



# Technological innovations and safety aspects of fermented table olives in Greece

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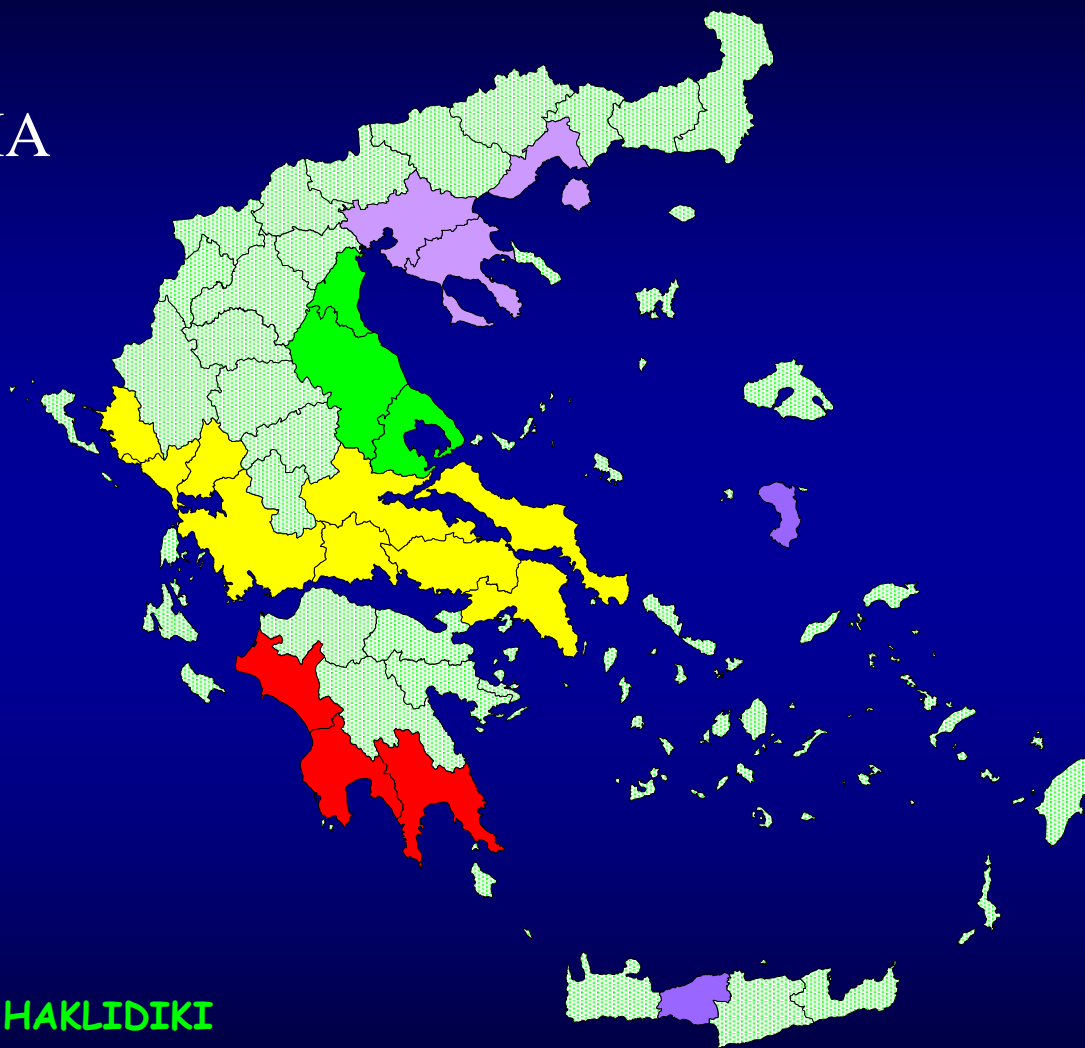


OLSZTYN, 13 Sept 2016



# Table olive varieties & cultivation areas

CONSERVOLIA  
HALKIDIKI  
THASOS  
KALAMON



■ 66% CONSERVOLIA

■ 12% CONSERVOLIA/ HAKLIDIKI

■ 11% KALAMON

■ 9% (HALKIDIKI / THASOS / THROUBOLIA)



# Trade preparations





# Basic Trade Preparations

(Olive Oil Council, Trade Standards Applying to Table Olives)



- Natural olives in brine (known as Greek type)



- Treated olives in brine (Known as Spanish style)



- Olives darkened by oxidation (Californian type)

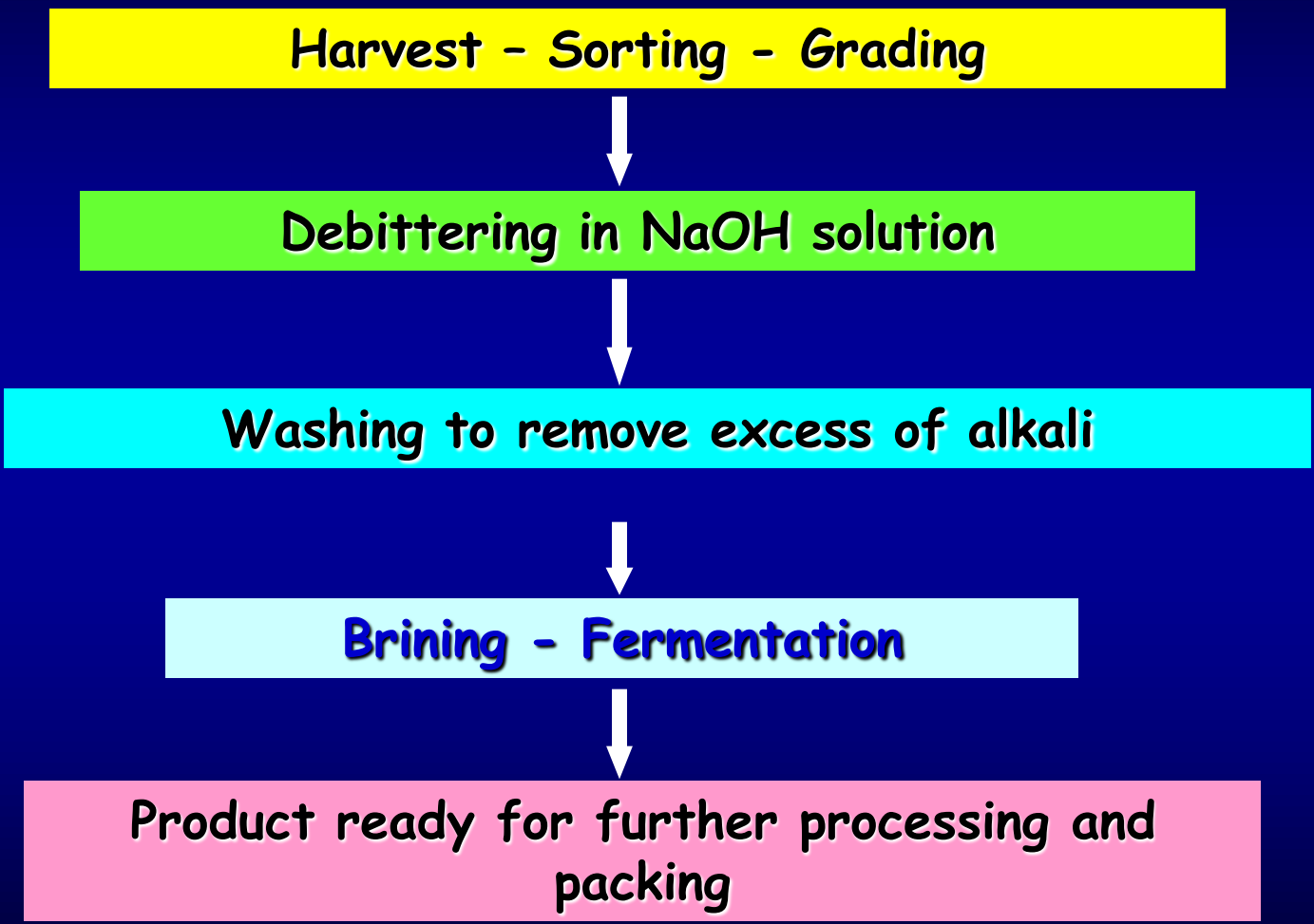


- Dehydrated and/or shrivelled olives

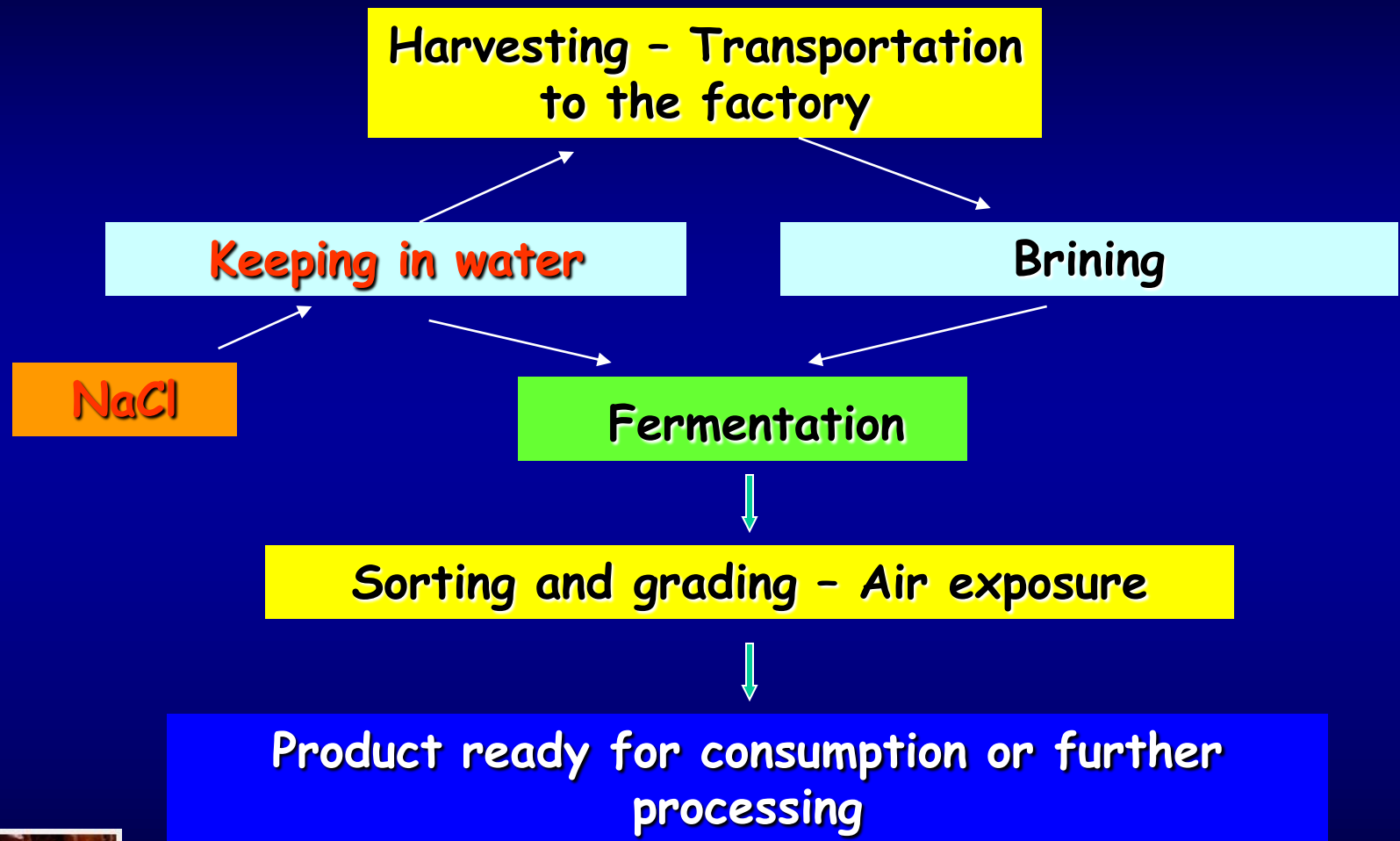




# Spanish-style green olives



# Naturally black olives in brine (Greek style)





# Olives darkened by oxidation (black ripe olives)

Harvesting - Transportation - Sorting

Preservation in brine  
(2.5-5.0% salt)

Debittering treatment  
with NaOH

Darkening process - Air oxidation

Colour stabilization (Ferrous lactate or gluconate) – Brine addition

Packing - Sterilization





# Dry salted olives

Harvest - Transportation to the factory



Sorting - Washing



Place in alternate layers with coarse salt (40%)



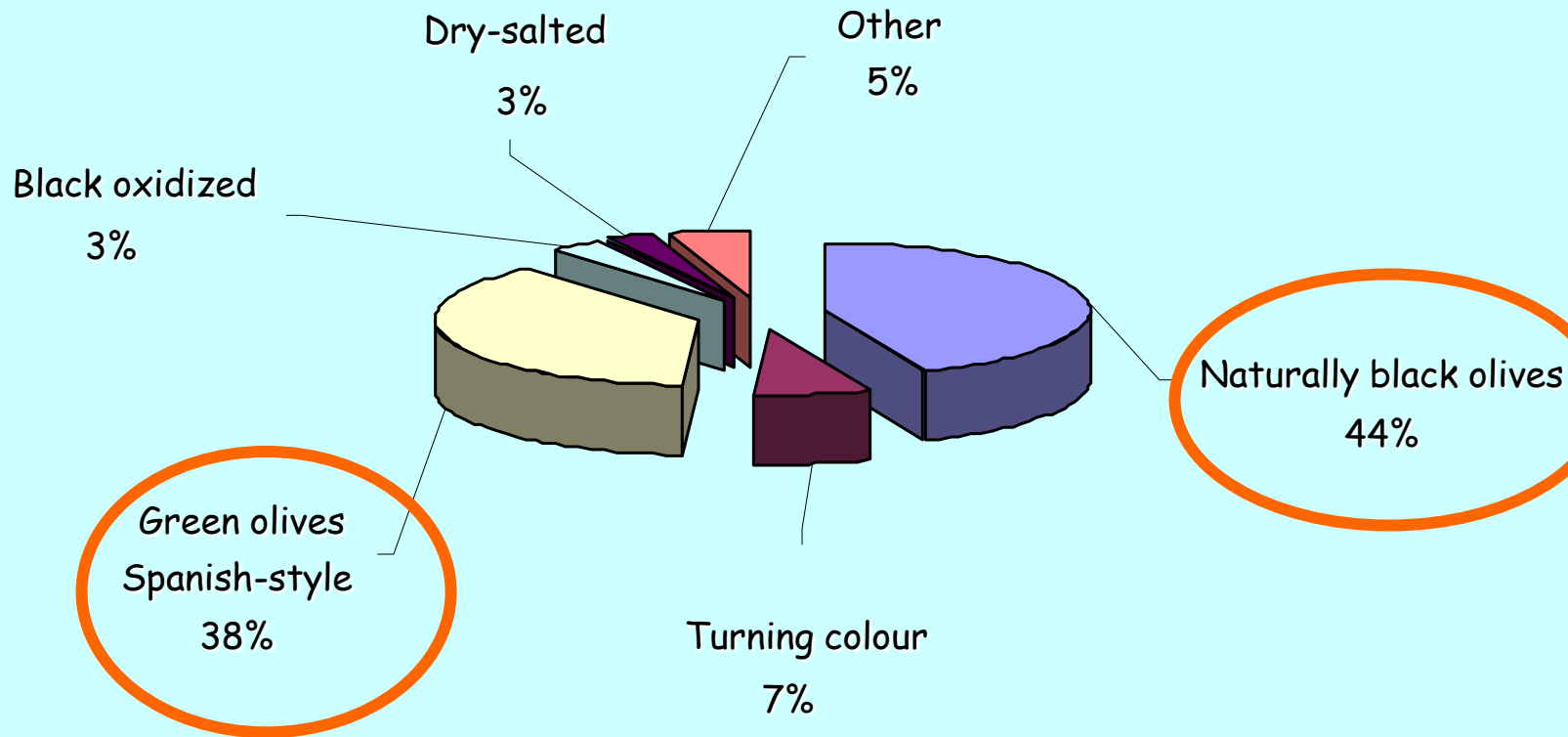
Solute loss - Shrivelling - Gradual debittering (curing)



Product ready for consumption (after 60-80 days)



# Production of different types of table olives





# Greek style olives (naturally black)





# Naturally black olives in brine

## (Greek-style table olives)

### Advantages:

- Natural processing with minimum input of chemicals
- Simple processing (traditional anaerobic method)
- Low energy consumption

### Disadvantages:

- Time consuming process (6-7 months)
- Possible damage to the crop before harvest due to early frosts



# Table olive fermentation

- **Fermentation** is a basic step in green and natural black table olive processing.
- It is undertaken by the **autochthonous microorganisms** present on the raw olive fruits.
- On immersion in the brine, a fraction of these microbes migrate in the brine and assimilates and fermentable material diffused from the olive flesh.
- Anaerobic conditions, salt concentration and the gradual decrease in pH have a selective role on microbial activity.
- Under normal conditions, **lactic acid bacteria and yeasts** dominate the process.
- Basic metabolic products: **Lactic acid, acetic acid and ethanol.**







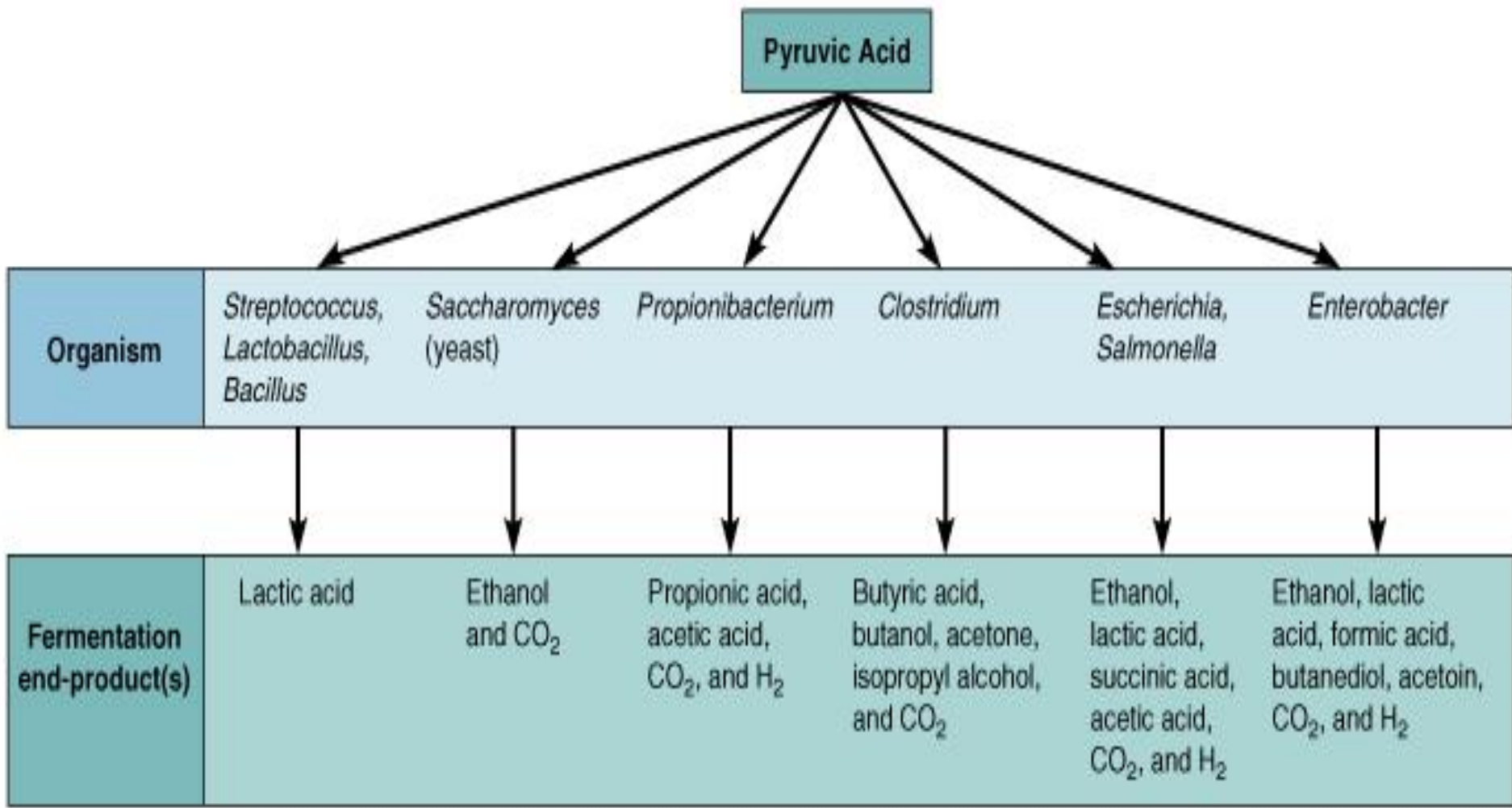
## For a successful fermentation

- Rapid dominance of the technological microbiota to minimize spoilage risk.
- Development of the appropriate physicochemical characteristics (**pH, acidity**) that will ensure the microbiological stability of the product during storage even at ambient temperature.
- Improvement of sensory characteristics.





# Final metabolic products



(b)



# Processing – traditional anaerobic method

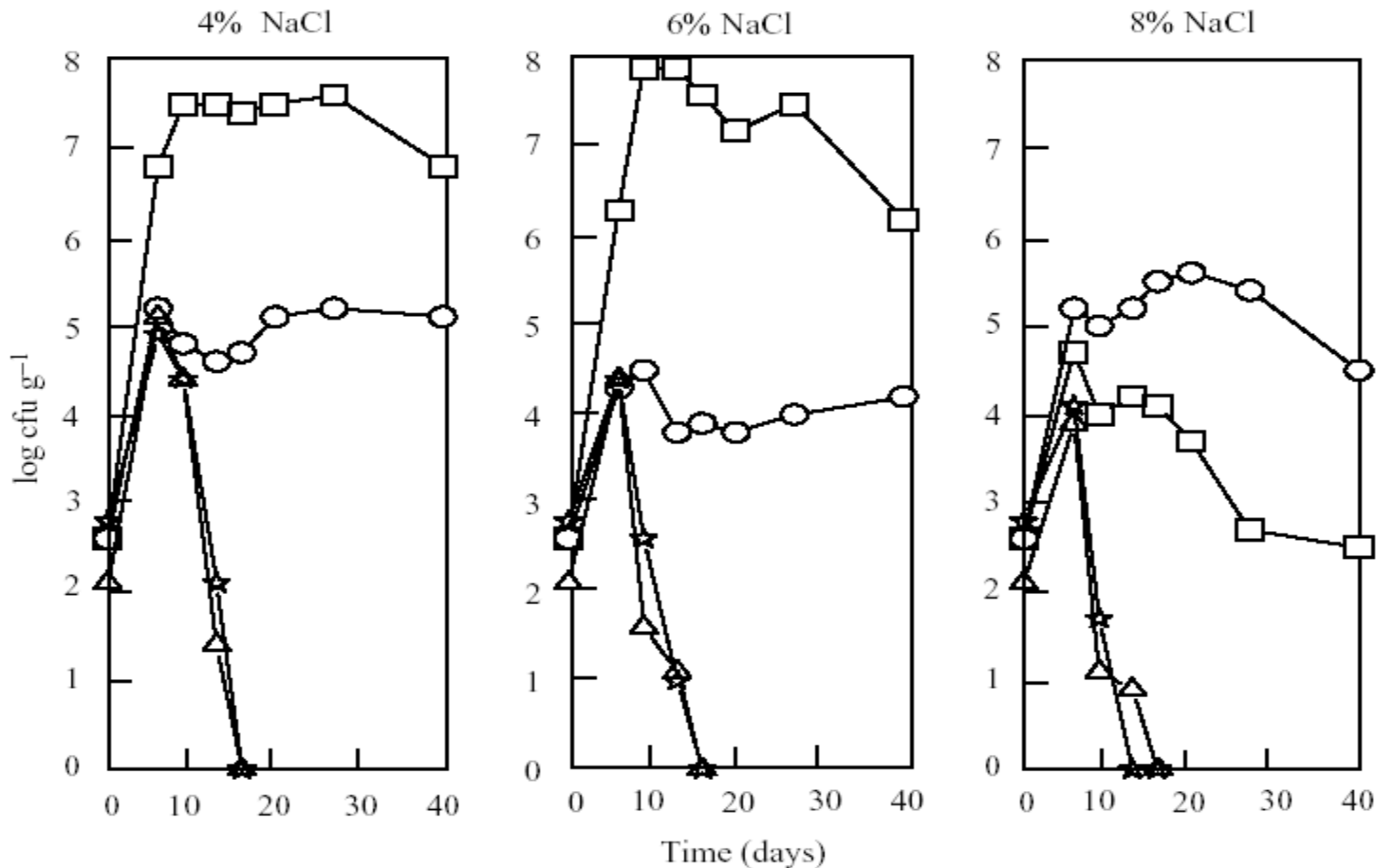
- Olives are placed directly in brine, 8-10% NaCl or even more
- Under these conditions, fermentation is carried out primarily by yeasts, gram-negative bacteria and sometimes lactic acid bacteria
- Fermentation is both alcoholic and lactic (to a lesser extend)
- The final product has pH 4,5-5,5 and titratable acidity 0,3-0,5% (expressed as lactic acid)



# Processing - new approach

- Olives are placed directly in brine at 6-7% NaCl, which is kept constant throughout fermentation
- These conditions favour the growth of lactic acid bacteria which become the dominant microbiota. Yeasts co-exist with lactic acid bacteria at lower population densities
- Fermentation is primarily lactic and alcoholic (to a lesser extent)
- The final product has pH 3,8-4,0 and titratable acidity 0,8-1,0% (expressed as lactic acid)
- After fermentation, NaCl is adjusted to 8% to avoid spoilage
- Brine acidification is usually carried out with lactic acid

# Effect of NaCl on population dynamics during fermentation at 25°C



-□- lactic acid bacteria, -O- yeasts, -△- enterobacteria -\*- pseudomonads



# Fermentation tanks







# Temperature control of fermentation tanks





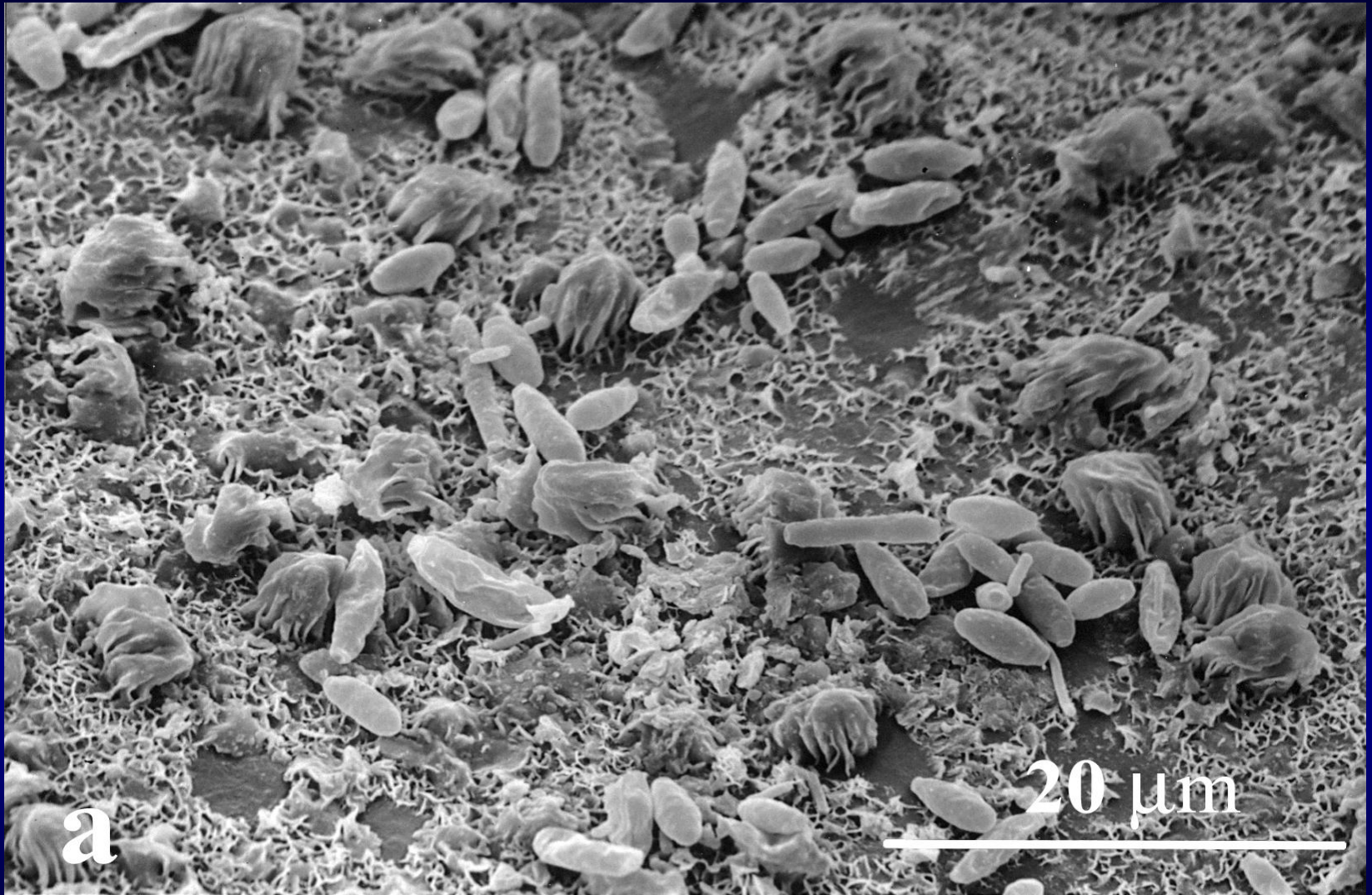
# Temperature control of fermentation tanks







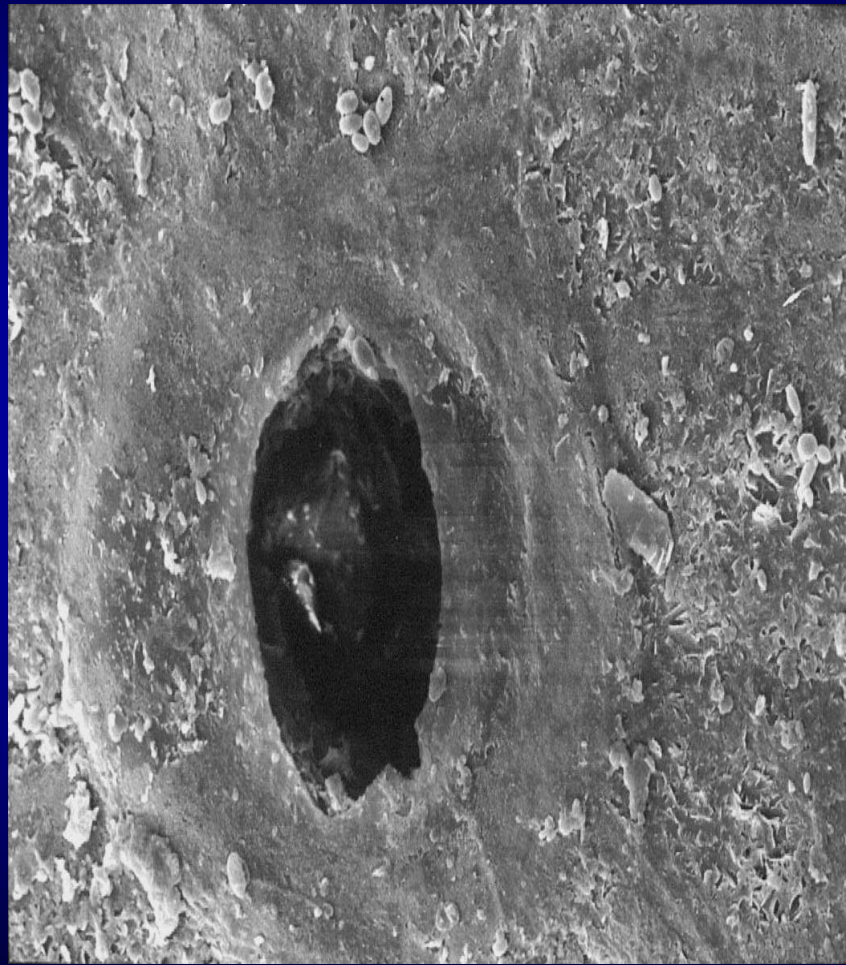
# Microorganisms on the surface of raw olives







# Stomata opening on raw olives



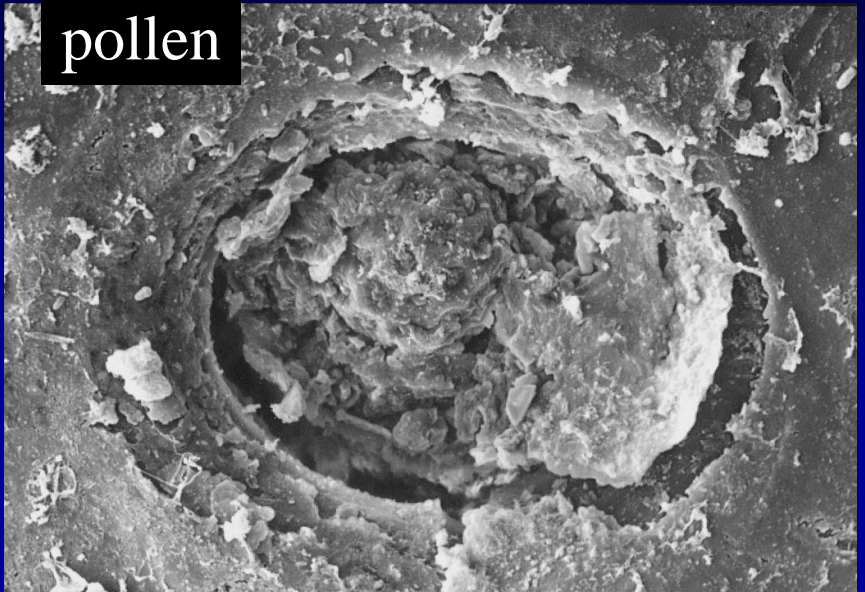


# Stomata opening blocked with:

earth



pollen



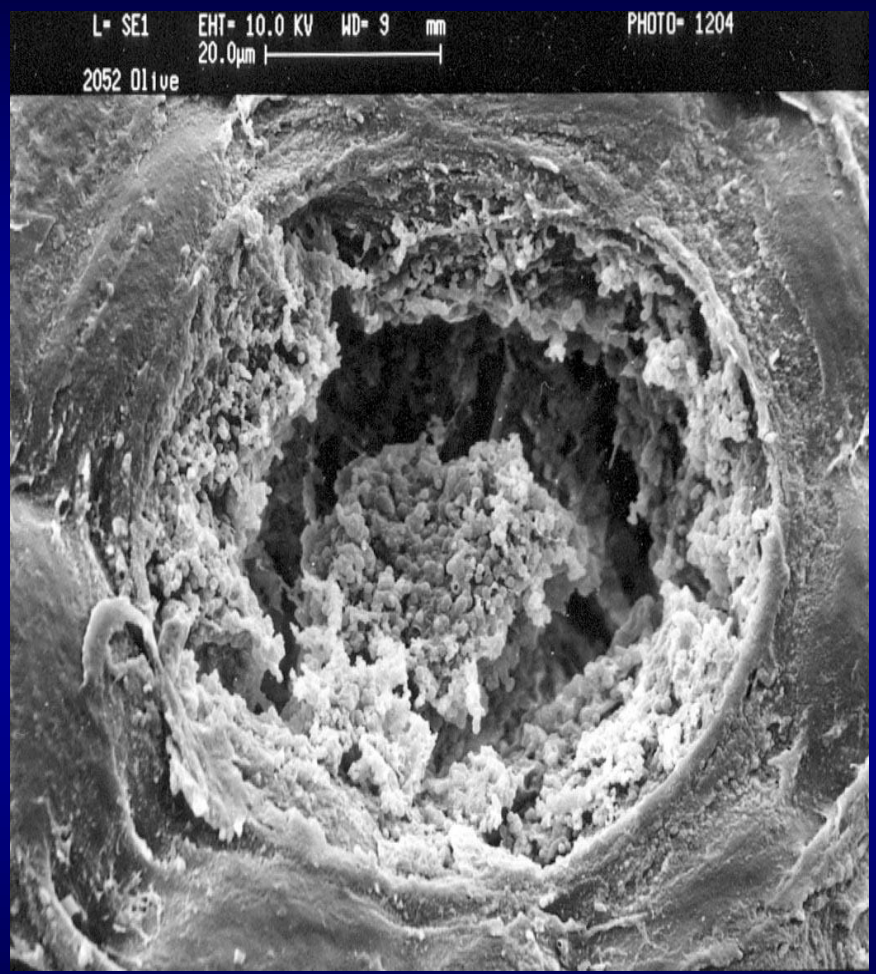
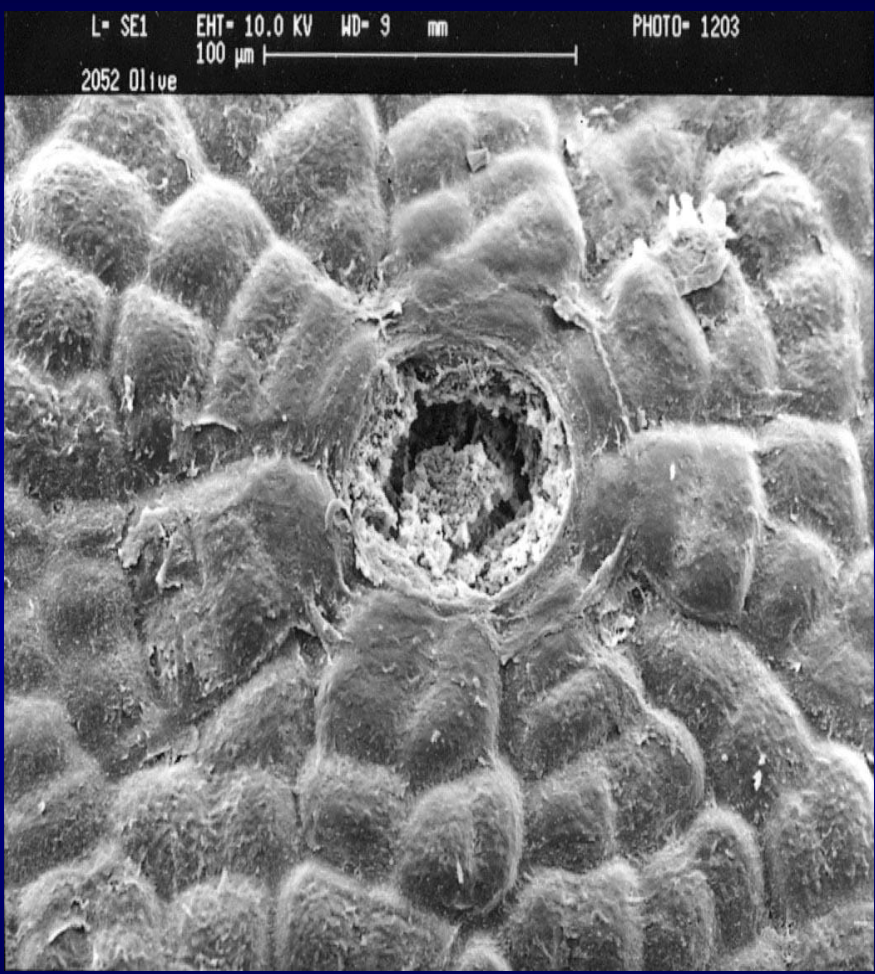
Fungal spore





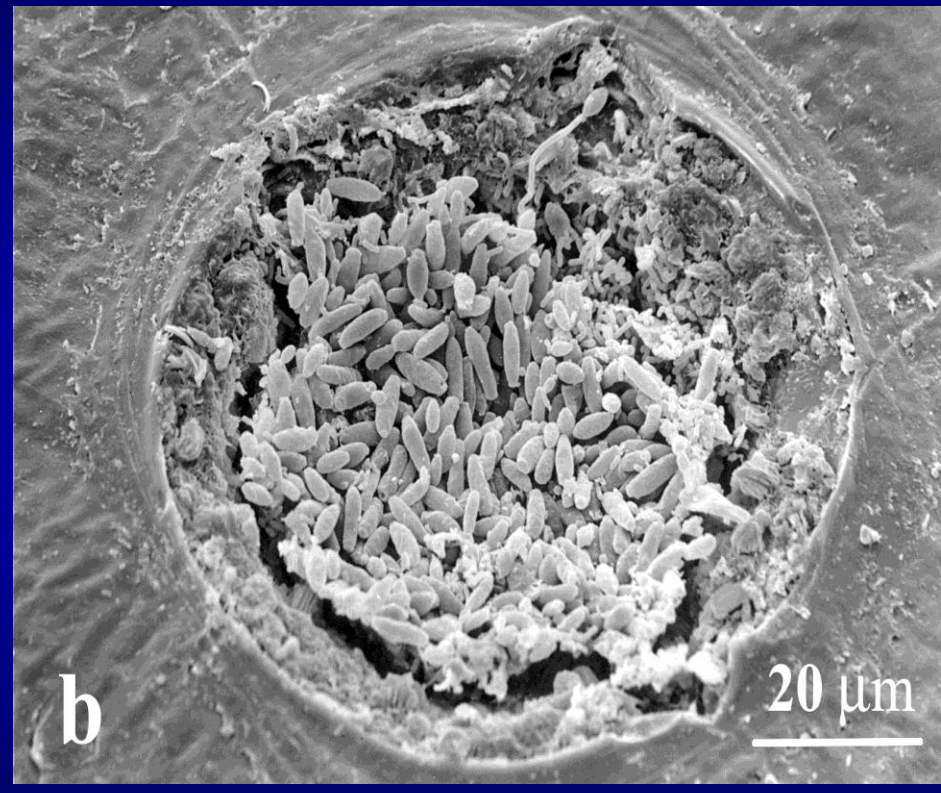
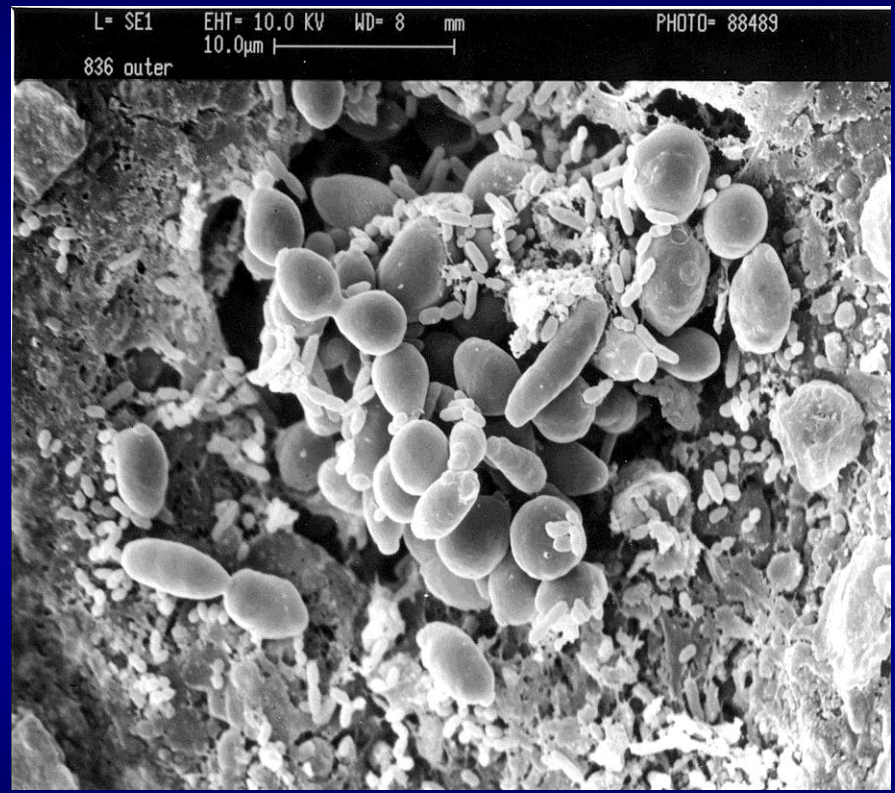


# Stomata opening on raw olives





# Spatial distribution of microorganisms during fermentation



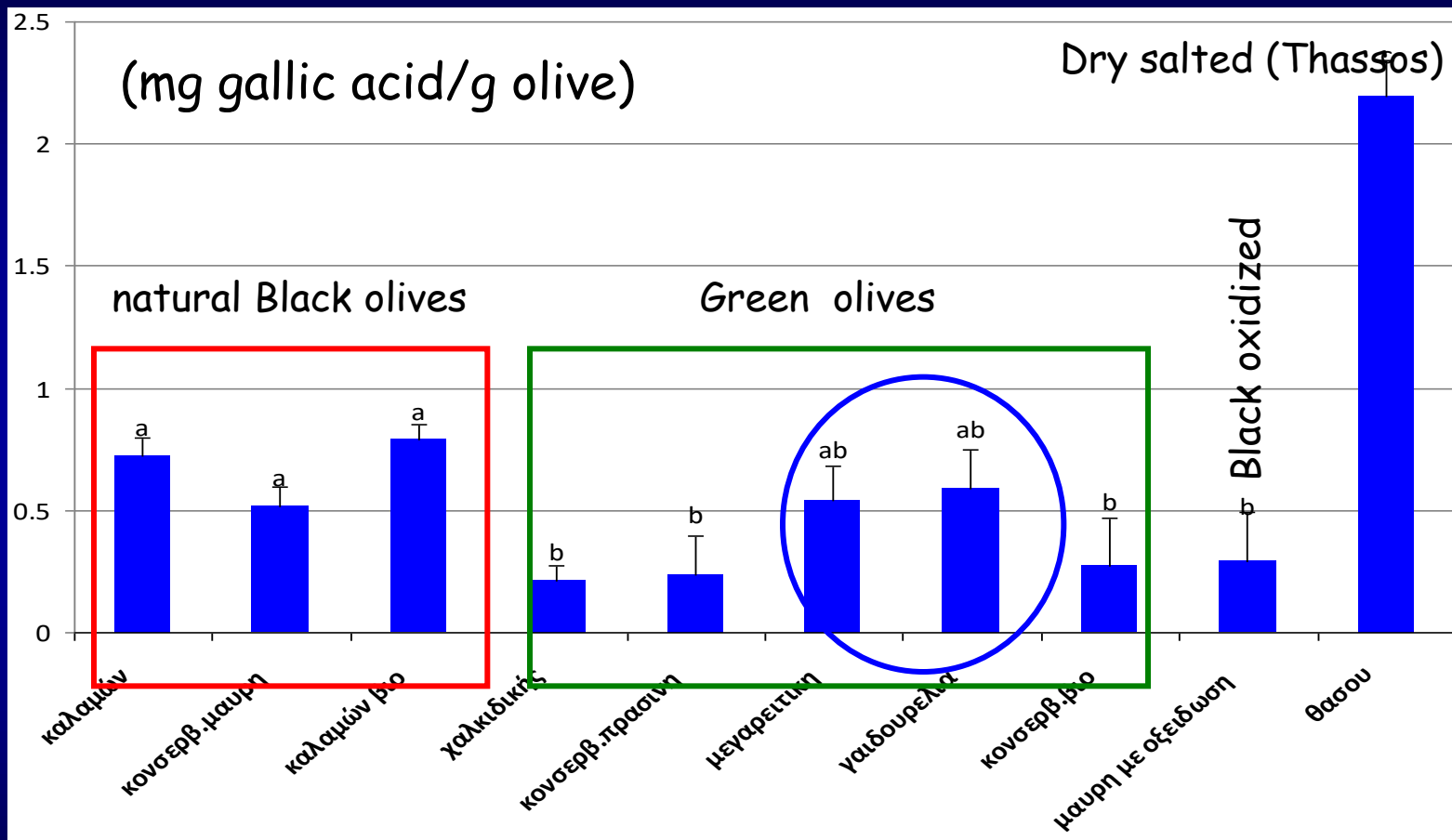




# Antioxidant potential of table olives

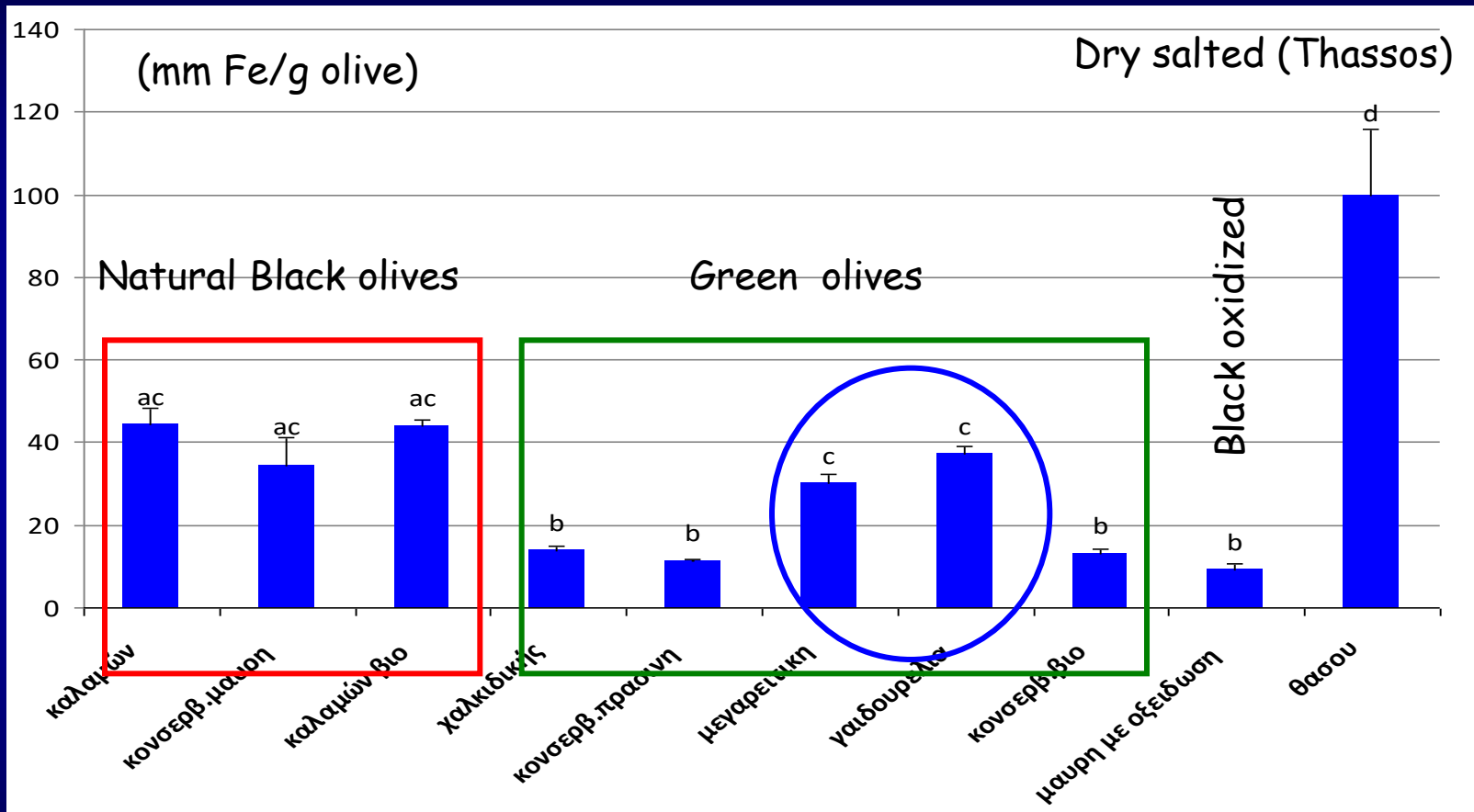


# Concentration of polyphenols in Greek table olives varieties





# In vitro antioxidant potential of Greek table olives varieties



Method FRAP (Ferric Reducing Antioxidant Power)





# Antioxidant potential of table olives compared to other fruits against 3 antioxidant indices - FRAP, TRAP, & TEAC

**TABLE 2**

*Ferric reducing-antioxidant power (FRAP), total radical-trapping antioxidant parameter (TRAP) and Trolox equivalent antioxidant capacity (TEAC) of fruit extracts<sup>1,2</sup>*

Fruit	FRAP		TRAP		TEAC	
	Value	Rank	Value	Rank	Value	Rank
	<i>(mmol Fe<sup>2+</sup>/kg FW<sup>3</sup>)</i>		<i>(mmol Trolox/kg FW)</i>			
Apple (red Delicious)	3.84	24	2.23	20	1.59	22
Apple (yellow Golden)	3.23	26	1.54	24	1.31	25
Apricot	4.02	23	2.29	19	1.44	24
Banana	2.28	28	1.05	27	0.64	30
Blackberry	51.53	1	21.01	1	20.24	1
Blueberry	18.61	9	9.30	7	7.43	10
Cherry	8.10	16	4.17	12	2.69	16
Clementine	8.88	15	2.74	16	3.10	14
Fig	5.82	20	2.06	21	2.47	18
Grape (black)	11.09	12	2.50	17	3.85	13
Grape (white)	3.25	25	1.59	23	2.48	17
Grapefruit (yellow)	10.20	13	4.04	13	3.05	15
Kiwi fruit	7.41	17	2.30	18	2.28	19
Loquat	2.70	27	1.73	22	0.75	27
Melon (cantaloupe)	5.73	21	0.95	28	1.20	26
Melon (honeydew)	2.27	29	1.12	26	0.65	29
Olive (black)	39.99	4	18.08	2	14.73	3
Olive (green)	24.59	6	14.64	3	10.43	7
Orange	20.50	5	5.65	4	8.74	9
Peach (yellow)	6.57	19	1.49	25	1.67	21
Pear	5.00	22	3.87	14	2.19	20
Pineapple	15.73	10	5.92	10	9.91	8
Plum (red)	12.79	11	8.09	9	5.11	11
Prickly pear	6.97	18	2.06	21	1.46	23
Raspberry	43.03	3	10.48	5	16.79	2
Redcurrant	44.86	2	12.14	4	14.05	4
Strawberry (cultivated)	22.74	7	8.56	8	10.94	6
Strawberry (wild)	28.00	5	10.34	6	11.34	5
Tangerine	9.60	14	2.76	15	4.16	12
Watermelon	1.13	30	0.46	29	0.69	28



# Functional table olives





FP7-SME-2008-2-243471



## "PROBIOLIVES"

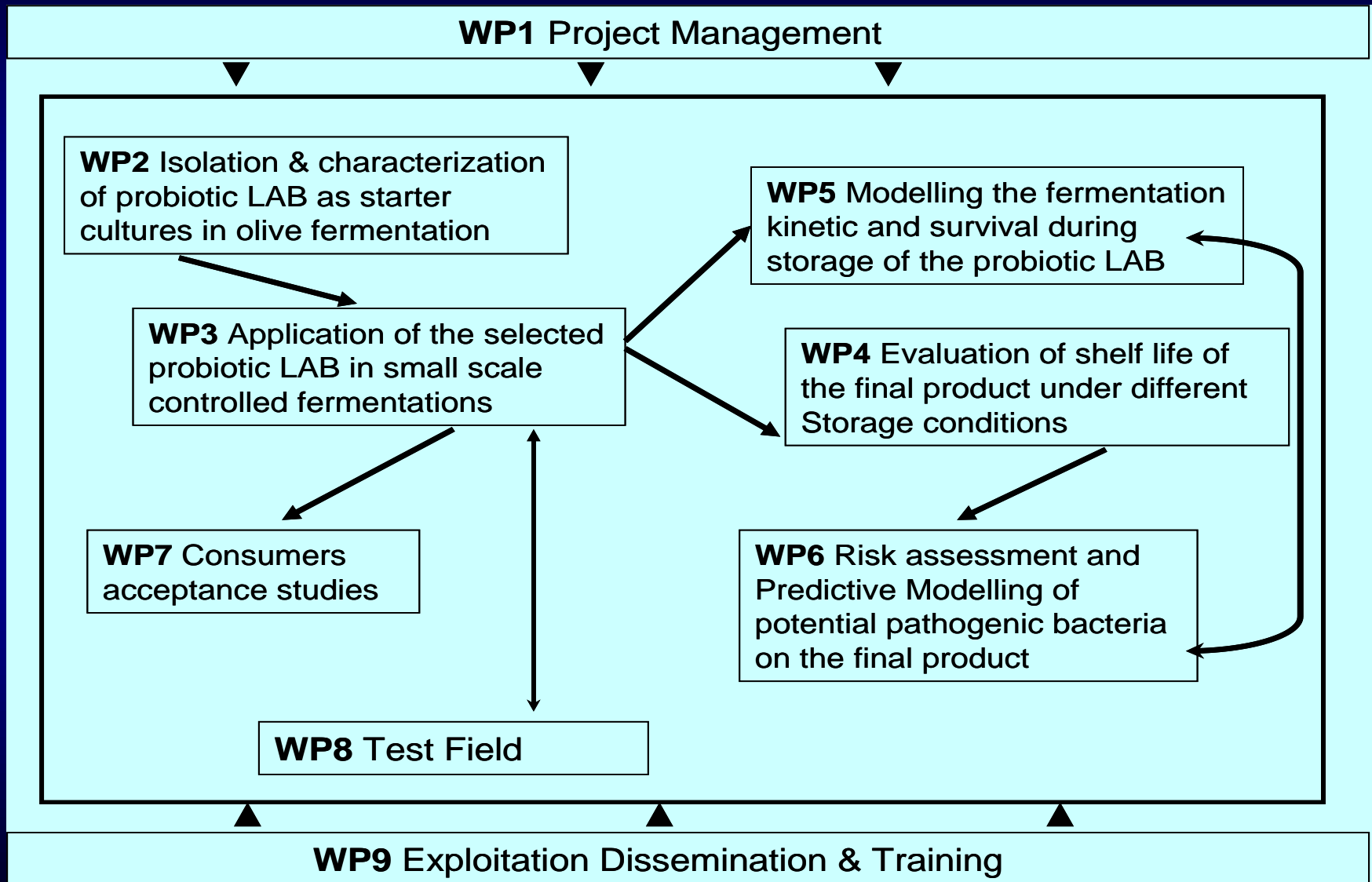
Table olive fermentation with selected strains of probiotic lactic acid bacteria. Towards a new functional food.

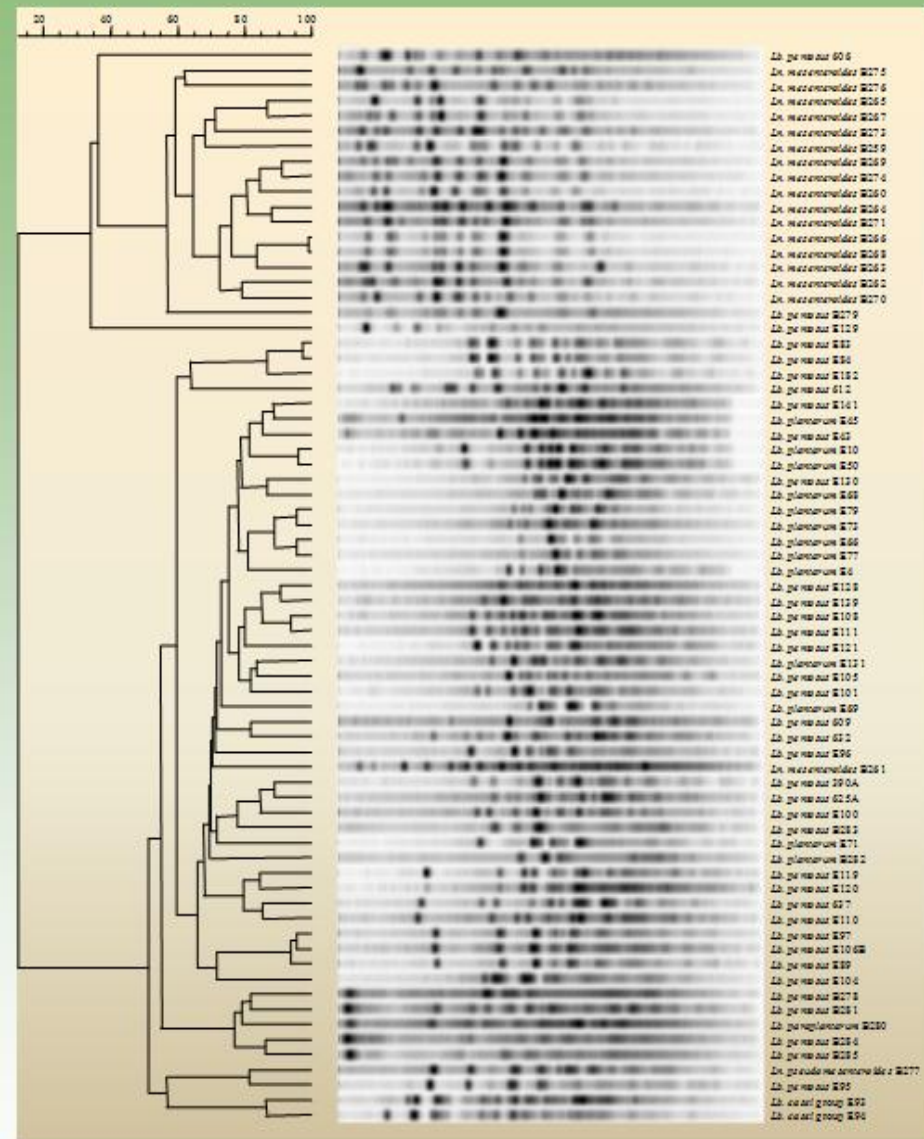
{GR, IT, S, P, Tu}

Coordinated by Dr. C. Tassou (ITAP- NAGREF)



# Activities-Targets of the project





71 different strains of LAB species isolated from Greek olives that contribute to fermentation



- 13 *Lactobacillus plantarum*
- 37 *Lb. pentosus*
- 1 *Lb. paraplantarum*
- 2 *Lb. casei* group (*Lb. casei*, *Lb. paracasei*)
- 17 *Leuconostoc mesenteroides*
- 1 *Ln. pseudomesenteroides*

From those 9 were found to possess

**PROBIOTIC PROPERTIES *IN VITRO***

Cluster analysis of PFGE *ApaI* digestion fragments of the different lactic acid bacteria strains recovered from olives and brine calculated by the unweighted average pair grouping method. The distance between the pattern of each strain is indicated by the mean correlation coefficient (r%).





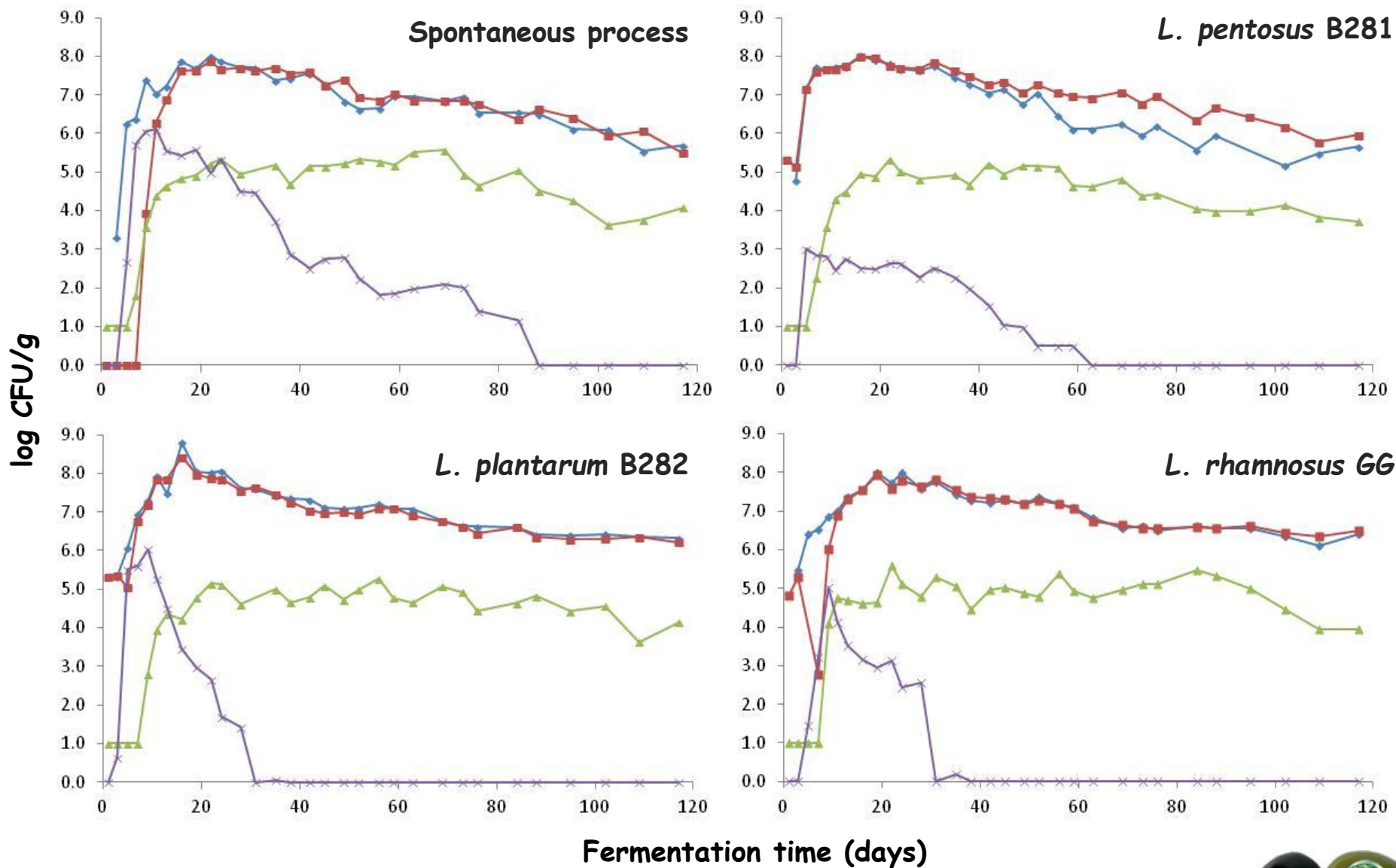
# Fermentation procedure

- ✓ **Olives:** Green olives Halkidiki variety
- ✓ **Brine:** 10 % (w/v) NaCl initial level
- ✓ **Fermentation process:** Spanish style processing
- ✓ **Fermentation treatments:**
  - ◇ Spontaneous process (control)
  - ◇ Inoculated process with *L. pentosus* B 281
  - ◇ Inoculated process with *L. plantarum* B 282
  - ◇ Inoculated process with *L. rhanmosus* GG
- ✓ **LAB strains were isolated from three different stages of the olive fermentation treatments (1, 56 and 117 days)**
- ✓ **Molecular tool:** Pulse Field Gel Electrophoresis (PFGE)





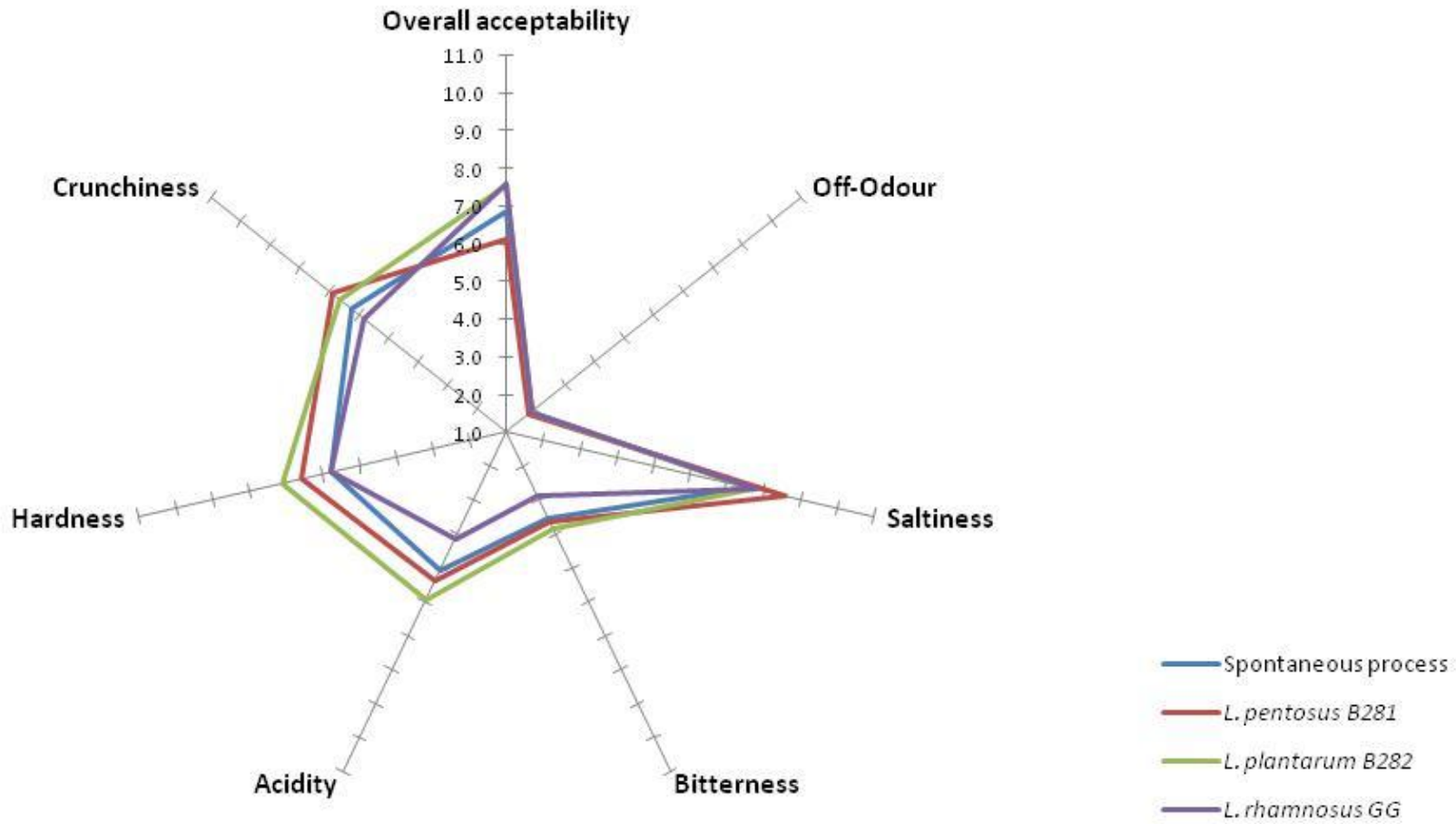
# Evolution of microbial association



◆ Total Viable Counts, ■ Lactic Acid Bacteria, ▲ Yeasts, × Enterobacteriaceae



# Organoleptic assessment





# Ecotrophelia 2012 - Probiotic olives





# Packaging of Probiotic olives

experimental procedure

- **Olives:** Green olives cv. Halkidiki
- **Packaging:** Plastic pouches (OPE 15  $\mu\text{m}$  / PE 80  $\mu\text{m}$ )
- **Storage temp.:** 4 and 20  $^{\circ}\text{C}$
- **Storage time:** 12 months
- **Composition:**
  - Fermented olives, 150 g
  - Brine 9%, 250 ml
  - Citric acid, 0.2 %
  - Ascorbic acid, 0.15 %
- **Packing Treatments:** Olives previously fermented by
  - (i) indigenous microbiota (spontaneous process)
  - (ii) *L. pentosus* B281
  - (iii) *L. plantarum* B282
  - (iv) mixture of both strains



# Survival rate of inoculated strains during storage according to molecular analysis



Inoculated strain	Fermentation time (days)	Survival rate	
		4°C	20°C
<i>L. pentosus</i> B281	1	90%	90%
	196	100%	80%
	357	93.75%	70%
<i>L. plantarum</i> B282	1	87.5%	87.5%
	196	96%	0%
	357	0%	0%
Mixed culture (B281 and B282)	1	90% B281/ 0% B282	90% B281/ 0% B282
	196	100% B281/ 0% B282	60% B281/ 0% B282
	357	95.6% B281/ 0% B282	50% B281/ 0% B282

# Production of probiotic olives at industrial scale

## Lye treatment

1.7 % NaOH (w/v) for about 10-12 hours



## Washing scheme

1<sup>st</sup> washing: 4 hours  
2<sup>nd</sup> washing: 8 hours



## Brining

10 % (w/v) NaCl  
0.1% lactic acid (95%)  
0.014 % HCl



## Debittering process



# Production of probiotic olives at industrial scale

## Inoculation



Fermentation in **12tn total capacity tank** (7-7.5tn olives and 4.5-5tn brine)

Initial **salt 10% w/v**

Acidification with **0.1% (v/v) lactic acid** and **0.014% (v/v) HCl**



After 24h of brining

*L. pentosus* B281  
culture

were added in the tank

**Final concentration of inoculum ap.  $10^7$  CFU/mL**



Fermentation was undertaken in outdoor conditions



# Survival rate of inoculated culture



Inoculated strain	Fermentation time (days)	Survival rate (PFGE)
<i>L. pentosus</i> B281	5	100%
	97	95.24%



# Table olives safety





# Survival of pathogens in fermented green olives

Fermented green olives



Discarding of old brine



Addition of fresh brine NaCl 6% (w/v)



Storage at 20°C

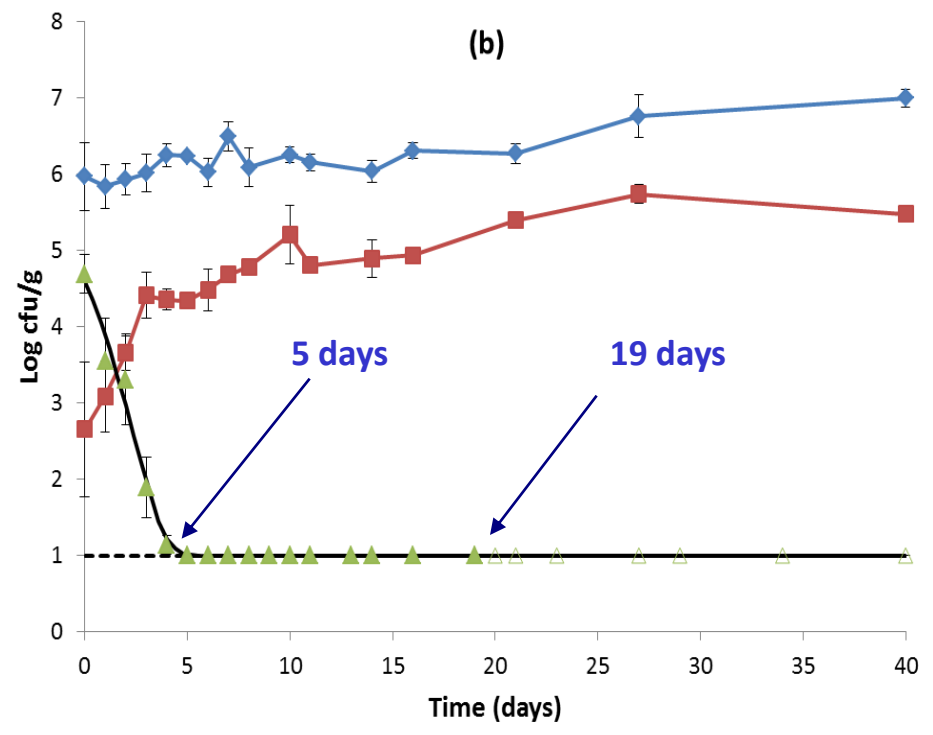
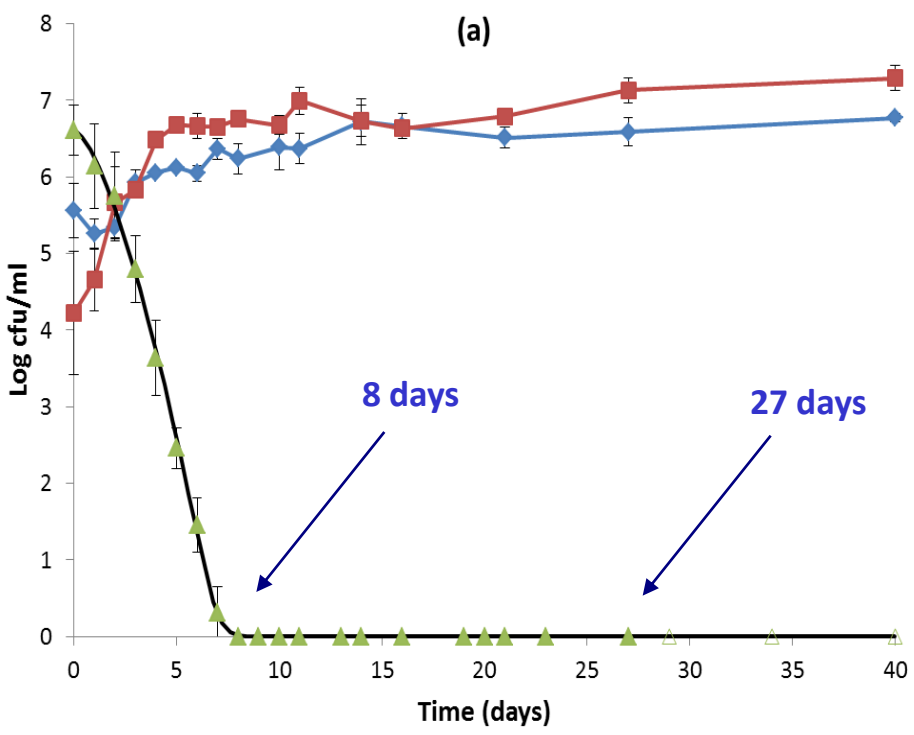
Addition of the pathogenic bacteria  
(Cocktail of 5 strains of each bacterium)

- *E. coli* O157:H7
- *Salmonella* Enteritidis
- *Listeria monocytogenes*





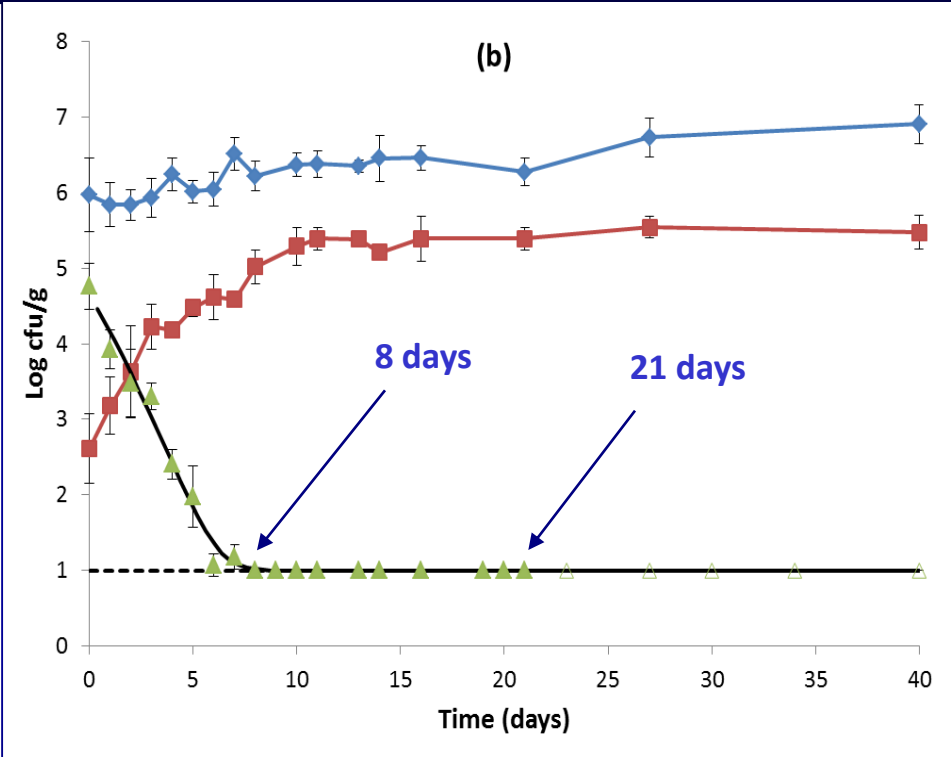
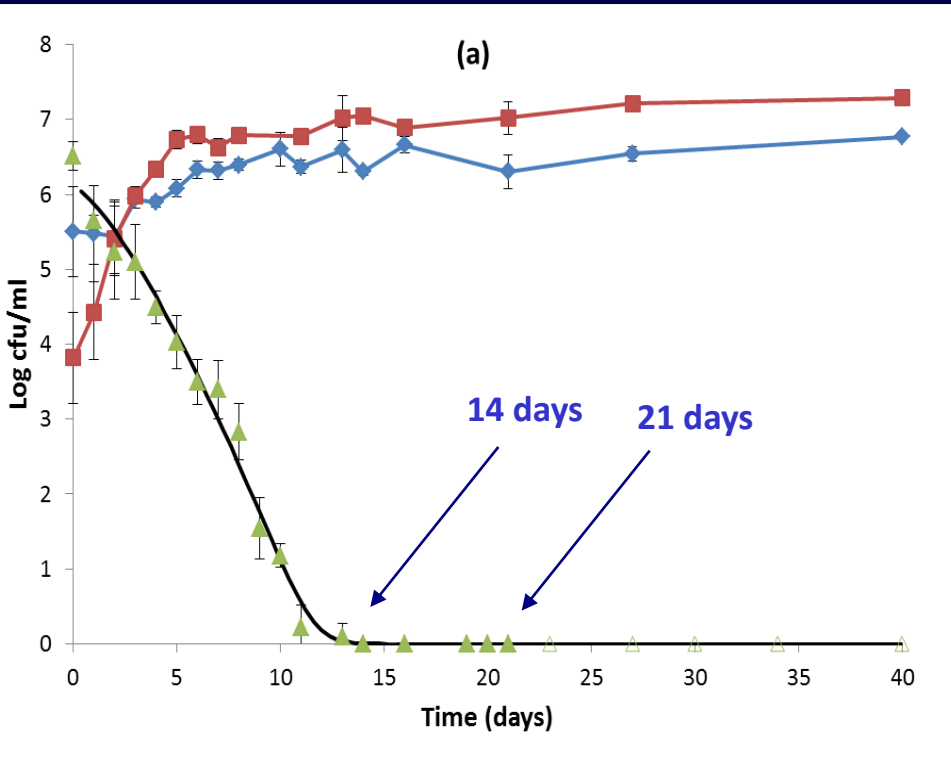
# Decline of *E.coli* O157:H7



Changes in the population of LAB (♦), yeasts (■) and *E. coli* O157:H7 (▲) in brine (a) and olive fruits (b), during storage of green table olives in pouches covered with brine at 20°C. (Δ): pathogen not detected after the enrichment method.



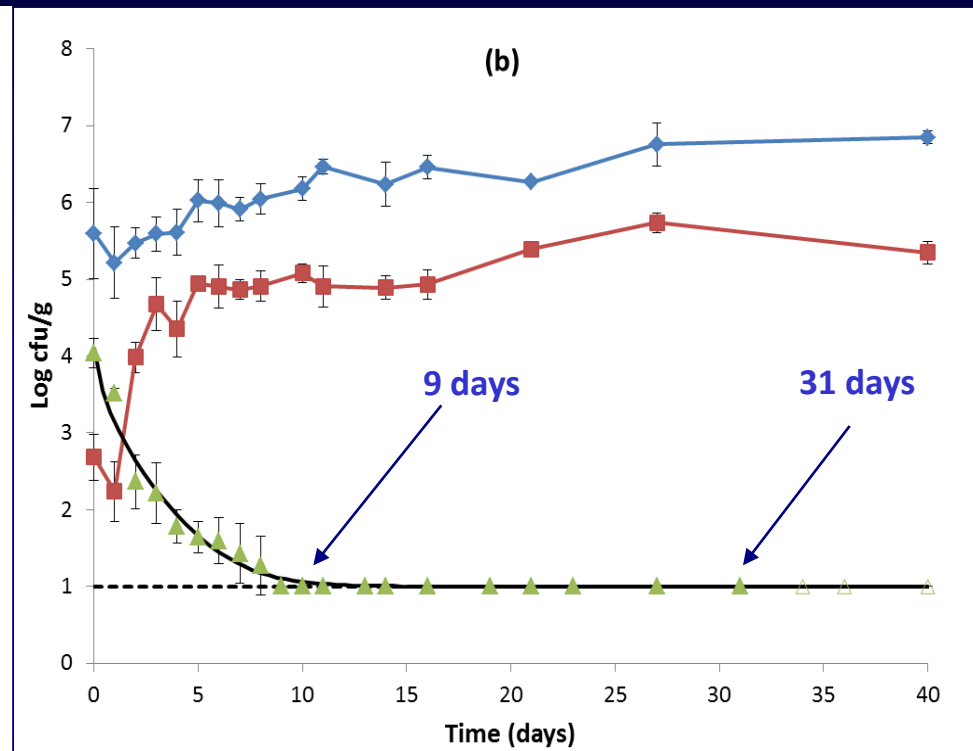
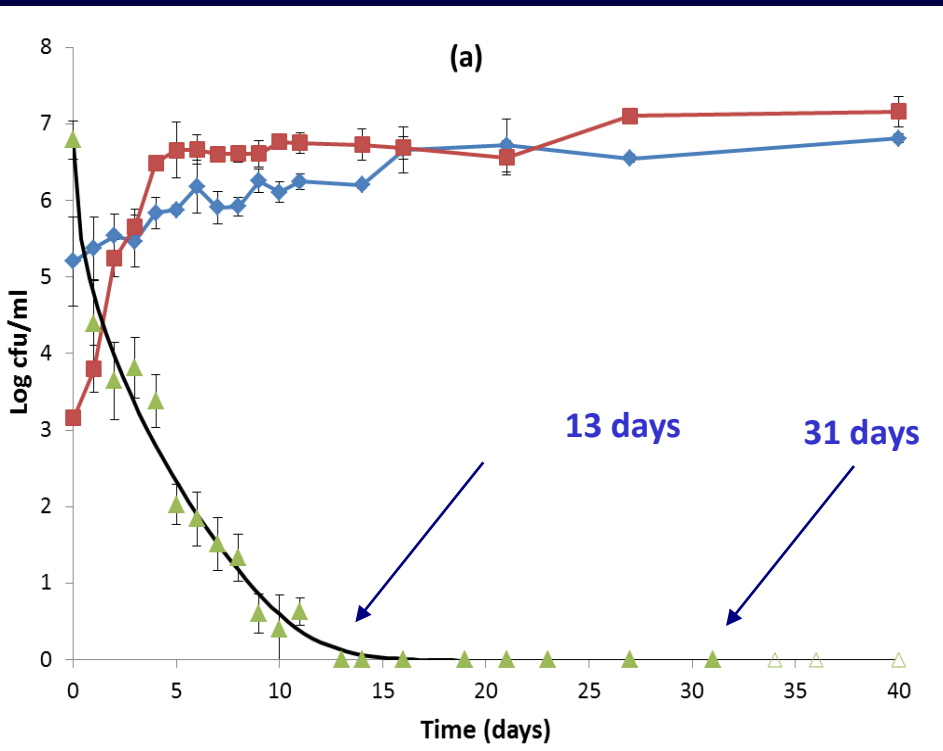
# Decline of *Salmonella* Enteritidis



Changes in the population of LAB (♦), yeasts (■) and *S. Enteritidis* (▲) in brine (a) and olive fruits (b), during storage of green table olives in pouches covered with brine at 20°C. (Δ): pathogen not detected after the enrichment method



# Decline of *Listeria monocytogenes*



Changes in the population of LAB (◆), yeasts (■) and *L. monocytogenes* (▲) in brine (a) and olive fruits (b), during storage of green table olives in pouches covered with brine at 20°C. (Δ): pathogen not detected after the enrichment method.

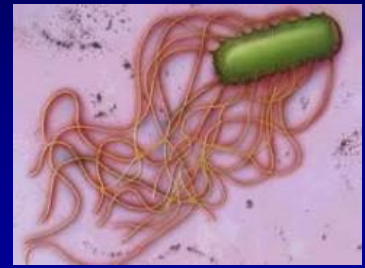
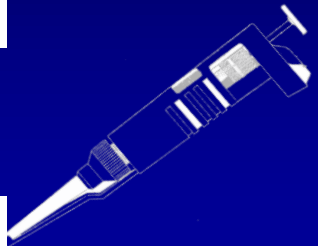


# Survival of pathogens in fermented black olives

Fermented black olives



Storage in air at  
20°C



Addition of the pathogenic bacteria

- *E. coli* O157:H7
- *Salmonella* Enteritidis
- *Salmonella* Typhimurium
- *Listeria monocytogenes*
- *Staphulococcus aureus*

# Decline of *Salmonella* Enteritidis and Typhimurium

**Table 1**

Populations of *Salmonella enterica* ser. Enteritidis and *Salmonella enterica* ser. Typhimurium recovered from inoculated natural black olives during storage at 4 and 20 °C.

S. Enteritidis Strain	T (°C)	Population (log CFU/g) on:								
		Day 0	Day 1	Day 2	Day 3	Day 5	Day 9	Day 12	Day 15	
B-56	4	4.6 ± 0.4 <sup>Aa</sup>	3.9 ± 0.2 <sup>Ba</sup>	nd	nd	nd	nd	nd	nd	
B-57		2.8 ± 0.4 <sup>b</sup>	nd	nd	nd	nd	nd	nd	nd	
ATCC 13076		4.1 ± 0.4 <sup>Ac</sup>	2.9 ± 0.4 <sup>Bb</sup>	nd	nd	nd	nd	nd	nd	
B-287		4.0 ± 0.5 <sup>Ac</sup>	2.2 ± 0.2 <sup>Bc</sup>	nd	nd	nd	nd	nd	nd	
Mixed culture		4.0 ± 0.2 <sup>Ac</sup>	3.2 ± 0.1 <sup>Bb</sup>	nd	nd	nd	nd	nd	nd	
B-56	20	4.6 ± 0.4 <sup>Aa</sup>	3.3 ± 0.2 <sup>Ba</sup>	nd	nd	nd	nd	nd	nd	
B-57		2.8 ± 0.4 <sup>b</sup>	nd	nd	nd	nd	nd	nd	nd	
ATCC 13076		4.1 ± 0.4 <sup>c</sup>	nd	nd	nd	nd	nd	nd	nd	
B-287		4.0 ± 0.5 <sup>c</sup>	nd	nd	nd	nd	nd	nd	nd	
Mixed culture		4.0 ± 0.2 <sup>Ac</sup>	3.5 ± 0.4 <sup>Ba</sup>	nd	nd	nd	nd	nd	nd	
<i>S. Typhimurium</i>										
B-137	4	4.6 ± 0.3 <sup>Aa</sup>	4.3 ± 0.1 <sup>Aa</sup>	nd	nd	nd	nd	nd	nd	
B-193		4.3 ± 0.2 <sup>Aa</sup>	3.4 ± 0.1 <sup>Bb</sup>	nd	nd	nd	nd	nd	nd	
B-194		4.5 ± 0.4 <sup>Aa</sup>	3.7 ± 0.2 <sup>Bb</sup>	nd	nd	nd	nd	nd	nd	
Mixed culture		4.7 ± 0.1 <sup>Aa</sup>	4.9 ± 0.1 <sup>Ac</sup>	nd	nd	nd	nd	nd	nd	
B-137	20	4.6 ± 0.3 <sup>Aa</sup>	3.5 ± 0.1 <sup>Ba</sup>	nd	nd	nd	nd	nd	nd	
B-193		4.3 ± 0.2 <sup>Aa</sup>	3.1 ± 0.3 <sup>Ba</sup>	nd	nd	nd	nd	nd	nd	
B-194		4.5 ± 0.4 <sup>Aa</sup>	3.0 ± 0.6 <sup>Ba</sup>	nd	nd	nd	nd	nd	nd	
Mixed culture		4.7 ± 0.1 <sup>a</sup>	nd	nd	nd	nd	nd	nd	nd	

nd: none detected (<2.0 log CFU/g of olives) by direct plating followed by enrichment where absence of the pathogen was observed (<1 CFU/25 g of olives).

Means with different capital letters in the same row are significantly different ( $P \leq 0.05$ ). Means with different lowercase letters in the same column are significantly different ( $P \leq 0.05$ ).



# Decline of *E. coli* O157:H7 and *S. aureus*

**Table 2**

Populations of *Escherichia coli* O157:H7 recovered from inoculated natural black olives during storage at 4 and 20 °C.

Strain	T (°C)	Population (log CFU/g) on:								
		Day 0	Day 1	Day 2	Day 3	Day 5	Day 9	Day 12	Day 15	
B-15	4	3.8 ± 0.2 <sup>Aa</sup>	4.9 ± 0.2 <sup>Ba</sup>	nd	nd	nd	nd	nd	nd	
B-16		4.3 ± 0.2 <sup>Aab</sup>	4.9 ± 0.1 <sup>Ba</sup>	nd	nd	nd	nd	nd	nd	
B-18		4.2 ± 0.1 <sup>Aab</sup>	4.5 ± 0.2 <sup>Aa</sup>	nd	nd	nd	nd	nd	nd	
Mixed culture		4.5 ± 0.1 <sup>Ab</sup>	4.5 ± 0.3 <sup>Aa</sup>	nd	nd	nd	nd	nd	nd	
B-15	20	3.8 ± 0.2 <sup>a</sup>	nd	nd	nd	nd	nd	nd	nd	
B-16		4.3 ± 0.2 <sup>ab</sup>	nd	nd	nd	nd	nd	nd	nd	
B-18		4.2 ± 0.1 <sup>ab</sup>	nd	nd	nd	nd	nd	nd	nd	
Mixed culture		4.5 ± 0.1 <sup>Ab</sup>	4.0 ± 0.5 <sup>A</sup>	nd	nd	nd	nd	nd	nd	

nd: none detected (<2.0 log CFU/g of olives) by direct plating followed by enrichment where absence of the pathogen was observed (<1 CFU/25 g of olives).

Means with different capital letters in the same row are significantly different ( $P \leq 0.05$ ). Means with different lowercase letters in the same column are significantly different ( $P \leq 0.05$ ).

**Table 4**

Populations of *S. aureus* recovered from inoculated natural black olives during storage at 4 and 20 °C.

Strain	T (°C)	Population (log CFU/g) on:								
		Day 0	Day 1	Day 2	Day 3	Day 5	Day 9	Day 12	Day 15	
B-95	4	5.0 ± 0.2 <sup>Aa</sup>	3.8 ± 0.6 <sup>Ba</sup>	nd	nd	nd	nd	nd	nd	
ATCC 6538		5.1 ± 0.2 <sup>Aa</sup>	3.5 ± 0.1 <sup>Bab</sup>	2.6 ± 0.1 <sup>Ba</sup>	nd	nd	nd	nd	nd	
B-135		5.0 ± 0.2 <sup>Aa</sup>	3.3 ± 0.2 <sup>Bbc</sup>	2.2 ± 0.3 <sup>Ca</sup>	nd	nd	nd	nd	nd	
Mixed culture		5.1 ± 0.2 <sup>Aa</sup>	2.9 ± 0.3 <sup>Bc</sup>	2.6 ± 0.2 <sup>Ba</sup>	nd	nd	nd	nd	nd	
B-95	20	5.0 ± 0.2 <sup>Aa</sup>	3.5 ± 0.1 <sup>Ba</sup>	nd	nd	nd	nd	nd	nd	
ATCC 6538		5.1 ± 0.2 <sup>Aa</sup>	3.3 ± 0.5 <sup>Ba</sup>	nd	nd	nd	nd	nd	nd	
B-135		5.0 ± 0.2 <sup>Aa</sup>	3.3 ± 0.3 <sup>Ba</sup>	nd	nd	nd	nd	nd	nd	
Mixed culture		5.1 ± 0.2 <sup>Aa</sup>	3.4 ± 0.3 <sup>Ba</sup>	nd	nd	nd	nd	nd	nd	

nd: none detected (<1.0 log CFU/g of olives) by direct plating.

Means with different capital letters in the same row are significantly different ( $P \leq 0.05$ ). Means with different lowercase letters in the same column are significantly different ( $P \leq 0.05$ ).



# Survival of *L. monocytogenes*

**Table 3**  
Populations of *Listeria monocytogenes* recovered from inoculated natural black olives during storage at 4 and 20 °C.

Strain	T (°C)	Population (log CFU/g) on:							
		Day 0	Day 1	Day 2	Day 3	Day 5	Day 9	Day 12	Day 15
B-128	4	5.6 ± 0.5 <sup>Aa</sup>	3.7 ± 0.1 <sup>Ba</sup>	+	+	+	+	+	+
B-129		5.1 ± 0.3 <sup>Aa</sup>	2.6 ± 0.4 <sup>Bb</sup>	+	+	+	+	+	+
B-131		5.3 ± 0.2 <sup>Aa</sup>	2.8 ± 0.3 <sup>Bb</sup>	+	+	+	+	+	+
Mixed culture		4.9 ± 0.4 <sup>Aa</sup>	3.6 ± 0.2 <sup>Ba</sup>	+	+	+	+	+	+
B-128	20	5.6 ± 0.5 <sup>Aa</sup>	4.4 ± 0.1 <sup>Ba</sup>	nd	nd	nd	nd	nd	nd
B-129		5.1 ± 0.3 <sup>Aa</sup>	2.7 ± 0.2 <sup>Bb</sup>	+	+	+	+	+	+
B-131		5.3 ± 0.2 <sup>Aa</sup>	2.3 ± 0.2 <sup>Bb</sup>	nd	nd	nd	nd	nd	nd
Mixed culture		4.9 ± 0.4 <sup>Aa</sup>	2.3 ± 0.4 <sup>Bb</sup>	nd	nd	nd	nd	nd	nd

nd: none detected (<2.0 log CFU/g of olives) by direct plating followed by enrichment where absence of the pathogen was observed (<1 CFU/25 g of olives).

+: enrichment positive.

Means with different capital letters in the same row are significantly different ( $P \leq 0.05$ ). Means with different lowercase letters in the same column are significantly different ( $P \leq 0.05$ ).



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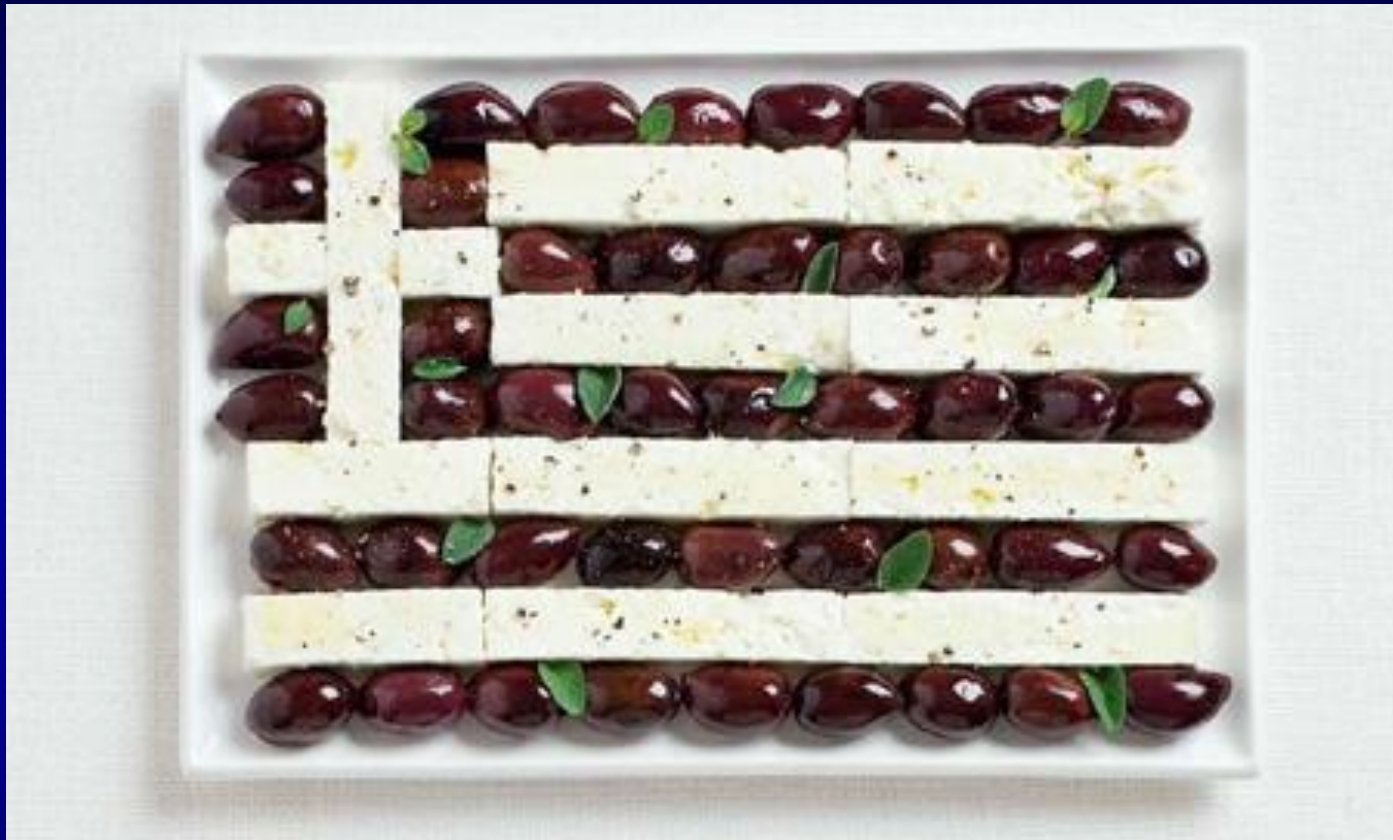
Review

## Types of traditional Greek foods and their safety

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**Thank you for your attention**