counts in contaminated units

Merguez sausages

Baseline (P=0.20)



Merguez sausages

Optimised starter cultures (P=0.02)

 The best intervention strategies could be ranked according to effectiveness for most of the food products

Example: *S. aureus* in Portuguese raw milk cheeses

Scenario	Bulk (log CFU/g)	Log10_ interventi on	µ_ref_mean	LAB ₀ (log CFU/g)	Concentration (median) (log CFU/g)	% Reduction
Baseline	N(-2.0, 0.1)	0	0.78	Pert(4.0, 4.5, 6.0)	3.799 (3.264 - 4.385)	-
#1: Addition of 1.0% spearmint extract powder to curd	N(-2.0, 0.1)	Pert(0.3, 0.8, 1.5)	0.78	Pert(4.0, 4.5, 6.0)	3.183 (2.556 - 3.800)	16%
#2: Thermisation of goat's raw milk	N(-3.5, 0.1)	0	0.78	Pert(4.0, 4.5, 6.0)	2.736 (2.211 - 3.231)	28%
#3: Use of ad-hoc starter culture	N(-2.0, 0.1)	0	0.70	Pert(7.0, 8.2, 9.0)	-0.193 (-0.755 - 0.515)	105%
#4: = (#2 & #3)	N(-3.5, 0.1)	0	0.70	Pert(7.0, 8.2, 9.0)	-1.097 (-1.4950.607)	130%

The ArtiSaneFood free tool

✓ The concept: To bring together all results allowing producers to store information/data of their processes and evaluate their safety



The ArtiSaneFood free tool







- Producers can introduce their own data from monitoring or controls of the end product
 - Create a company
 - Register members of the company
 - Associate products to the company
 - Insert data on a lot basis for analysis of microbial and physicochemical attributes for their foods

Database

ArtiSaneFood

serrano cheese - Ursula Gonzales Barron

📕 Company Info

- A Products
- 🖽 Batch Data
- 💄 User Info
- II Data Analysis



Company Name	
serrano cheese	
Address 1	
bragança	

Cancel		

Submit

Company colaborators:

ubarron@ipb.pt

<u>Log Out</u>

Country

Portugal	~
City	
bragança	

Save

Database

ArtiSaneFood

serrano cheese - Ursula Gonzales Barron

🛱 Company Info

A Products

🖽 Batch Data

💄 User Info

II Data Analysis

Products

Queijo de Cabra	Open	Edit
Serrano cheese	Open	Edit
Alheira	Open	Edit
Alheira de Vinhais	Open	Edit

<u>Log Out</u>

Add new

Database

ArtiSaneFood

ano cheese - Ursula zales Barron						Export Data	New Batch
ompany Info							
		Product	Batch ID	Data Type	Production Date	Insertion Date	
Products	0	Serrano cheese	Batch1	Individual	2022-10-31	26/07/2023	
atab Data	1	Serrano cheese	Batch2	Individual	2022-11-16	04/02/2023	•••
alth Vala	2	Serrano cheese	Batch3	Mean	2022-11-14	17/11/2022	
ser Info	3	Serrano cheese	Batch4	Mean	2022-11-16	18/11/2022	
ata Analycic	4	Serrano cheese	Batch 3	Mean	2023-01-20	06/02/2023	
	5	Serrano cheese	C1	Individual	16/11/2020	28/07/2023	
	6	Serrano cheese	C2	Individual	23/11/2020	28/07/2023	
	7	Serrano cheese	C3	Individual	02/12/2020	28/07/2023	
	8	Serrano cheese	C4	Individual	22/03/2021	28/07/2023	

<u>Log Out</u>

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Search

1/1 • •



General Data

Batch ID	Batch1
Product	Serrano cheese
Batch Size	100
Number of samples	2
Trial	Yes
Production Date	2022-10-31
Insertion Date	09/06/2023

Samples

Sample 1 Sample 2

Microbiology	Nutritional			
Bacteria	Value	Units	Bacteria	Detected
Mesophiles	3.4	(log CFU/g)	Salmonella	0
LAB	2.1	(log CFU/g)	Listeria	1
Coliforms	4.3	(log CFU/g)		
E. coli	0.6	(log CFU/g)		
Staphylococci	-	(log CFU/g)		
Bacillus cereus	-	(log CFU/g)		
S. aureus	-	(log CFU/g)		
L. monocytogenes	-	(log CFU/g)		



Our Models





ArtiSaneR 🛛 🖽 Data 🔟 Control charts 🐇 About

Select inputs

Select product:	
Serrano cheese	
Select bacteria:	

S_aureus





Select inputs

Control

charts

shiny

3-2

Select product:		Datab aumon		bi e e			X-Bar C	ontrol Chart	R Control	Chart			
Serrano cheese	•	Batch summ			Drint		Х-Ваг С	ontrol Chart:	shows the p	rocess variation over t	ime.		
Select bacteria:		Show 10	v entrie	er PDP PS	Planc								
C_perfringens	•		Se	earch:			tistics 2.0						
		Batch 🔶	n 🔶	Mean ≬	SD ≬	Range 🔶	ary sta						
		All	Al	All	All	All	3 summa 1.5					U(
		C1	5	2.343	0.1961	0.4750	Group 1.0				•	LC	CL
		C2	5	0.9926	0.4032	0.7780		C1		C2	C3	C4	
		C3	5	0.8194	0.1649	0.3010		Number of grou	ps = 4	Batch			
		C4	5	0.7592	0.1346	0.3010		Center = 1.2286 StdDev = 0.199	6 3766	LCL = 0.9611082 UCL = 1.496092	Number beyond limits = 3 Number violating runs = 0		
		Showing 1 to	o 4 of 4 er	Itries	Previous	1 Next							



ArtiSaneR 🛛 🖽 Data 🔟 Control charts About

Select inputs

Select product:	
Merguez	
Select bacteria:	

Mesophiles

-

 \mathbf{T}

Batch sum	mary statis	tics			
Сору (CSV Exce	el PDF	Print		
Show 10	∼ entrie	s			
	Se	earch:			
Batch 💧	n 🔶	Mean ≬	SD ≬	Range	<u>a</u>
All	Al	All	All	All	
P1	10	8.277	0.2559	0.76	508
P2	4	7.094	1.641	3.3	356
P3	11	7.985	0.6208	1.6	52
P4	10	5.219	0.4720	1.2	215
Showing 1	to 8 of 8 en	tries			
			Previous	1 Ne	ext





Open the link:

https://arti-sane-foodfrontend.vercel.app/

And try by yourselves!

Press Update after input change

Select input

Weight of cheese (g):							
300		500				1	,000,
-	.1	Q	-1	-1	-1	11	- P
300	400	500	600	700	800	900	1,000



PROCESS RISK MODELS: Simulation Model for **Cheese-Making Process**



Model

The model estimates the prevalence and concentration of Listeria monocytogenes along the cheesemaking stages, which include:

1. milk in vat and coagulation; 2. moulding of cheeses; 3. effect of bio-intervention strategy; and 4. maturation of cheeses.

This is a generic model that has been customised for goat's raw milk cheese (IPB), goat's soft cheese (UCO), Lben fermented milk (ISBST/UMA) and **Jben* cheese (UIZ)



Model functions

The model consists of four functions:

- 1. Lot generation: it generates a vector of contaminated lots from information on the parameters of a normal distribution representing the microbial counts in the contaminated milk that will be used for cheese making.
- 2. Moulding: it models the pressing of coagulated milk to discard whey; and the accommodation of curd into moulds. No cross-contamination due to handling is assumed during moulding; however, randomness is given to the distribution of bacteria among recently-pressed cheese units from the bulk of milk curd.
- 3. Biointervention: it should be used only when a plant extract is added into milk or milk curd, and assumes that it would cause a reduction in the pathogen population right after moulding.
- 4. Ripening: it simulates the growth of the pathogen and the lactic acid bacteria in cheese during maturation based on Jameson effect primary

🛛 Introduction 🔐 Batter 🖉 Stuffing 🏓 Biointervention 🏦 Maceration 🛞 Maturation & Packaging 😤 About ARTISANER

Press Update after input change Select inputs Model Number of lots sampled 200 500 1,500 **Territoria** 400 600 800 1.000 1.200 1.400 1.500

Number of units in a lot

200

Terri Christer tradicitient 200 400 600 800 1000 1200 1400

Sausage weight (g)

300 200 250 300

PROCESS RISK MODELS: Simulation Model for Sausage-Making Process

The model estimates the prevalence and concentration of Salmonella spp., Staphylococcus aureus or Listeria monocytogenes along the sausage-making stages, which include:

- 1. mixing of ingredients to prepare batter;
- 2. stuffing and potential cross-contamination;
- 3. effect of bio-intervention strategy;
- 4. maceration or early stage of fermentation;
- 5. maturation or curing; and 6. packaging.

This is a generic model that has been customised for alheira sausage (IPB), salchichon (UCO), and Merguez sausage (UIZ and ISBST/UMA).



Model functions

The model consists of six functions:

- 1. Lot generation: it generates contaminated lots of sausage batter, whose prevalence and concentrations are dictated by the concentrations and prevalences of two major ingredients, such as meat and fat, and the mixed spices, taking into account the proportions in the formulation.
- 2. Stuffing: it simulates the potential cross-contamination that can take place by stuffing batter into contaminated casings, taking into account each of the following events: 2.1. cross-contamination occurring in lots of sausage batter already contaminated; 2.2. contamination occurring in lots of sausage batter that were not contaminated; 2.3. no crosscontamination occurring in lots of sausage batter already contaminated; and 2.4. no cross-contamination occurring in lots of sausage batter that were not contaminated.
- 3. Biointervention: it should be used either when a plant extract or a starter culture is added into batter, and assumes that it would cause a reduction in the pathogen population right after stuffing.

🚈 ARTISANER 🛛 🖈 Introduction 🎰 Batter 🖉 Stuffing 🏂 Biointervention 🏛 Maceration 🛞 Maturation & Packaging 💒 About



Proportion of main ingredient 1 (%)





🛿 Introduction 🍿 Batter 🖸 Stuffing 🏂 Biointervention 🏦 Maceration 🛠 Maturation & Packaging 💒 About ARTISANER

Select inputs

Set a random seed

299 200 210 220 230 240 250 260 270 280 290 299

Probability of having casings contaminated on a lot basis



Numbers of microbes on the internal surface of a single casing (CFU)



Dispersion factor of the Beta distribution



Variab	ility of mean o	ontamination between lots after stuffing)	Variability of contaminated sausages after stuffing				
Preva [1] (alence of con 0.69336	taminated lots after stuffing		Prevalence of contaminated sausages after stuffing [1] 0.6921064				
Sumr	mary statistics	Counts distribution (CFU/g)		Summary statistics	Counts distribution (CFU/g)			
	Statistics	Counts (CFU/g)	Counts (log10 CFU/g) ≬		•			
1	Minimum	0.1308	-0.8833	4000	8			
2	pct 2.5th	0.1327	-0.8771		5000			
3	Mean	275.3	1.889	3000	60000			
4	Median	397.6	2.599	2000				
5	pct 97.5th	548.8	2.739		30000			
6	Maximum	597.7	2.777	1000				
				0-0.8-0.4	4 0.0 0.4 0.8 0 1000 2000 3000 4000			

🛿 Introduction 🅁 Batter 🖉 Stuffing 🏂 Biointervention 🏦 Maceration 🛞 Maturation & Packaging 🎂 About ARTISANER Variability of mean contamination between lots before maceration Variability of contaminated sausages before maceration Select inputs prevalence of contaminated lots before maceration Prevalence of contaminated sausages before maceration Set a random seed [1] 0.69336 [1] 0.6921064 399 Summary statistics Counts distribution (CFU/g) Summary statistics Counts distribution (CFU/g) 300 310 320 330 340 350 360 370 380 390 399 Minimum log10 reduction Statistics Counts (CFU/g) Counts (log10 CFU/g) 5 4000 Minimum 0.1308 -0.8833 1 0 0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 90000 Mode log10 reduction pct 2.5th 0.1327 -0.8771 2 5 3000 275.3 Mean 1.889 3 0 0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 60000 Median 397.6 2.599 4 2000 Maximum log10 reduction 5 pct 97.5th 548.8 2.739 30000 1.5 2 2.5 3 3.5 4 4.5 5 0 0.5 1000 597.7 2.777 6 Maximum Update 0 -0.8 -0.4 0.0 0.4 0.8 1000 2000 3000 4000 0

🛿 Introduction 🌐 Batter 🖉 Stuffing 🏂 Biointervention 🚊 Maceration 🛞 Maturation & Packaging 💒 About ARTISANER

Select inputs Set a random seed <u>_________________________________</u> 400 410 420 430 440 450 460 470 480 490 49

Maximum population density (log10 CFU/g)

4					7.5			10
		•		-)Ŧ	111	111	Т
4	4.75	5.5	6.25	7	7.75	8.5	9.25	10
Mean exponential growth								

Mean exponential growth rate at 5 °C (1/h)

0.036					1.2
\bigcirc					
0.01	0.248	0.486	0.724	0.962	1.2

St. error of the mean exponential growth rate at 5 °C (1/h)

700 0.96 0 0.120.24 0.48 0.72 1.2

1.2

Nominal minimum

Variability of mean contamination between lots after maceration							
Prevalence of contaminated lots after maceration [1] 0.69336							
Summ	nary statistics	Counts distribution (log10 CFU/g)					
	Statistics	Counts (CFU/g)	Counts (log10 CFU/g) 🔶				
1	Minimum	0.2589	-0.5869				
2	pct 2.5th	0.6814	-0.1666				
3	Mean	1.101e+4	3.025				
4	Median	1,995	3.300				
5	pct 97.5th	8.056e+4	4.906				
6	Maximum	4.793e+5	5.681				

Variability of contaminated sausages after maceration

Prevalence of contaminated sausages after maceration [1] 0.6921064



ARTISANER 🛿 Introduction 🕁 Batter Ø Stuffing 😕 Biointervention 🏛 Maceration 🛠 Maturation & Packaging 💒 About Variability of mean contamination between lots Variability of contamination in the packs Select inputs Prevalence of contaminated lots Prevalence of contaminated packs Set a random seed [1] 0.69336 [1] 0.69336 599 Lotter to to to to to to to to Counts distribution (log10 CFU/g) Counts distribution (log10 CFU/g) Summary statistics Summary statistics 500 510 520 530 540 550 560 570 580 590 599 Sausages pH at the start of Statistics Counts (CFU/g) Counts (log10 CFU/g) 8000 maturation 7 3.5 Minimum 0.02901 -1.537 1 3.5 3.9 4.3 4.7 5.1 5.5 5.9 6.3 6.7 7 pct 2.5th 0.1179 6000 -0.9285 2 Maturation time at low temperature (h) Mean 2,299 2.292 3 2 240 4000 Median 350.4 2.545 4 120 144 168 192 216 240 48 72 96 pct 97.5th 1.352e+4 4.131 5 Log10 of reference D (day) at 2000 pHref=7.0 Maximum 1.067e+5 5.028 6 -2 0 0.3 0.6 0.9 1.2 1.5 1.8 2.1 2.4 2.7 3 St. dev. of log10 of reference -0.4 0 2 -0.8 0.0 0.4 0.8 -2 4







ArtiSaneFood - Innovative bio-interventions and risk modelling approaches for ensuring microbial safety and quality of Mediterranean artisanal fermented foods

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European Commissior







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