

PROGRAMMA OPERATIVO REGIONALE FSE 2014-2020



REGIONE DEL VENETO



Miglioramento della qualità dei vini passiti mediante
applicazione di chitosano e lieviti non-*Saccharomyces* in fruttajo

***Approccio microbiologico mediante applicazione di una sospensione di cellule
del lievito *Starmerella bacillaris* sui grappoli posti in fruttajo***

Dott. Vinícius da Silva Duarte (Università degli Studi di Padova)



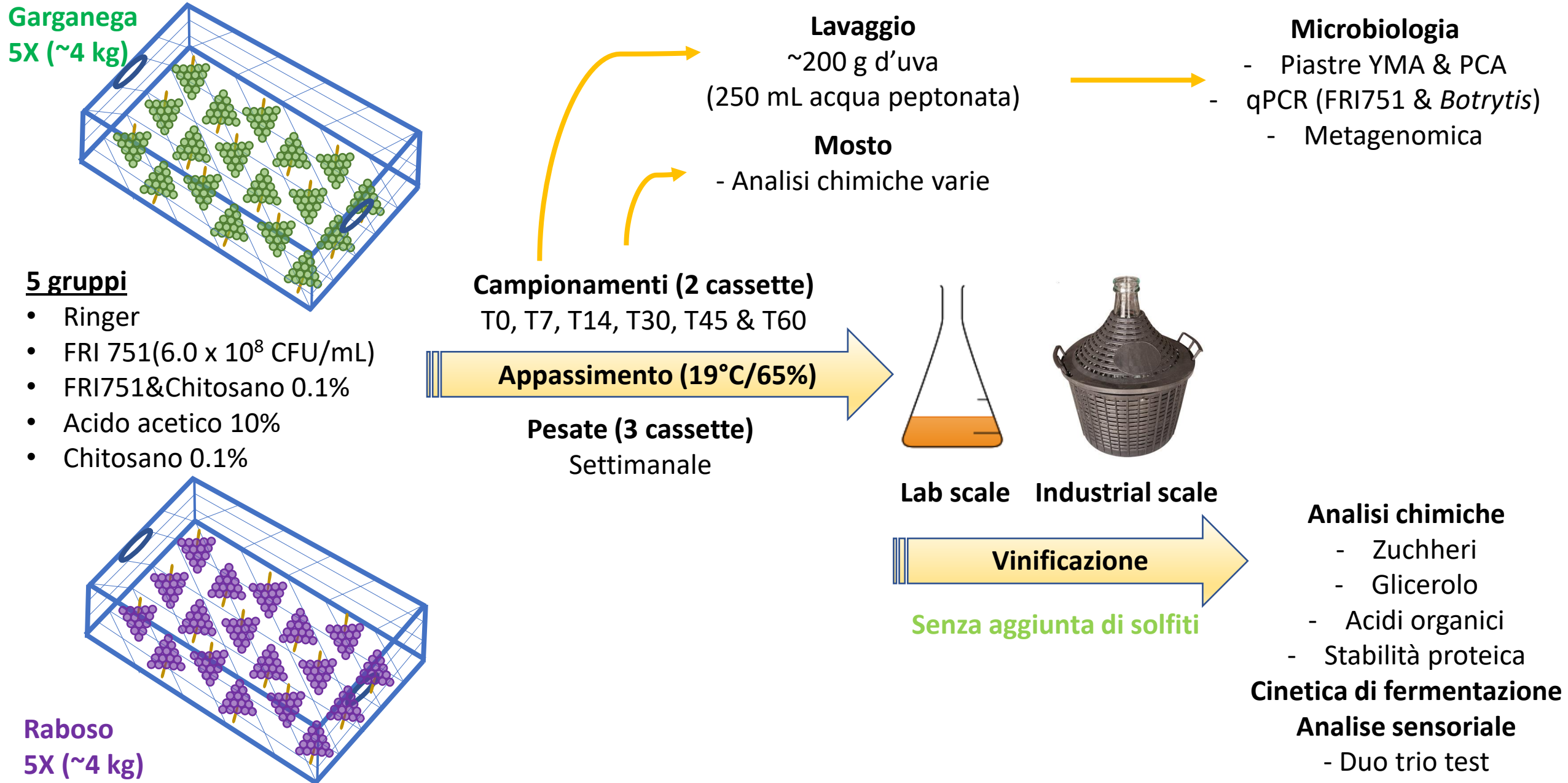
SOAVE
CONSORZIO TUTELA



BONOTTO DELLE TEZZE



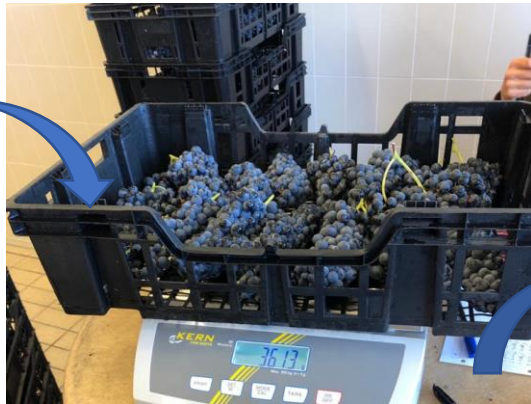
Disegno sperimentale



Dall'uva al vino



Arrivo dell'uva



Pesaggio dell'uva



Spruzzo del lievito



Asciugatura



Appassimento

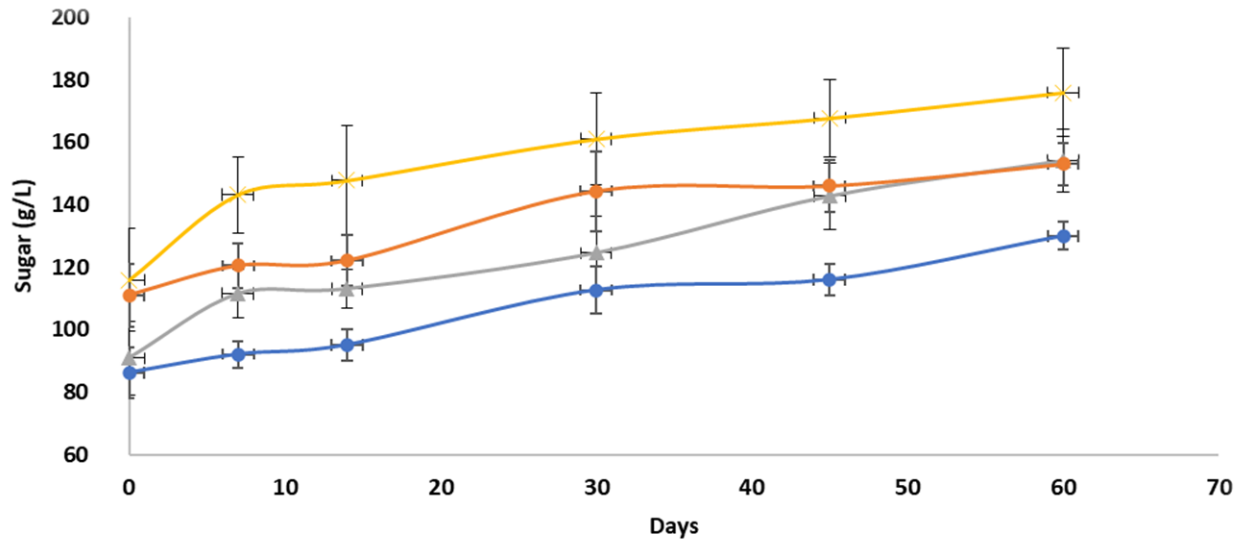
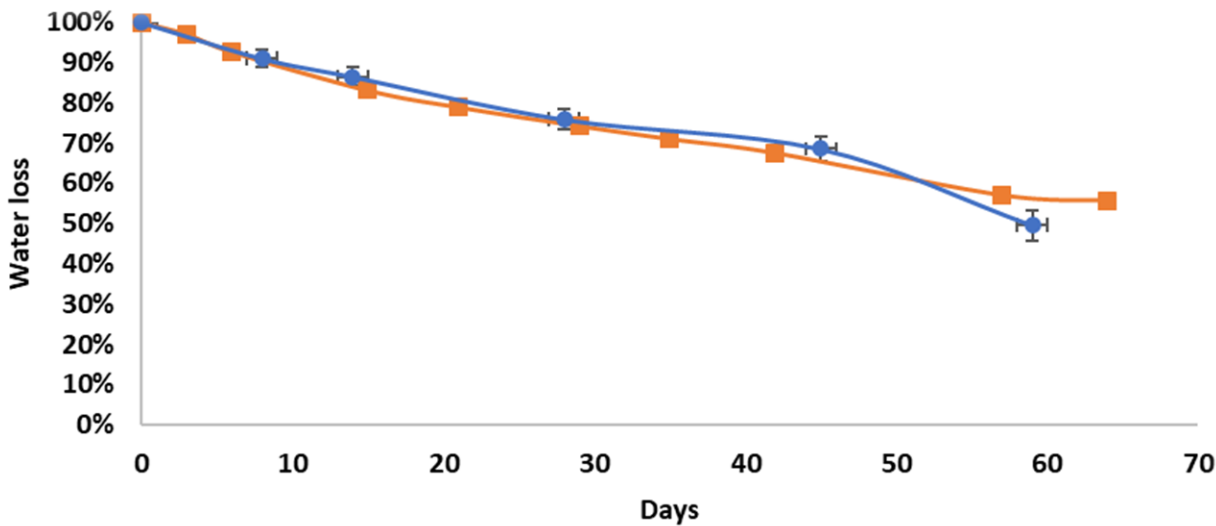


Pigiatura e diraspatura



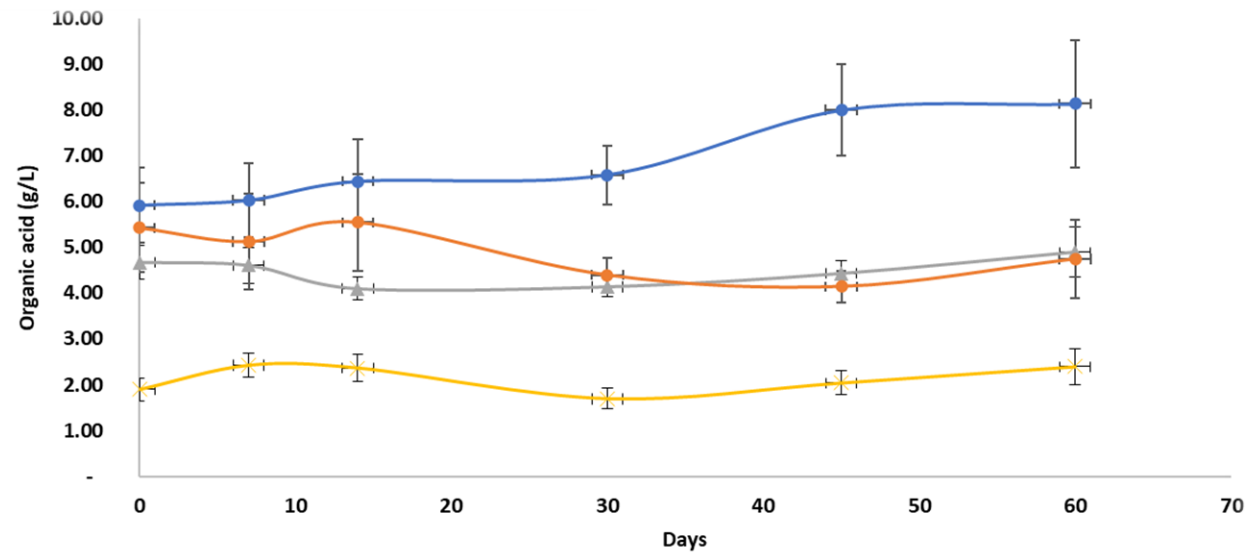
Vinificazione in rosso

Andamento dell'appassimento



—■— Garganega —●— Raboso

—▲— Glucose_Garganega —x— Fructose_Garganega —●— Glucose_Raboso —●— Fructose_Raboso

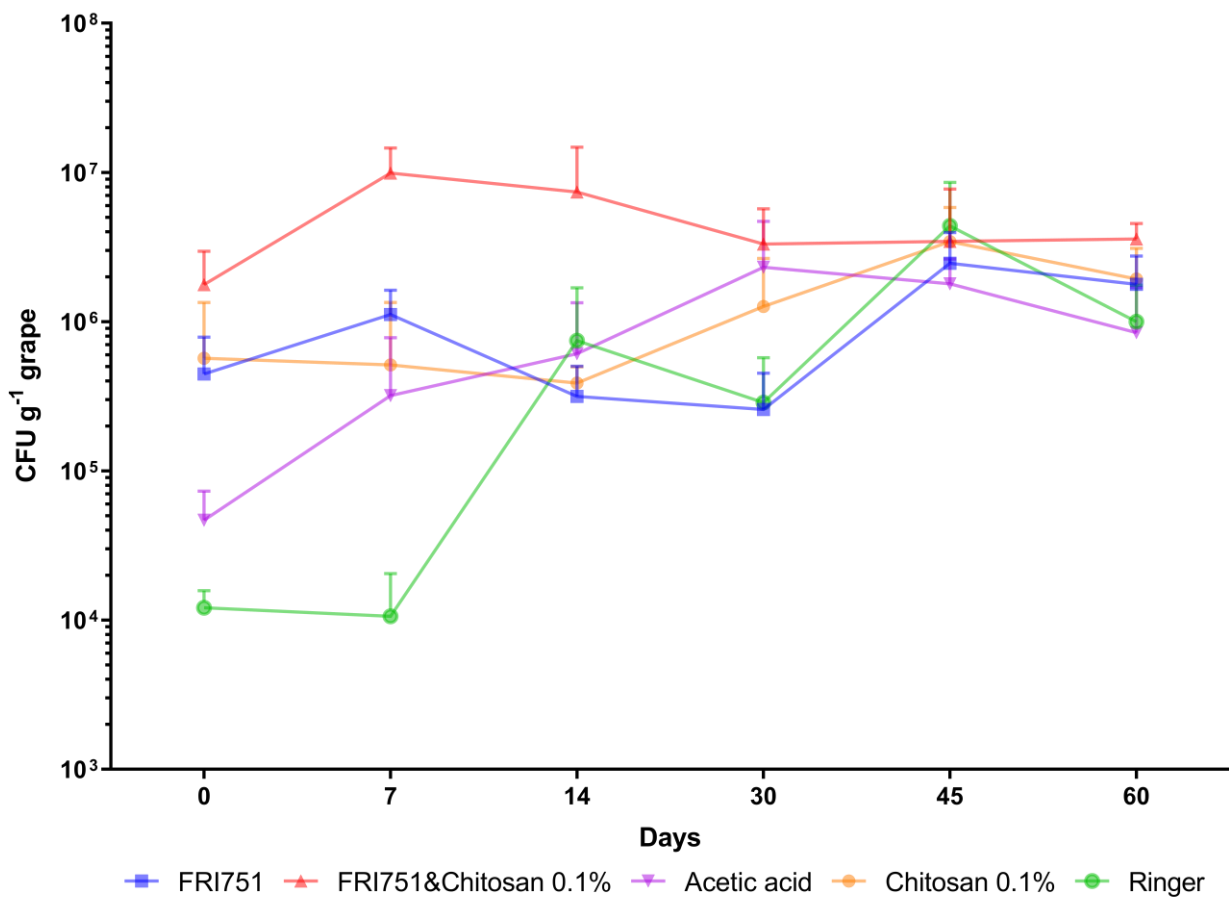


—▲— Tartaric acid_garganega —x— Malic acid_garganega —●— Tartaric acid_Raboso —●— Malic acid_Raboso

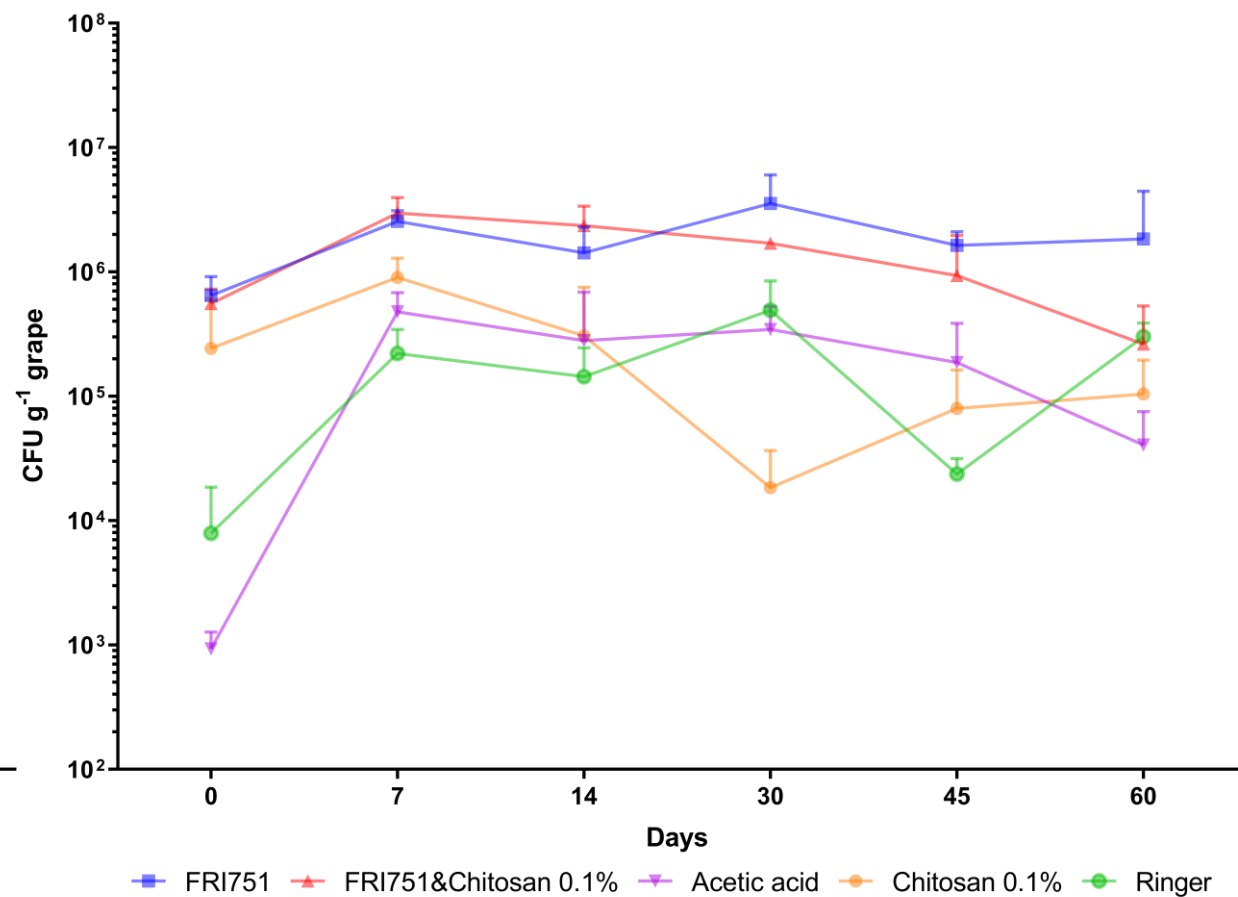
Appassimento – Lieviti (YMA)

Lavaggio (250 mL) → YMA → CFU/g di grappolo d'uva

Garganega



Raboso



qPCR – Procedura

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Genome > Genome Information by Organism

Starmarella bacillaris

Organism name (common or scientific) or Accession (Assembly, BioProject or replicon) ... Search

Download Reports from FTP site

Organism Overview: Genome Assembly and Annotation report (5); Organelles (1)

Choose Columns

#	Organism Name	Organism Groups	Strain	BioSample	BioProject	Assembly	Level	Size(Mb)	GC%	WGS	Scaffolds	CDS	Release Date	FTP
1	Starmarella bacillaris	Eukaryota;Fungi;Ascomycetes	PAS13	SAMN06434767	PRJNA376556	GCA_002094175.1		9.33	39.50	MWP01	79		17-Apr-2017	G
2	Starmarella bacillaris	Eukaryota;Fungi;Ascomycetes	FR751	SAMN06446006	PRJNA376556	GCA_002024125.1		9.27	39.50	MWSF01	111		06-Mar-2017	G
3	Starmarella bacillaris	Eukaryota;Fungi;Ascomycetes	NP2	SAMN07482632	PRJNA367461	GCA_002270425.1		9.31	39.40	NQLE01	117		25-Aug-2017	G
4	Starmarella bacillaris	Eukaryota;Fungi;Ascomycetes	CBS 9494	SAMN09428497	PRJNA476211	GCA_003260575.1		9.29	39.50	QLK001	200		22-Jun-2018	G
5	Starmarella bacillaris	Eukaryota;Fungi;Ascomycetes	PYCC 3044	SAMN07581196	PRJNA416493	GCA_003033765.1		9.36	39.40	PEO001	195		04-Apr-2018	G

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View 1 - 5 of 5

Specie d'interesse



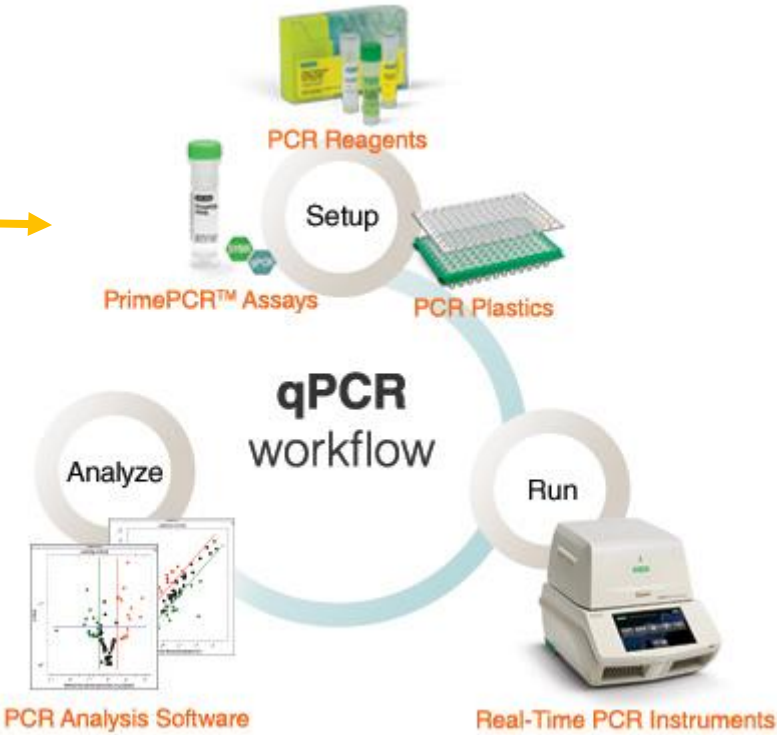
Primer design

Using Primer BLAST

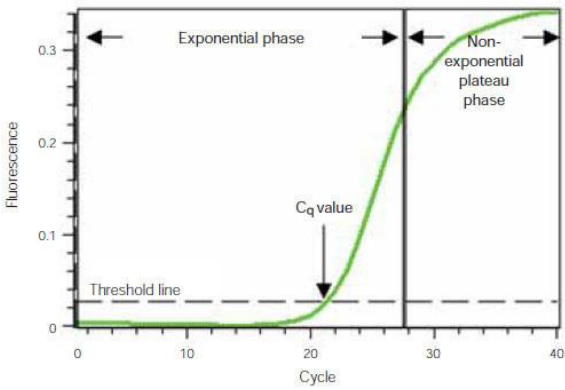
How to use Primer BLAST in Sequence Viewer



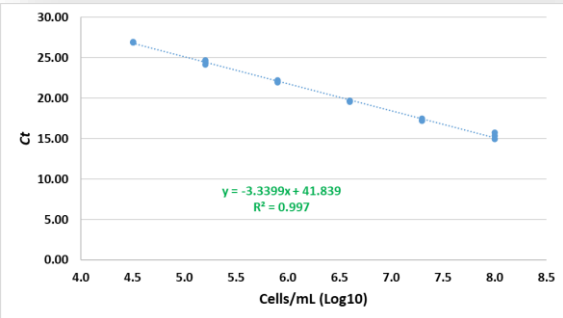
Reazione



Cicli soglia



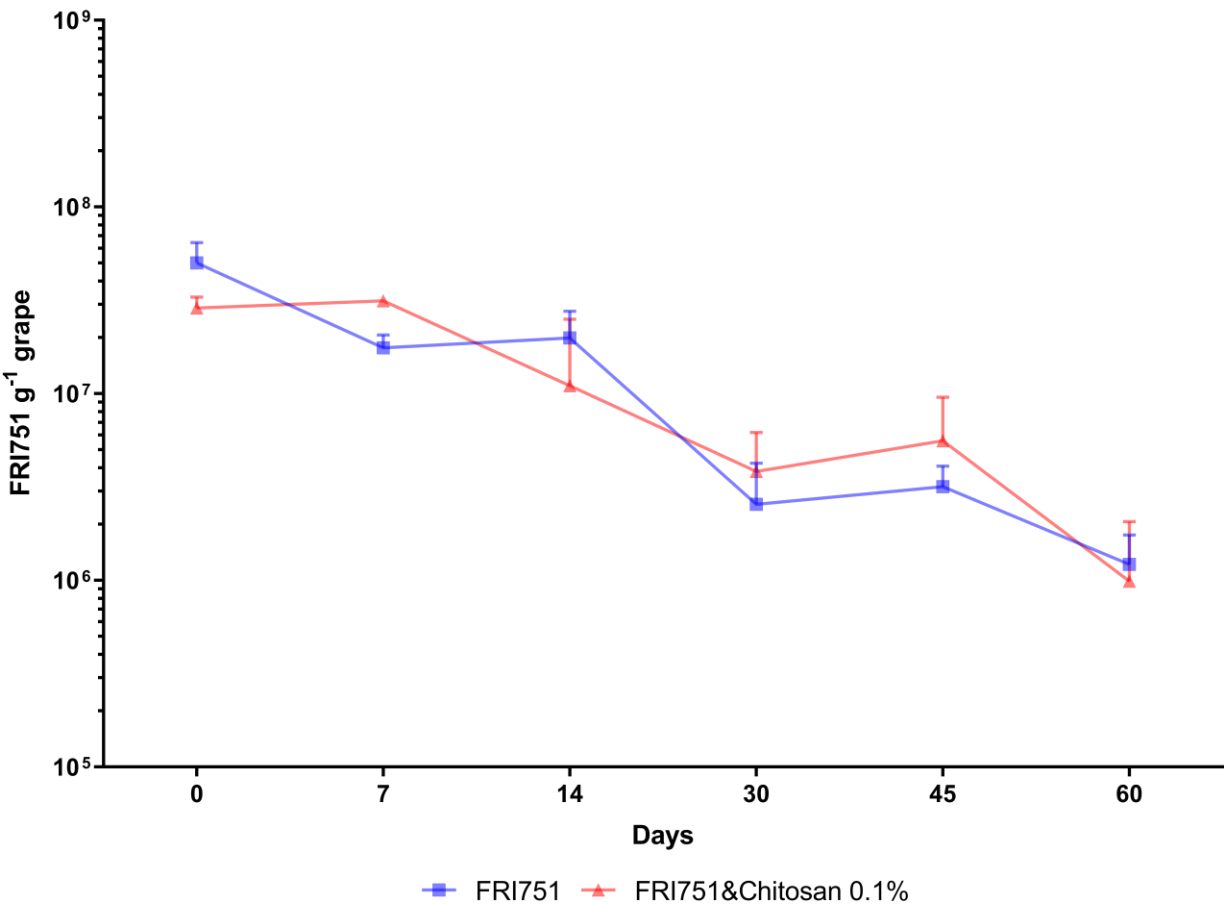
Retta di Taratura



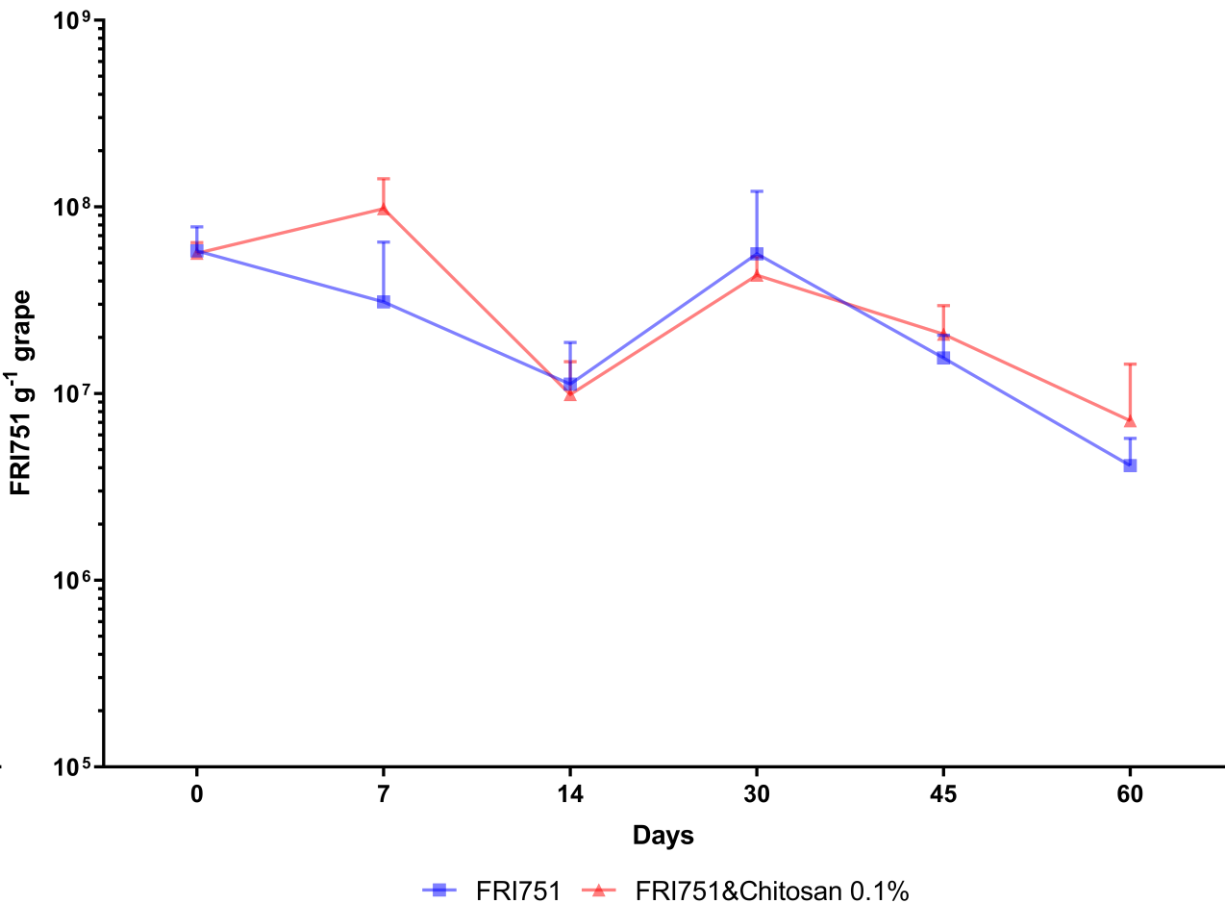
Appassimento – qPCR *S. bacillaris* FRI751

Lavaggio (250 mL) → arricchimento cellulare (4500 rpm, 10 min) → estrazione di DNA totale → FRI751 VSb (103 bp)

Garganega



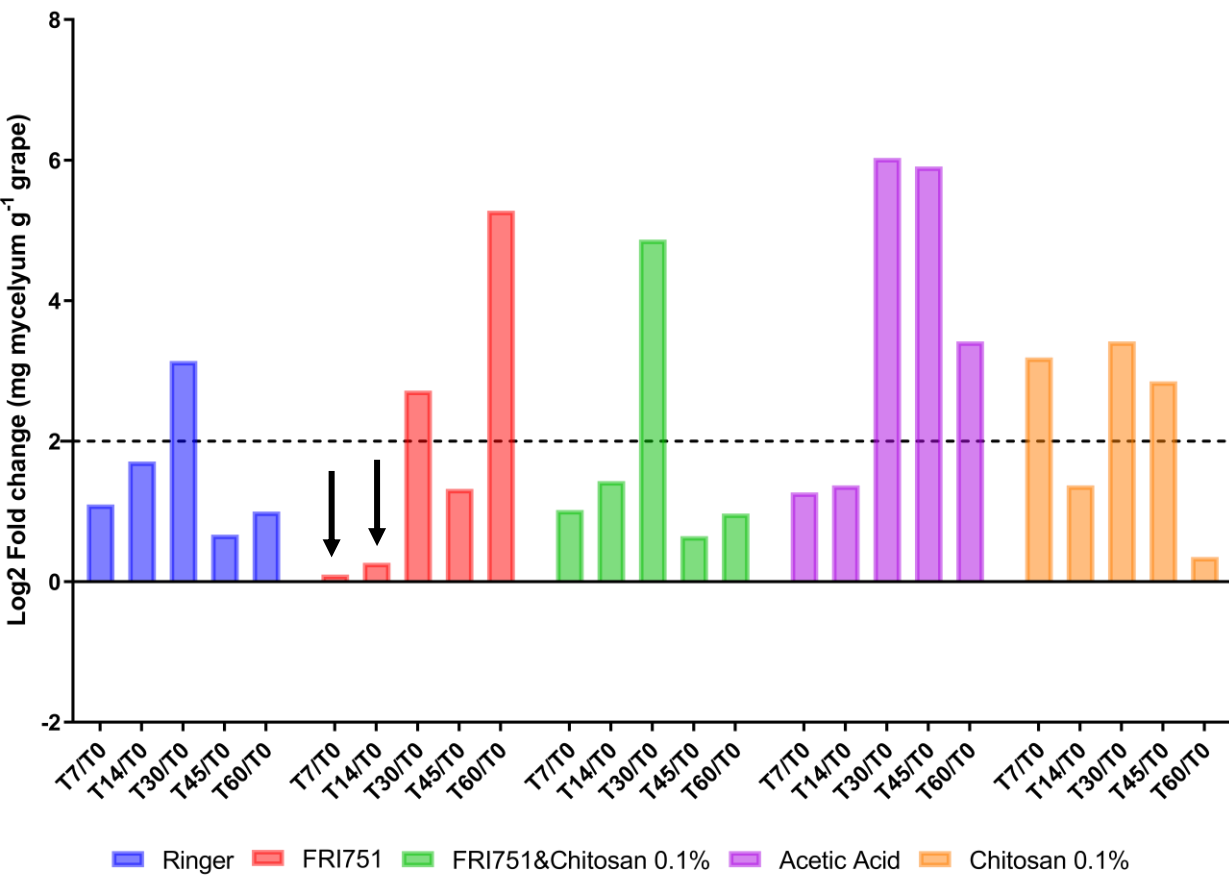
Raboso



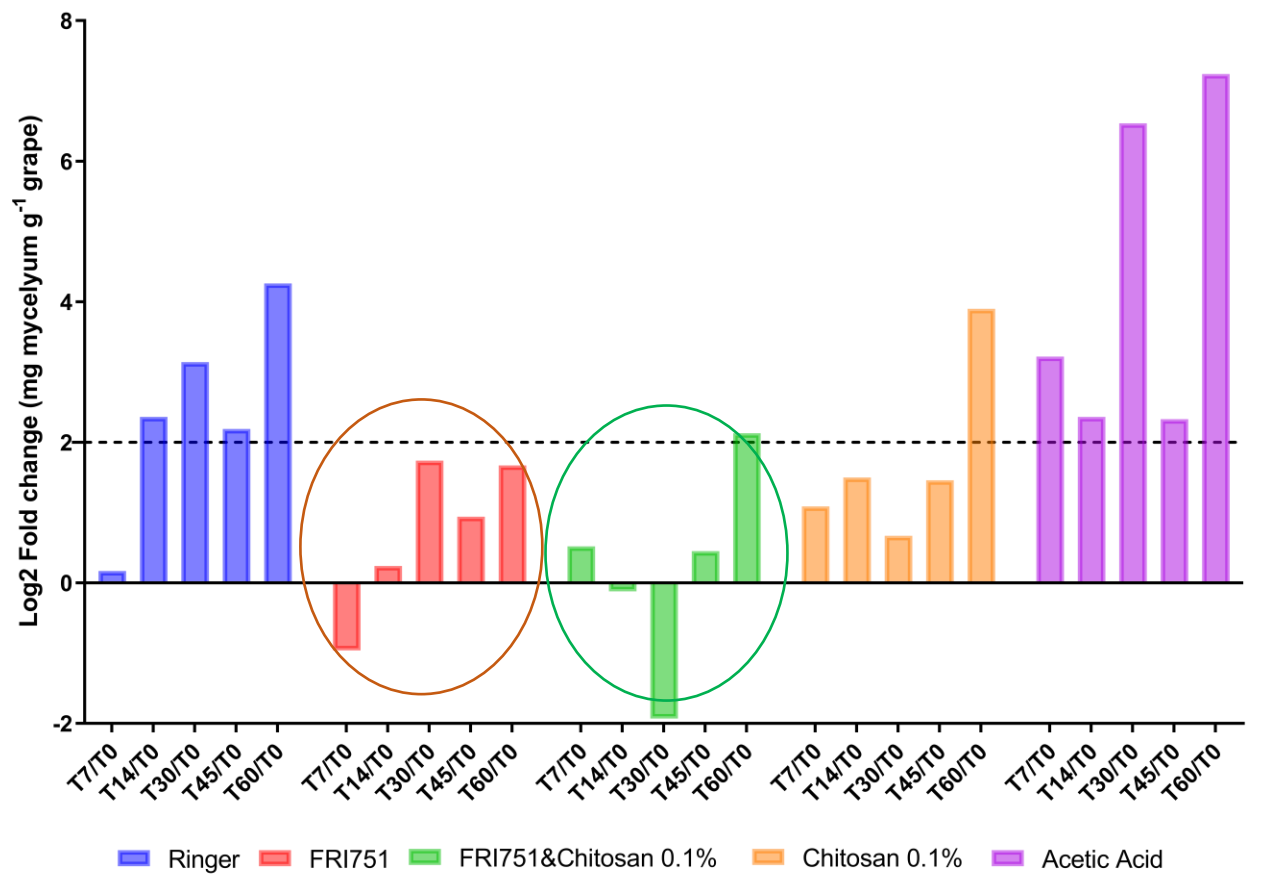
Appassimento – qPCR *Botrytis cinerea*

Lavaggio (250 mL) → arricchimento cellulare (4500 rpm, 10 min) → estrazione di DNA totale → *B. cinerea* Bc3F/Bc3R (95 bp) Suarez *et al.*, 2005

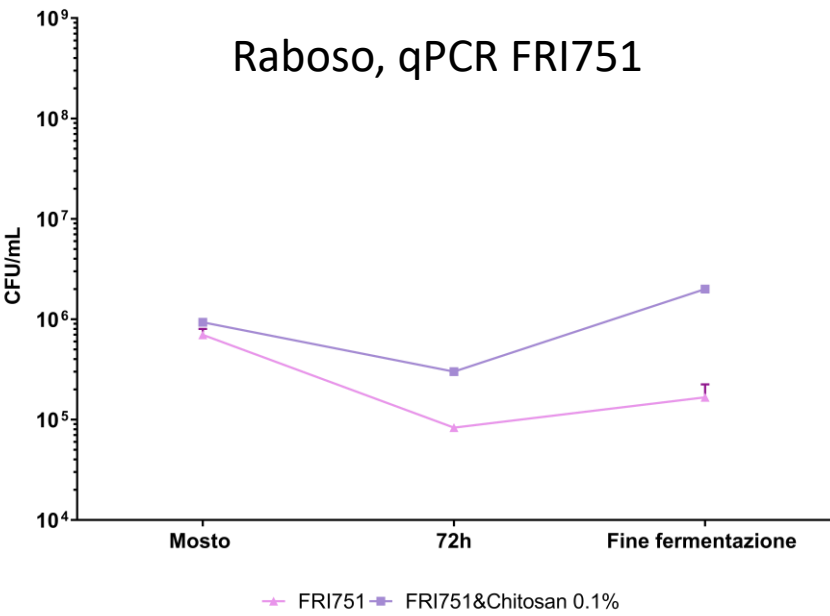
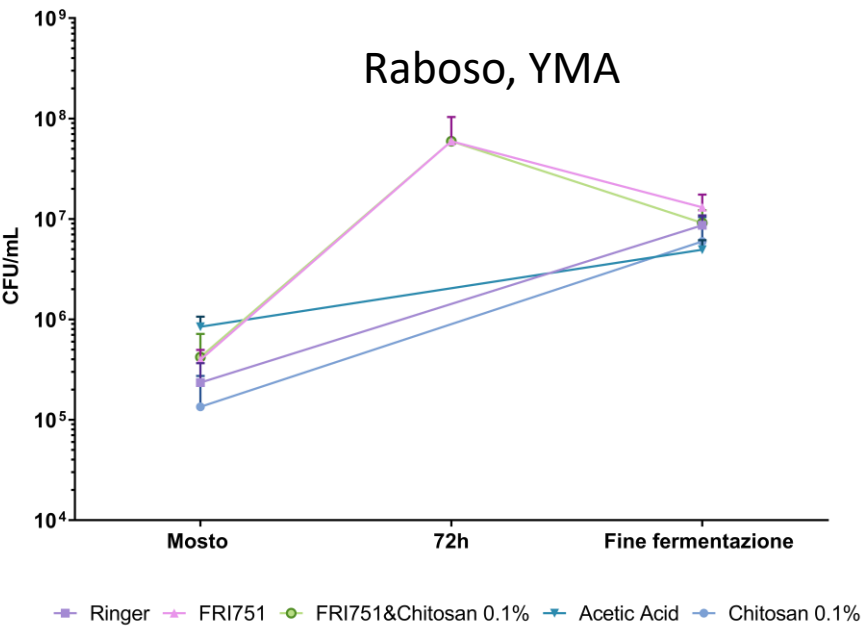
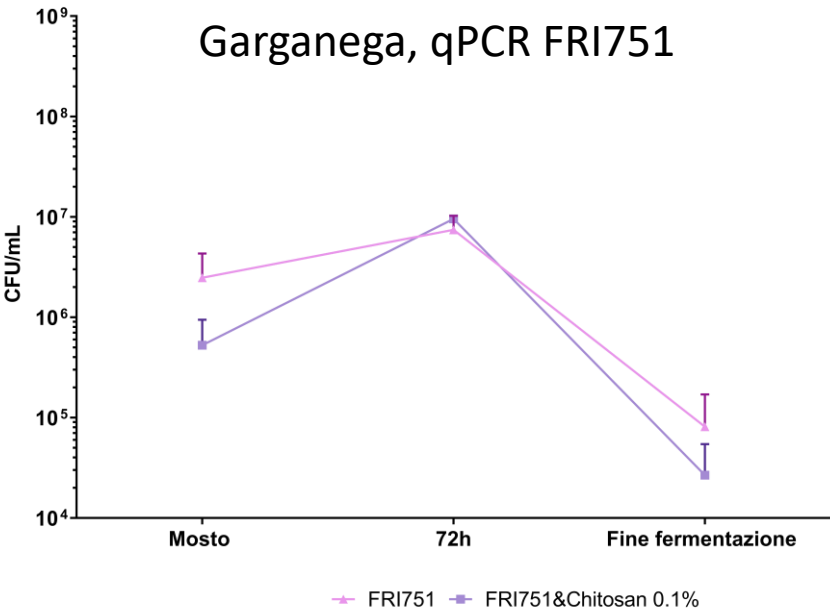
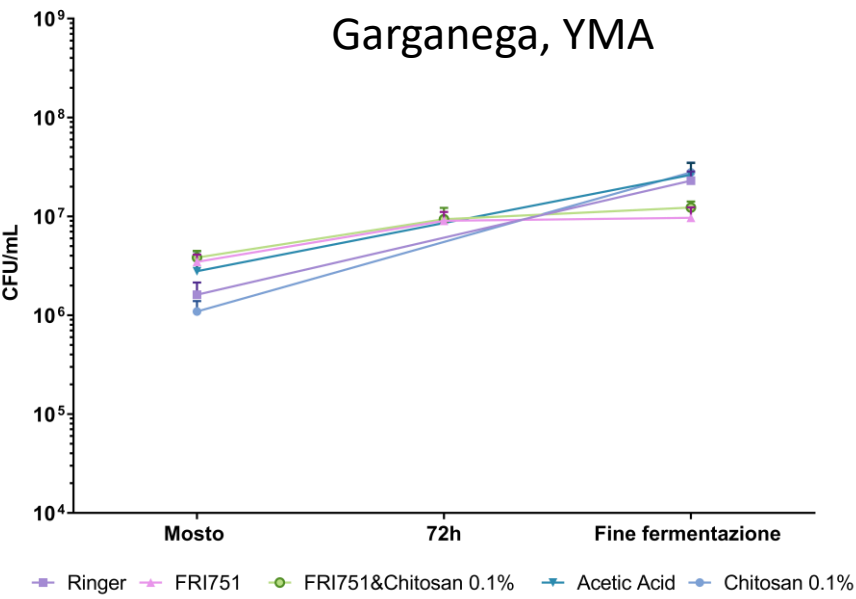
Garganega



Raboso

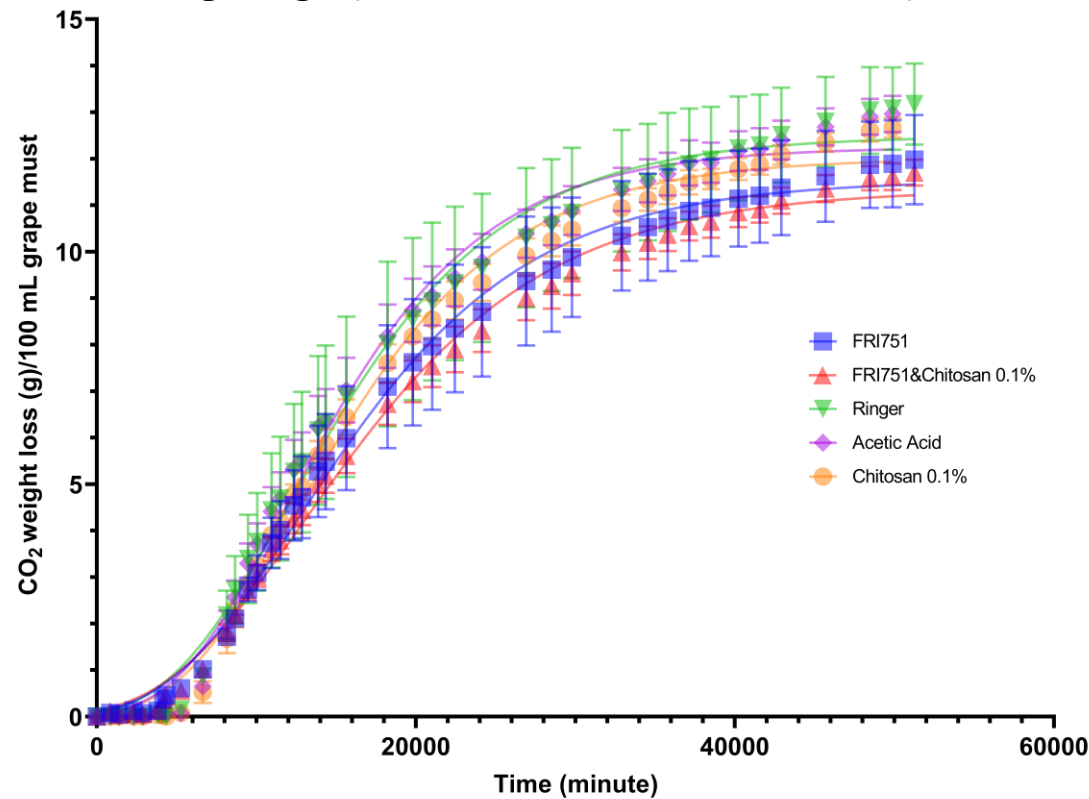


Vinificazione – Lab scale, Lieviti & qPCR FRI751



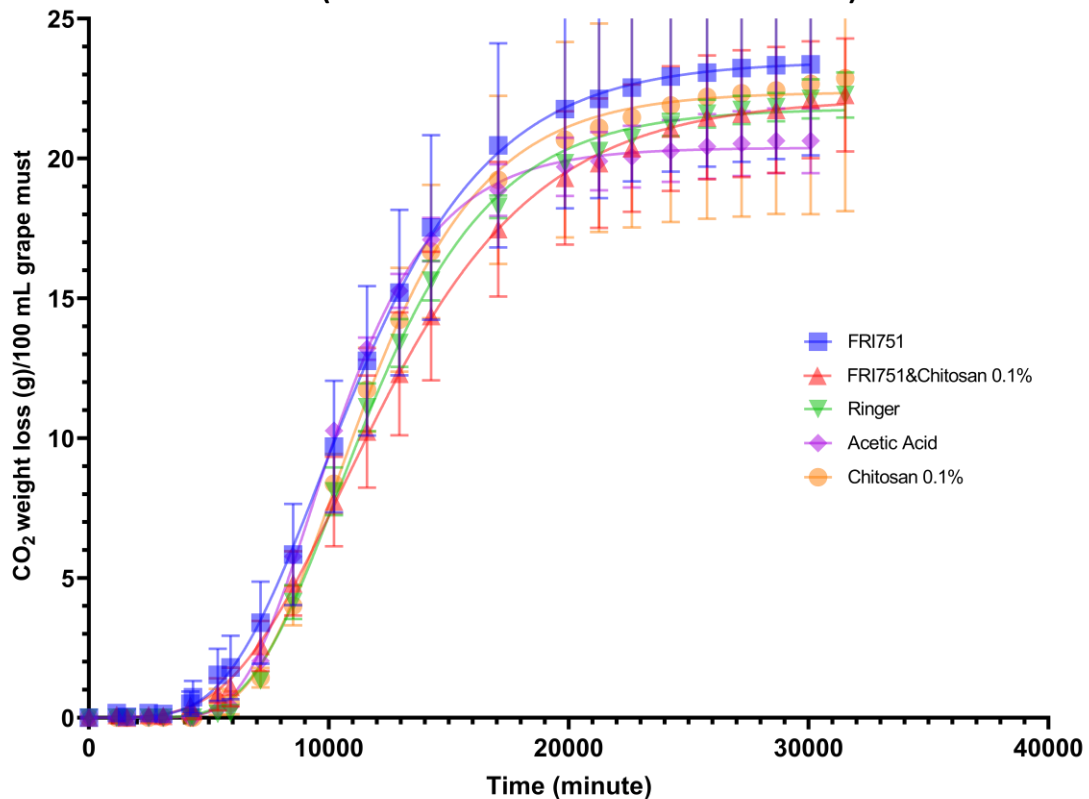
Vinificazione – Lab scale

Garganega (*S. cerevisiae*: Excellence TXL)



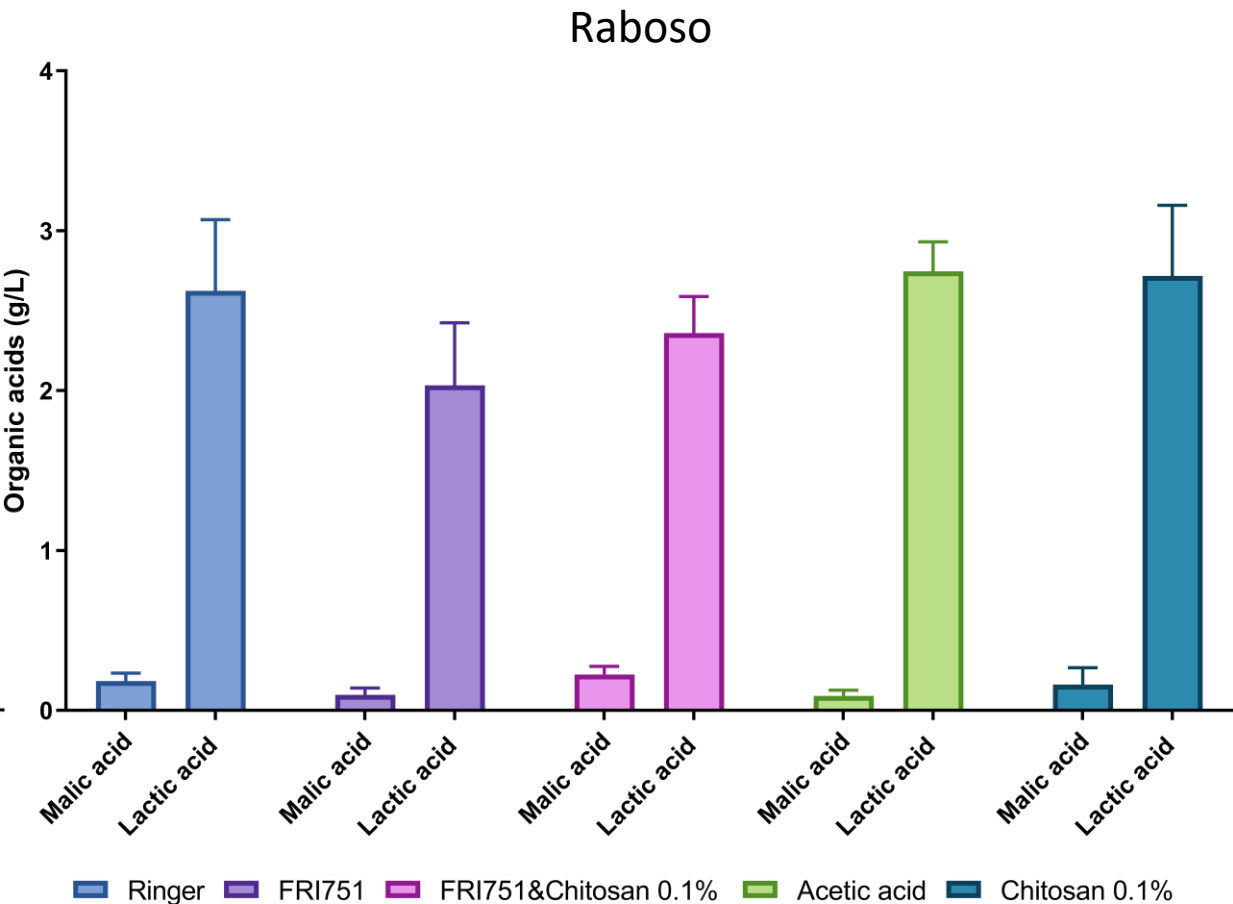
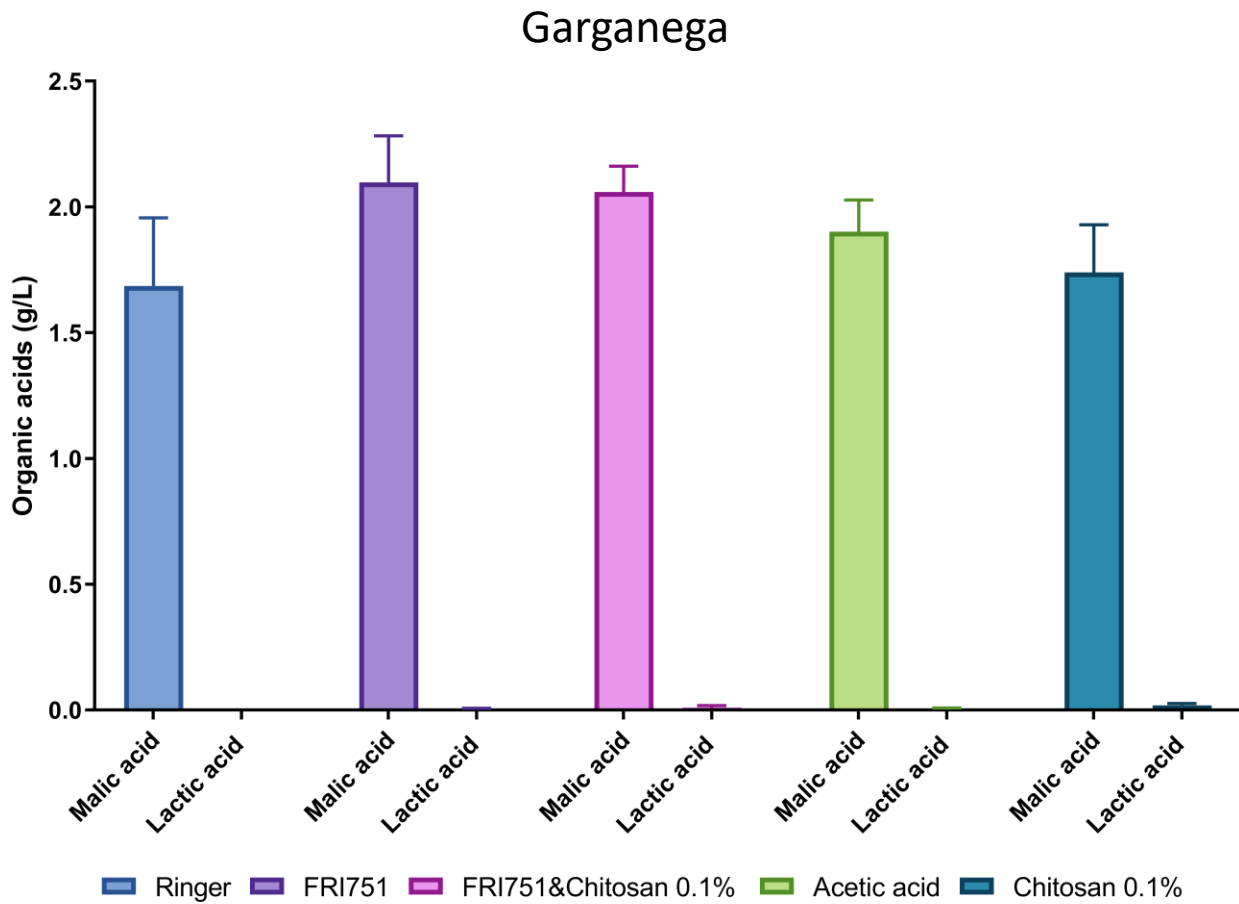
Group	Glucose (g/L)	Fructose (g/L)	Glycerol (g/L)	Ethanol (% v/v)	Residual sugar (g/L)
Ringer_Mosto	149 ± 6.6	154.5 ± 6.5	*	*	
Ringer_FF	0.0 ± 0.0	40.8 ± 9.0	13.3 ± 1.0	19.8 ± 1.0	40.8 g/L
FRI751_Mosto	163.6 ± 2.3	171.0 ± 0.3	*	*	
FRI751_72h	166.2 ± 2.3	173.1 ± 2.5	*	*	
FRI751_FF	35.0 ± 19.1	60.3 ± 4.0	15.8 ± 0.2	18.8 ± 1.2	95.3 g/L
FRI751&Chito_Mosto	158.2 ± 0.6	170.2 ± 1.3	*	*	
FRI751&Chito_48h	163.1 ± 2.9	172.3 ± 3.6	*	*	
FRI751&Chito_FF	33.4 ± 6.9	56.9 ± 9.0	16.8 ± 0.2	17.9 ± 0.9	90.3 g/L
Acetic Acid_Mosto	151.4 ± 3.4	162.6 ± 2.5	*	*	
Acetic Acid_FF	6.5 ± 9.2	54.4 ± 14.0	14.7 ± 0.3	19.1 ± 0.5	60.9 g/L
Chitosan 0.1%_Mosto	150.8 ± 3.3	159.2 ± 3.2	*	*	
Chitosan 0.1%_FF	18.6 ± 2.5	71.7 ± 10.4	14.8 ± 0.9	18.6 ± 0.2	90.3 g/L

Raboso – (*S. cerevisiae*: Anchor NT50)



Group	Glucose (g/L)	Fructose (g/L)	Glycerol (g/L)	Ethanol (% v/v)	Residual sugar (g/L)
Ringer_Mosto	163.7 ± 6.7	184.1 ± 6.5	*	*	
Ringer_FF	32.8 ± 7.7	87.1 ± 15.1	17.7 ± 0.6	15.6 ± 2.3	119.9 g/L
FRI751_Mosto	159.3 ± 11.5	177.5 ± 10.8	*	*	
FRI751_72h	159.8 ± 11.8	175.8 ± 15.1	*	*	
FRI751_FF	17.1 ± 11.2	39.7 ± 27.7	16.6 ± 0.5	16.4 ± 0.2	56.8 g/L
FRI751&Chito_Mosto	156.5 ± 4.2	170.4 ± 5.7	*	*	
FRI751&Chito_48h	166.1 ± 5.9	179.7 ± 10.5	*	*	
FRI751&Chito_FF	27.4 ± 10	53.5 ± 13.9	14.9 ± 0.4	15.8 ± 1.0	80.9 g/L
Acetic Acid_Mosto	154 ± 3.4	174.5 ± 5.3	*	*	
Acetic Acid_FF	18.2 ± 2.5	56.7 ± 5.4	16.5 ± 0.7	18.8 ± 1.5	74.9 g/L
Chitosan 0.1%_Mosto	168.8 ± 2.3	190.7 ± 5.1	*	*	
Chitosan 0.1%_FF	27.6 ± 2.9	78.5 ± 1.6	15.4 ± 0.8	15.2 ± 2.6	90.3 g/L

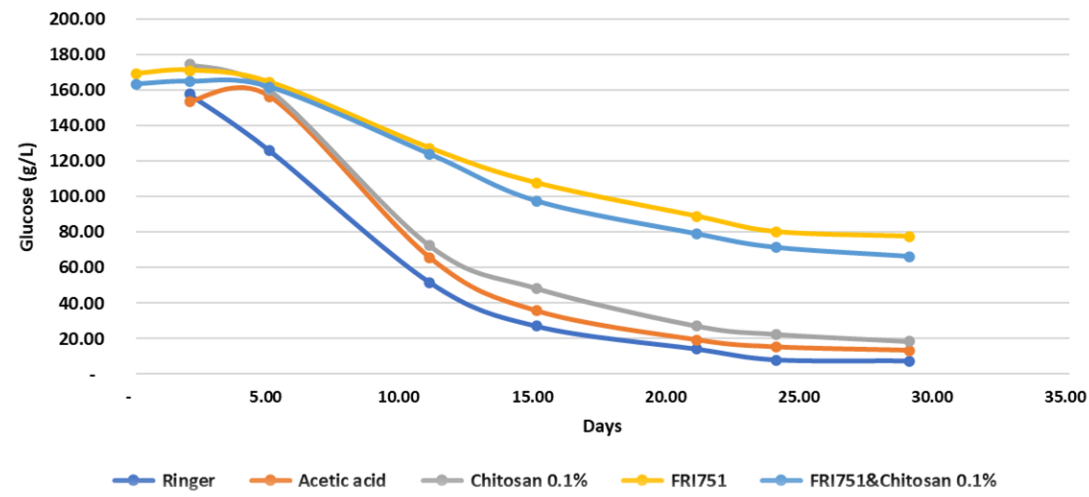
Vinificazione – Lab scale, Malolattica



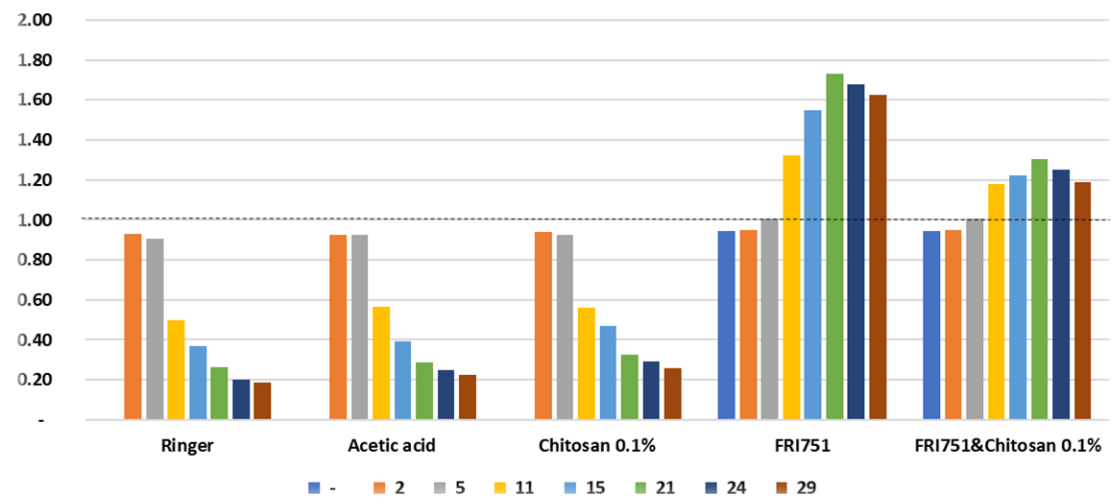
Vinificazione – Industrial scale

Garganega

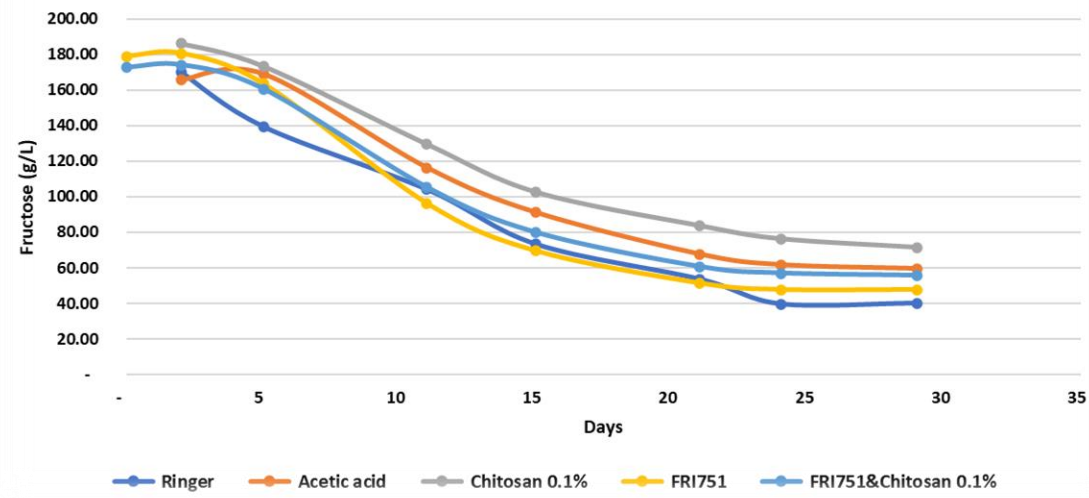
Tendenze glucosio



Rapporto glucosio/fruttosio



Tendenze fruttosio

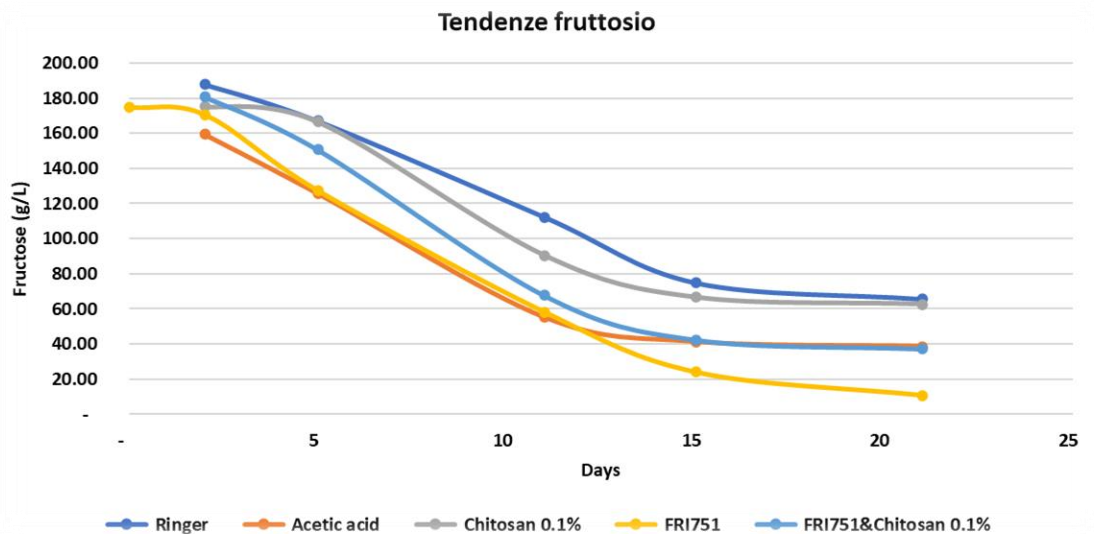
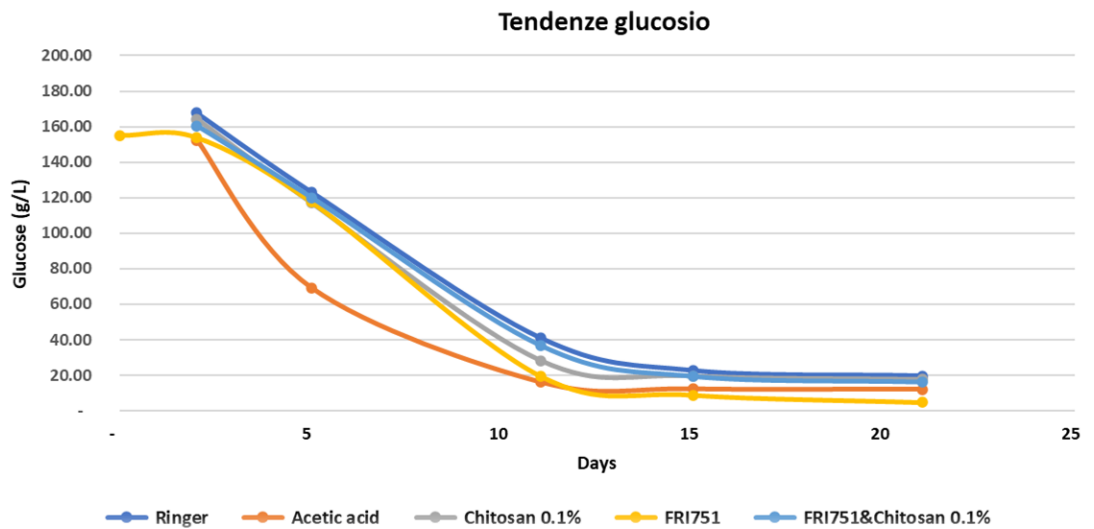


Tendenze glicerolo (g/L)

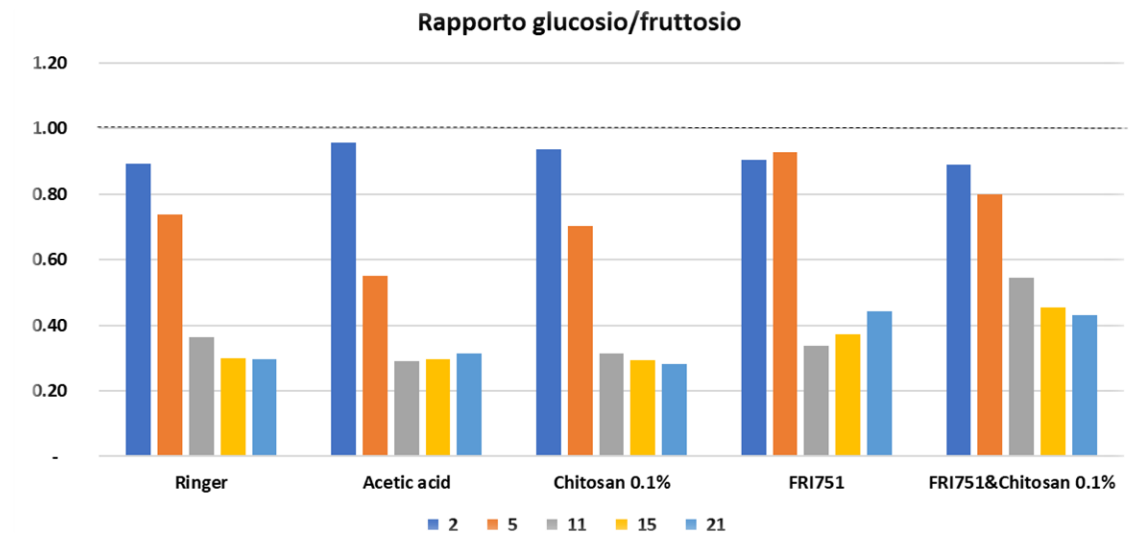
Days	Ringer	Acetic acid	Chitosan 0.1%	FRI751	FRI751&Chitosan 0.1%
0	0	0	0	0	0
2	0	0	0	0	0
5	0	0	0	0	0
11	10.7	12.4	12.2	13.8	12.2
15	13.5	14.6	14.5	16.4	15.1
21	15.5	15.7	15.2	18.3	17.2
24	14.6	14.7	15.2	19.0	17.9
30	15.5	15.7	15.6	19.3	17.4

Fine fermentazione

Vinificazione – Industrial scale



Raboso



Tendenze glicerolo (g/L)

Days	Ringer	Acetic acid	Chitosan 0.1%	FRI751	FRI751&Chitosan 0.1%
0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0
5	0.0	0.0	8.6	10.1	7.6
11	17.4	18.9	18.3	18.0	17.7
15	17.1	19.1	17.3	18.5	17.2

Fine fermentazione

Vinificazione – Stabilità proteica

Garganega

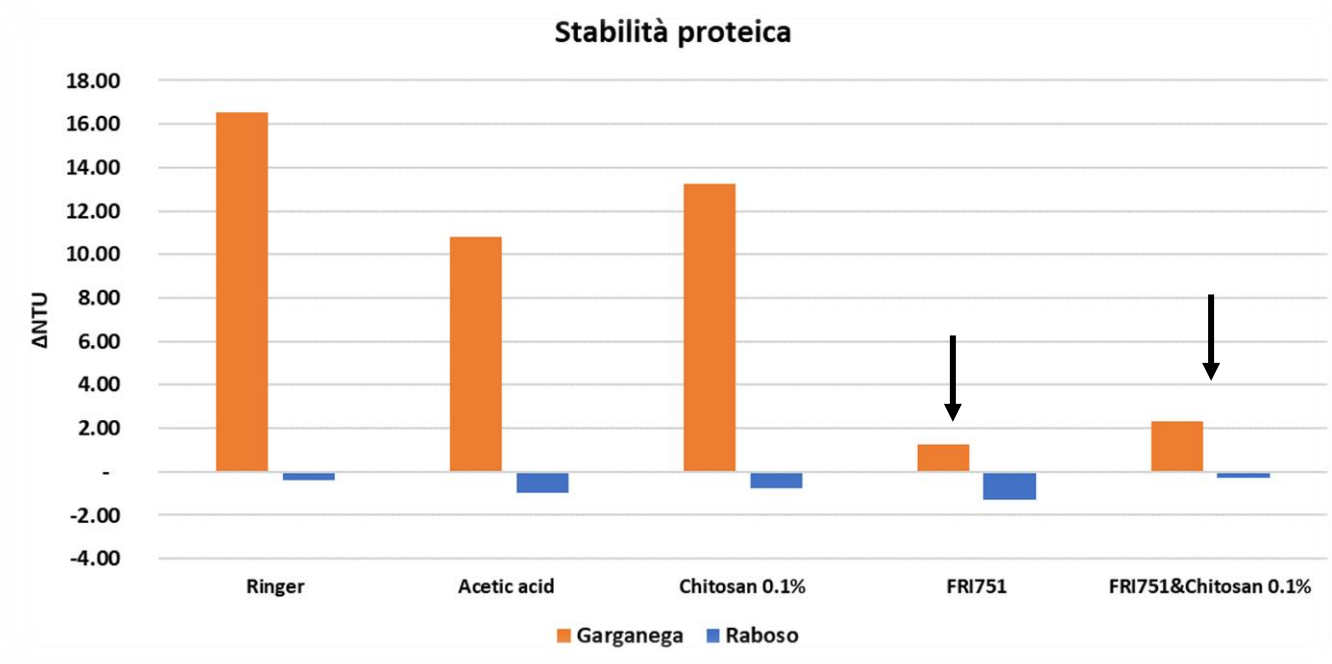
Group	Protein Stability (Δ NTU)	Tartaric Stability (Δ NTU)
Ringer_FF	64.7 $\pm$ 27.9	21.3 $\pm$ 5.5
FRI751_FF	19.2 $\pm$ 13.0	22.0 $\pm$ 4.4
FRI751&Chito_FF	28.2 $\pm$ 21.4	27.7 $\pm$ 13.3
Acetic Acid_FF	50.2 $\pm$ 12.3	19.3 $\pm$ 2.5
Chitosan 0.1%_FF	60.5 $\pm$ 11.5	18.7 $\pm$ 2.5

$p > 0.05$

Raboso

Group	Protein Stability (Δ NTU)	Tartaric Stability (Δ NTU)
Ringer_FF	131.4 $\pm$ 42.2	17.0 $\pm$ 1.7
FRI751_FF	147.8 $\pm$ 55.7	17.3 $\pm$ 0.6
FRI751&Chito_FF	110.5 $\pm$ 35.1	18.0 $\pm$ 2.0
Acetic Acid_FF	518.3 $\pm$ 390.5	17.3 $\pm$ 0.6
Chitosan 0.1%_FF	106.1 $\pm$ 50.8	17.0 $\pm$ 4.0

$p > 0.05$



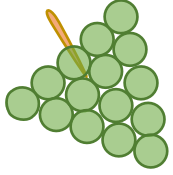
Vinificazione – Industrial scale, vino finito

Garganega							
Gruppi	Etanolo	pH	Acidità totale	Acido lattico	Acido malico	Acido acetico	Acetaldeide
Ringer	16,13 ± 0,13	3.57	6,83 ± 0,047	0,032 ± 0,0032	1,879 ± 0,019	0,851 ± 0,008	28,66 ± 0,577
Acetic acid	15,67 ± 0,035	3.64	7,03 ± 0,047	0,033 ± 0,0035	2,07 ± 0,016	0,933 ± 0,005	27,33 ± 1,52
Chitosan 0.1%	15,22 ± 0,10	3.60	7,16 ± 0,094	0,034 ± 0,005	1,935 ± 0,009	1,028 ± 0,0056	24,66 ± 0,577
FRI751	13,70 ± 0,26	3.58	7,56 ± 0,23	0,022 ± 0,0045	2,281 ± 0,001	0,825 ± 0,005	29 ± 1,73
FRI751&Chitosan 0.1%	13,61 ± 0,19	3.55	7,56 ± 0,094	0,023 ± 0,0051	2,24 ± 0,092	0,841 ± 0,016	29,66 ± 1,52

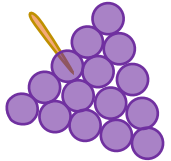
Raboso							
Gruppi	Etanolo	pH	Acidità totale	Acido lattico	Acido malico	Acido acetico	Acetaldeide
Ringer	16,15 ± 0,117	3.73	9,6	2,7495 ± 0,062	0,14 ± 0,0005	1,21 ± 0,0134	60 ± 6,24
Acetic acid	17,15 ± 0,13	3.63	9,6	2,0625 ± 0,0021	0,111 ± 0,0035	0,855 ± 0,0175	43,66 ± 9,07
Chitosan 0.1%	17,41 ± 0,122	3.72	9,53	2,068 ± 0,035	0,12 ± 0,0025	1,24 ± 0,0243	39 ± 7,54
FRI751	17,92 ± 0,0577	3.47	9,46	1,8975 ± 0,0148	0,112 ± 0,0005	0,81 ± 0,0173	59,66 ± 4,04
FRI751&Chitosan 0.1%	17,37 ± 0,11	3.43	10,63	1,6825 ± 0,0049	0,23 ± 0,001	1,2 ± 0,0313	44,33 ± 0,57

Duo trio test, $p > 0.05$: Gli odori non sono diversi

Vinificazione – Conclusione



- *Starmerella bacillaris* FRI751 è stata rilevata durante tutto il periodo d'appassimento;
- FRI751, associato o meno al chitosano, contribuisce al controllo di *B. cinerea* nei primi 15 giorni di appassimento;
- FRI751 ha fornito una maggiore stabilità proteica al vino prodotto, oltre ad aumentare la concentrazione di glicerolo.



- FRI751, associato o meno al chitosano, contribuisce in modo significativo al controllo di *B. cinerea* durante tutto l'appassimento;
- Non è stato riscontrato nessun apporto di FRI751 durante il processo di vinificazione.

PROGRAMMA OPERATIVO REGIONALE FSE 2014-2020



Ringraziamento

