

# **Prove di fermentazione controllata di olive da tavola (*Olea europaea* L.) cv. *Leccino* con colture starter di ceppi selezionati di batteri lattici (LAB) e lieviti killer**

**Grazia Federica Bencresciuto, Claudio Mandalà, Carmela Anna Migliori e Laura Bardi**

***CREA-IT, Torino***

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Le olive da tavola sono ricche in composti fenolici con proprietà antiossidanti, antinfiammatorie, antibatteriche.



Oleuropeina e ligstroside  $\xrightarrow[\text{Esterasi}]{\beta\text{-glucosidasi}}$  Acido elenoico, idrossitirosolo (3,4 DHPEA) e tirosolo (p-HPEA)

### *Popolazioni microbiche autoctone coinvolte nella fermentazione naturale*

#### Lattobacilli

*L. plantarum*, *L. pentosus*,  
*L. paracasei*, *L. casei* e  
*L. paraplantarum*



- deamarizzazione
- acidificazione salamoia
- diminuzione pH
- prolungata shelf-life

#### Lieviti

*D. hansenii*,  
*T. delbrueckii*, *K. lactis* e  
*S. cerevisiae*



- deamarizzazione
- proprietà organolettiche
- favoriscono LAB

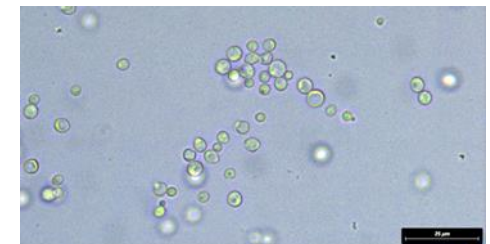
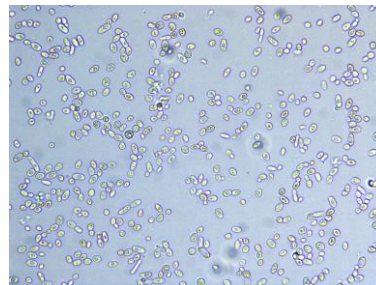
## Impiego colture starter


- Abbreviare i tempi di fermentazione
- Standardizzare il processo
- Migliorare le proprietà organolettiche e nutrizionali
- Prevengono spoilage

*Lactobacillus plantarum*  
*Lactobacillus pentosus*


*Wickerhamomyces anomalus*  
*Saccharomyces cerevisiae*

Capacità Killer





*Wickerhamomyces  
anomalus*



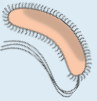
*Lactobacillus  
plantarum*

Consociazione

Successione



*Saccharomyces  
cerevisiae*



*Lactobacillus  
plantarum*

Consociazione

Successione



Tesi a confronto con fermentazione spontanea



- ✓ Deamarizzazione delle olive
- ✓ Ottenere un prodotto finale evitando pretrattamenti, sale eccessivo e ulteriori additivi e conservanti

## Schema sperimentale

### Tesi:

- (i) fermentazione spontanea (SP)
- (ii) *L. plantarum* (LP)
- (iii) *W. anomalus* (WA)
- (iv) consociazione *L. plantarum* + *S. cerevisiae* (MIX1)
- (v) successione *L. plantarum* + *S. cerevisiae* (LY1)
- (vi) consociazione *L. plantarum* + *W. anomalus* (MIX2)
- (vii) successione di *L. plantarum* + *W. anomalus* (LY2)

### Ceppi:

#### • *L. plantarum* B51:

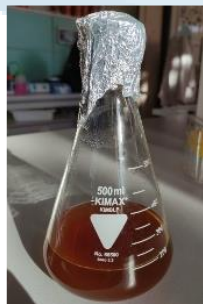
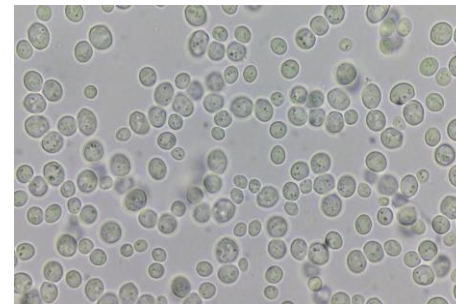
Collezione di Microrganismi della sede CREA-IT Pescara, unica detentrica.

#### • *S. cerevisiae* Sc24 e *W. anomalus* Wa1:

Collezione di Microrganismi dell'Università degli Studi di Sassari, unica detentrica.

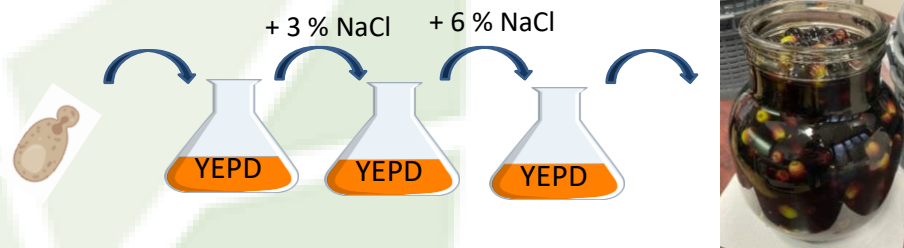


Olive cv. *Leccino*



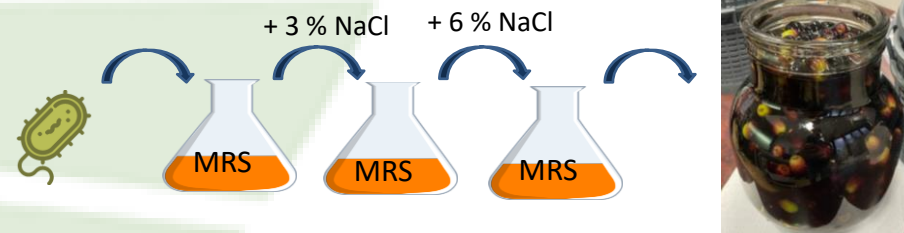
## Adattamento delle precolture in NaCl

Precoltura di *S. cerevisiae* Sc24 e *W. anomalus* Wa1



**Messa in salamoia**  
(8 %NaCl),  $10^6$  CFU/mL

Precoltura *L. plantarum* B51



**Messa in salamoia**  
(8 %NaCl),  $10^6$  CFU/mL

### Condizioni di fermentazione

- 2,5 kg di olive in damigiane sterili con 2 L di salamoia sterile (8% NaCl)
- Tesi in triplicato
- T ambiente: 19°C-25°C
- 188 giorni

## Campionamenti



Salamoia

Olive

Analisi  
microbiologiche

- Lattobacilli
- Lieviti e muffe
- Enterobatteri

Profilo chimico

- Polifenoli
- Zuccheri fermentescibili
- Acidi organici

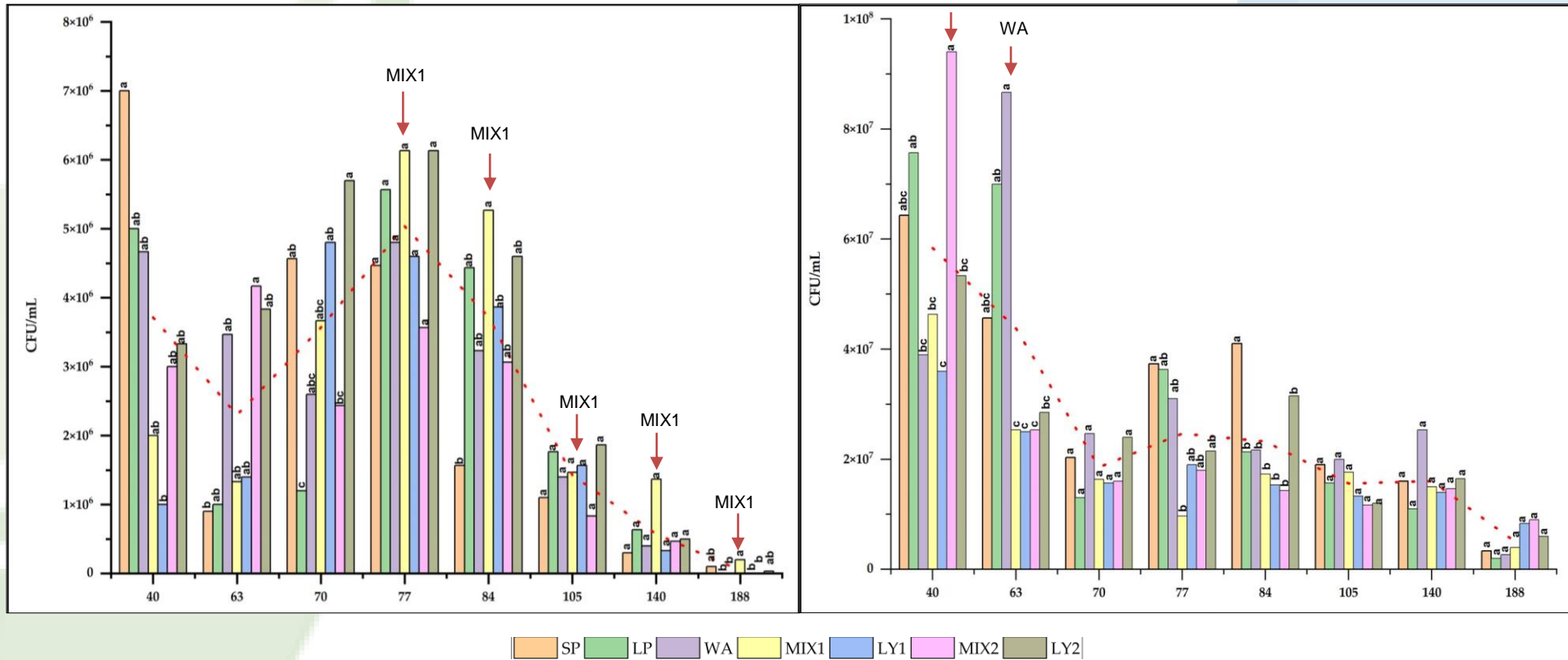
Profilo chimico

- Polifenoli
- Zuccheri fermentescibili
- Acidi organici



## Lieviti

## Lattobacilli

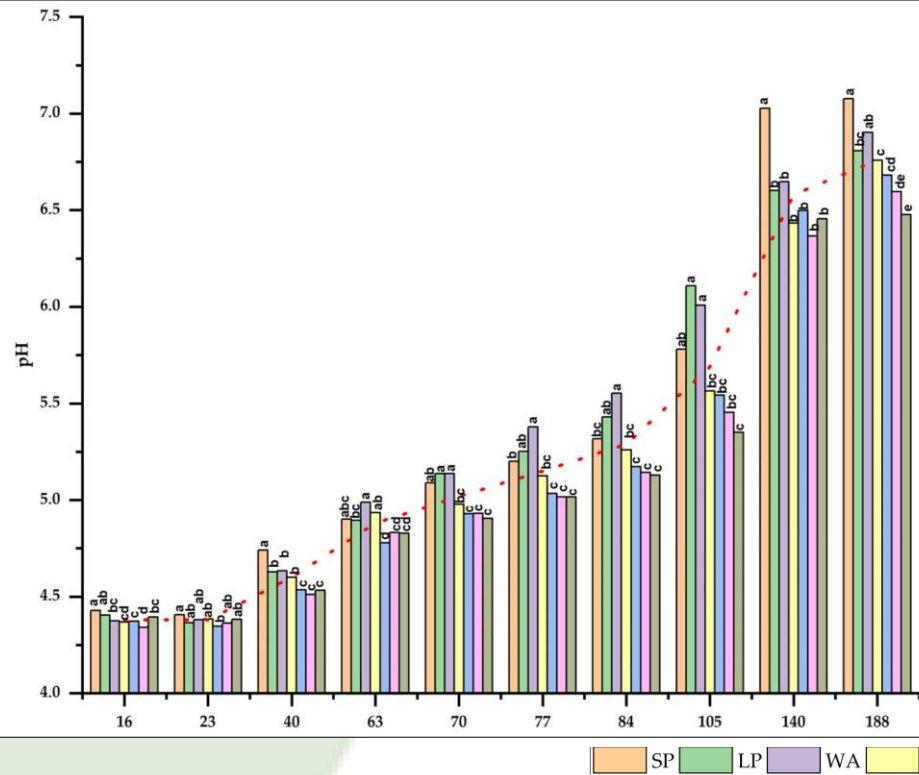


Migliore sinergia tra *S. cerevisiae* e LAB  
(MIX1)

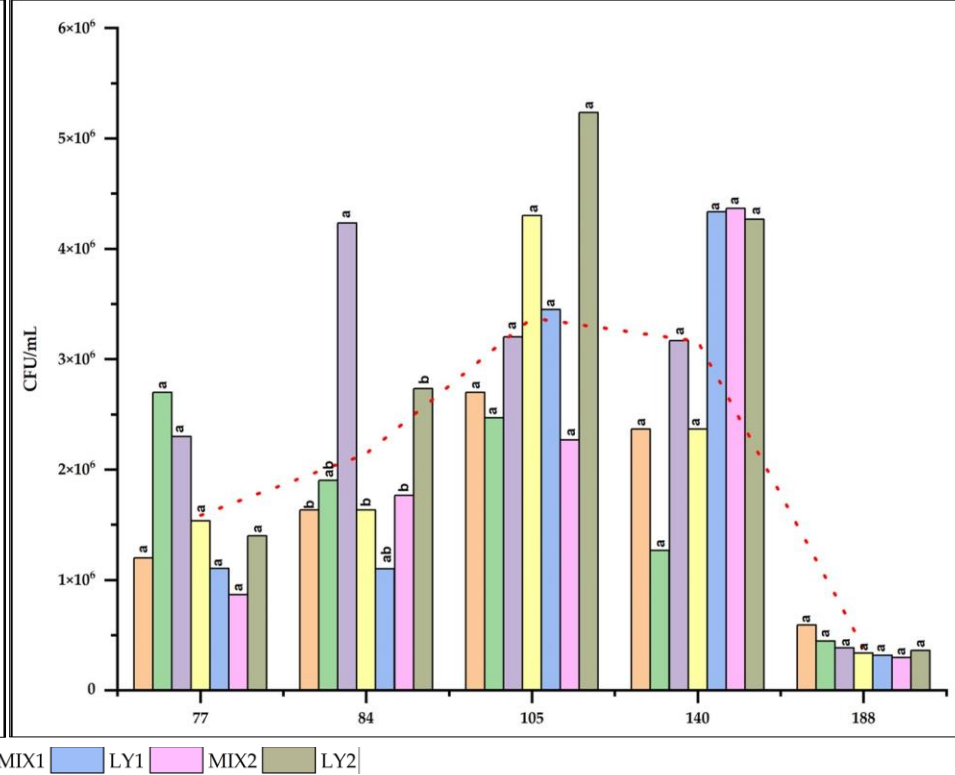
Effetto positivo di *W. anomalus* in MIX2 e WA



## pH



## Enterobatteri



Differenze significative tra tesi inoculate e fermentazione spontanea

Sviluppo anomalo di enterobatteri associabile a un andamento insolito di pH

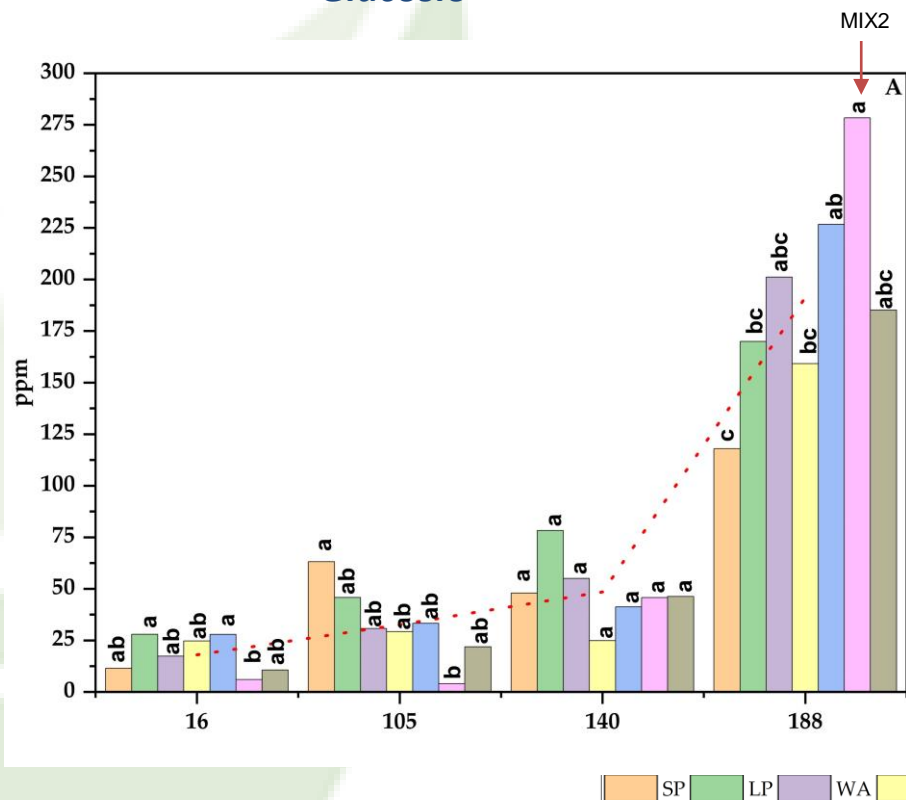
Olive grezze: glucosio rappresentava lo zucchero più abbondante (9477.74 ppm) seguito dal fruttosio (615.80 ppm). Tra i polialcoli, il mannitolo concentrazione più alta (3619.97 ppm), sorbitolo molto inferiore (92.41 ppm).

## Glucosio e fruttosio in polpa

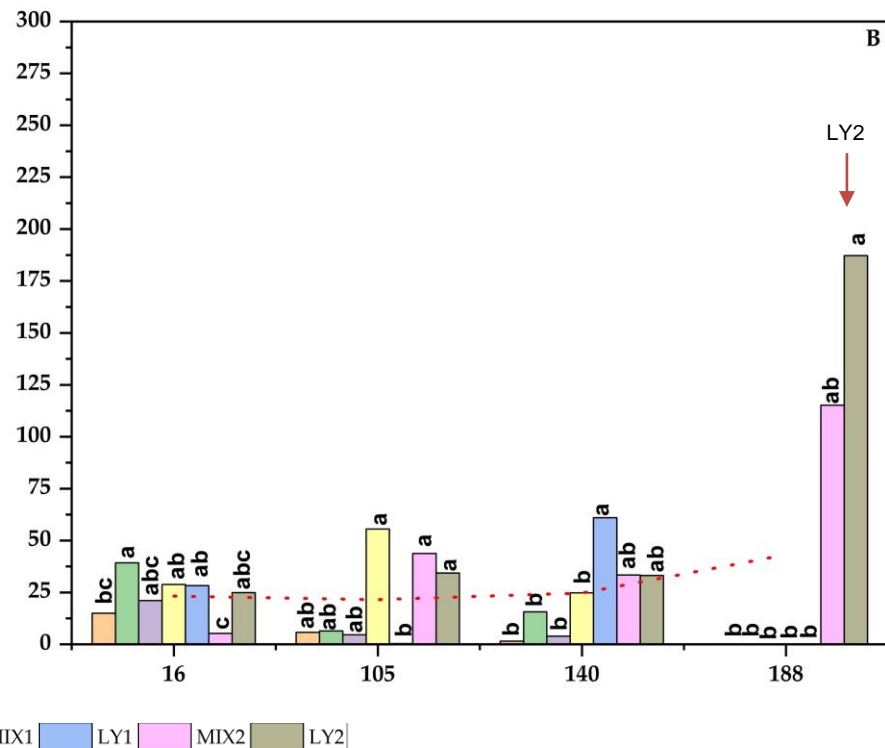
Giorno	23	40	105	140	188
<b>Tesi</b>	<b>Glucosio (ppm)</b>				
SP	1336.53 ± 30.22 <sub>f</sub>	264.96 ± 5.08 <sub>c</sub>	5.14 ± 4.06 <sub>ab</sub>	225.35 ± 64.80 <sub>a</sub>	0.00 ± 0.00 <sub>b</sub>
LP	2678.28 ± 89.35 <sub>ab</sub>	589.65 ± 138.75 <sub>b</sub>	0.73 ± 0.73 <sub>b</sub>	20.60 ± 1.27 <sub>b</sub>	0.00 ± 0.00 <sub>b</sub>
WA	2370.15 ± 74.86 <sub>cd</sub>	886.36 ± 209.44 <sub>a</sub>	0.00 ± 0.00 <sub>b</sub>	18.90 ± 3.25 <sub>b</sub>	17.17 ± 7.52 <sub>a</sub>
MIX1	2091.05 ± 104.84 <sub>e</sub>	231.74 ± 17.91 <sub>c</sub>	0.00 ± 0.00 <sub>b</sub>	12.64 ± 0.94 <sub>b</sub>	9.27 ± 2.12 <sub>ab</sub>
LY1	2730.10 ± 38.10 <sub>a</sub>	172.82 ± 7.96 <sub>c</sub>	0.00 ± 0.00 <sub>b</sub>	4.93 ± 0.72 <sub>b</sub>	3.80 ± 0.55 <sub>b</sub>
MIX2	2472.06 ± 34.42 <sub>bc</sub>	217.64 ± 18.48 <sub>c</sub>	1.38 ± 0.20 <sub>b</sub>	0.00 ± 0.00 <sub>b</sub>	0.00 ± 0.00 <sub>b</sub>
LY2	2204.15 ± 108.27 <sub>de</sub>	212.25 ± 3.30 <sub>c</sub>	10.17 ± 4.36 <sub>a</sub>	2.54 ± 0.37 <sub>b</sub>	0.00 ± 0.00 <sub>b</sub>
	<b>Fruttosio (ppm)</b>				
SP	425.31 ± 11.99 <sub>ab</sub>	234.09 ± 3.06 <sub>a</sub>	1.05 ± 0.87 <sub>b</sub>	37.69 ± 14.64 <sub>a</sub>	0.00 ± 0.00 <sub>c</sub>
LP	366.63 ± 33.94 <sub>ab</sub>	377.97 ± 52.56 <sub>a</sub>	5.62 ± 0.87 <sub>b</sub>	1.92 ± 0.56 <sub>b</sub>	0.00 ± 0.00 <sub>c</sub>
WA	379.57 ± 13.07 <sub>ab</sub>	248.65 ± 114.49 <sub>a</sub>	0.00 ± 0.00 <sub>b</sub>	0.00 ± 0.00 <sub>b</sub>	17.52 ± 2.73 <sub>b</sub>
MIX1	468.63 ± 59.45 <sub>a</sub>	60.56 ± 7.77 <sub>b</sub>	0.00 ± 0.00 <sub>b</sub>	3.29 ± 0.73 <sub>b</sub>	44.27 ± 3.28 <sub>a</sub>
LY1	374.99 ± 7.21 <sub>ab</sub>	83.75 ± 9.93 <sub>b</sub>	0.00 ± 0.00 <sub>b</sub>	2.91 ± 0.69 <sub>b</sub>	6.24 ± 2.15 <sub>c</sub>
MIX2	359.85 ± 12.16 <sub>ab</sub>	42.41 ± 20.16 <sub>b</sub>	21.93 ± 6.53 <sub>b</sub>	12.01 ± 4.14 <sub>b</sub>	0.00 ± 0.00 <sub>c</sub>
LY2	287.43 ± 118.51 <sub>b</sub>	53.84 ± 8.28 <sub>b</sub>	63.09 ± 19.98 <sub>a</sub>	20.09 ± 10.27 <sub>ab</sub>	0.00 ± 0.00 <sub>c</sub>

## Zuccheri in salamoia

### Glucosio



### Fruttosio



Glucosio e fruttosio sono stati trovati in concentrazioni più elevate durante l'ultimo campionamento rispettivamente nelle tesi **MIX2** e **LY2**.

## Mannitolo ed etanolo in polpa

Giorno	23	40	105	140	188
<b>Tesi</b>	<b>Mannitolo (ppm)</b>				
SP	2841.82 ± 184.23 <sub>bc</sub>	330.47 ± 15.47 <sub>b</sub>	1.03 ± 0.52 <sub>a</sub>	107.09 ± 13.69 <sub>b</sub>	66.47 ± 20.02 <sub>b</sub>
LP	2721.22 ± 443.95 <sub>c</sub>	881.01 ± 406.13 <sub>a</sub>	0.00 ± 0.00 <sub>b</sub>	0.00 ± 0.00 <sub>d</sub>	666.53 ± 20.87 <sub>a</sub>
WA	4248.15 ± 117.43 <sub>a</sub>	533.65 ± 12.23 <sub>b<sub>ab</sub></sub>	0.00 ± 0.00 <sub>b</sub>	0.00 ± 0.00 <sub>d</sub>	608.07 ± 111.80 <sub>a</sub>
MIX1	4205.16 ± 192.19 <sub>a</sub>	613.97 ± 89.47 <sub>ab</sub>	0.00 ± 0.00 <sub>b</sub>	99.60 ± 10.39 <sub>b</sub>	123.15 ± 16.39 <sub>b</sub>
LY1	3505.10 ± 184.46 <sub>ab</sub>	460.93 ± 31.92 <sub>ab</sub>	0.00 ± 0.00 <sub>b</sub>	66.89 ± 5.74 <sub>c</sub>	103.76 ± 19.62 <sub>b</sub>
MIX2	3146.29 ± 29.75 <sub>bc</sub>	445.93 ± 51.91 <sub>ab</sub>	0.00 ± 0.00 <sub>b</sub>	166.02 ± 6.45 <sub>a</sub>	0.00 ± 0.00 <sub>b</sub>
LY2	2539.49 ± 361.20 <sub>c</sub>	466.11 ± 72.69 <sub>ab</sub>	0.00 ± 0.00 <sub>b</sub>	183.03 ± 9.68 <sub>a</sub>	43.10 ± 7.92 <sub>b</sub>
	<b>Etanolo (ppm)</b>				
SP	0.00 ± 0.00	0.00 ± 0.00	467.75 ± 132.03 <sub>d</sub>	0.00 ± 0.00	0.00 ± 0.00 <sub>b</sub>
LP	0.00 ± 0.00	0.00 ± 0.00	822.96 ± 32.33 <sub>ab</sub>	0.00 ± 0.00	0.00 ± 0.00 <sub>b</sub>
WA	0.00 ± 0.00	0.00 ± 0.00	851.71 ± 78.11 <sub>a</sub>	0.00 ± 0.00	0.00 ± 0.00 <sub>b</sub>
MIX1	0.00 ± 0.00	0.00 ± 0.00	598.26 ± 50.76 <sub>bcd</sub>	0.00 ± 0.00	0.00 ± 0.00 <sub>b</sub>
LY1	0.00 ± 0.00	0.00 ± 0.00	773.19 ± 40.86 <sub>abc</sub>	0.00 ± 0.00	0.00 ± 0.00 <sub>b</sub>
MIX2	0.00 ± 0.00	0.00 ± 0.00	576.26 ± 23.88 <sub>cd</sub>	0.00 ± 0.00	41.49 ± 5.33 <sub>a</sub>
LY2	0.00 ± 0.00	0.00 ± 0.00	535.13 ± 102.12 <sub>d</sub>	0.00 ± 0.00	0.00 ± 0.00 <sub>b</sub>

Azzeramento mannitolo  
Comparsa etanolo

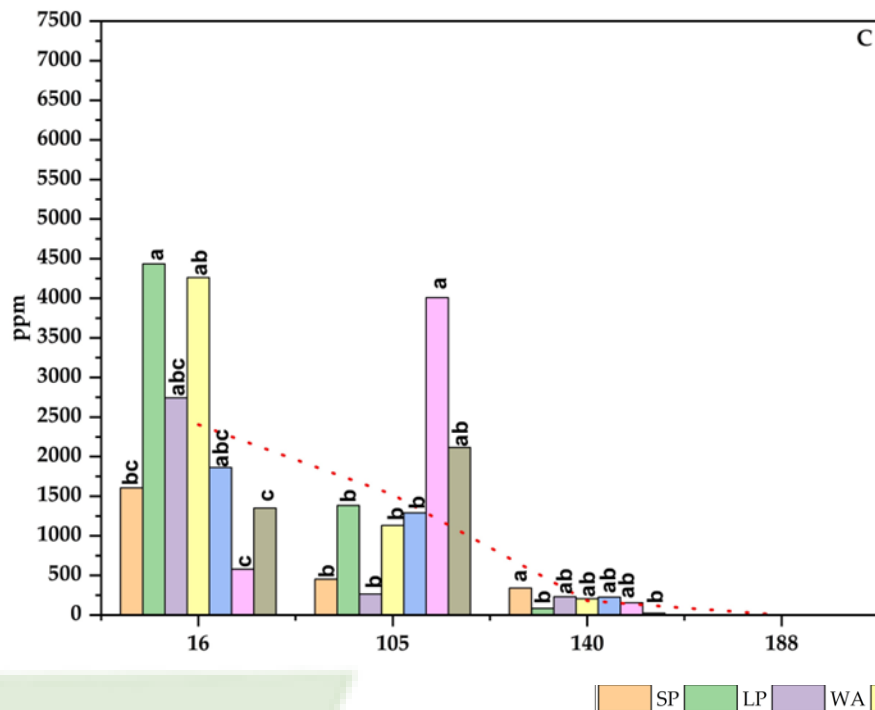
**Giorno 105**



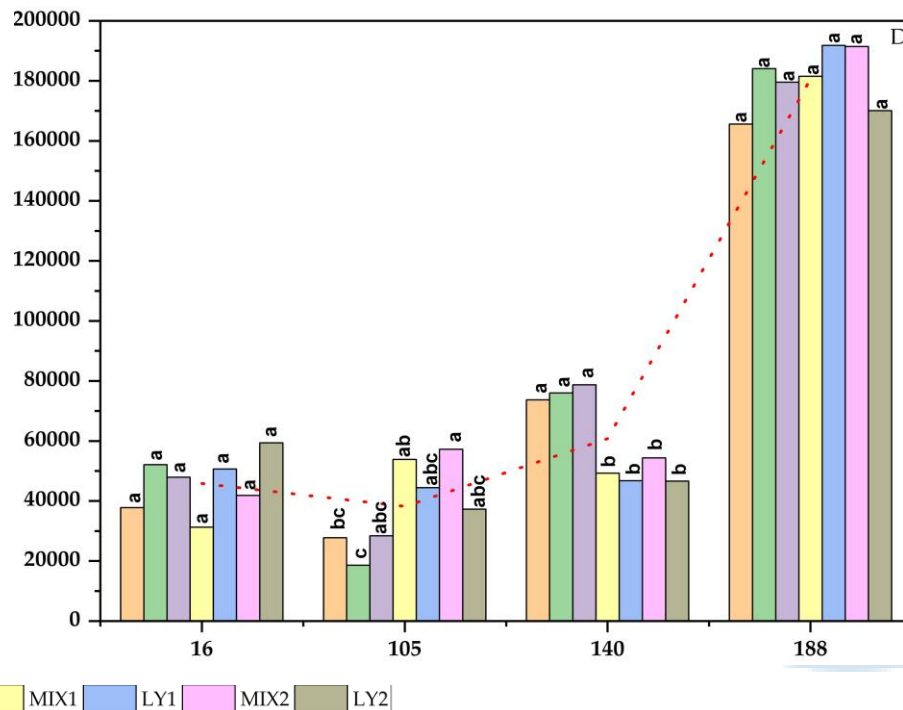
Enterobatteri, lieviti,  
usano mannitolo

## Mannitolo ed etanolo in salamoia

### Mannitolo



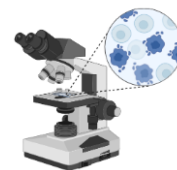
### Etanolo



**Mannitolo:** elevate concentrazioni associabili a presenza di enterobatteri e muffe in aumento dal giorno 63, tra cui *Fusarium spp.*

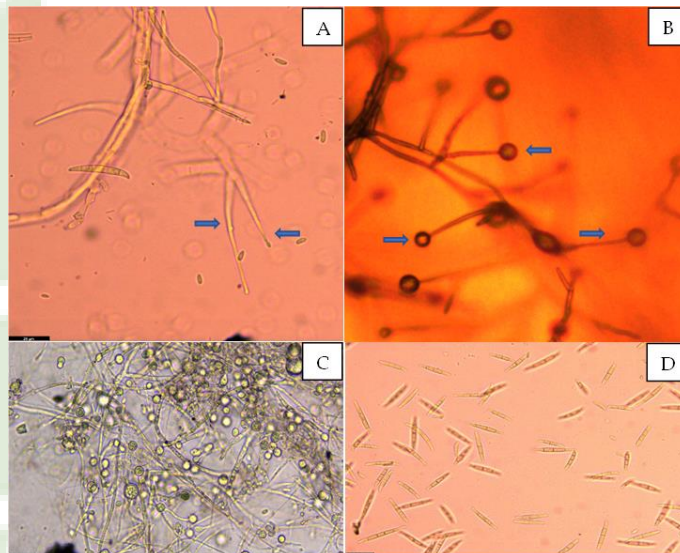
**Etanolo:** aumento crescente in salamoia, associabile a enterobatteri e muffe

## Identificazione *Fusarium solani*



### Morfologia di *Fusarium solani*

- A: Monofialidi lunghi
- B: False teste del micelio
- C: Clamidospore prodotte in PDA
- D: Macroconidi da sporodochi



### Valori texture

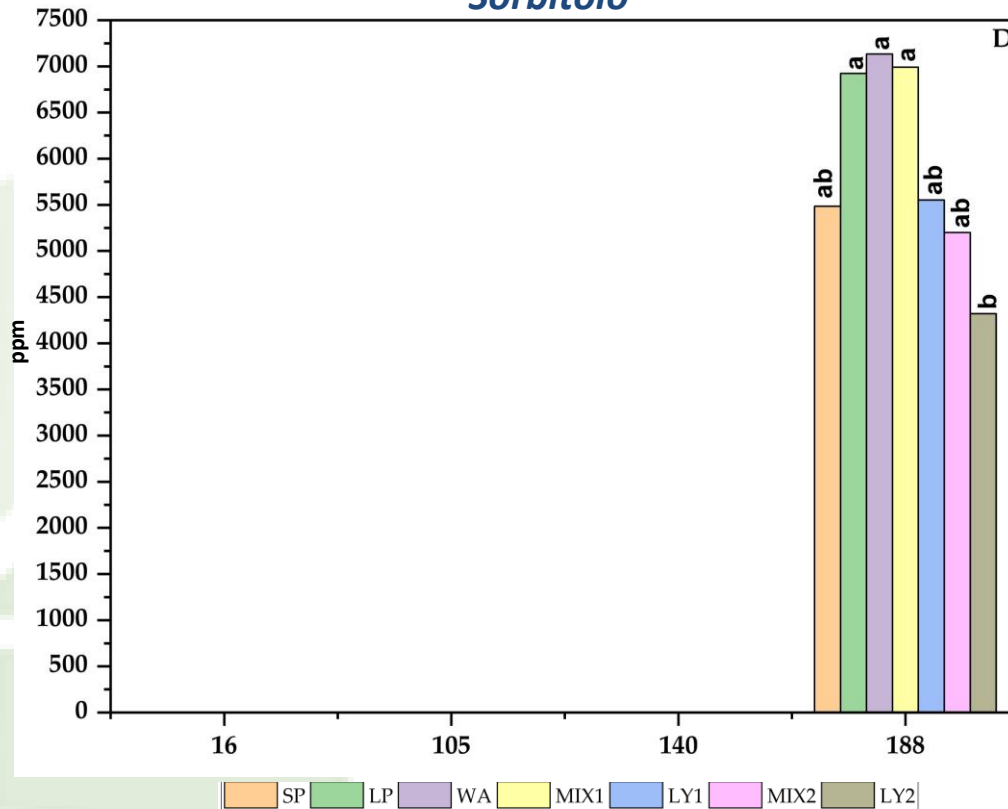
Treatments	Hardness (g)	Springiness (mm)	Cohesiveness	Gumminess (g)	Chewiness (g)					
SP	286.35 ± 11.47	ab	0.66 ± 0.01	ab	0.50 ± 0.01	a	142.46 ± 5.45	abc	96.79 ± 4.79	bc
LP	315.20 ± 17.71	ab	0.67 ± 0.01	ab	0.50 ± 0.01	a	155.40 ± 8.30	ab	126.90 ± 6.43	a
WA	334.29 ± 12.26	ab	0.66 ± 0.01	ab	0.48 ± 0.01	a	158.83 ± 5.65	ab	107.31 ± 4.31	abc
MIX1	337.11 ± 10.55	a	0.69 ± 0.01	a	0.48 ± 0.01	a	162.80 ± 5.14	a	116.23 ± 3.90	ab
LY1	321.51 ± 13.74	ab	0.69 ± 0.01	a	0.48 ± 0.01	a	155.81 ± 6.29	abc	115.08 ± 4.54	ab
MIX2	277.62 ± 11.61	b	0.61 ± 0.01	c	0.48 ± 0.01	a	130.37 ± 5.39	c	81.52 ± 4.04	c
LY2	314.41 ± 23.30	ab	0.65 ± 0.01	cb	0.47 ± 0.01	a	134.42 ± 5.75	bc	101.06 ± 6.45	abc

**MIX1** maggiori valori di hardness e gumminess

Giorno	23	40	105	140	188
<b>Sorbitolo (ppm)</b>					
SP	37.29 ± 13.06 <sub>ab</sub>	125.09 ± 3.96 <sub>bcd</sub>	0.13 ± 0.08 <sub>a</sub>	808.75 ± 39.18 <sub>a</sub>	90.20 ± 9.68 <sub>c</sub>
LP	27.99 ± 5.01 <sub>ab</sub>	97.59 ± 0.44 <sub>cd</sub>	0.00 ± 0.00 <sub>b</sub>	321.37 ± 41.25 <sub>bc</sub>	46.20 ± 5.41 <sub>d</sub>
WA	36.42 ± 5.99 <sub>ab</sub>	59.67 ± 27.49 <sub>d</sub>	0.00 ± 0.00 <sub>b</sub>	402.61 ± 54.24 <sub>bc</sub>	113.06 ± 0.20 <sub>b</sub>
MIX1	66.90 ± 16.76 <sub>a</sub>	211.87 ± 39.55 <sub>b</sub>	0.00 ± 0.00 <sub>b</sub>	235.62 ± 75.17 <sub>c</sub>	198.39 ± 8.32 <sub>a</sub>
LY1	18.58 ± 7.61 <sub>b</sub>	201.85 ± 10.38 <sub>bc</sub>	0.00 ± 0.00 <sub>b</sub>	432.52 ± 46.01 <sub>b</sub>	0.00 ± 0.00 <sub>e</sub>
MIX2	16.25 ± 2.69 <sub>b</sub>	337.44 ± 84.51 <sub>a</sub>	0.00 ± 0.00 <sub>b</sub>	290.22 ± 88.32 <sub>bc</sub>	16.42 ± 4.77 <sub>e</sub>
LY2	41.03 ± 26.42 <sub>ab</sub>	177.08 ± 8.37 <sub>bc</sub>	0.00 ± 0.00 <sub>b</sub>	479.53 ± 86.09 <sub>b</sub>	10.22 ± 4.86 <sub>e</sub>
<b>Glicerolo (ppm)</b>					
SP	0.00 ± 0.00	0.00 ± 0.00 <sub>c</sub>	0.00 ± 0.00	1342.62 ± 287.81 <sub>a</sub>	2362.26 ± 496.59 <sub>a</sub>
LP	0.00 ± 0.00	0.00 ± 0.00 <sub>c</sub>	0.00 ± 0.00	1331.19 ± 52.87 <sub>a</sub>	1487.78 ± 150.80 <sub>bc</sub>
WA	0.00 ± 0.00	470.96 ± 156.54 <sub>b</sub>	0.00 ± 0.00	1353.50 ± 156.54 <sub>a</sub>	950.54 ± 137.67 <sub>cd</sub>
MIX1	0.00 ± 0.00	740.64 ± 228.03 <sub>b</sub>	0.00 ± 0.00	1726.35 ± 49.04 <sub>a</sub>	1792.01 ± 77.12 <sub>ab</sub>
LY1	0.00 ± 0.00	880.76 ± 74.01 <sub>ab</sub>	0.00 ± 0.00	1681.73 ± 207.55 <sub>a</sub>	450.85 ± 32.96 <sub>de</sub>
MIX2	0.00 ± 0.00	1221.38 ± 274.93 <sub>a</sub>	0.00 ± 0.00	1345.29 ± 137.99 <sub>a</sub>	0.00 ± 0.00 <sub>e</sub>
LY2	0.00 ± 0.00	858.80 ± 73.42 <sub>ab</sub>	0.00 ± 0.00	1368.59 ± 205.04 <sub>a</sub>	0.00 ± 0.00 <sub>e</sub>

## Sorbitolo in salamoia

### Sorbitolo



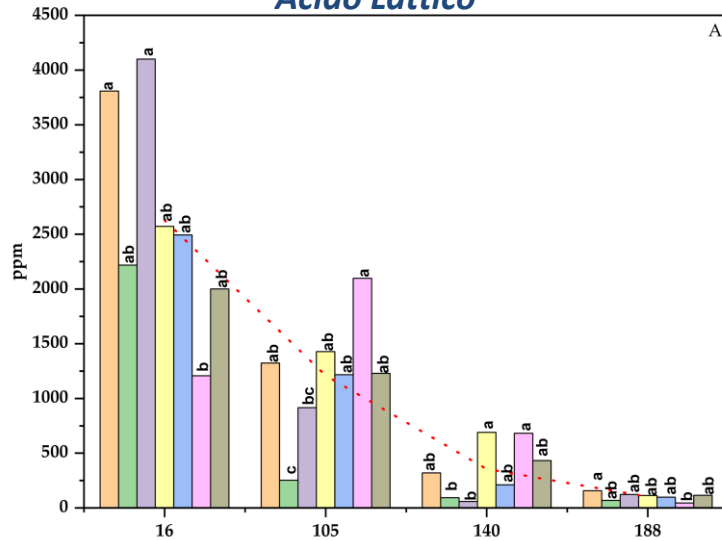
### Sorbitolo:

- ✓ Compare a fine fermentazione
- ✓ Lieviti presenti nelle olive come *C. boidinii* e *S. cerevisiae* accumulano sorbitolo in cellule come protezione dallo stress osmotico

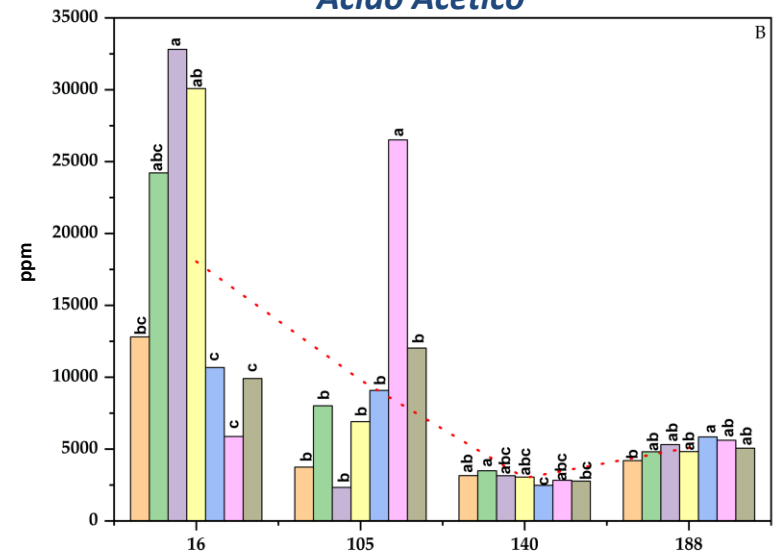


## Metaboliti in salamoia

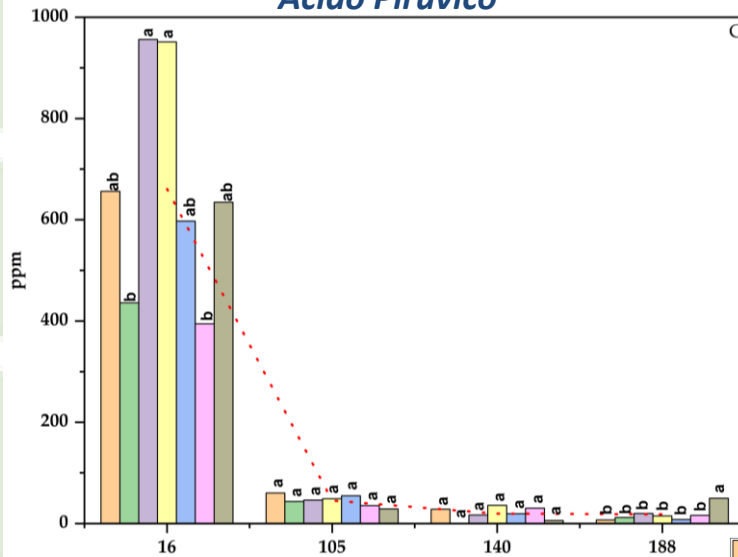
### Acido Lattico



### Acido Acetico



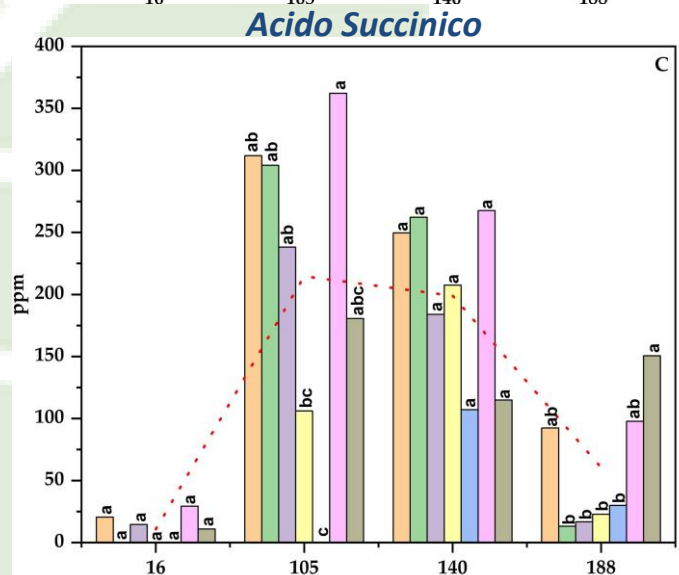
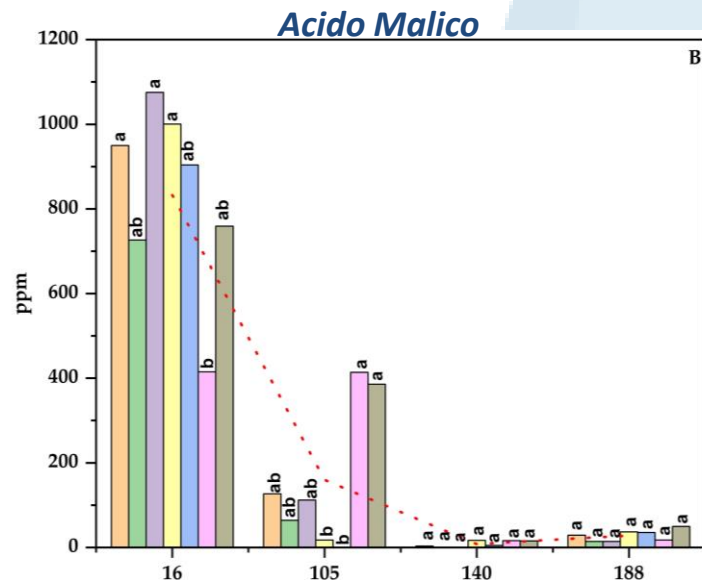
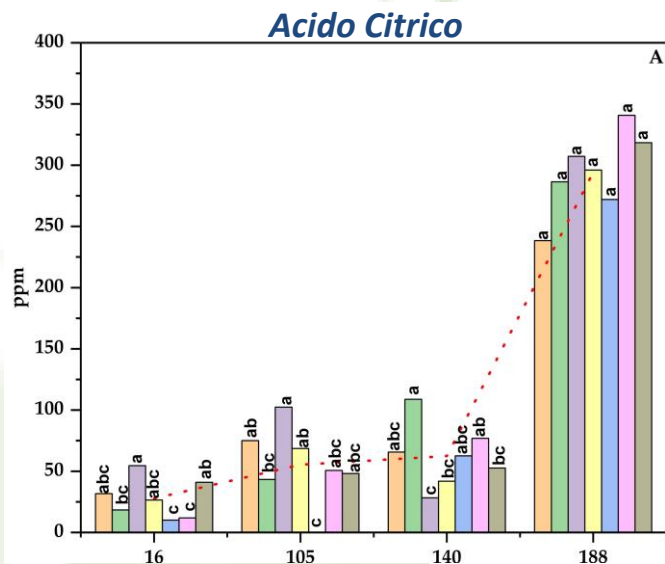
### Acido Piruvico



- Diminuzione acidi organici
- Correlazione con innalzamento pH

SP LP WA MIX1 LY1 MIX2 LY2

## Metaboliti in salamoia

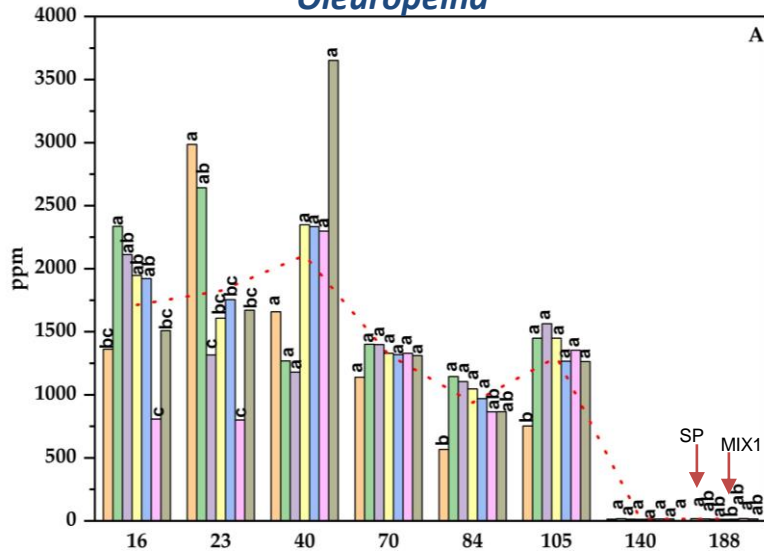


- Citrato → prodotto da muffe
- Acido malico → metabolismo LAB
- Acido succinico → *Enterobacteriaceae*, *Propionobacteriaceae*

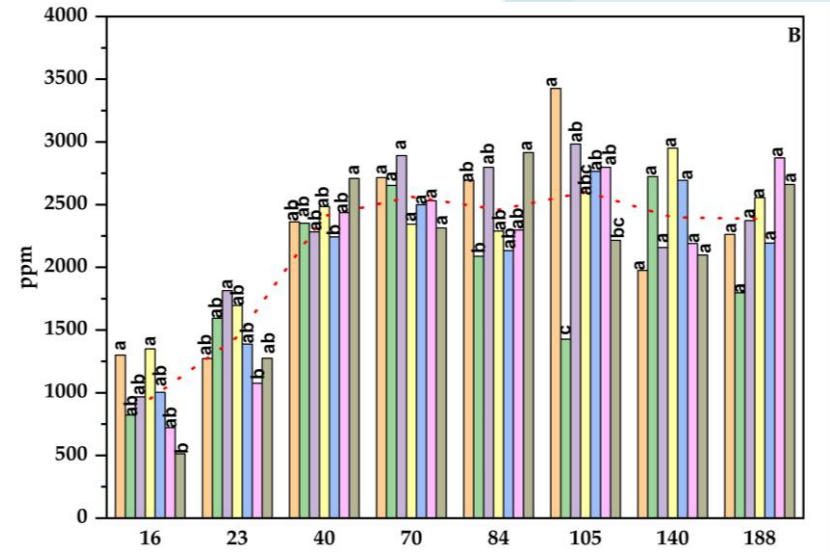
SP LP WA MIX1 LY1 MIX2 LY2

## Polifenoli in salamoia

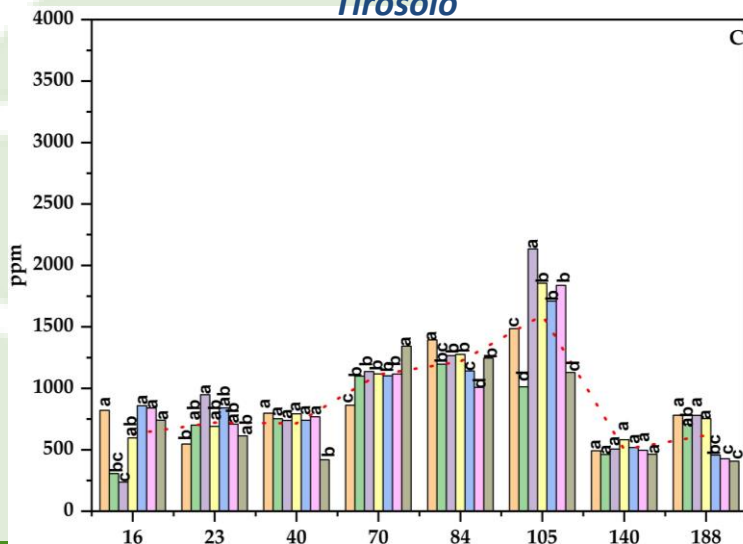
### Oleuropeina



### Idrossitiroso



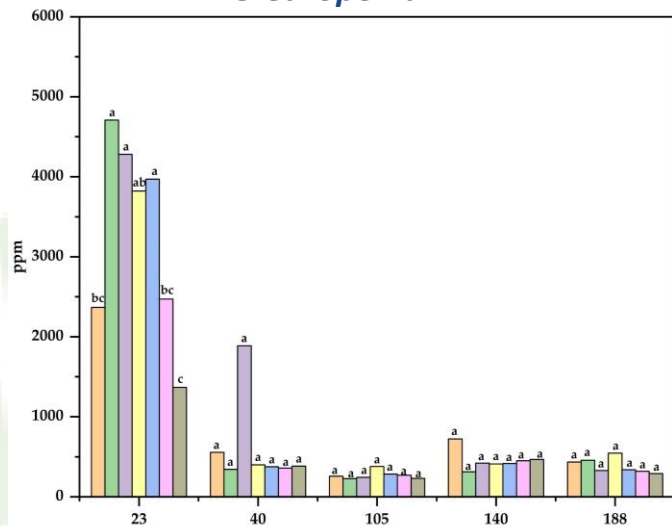
### Tirosolo



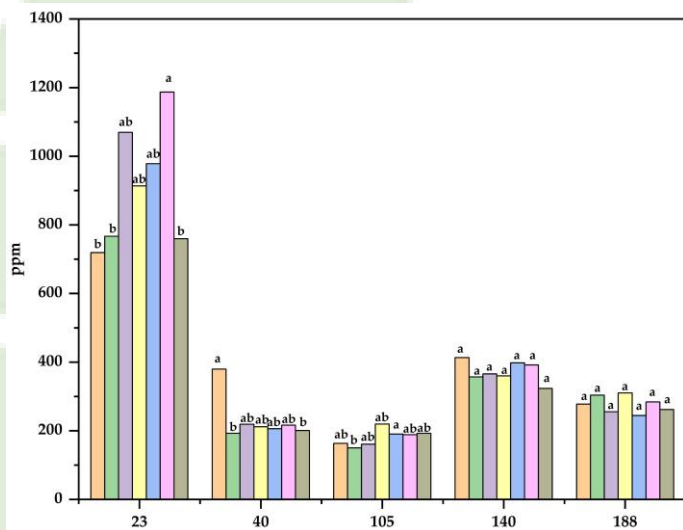
- ✓ Abbattimento oleuropeina
- ✓ Effetto positivo di *S. cerevisiae* sull'idrolisi dell'oleuropeina (**MIX1**)
- ✓ Concentrazioni più alte in **SP**

SP LP WA MIX1 LY1 MIX2 LY2

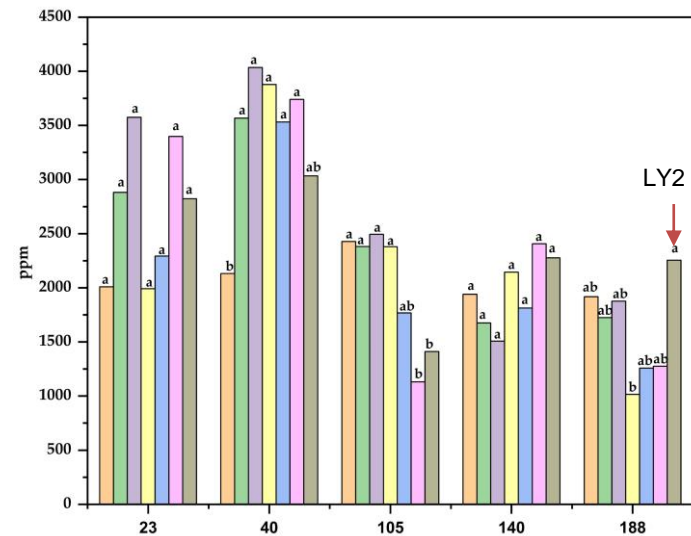
### Oleuropeina



### Tirosolo



### Idrossitirosolo



- ✓ Abbattimento oleuropeina
- ✓ Effetto sinergico di LAB e *W. anomalous* (LY2)
- ✓ Idrossitirosolo: attività antiossidante, anti-radicalica, potenziale nutraceutico

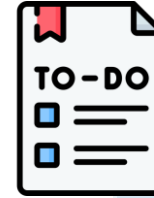
SP LP WA MIX1 LY1 MIX2 LY2



- Tutte le tesi hanno raggiunto rapidamente valori bassi di pH e un buon livello di deamarizzazione
- Sinergia positiva tra LAB e lieviti inoculati nell'abbassamento di pH
- Sinergia inoculo lieviti e LAB, presenza significativa nel prodotto finale di idrossitirosolo



- Durante la fermentazione progressivo aumento del pH ed una crescita eccessiva di microrganismi indesiderati.
- Crescita eccessiva di microrganismi indesiderati → alte temperature invernali e disponibilità mannitolo



- ✓ Il possibile ruolo del cambiamento climatico sulla qualità e sicurezza degli alimenti fermentati, potrebbe essere oggetto di studi successivi
- ✓ Studi sulle diverse combinazioni di ceppi di lieviti e lattobacilli selezionati per ottenere prodotti con maggiore concentrazione di idrossitiroso








*fermentation*



Article

### Assessment of Starters of Lactic Acid Bacteria and Killer Yeasts: Selected Strains in Lab-Scale Fermentations of Table Olives (*Olea europaea* L.) cv. *Leccino*

Grazia Federica Bencresciuto <sup>1</sup>, Claudio Mandalà <sup>1</sup>, Carmela Anna Migliori <sup>1</sup>, Giovanna Cortellino <sup>2</sup>,  
Maristella Vanoli <sup>2</sup> and Laura Bardi <sup>1,\*</sup>

# Grazie per l'attenzione