

# Advances in natural black table olive processing in Greece

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1<sup>st</sup> International Olive Conference  
Table Olives: Pursuing Innovation - Exploring Trends  
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# Trade Preparations

*(Trade Standards Applying to Table Olives, COI/OT/NC no. 1, December 2004)*

- Treated olives in brine
- Natural olives in brine
- Olives darkened by oxidation
- Dehydrated and/or shriveled olives
- Specialities

# Table olive varieties for natural black olive processing



**Conservolea  
variety**

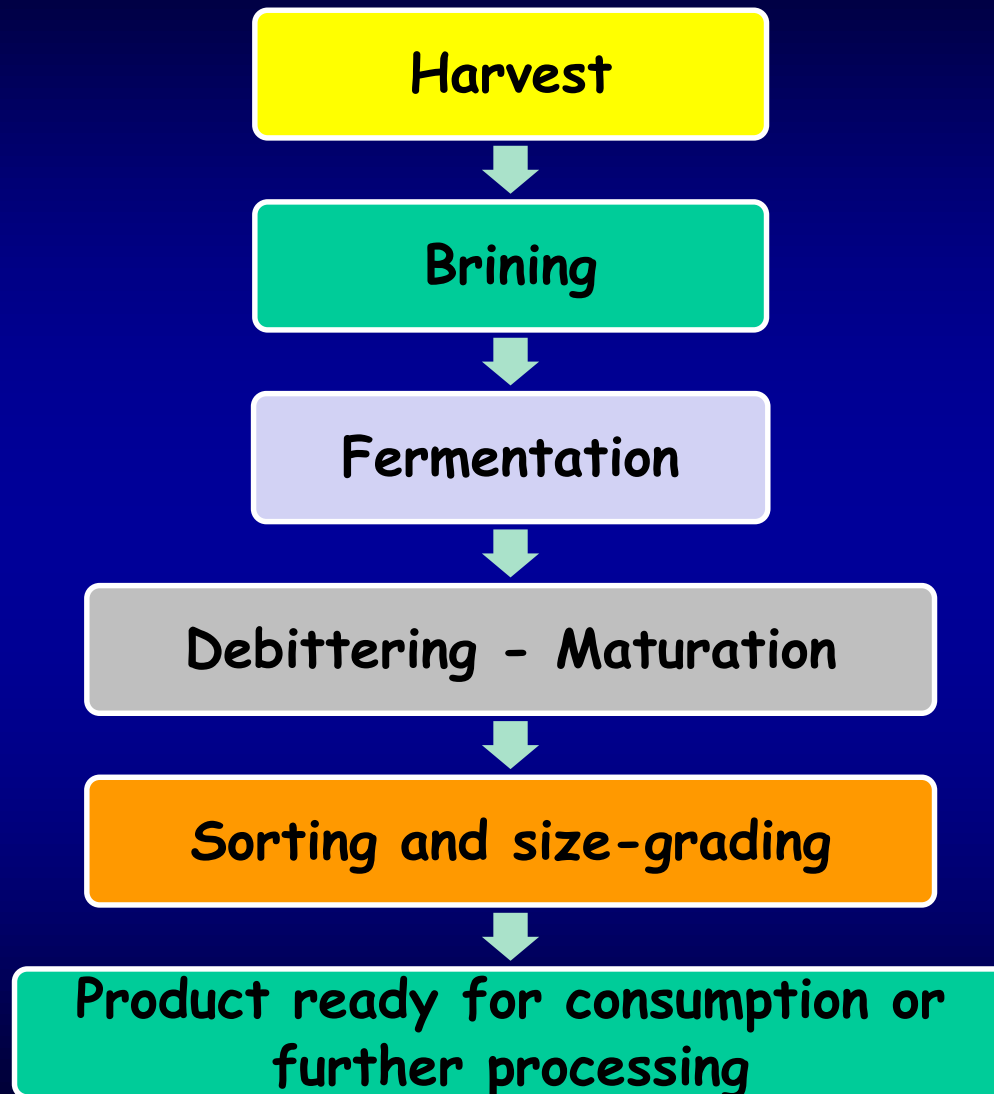


**Kalamata  
variety**



**Thassos  
variety**

# Natural black olives in brine (Greek style)



# Natural 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)

Garrido-Fernández, A., Fernández-Díez, M.J., Adams, M.R. (1997) Table Olives: Production and Processing. Chapman & Hall.

# Role of salt in table olive processing

- Controls processing through the selection of the dominant microbiota that will eventually drive fermentation.
- Inhibits undesirable spoilage and pathogenic bacteria ensuring the microbiological safety of the final product.
- Results in the debittering of natural black olives.
- Contributes (due to osmosis) to the diffusion of fermentable substrates from the olive's mesocarp to the brine.
- Improves the sensory characteristics of table olives.

# Processing - traditional anaerobic method

- Olives are placed directly in brine, 8-10% NaCl or even higher
- Under these conditions, fermentation is carried out primarily by yeasts, gram-negative bacteria and sometimes by lactic acid bacteria
- Fermentation is both alcoholic and lactic (to a lesser extent)
- The final product has pH 4.3-4.5 and titratable acidity 0.2-0.4% (expressed as lactic acid)

# Processing - new approach

- Olives are placed directly in 6-7% NaCl brine
- 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 0.6-1.0% (expressed as lactic acid)

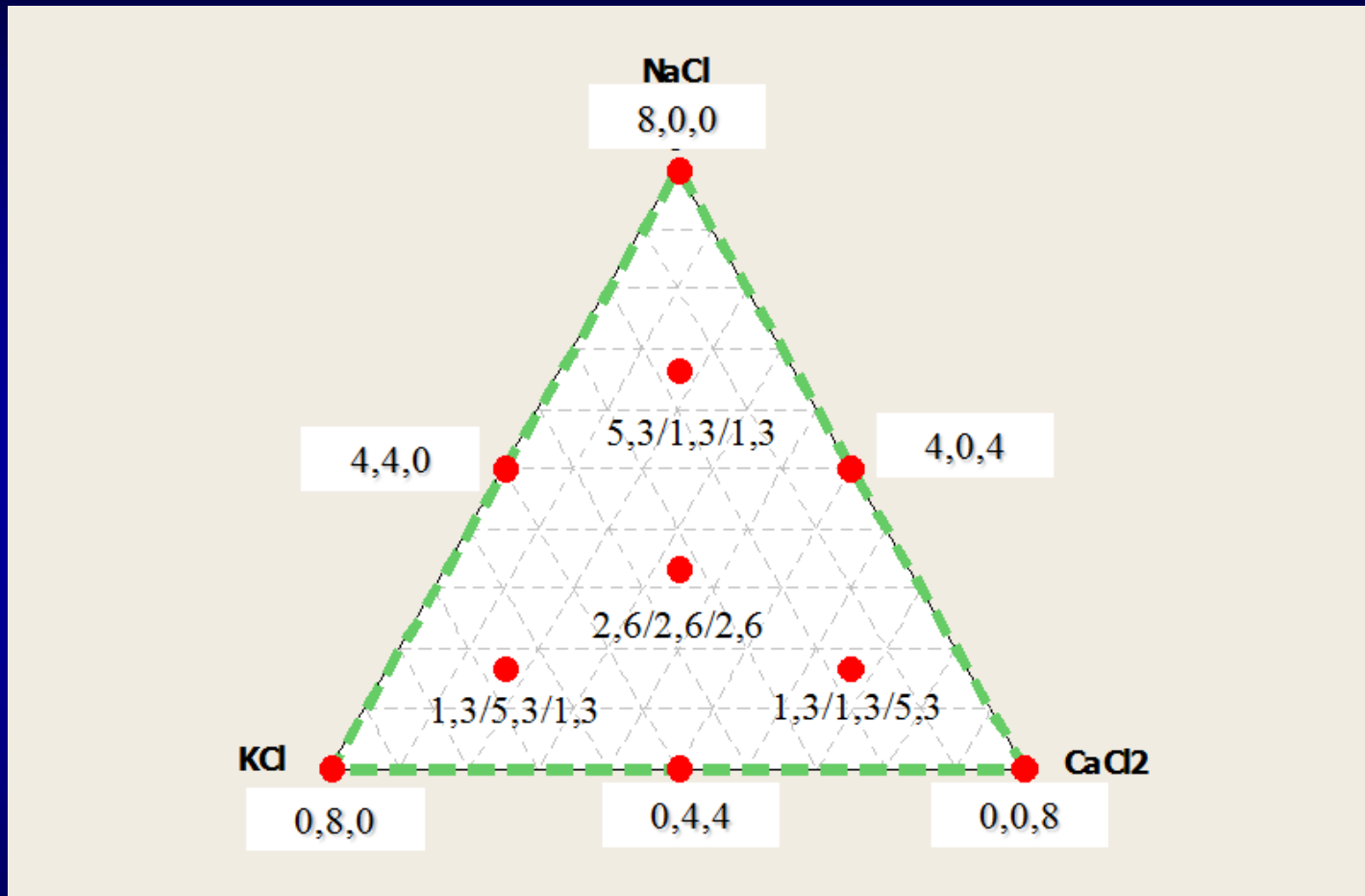
# Problem - excessive consumption of salt

- Sodium intake limit for adults **2.0 g/day** or **5 g NaCl/day** (WHO, 2012).
- 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.
- Salt has deep roots in Greek cuisine since the times when the Greek households had limited ways to preserve food.
- Refrigeration has reduced the need to use salt in food preservation but the preference of consuming high salt food has maintained through the generations ([www.efet.gr](http://www.efet.gr))

# Reduced salt black olives

- Reduced salt fermentation of natural black olives has been reported in the literature.
- Tsapatsaris, S., Kotzekidou, P. (2004) Application of a central composite design and response surface methodology to the fermentation of olive juice by *Lactobacillus plantarum* and *Debaryomyces hansenii*. *International Journal of Food Microbiology* 95, 157-168.
- Kaltsa, A., Papaliaga, D., Papaioannou, E., Kotzekidou, P. (2015) Characteristics of oleuropeinolytic strains of *Lactobacillus plantarum* group and influence on phenolic compounds in table olives elaborated under reduced salt conditions. *Food Microbiology* 48, 58-62.
- Tataridou, M., Kotzekidou, P. (2015) Fermentation of table olives by oleuropeinolytic starter culture in reduced salt brines and inactivation of *Escherichia coli* O157:H7 and *Listeria monocytogenes*. *International Journal of Food Microbiology* 208, 122-130.
- Mantzouridou, F., Tsimidou, M.Z. (2011) Microbiological quality and biophenol content of hot air-dried Thassos cv. table olives upon storage. *European Journal of Lipid Science and Technology* 113, 786-795.
- Kanavouras, A., Gazouli, M., Tzouvelekis, L., Petrakis, C. (2005) Evaluation of black table olives in different brines. *Grasas y Aceites* 56, 106-115.

# NaCl substitution - Mixture experiments with potassium chloride and calcium chloride



Augmented simplex lattice design of 2<sup>nd</sup> order

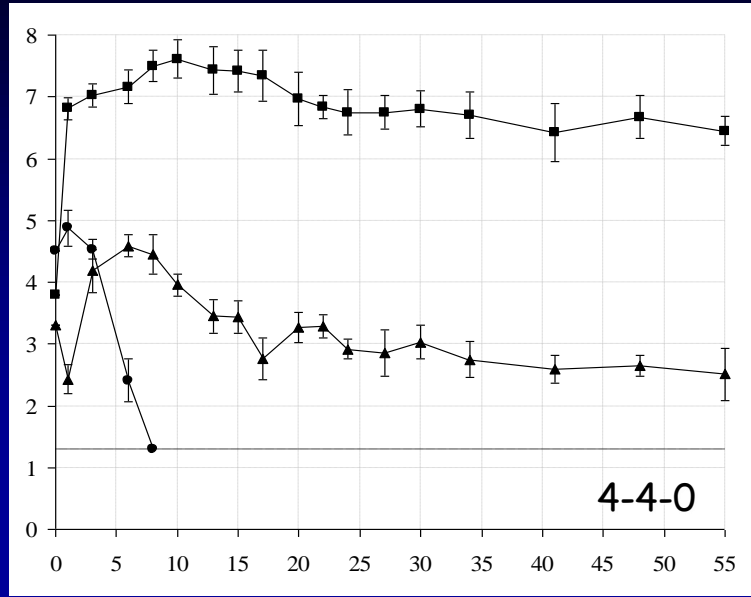
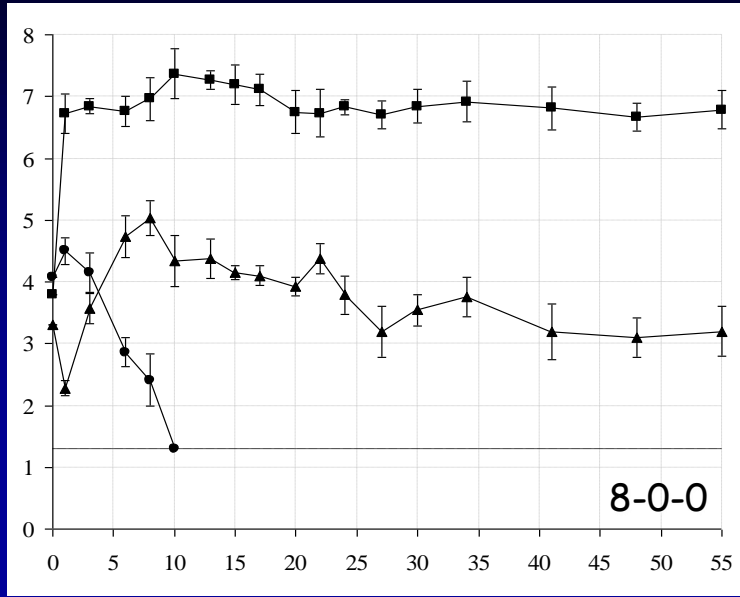
# Substitution of salt - Issues to be addressed

- **Question 1:** Is there a normal fermentation procedure with partial/total substitution of salt?
- **Question 2:** Do olives maintain acceptable sensory characteristics?

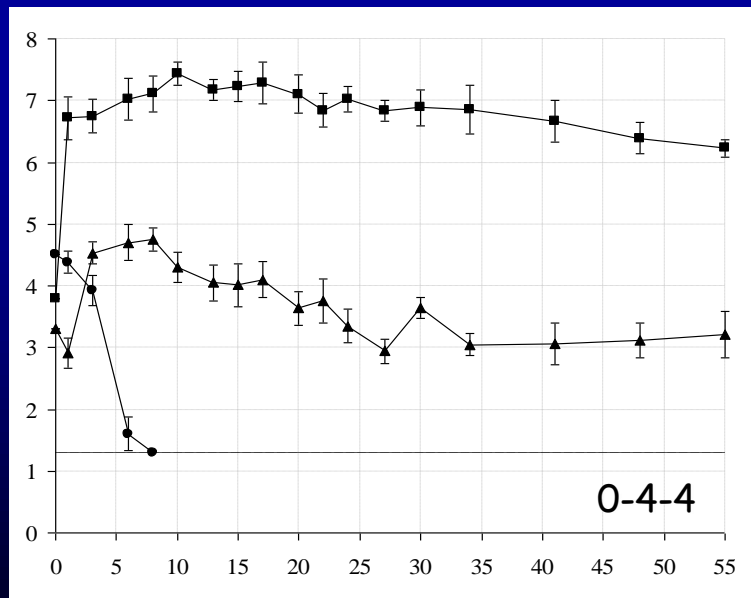
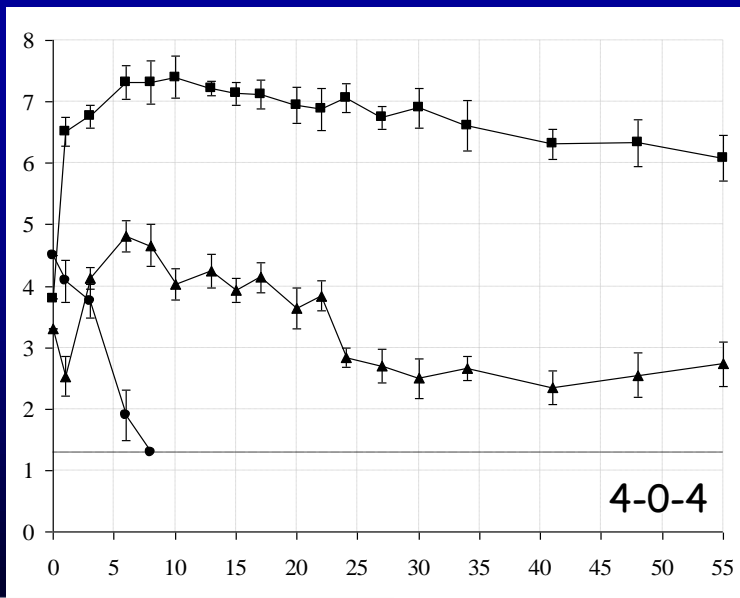


# Microbiological changes of selected fermentations

$\log_{10}$  CFU/ml

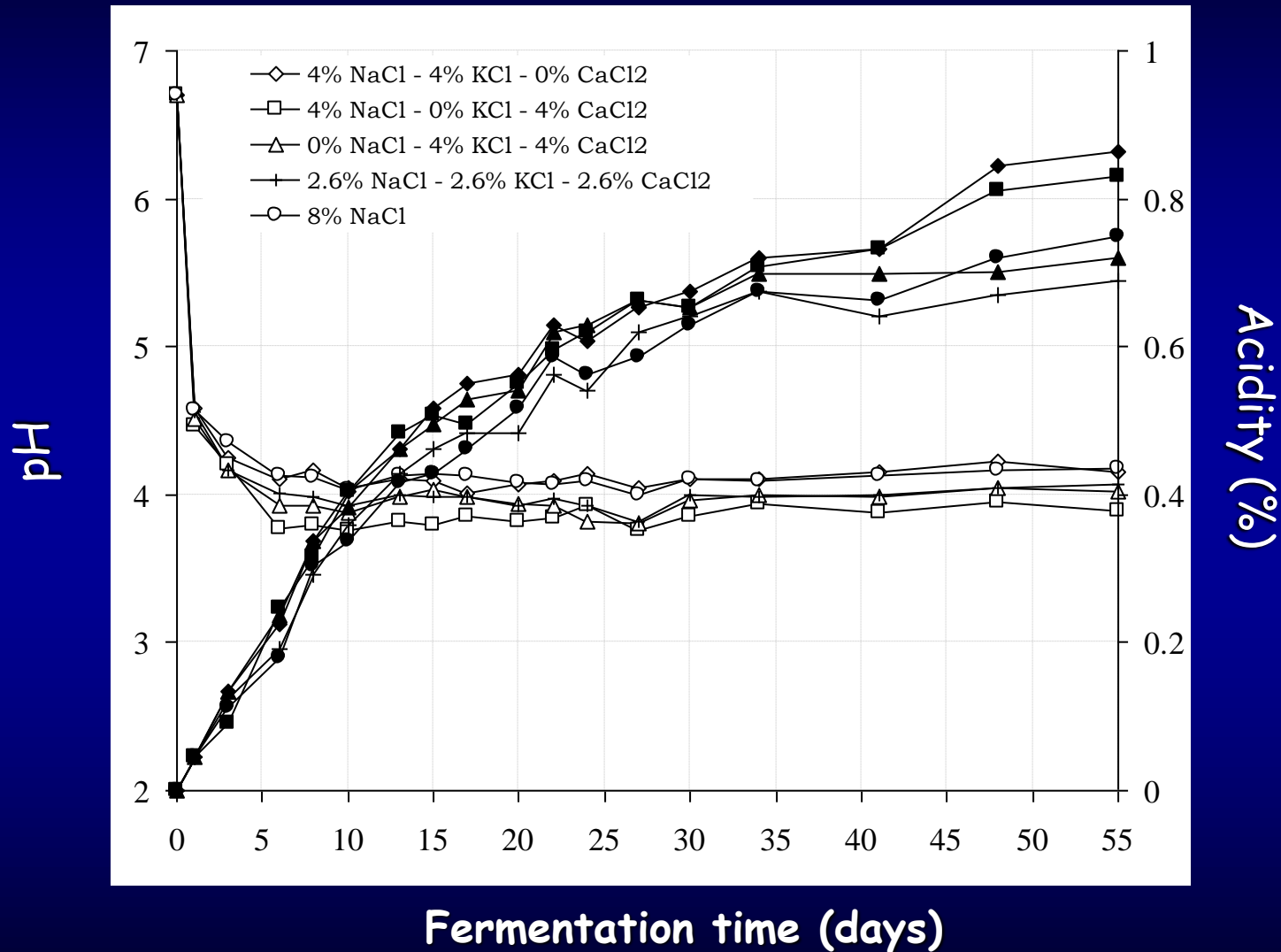


$\log_{10}$  CFU/ml

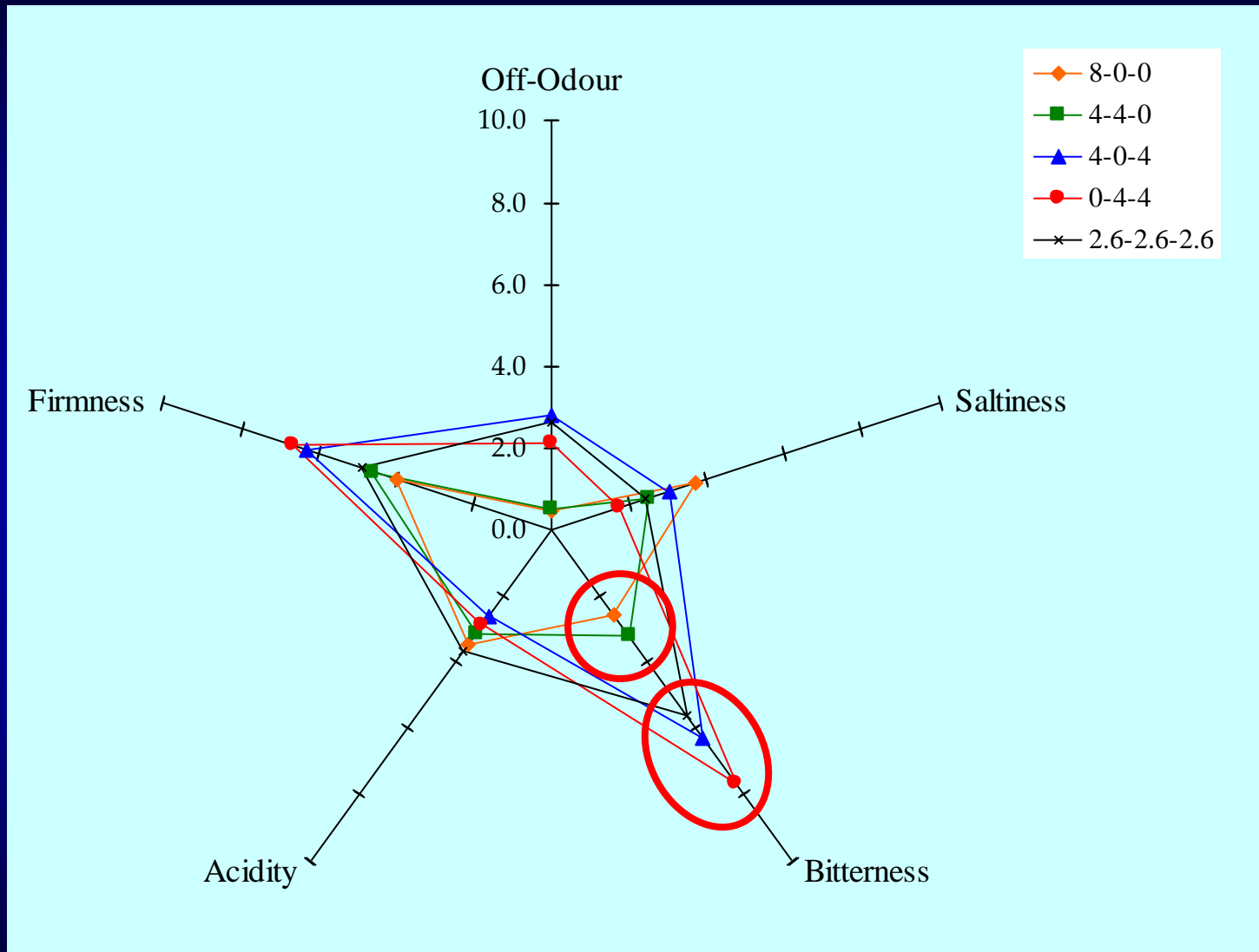


Fermentation time (days)

# Changes in pH and acidity of selected fermentations



# Sensory profile of selected fermentations

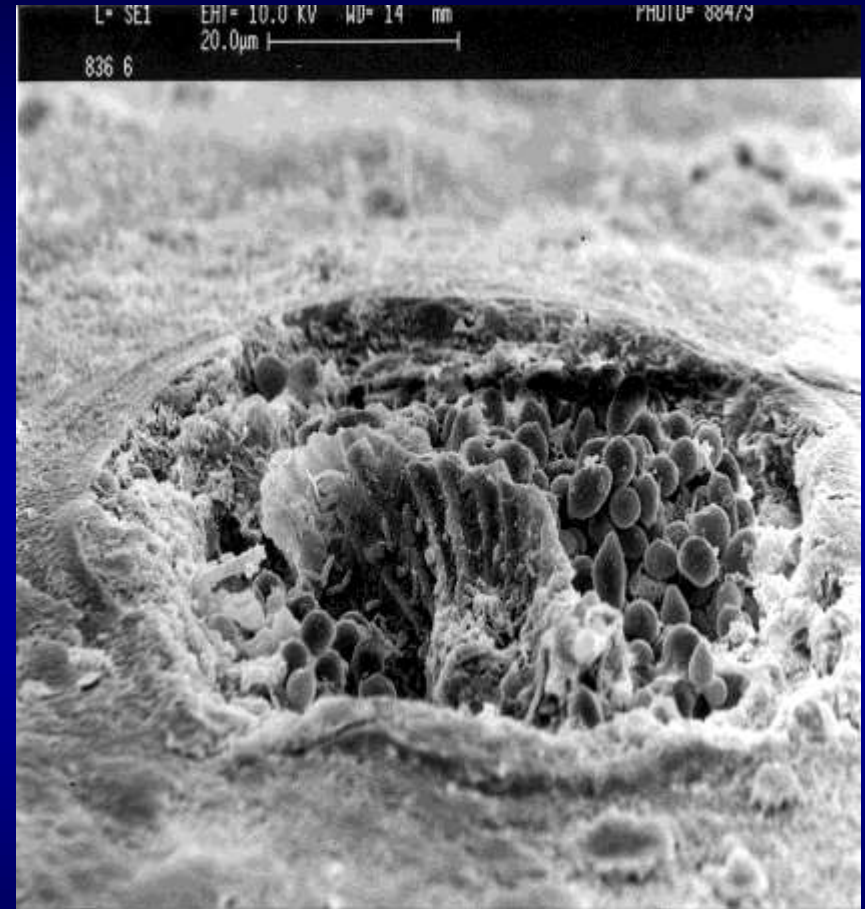
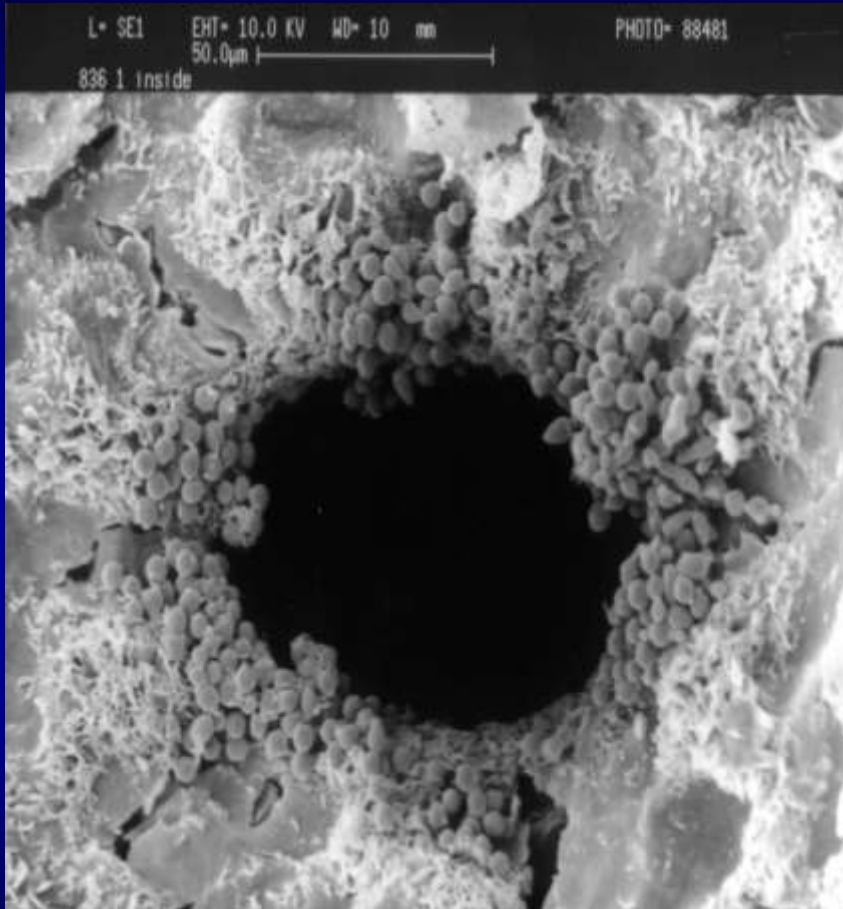




# Natural black olives as functional food

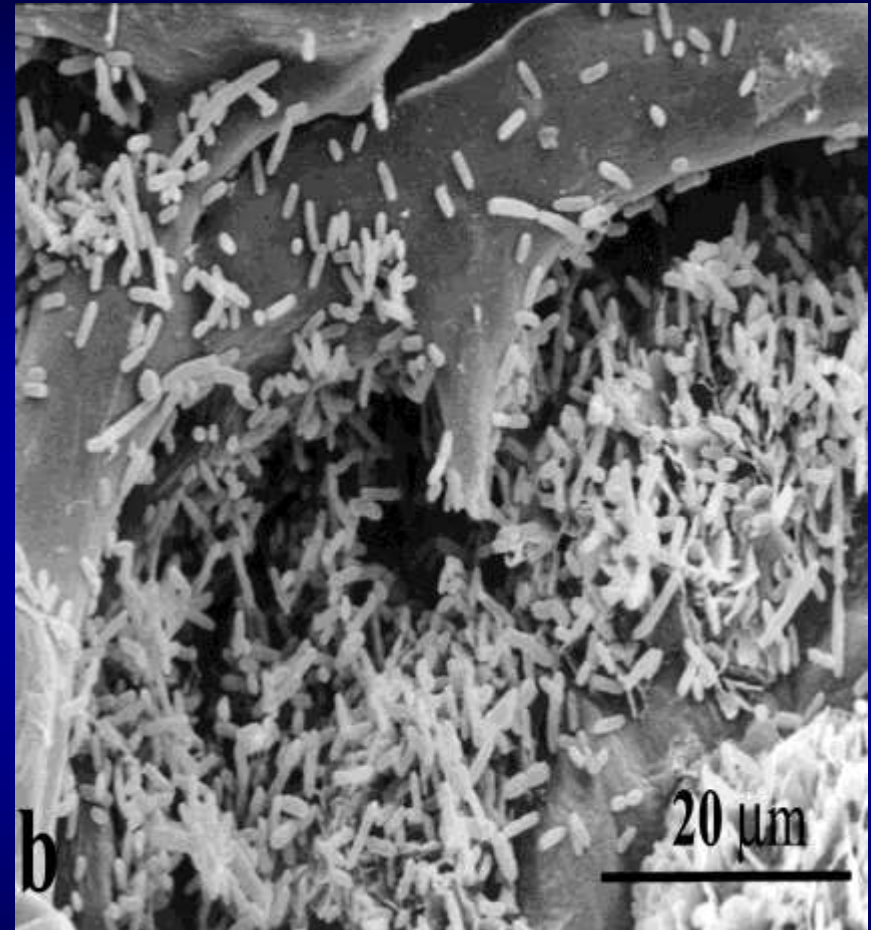


# Attachment of microorganisms on fermented *Conservolea* natural black olives



Nychas, G.-J.E., Panagou, E.Z., Parker, M.L., Waldron, K.W., Tassou, C.C. (2002) Microbial colonisation of naturally black olives during fermentation and associated biochemical activities in the cover brine, *Letters in Applied Microbiology* 34, 173-177.

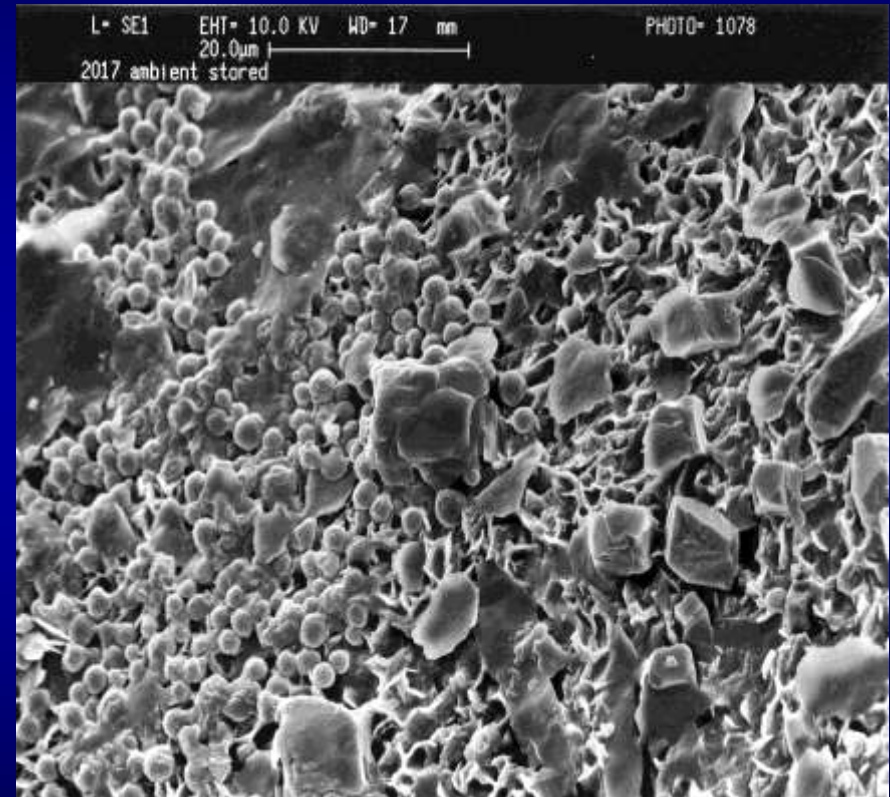
# Presence of microorganisms in substomal cavities of fermented *Conservolea* table olives



Nychas, G.-J.E., Panagou, E.Z., Parker, M.L., Waldron, K.W., Tassou, C.C. (2002) Microbial colonisation of naturally black olives during fermentation and associated biochemical activities in the cover brine, *Letters in Applied Microbiology* 34, 173-177.



# Spatial distribution of microorganisms on Thassos dry-salted olives



Tassou, C.C., Panagou, E.Z., Nychas, G.-J.E. (2010) Microbial colonization of naturally fermented olives. In: Preedy, V.R, Watson, R.R. (eds.) *Olives and Olive Oil in Health and Disease Prevention*. Academic Press, London, UK.

# Technological and functional traits of the technological microbiota of olives

Properties	Desirable	Undesirable
Technological	Ability to survive/grow in different salt concentrations; Ability to survive/grow on high and low pH values; Ability to attach on olive epidermis	Production of CO <sub>2</sub> ; Assimilation of lactic acid; Production of mycotoxins and biogenic amines
Probiotic	Survive in conditions simulating the passage through gastrointestinal tract; Ability to adhere and colonize the epithelial cells; Antimicrobial activity against pathogens; Ability to aggregate with pathogens	
Functional	Bio-assimilation of phenolic compounds such as oleuropein; Production of vitamins; Antimicrobial activity	
Enzymatic activity	Esterase; Lipase; Catalase; Phytase; Alkaline/acid phosphatase; β-glucosidase	Proteolytic and Xylanolytic activity

Bonatsou, S., Tassou, C.C., Panagou, E.Z., Nychas, G.-J.E. (2017) Table olive fermentation using starter cultures with multifunctional potential. *Microorganisms* 5, 30.



Contents lists available at SciVerse ScienceDirect

## LWT - Food Science and Technology

journal homepage: [www.elsevier.com/locate/lwt](http://www.elsevier.com/locate/lwt)



### Research note

## Molecular characterization of lactic acid bacteria isolated from industrially fermented Greek table olives

Agapi I. Doulgeraki<sup>a</sup>, Paraskevi Pramateftaki<sup>b</sup>, Anthoula A. Argyri<sup>b</sup>, George-John E. Nychas<sup>a</sup>, Chrysoula C. Tassou<sup>b</sup>, Efstathios Z. Panagou<sup>a,\*</sup>

<sup>a</sup>Laboratory of Microbiology and Biotech

<sup>b</sup>Hellenic Agricultural Organisation 'Dem

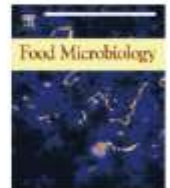
Food Microbiology 33 (2013) 282–291



Contents lists available at SciVerse ScienceDirect

## Food Microbiology

journal homepage: [www.elsevier.com/locate/fm](http://www.elsevier.com/locate/fm)



## Selection of potential probiotic lactic acid bacteria from fermented olives by *in vitro* tests

Anthoula A. Argyri<sup>a</sup>, Georgia Zoumpopoulou<sup>b</sup>, Kimon-Andreas G. Karatzas<sup>c</sup>, Effie Tsakalidou<sup>b</sup>, George-John E. Nychas<sup>d</sup>, Efstathios Z. Panagou<sup>d</sup>, Chrysoula C. Tassou<sup>a,\*</sup>

<sup>a</sup>Hellenic Agricultural Organisation 'DEMETER', Institute of Technology of Agricultural Products, Sof. Venizelou 1, 14123 Lycovrissi, Attikis, Greece

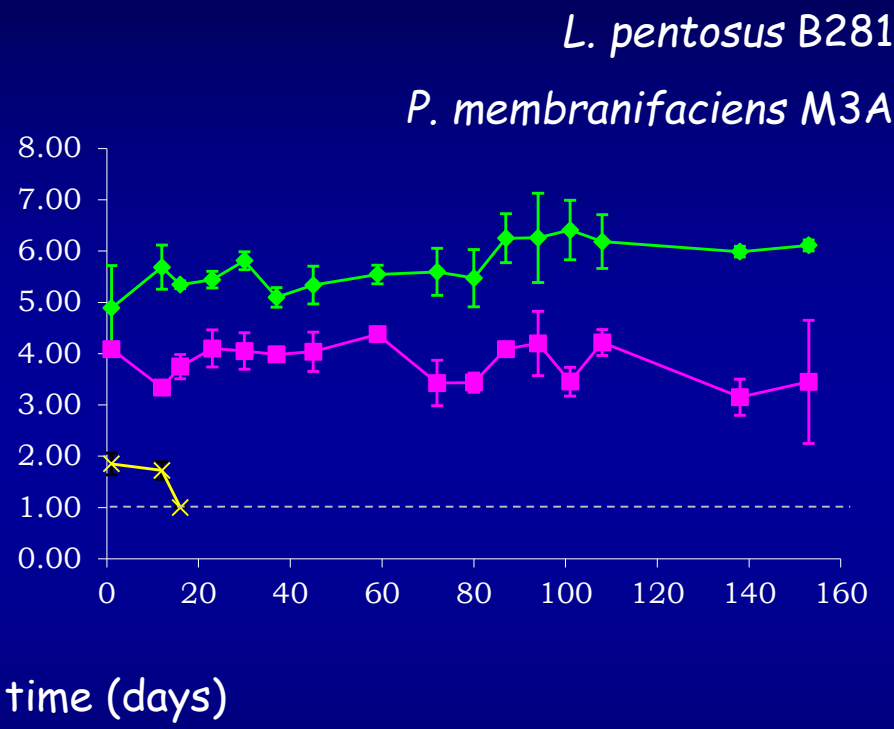
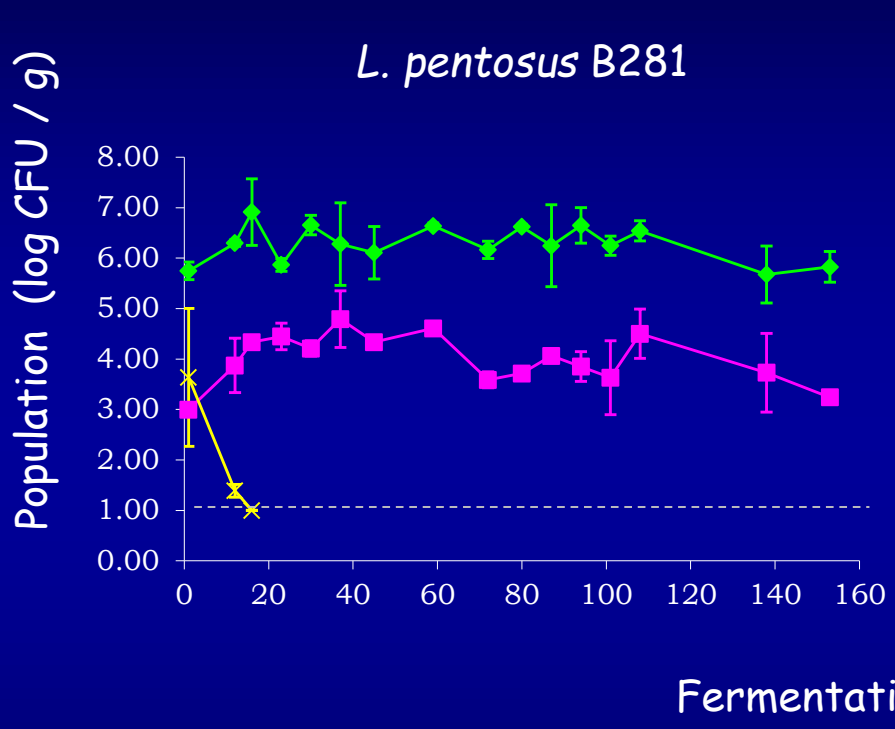
<sup>b</sup>Agricultural University of Athens, Dept. of Food Science and Technology, Lab of Dairy Research, Iera Odos 75, 11855 Athens, Greece

<sup>c</sup>University of Reading, Dept. of Food and Nutritional Sciences, Whiteknights, Reading, Berkshire RG6 6AH, UK

<sup>d</sup>Agricultural University of Athens, Dept. of Food Science and Technology, Lab of Microbiology and Biotechnology of Foods, Iera Odos 75, 11855 Athens, Greece



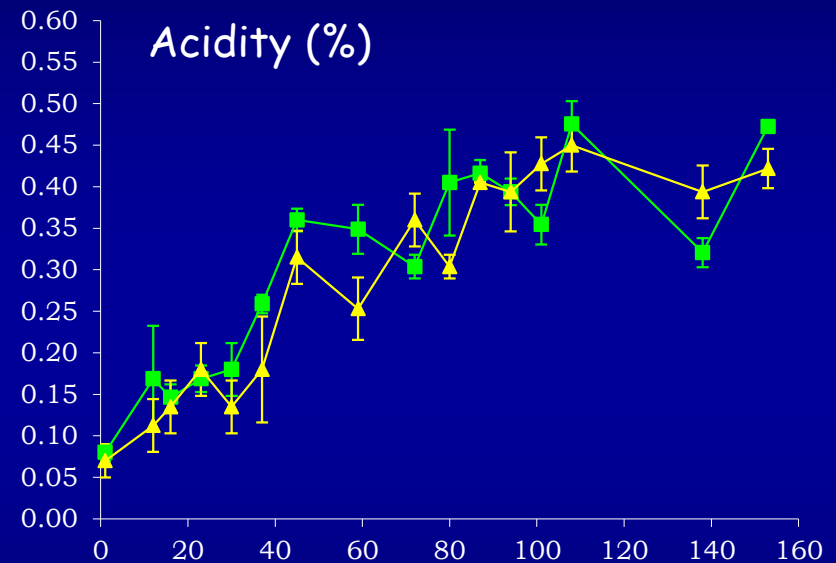
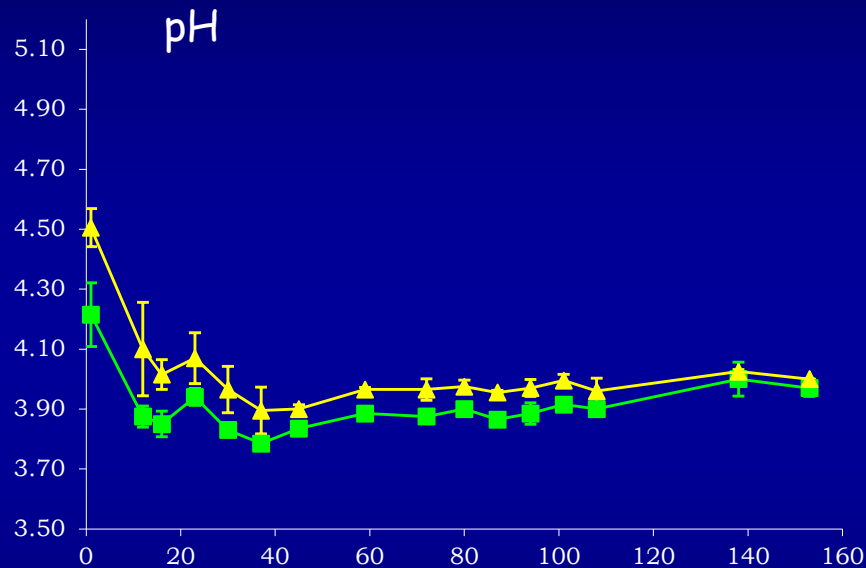
# Fermentation of *Conservolea* black olives using a multifunctional *Lactobacillus pentosus* B281 starter culture



LAB (♦), Yeasts (■), *Enterobacteriaceae* (×)

Grounta, A., Doulgeraki, A., Nychas, G-J.E., Panagou, E.Z. (2016) Biofilm formation on *Conservolea* natural black olives during single and combined inoculation with a functional *Lactobacillus pentosus* starter culture. *Food Microbiology* 56, 35-44.

# Fermentation of *Conservolea* black olives using a multifunctional *Lactobacillus pentosus* starter culture



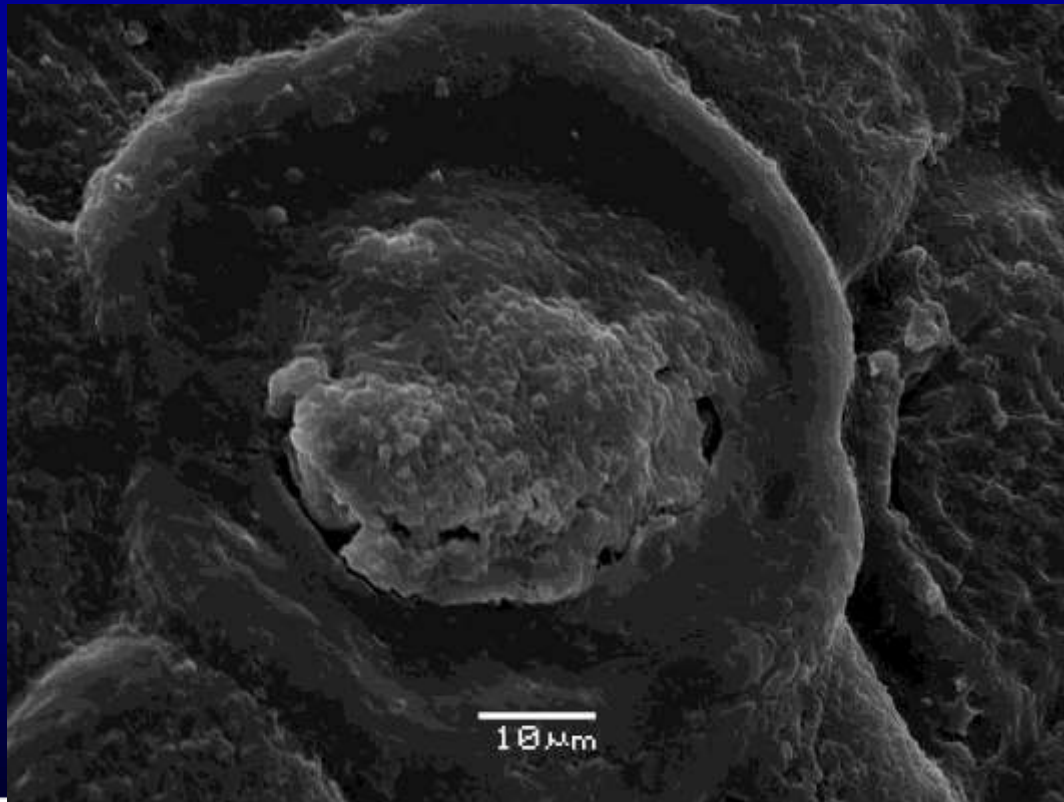
Fermentation time (days)

*L. pentosus* B281 (■), *L. pentosus* B281 και *P. membranifaciens* M3A(▲)

Grounta, A., Doulgeraki, A., Nychas, G-J.E., Panagou, E.Z. (2016) Biofilm formation on *Conservolea* natural black olives during single and combined inoculation with a functional *Lactobacillus pentosus* starter culture. *Food Microbiology* 56, 35-44.

# Survival of inoculated *Lactobacillus pentosus* starter culture

Inoculated process	Fermentation time (days)	<i>L. pentosus</i> B281		<i>P. membranifaciens</i> M3A	
		No of isolates	Survival rate	No of isolates	Survival rate
<i>L. pentosus</i> B281	1	15	15/15 (100%)	—	—
	72	23	23/23 (100%)	—	—
	153	24	24/24 (100%)	—	—
<i>L. pentosus</i> B281 & <i>P. membranifaciens</i> M3A	1	10	10/10 (100%)	22	22/22 (100%)
	72	17	17/17 (100%)	19	8/19 (42%)
	153	17	17/17 (100%)	12	0/12 (0%)



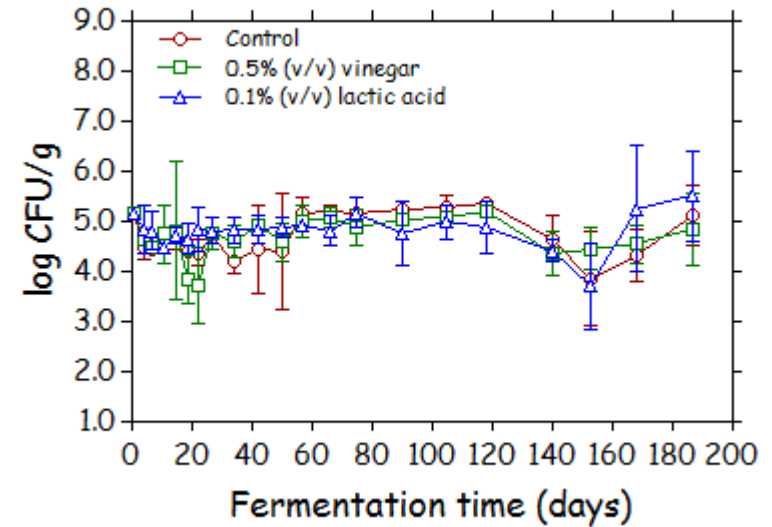
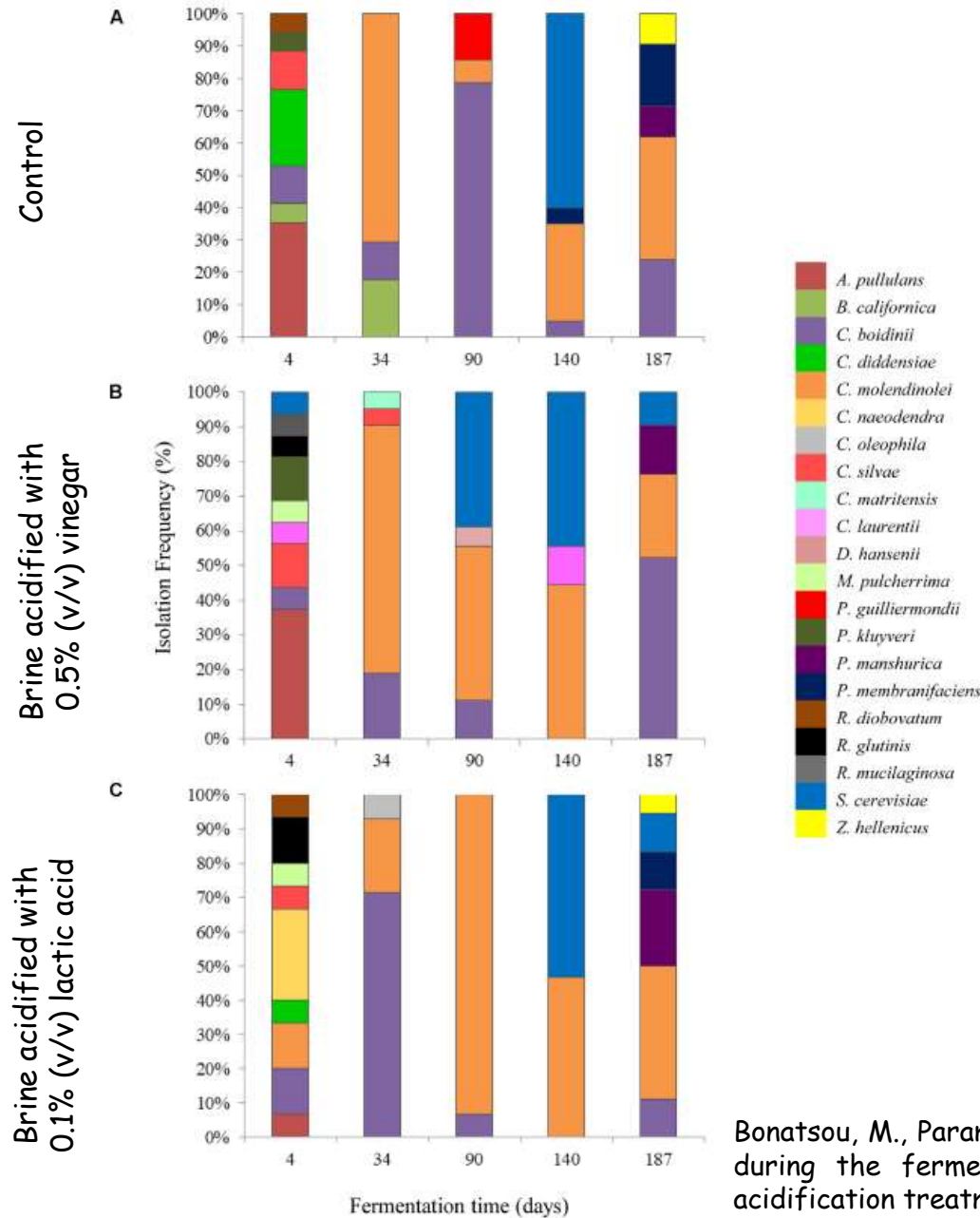
## Yeasts in table olive fermentation

- Arroyo-López, F.N., Querol, A., Bautista-Gallego, J., Garrido-Fernández, A. (2008) Role of yeasts in table olive production. *Int. J. Food Microbiol.* 128, 189-196.
- Arroyo-López, F.N., Romero-Gil, V., Bautista-Gallego, J., Rodríguez-Gómez, F., Jiménez-Díaz, R., García-García, P., Querol, A., Garrido-Fernández, A. (2012) Yeasts in table olive processing: Desirable or spoilage microorganisms? *Int. J. Food Microbiol.* 160, 42-49.
- Bevilacqua, A., Corbo, M.R., Sinigaglia, M. (2012) Selection of yeasts as starter cultures for table olives: a step-by-step procedure. *Front. Microbiol.* 3, 194.

## Yeasts in Greek-style table olives

- Kotzekidou, P. (1997) Identification of yeasts from black olives in rapid system microtitre plates. *Food Microbiol.* 14, 609-616.
- Psani, M., Kotzekidou, P. (2006) Technological characteristics of yeasts strains and their potential as starter adjuncts in Greek-style black olive fermentation. *World J. Microbiol. Biotechnol.* 22, 1329-1336.

# Evolution of yeast consortia during fermentation of Kalamata black olives



## Dominant yeast species at the end of fermentation

- ✓ *Candida boidinii*
- ✓ *Candida molendinolei*
- ✓ *Saccharomyces cerevisiae*
- ✓ *Pichia membranifaciens* (control)
- ✓ *Pichia manshurica* (acidified brines)

Bonatsou, M., Paramithiotis, S., Panagou, E.Z. (2018) Evolution of yeast consortia during the fermentation of Kalamata natural black olives upon two initial acidification treatments. *Frontiers in Microbiology* 8, 2673.

# Probiotic and technological traits of yeasts from Kalamata black olive fermentation

Yeast strain	Number of isolates	Code
<i>Rhodotorula glutinis</i>	3	Y1, Y2, Y3
<i>Citeromyces matrinensis</i>	1	Y4
<i>Pichia kluyveri</i>	2	Y5, Y6
<i>Cystofilobasidium bisporeidii</i>	1	Y7
<i>Candida naeodendra</i>	2	Y8, Y9
<i>Candida diddensiae</i>	3	Y10, Y11, Y12
<i>Metschnikowia pulcherrima</i>	3	Y13, Y14, Y15
<i>Rhodotorula mucilaginosa</i>	1	Y16
<i>Pichia manshurica</i>	3	Y17, Y18, Y19
<i>Pichia guilliermondii</i>	2	Y21, Y22
<i>Zygoascus hellenicus</i>	3	Y23, Y24, Y25
<i>Candida boidinii</i>	6	Y26, Y27, Y28, Y29, Y30, Y31
<i>Saccharomyces cerevisiae</i>	6	Y32, Y33, Y34, Y35, Y36, Y37
<i>Aureobasidium pullulans</i>	6	Y38, Y39, Y40, Y41, Y42, Y43
<i>Candida molendinolei</i>	7	Y44, Y44, Y45, Y46, Y47, Y48, Y49, Y50

## Probiotic potential (49 yeast strains)

- *In vitro* survival in simulated gastric and pancreatic digestions
- Surface adhesion to the intestinal cell line Caco-2
- Hydrophobicity
- Auto-aggregation
- Haemolytic activity

## Technological characteristics (49 yeast strains)

- Enzymatic activity
- Susceptibility to diverse salt levels (0-250 g/L) under different pH values (3.5, 5.0, and 6.5)

Bonatsou, M., Karamouza, M., Zoumpopoulou, G., Mavrogonatou, E., Kletsas, D., Papadimitriou, K., Tsakalidou, E., Nychas, G.-J.E., Panagou, E.Z. (2018) Evaluating the probiotic potential and technological characteristics of yeasts implicated in cv. Kalamata black olive fermentation. *International Journal of Food Microbiology* 271, 48-59.

# Probiotic and technological traits of yeasts from Kalamata black olive fermentation

*M. pulcherima* (Y13)  
*S. cerevisiae* (Y36)  
*A. pullulans* (Y39)

} > 90%

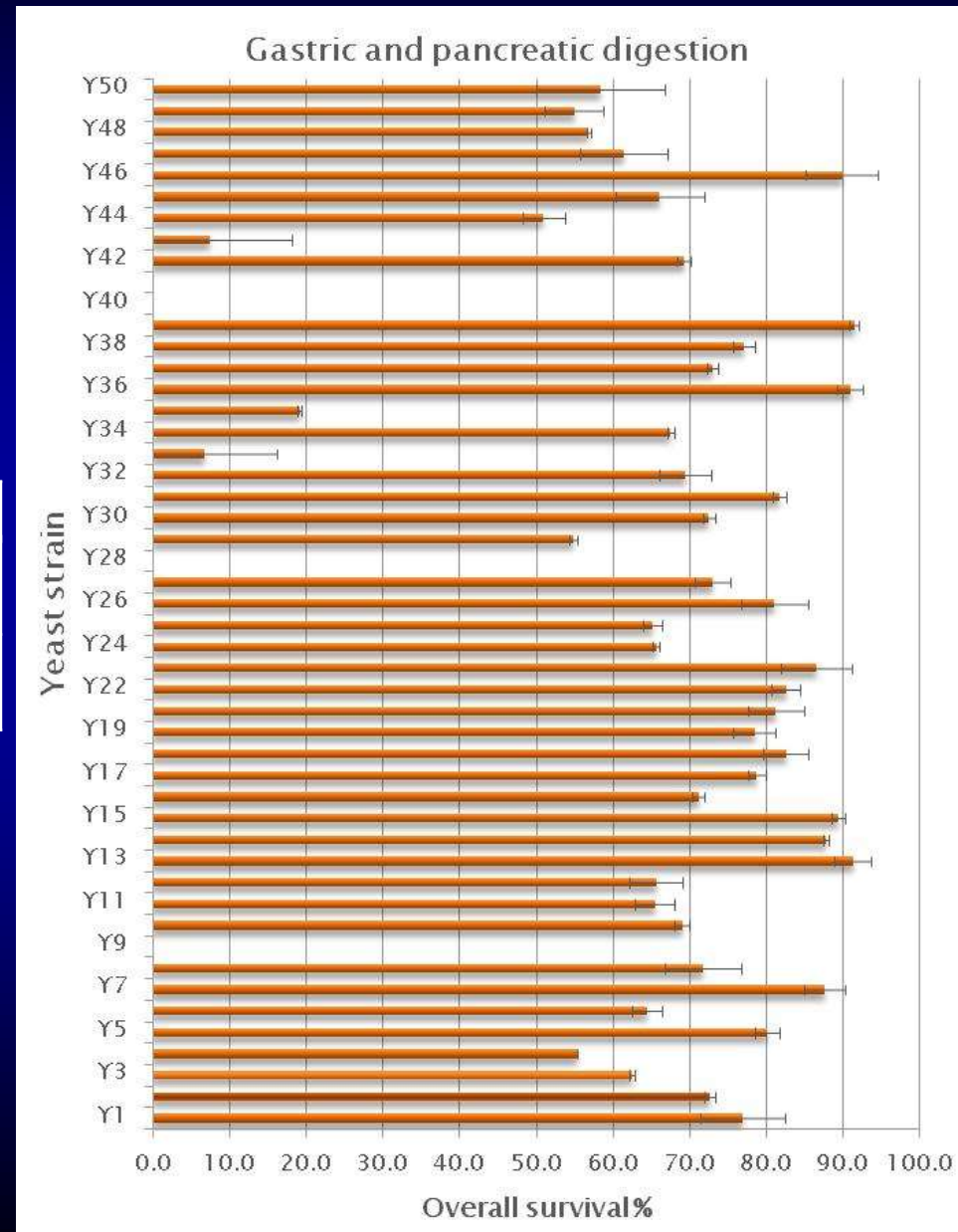
24/49 yeast strains > 70%

## Resistance to salt level

	pH 3.5	pH 5.0	pH 6.5
NIC (g/L)	13.0-102.1	7.1-93.7	11.7-98.1
MIC (g/L)	69.1-207.2	74.2-210.2	88.5-279.0

## The highest tolerance:

- pH 3.5: *S. cerevisiae* (Y33, Y35) and *A. pullulans* (Y39)
- pH 5.0: *C. naeodendra* (Y9) and *P. guilliermondii* (Y21, Y22)
- pH 6.5: *R. glutinis* (Y7), *M. pulcherrima* (Y22), and *S. cerevisiae* (Y37)



# Probiotic and technological traits of yeasts from Kalamata black olive fermentation

Traits	Number of Yeast Strains	Functional Role
Desirable		
Auto-Aggregation Hydrophobicity	All	Contributes to biofilm formation and adhesion ability
Adhesion to <i>Caco2</i> cells	2	Antimicrobial activity against pathogens
Enzymatic Activity		
alkaline phosphatase	11	Cleaves phosphate groups from phosphorylated compounds facilitating transport across membranes and providing the cell with a source of inorganic phosphate at time of phosphate starvation
acid phosphatase	48	Hydrolyzes phosphate esters and liberates phosphate from phytic acid
$\beta$ -glucosidase	27	Bioassimilates oleuropein and leads to the natural debittering of the olives
$\alpha$ -galactosidase	11	Hydrolyzes carbohydrates, which are found in plants, and cannot be metabolized by humans. Thus, prevents from intestinal disturbances
Esterase ( $C_4$ ) Esterase lipase ( $C_8$ )	All	Contributes to overall aroma of the olives by the catabolism of free fatty acids (FFAs) and alcohols of esters which are important flavor precursors of catabolic reactions
Undesirable		
Haemolytic activity	None	

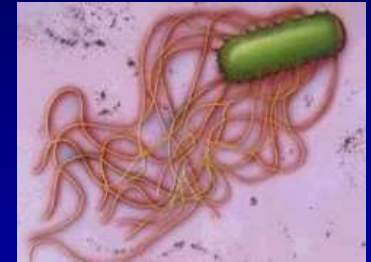
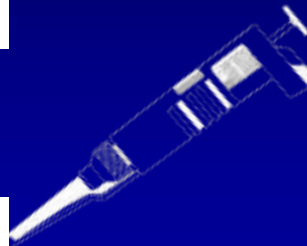


# Safety of natural black table olives



# Survival of pathogens in fermented *Conservolea* black olives

Fermented black olives  
(salt 6.0%, pH 3.95)



Addition of the pathogenic bacteria

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

Storage in air at 4°C and 20°C

Grounta, A., Nychas, G.-J.E., Panagou, E.Z. (2013) Survival of food-borne pathogens on natural black olives after post-processing contamination, *International Journal of Food Microbiology* 161, 197-202.

# 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 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	nd
B-57		2.8 ± 0.4 <sup>b</sup>	nd	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	nd
B-287		4.0 ± 0.5 <sup>Ac</sup>	2.2 ± 0.2 <sup>Bc</sup>	nd	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	nd
B-56	20	4.6 ± 0.4 <sup>Aa</sup>	3.3 ± 0.2 <sup>Ba</sup>	nd	nd	nd	nd	nd	nd	nd
B-57		2.8 ± 0.4 <sup>b</sup>	nd	nd	nd	nd	nd	nd	nd	nd
ATCC 13076		4.1 ± 0.4 <sup>c</sup>	nd	nd	nd	nd	nd	nd	nd	nd
B-287		4.0 ± 0.5 <sup>c</sup>	nd	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	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	nd
B-193		4.3 ± 0.2 <sup>Aa</sup>	3.4 ± 0.1 <sup>Bb</sup>	nd	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	nd
Mixed culture		4.7 ± 0.1 <sup>Aa</sup>	4.9 ± 0.1 <sup>Ac</sup>	nd	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	nd
B-194	4.5 ± 0.4 <sup>Aa</sup>		3.0 ± 0.6 <sup>Ba</sup>	nd	nd	nd	nd	nd	nd	nd
Mixed culture	4.7 ± 0.1 <sup>a</sup>		nd	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 <sup>Aa</sup>	4.9 ± 0.2 <sup>Ba</sup>	nd	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	nd
B-18		4.2 ± 0.1 <sup>Aab</sup>	4.5 ± 0.2 <sup>Aa</sup>	nd	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	nd
B-15	20	3.8 ± 0.2 <sup>a</sup>	nd	nd	nd	nd	nd	nd	nd	nd
B-16		4.3 ± 0.2 <sup>ab</sup>	nd	nd	nd	nd	nd	nd	nd	nd
B-18		4.2 ± 0.1 <sup>ab</sup>	nd	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

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	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	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	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	nd
B-95	20	5.0 ± 0.2 <sup>Aa</sup>	3.5 ± 0.1 <sup>Ba</sup>	nd	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	nd
B-135		5.0 ± 0.2 <sup>Aa</sup>	3.3 ± 0.3 <sup>Ba</sup>	nd	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

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

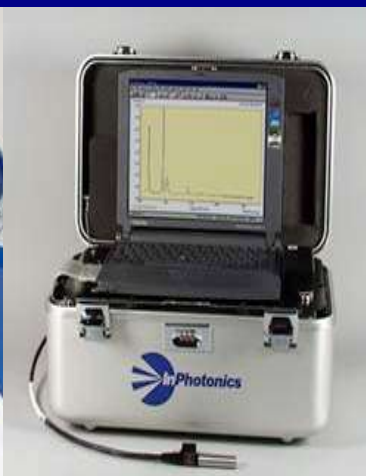
nd: none detected (<2.0 log CFU/g of olives) by direct plating followed by enrichment where absence of the pathogen was observed (<1 CFU/25 g of olives).

+: enrichment positive.

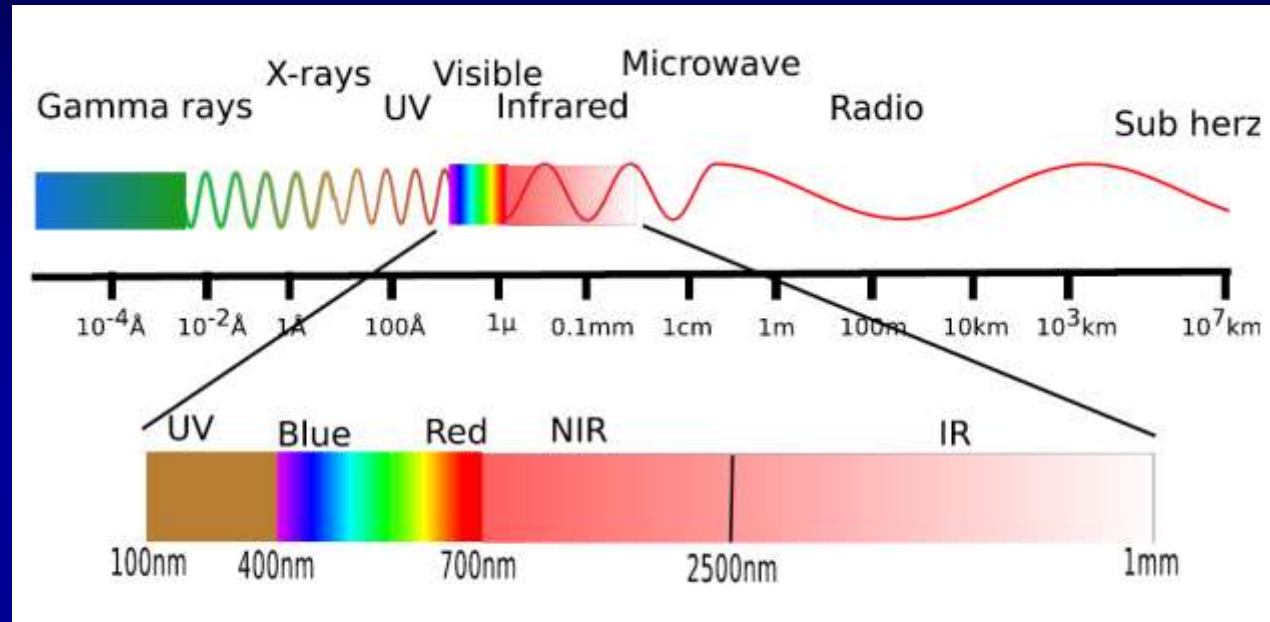
Means with different capital letters in the same row are significantly different ( $P \leq 0.05$ ). Means with different lowercase letters in the same column are significantly different ( $P \leq 0.05$ ).



# Perspectives for rapid and non-invasive determination of table olive quality



# Multispectral imaging of table olives



- When electromagnetic radiation interacts with an object some frequencies are absorbed and some are reflected.
- The reflection depends on the chemistry of the surface of the object it interacts with.

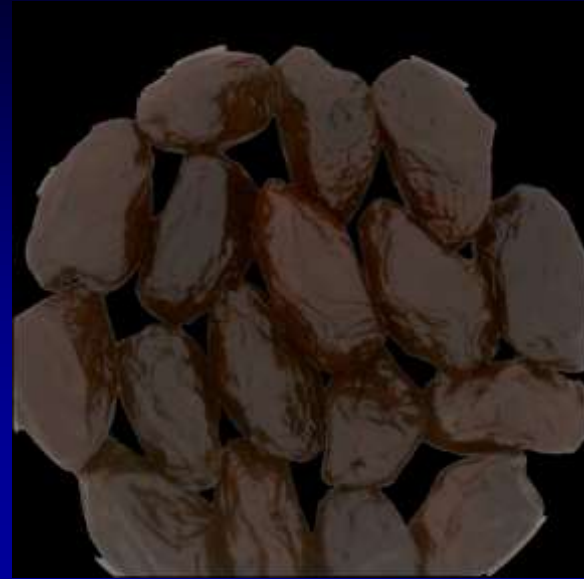
# VideometerLab



Wavelength (nm)	Color	Compound
405	UV	Fluorescence
435	UB	Chlorophyll A
450	Blue	Riboflavin, Chlorophyll B, Beta-carotene
470	Blue	RGB blue
505	Blue-Green	RGB green, Metmyoglobin
525	Green	RGB green
570	Green	Myoglobin
590	Yellow	Oxymyoglobin
630	Red	RGB red, Metmyoglobin (weak)
645	Red	Chlorophyll B
660	Red	Oxidation, Chlorophyll A
700	Red	Oxidation
850	NIR	Baseline
870	NIR	Baseline
890	NIR	Fat shoulder (instauration)
910	NIR	Protein (C-H)
940	NIR	Fat
970	NIR	Water

# Raw and segmented images of dry-salted cv. Thassos olives

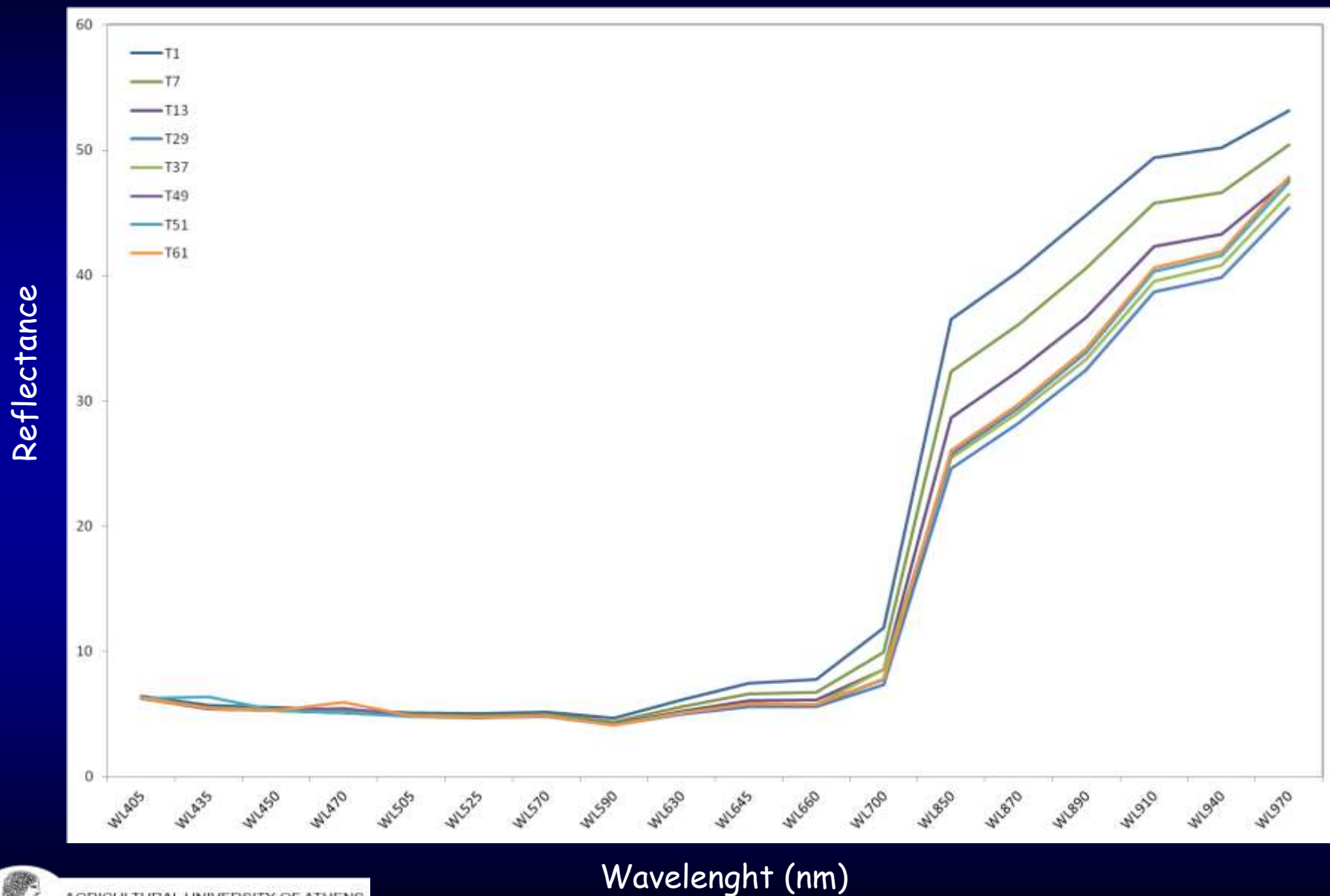
t= 0 days



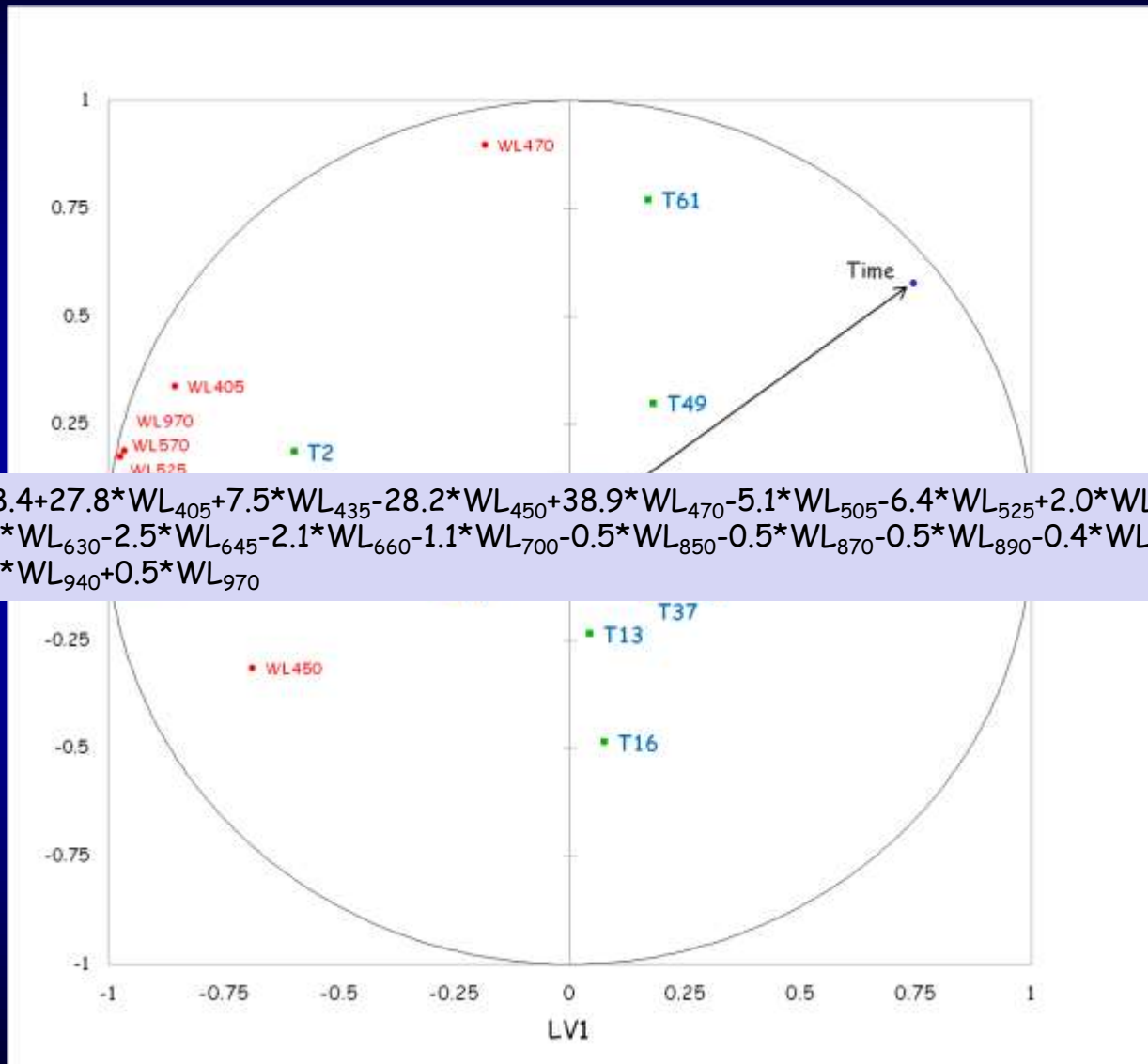
t= 60 days



# Raw and segmented images of dry-salted cv. Thassos olives

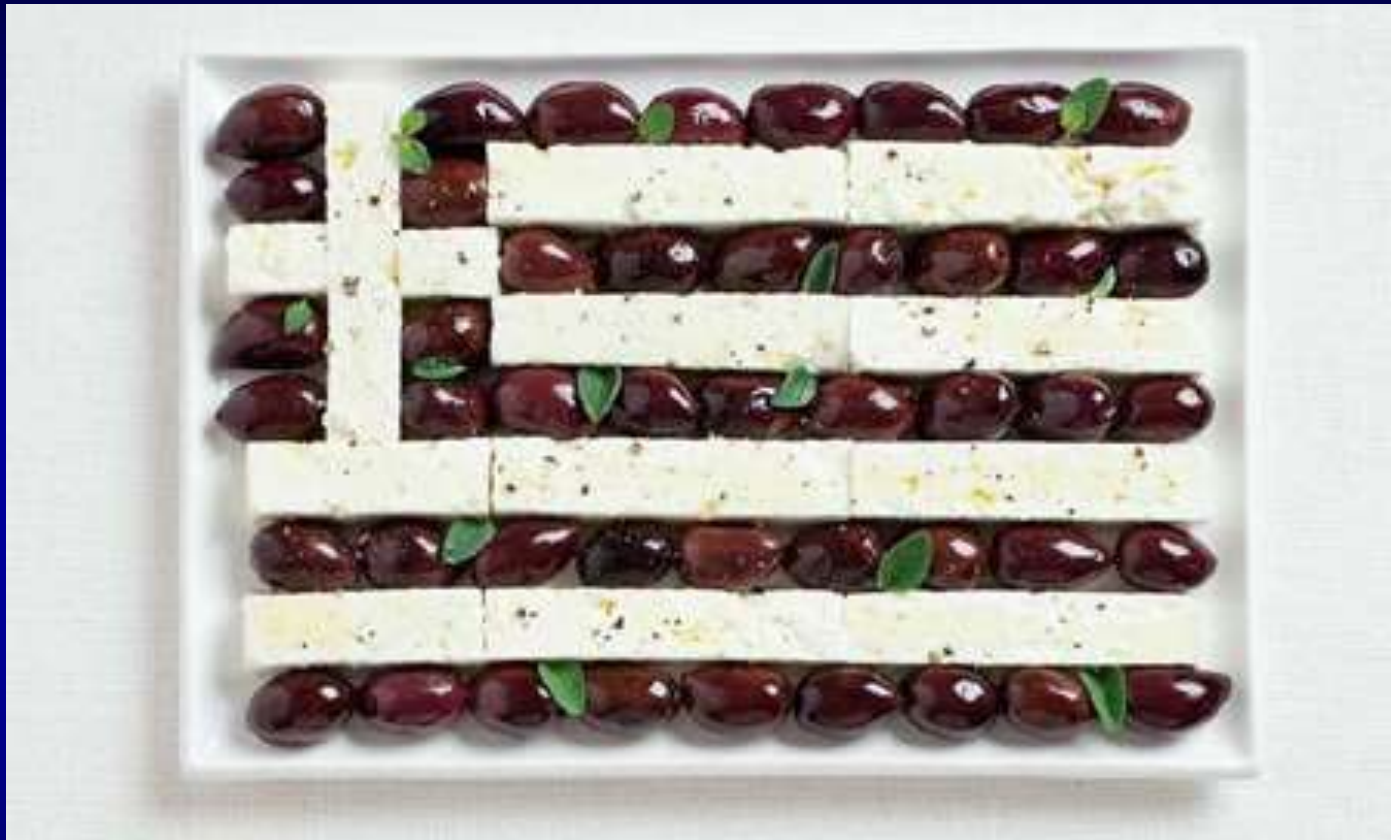


# Multispectral image analysis of dry-salted cv.Thassos olives (PLS-R)



$$\text{Storage time} = -48.4 + 27.8 \cdot \text{WL}_{405} + 7.5 \cdot \text{WL}_{435} - 28.2 \cdot \text{WL}_{450} + 38.9 \cdot \text{WL}_{470} - 5.1 \cdot \text{WL}_{505} - 6.4 \cdot \text{WL}_{525} + 2.0 \cdot \text{WL}_{570} - 8.5 \cdot \text{WL}_{590} - 3.2 \cdot \text{WL}_{630} - 2.5 \cdot \text{WL}_{645} - 2.1 \cdot \text{WL}_{660} - 1.1 \cdot \text{WL}_{700} - 0.5 \cdot \text{WL}_{850} - 0.5 \cdot \text{WL}_{870} - 0.5 \cdot \text{WL}_{890} - 0.4 \cdot \text{WL}_{910} - 0.3 \cdot \text{WL}_{940} + 0.5 \cdot \text{WL}_{970}$$





**Thank you for your attention**

