New protocols for the production of high quality table olives

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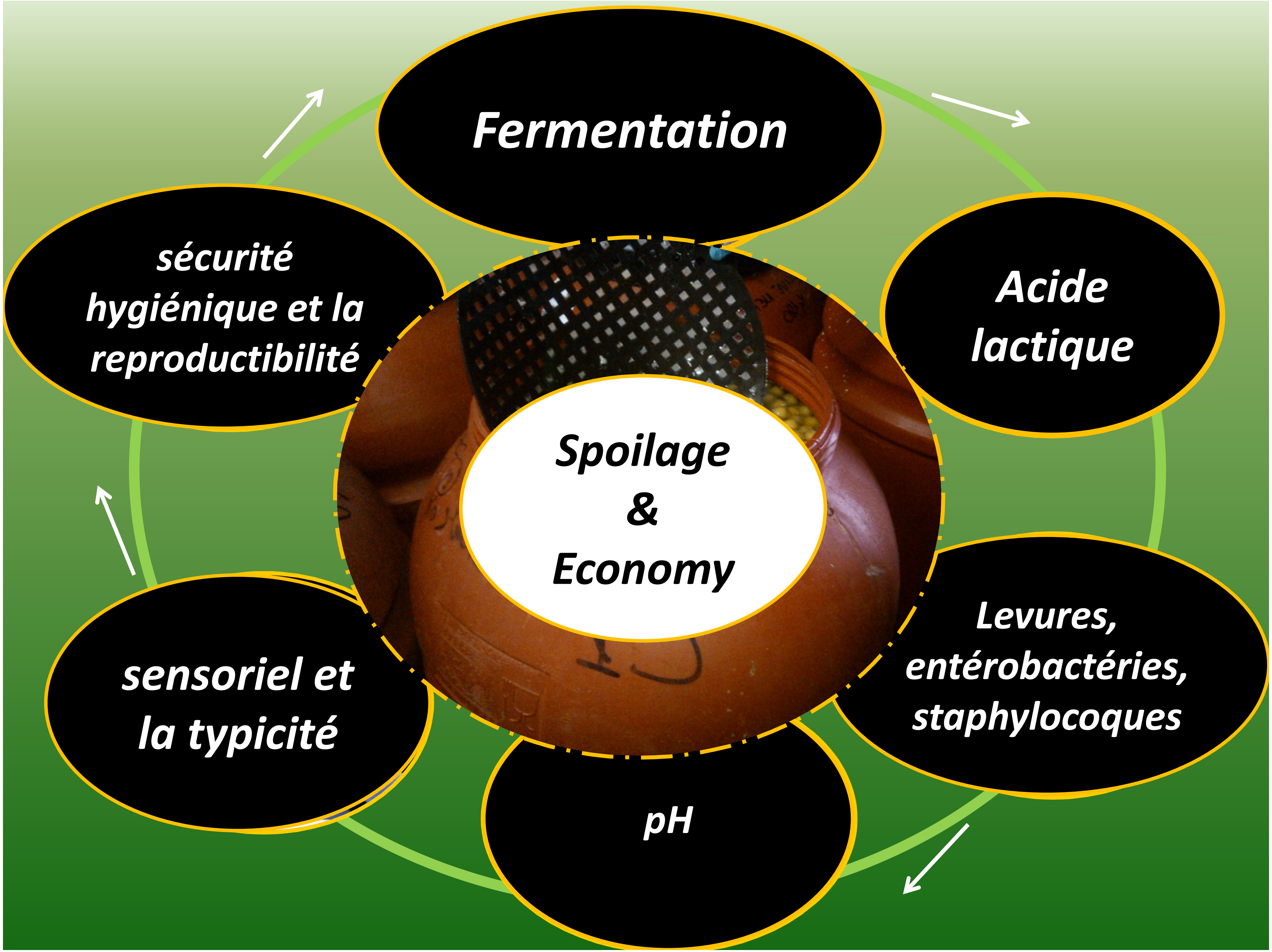

Fermentation
Acide lactique
Levures, entérobactéries, staphylocoques
pH
sensoriel et la typicité
sécurité hygiénique et la reproductibilité
Spoilage & Economy

Les olives de table & la production mondiale

2.550 MILLIONS ton per year

(IOOC, 2014/15 estimated data)

34%
Spain-Greek-Italy

Spain 515000 ton

Greek 235000 ton

Italy 79500 ton

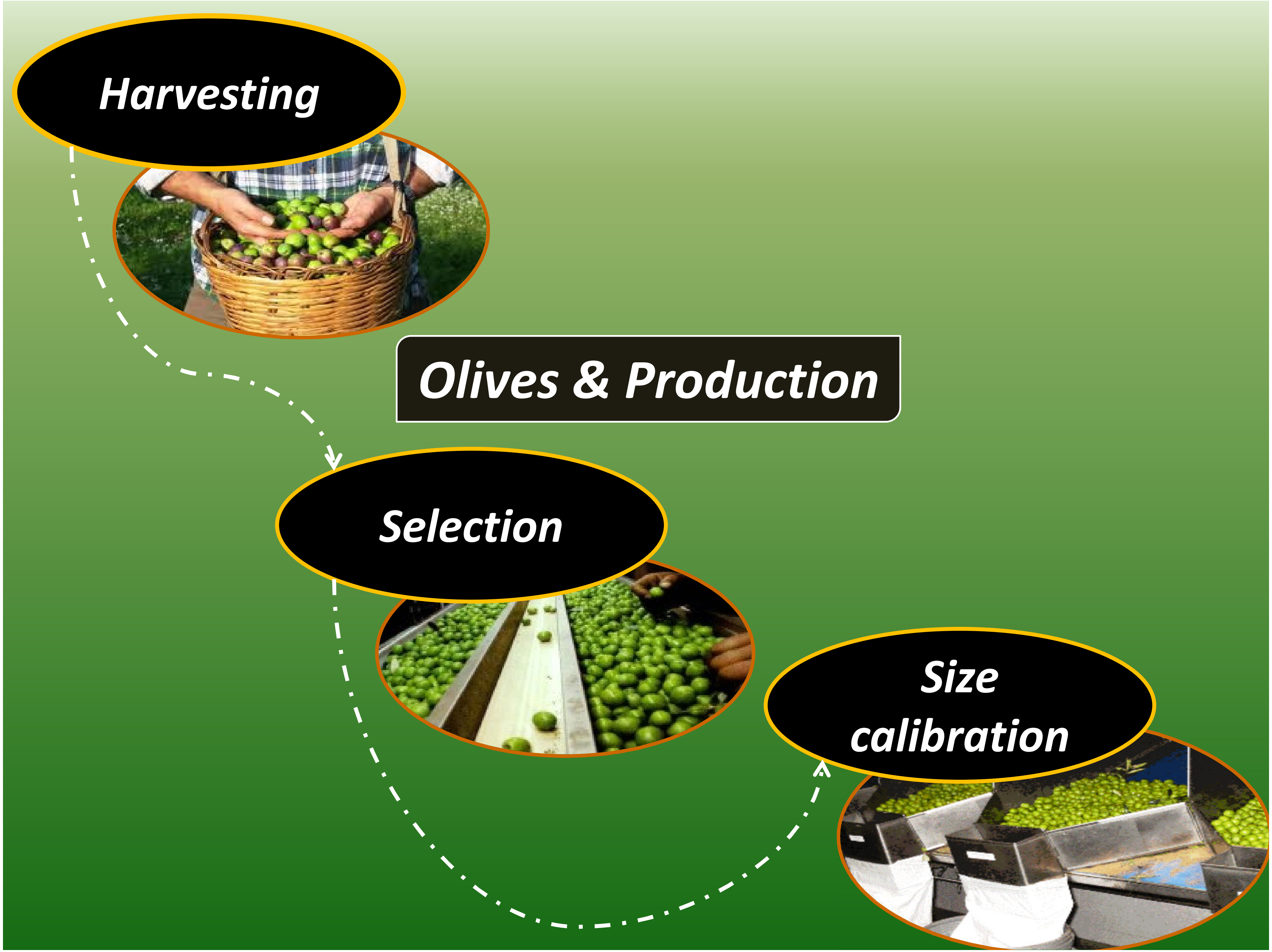
Portugal 16800 ton

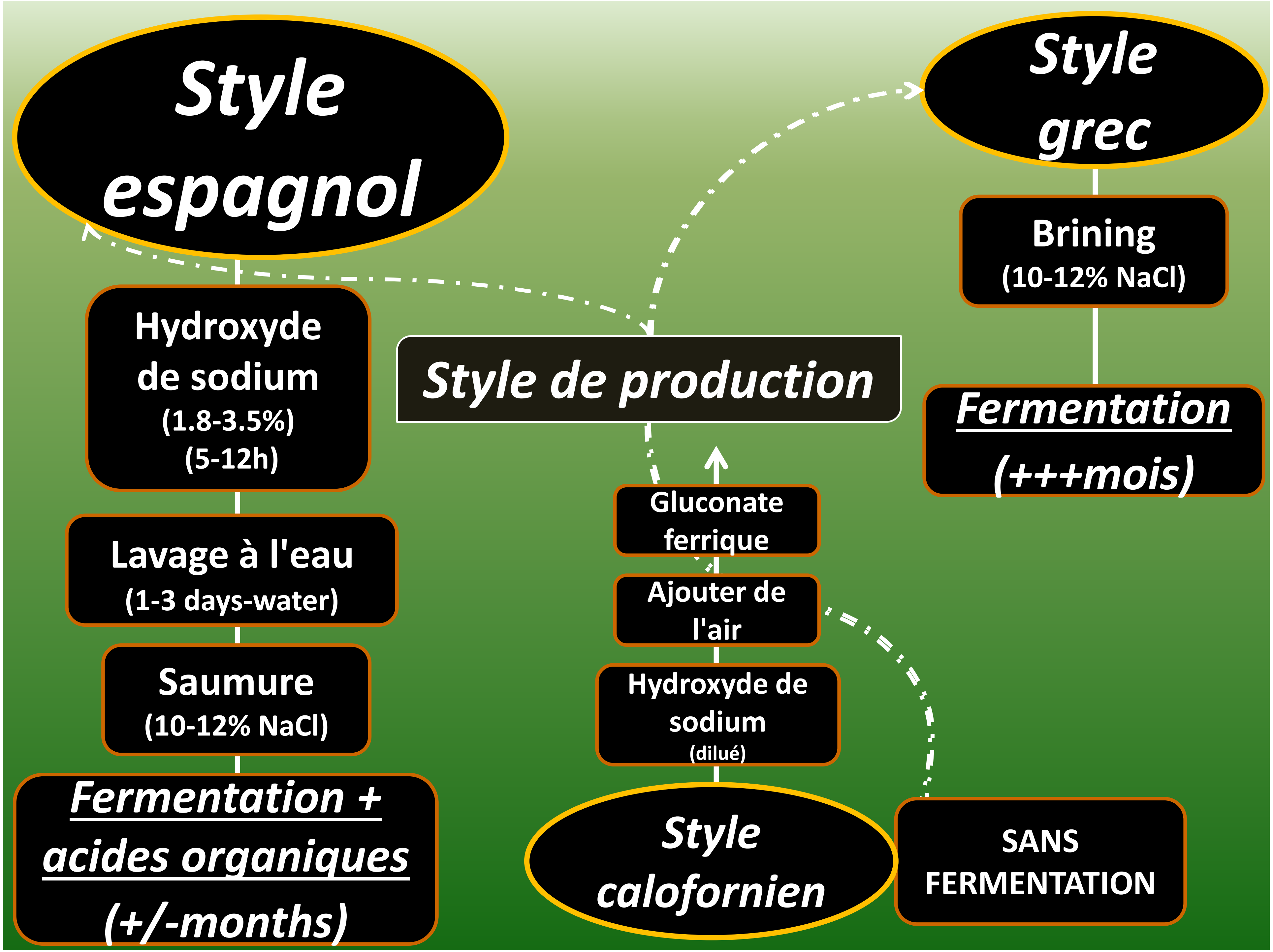
French 1100 ton

Les ol

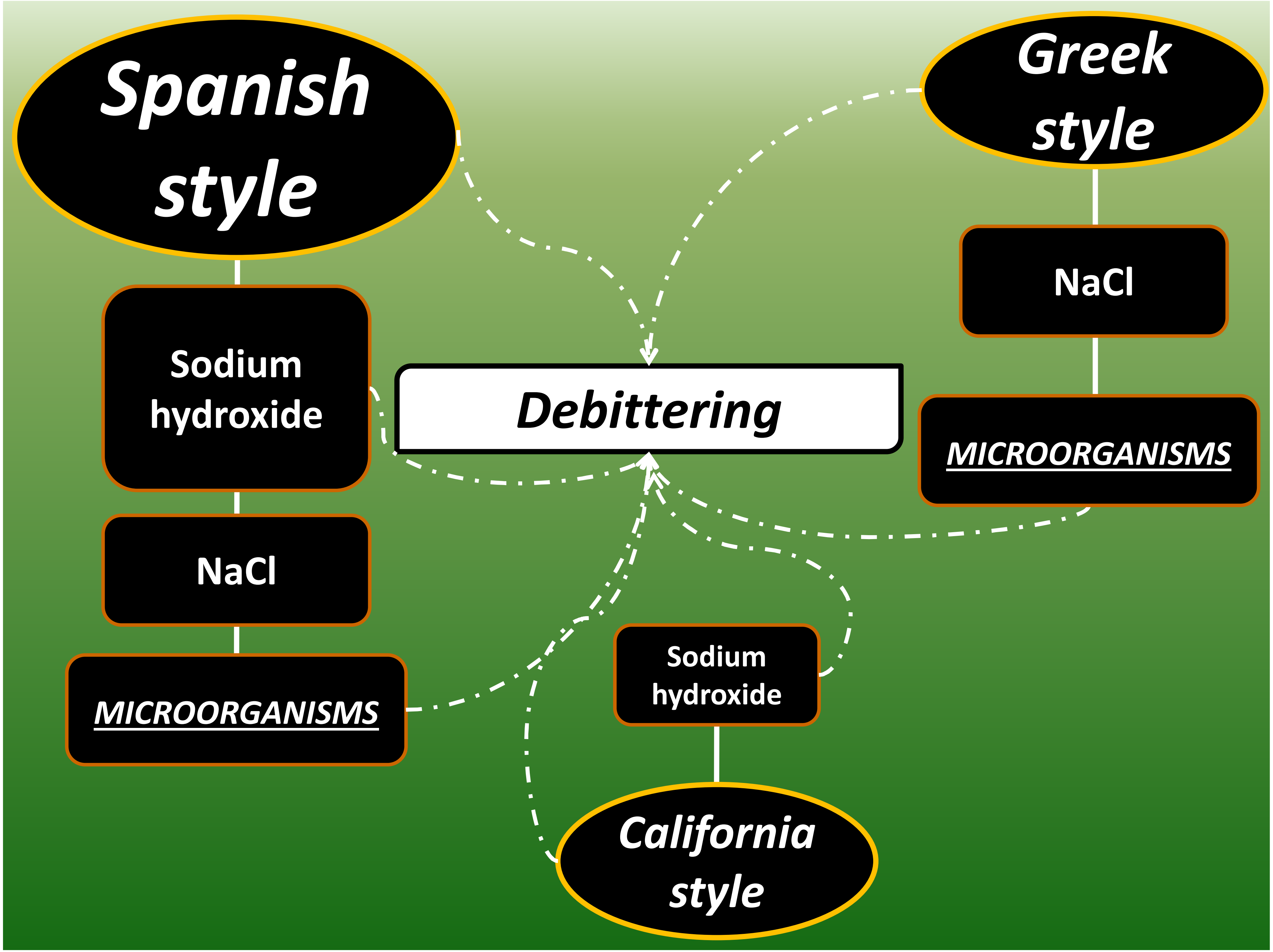
ket

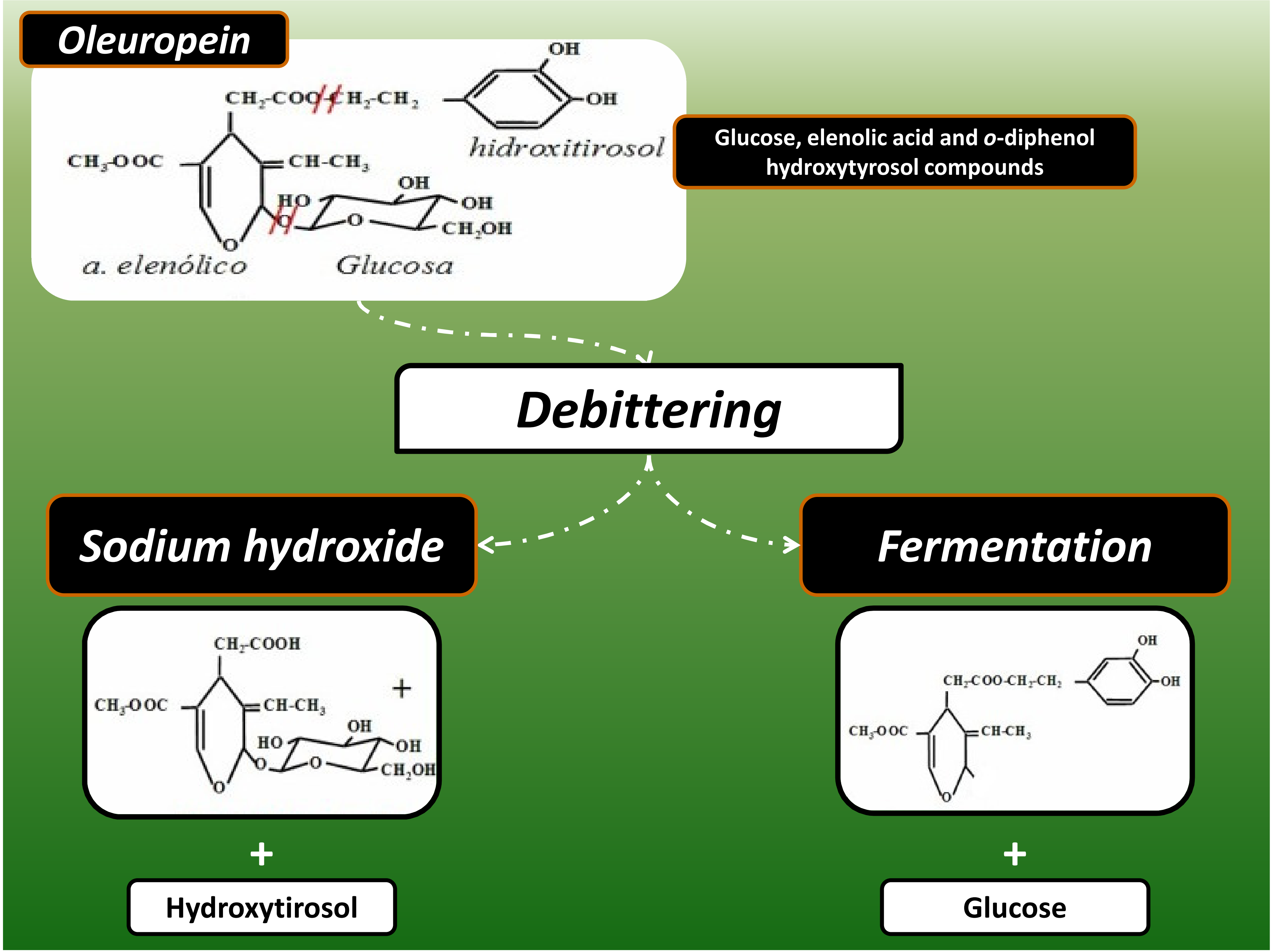
Les olive... ...arket


Olives & Production
Harvesting
Selection
Size calibration



Style espagnol
Hydroxyde de sodium
(1.8-3.5%)
(5-12h)
Lavage à l'eau
(1-3 days-water)
Saumure
(10-12% NaCl)
Fermentation + acides organiques
(+/-months)
Style de production
Gluconate ferrique
Ajouter de l'air
Hydroxyde de sodium
(dilué)
Style calofornien
SANS FERMENTATION
Style grec
Brining
(10-12% NaCl)
Fermentation
(+++mois)


Spanish style
Sodium hydroxide
NaCl
MICROORGANISMS
Debittering
Greek style
NaCl
MICROORGANISMS
Sodium hydroxide
California style


Oleuropein
hidroxitirosol
a. elenólico
Glucosa
Glucose, elenolic acid and o-diphenol hydroxytyrosol compounds
Debittering
Sodium hydroxide
Fermentation
Hydroxytirosol
Glucose

Fiberglass tank to ferment olive in brine

Spanish/Greek style

Tank to ferment olive in brine

Spanish/Greek style

Tank to ferment olive in brine

Spanish/Greek style

Tank to ferment olive in brine

Spanish/Greek style

Transfer of olives from tank after fermentation

Spanish/Greek style

Transfer of olives from tank after fermentation

Spanish/Greek style

Transfer of olives from tank after fermentation

Spanish/Greek style

Calibration of size of fermented olive

Spanish/Greek style

Fermented olives
«Nocelllara del Belice olive cultivar»

Spanish/Greek style

Distribution of fermented olives

Spanish/Greek style

Fermented GREEN olives

Spanish/Greek style

Fermented BLACK olives

Spanish/Greek style

Addition of alkaline solution and iron salts

California style

Transfer into tank for the oxidation

California style

Tank for the oxidation

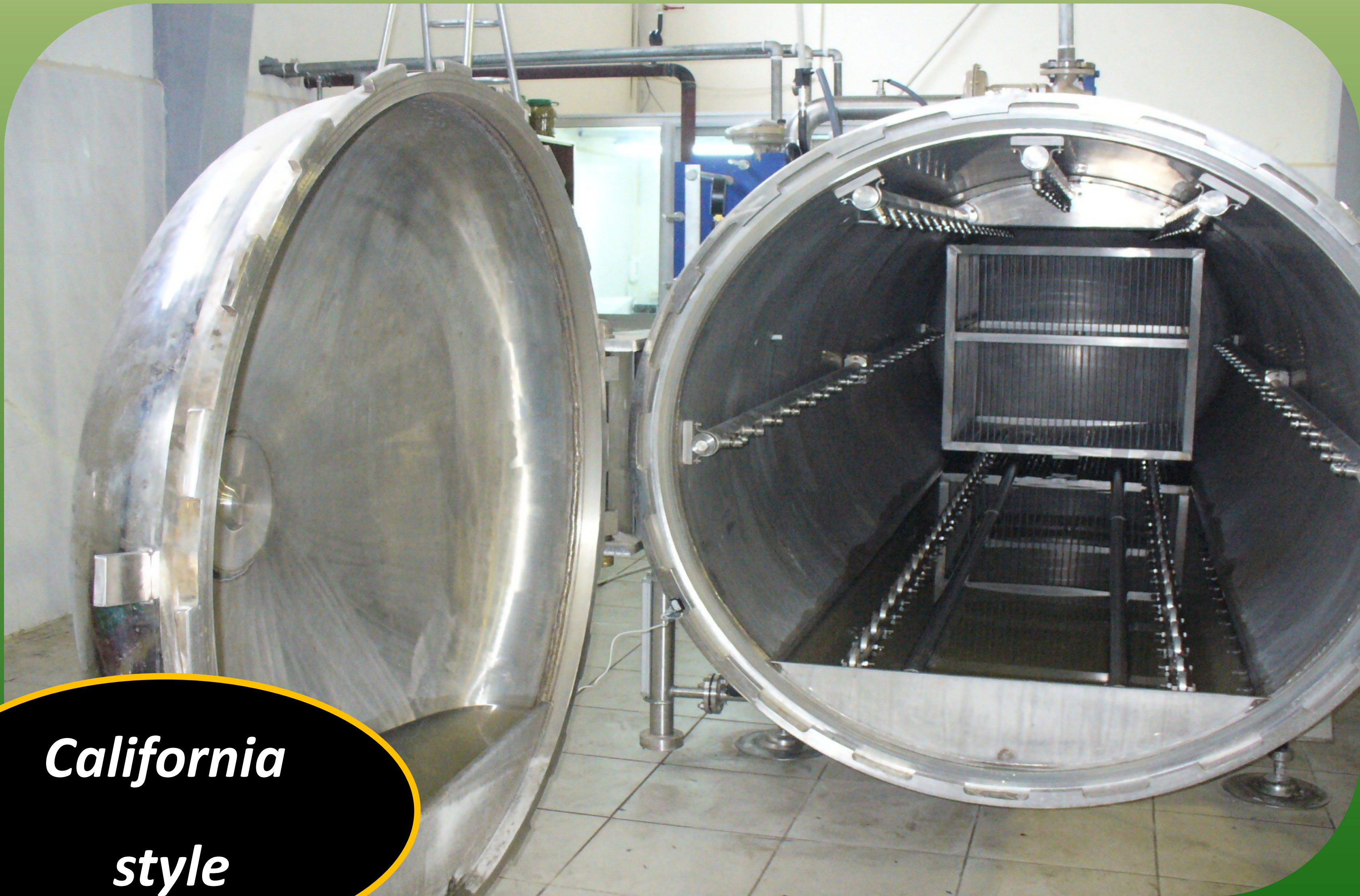
California style

Sterilization

California

style

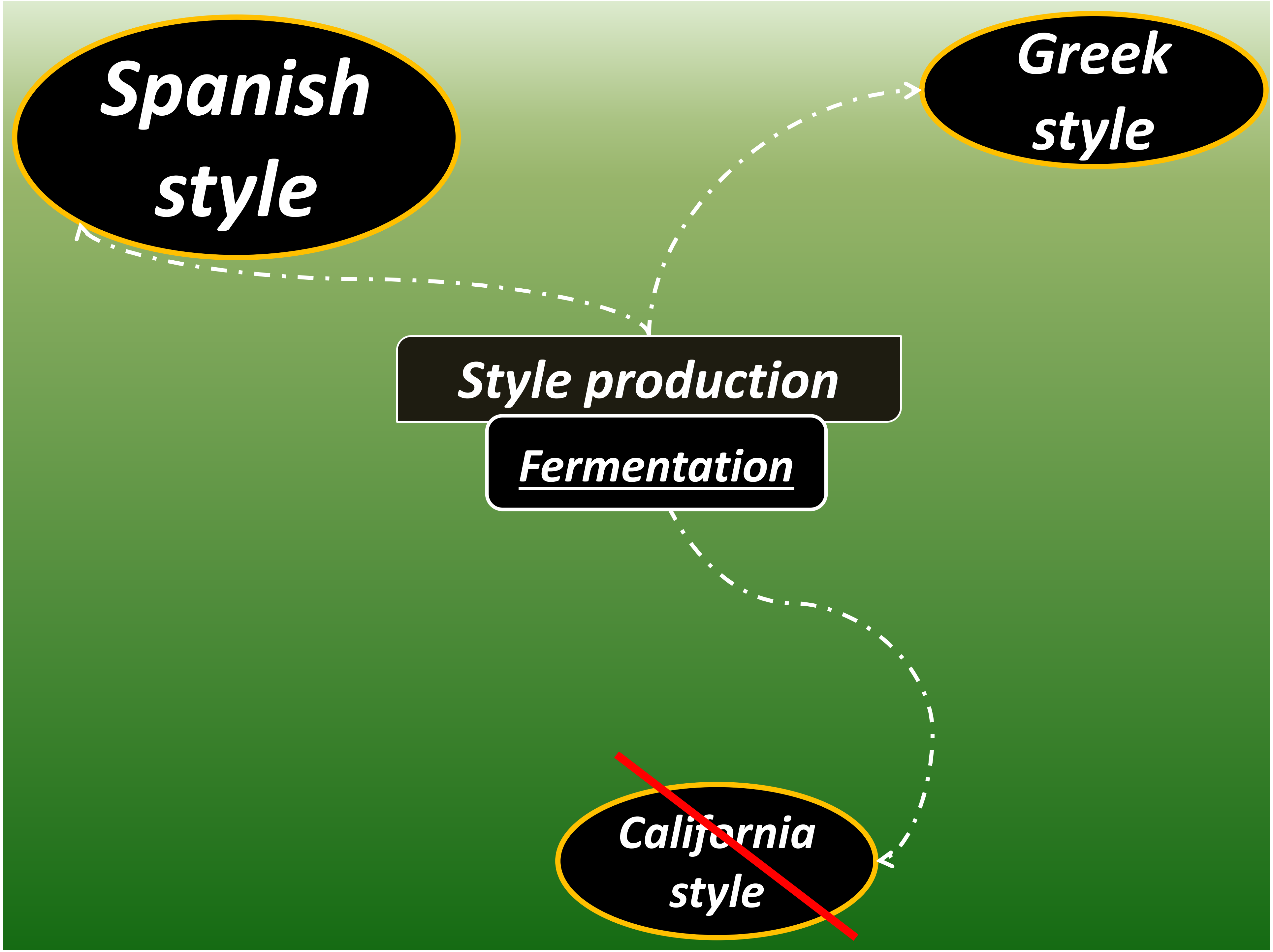
Sterilization



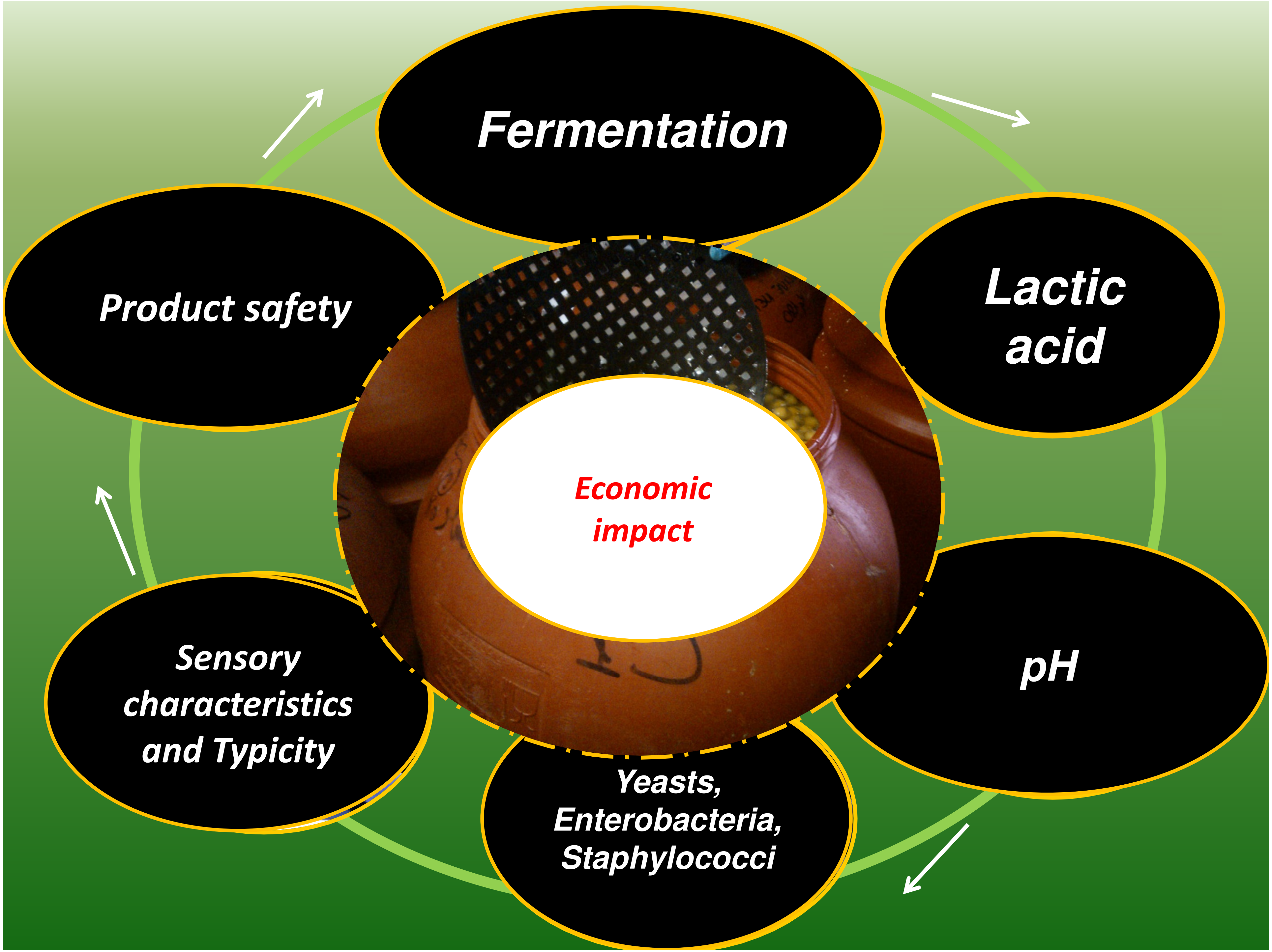
California style

Pastorization

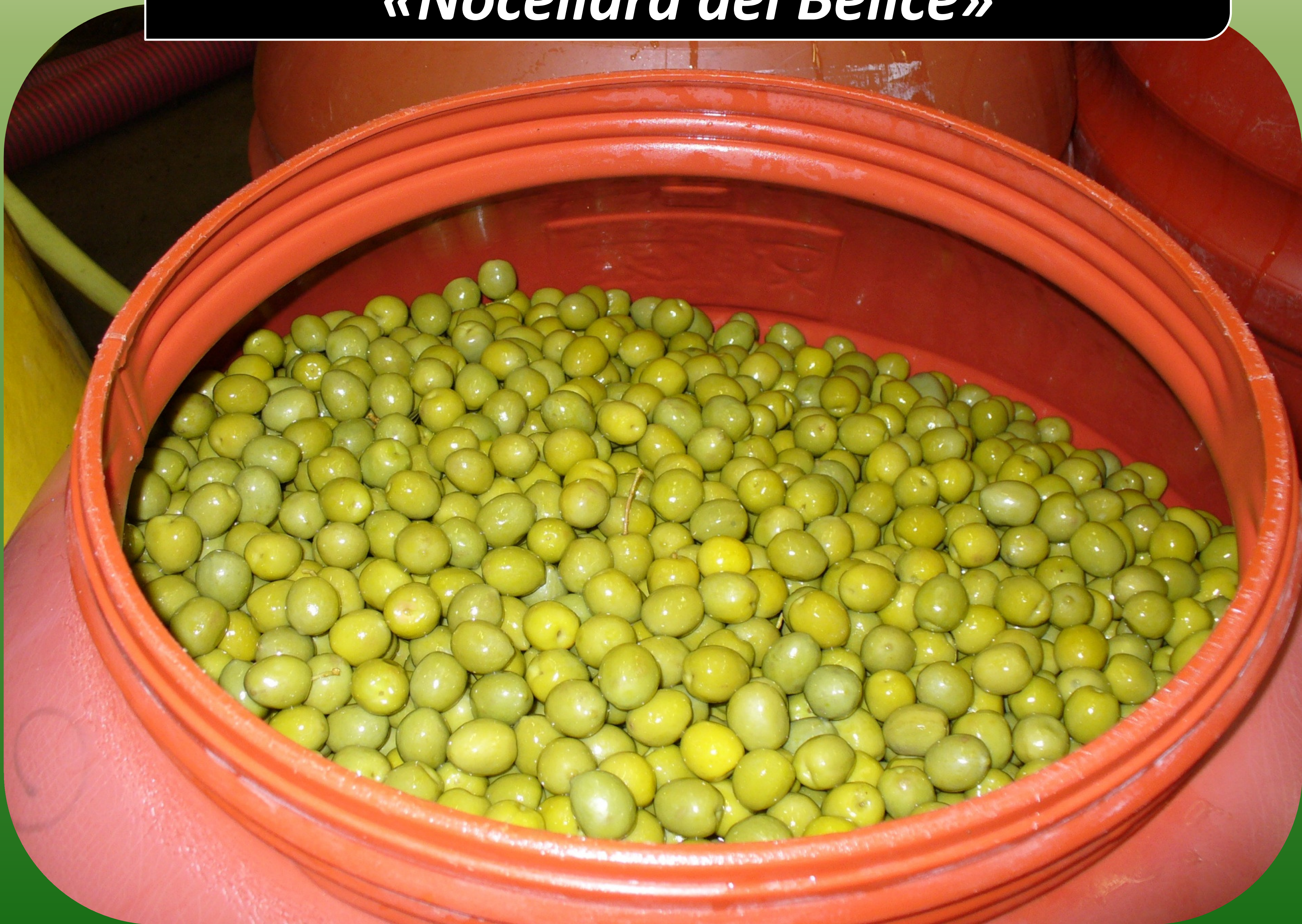
California style

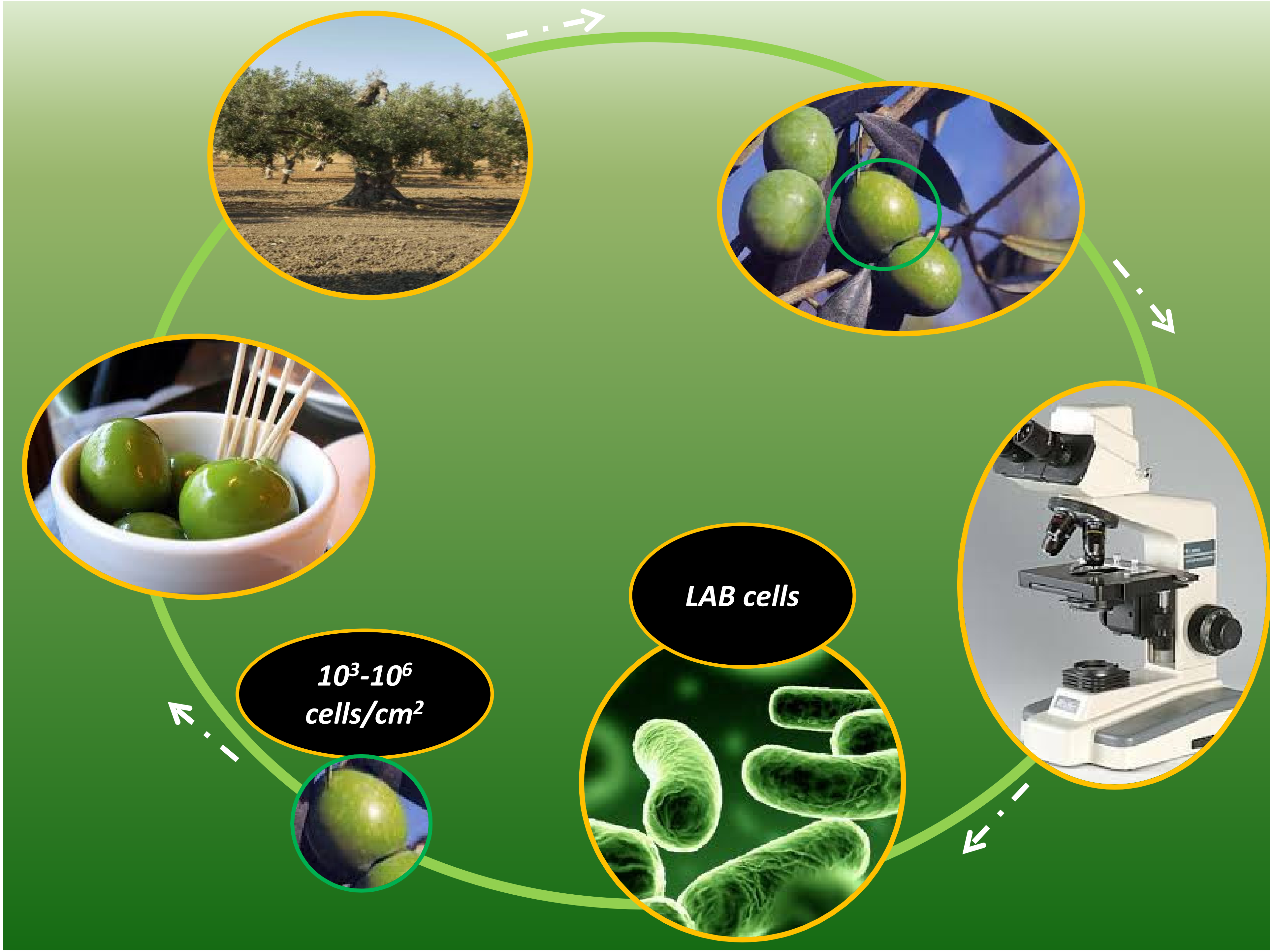


Spanish style
Greek style
Style production
Fermentation
California style


Fermentation
Lactic acid
pH
Yeasts, Enterobacteria, Staphylococci
Sensory characteristics and Typicity
Product safety
Economic impact

Fermented olives «Nocellara del Belice»




LAB cells
10^3-10^6 cells/cm^2

Microorganisms

Lactic acid bacteria

Lactobacillus spp.
Enterococcus spp.
Pediococcus spp.
Leuconostoc spp.

Yeasts

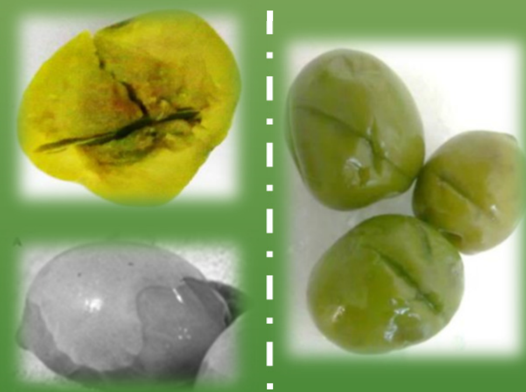
Saccharomyces spp.

Pichia spp.

Rhodotorula spp.

Microrganismi pro-tecnologici

Alterative or pathogenic



Pseudomonas spp.
Staphylococcus spp.
Enterobacteriaceae
Bacillus spp.

Lactic acid bacteria and Yeasts

Lactobacillus plantarum

Lactobacillus pentosus

Sugars

- *Saccharomyces cerevisiae*
- *Wickerhamomyces anomalus*
- *Candida boidinii*
- *Candida diddensiae*
- *Pichia galeiformis*
- *Pichia membranefaciens*
- *Kluyveromyces lactis*

Homofermentants (Lactic acid)

Heterofermentants :

- **Facultative (lactic acid, ethanol, acetic acid)**
- **Obligated (+CO_2)**

Ethanol and CO_2 production

Role of yeasts

Protechnological

Improved organoleptic characteristics of the fruit (glycerol, ethanol, alcohols, esters and other volatile organic compounds)

Polyphenol degradation (β-glucosidase)

Biocontrol agents

Antioxidant action (prevents oxidation of fatty acids and formation of hydrogen peroxide)

The use of yeast starter cultures is limited or almost absent !!!!

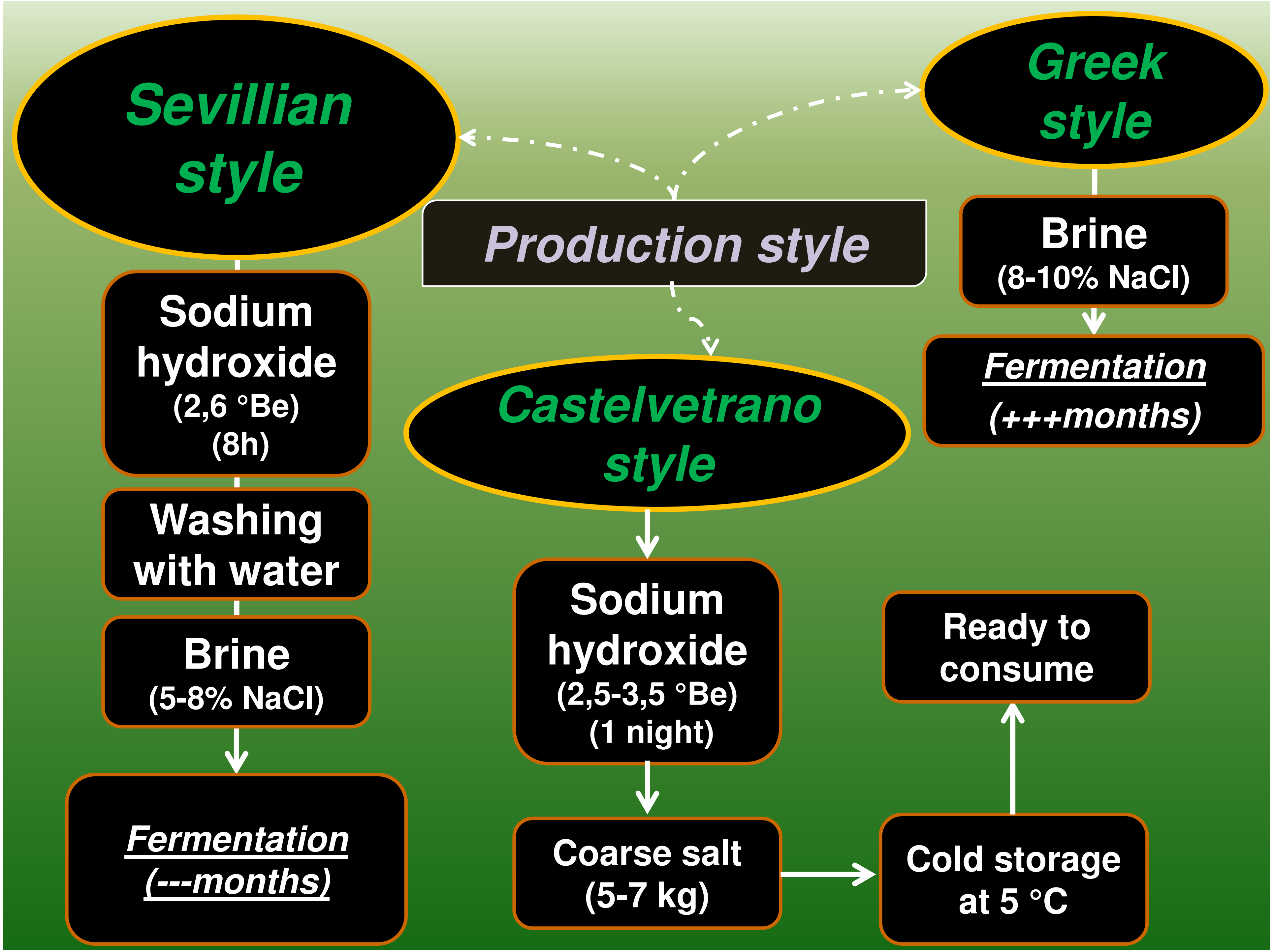
Alterative

Yeasts > LAB

Lower aromaticity (low production of volatile organic compounds)

Gas pocket o Alambrado (CO_2 accumulation)

Consistency of pulp (polygalacturonase production)



Sevillian style
Production style
Greek style
Sodium hydroxide (2,6 °Be) (8h)
Washing with water
Brine (5-8% NaCl)
Fermentation (---months)
Castelvetrano style
Sodium hydroxide (2,5-3,5 °Be) (1 night)
Coarse salt (5-7 kg)
Cold storage at 5 °C
Ready to consume
Brine (8-10% NaCl)
Fermentation (+++months)

Spontaneous fermentation

High microbial biodiversity

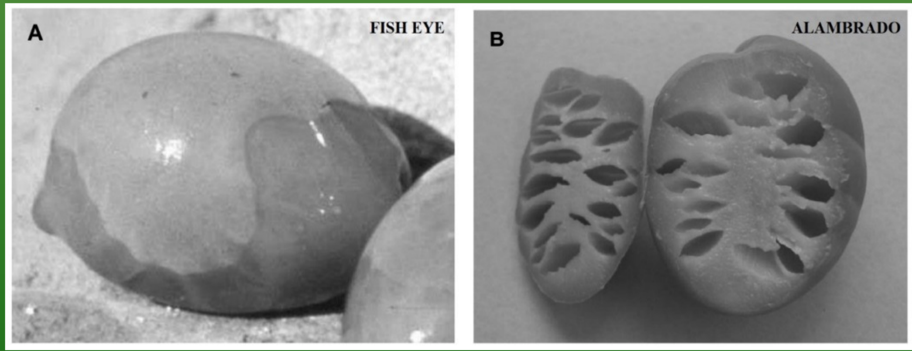
High sensory complexity

Sevillian style	***Lb. pentosus, Lb. plantarum*** ***Leuconostoc mesenteroides***
Greek style	***C. boidinii, C. diddensiae, C. membranaefaciens, Kluyveromyces lactis, Pichia kluyveri, Pichia membranaefaciens , Rhodotolura glutinis***

Processi imprevedibili

- **ZAPATERA (*Propionibacterium* sp)**
- **PULP SOFTENING (*B. pumilus*)**
- **ANOMALOUS FERMENTATIONS (off-flavours)**
- **FISH-EYES (o GAS POCKET)**
- **ALAMBRADO (fessurazione polpa)**

Danni economici (40%)



Spontaneous fermentation

Distribution of microbial groups

1st

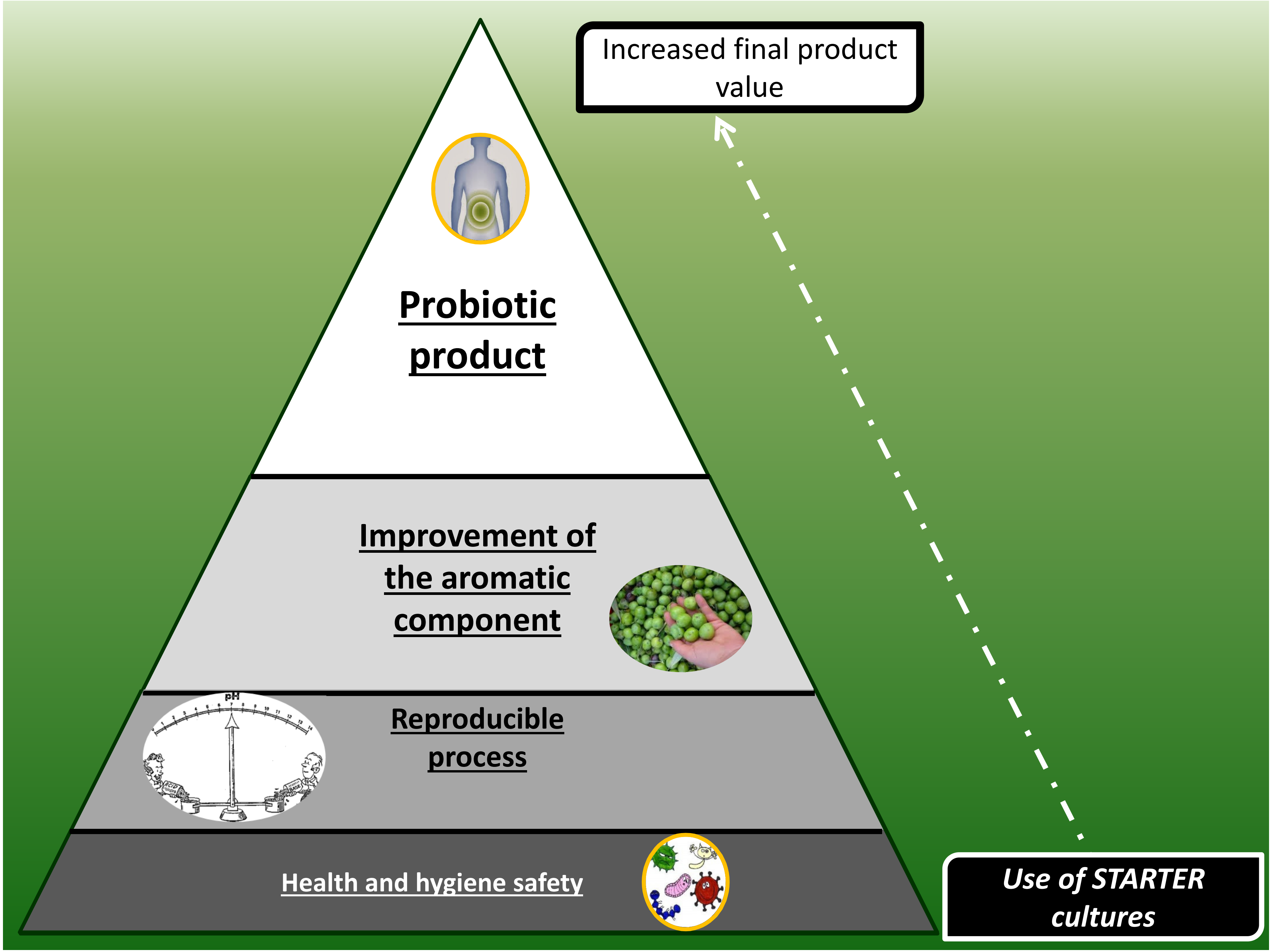
pH: 8.5-7.0
> *Enterobacteriaceae*

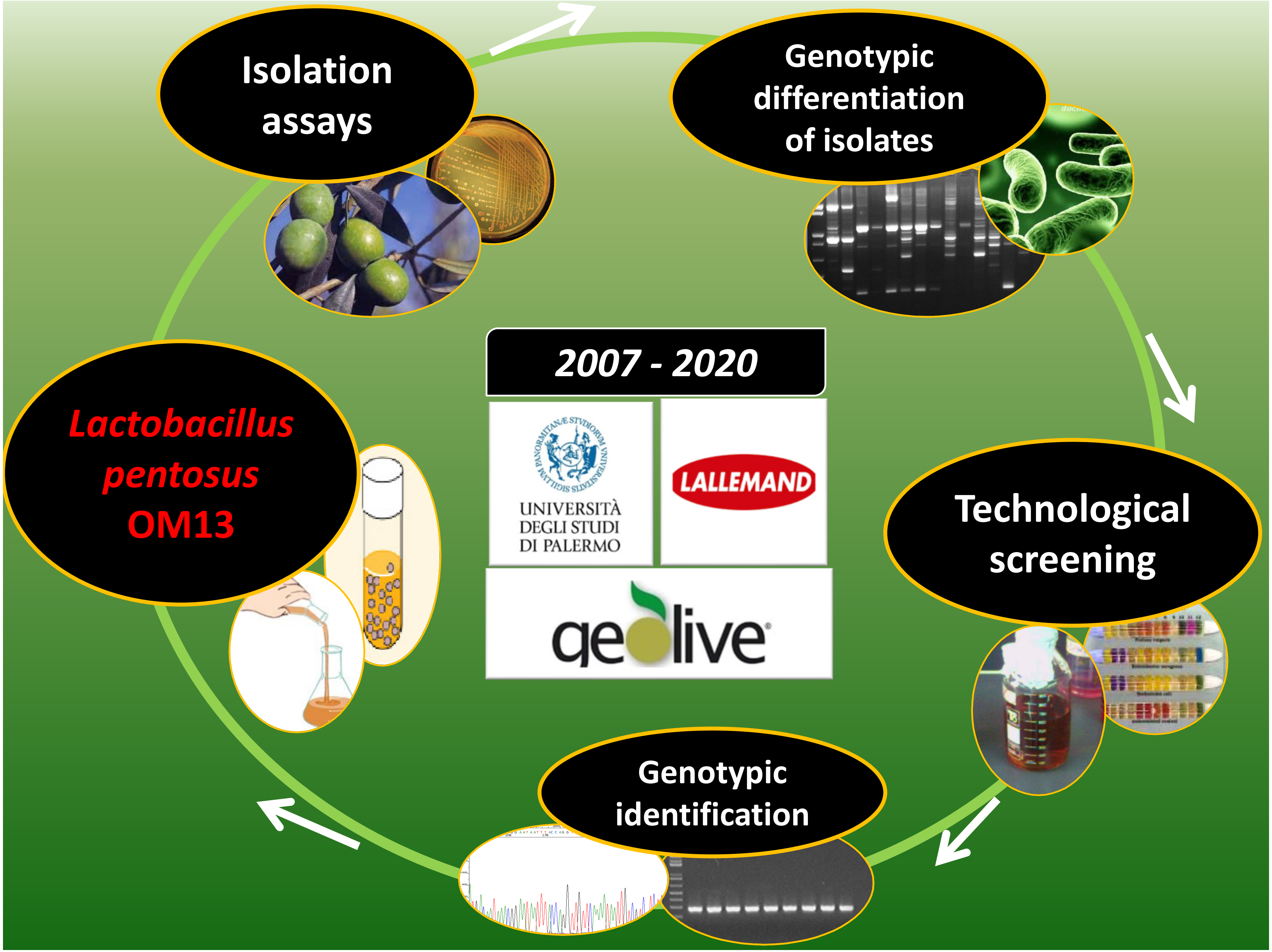
2st

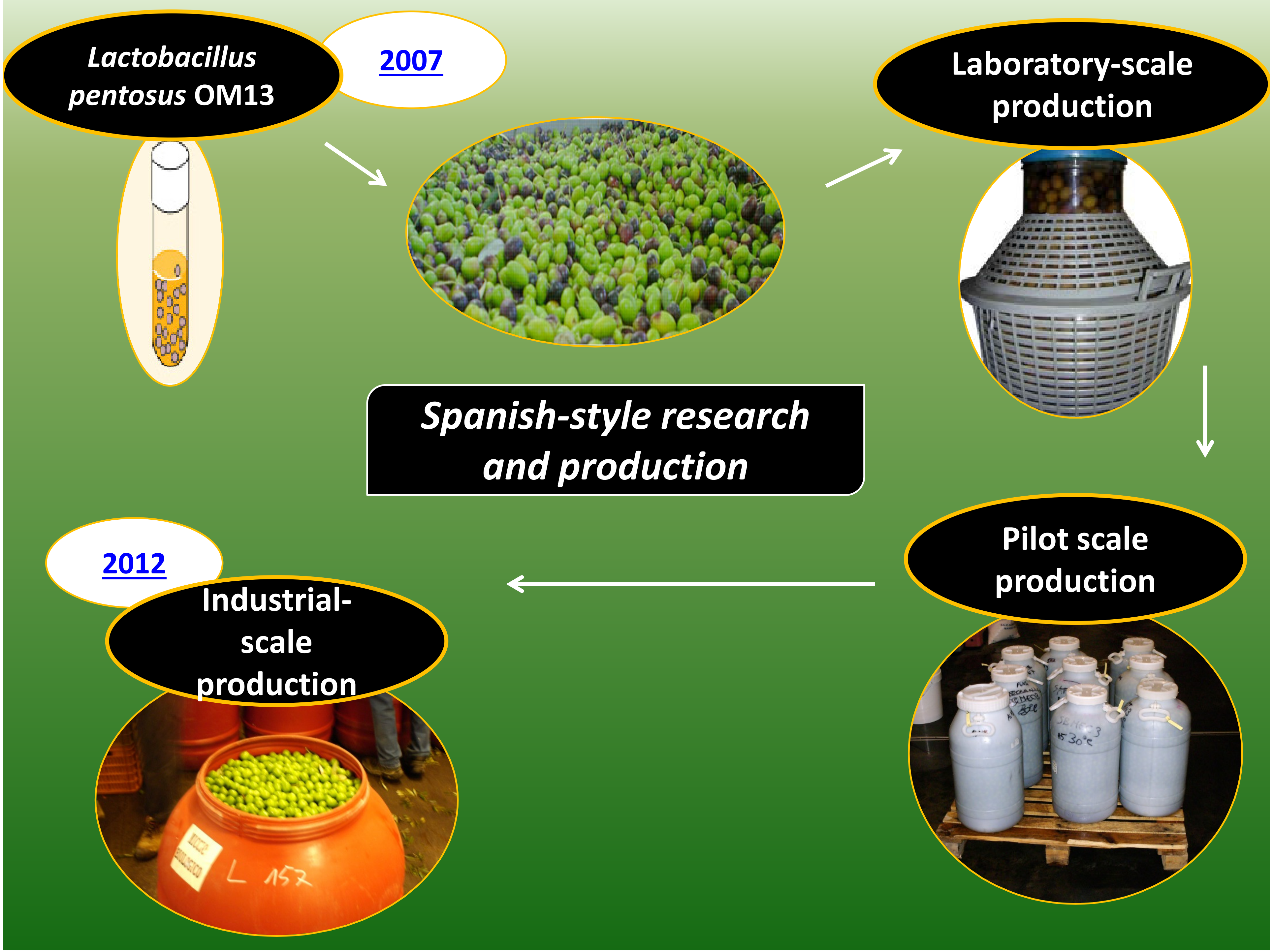
pH: 6.0-5.0
> LAB

3st

pH: 5.0-4,0 e T°<15
< LAB
> Yeasts


Increased final product value
Probiotic product
Improvement of the aromatic component
Reproducible process
Health and hygiene safety
Use of STARTER cultures


Isolation assays
Genotypic differentiation of isolates
Technological screening
Genotypic identification
Lactobacillus pentosus OM13
2007 - 2020
UNIVERSITÀ DEGLI STUDI DI PALERMO
LALLEMAND
geolive


Lactobacillus pentosus OM13
2007
Laboratory-scale production
Spanish-style research and production
Pilot scale production
2012
Industrial-scale production

1

pH

How to manage?

2

Inoculum starter strain

When to do it?

3

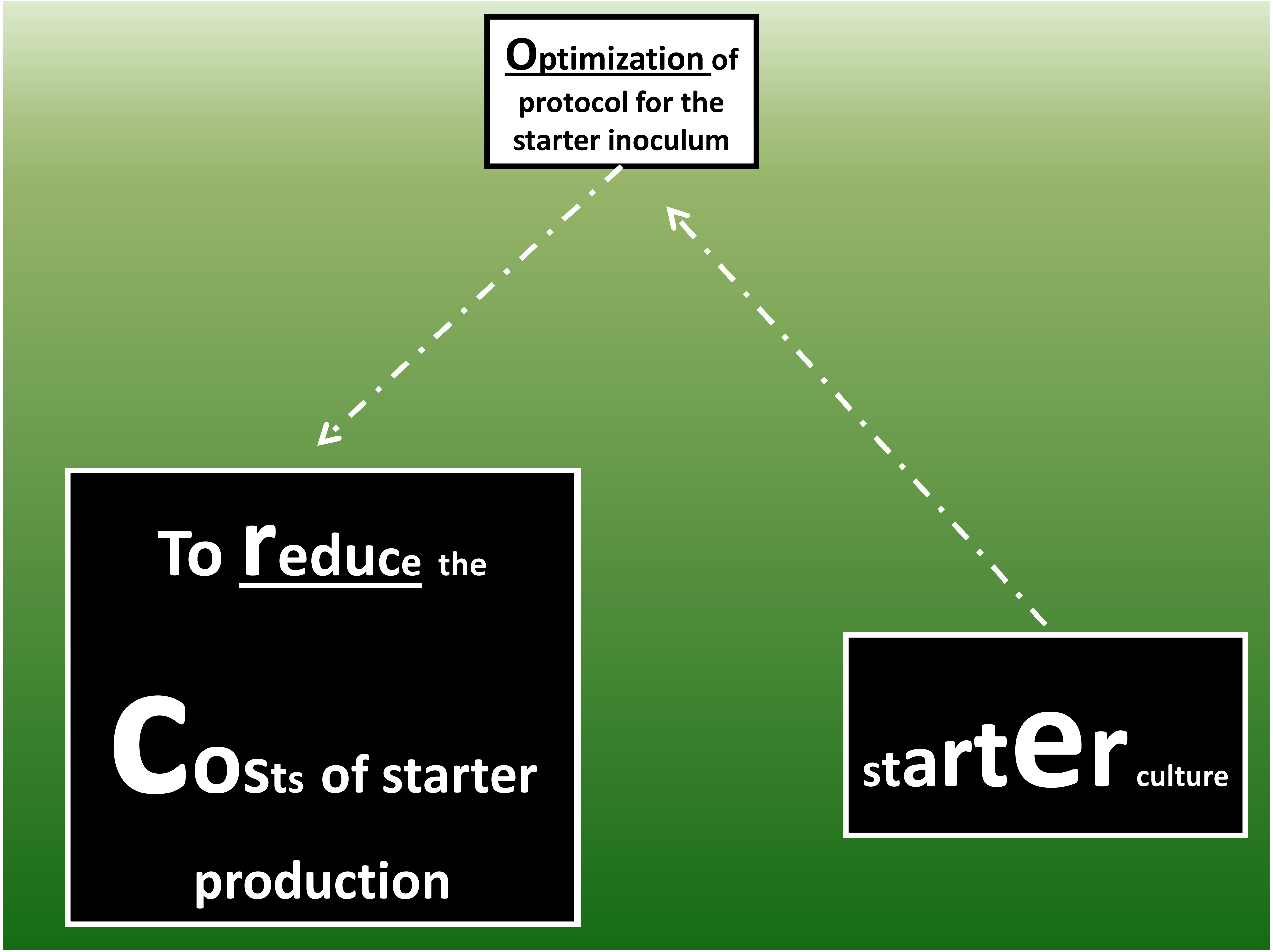
Fermentation

How to optimise it?

4

Economic losses

- Safety of table olives
- Sensory quality

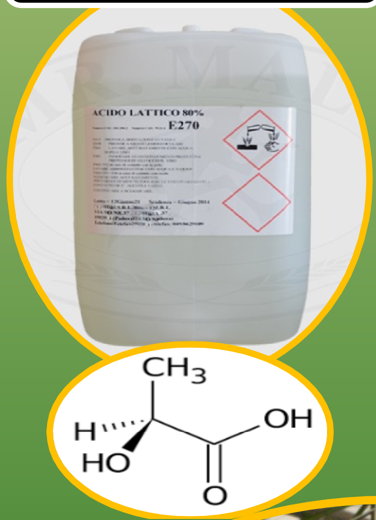


Optimization of protocol for the starter inoculum
To reduce the
Costs of starter production
starter culture

Lallemand

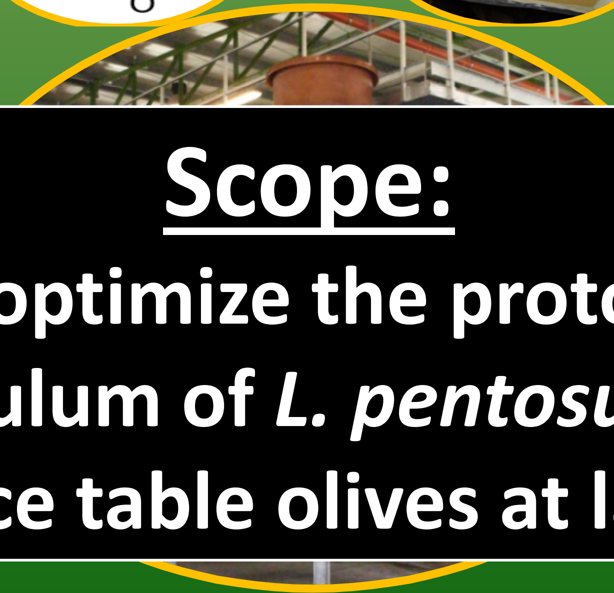
L. pentosus OM13

Lactic acid



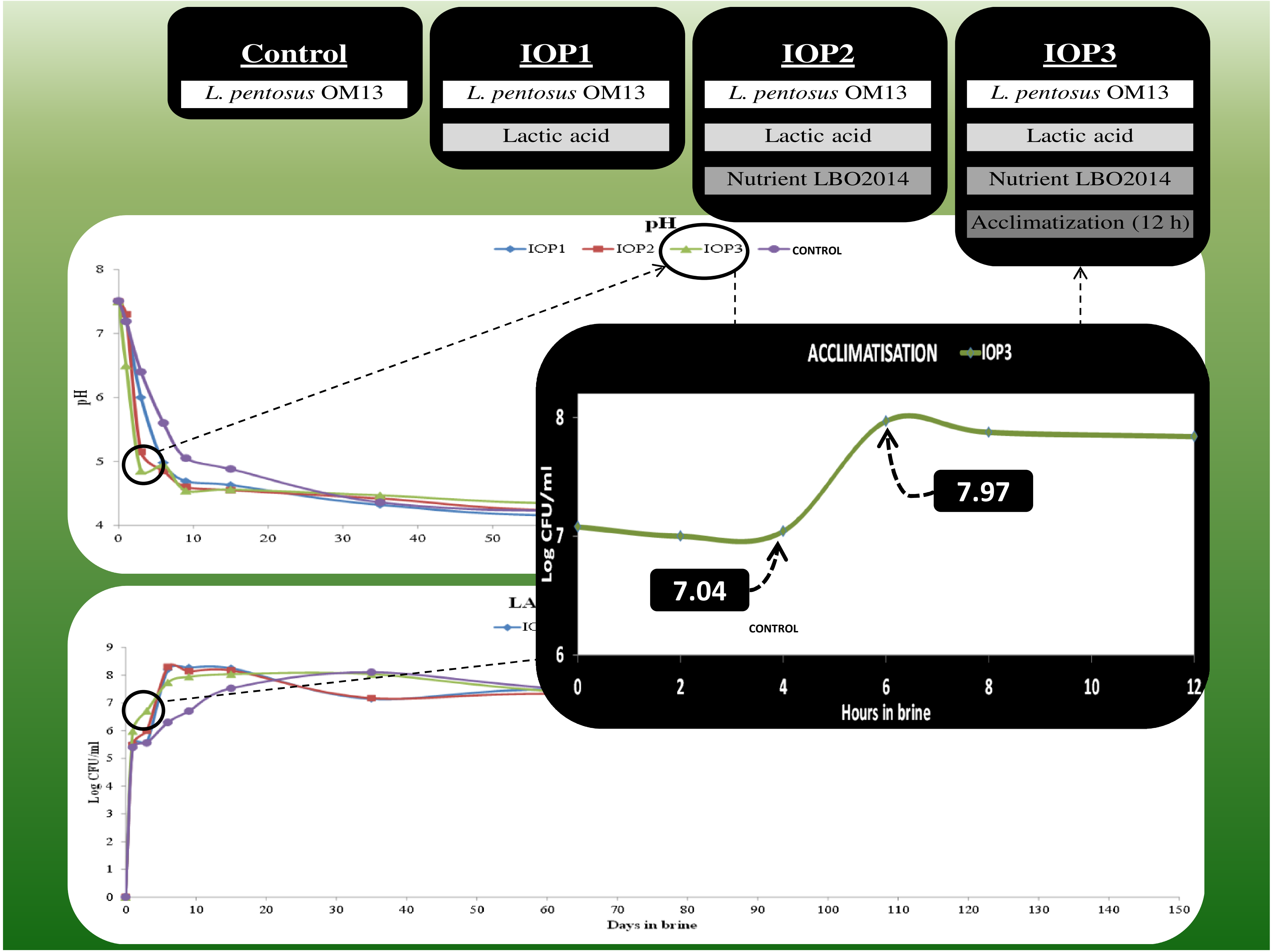
Nutrient LPO2014

Acclimatisation



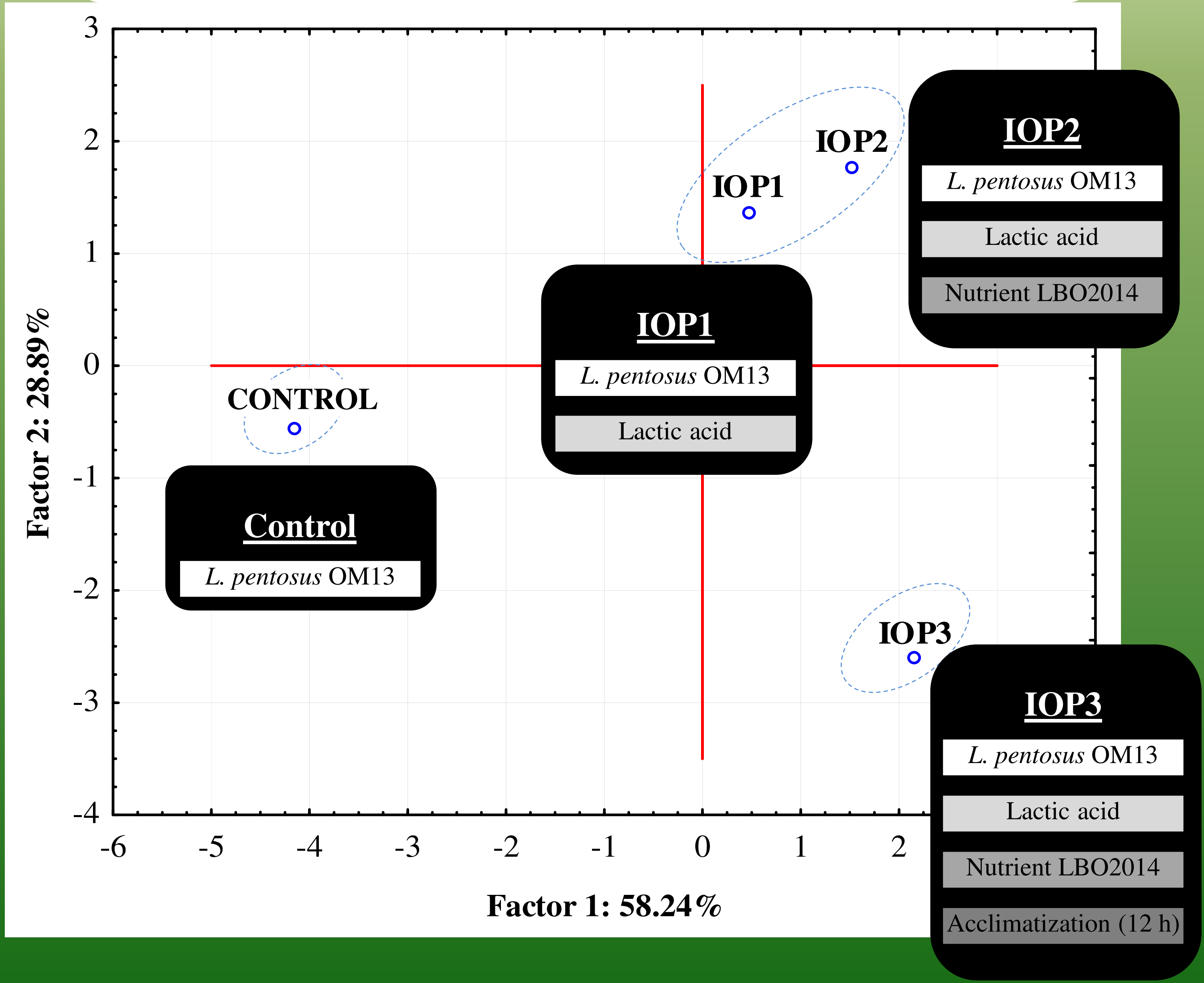
<u>Scope:</u>

to optimize the protocol
for inoculum of *L. pentosus* OM13
to produce table olives at large-scale

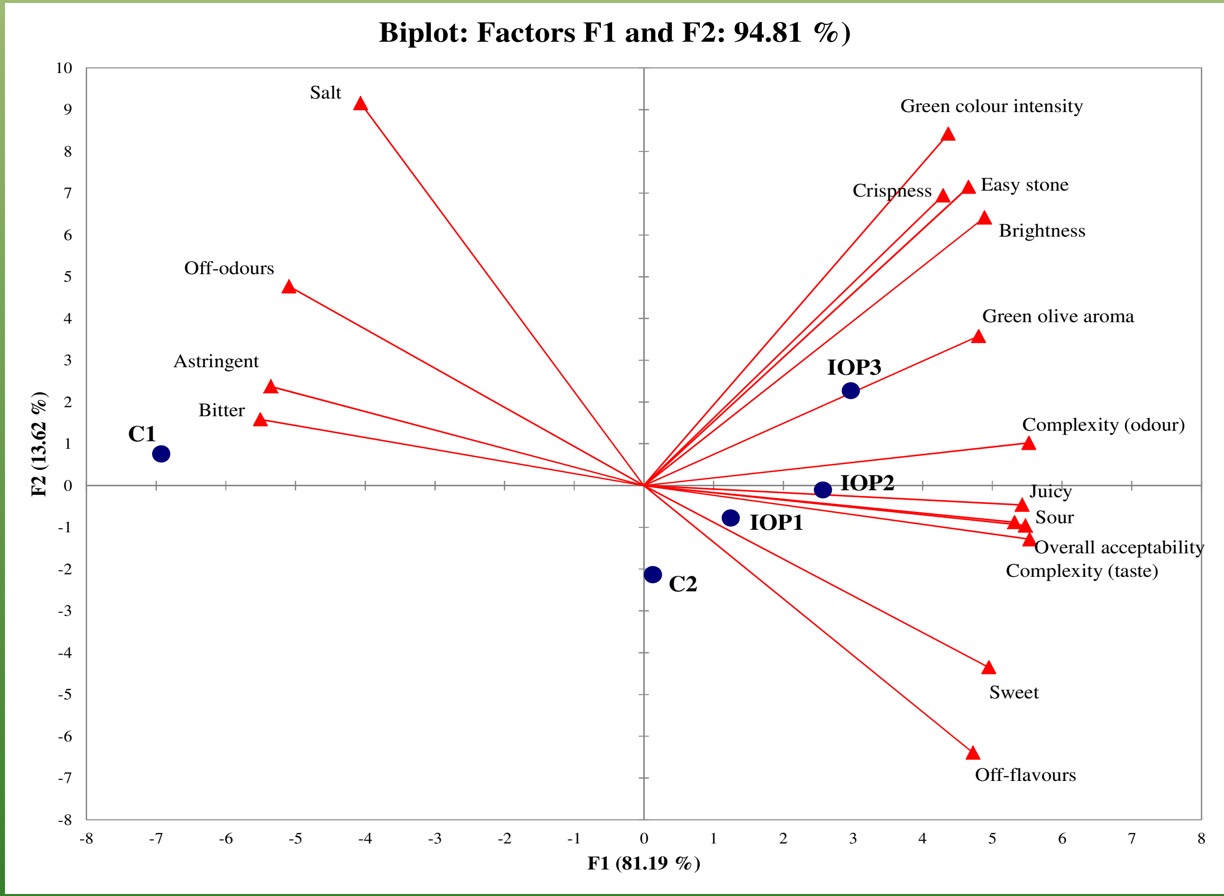


Control
L. pentosus OM13
IOP1
L. pentosus OM13
Lactic acid
IOP2
L. pentosus OM13
Lactic acid
Nutrient LBO2014
IOP3
L. pentosus OM13
Lactic acid
Nutrient LBO2014
Acclimatization (12 h)
pH
IOP1
IOP2
IOP3
CONTROL
ACCLIMATISATION
IOP3
Log CFU/ml
7.97
7.04
CONTROL
Hours in brine
Log CFU/ml
Days in brine

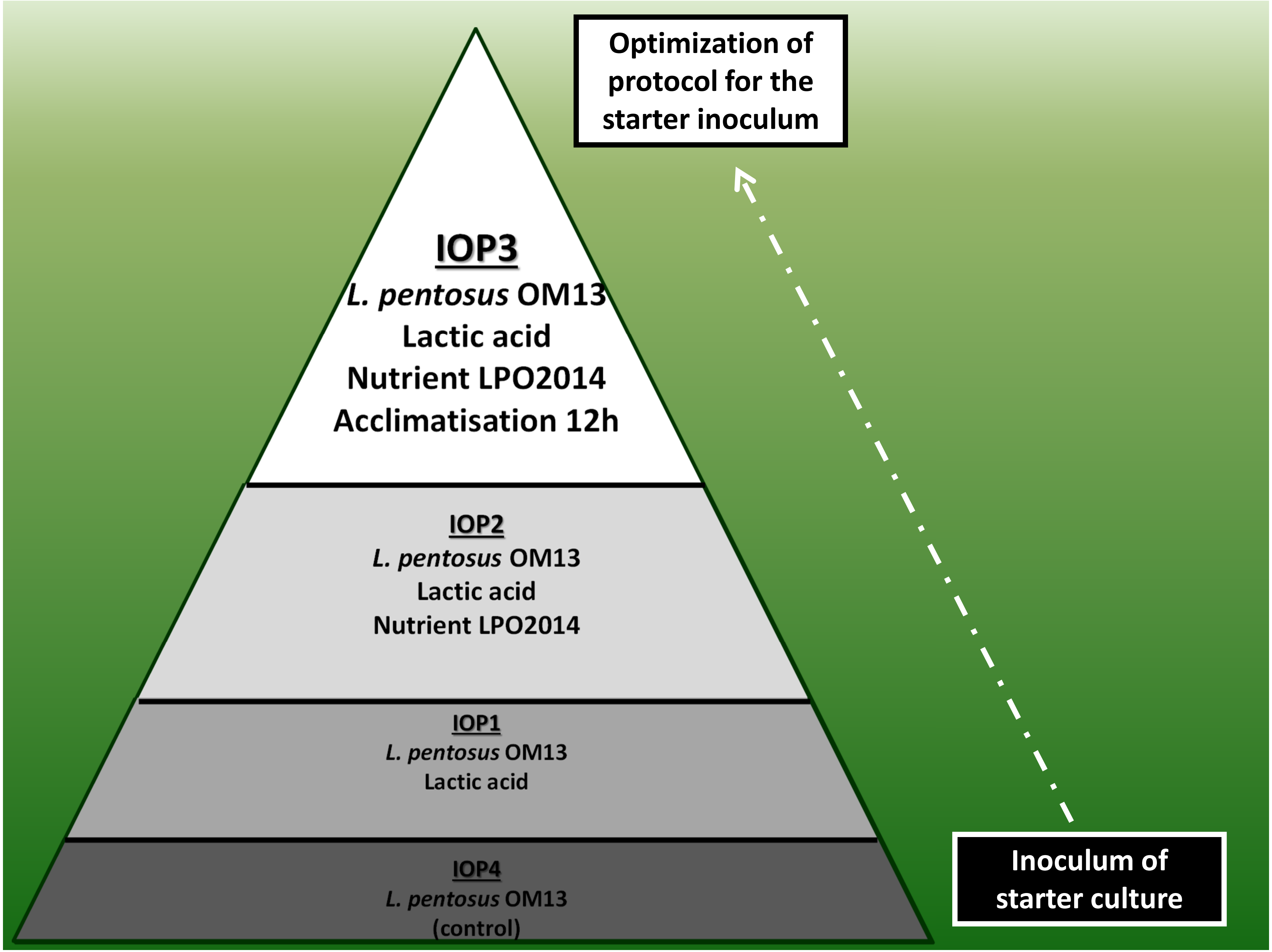
PRINCIPAL COMPONENT ANALYSIS
To evaluate differences among trials



PCA for sensory data of table olives at the end of process (195 day). Biplot graphs show relationships among factors, variables and trials.



The codes IOP1, IOP2 and IOP3 refer to the experimental trials; the codes C1 and C2 refer to the control trials.


Optimization of protocol for the starter inoculum
IOP3
L. pentosus OM13
Lactic acid
Nutrient LPO2014
Acclimatisation 12h
IOP2
L. pentosus OM13
Lactic acid
Nutrient LPO2014
IOP1
L. pentosus OM13
Lactic acid
IOP4
L. pentosus OM13
(control)
Inoculum of starter culture

Stages of research activities aimed at improving fermentation conditions

2007: Research demonstrating the validity of *L. pentosus* OM13.

.

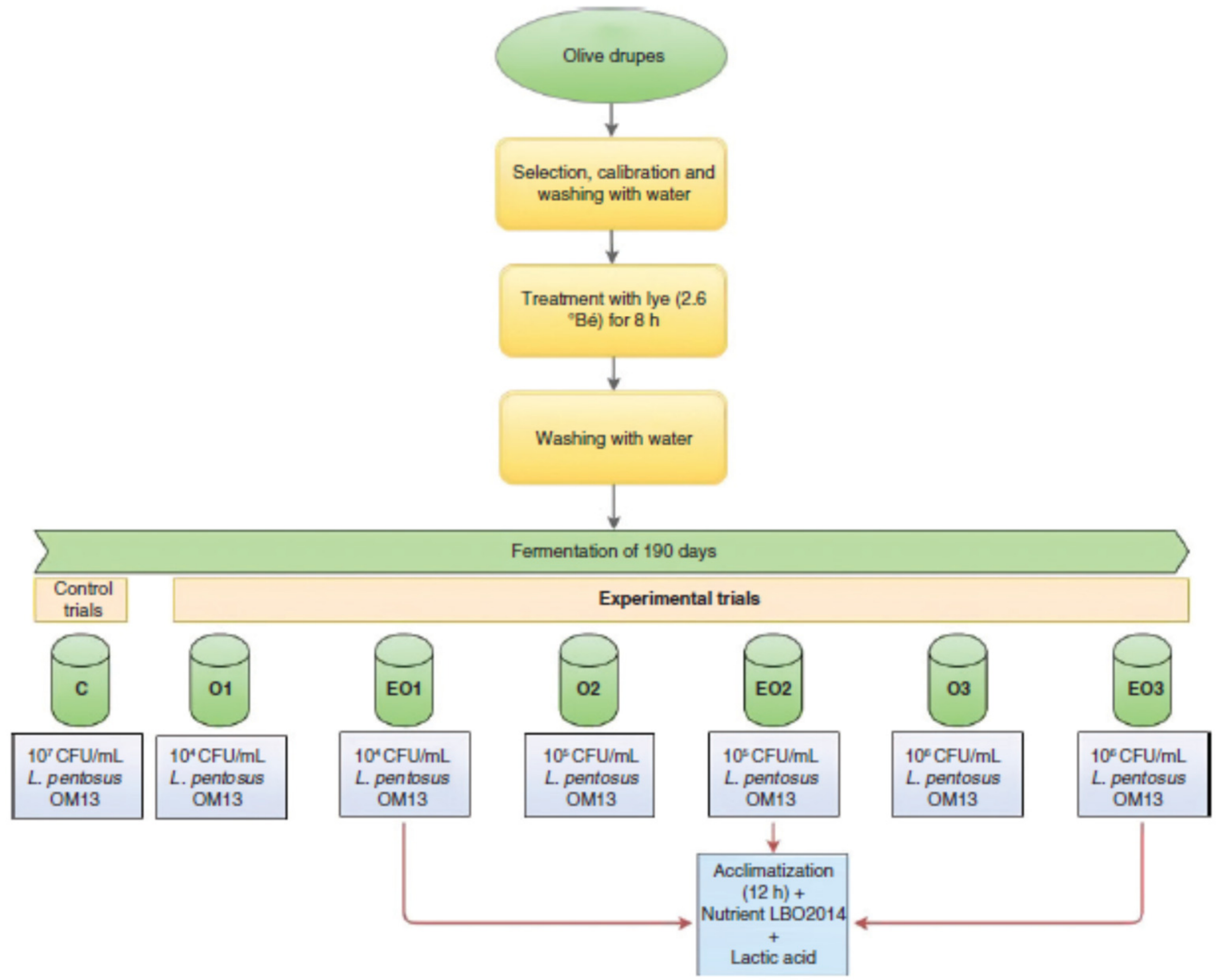
2013: Study demonstrates the validity of using a nutrient and activator.

2016: Approaches to improve the growth of the starter lactic acid bacterium OM13 during the early stages of green Spanish-style table olive production.

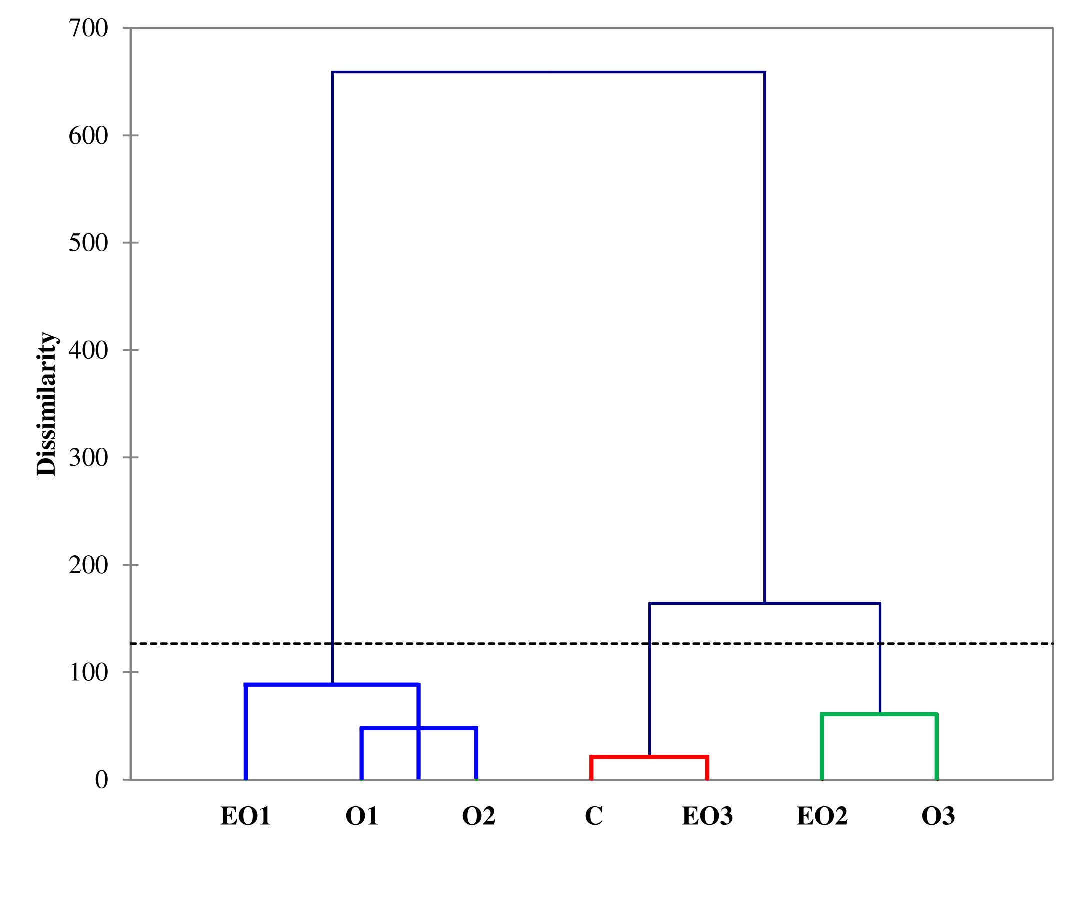
2017: Development of an innovative protocol for optimising the fermentation process using nutrients, activator and strain acclimatisation.

2018-19: Use of *L. pentosus* OM13 in Italy and Spain for the production of fermented olives through the Sevillian style.

Approaches to improve the growth of the starter lactic acid bacterium OM13 during the early stages of green Spanish-style table olive production.



Approaches to improve the growth of the starter lactic acid bacterium OM13 during the early stages of green Spanish-style table olive production.



Dendrogram resulting from agglomerative hierarchical clustering analysis based on values of pH and microbial populations.

The cell acclimatization procedure was found to improve the dominance of starter OM13 even just after the inoculum into brine, when its population levels were 2 Log cycles lower than that commonly reached during standard fermentation.

The treatment EO3 showed characteristics similar to those obtained for trial C. This trend was also confirmed by the results from the sensory analysis, since trials C, EO2 and EO3 showed high values of preference and satisfaction, as well as by AHC results, which indicated that trials C and EO3 were closely related to the population of LAB.

Acclimatisation

Technique used in oenology

LALLEMAND

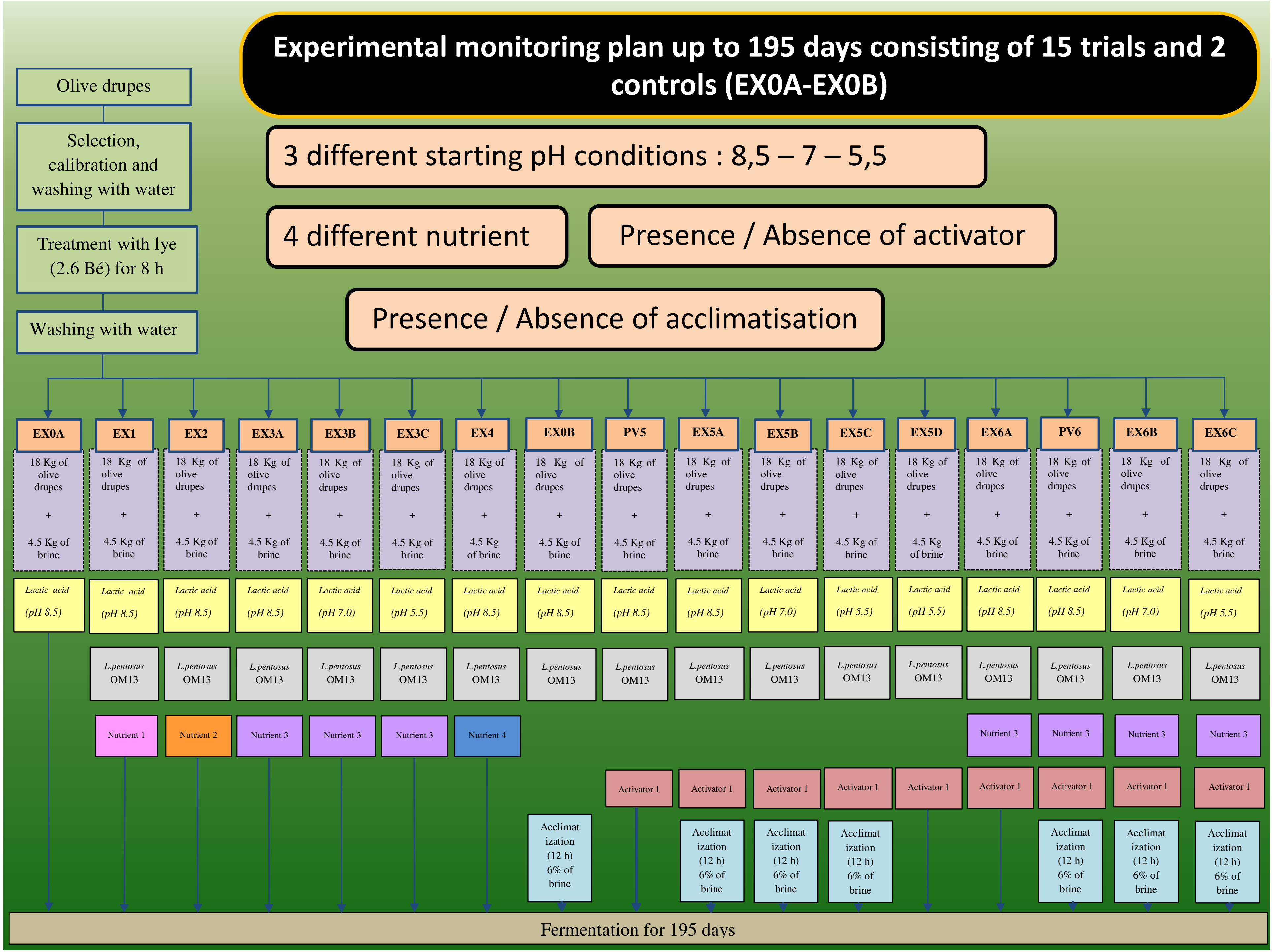
Extensively used in oenology

Commercial nutrient

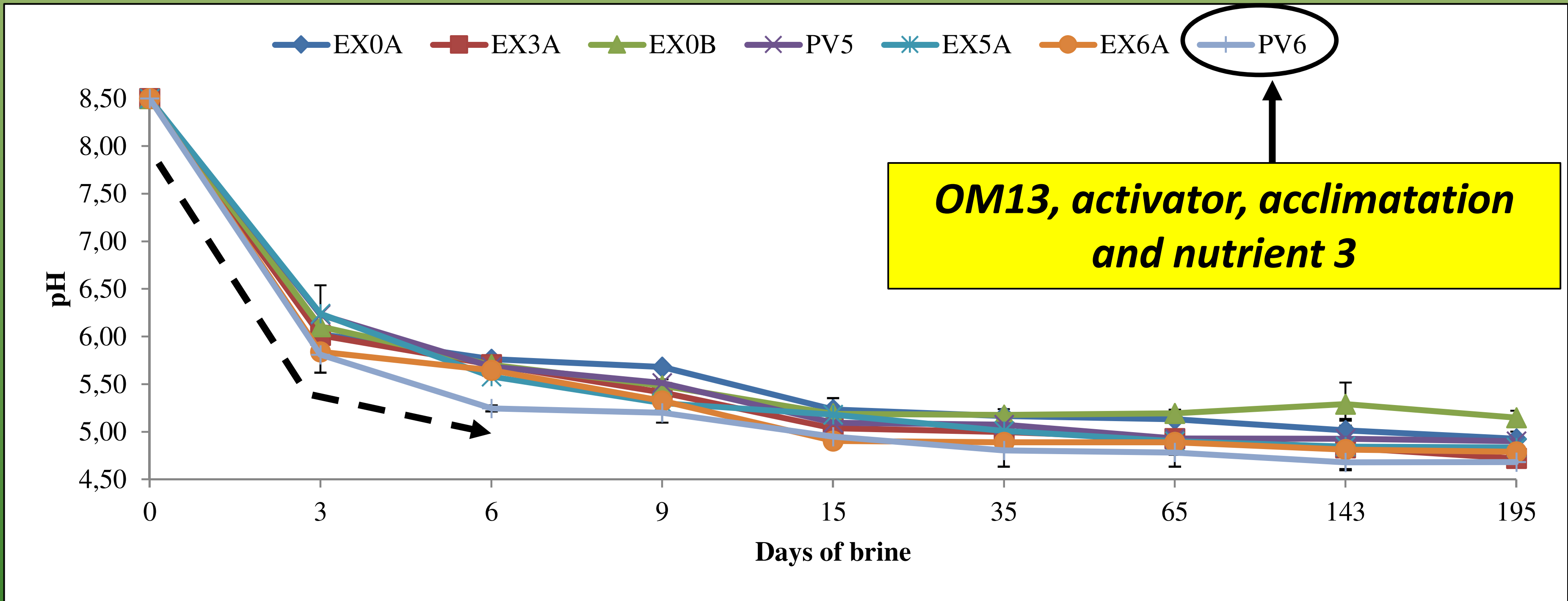
Composed of sugars, aminoacids and vitamins

Increases the viability and efficiency of the starter strain

Reduces fermentation time


Experimental monitoring plan up to 195 days consisting of 15 trials and 2 controls (EX0A-EX0B)
3 different starting pH conditions : 8,5 – 7 – 5,5
4 different nutrient
Presence / Absence of activator
Presence / Absence of acclimatisation
Olive drupes
Selection, calibration and washing with water
Treatment with lye (2.6 Bé) for 8 h
Washing with water
EX0A
EX1
EX2
EX3A
EX3B
EX3C
EX4
EX0B
PV5
EX5A
EX5B
EX5C
EX5D
EX6A
PV6
EX6B
EX6C
18 Kg of olive drupes + 4.5 Kg of brine
Lactic acid (pH 8.5)
Lactic acid (pH 7.0)
Lactic acid (pH 5.5)
L.pentosus OM13
Nutrient 1
Nutrient 2
Nutrient 3
Nutrient 4
Activator 1
Acclimatization (12 h) 6% of brine
Fermentation for 195 days

Kinetics of brine acidification



EX0A = spontaneous

EX3A = pH 8.5+OM13+N3

EX5A = pH 8,5+OM13+activator+acclimatation

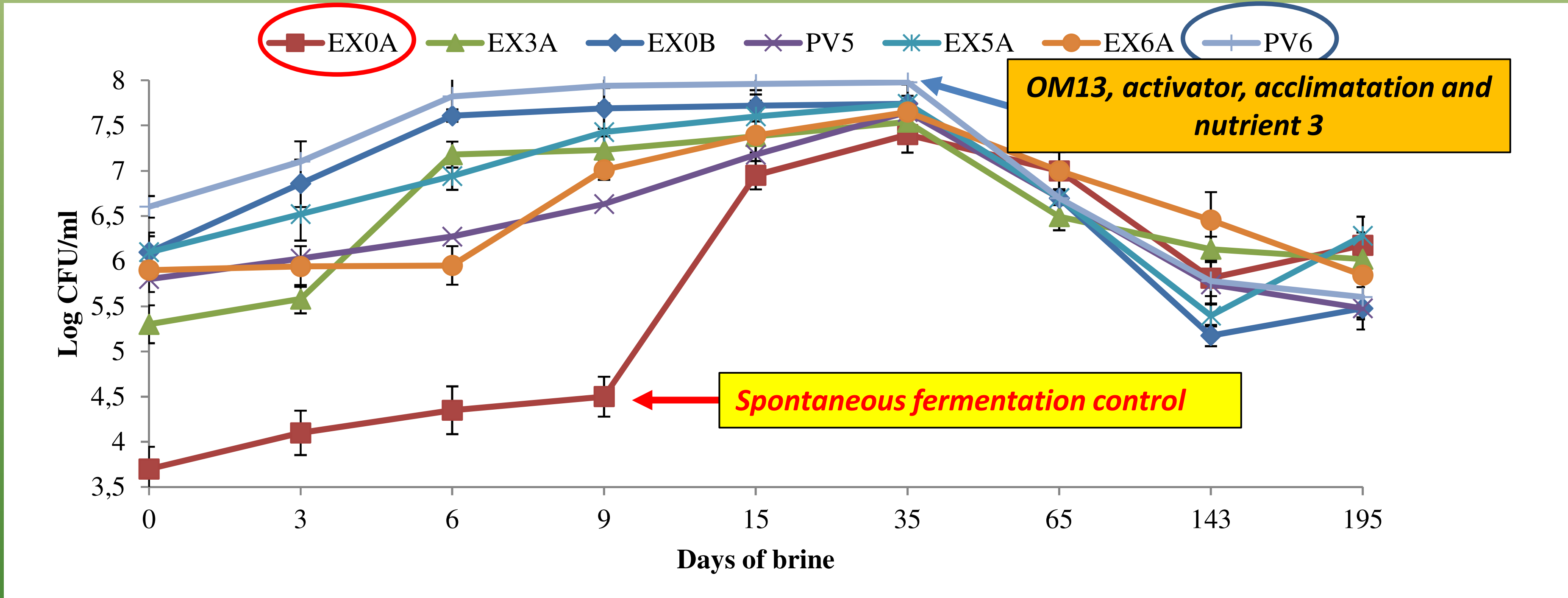
EX0B = pH 8.5 + OM13 + acclimatation

PV5 = pH 8.5+OM13+activator

PV6 = pH 8.5+OM13+activator+N3+acclimatation

EX6A=pH 8.5+OM13+activator+N3

Batteri lattici



EX0A = spontaneous

EX3A = pH 8.5+OM13+N3

EX5A = pH 8,5+OM13+activator+acclimatation

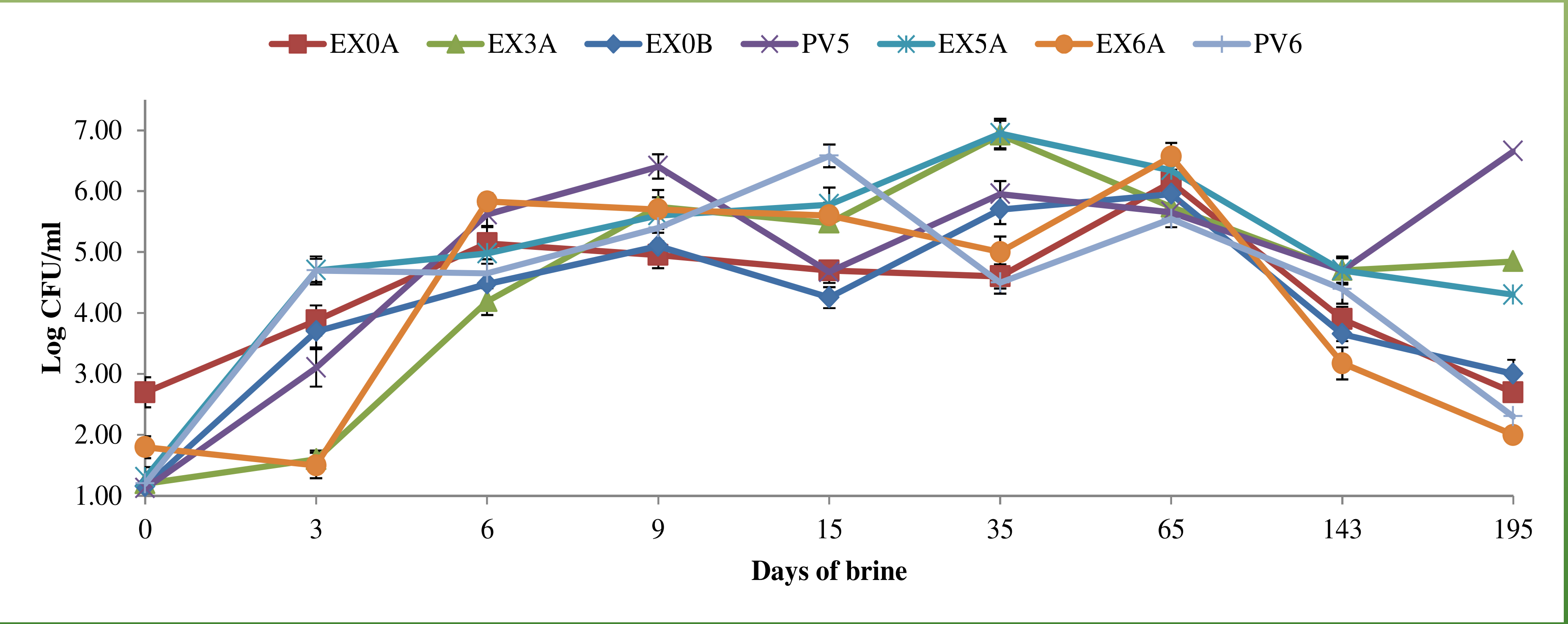
EX0B = pH 8.5 + OM13 + acclimatation

PV5 = pH 8.5+OM13+activator

PV6 = pH 8.5+OM13+activator+N3+acclimatation

EX6A=pH 8.5+OM13+activator+N3

Yeasts



EX0A = spontaneous

EX3A = pH 8.5+OM13+N3

EX5A = pH 8,5+OM13+activator+acclimatation

EX0B = pH 8.5 + OM13 + acclimatation

PV5 = pH 8.5+OM13+activator

PV6 = pH 8.5+OM13+activator+N3+acclimatation

EX6A=pH 8.5+OM13+activator+N3

Distribution of yeast species

Species	Trials						
	EX0A	EX3A	EX0B	PV5	EX5A	EX6A	PV6
Candida aaseri	■						
Candida atlantica	■						
Candida boidinii	■	■	■		■		■
Debaryomyces hansenii	■	■	■	■	■	■	■
Debaryomyces vindobonensis							■
Kluyveromyces lactis					■	■	
Wickerhamomyces anomalus	■	■		■	■	■	■
Yamadazyma olivae	■		■	■			
Yamadazyma takamatsuzukensis		■					

EX0A = spontaneous

EX3A = pH 8.5+OM13+N3

EX5A = pH 8,5+OM13+activator+acclimatation

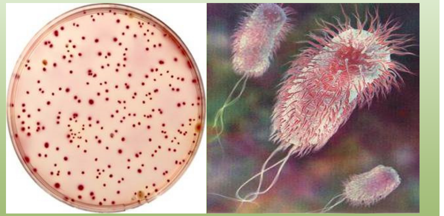
EX0B = pH 8.5 + OM13 + acclimatation

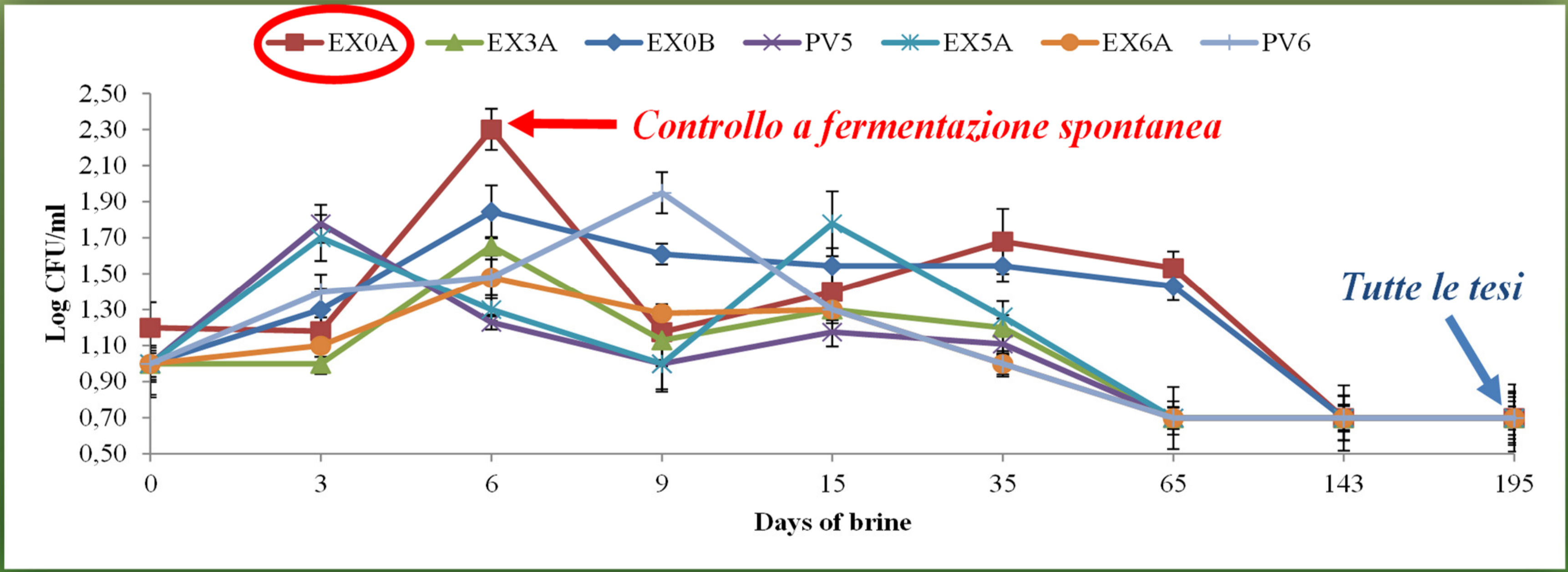
PV5 = pH 8.5+OM13+activator

PV6 = pH 8.5+OM13+activator+N3+acclimatation

EX6A=pH 8.5+OM13+activator+N3

Enterobacteriaceae





EX0A = spontaneous

EX3A = pH 8.5+OM13+N3

EX5A = pH 8,5+OM13+activator+acclimatation

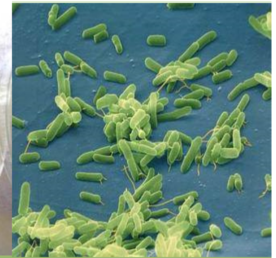
EX0B = pH 8.5 + OM13 + acclimatation

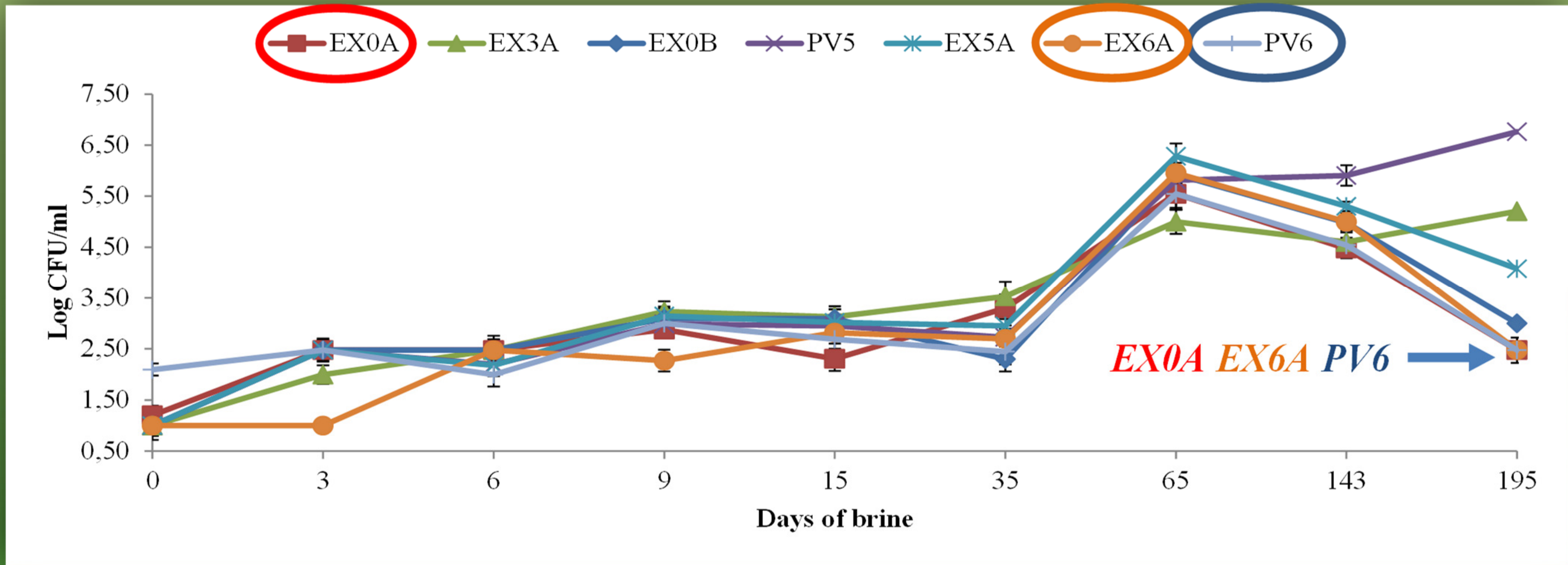
PV5 = pH 8.5+OM13+activator

PV6 = pH 8.5+OM13+activator+N3+acclimatation

EX6A=pH 8.5+OM13+activator+N3

Pseudomonadaceae





EX0A = spontaneous

EX3A = pH 8.5+OM13+N3

EX5A = pH 8,5+OM13+activator+acclimatation

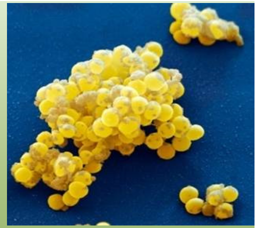
EX0B = pH 8.5 + OM13 + acclimatation

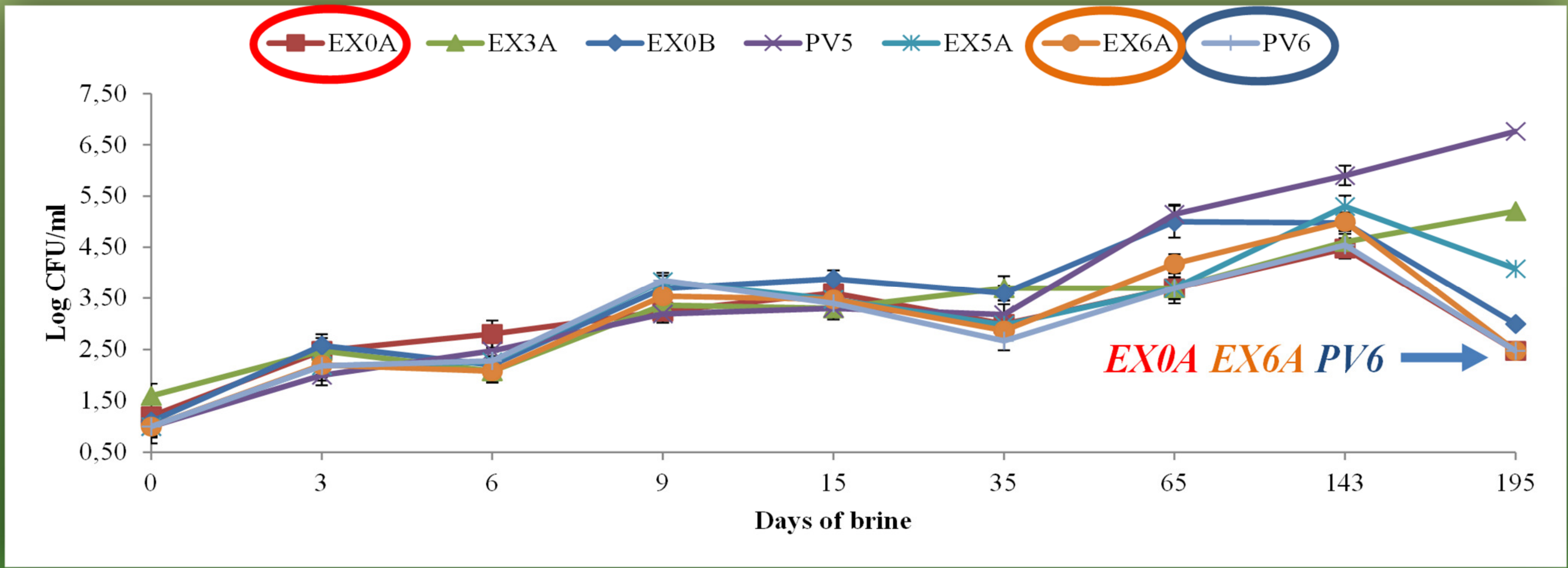
PV5 = pH 8.5+OM13+activator

PV6 = pH 8.5+OM13+activator+N3+acclimatation

EX6A=pH 8.5+OM13+activator+N3

Staphylococcaceae





EX0A = spontaneous

EX3A = pH 8.5+OM13+N3

EX5A = pH 8,5+OM13+activator+acclimatation

EX0B = pH 8.5 + OM13 + acclimatation

PV5 = pH 8.5+OM13+activator

PV6 = pH 8.5+OM13+activator+N3+acclimatation

EX6A=pH 8.5+OM13+activator+N3

Sensory analysis

EX0A-EX6A-PV6

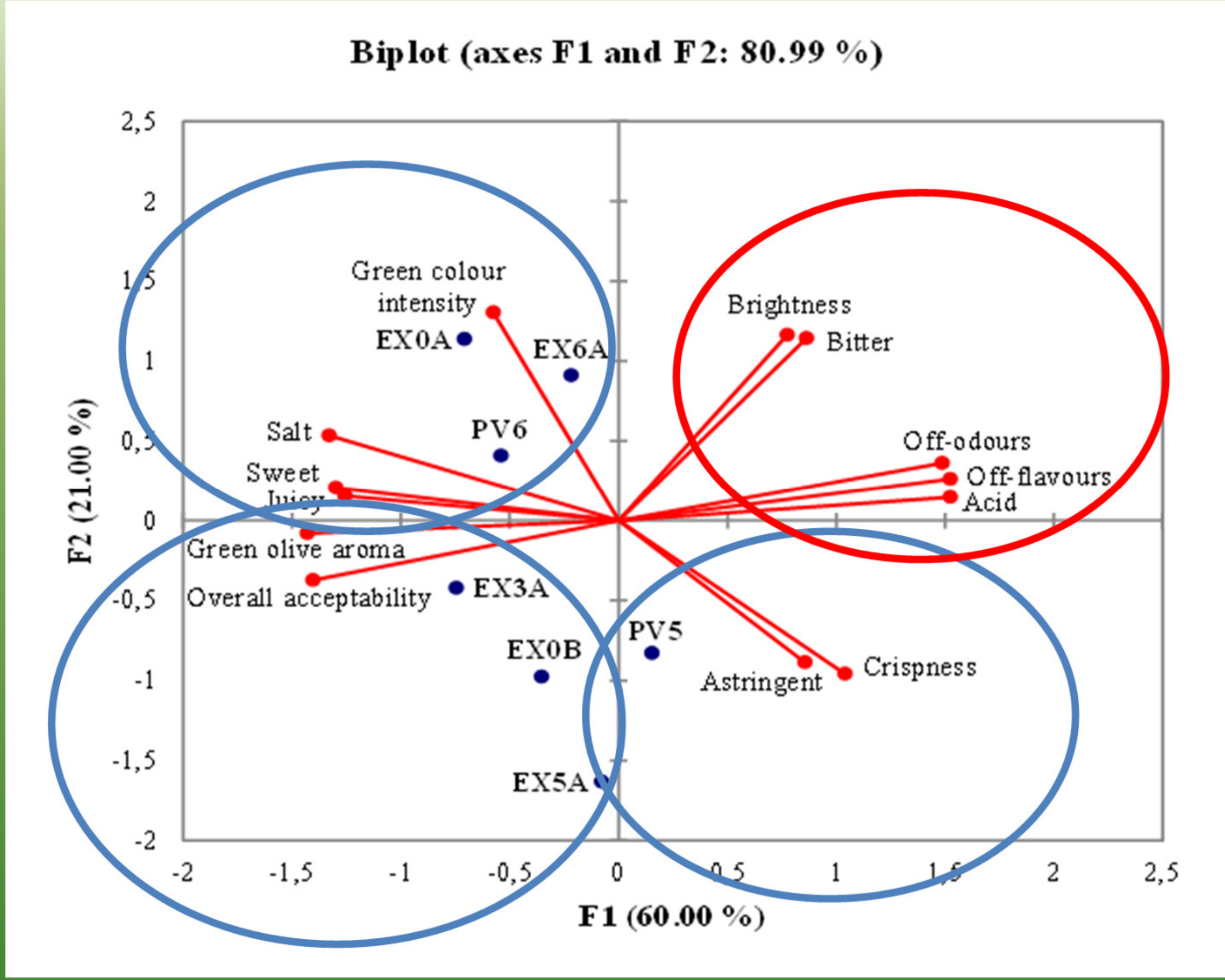
- Green colour intensity
- Salty
- Sweetness
- Juiciness

EX3A-EX0B-EX5A

- Aroma of green olives
- Overall satisfaction

PV5

- Astringency
- Crispness



EX0A = spontaneous

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EX5A = pH 8,5+OM13+activator+acclimatation

EX0B = pH 8.5 + OM13 + acclimatation

PV5 = pH 8.5+OM13+activator

PV6 = pH 8.5+OM13+activator+N3+acclimatation

EX6A=pH 8.5+OM13+activator+N3

Conclusions

The use of the starter with acclimatisation, activator and nutrient 3 ensured a rapid lowering of the pH

Acidification ensured the hygienic safety of the product

Sensory analysis showed a total absence of unpleasant tastes and odours

The study has a high potential for industrial applicability

Cost optimisation

Olive drupes "Manzanilla"

↓

Selection, calibration and washing with water

↓

Treatment with lye (2.1-2.6 °Bé) for 6-8 h and washing with water (n.1 rinsing for 12 hours)

↓

Acidification with HCl

Analysis at time (days): Before inoculation, 24 h since inoculation, 10-15 days of inoculation, 29-32 days of inoculation, 60 days of inoculation, 90 days of inoculation

EXPERIMENTAL TRIALS

A	B	C	D	E
5 bags Crispy without Activator	5 bags Crispy with Activator	3 bags Crispy without Activator	3 bags Crispy with Activator	Control Starter and factory production protocol
pH = 8,40	**pH = 8,40**	**pH = 8,40**	**pH = 8,40**	**pH = 6,70**

Results: Lactic Acid Bacteria

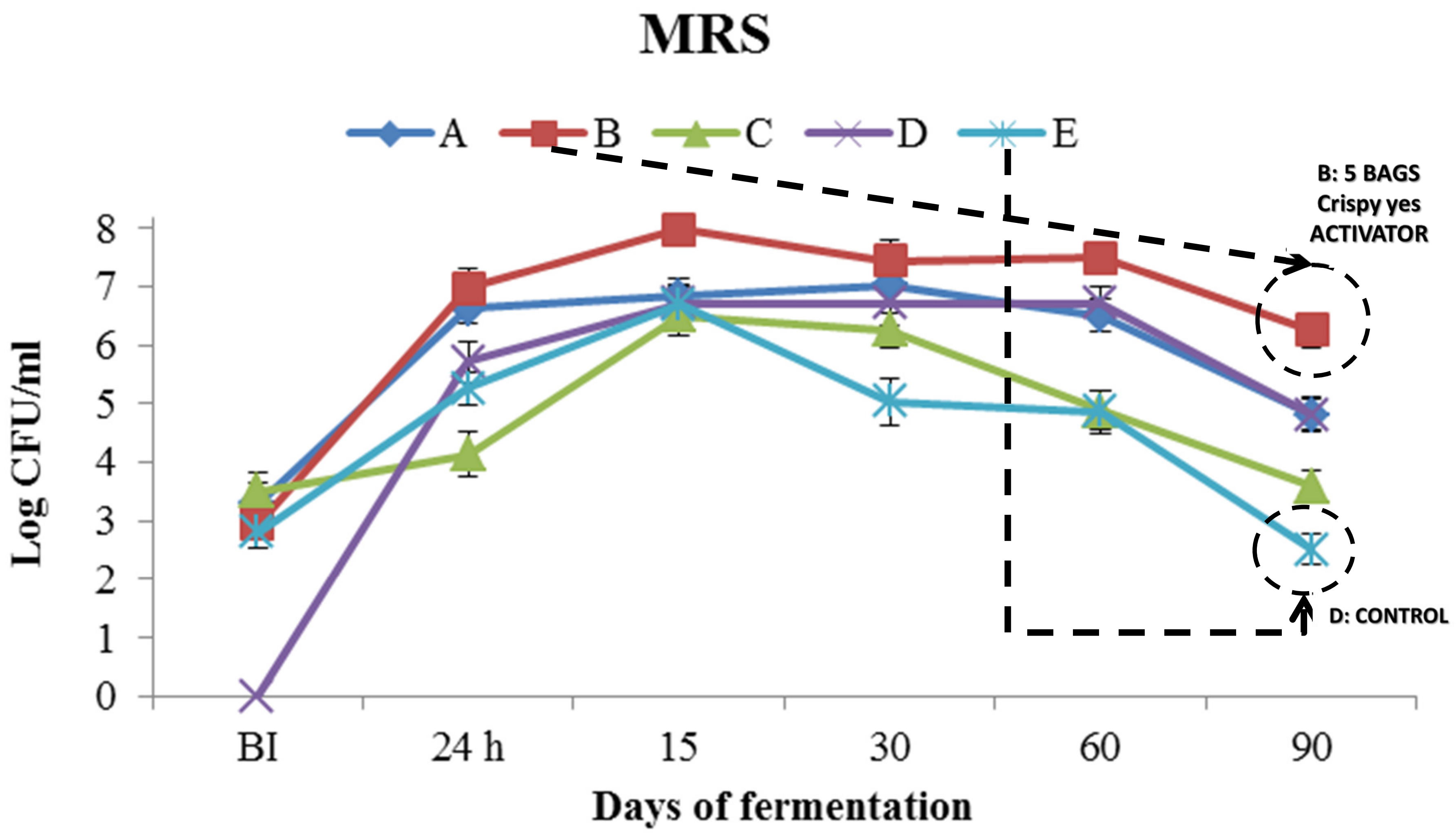
A: 5 BAGS Crispy no activator

C: 3 BAGS Crispy no activator

B: 5 BAGS Crispy yes activator

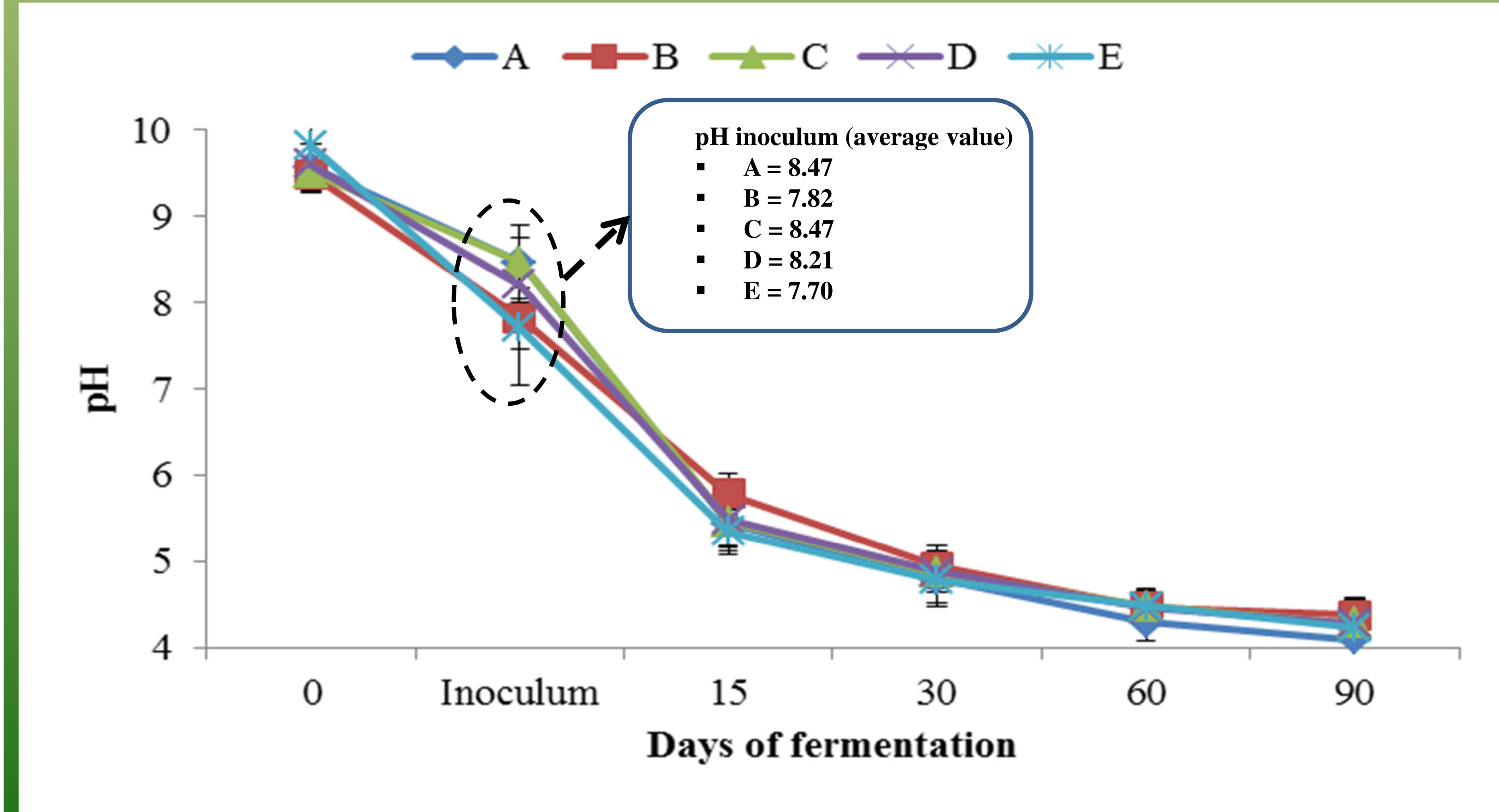
D: 3 BAGS Crispy yes activator

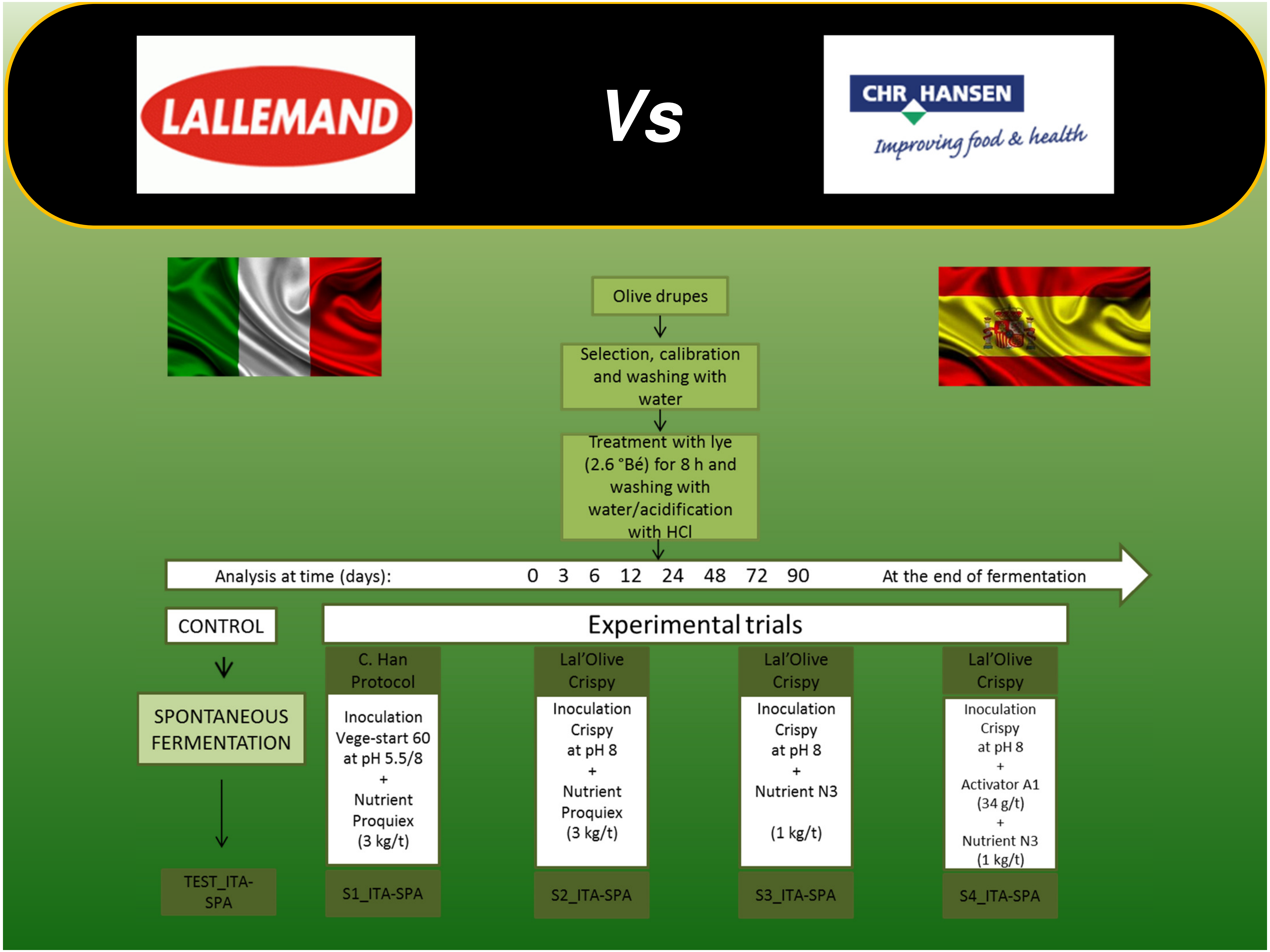
E: Control



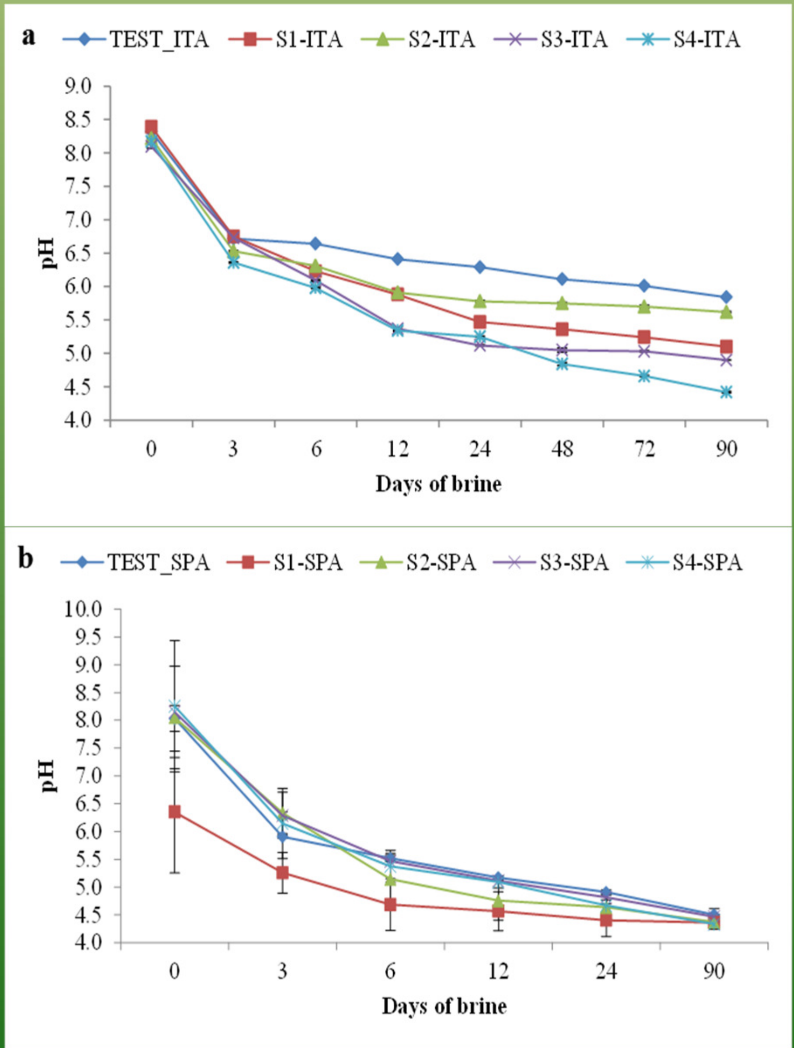
Results: pH

A: 5 BAGS Crispy no activator
C: 3 BAGS Crispy no activator
E: Control
B: 5 BAGS Crispy yes activator
D: 3 BAGS Crispy yes activator

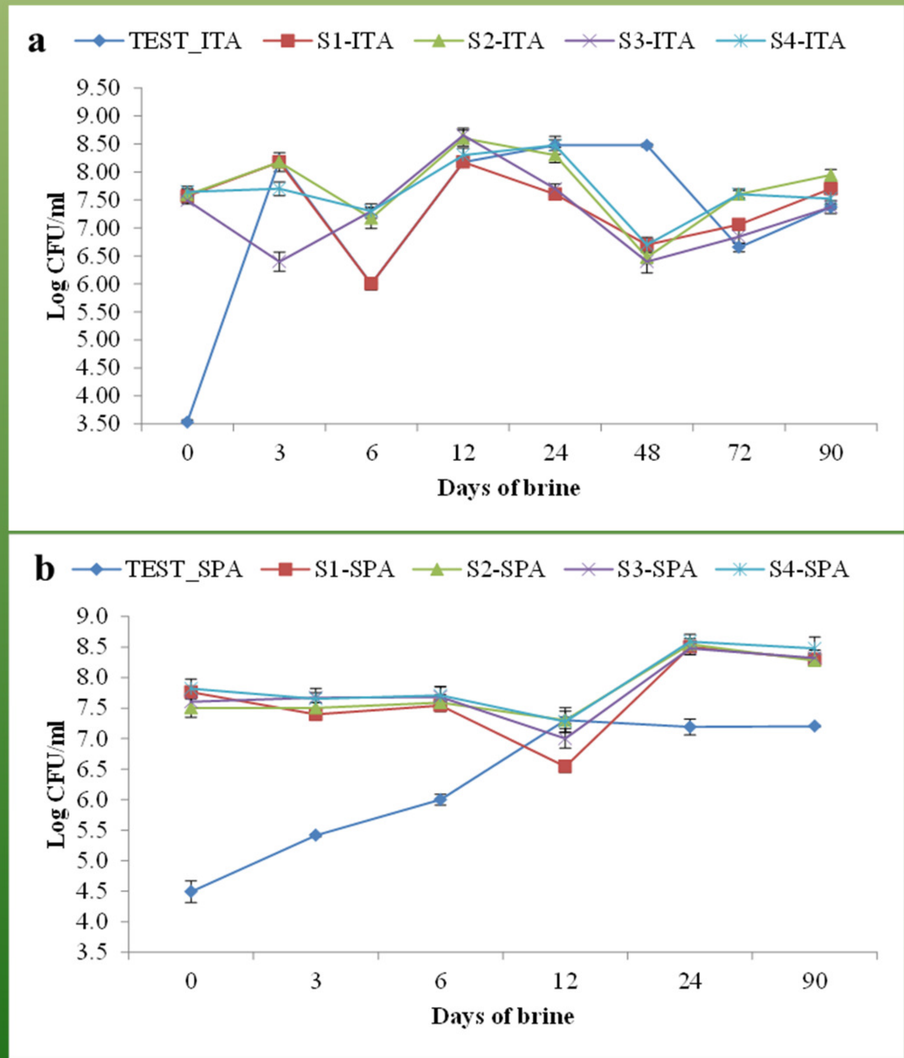



LALLEMAND
Vs
CHR HANSEN
Improving food & health
Olive drupes
Selection, calibration and washing with water
Treatment with lye (2.6 °Bé) for 8 h and washing with water/acidification with HCl
Analysis at time (days): 0 3 6 12 24 48 72 90 At the end of fermentation
CONTROL
SPONTANEOUS FERMENTATION
TEST_ITA-SPA
Experimental trials
C. Han Protocol
Inoculation Vege-start 60 at pH 5.5/8 + Nutrient Proquiex (3 kg/t)
S1_ITA-SPA
Lal'Olive Crispy
Inoculation Crispy at pH 8 + Nutrient Proquiex (3 kg/t)
S2_ITA-SPA
Lal'Olive Crispy
Inoculation Crispy at pH 8 + Nutrient N3 (1 kg/t)
S3_ITA-SPA
Lal'Olive Crispy
Inoculation Crispy at pH 8 + Activator A1 (34 g/t) + Nutrient N3 (1 kg/t)
S4_ITA-SPA

pH


a
TEST_ITA
S1-ITA
S2-ITA
S3-ITA
S4-ITA
pH
Days of brine
0
3
6
12
24
48
72
90
b
TEST_SPA
S1-SPA
S2-SPA
S3-SPA
S4-SPA
pH
Days of brine
0
3
6
12
24
90

LAB


a
TEST_ITA
S1-ITA
S2-ITA
S3-ITA
S4-ITA
Log CFU/ml
Days of brine
0
3
6
12
24
48
72
90
b
TEST_SPA
S1-SPA
S2-SPA
S3-SPA
S4-SPA
Log CFU/ml
Days of brine
0
3
6
12
24
90

LALLEMAND Vs CHR HANSEN Improving food & health

This research activity has clarified the differences in the use of Lal'Olive Crispy *L. pentosus* OM13 and Bactoferm® Vege-Start 60 for the production of olives with the Sevillian method.

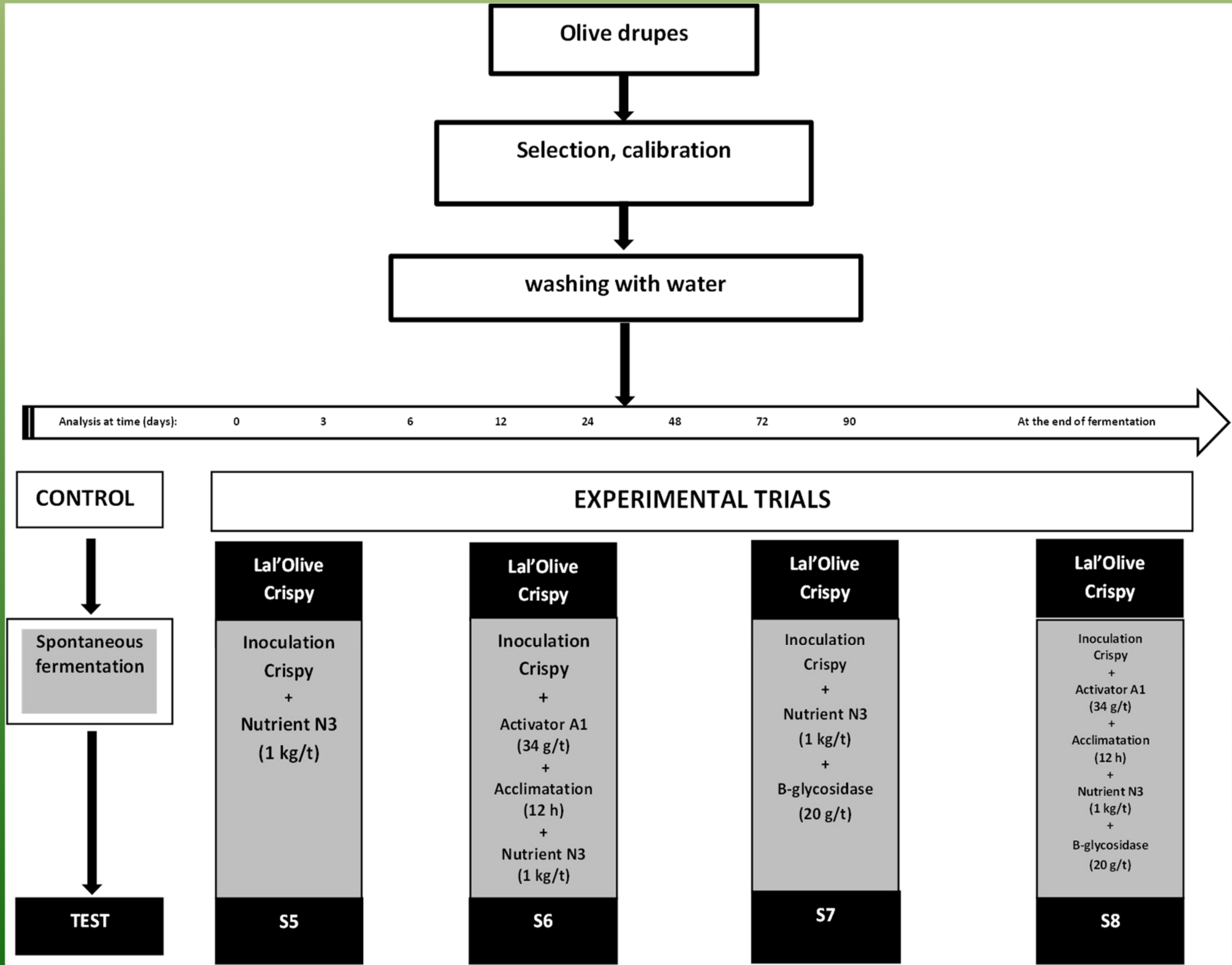
Compared to spontaneous productions, both starter strains caused a rapid decrease in the pH of the brine during the 90 days of fermentation, constantly improving the safety of the product.

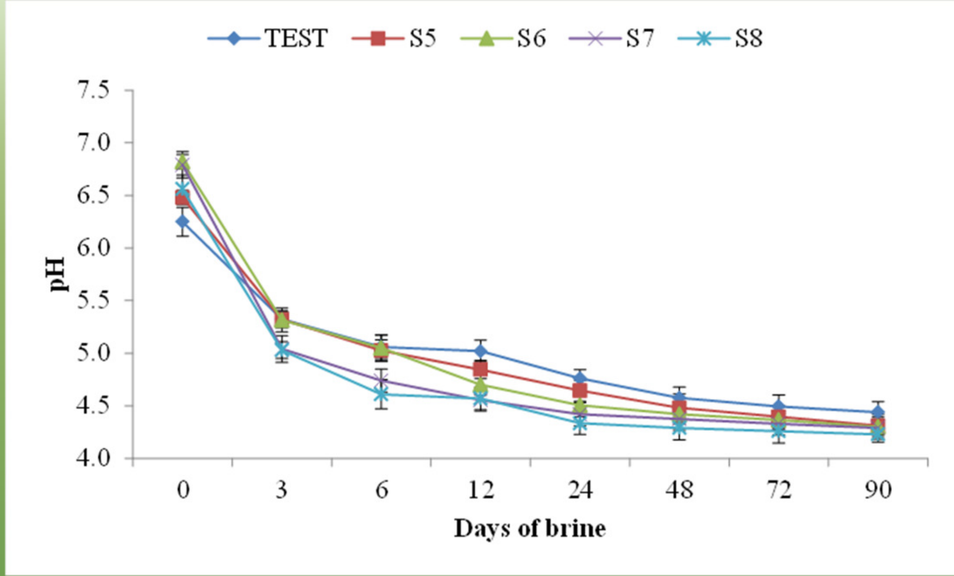
Depending on the production site (Italy or Spain), differences were observed in relation to the starter strain used. Most likely these differences are related to the different processing technology

Presumably the temperatures of the fermentation process as well as the presence/absence and number of the washing s with water of the olives after deamarizing treatment with soda determined different conditions which influence the starters during the fermentation process.

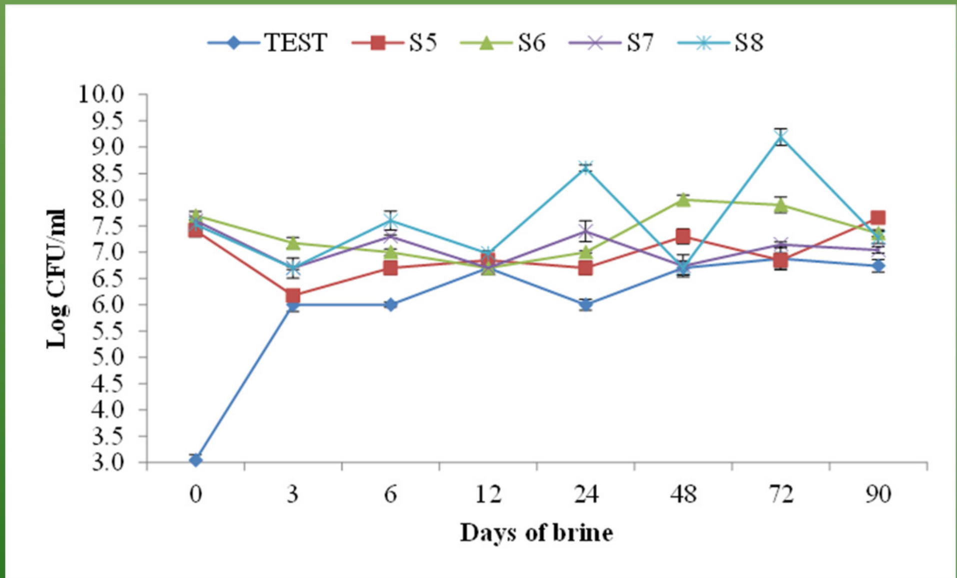
Olives fermented with Lal'Olive Crispy *L. pentosus* OM13 achieved higher overall satisfaction values.

Use of enzymes instead of soda ash for debittering table olives

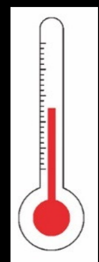




β-glycosidase does not influence the acification dynamics of the table olive fermentation process



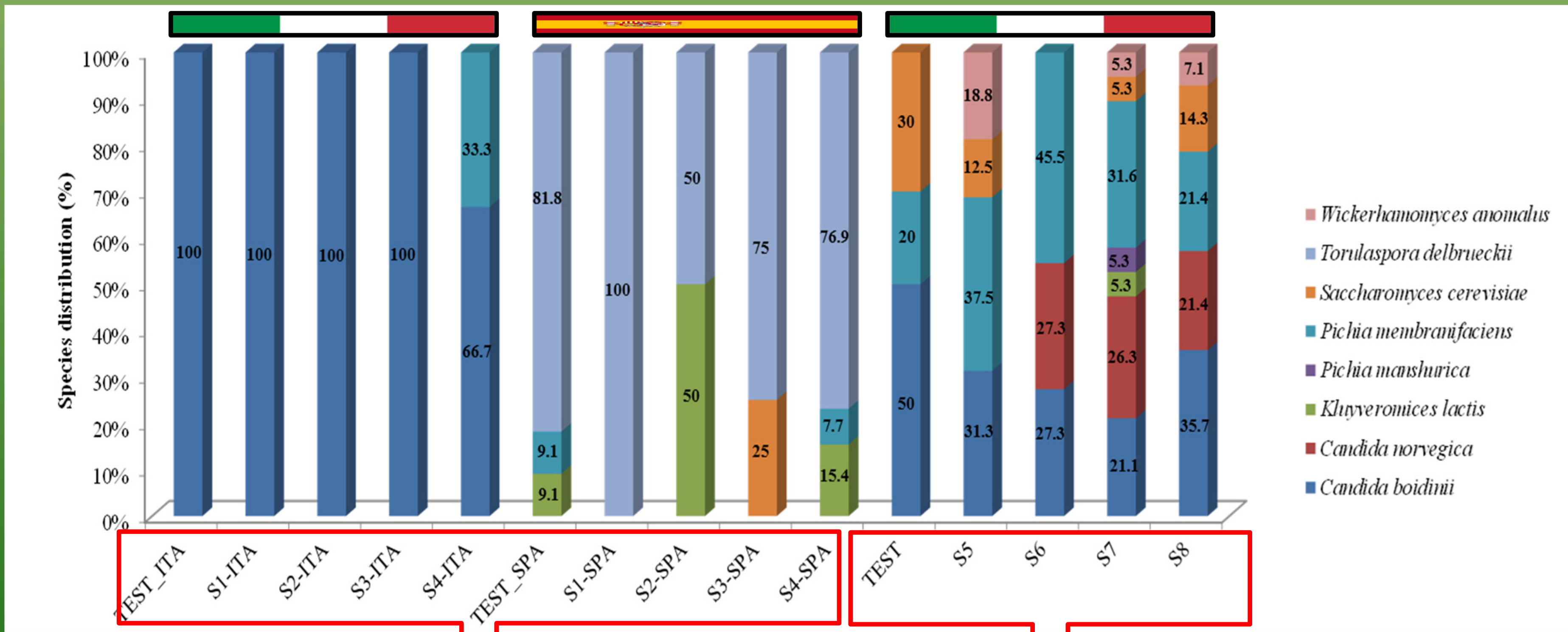
LAB growth follows the normal trend observed in experimental productions without the use of β -glycosidase



Temperature is an important factor for regulating enzyme activity.

Yeasts: alterative or protechnological?

Yeast biodiversity



Sevillian style < biodiversity

Greek style > biodiversity

Health aspects

Is eating olives good for you?

Probiotic olives
Rich in Lactobacilli and Bifidobacteria

Maintaining and improving the balance of intestinal microbial populations

It improves the well-being of the body by strengthening its natural defences

Lactobacillus pentosus
(Lactose and complex sugars digestibility)

Antioxidants
- Cardiovascular system protection
- Prevention of cholesterol formation

Unsaturated fatty acid
- Regulate blood cholesterol levels
- Reduced risk of heart disease
- Positive effects on the liver
- Protection of brain cells against ageing

Vitamina E
- Prevention of cancer formation

=

Thank you for your attention