AGRICULTURAL UNIVERSITY OF ATHENS
DEPARTMENT OF FOOD SCIENCE AND HUMAN NUTRITION
LAB. OF MICROBIOLOGY AND BIOTECHNOLOGY OF FOODS

Table olives processing in Greece

Dr Efstathios Z. Panagou

Aix en Provence, 27 March 2015

Table olive varieties

Primary sector - Importance

- Table olive producers: 50.000-60.000
- Cultivated olive trees: 25-30.000.000
- Cultivated area: 153.000 ha
- Overall production: 200.000 tonnes
- Processed volume: 90-100.000 tonnes

Table olive cultivation areas

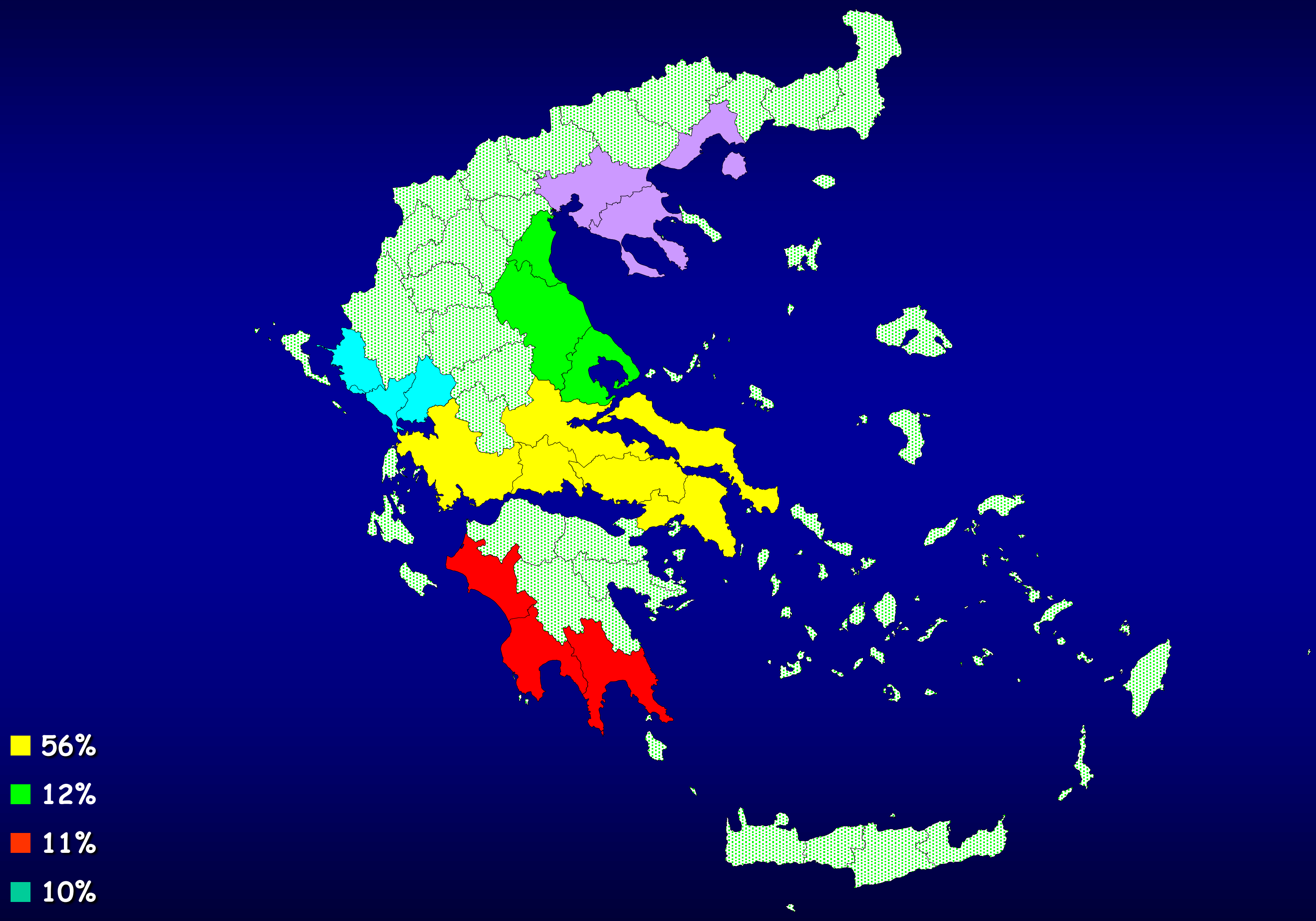


Table olive varieties- Conservolea

- Amounts to 51% of total olive production in Greece
- Average size: 180-200 fruits/kg
- Processed as Spanish-style green and naturally black olives
- Flesh-to-pit ratio: 8:1
- Oil content: 20-25% (w.b.)
- Fermentable material: 2-3% (w.b.)
- Similar to Manzanilla

Table olive varieties- Halkidiki

- Amounts to 26% of total olive production in Greece
- Average size: 120-140 fruits/kg
- Processed as Spanish-style green olives
- Flesh-to-pit ratio: 10:1
- Oil content: 19-20% (w.b.)
- Similar to Gordal

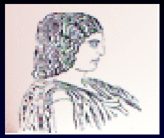
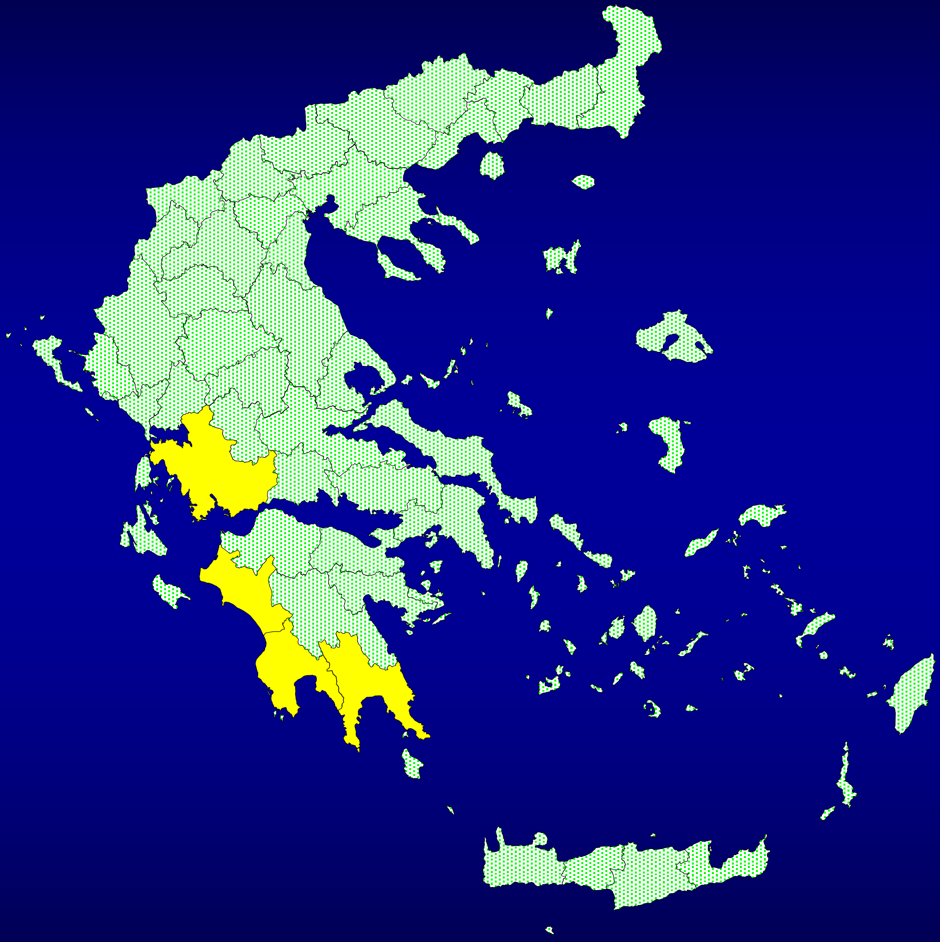


Table olive varieties- Kalamon



- Amounts to 20% of total olive production in Greece
- Average size: 220-240 fruits/kg
- Processed as naturally black olives
- Flesh-to-pit ratio: 8:1
- Oil content: 25% (w.b.)
- Fermentable material 3.1-3.5% (w.b.)

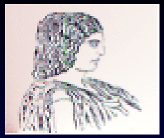
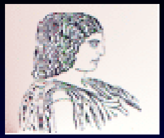


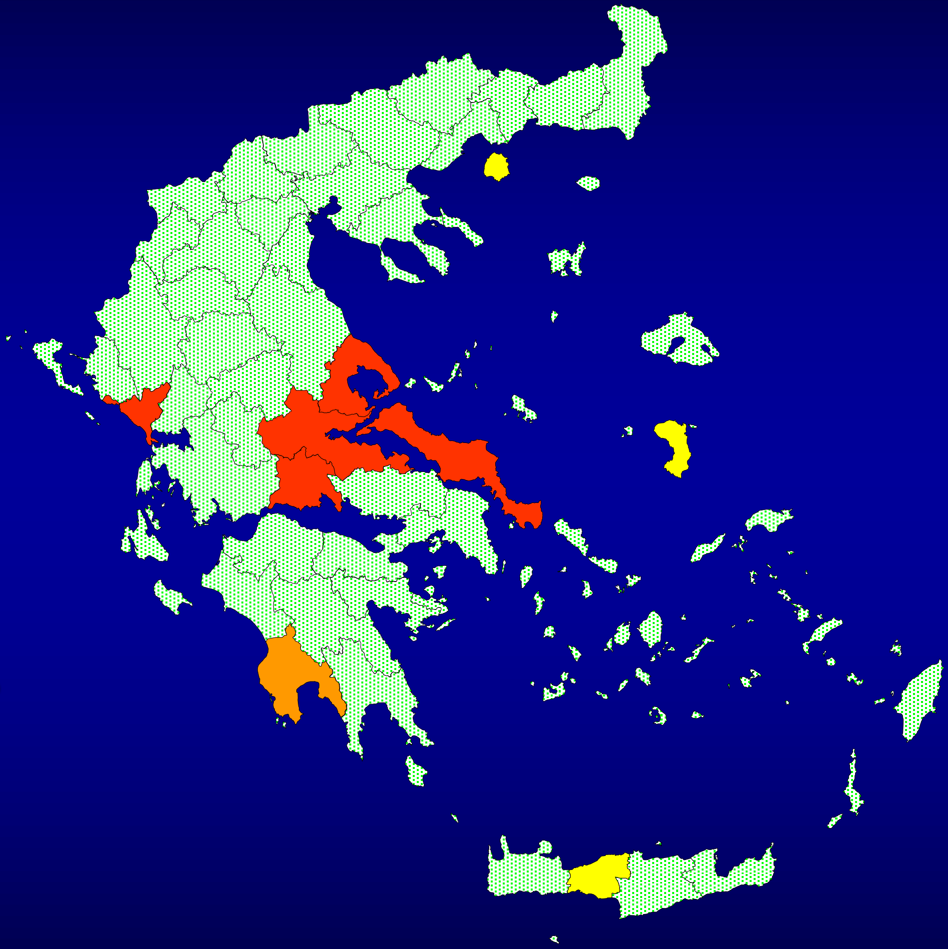
Table olive varieties- Thassos

- Processed as dry-salted olives
- Flesh-to-pit ratio: 6:1
- Oil content: 26% (w.b.)
- Fermentable material 3.5% (w.b.)
- Limited interest in the international market
- Consumed locally

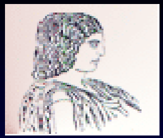


Protected Destination of Origin (PDO) table olives

- Kalamata olives
- Conservolea Amfissa
- Conservolea Arta
- Conservolea Atalanti
- Conservolea Rovies
- Conservolea Stylida
- Conservolea Pilion, Volos
- Thrubolea Thassos
- Thrubolea Chios
- Thrubolea Ambadias, Rethymno, Crete



Trade preparations



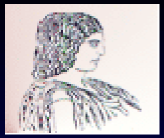
Basic Trade Preparations
(Olive Oil Council, Trade Standards Applying to Table Olives)

- 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

Harvest – Sorting - Grading

↓

Debittering in NaOH solution

↓

Washing to remove excess of alkali

↓

Brining - Fermentation

↓

Product ready for further processing and packing

Naturally black olives in brine (Greek style)

Harvesting – Transportation to the factory

Keeping in water

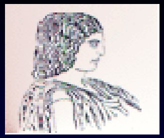
Brining

NaCl

Fermentation

Sorting and grading – Air exposure

Product ready for consumption or further processing



Olives darkened by oxidation (black ripe olives)

Harvesting – Transportation - Sorting

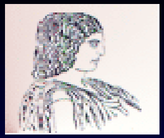
Preservation in brine (2.5-5.0% salt)

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%)

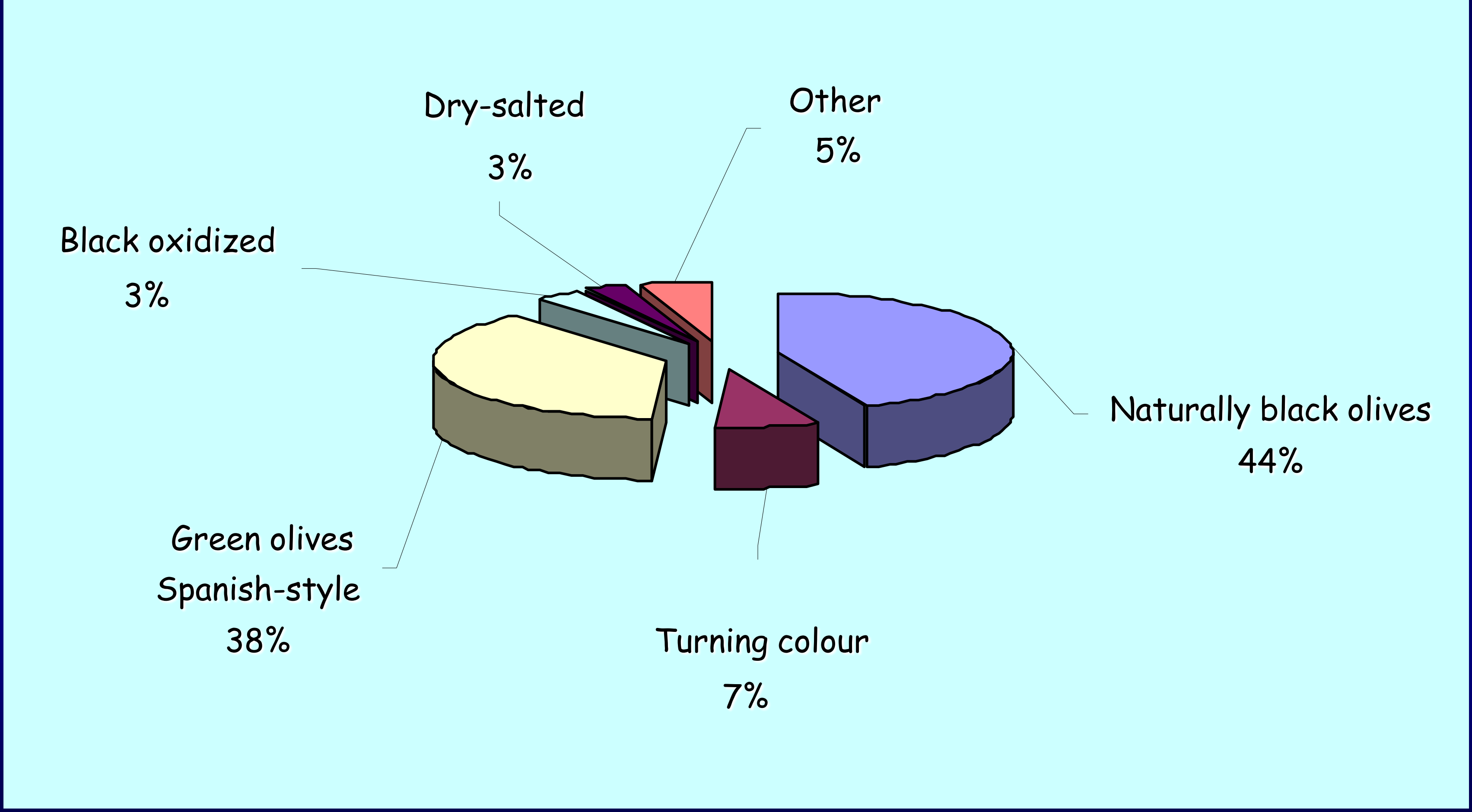
↓

Solute loss – Shrivelling – Gradual debittering (curing)

↓

Product ready for consumption (after 60-80 days)

Production of different types of table olives



The table olive sector

Table olive processing

Table olive processing takes place in:

- Small-scale farmers' installations
- Cooperative owned installations (20)
- SMEs (50)

Overall capacity: 100-110.000 tonnes

SMEs with exporting orientation are organised in the Panhellenic Association of Table Olive Producers, Packers and Exporters (PEMETE) to support the product in domestic and international market

Table olive production in Greece, 2003-2011 (x1000 tonnes)

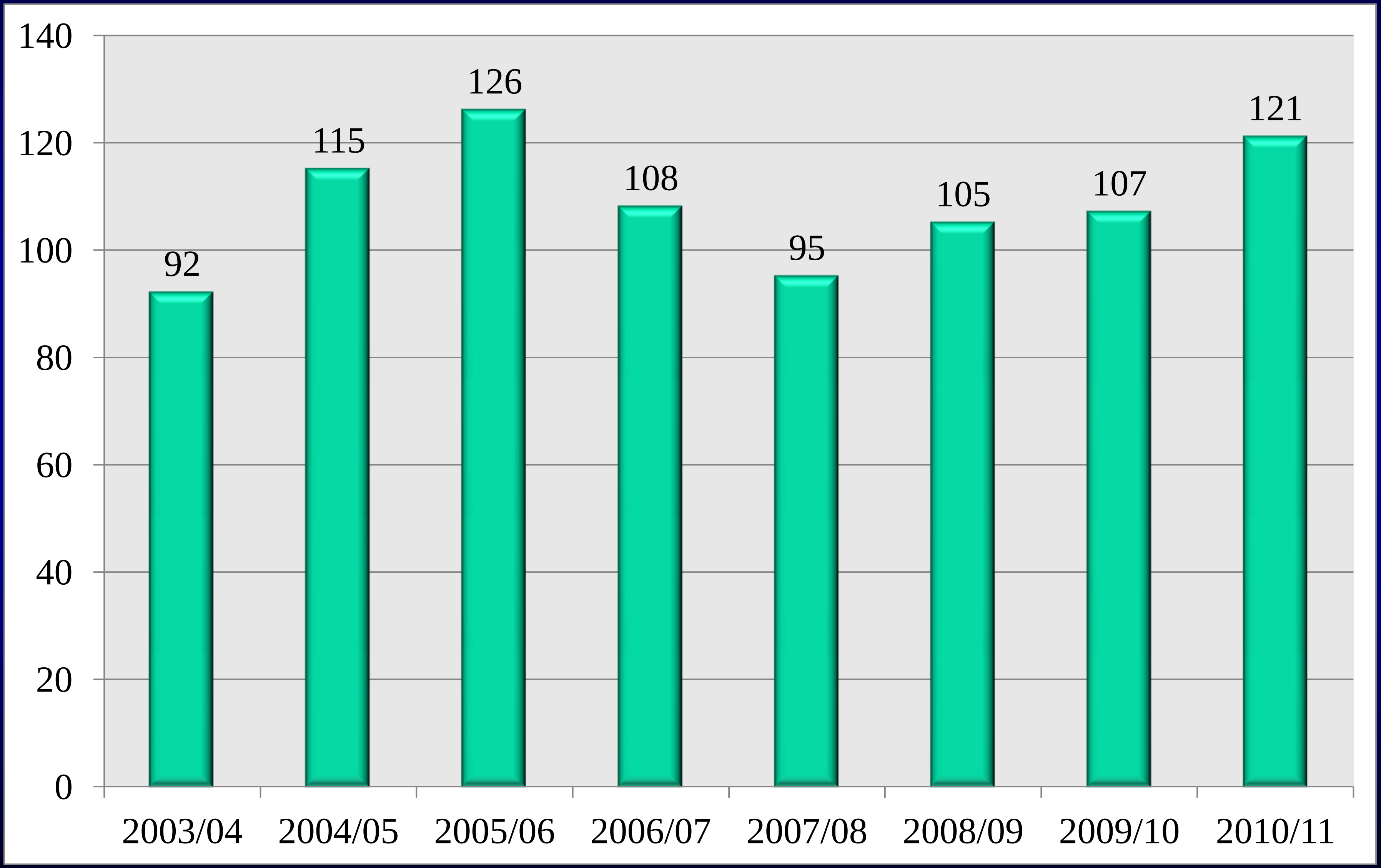
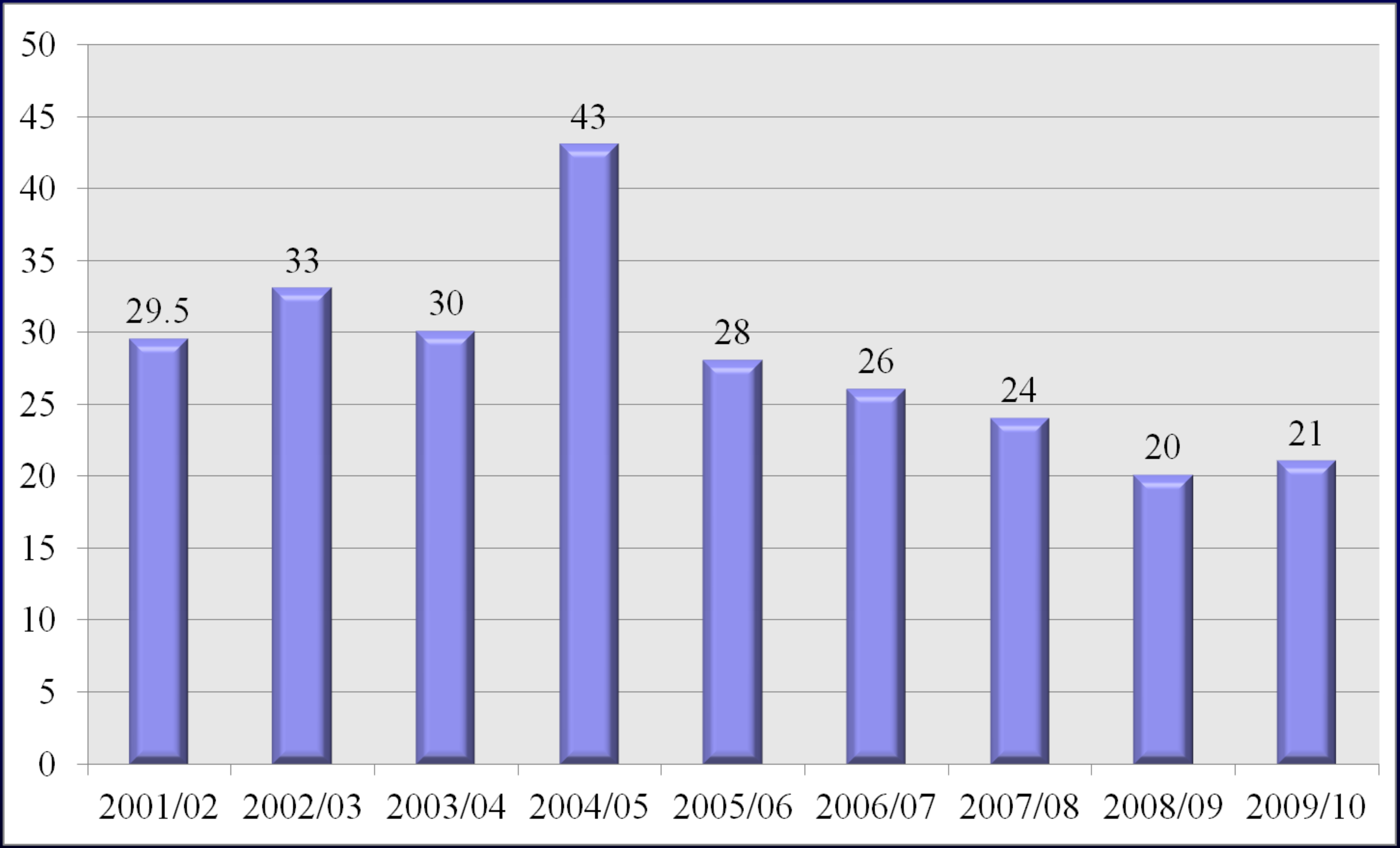


Table olive consumption in Greece, 2001-2010 (x1000 tonnes)



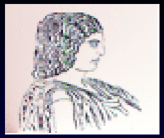


Table olive consumption characteristics

Consumers preference:

- 65-70% naturally black olives
- 20% green olives Spanish-style
- 10-15% other types (e.g. dry-salted olives)

Demand for table olives (2008)

- Urban areas: 260 g/month/household
- Rural areas: 427 g/month/household
- Average expenditure/household 1.15 € (urban areas) and 1.48 (rural areas)

The value of domestic market in 2009/10 was estimated to 41 million euros

Source: ICAP GROUP (2011), Table olives

Table olives – from the farm to the consumer

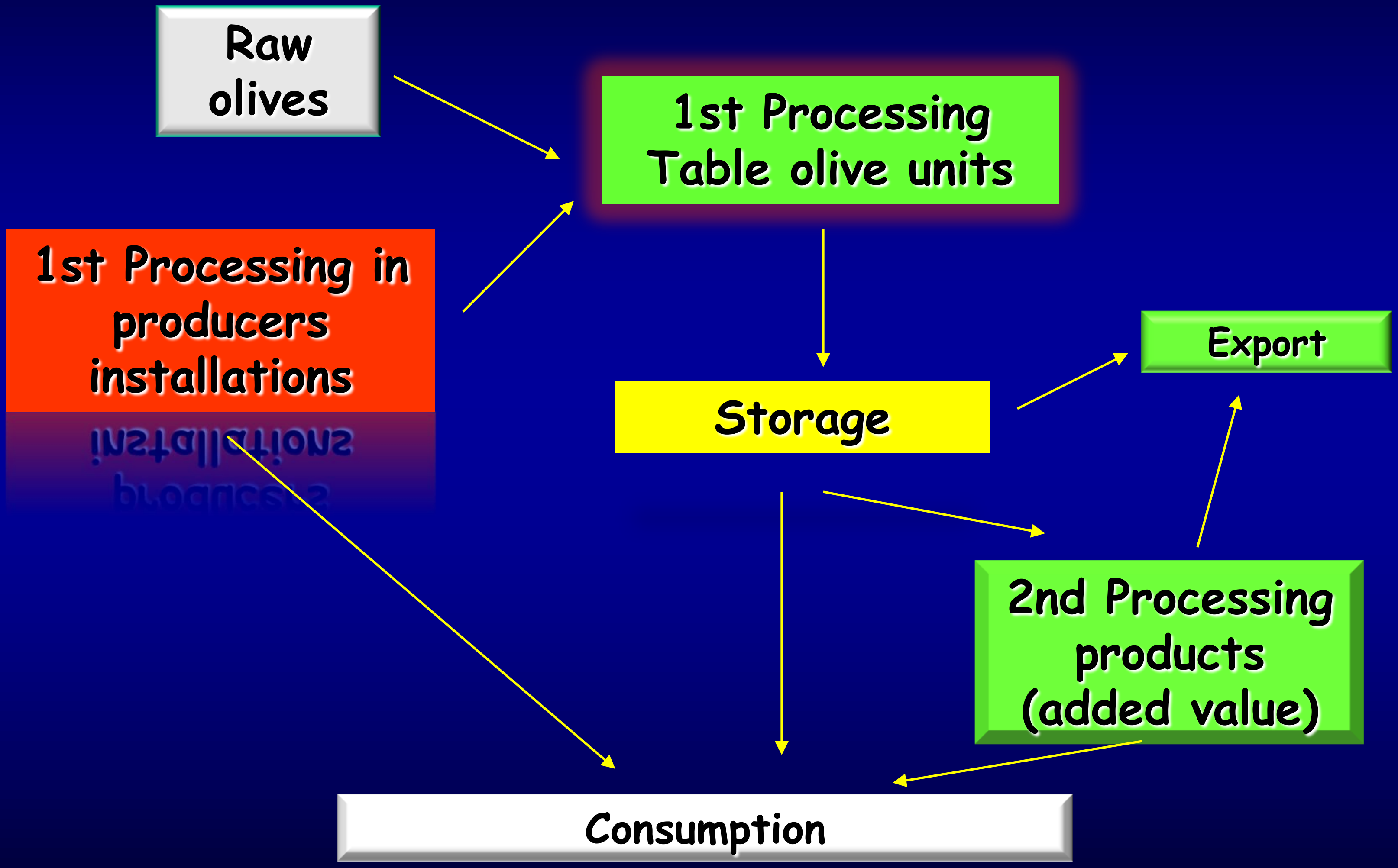


Table olive marketing in Greece

Open Markets

Central and Regional Food Stores

Catering centers

Consumer

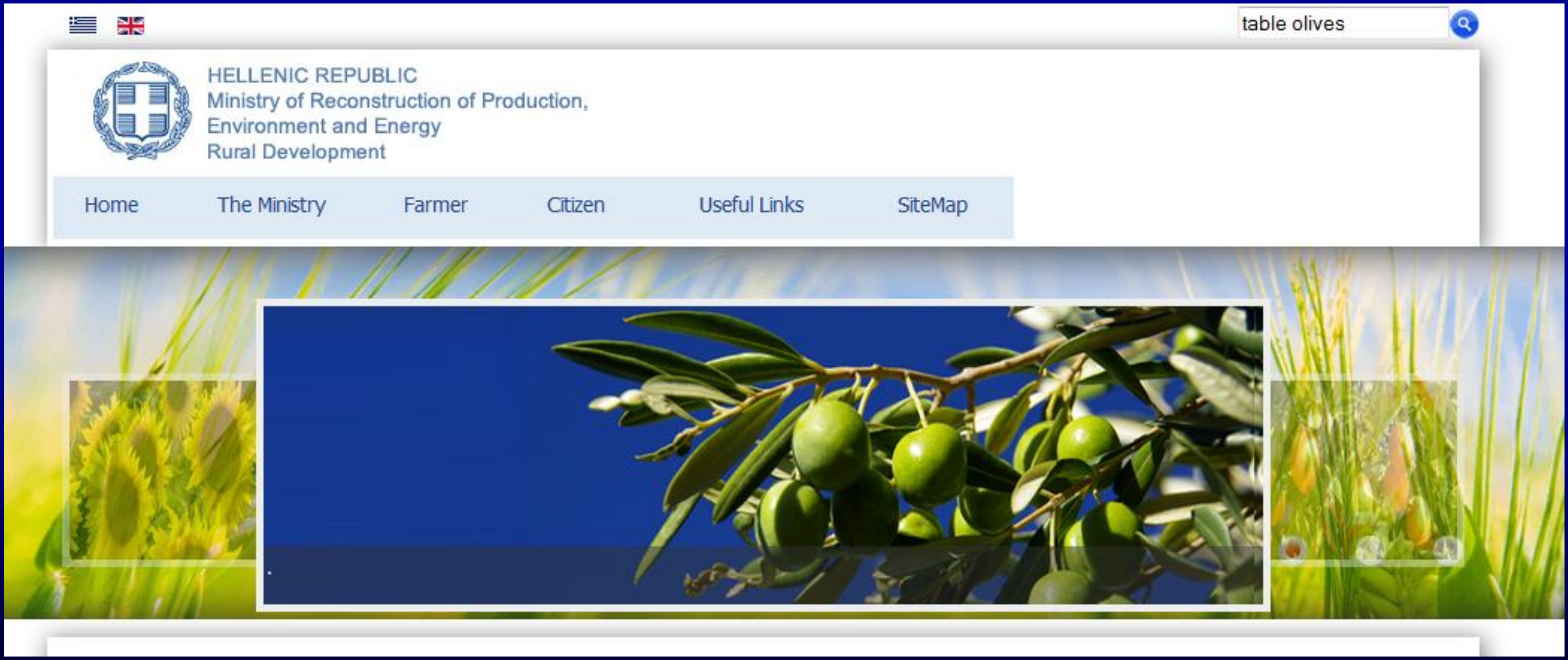
The consumption of packaged and standardized products in Greece is generally low (<10%)

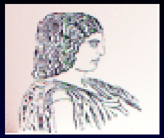
Table olive marketing in Greece

Retail outlets

Quality control of table olives in Greece
Inspection authorities

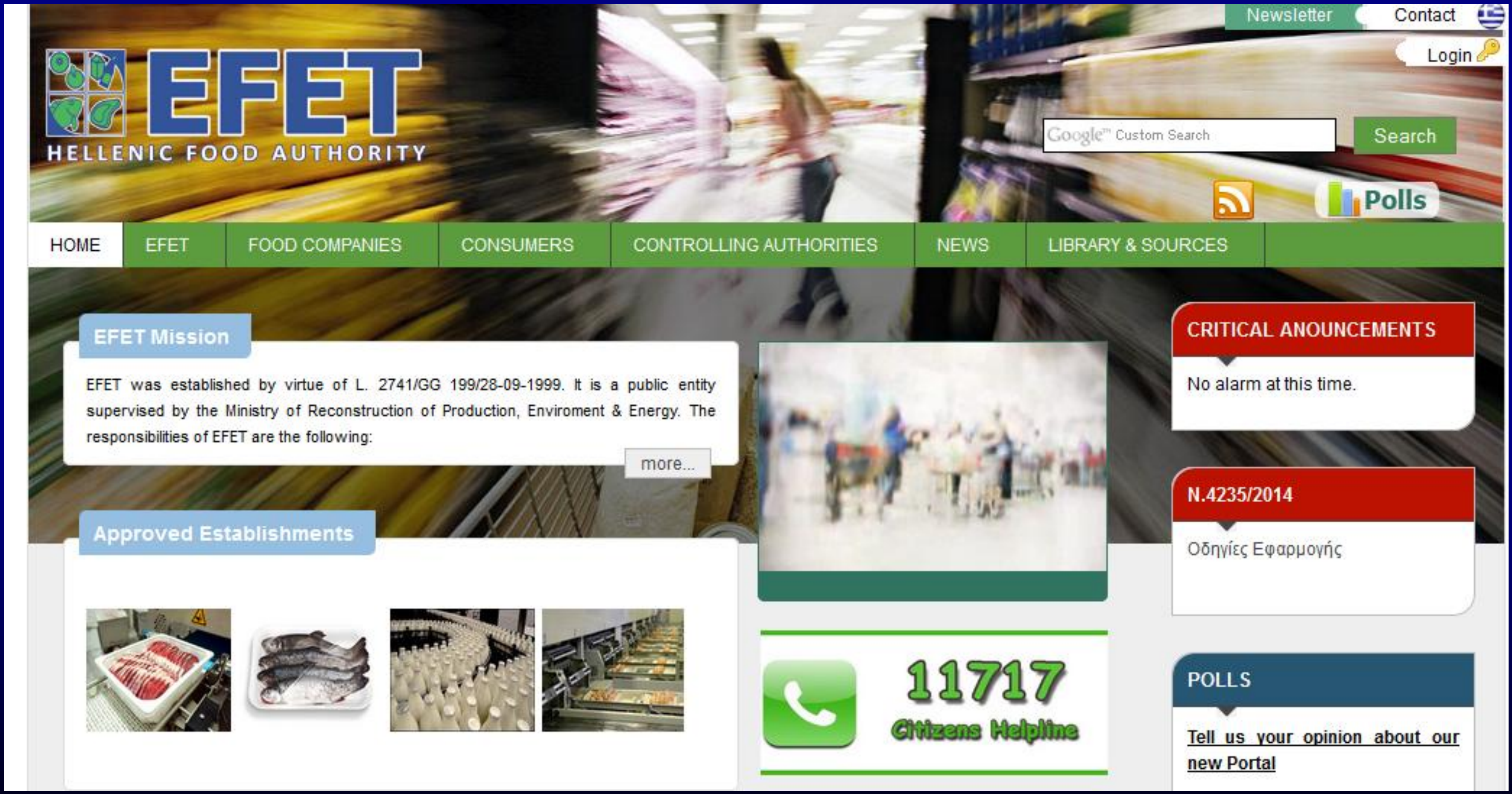
- **Ministry for Agriculture, based on the Presidential Decree 221/79 "*…for standardization, packaging and quality control of table olives destined for exportation*"**

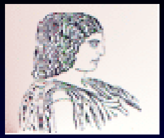




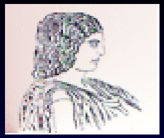
Quality control of table olives in Greece
Inspection authorities

- Table olives destined for the domestic market are inspected by EFET (Hellenic Food Authority)





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

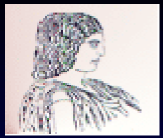


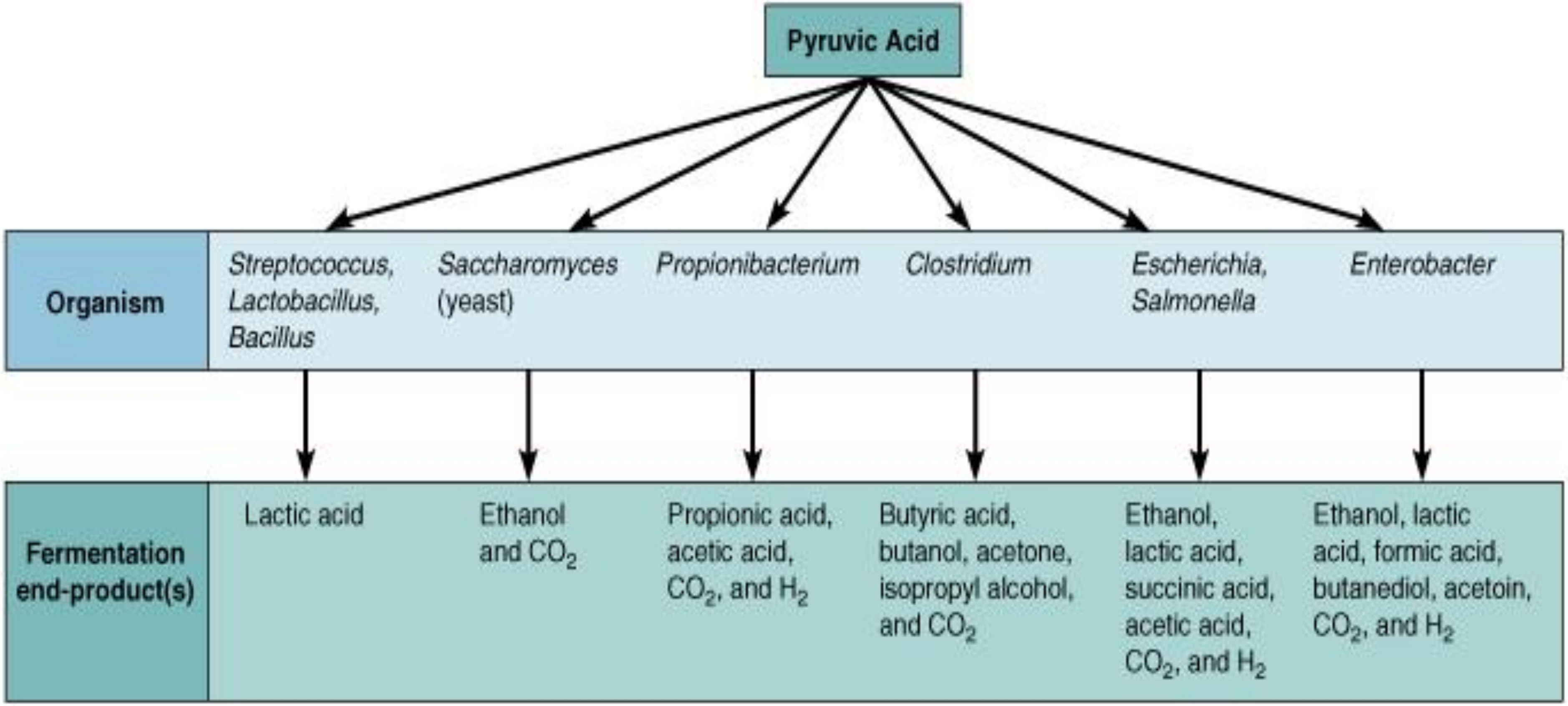
Table olive fermentation

- 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.

Characteristics of 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



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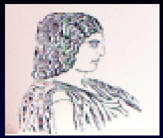


Influence of green olive and black oxidised olive processing on olive composition

Treatment		Changes in composition
Brining or immersion in acidic solution	Prior storage	Slow loss of sugars and polyphenols. Formation of organic acids, ethanol, and other aromatic compounds.
Alkali treatments, washing and oxidation	Darkening	Hydrolysis of oleuropein. Loss of sugars and organic acids. Polymerisation of polyphenols (caffeic and hydroxytyrosol). Loss of soluble components
Addition of new brine	Packing Sterilisation	Iron adsorption Loss of texture
	Shelf life	None under normal conditions

Main changes in Greek natural black olives in brine

Treatment		Changes in composition
Addition of brine	Brining	Slow loss of sugars, organic acids, polyphenols, minerals and other soluble components
Correction of salt content and pH	Fermentation	Formation of organic acids, ethanol, acetaldehyde, ethyl acetate, etc.
Addition of new brine	Packing	New dilution of soluble components
	Shelf life	None under normal conditions

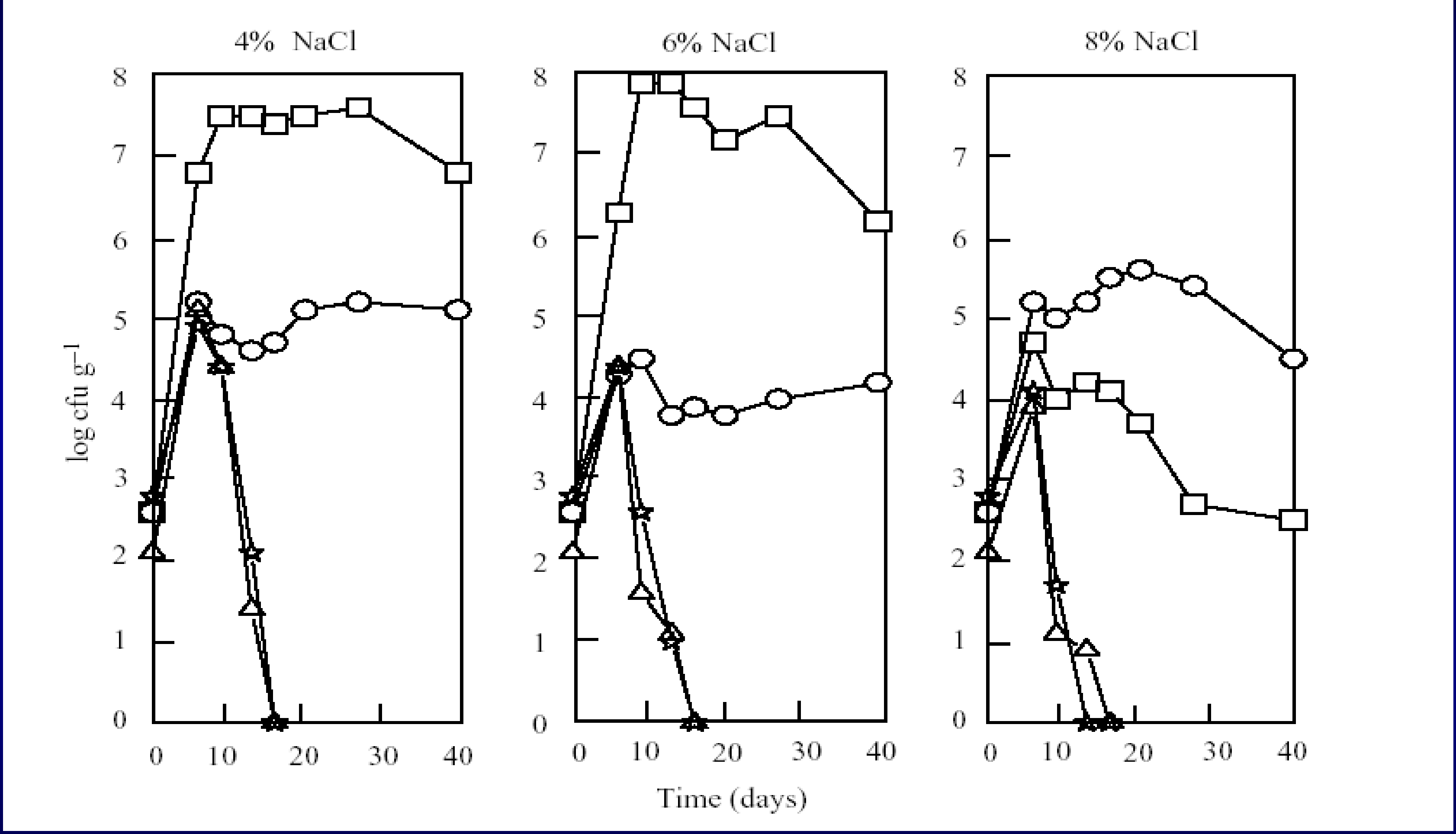
Processing – traditional anaerobic method

- 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 extend)
- 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

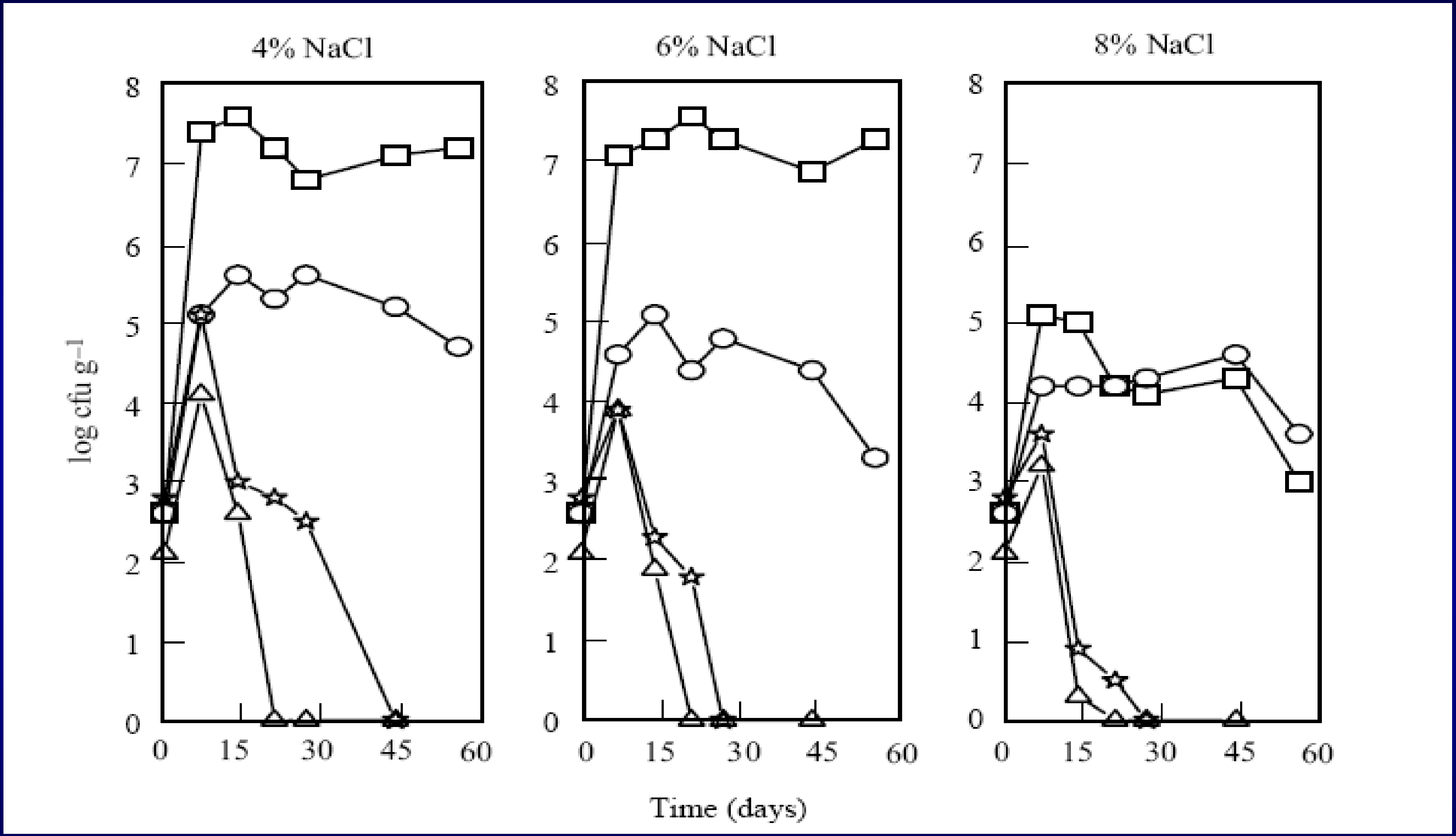
Effect of NaCl on population dynamics during fermentation at 25°C



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

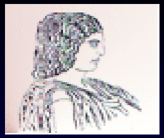
Tassou, C.C., Panagou, E.Z. and Katsaboxakis, K.Z. (2002) Microbiological and physicochemical changes of naturally black olives fermented at different temperatures and NaCl levels in the brines, *Food Microbiology* 19:605-615.

Effect of NaCl on population dynamics during fermentation at 18°C

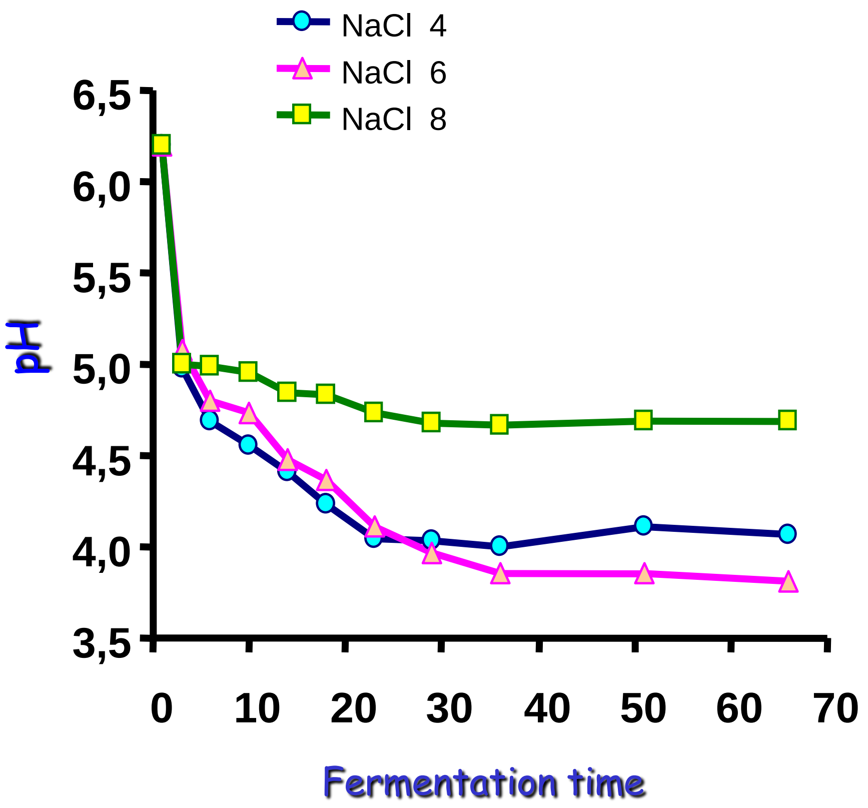


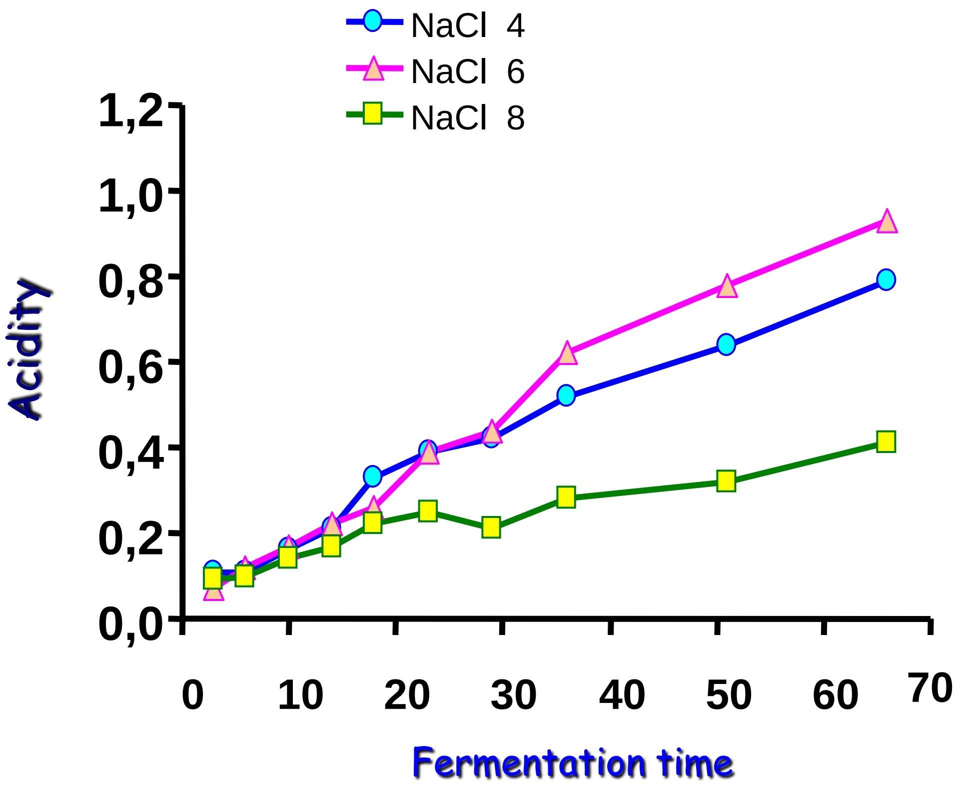
-□- lactic acid bacteria, -O- yeasts, -△- enterobacteria, -*- pseudomonads

Tassou, C.C., Panagou, E.Z. and Katsaboxakis, K.Z. (2002) Microbiological and physicochemical changes of naturally black olives fermented at different temperatures and NaCl levels in the brines, *Food Microbiology* 19:605-615.



Effect of NaCl level on pH and titratable acidity profile during fermentation at 25°C





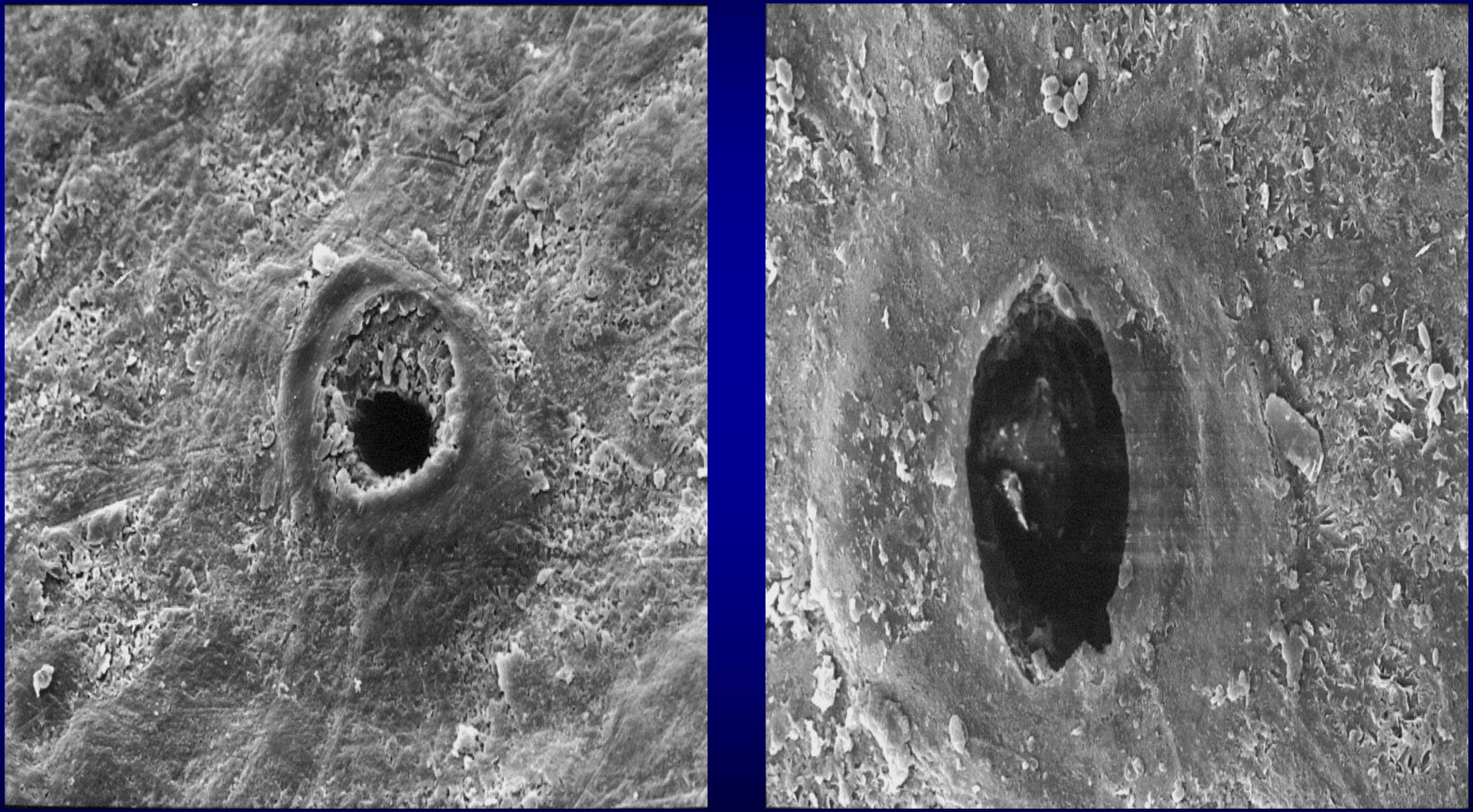
Fermentation tanks

Temperature control of fermentation tanks

Temperature control of fermentation tanks

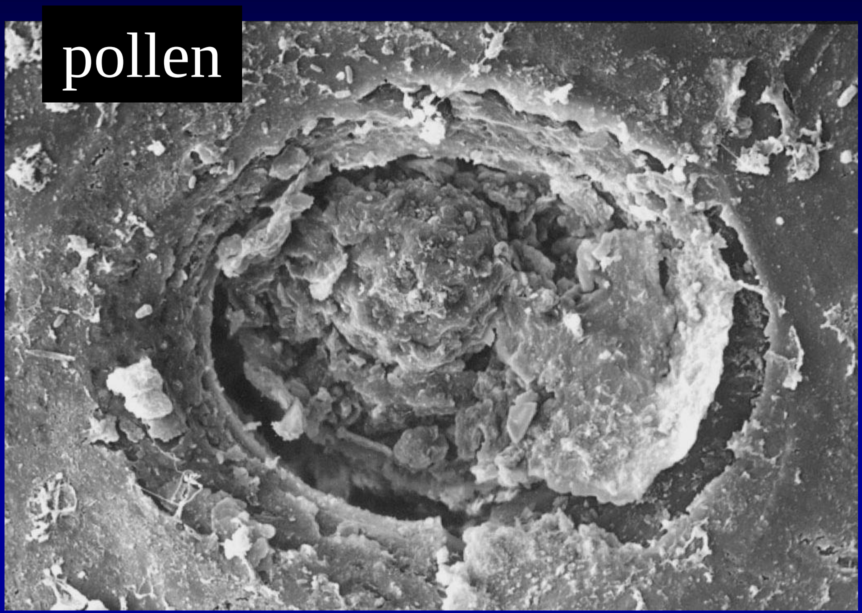
Microorganisms on the surface of raw olives

Stomata opening on raw olives

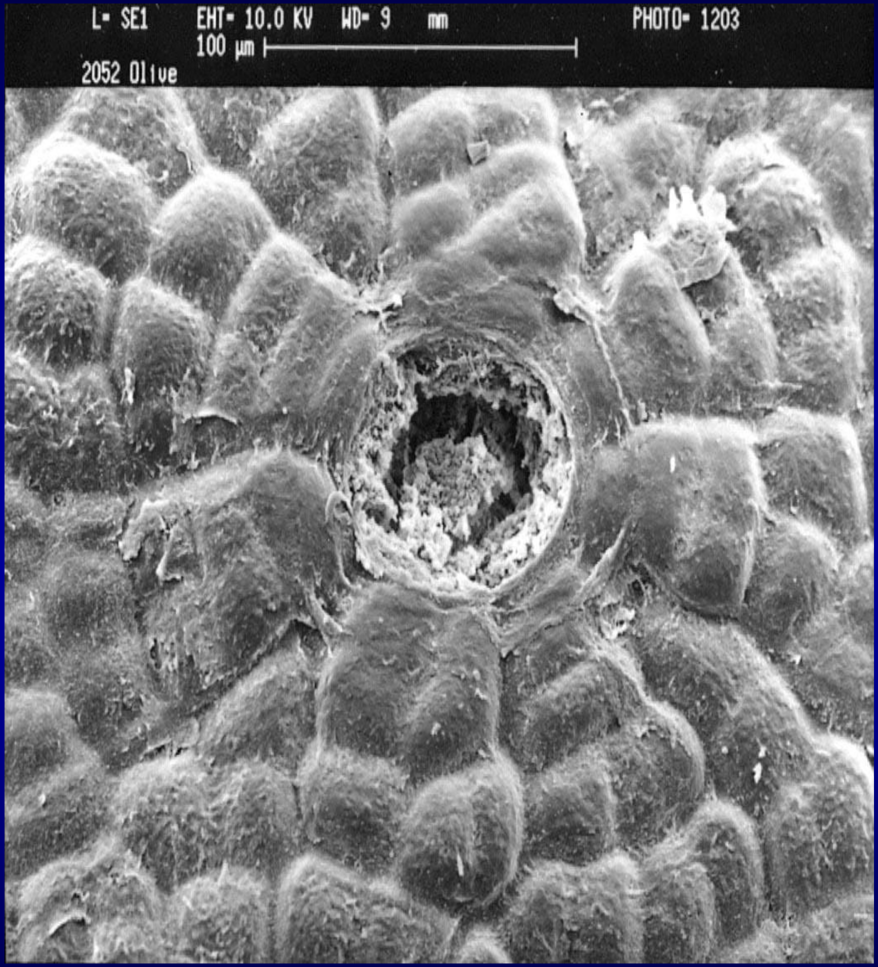


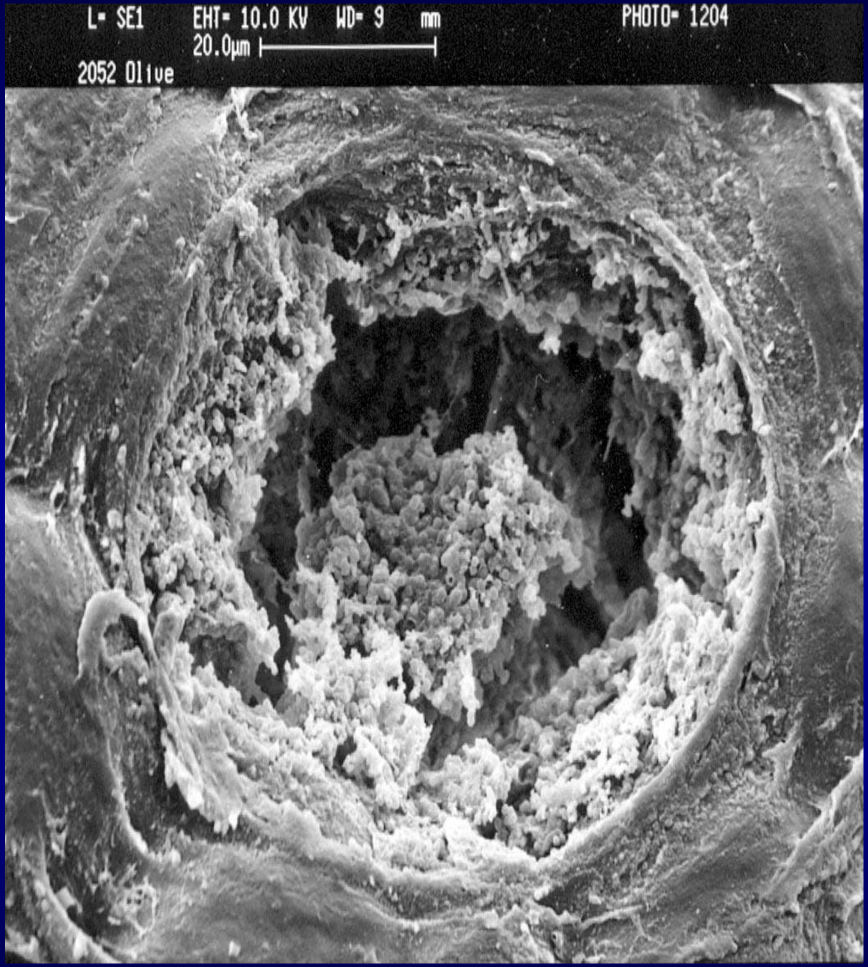
Photos: Dr Mary Parker, Institute of Food Research, Norwich, UK

Stomata opening blocked with:



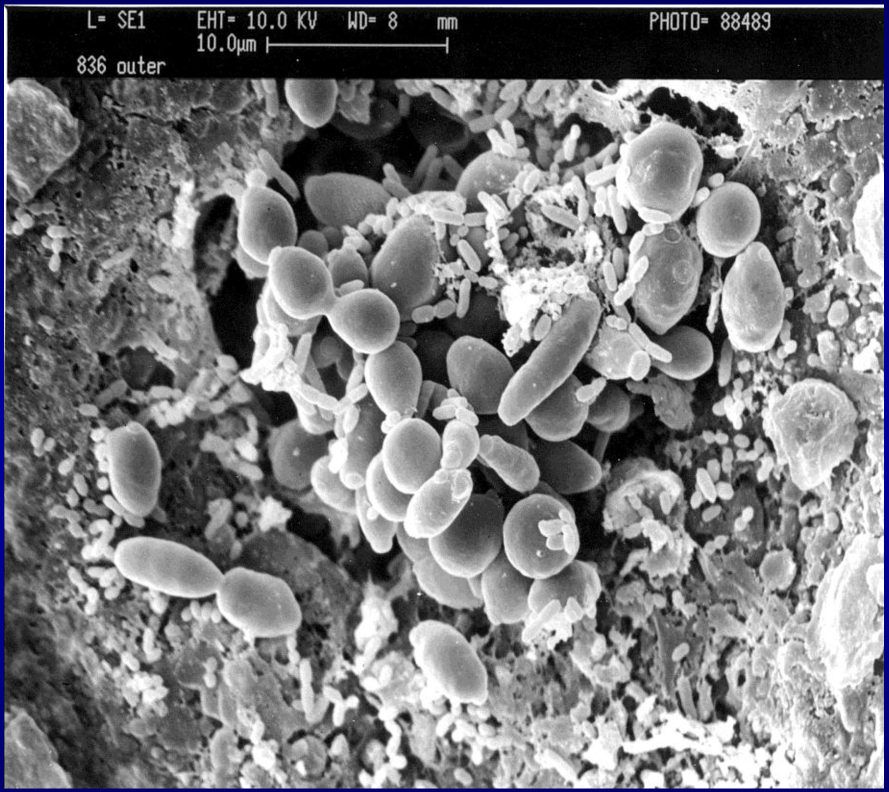
Stomata opening on raw olives

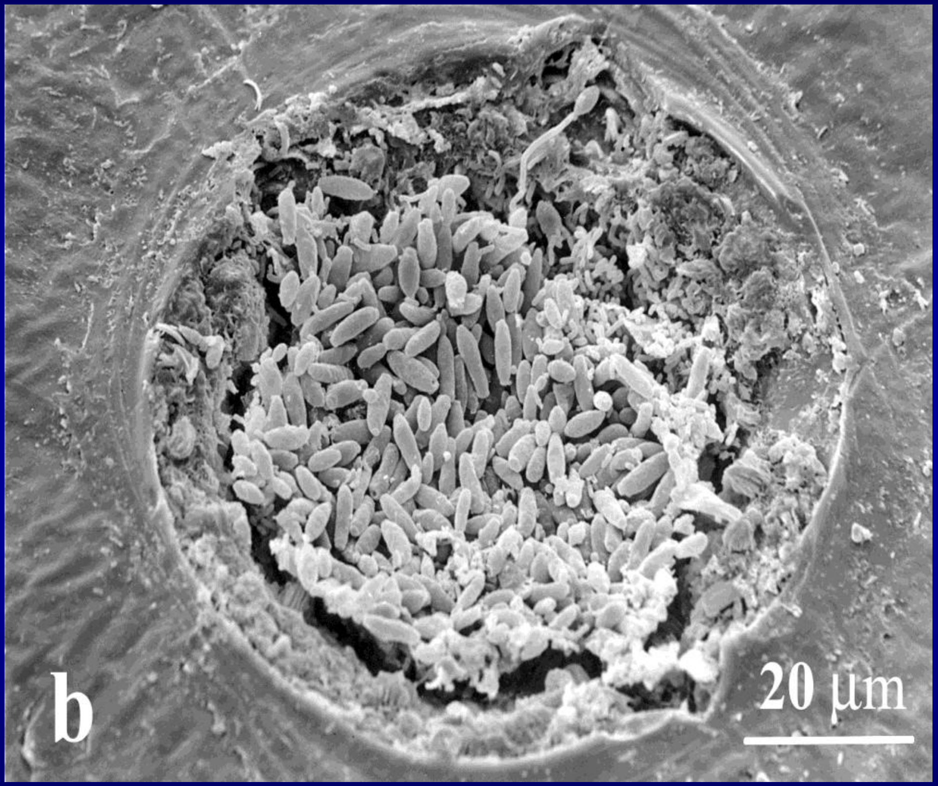




Photos: Dr Mary Parker, Institute of Food Research, Norwich, UK

Spatial distribution of microorganisms during fermentation

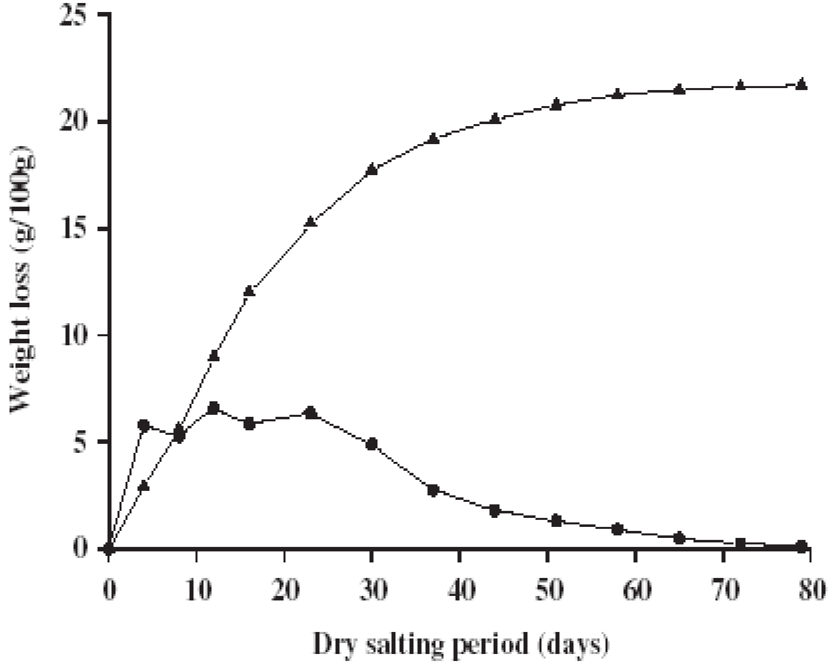


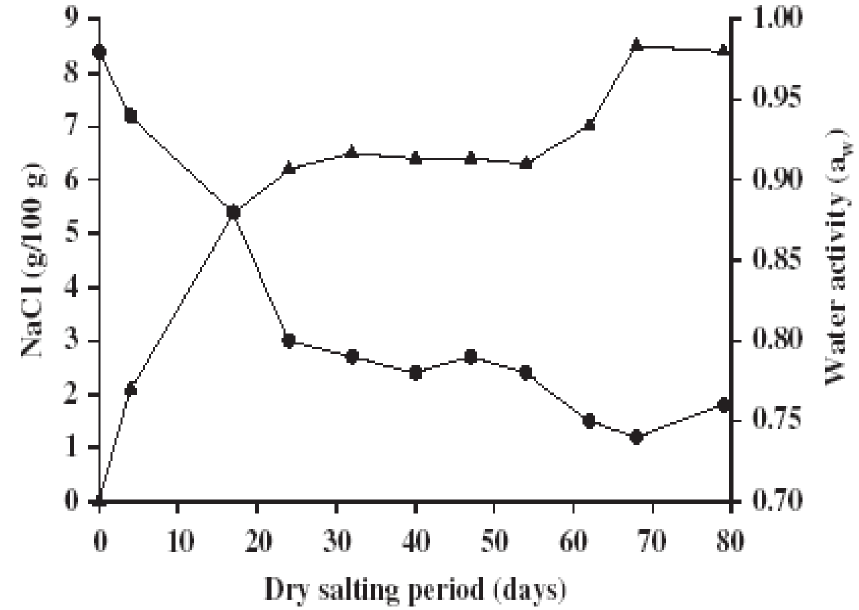


Photos: Dr Mary Parker, Institute of Food Research, Norwich, UK

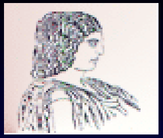
Dry salted naturally black olives

Dry salting process





E. Z. Panagou (2006) Greek dry-salted olives: Monitoring the dry-salting process and subsequent physicochemical and microbiological profile during storage at 4 and 20°C, *Lebensmittel-Wissenchaft und-Technologie* 39:323-330.



Dry salting process

Microorganism	Dry salting period (days)				
	0	20	40	60	80
Total viable counts	6.5±0.7	5.9±0.4	4.7±0.6	5.6±0.5	6.0±0.4
Lactic acid bacteria	4.1±0.3	<1	<1	<1	<1
Yeasts	5.7±0.6	5.6±0.2	4.7±0.5	5.6±0.4	6.0±0.5
Enterobacteria	3.7±0.9	<1	<1	<1	<1
Pseudomonads	4.0±0.5	<10	<10	<10	<10

- Initial microbiota consists of lactic acid bacteria, yeasts and gram negative bacteria
- Salt exerts a selective action resulting in the survival of salt tolerant yeasts

E. Z. Panagou (2006) Greek dry-salted olives: Monitoring the dry-salting process and subsequent physicochemical and microbiological profile during storage at 4 and 20°C, *Lebensmittel-Wissenchaft und-Technologie* 39:323-330.

Characteristics of the final product

- pH: 4.9-5.2
- Sodium chloride content in the flesh: 8.5-10.0 %
- Water activity: 0.75-0.85 (depending on the duration of the process)
- Reducing sugars: ~ 2%
- Dominant microbiota: salt tolerant yeasts (Candida famata)

Olives are marketed without brine (in dry) and are thus susceptible to fungal spoilage. There is external (visible) and internal (invisible) growth of mycellium.

Dry salting process

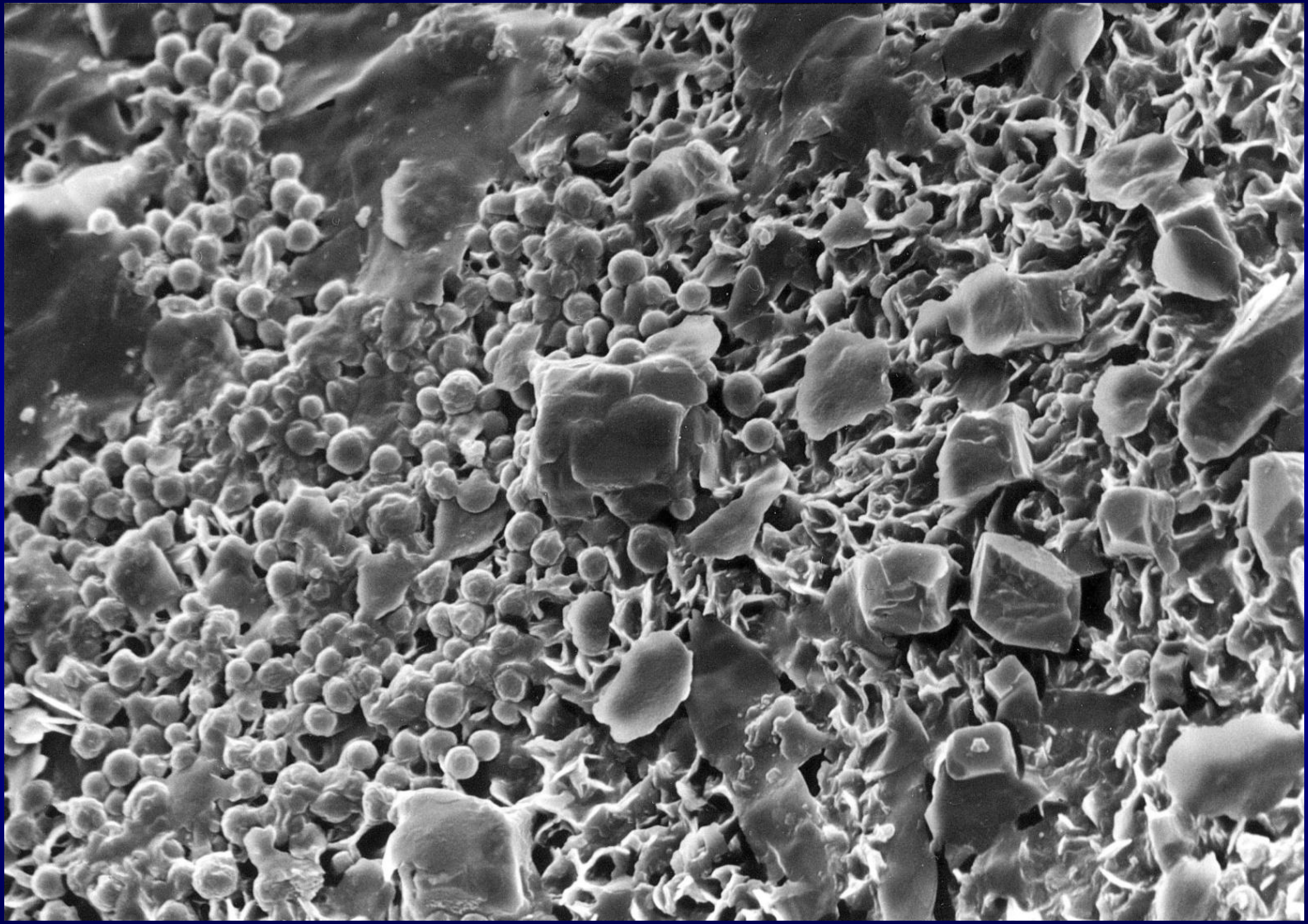
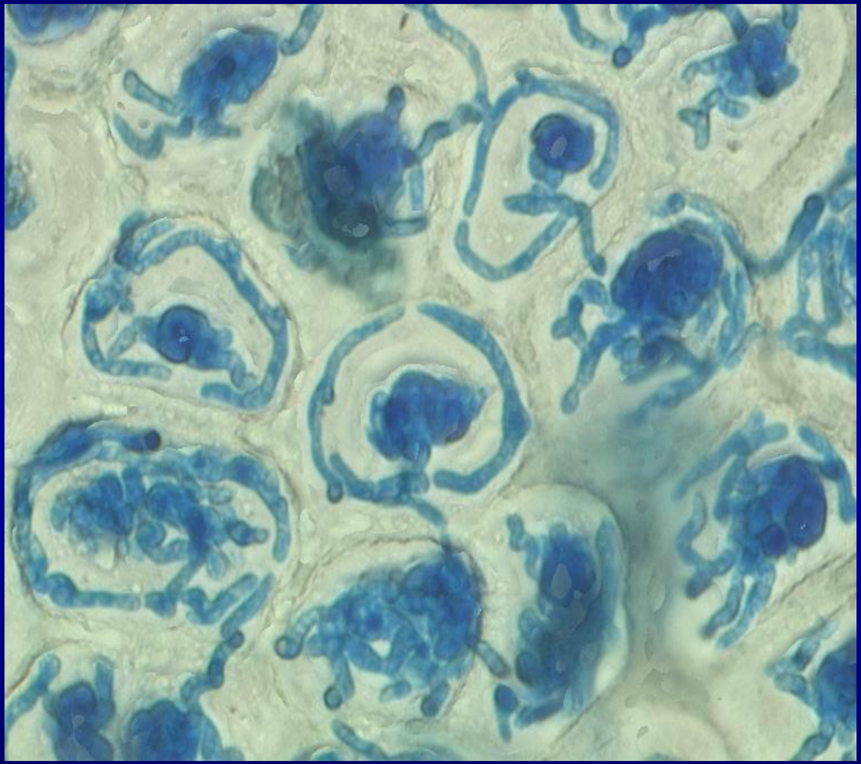
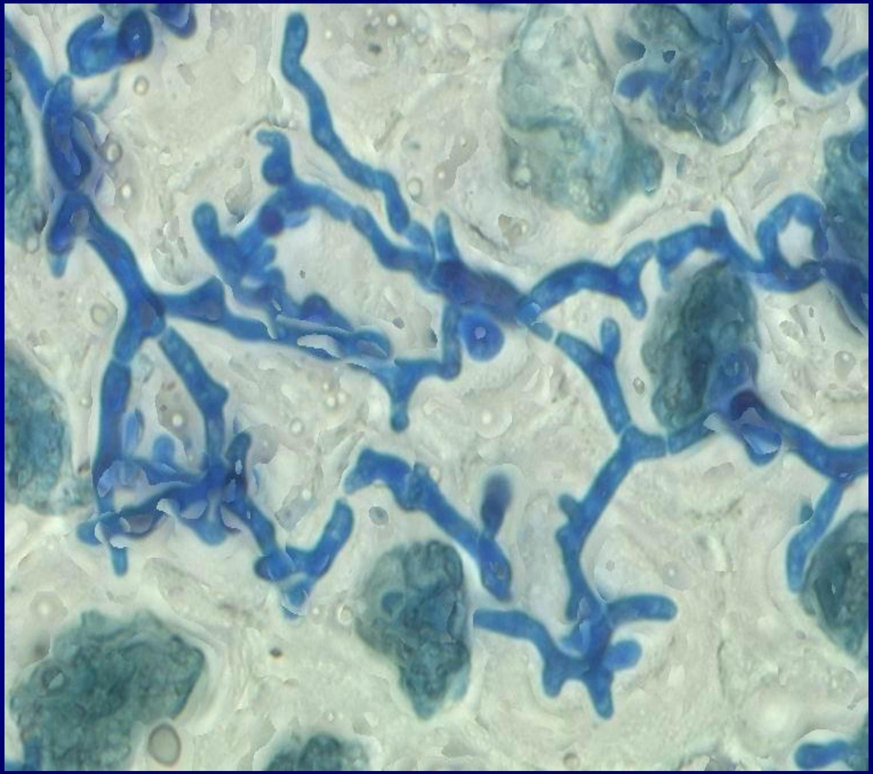


Photo: Dr Mary Parker, Institute of Food Research, Norwich, UK

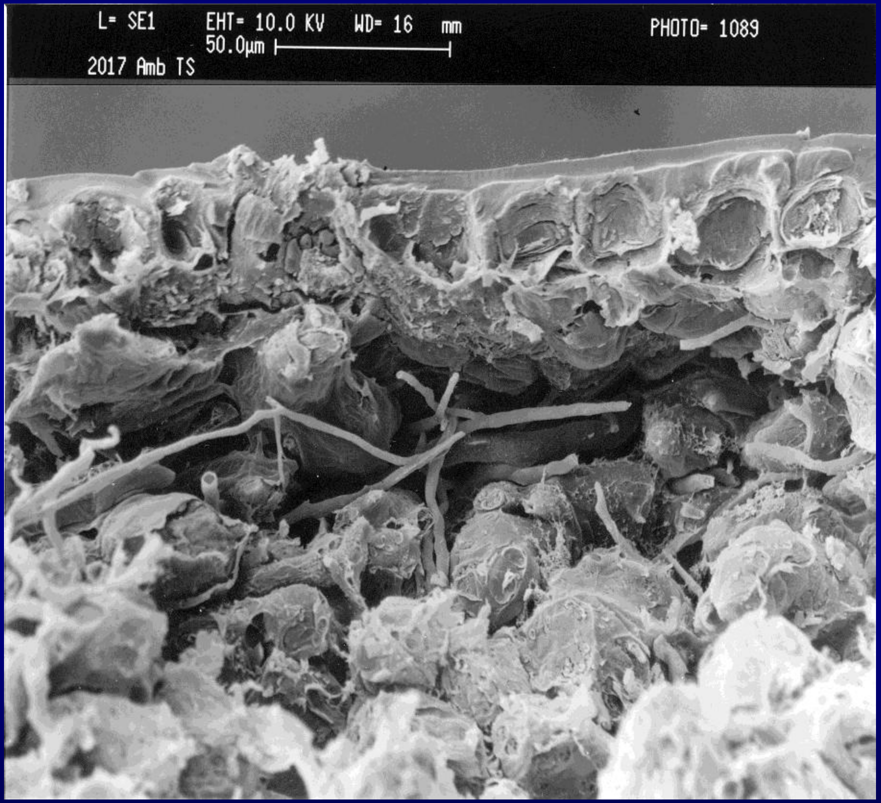
Growth of internal mycelium





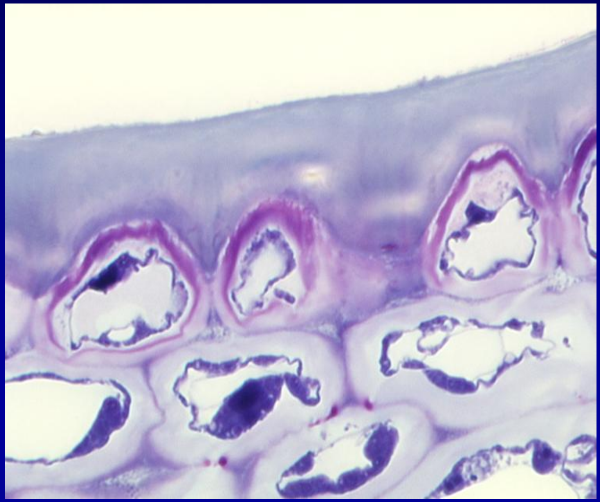
Photos: Dr Mary Parker, Institute of Food Research, Norwich, UK

Growth of internal mycelium under SEM

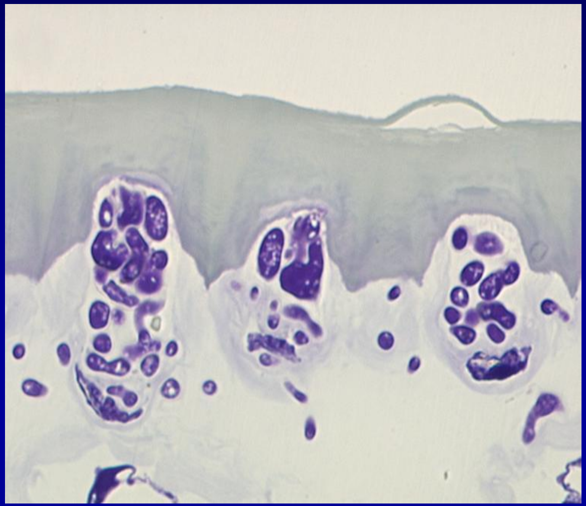


Photos: Dr Mary Parker, Institute of Food Research, Norwich, UK

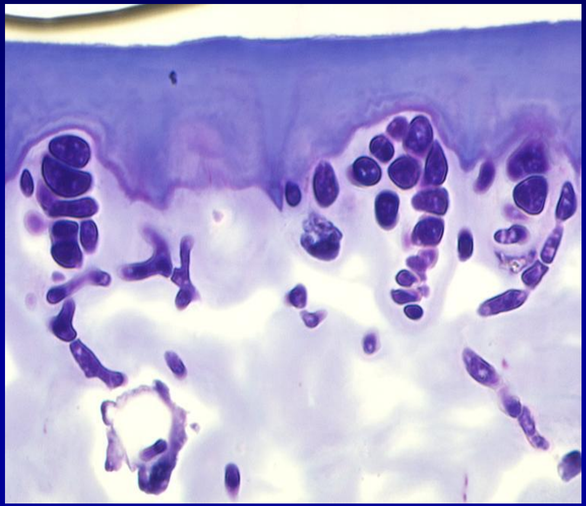
Treatments to minimise the extend of fungal growth



$100\%\ CO_2$



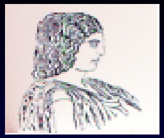
$100\%\ N_2$



Dip in 1% (w/v) potassium sorbate for 10 min

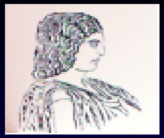
Photos: Dr Mary Parker, Institute of Food Research, Norwich, UK

Reduced salt natural black olives



Problem – excessive consumption of salt

- Sodium intake limit 2.4 g/day or 6 g NaCl/day (WHO, 2007).
- In many industrialized countries sodium intake ranges between 3600-4800 mg/day
- 75% of sodium intake comes from processed food, 10-12% is naturally occurring in foods, and 10-15% comes from food cooking or at the table.
- There is danger for (hypertension, strokes, cardiovascular diseases).
- Sodium intake reduction has the same importance as fat and sugar intake reduction.

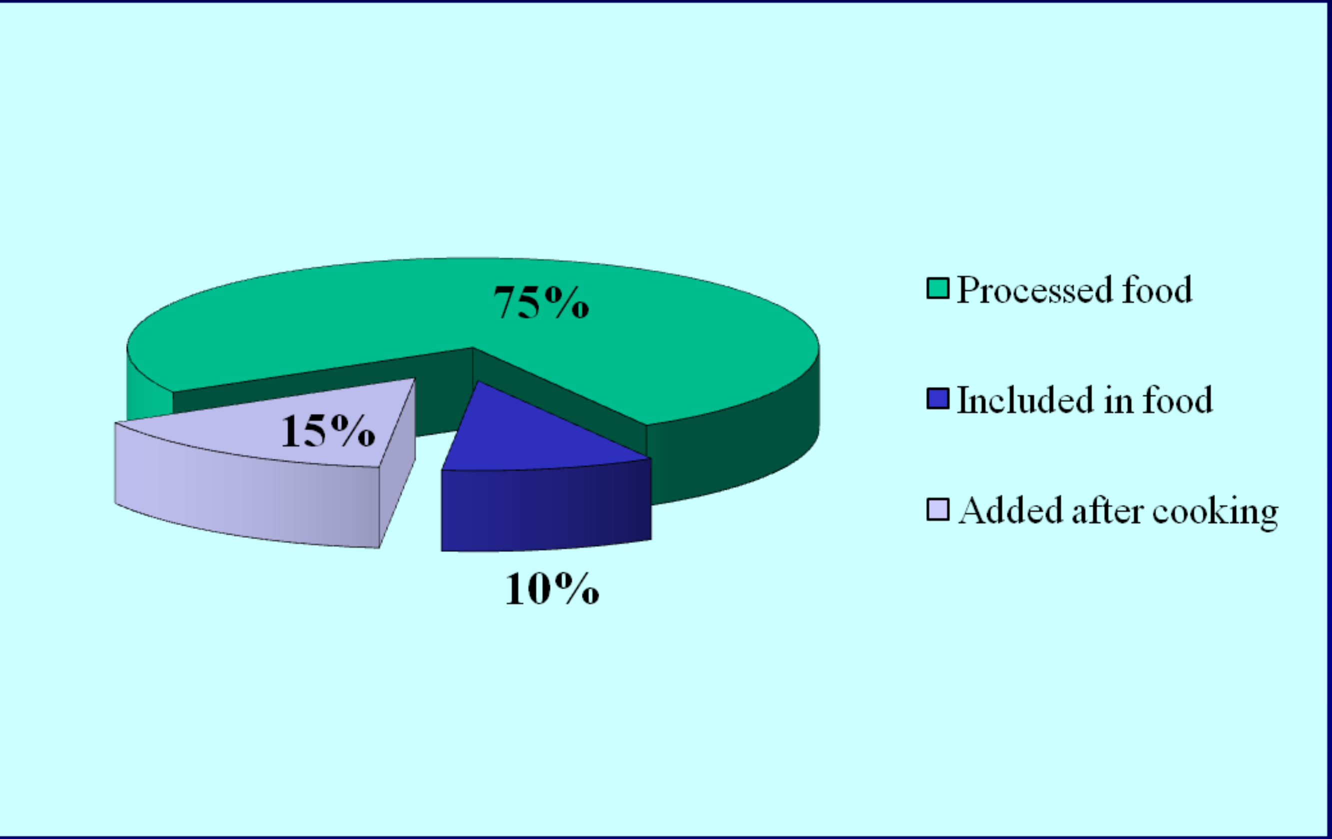


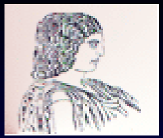
Minimum salt content of various trade preparations

Preparation	Minimum sodium chloride content %			Maximum pH limit			Minimum lactic acidity % lactic acid		
	SCC, MAT	PR, R	P, S	SCC, MAT	PR, R	P, S	SCC, MAT	PR, R	P, S
Treated olives	5	4	GMP	4.0	4.0	4.3	0.5	0.4	GMP
Natural olives	6	6	GMP	4.3	4.3	4.3	0.3	0.3	GMP
Dehydrated and/or shrivelled olives	10	10	GMP	GMP	GMP	GMP	GMP	GMP	GMP
Olives darkened by oxidation	GMP	GMP	GMP	GMP	GMP	GMP	GMP	GMP	GMP

PROPOSED DRAFT REVISION TO THE CODEX STANDARD FOR TABLE OLIVES

Sources of salt intake

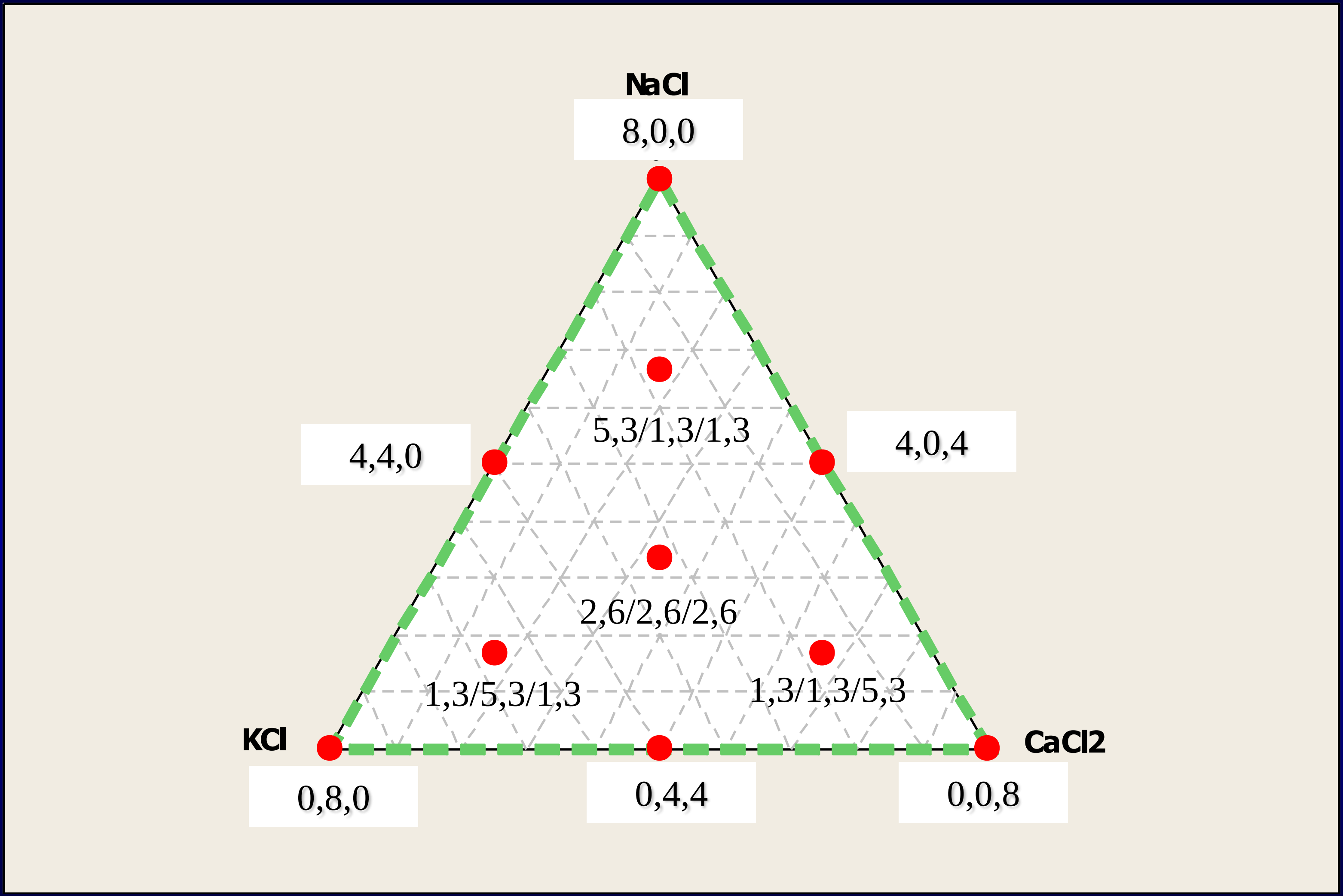




NaCl substitution – Mixture experiments with potassium chloride and calcium chloride

Fermentation	NaCl (%)	KCl (%)	$CaCl_2$ (%)
1	8	0	0
2	4	4	0
3	4	0	4
4	0	8	0
5	0	4	4
6	0	0	8
7	2,66	2,66	2,66
8	5,33	1,33	1,33
9	1,33	5,33	1,33
10	1,33	1,33	5,33

Mixture experiments with sodium chloride, potassium chloride and calcium chloride

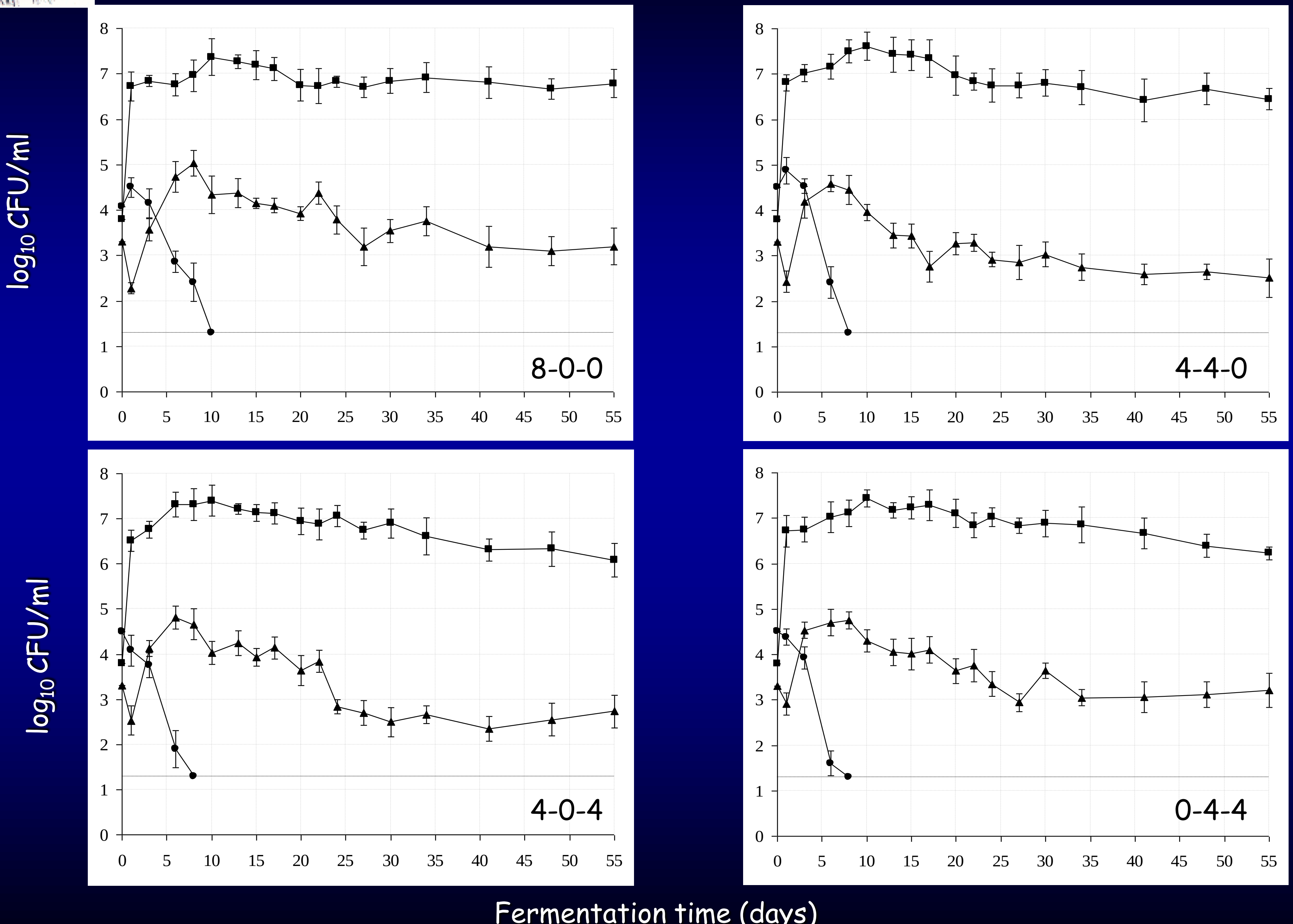


Partial substitution of salt

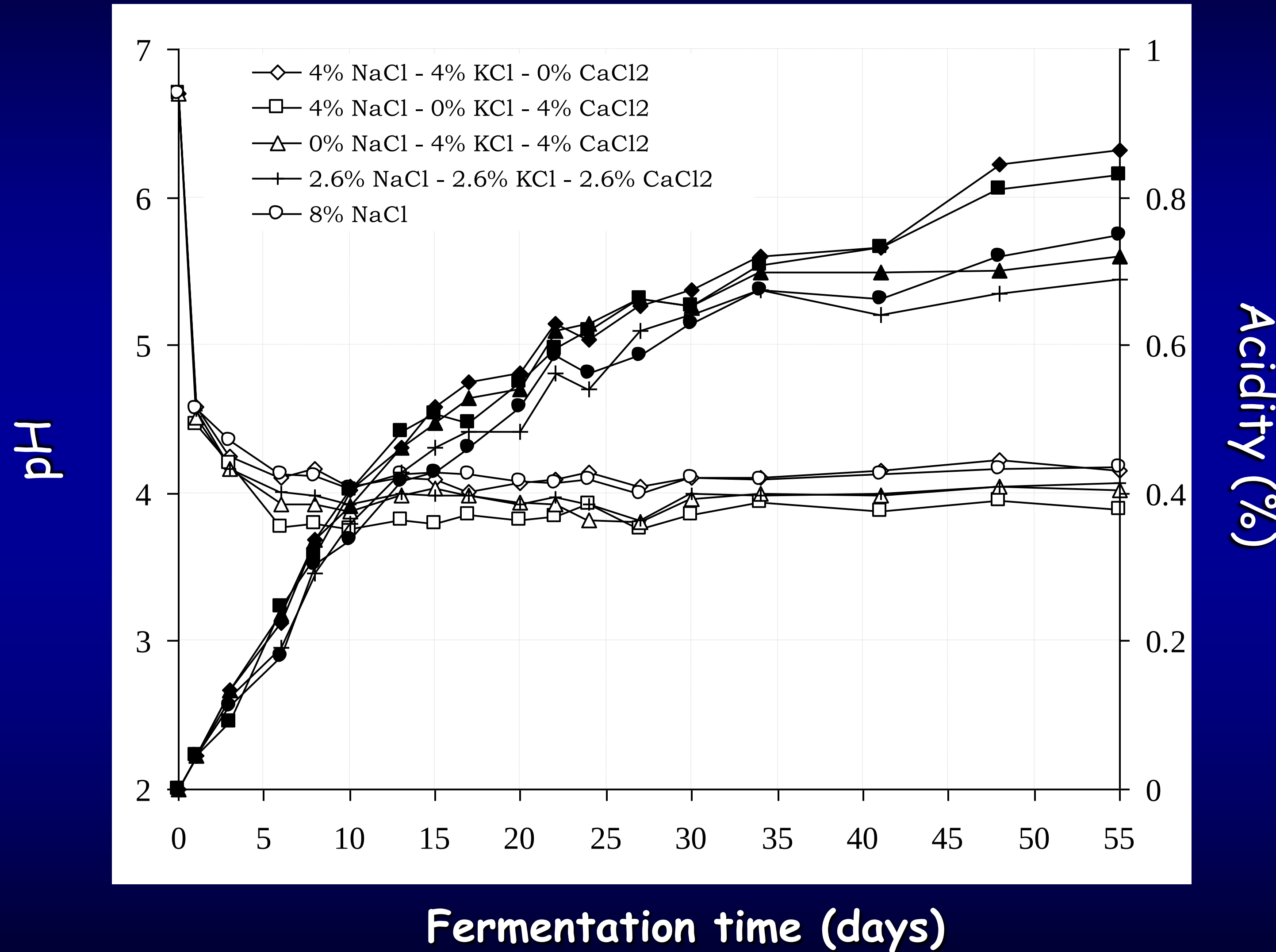
- Question 1: Is there a normal fermentation procedure with partial/total substitution of salt?

- Question 2: Do olives maintain acceptable sensory characteristics?

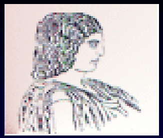
Microbiological changes of selected fermentations

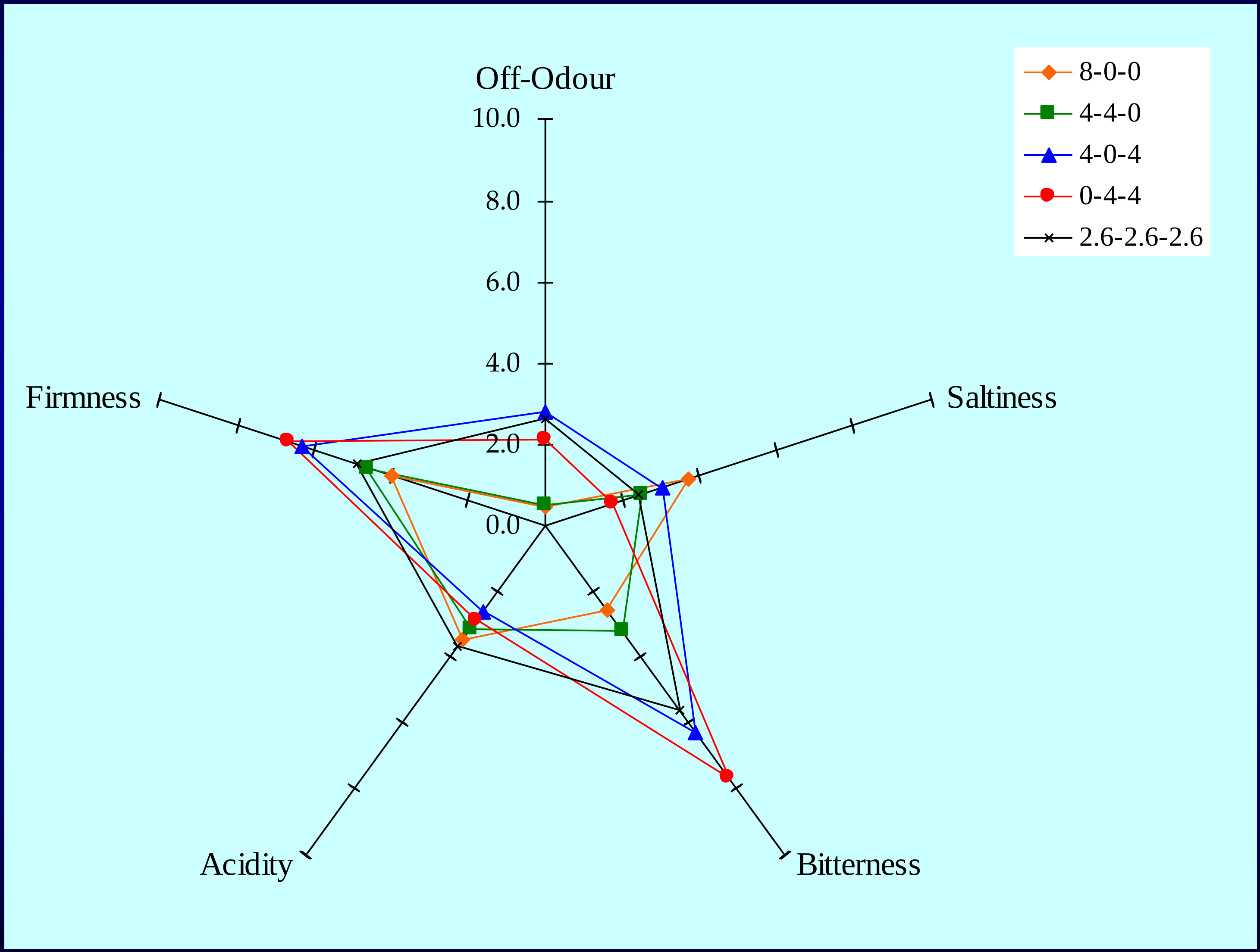


Changes in pH and acidity of selected fermentations



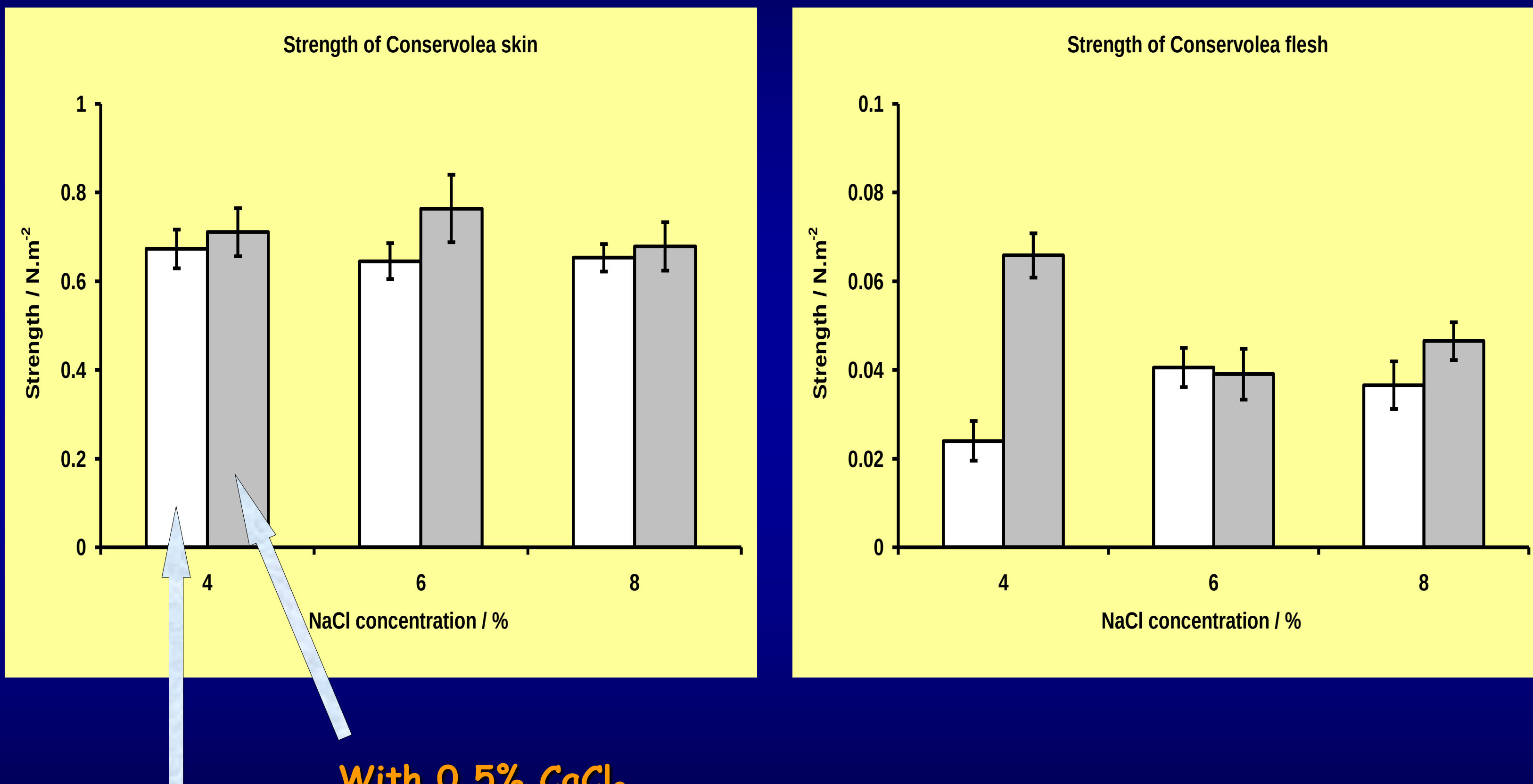
Sensory profile of selected fermentations





Texture improvement with the use of calcium chloride

Texture improvement – Addition of 0.5% $CaCl_2$

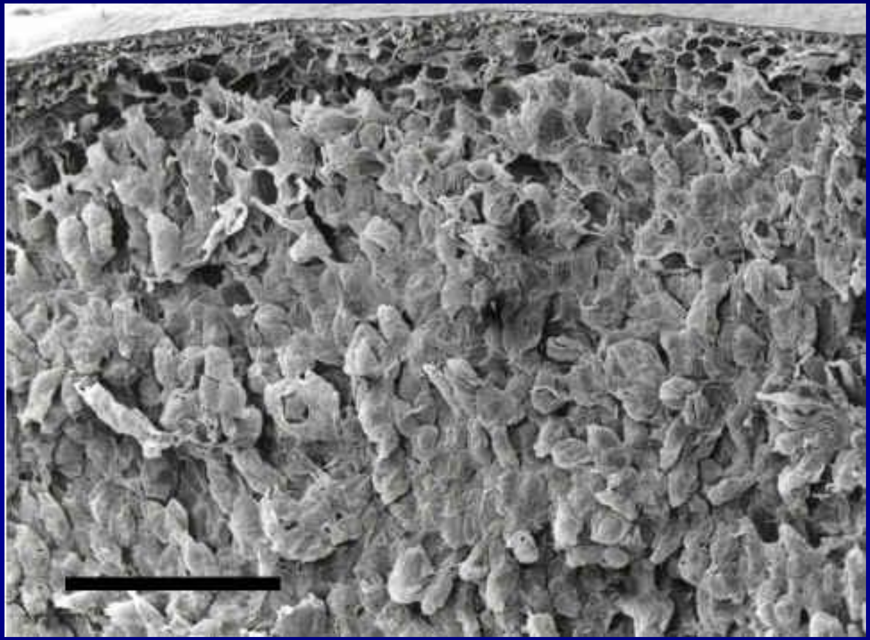


With 0.5% $CaCl_2$

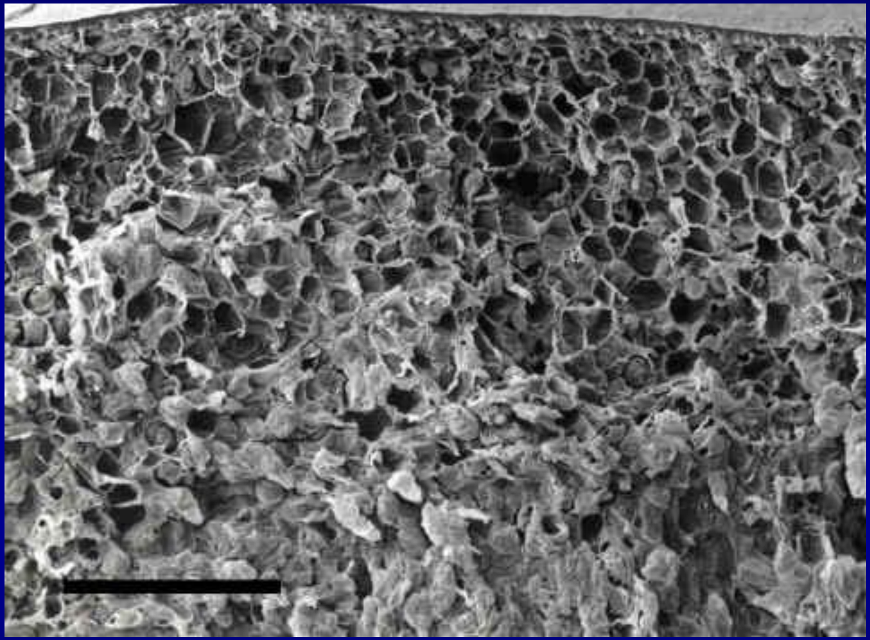
Without $CaCl_2$

C.C. Tassou, C.Z. Katsaboxakis, D.M.R. Georget, M.L. Parker, K.W. Waldron, A.C. Smith, E.Z. Panagou (2007). Effect of calcium chloride on mechanical properties and microbiological characteristics of cv. Conservolea naturally black olives fermented at different sodium chloride levels. *J. Sci. Food. Agric., 87`:1123-1131.*

SEM image of skin and outer flesh of Conservolea olive fermented in 4% NaCl with/without 0.5% $CaCl_2$



Without $CaCl_2$

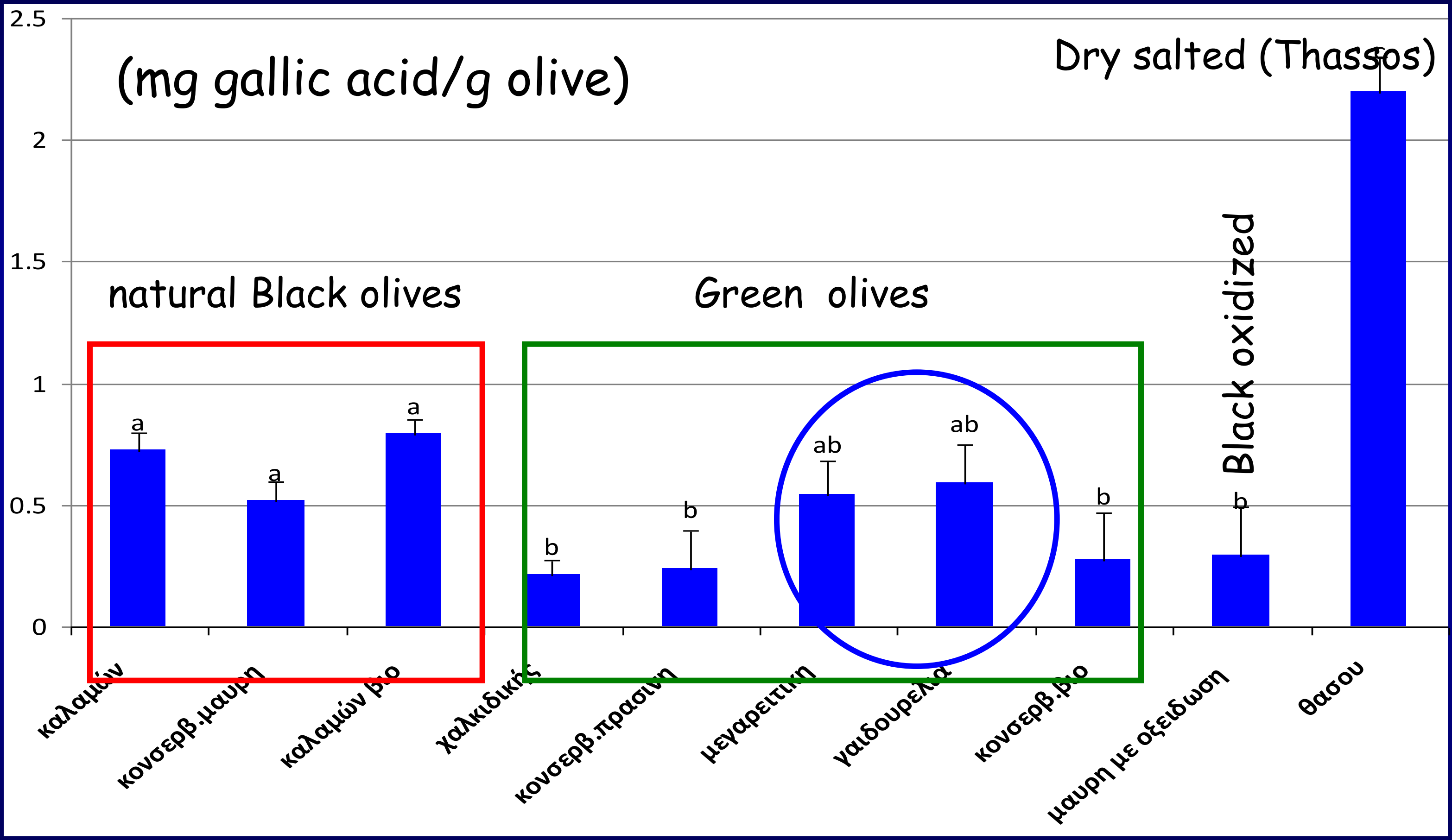


With 0.5% $CaCl_2$

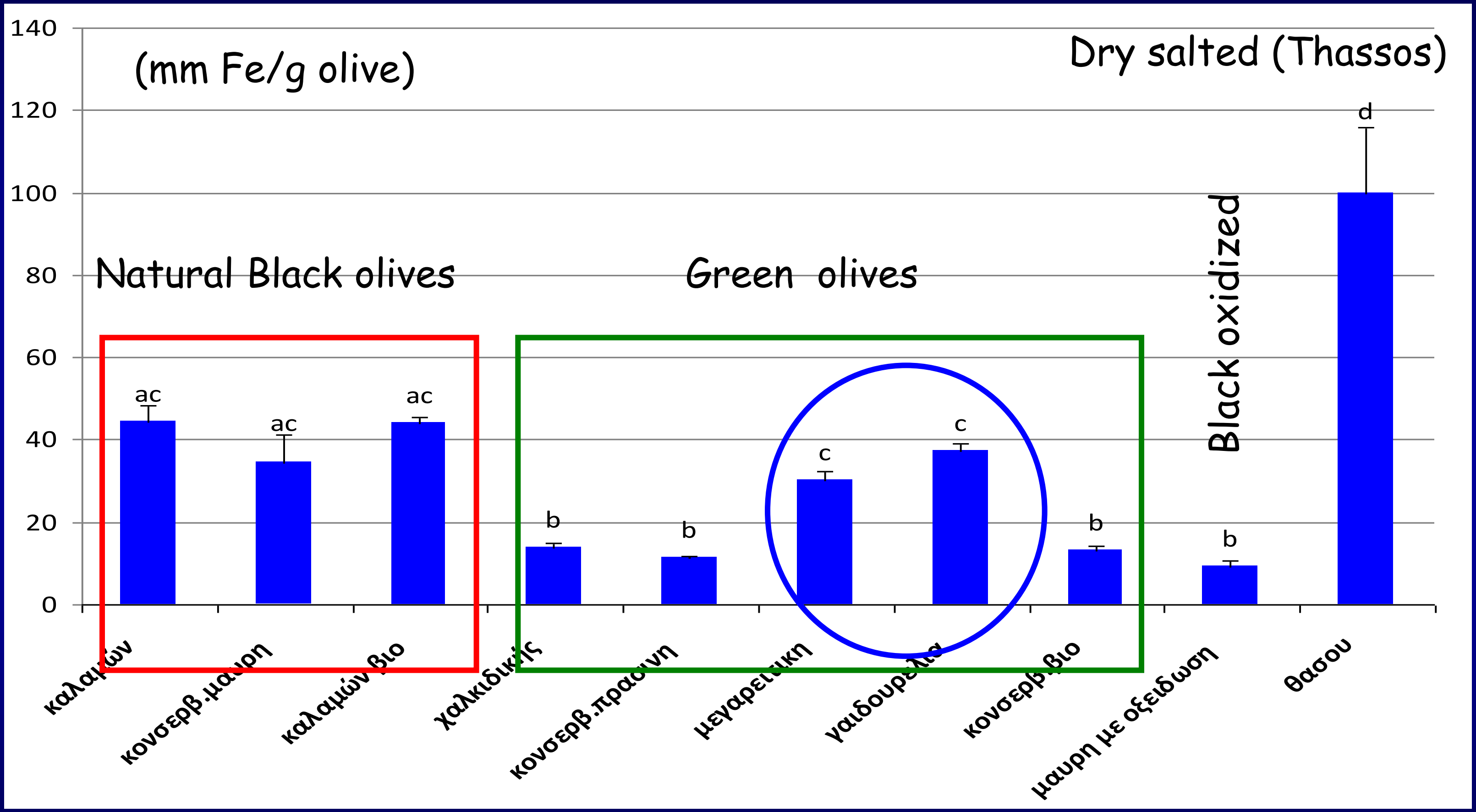
C.C. Tassou, C.Z. Katsaboxakis, D.M.R. Georget, M.L. Parker, K.W. Waldron, A.C. Smith, E.Z. Panagou (2007). Effect of calcium chloride on mechanical properties and microbiological characteristics of cv. Conservolea naturally black olives fermented at different sodium chloride levels. J. Sci. Food. Agric., 87:1123-1131.

Antioxidant potential of table olives

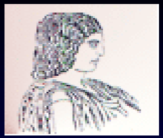
Concentration of polyphenols in Greek table olives varieties



In vitro antioxidant potential of Greek table olives varieties



Method FRAP (Ferric Reducing Antioxidant Power)



Antioxidant potential of table olives compared to other fruits

TABLE 2

Ferric reducing-antioxidant power (FRAP), total radical-trapping antioxidant parameter (TRAP) and Trolox equivalent antioxidant capacity (TEAC) of fruit extracts[1,2]

Fruit	FRAP Value	FRAP Rank	TRAP Value	TRAP Rank	TEAC Value	TEAC Rank
	(mmol Fe^{2+}/kg FW[3])		*(mmol Trolox/kg FW)*			
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	8	5.65	11	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

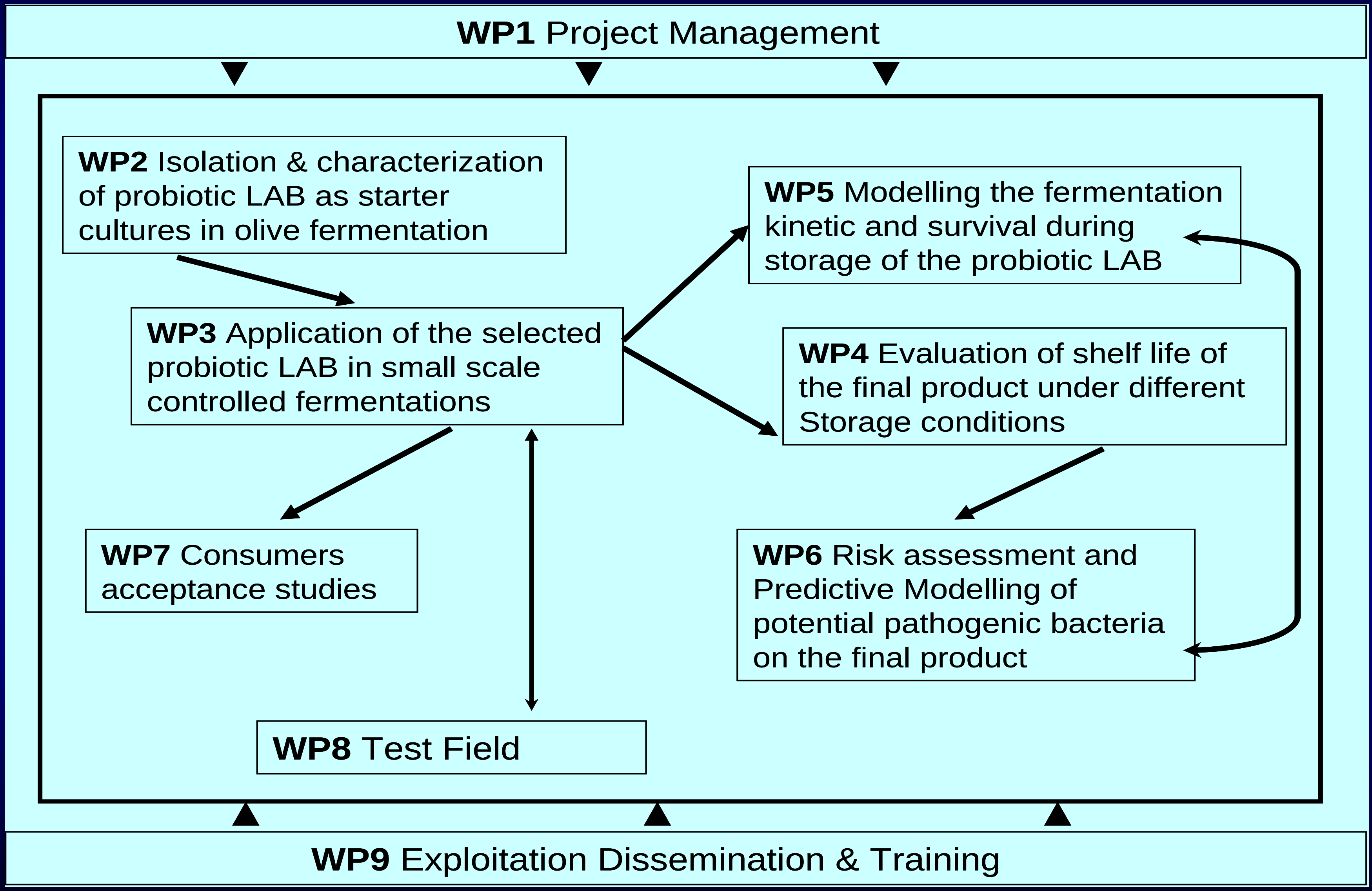
Functional table olives

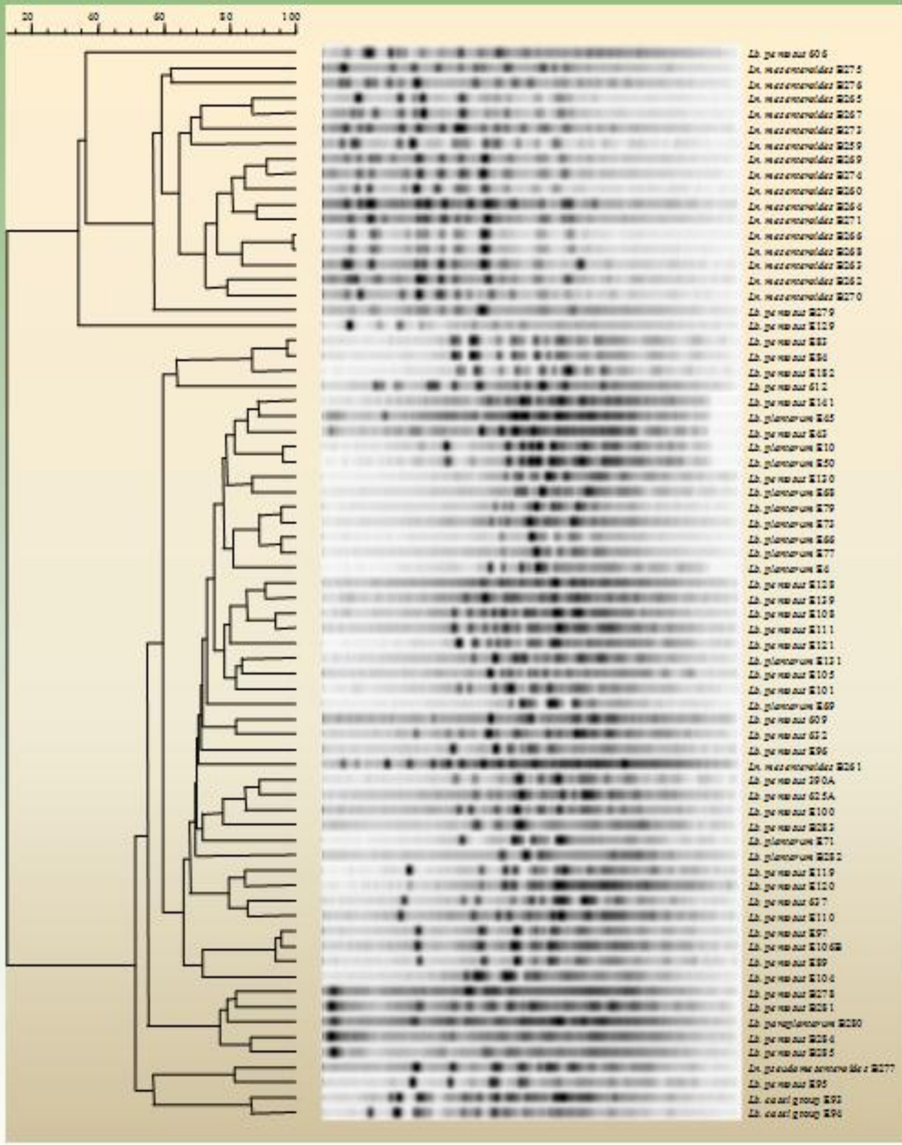
FP7-SME-2008-2-243471

"PROBIOLIVES"

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

Activities-Targets of the project





Cluster analysis of PFGE *Apa*I 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%).

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 posses

PROBIOTIC PROPERTIES *IN VITRO*

Selected strains with probiotic potential according to *in vitro* tests in comparison with the *Lb. casei* Shirota, and *Lb. rhamnosus GG*

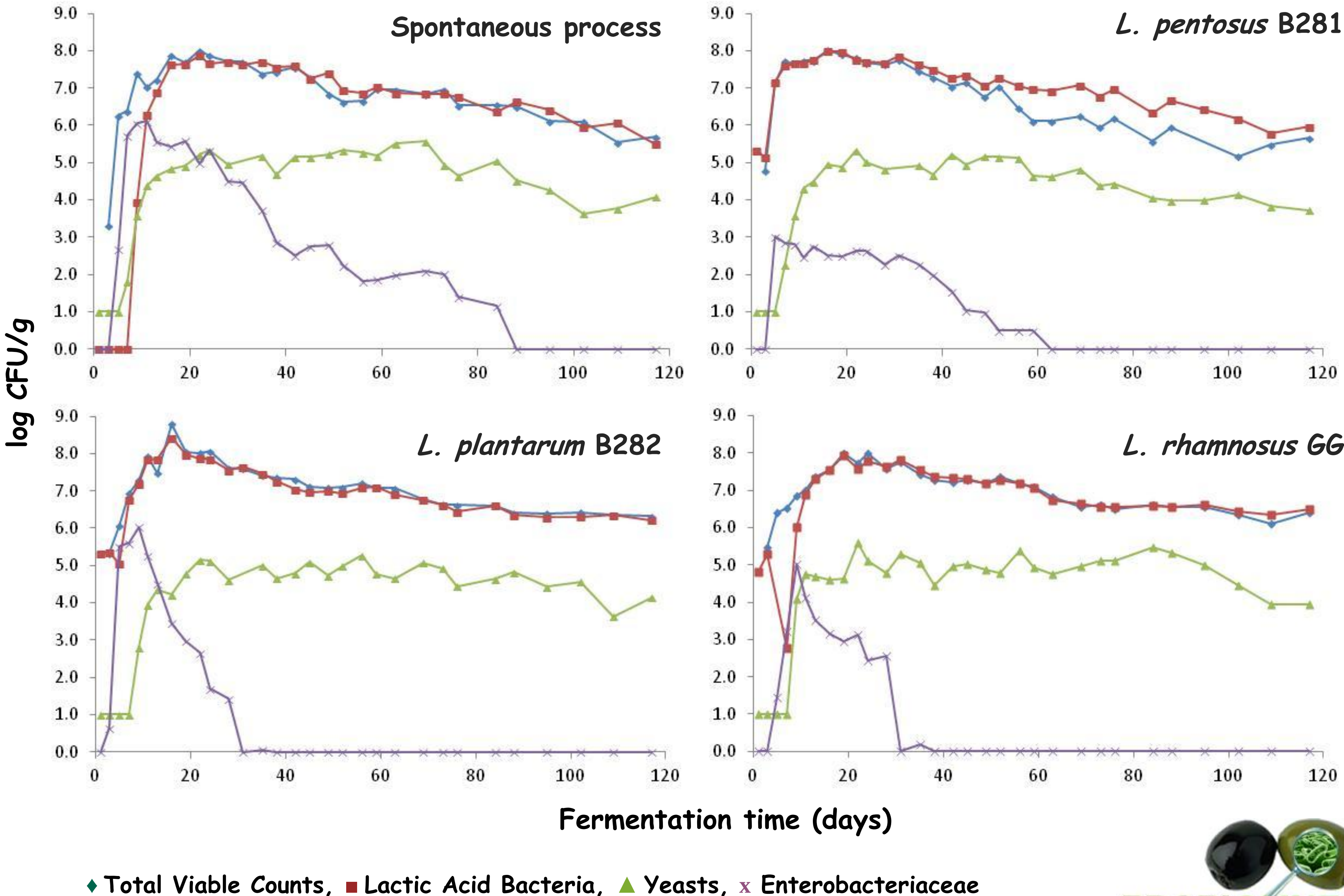
Strains	Test					
	Low pH (SR%)[a]	Bile salts (SR%)[b]	Bile salts hydrolysis	Haemolytic activity[d]	Antibiotic resistance[e]	Caco-2 (Adherence %)
***Lb. pentosus* B281**	**95.64**	**94.78**	**0**[c]	**α**	**K, C, S**	**37.21**
***Lb. pentosus* E97**	**89.69**	**96.79**	**0**	**γ**	**K, C, S**	**39.76**
***Lb. pentosus* E104**	**92.52**	**97.64**	**0**	**γ**	**K, G**	**33.72**
***Lb. pentosus* E108**	**91.08**	**100.59**	**0**	**γ**	**K, A**	**60.78**
***Lb. plantarum* B282**	**87.79**	**100.09**	**1**	**γ**	**K, G, E**	**68.94**
***Lb. plantarum* E10**	**89.95**	**98.67**	**1**	**γ**	**K, G**	**44.75**
***Lb. plantarum* E69**	**98.36**	**100.02**	**0**	**γ**	**K, G**	**30.51**
***Lb. paracasei* subs. *paracasei* E93**	**89.41**	**96.55**	**0**	**γ**	**K, G, S**	**41.92**
***Lb. paracasei* subs. *paracasei* E94**	**82.75**	**88.80**	**0**	**γ**	**K, G, S**	**74.02**
***Lb. casei* Shirota**	**82.83**	**100.20**	**0**	**γ**	**S, E, P, T, C**	**31.41**

[a] survival rate after 3h in low pH, [b] survival rate after 4h in bile salts, [c] 0, no hydγrolysis; 1, partial hydrolysis.
[d] a-haemolysis, γ-haemolysis, [e] A: ampicillin, V: vancomycin, G: gentamycin, K: kanamycin, S: streptomycin, P: penicillin, E: erythromycin, T: tetracycline, C: chloramphenicol

Fermentation procedure

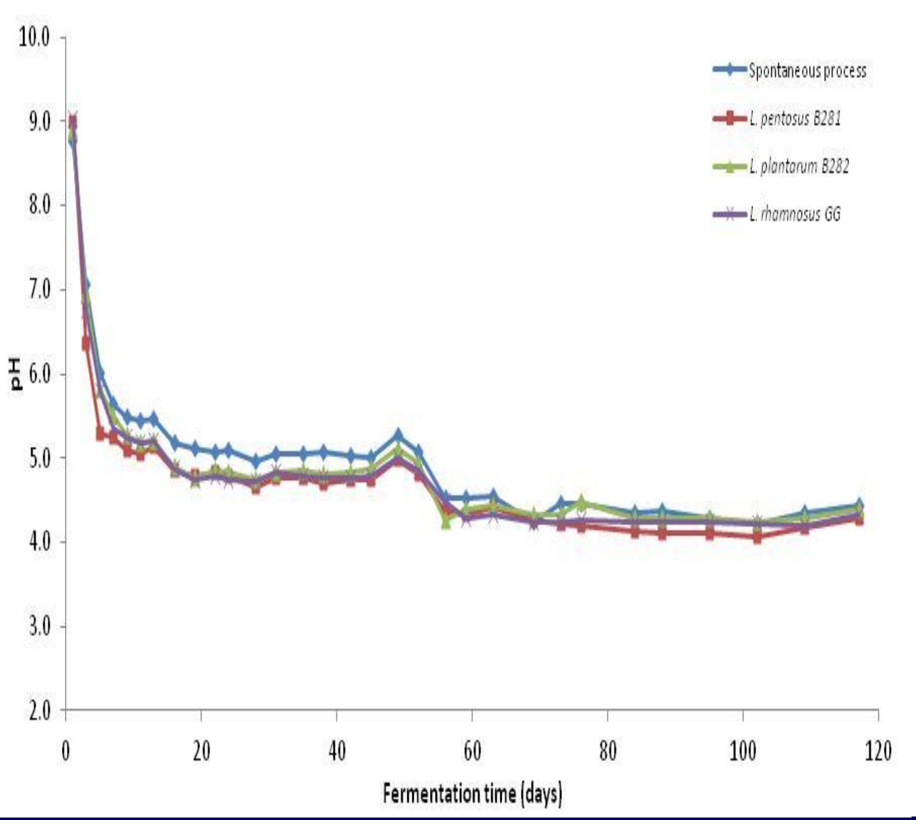
- ✓ Olives: Green olives Halkidiki variety
- ✓ Brine: 10 % (w/v) NaCl initial level
- ✓ Fermentation process: Spanish style processing
- ✓ Fermentation treatments:
  - Spontaneous process (control)
  - Inoculated process with *L. pentosus* B 281
  - Inoculated process with *L. plantarum* B 282
  - Inoculated 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)

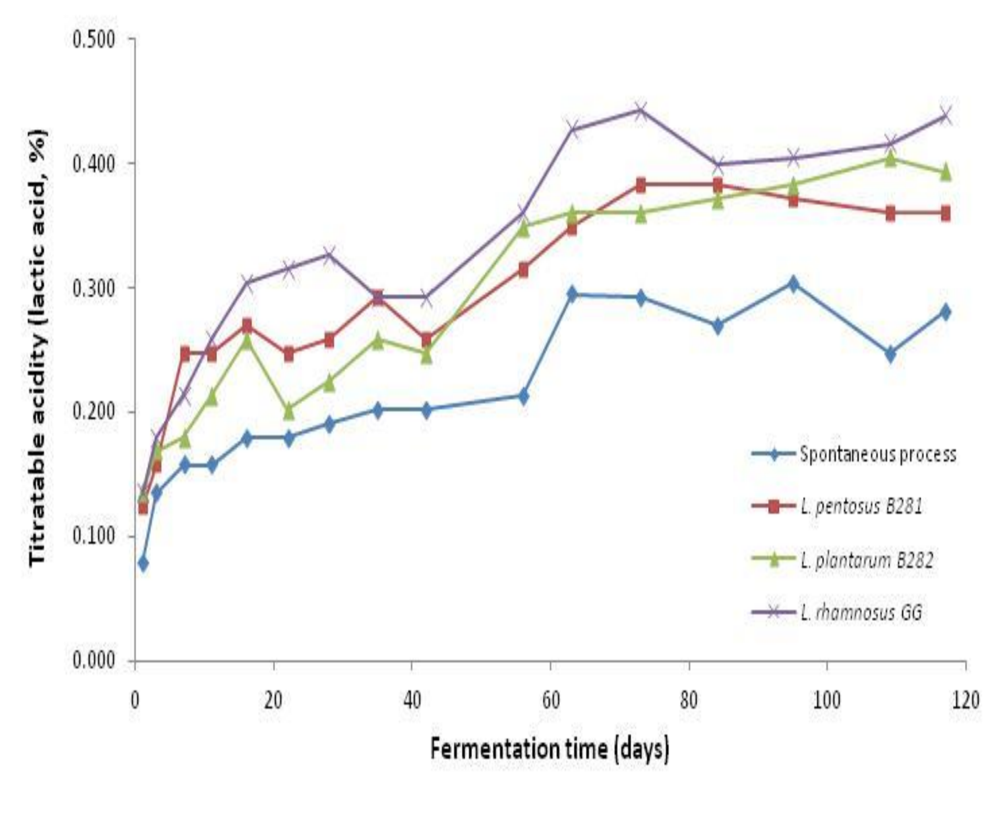
Evolution of microbial association



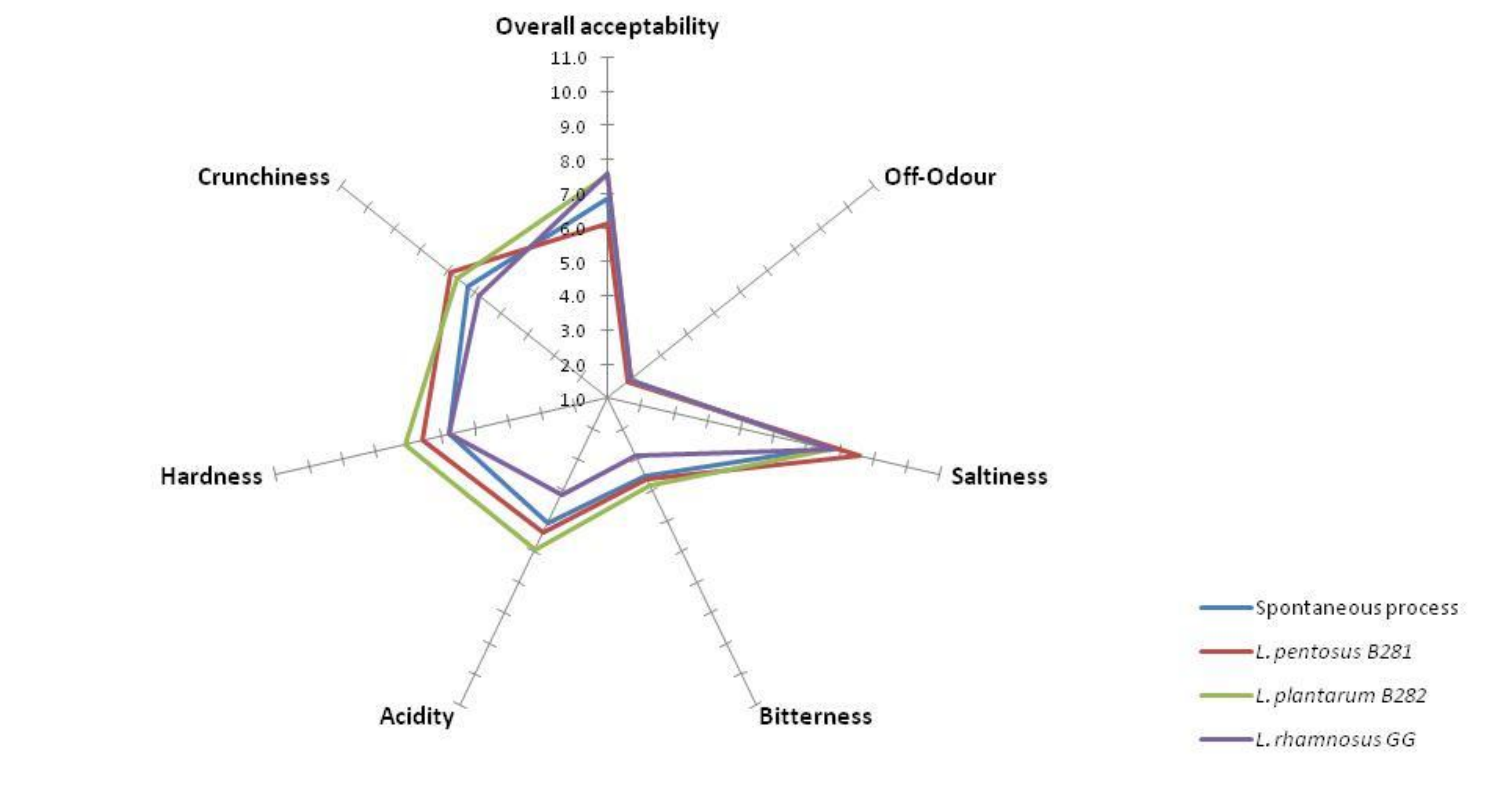
PROBIOLIVES

Evolution of pH / acidity





Organoleptic assessment



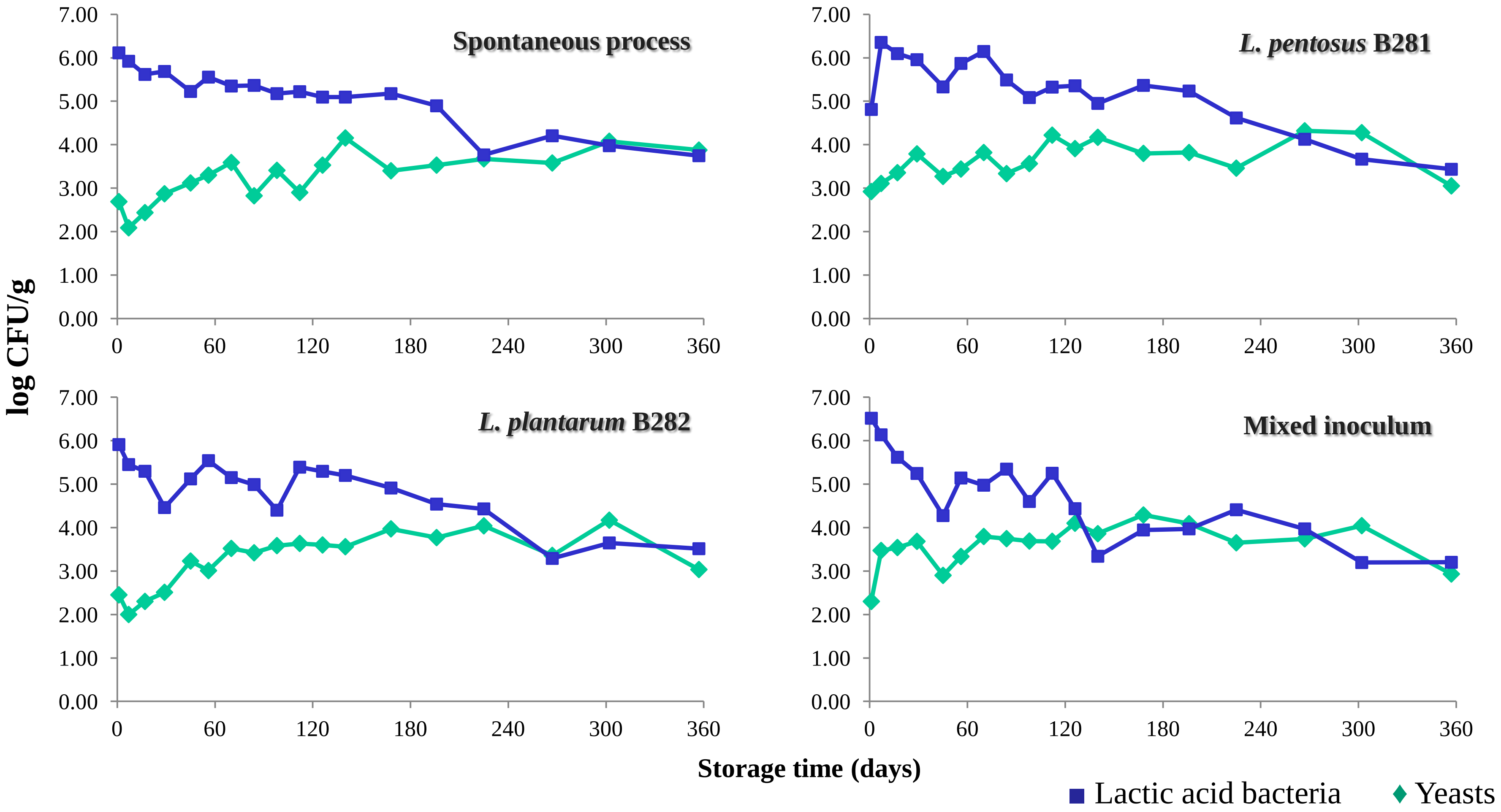
Ecotrophelia 2012 – Probiotic olives

Packaging of Probiotic olives

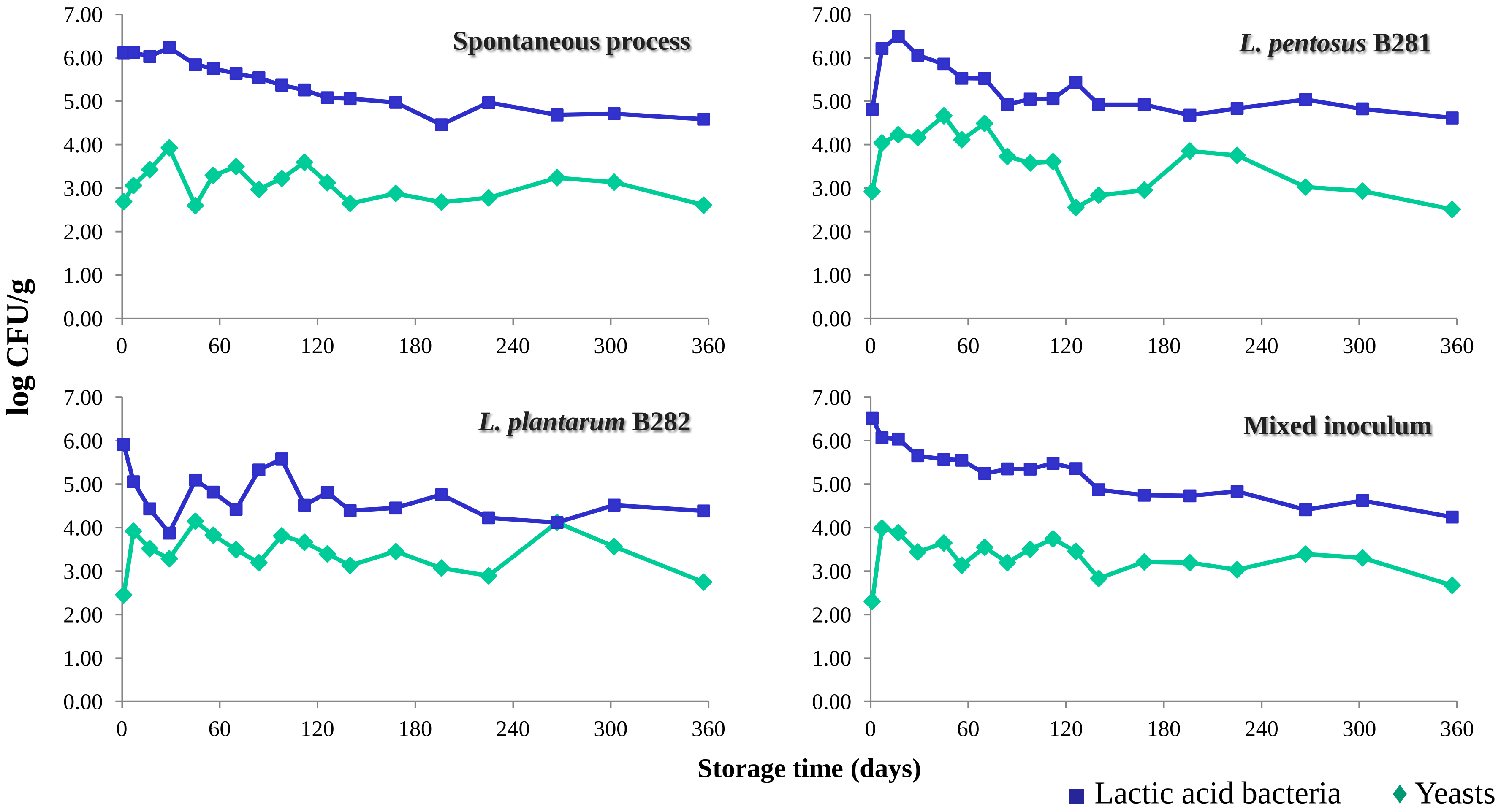
Experimental procedure

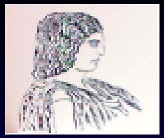
- **Olives:** Green olives cv. Halkidiki
- **Packaging:** Plastic pouches (OPE 15 μm / PE 80 μm)
- **Storage temp.:** 4 and 20 °C
- **Storage time:** 12 months
- **Composition:** - Fermented olives, 150 g
  - Brine 9%, 250 ml
  - Citric acid, 0.2 %
  - Ascorbic acid, 0.15 %
- **Packing Treatments:**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

Evolution of microbial association at 4°C



Evolution of microbial association at 20°C





Survival rate of inoculated strains during storage according to molecular analysis

		Survival rate	
Inoculated strain	**Fermentation time (days)**	**4°C**	**20°C**
***L. pentosus* B281**	1	90%	90%
	196	100%	20%
	357	93.75%	70%
***L. plantarum* 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

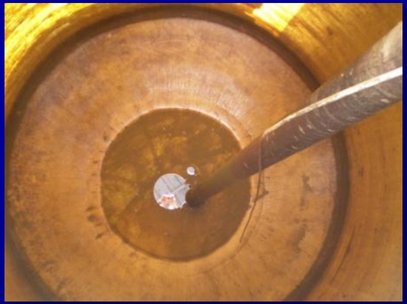
Washing scheme

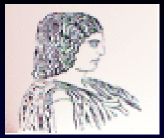
1st washing: 4 hours
2nd washing: 8 hours

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**

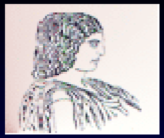
Acidification with **0.1% (v/v) lactic acid** and **0.014% (v/v) HCl**

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

Evolution of microbial association



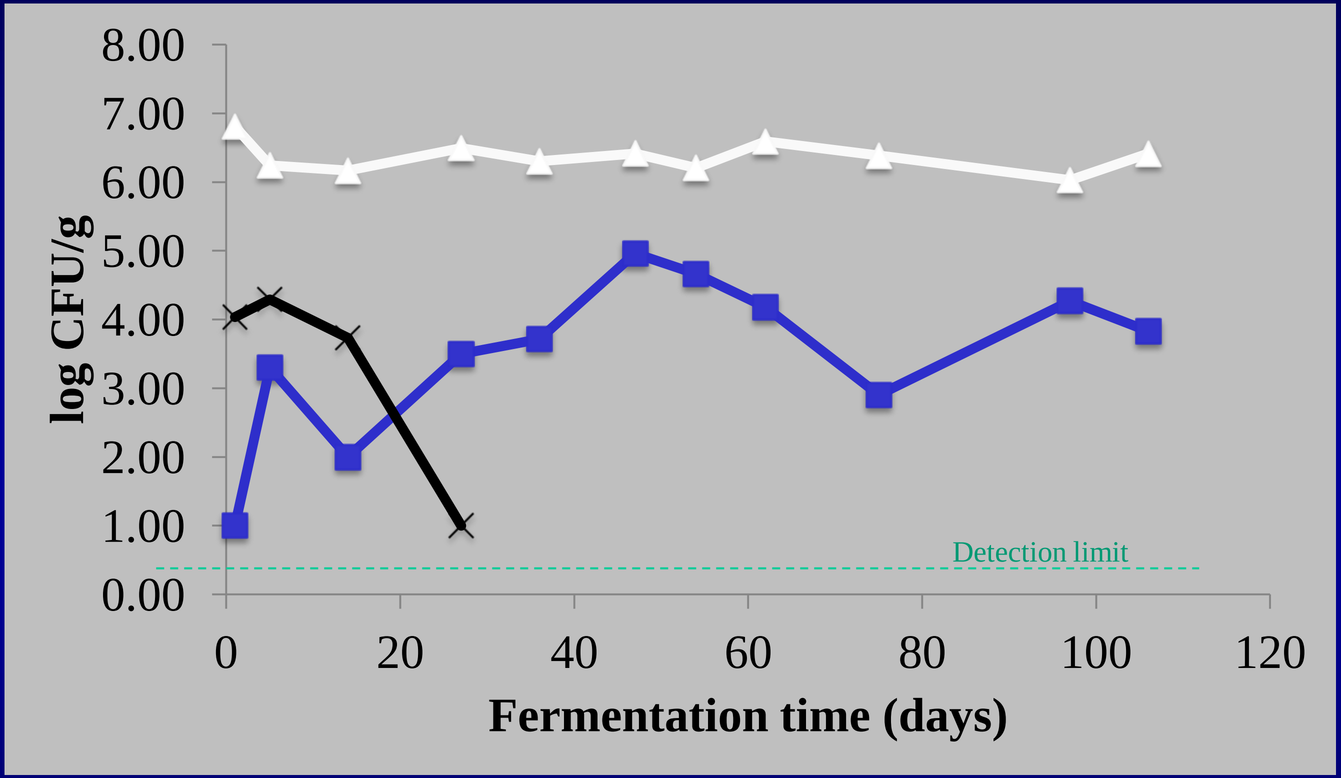
Microbiological *analysis*

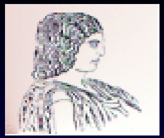
Lactic acid bacteria

(▲)

Yeasts (■)

***Enterobacteriaceae* (×)**





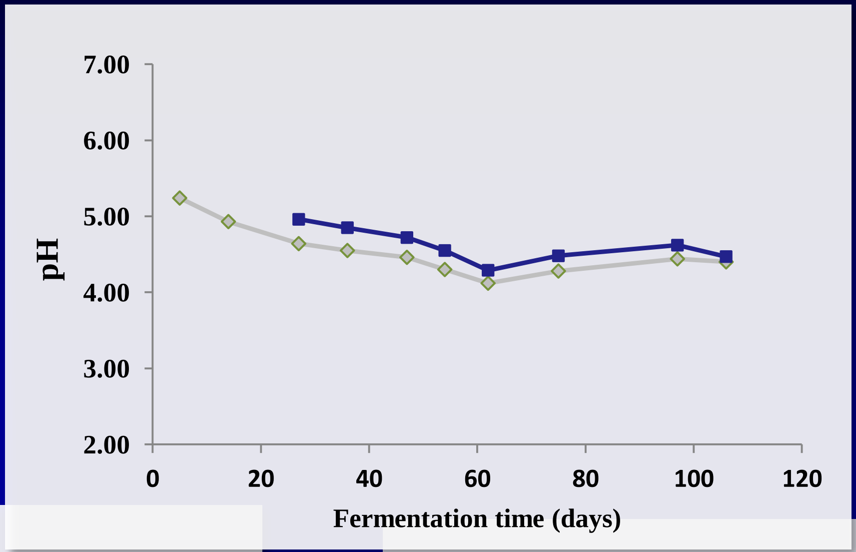
Changes in pH, acidity, salt level

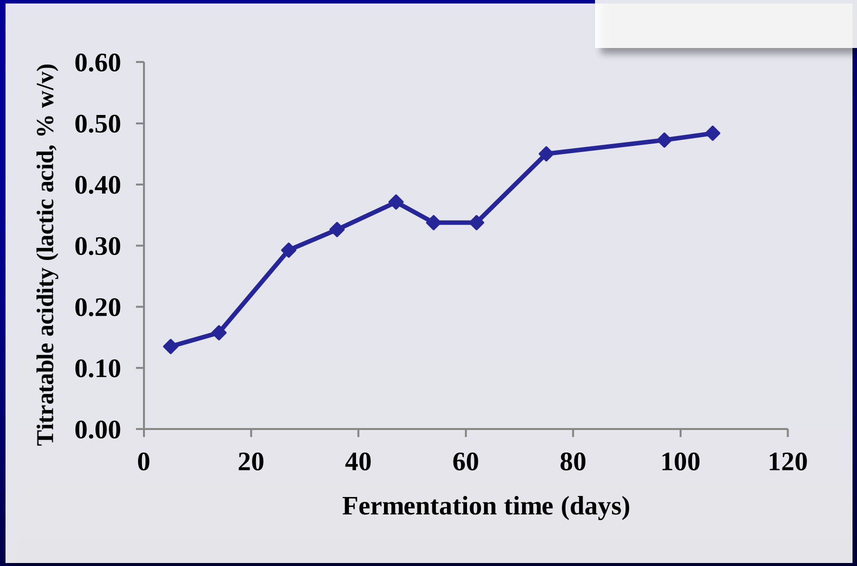
***Physicochemical** analysis*

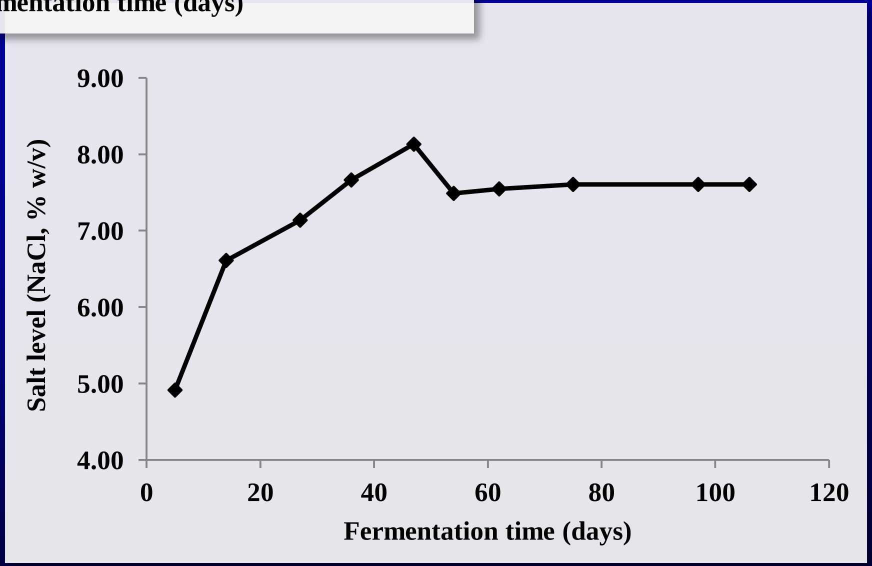
pH (brine ♦/ olive ■)

Salt level (brine)

Titratable acidity (brine)







Survival rate of inoculated culture

Inoculated strain	Fermentation time (days)	Survival rate (PFGE)
L. pentosus B281	5	**100%**
	97	**95.24%**

Table olive packaging

Packaging

Traditional

Glass / metal containers

New methods

- Multi-laminated pouches
- Rigid plastic containers
- Modified atmospheres

Thermal pasteurization

Glass filling (375 ml)

Brine addition (65°C)

Close and air removal

Outlet pasteurization (60°C)

Pasteurization (80°C/15-20 min)

Inlet pasteurization (30°C)

Total processing time 60 min

Packaging in bulk

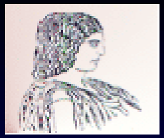
Packaging in pouches with brine and vacuum

Packaging in pouches with brine and vacuum

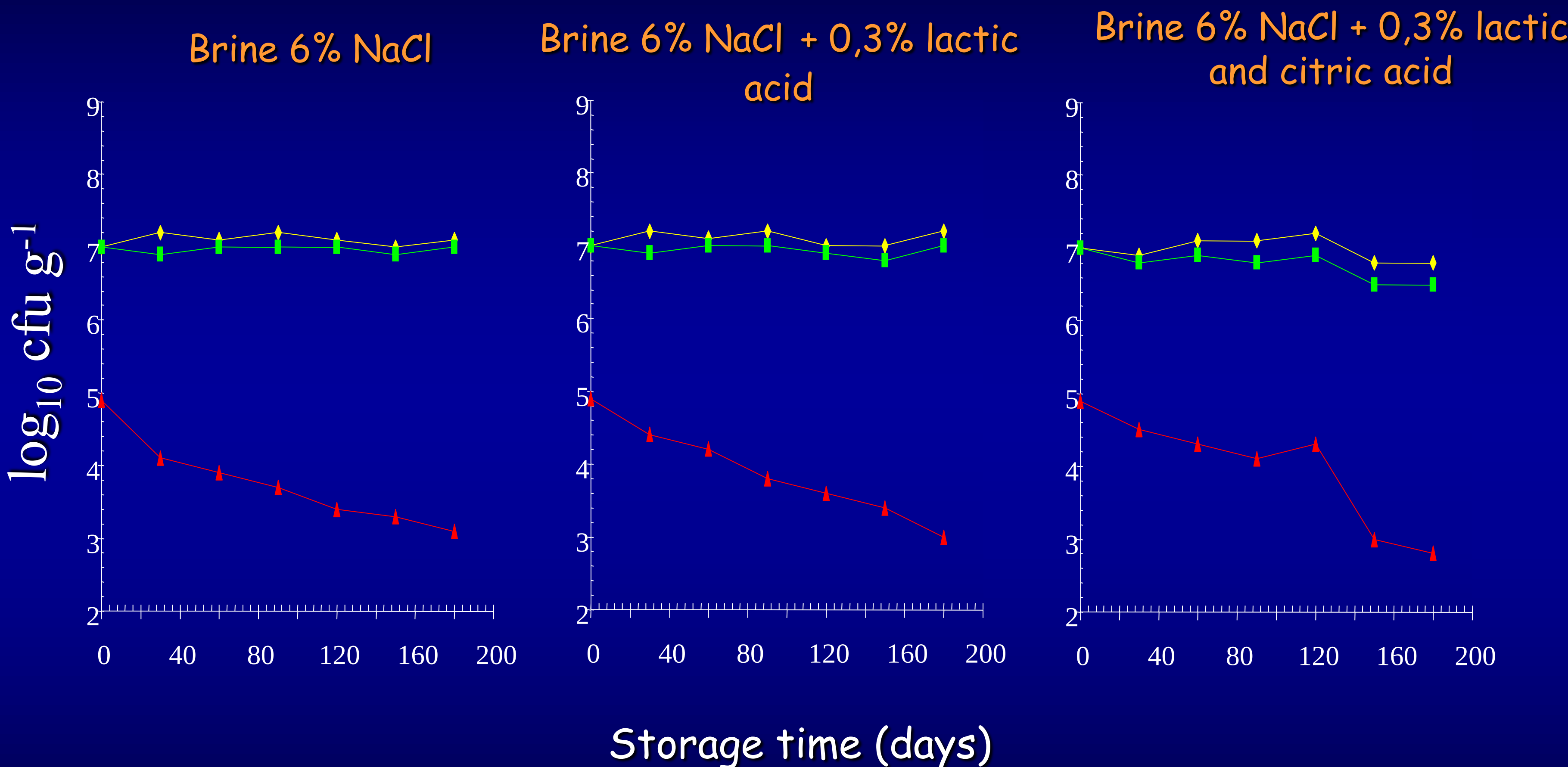
- ✓ **Olives:** Green olives “Conservolea”
- ✓ **Packaging:** HDPE 60 μm
- ✓ **Temperature:** 20 °C
- ✓ **Storage time:** 180 days

Packages

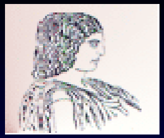
- Brine 6 % NaCl
- Brine 6 % NaCl + 0,3 % lactic acid
- Brine 6 % NaCl + 0,3 % lactic acid, 0,3 % citric acid



Changes in the population of microbes



Yeasts, Lactic acid bacteria, Total viable counts



Changes in the pH of olives and brine

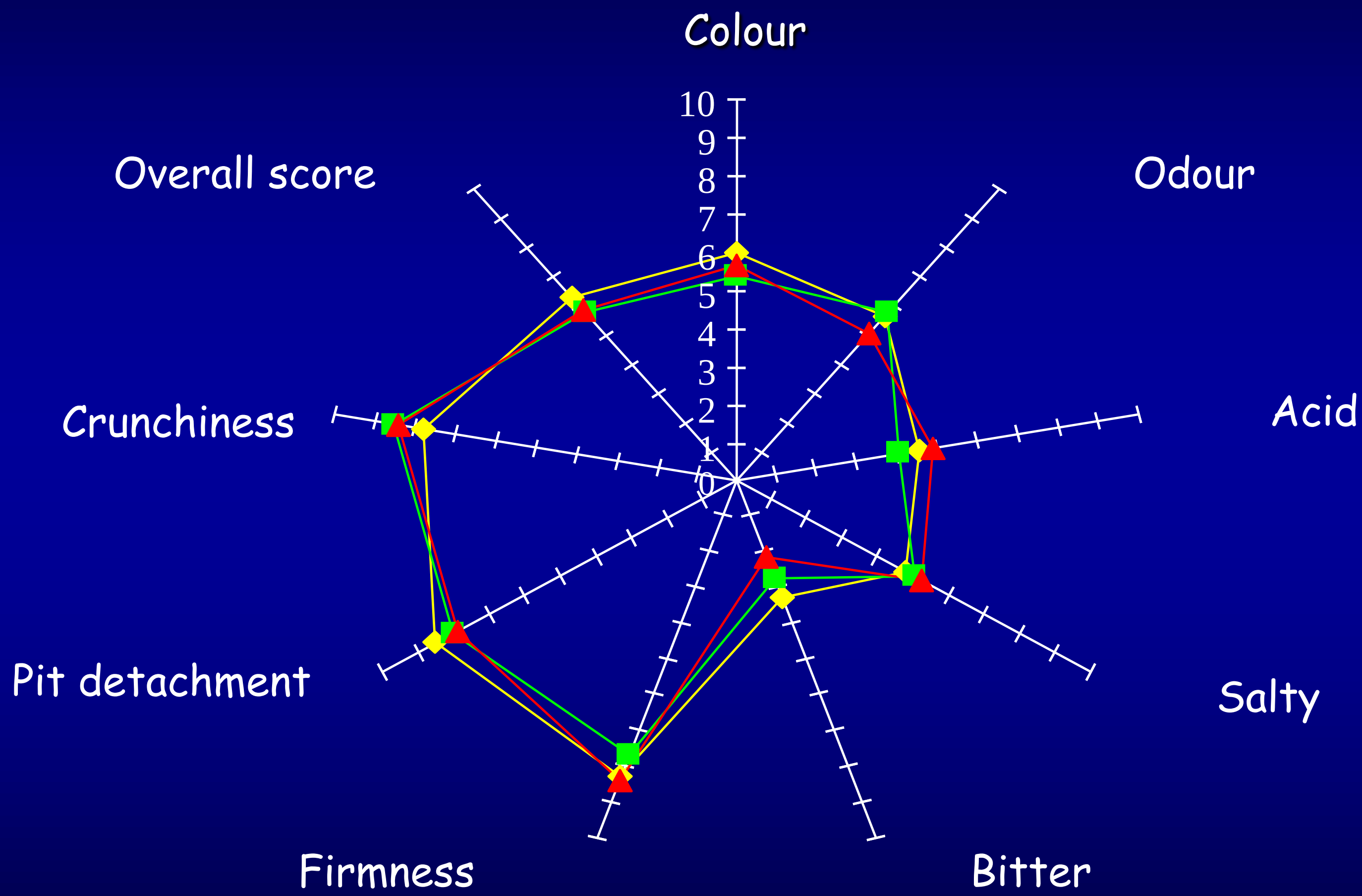
Time (days)	A		B		C	
	pH (olives)	pH (brine)	pH (olives)	pH (brine)	pH (olives)	pH (brine)
0	4,1	7,2	4,1	2,6	4,1	2,1
30	4,1	4,0	4,0	3,5	3,5	3,4
60	4,2	4,0	3,7	3,5	3,6	3,4
90	4,2	4,1	3,8	3,6	3,6	3,5
120	4,3	4,1	3,9	3,8	3,7	3,6
150	4,3	4,1	3,9	3,8	3,7	3,6
180	4,3	4,1	3,9	3,8	3,7	3,6

A: Brine 6% NaCl

B: Brine 6% NaCl + 0,3% lactic acid

C: Brine 6% NaCl + 0,3% lactic and citric acid

Sensory evaluation



Brine 6% NaCl
Brine 6% NaCl + 0,3 % lactic acid
Brine 6% NaCl + 0,3 % lactic and 0,3 % citric acid

Packaging in pouches under modified atmospheres

- ✓ **Olives:** Green olives "Conservolea"
- ✓ **Packaging:** HDPE 60 μm
- ✓ **Temperature:** 20 °C
- ✓ **Storage time:** 180 days

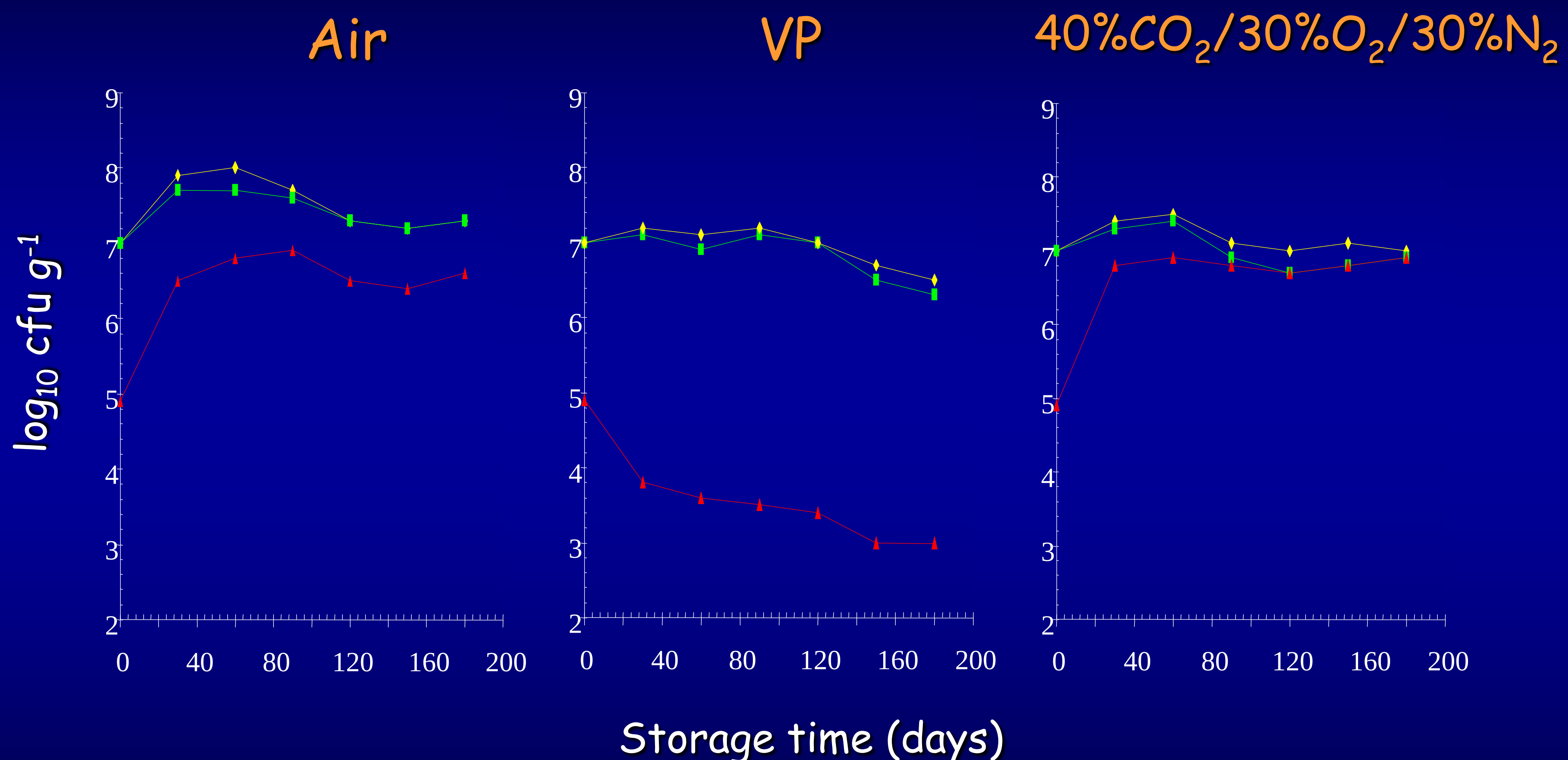
Packages

→ Aerobic storage (control)

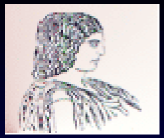
→ Vacuum packing

→ 40 % CO_2/ 30% O_2 / 30 % N_2

Changes in the population of microbes



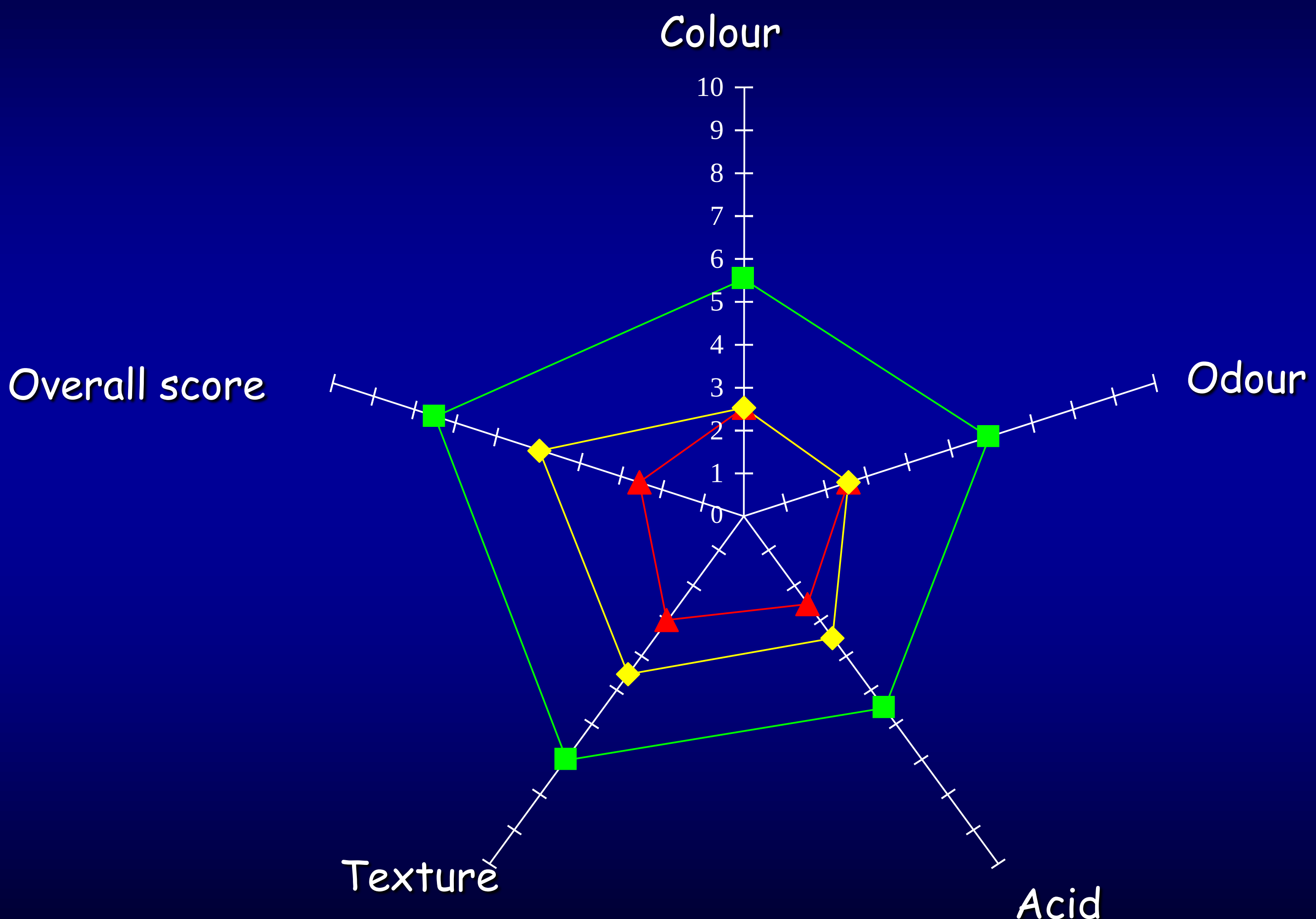
Yeasts, Lactic acid bacteria, Total viable counts



Changes in pH and texture of olives

Time (days)	Air		VP		MAP	
	Texture (N/g)	pH	Texture (N/g)	pH	Texture (N/g)	pH
0	42,3	4,1	42,3	4,1	42,3	4,1
30	37,1	4,3	40,2	4,2	38,5	4,4
60	32,6	4,2	38,7	4,2	25,7	4,2
90	28,1	4,3	36,3	4,3	32,1	4,3
120	23,5	4,3	34,1	4,4	30,8	4,2
150	21,6	4,3	32,7	4,4	27,1	4,4
180	14,0	4,3	31,6	4,4	25,8	4,4

Sensory evaluation



Air, VP, $40\%CO_2/30\%O_2/30\%N_2$

Table olives safety

Survival of pathogens in fermented green olives

Fermented green olives

Discarding of old brine

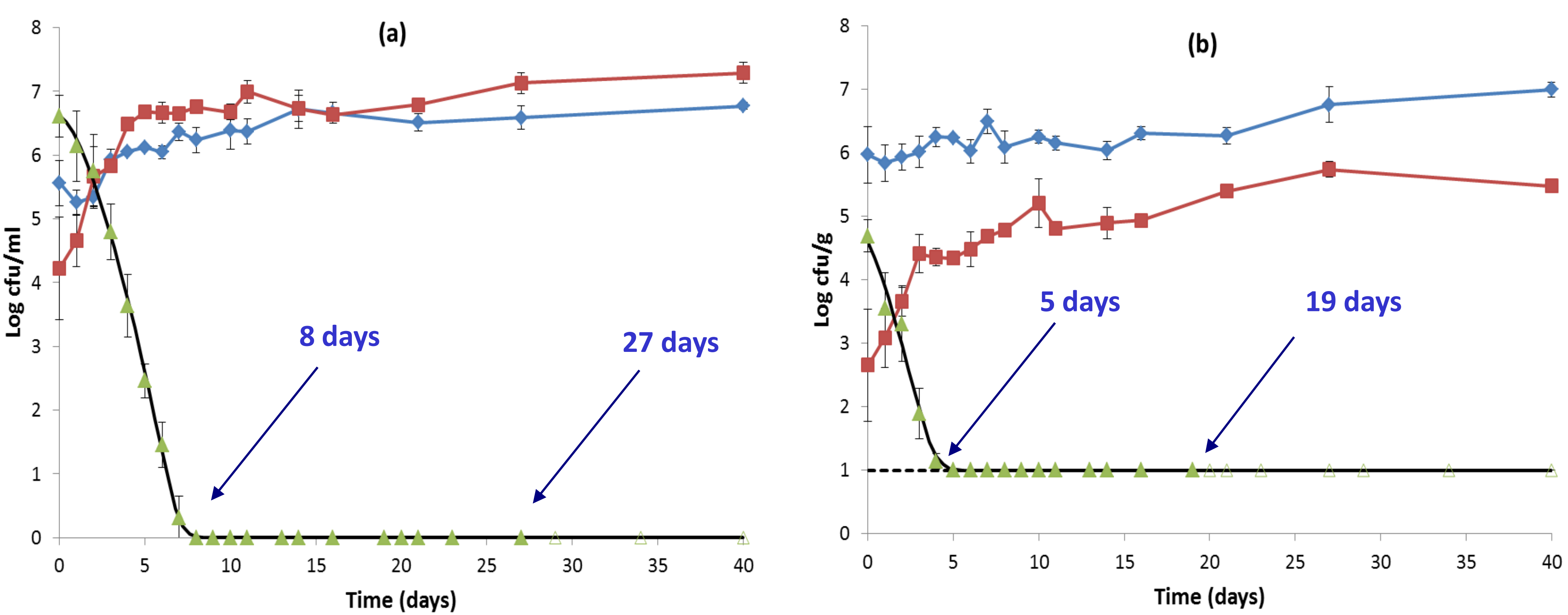
Addition of fresh brine NaCl 6% (w/v)

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

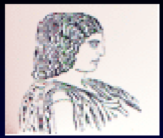
- *E. coli* O157:H7
- *Salmonella* Enteritidis
- *Listeria monocytogenes*

Storage at 20°C

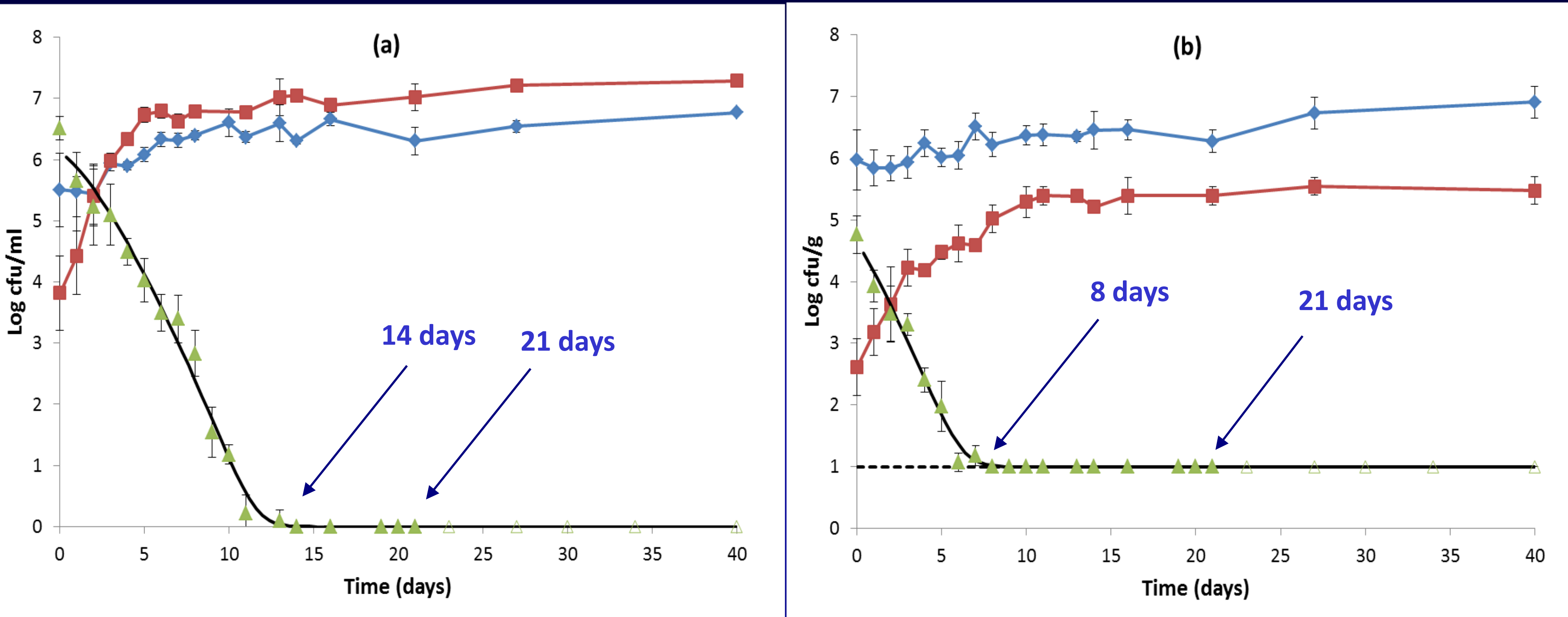
Survival of *E.coli* O157:H7



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.

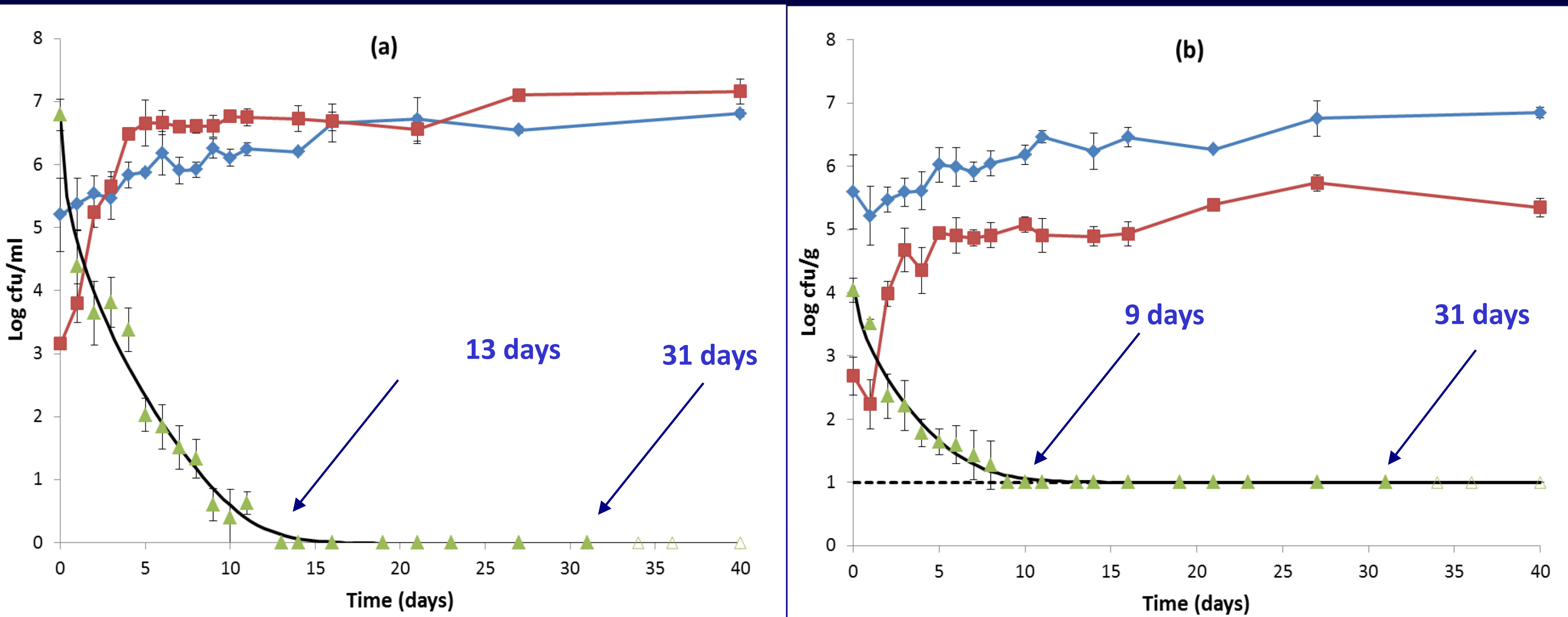


Survival of *Salmonella* Enteritidis



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

Survival 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.

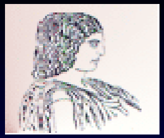
Survival of pathogens in fermented black olives

Fermented black olives

Addition of the pathogenic bacteria

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

Storage in air at 20°C



Survival 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	T (°C)	Population (log CFU/g) on:							
Strain		Day 0	Day 1	Day 2	Day 3	Day 5	Day 9	Day 12	Day 15
B-56	4	4.6 ± 0.4^{Aa}	3.9 ± 0.2^{Ba}	nd	nd	nd	nd	nd	nd
B-57		2.8 ± 0.4^{b}	nd	nd	nd	nd	nd	nd	nd
ATCC 13076		4.1 ± 0.4^{Ac}	2.9 ± 0.4^{Bb}	nd	nd	nd	nd	nd	nd
B-287		4.0 ± 0.5^{Ac}	2.2 ± 0.2^{Bc}	nd	nd	nd	nd	nd	nd
Mixed culture		4.0 ± 0.2^{Ac}	3.2 ± 0.1^{Bb}	nd	nd	nd	nd	nd	nd
B-56	20	4.6 ± 0.4^{Aa}	3.3 ± 0.2^{Ba}	nd	nd	nd	nd	nd	nd
B-57		2.8 ± 0.4^{b}	nd	nd	nd	nd	nd	nd	nd
ATCC 13076		4.1 ± 0.4^{c}	nd	nd	nd	nd	nd	nd	nd
B-287		4.0 ± 0.5^{c}	nd	nd	nd	nd	nd	nd	nd
Mixed culture		4.0 ± 0.2^{Ac}	3.5 ± 0.4^{Ba}	nd	nd	nd	nd	nd	nd
S. Typhimurium									
B-137	4	4.6 ± 0.3^{Aa}	4.3 ± 0.1^{Aa}	nd	nd	nd	nd	nd	nd
B-193		4.3 ± 0.2^{Aa}	3.4 ± 0.1^{Bb}	nd	nd	nd	nd	nd	nd
B-194		4.5 ± 0.4^{Aa}	3.7 ± 02^{Bb}	nd	nd	nd	nd	nd	nd
Mixed culture		4.7 ± 0.1^{Aa}	4.9 ± 0.1^{Ac}	nd	nd	nd	nd	nd	nd
B-137	20	4.6 ± 0.3^{Aa}	3.5 ± 0.1^{Ba}	nd	nd	nd	nd	nd	nd
B-193		4.3 ± 0.2^{Aa}	3.1 ± 0.3^{Ba}	nd	nd	nd	nd	nd	nd
B-194		4.5 ± 0.4^{Aa}	3.0 ± 0.6^{Ba}	nd	nd	nd	nd	nd	nd
Mixed culture		4.7 ± 0.1^{a}	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 ($P \leq 0.05$).

Survival 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.

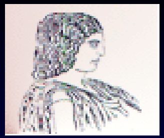
Strain	T (°C)	Population (log CFU/g) on:							
		Day 0	Day 1	Day 2	Day 3	Day 5	Day 9	Day 12	Day 15
B-15	4	3.8 ± 0.2^{Aa}	4.9 ± 0.2^{Ba}	nd	nd	nd	nd	nd	nd
B-16		4.3 ± 0.2^{Aab}	4.9 ± 0.1^{Ba}	nd	nd	nd	nd	nd	nd
B-18		4.2 ± 0.1^{Aab}	4.5 ± 0.2^{Aa}	nd	nd	nd	nd	nd	nd
Mixed culture		4.5 ± 0.1^{Ab}	4.5 ± 0.3^{Aa}	nd	nd	nd	nd	nd	nd
B-15	20	3.8 ± 0.2^{a}	nd	nd	nd	nd	nd	nd	nd
B-16		4.3 ± 0.2^{ab}	nd	nd	nd	nd	nd	nd	nd
B-18		4.2 ± 0.1^{ab}	nd	nd	nd	nd	nd	nd	nd
Mixed culture		4.5 ± 0.1^{Ab}	4.0 ± 0.5^{A}	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^{Aa}	3.8 ± 0.6^{Ba}	nd	nd	nd	nd	nd	nd
ATCC 6538		5.1 ± 0.2^{Aa}	3.5 ± 0.1^{Bab}	2.6 ± 0.1^{Ba}	nd	nd	nd	nd	nd
B-135		5.0 ± 0.2^{Aa}	3.3 ± 0.2^{Bbc}	2.2 ± 0.3^{Ca}	nd	nd	nd	nd	nd
Mixed culture		5.1 ± 0.2^{Aa}	2.9 ± 0.3^{Bc}	2.6 ± 0.2^{Ba}	nd	nd	nd	nd	nd
B-95	20	5.0 ± 0.2^{Aa}	3.5 ± 0.1^{Ba}	nd	nd	nd	nd	nd	nd
ATCC 6538		5.1 ± 0.2^{Aa}	3.3 ± 0.5^{Ba}	nd	nd	nd	nd	nd	nd
B-135		5.0 ± 0.2^{Aa}	3.3 ± 0.3^{Ba}	nd	nd	nd	nd	nd	nd
Mixed culture		5.1 ± 0.2^{Aa}	3.4 ± 0.3^{Ba}	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 ($P \leq 0.05$).

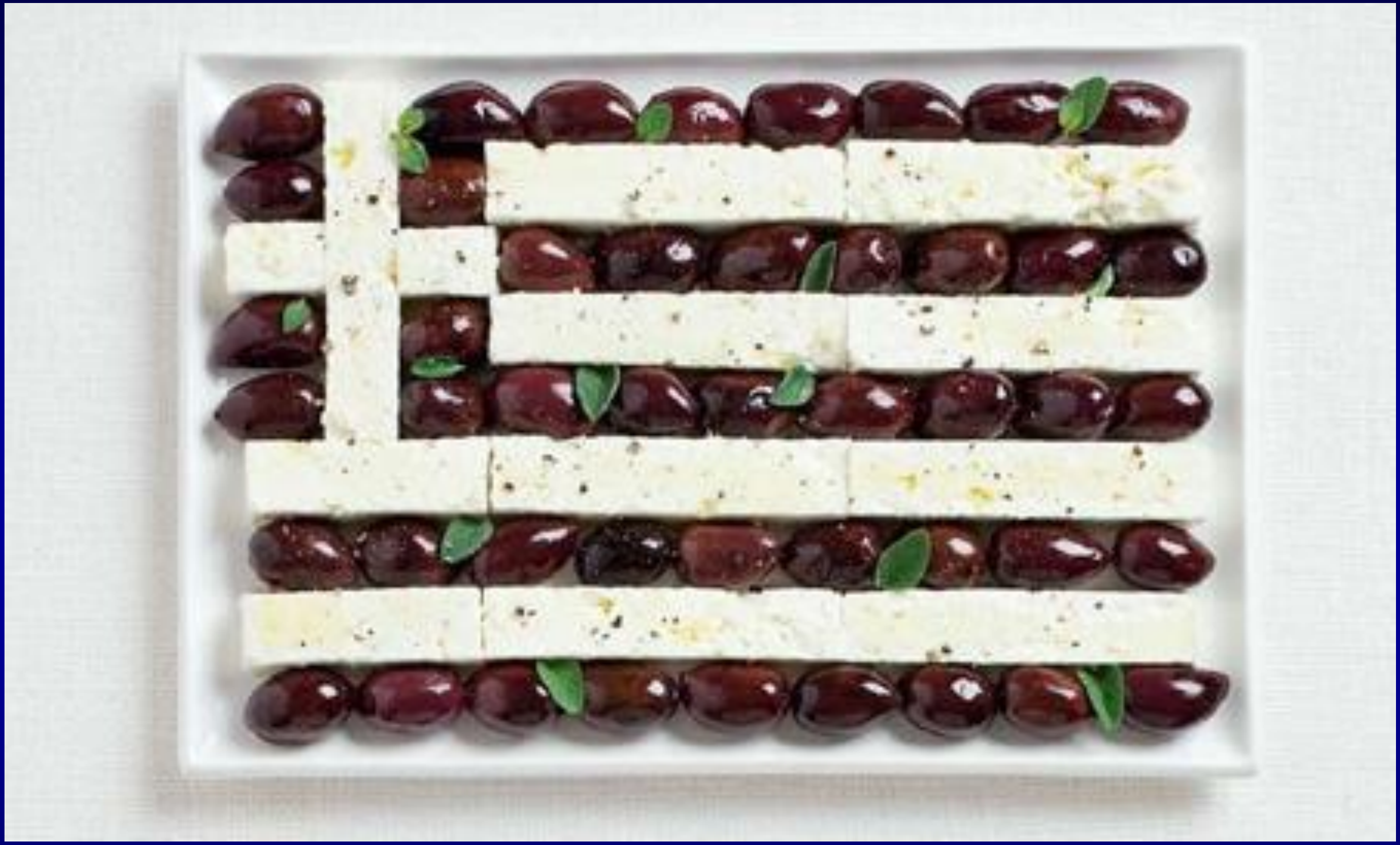


Survival of *L. monocytogenes*

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^{Aa}	3.7 ± 0.1^{Ba}	+	+	+	+	+	+
B-129		5.1 ± 0.3^{Aa}	2.6 ± 0.4^{Bb}	+	+	+	+	+	+
B-131		5.3 ± 0.2^{Aa}	2.8 ± 0.3^{Bb}	+	+	+	+	+	+
Mixed culture		4.9 ± 0.4^{Aa}	3.6 ± 0.2^{Ba}	+	+	+	+	+	+
B-128	20	5.6 ± 0.5^{Aa}	4.4 ± 0.1^{Ba}	nd	nd	nd	nd	nd	nd
B-129		5.1 ± 0.3^{Aa}	2.7 ± 0.2^{Bb}	+	+	+	+	+	+
B-131		5.3 ± 0.2^{Aa}	2.3 ± 0.2^{Bb}	nd	nd	nd	nd	nd	nd
Mixed culture		4.9 ± 0.4^{Aa}	2.3 ± 0.4^{Bb}	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 ($P \leq 0.05$).



Thank you for your attention