

# SANGOVESE E QUERCETINA: I RISULTATI DEL PROGETTO QUE-STAB

Francesca Borghini



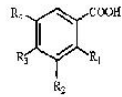
Il progetto QUE-STAB è stato realizzato con il co-finanziamento del PSR 2014-2020 Regione Toscana - Sottomisura 16.2



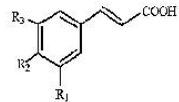
Regione Toscana



# Polifenoli

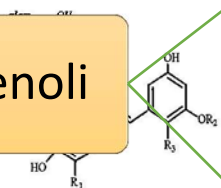


| Benzoid acid     | R <sub>1</sub> | R <sub>2</sub>   | R <sub>3</sub> | R <sub>4</sub>   |
|------------------|----------------|------------------|----------------|------------------|
| p-Hydroxybenzoic | H              | H                | OH             | H                |
| Protocatechic    | H              | OH               | OH             | H                |
| Vanilic          | H              | OCH <sub>3</sub> | OH             | H                |
| Galic            | H              | OH               | OH             | OH               |
| Syringic         | H              | OCH <sub>3</sub> | OH             | OCH <sub>3</sub> |
| Salicic          | OH             | H                | H              | H                |
| Gallic           | OH             | H                | H              | OH               |



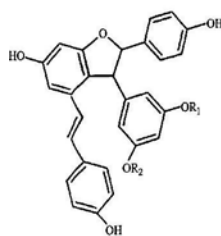
| Hydroxycinnamic acid | R <sub>1</sub>   | R <sub>2</sub> | R <sub>3</sub>   |
|----------------------|------------------|----------------|------------------|
| p-Coumaric           | H                | OH             | H                |
| Caffeic              | OH               | OH             | H                |
| Ferulic              | OCH <sub>3</sub> | OH             | H                |
| Sinapic              | OCH <sub>3</sub> | OH             | OCH <sub>3</sub> |

Polifenoli

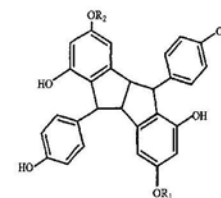


| Flavanoli                                | R <sub>1</sub> | R <sub>2</sub> | R <sub>3</sub> |
|--|----------------|----------------|----------------|
| Trans-resveratrol                        | H              | H              | H              |
| Trans-resveratrol-3-O-glucoside (piceid) | H              | glc            | H              |
| Trans-resveratrol-2-C-glucoside          | H              | H              | glc            |
| Trans-astringin                          | OH             | glc            | H              |

Non Flavonoidi



| Stilbeni                      | R <sub>1</sub> | R <sub>2</sub> |
|-------------------------------|----------------|----------------|
| Trans-p-Viniferin-diglucoside | glc            | glc            |



| Dimeric stilbene          | R <sub>1</sub> | R <sub>2</sub> |
|---------------------------|----------------|----------------|
| Pallidol                  | H              | H              |
| Pallidol-3-O-glucoside    | glc            | H              |
| Pallidol-3,3'-diglucoside | glc            | glc            |

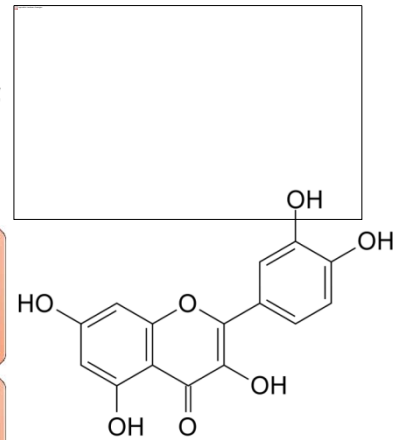
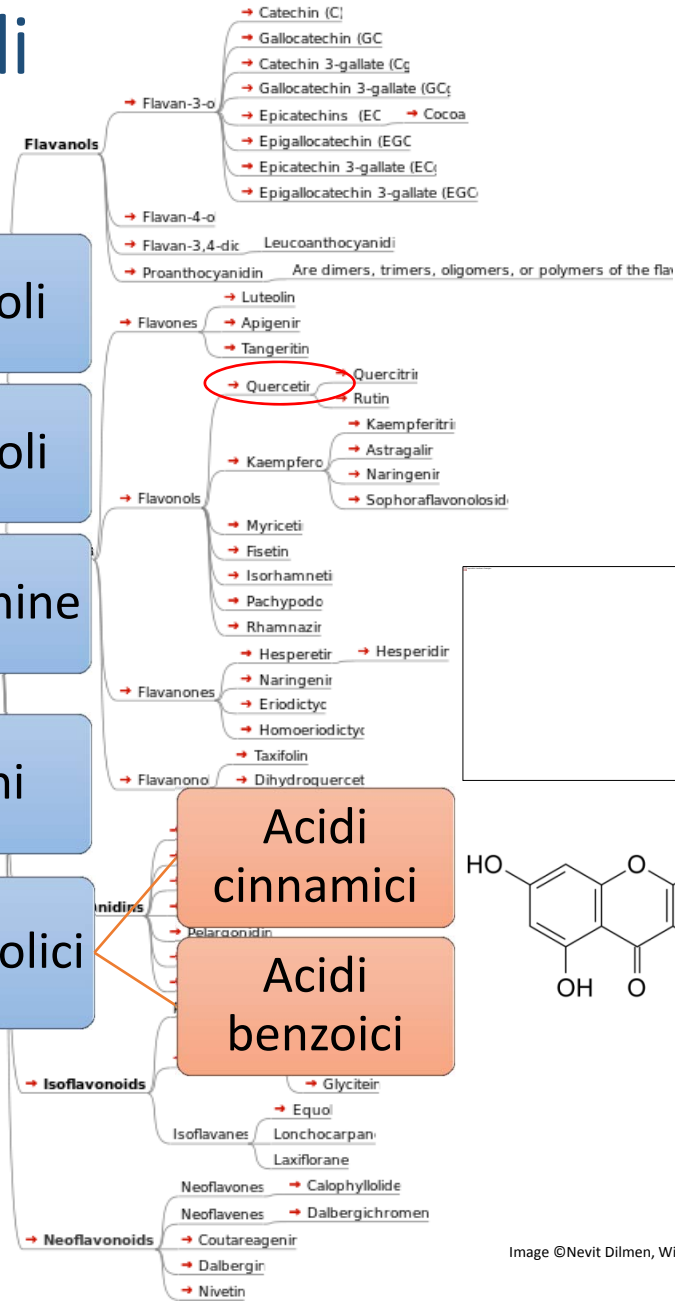
Flavanoli

Flavonoli

Antocianine

Stilbeni

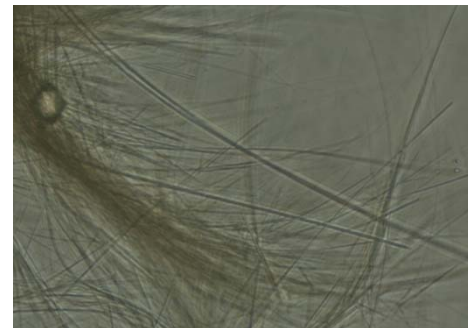
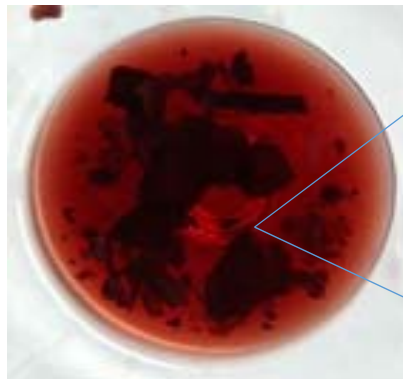
Acidi fenolici



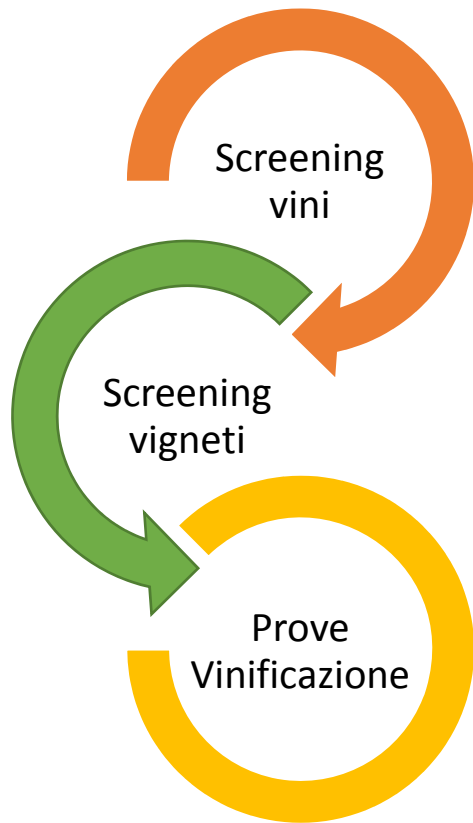
# Flavonoli nel Sangiovese



- Uve: netta prevalenza di glucoside della Quercetina, fino a 200 mg/kg
- Rilascio durante la macerazione, fino al 50% e più del contenuto
- Idrolisi del glucoside → rilascio dell'aglicone (prima soprattutto enzimatica e poi solo chimica)
- Precipitazione dell'aglicone, attraverso un lento accrescimento degli aggregati (microfiltrabili → n/microfiltrabili → precipitabili)



# Progetto QUESTAB

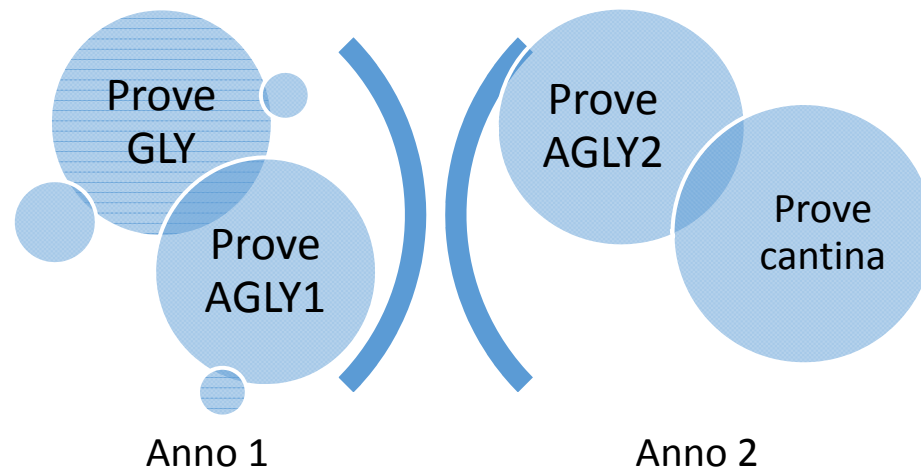


**COL D'ORCIA MONTALCINO** **ISV EA** **CONSORZIO DEL SANGIOVESE DI MONTALCINO**

## QUERCETINA & VINO

UNA QUALITA' DEL SANGIOVESE PER IL CONSUMATORE,  
UNA SFIDA TECNOLOGICA PER L'ENOLOGO

Venerdì 15 giugno 2018, ore 9.00 • Teatro Municipale di Montalcino



## 1. IFTR-UV-Vis

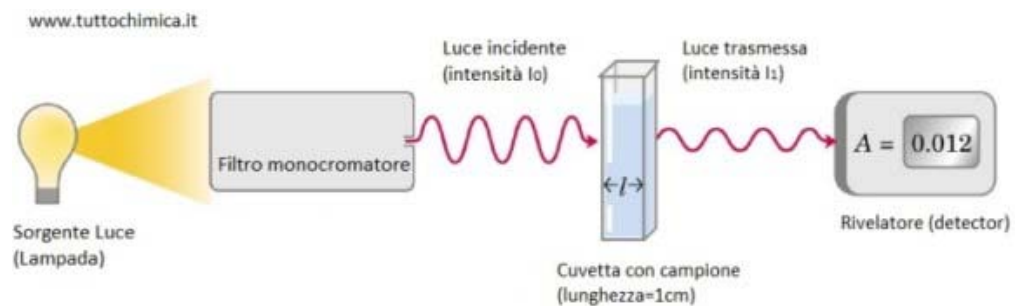
- Grado alcolico, zuccheri (Glucosio+Fruttosio), densità, dry extract.
- Acidità totale, pH, acidità volatile.
- Acidi organici: malico, lattico, tartarico, gluconico, succinico, citrico.



## 2. Solforose: OIV MA-AS323-04B

## 3. AWRI: fenoli totali, tannini, antociani

## 4. Determinazione caratteristiche cromatiche: trasmittanza (CIELab) e assorbanza UV-Vis



5. HPLC-PDA: analisi antociani e flavonoli
6. HPLC-HRMS: profilo polifenoli non antocianici

## Parametri Cromatografici

Colonna: Kinetex 1.7  $\mu\text{m}$  F5 100° 100\*2.1mm  
T colonna: 40°C  
Flusso: 0.4 ml/min  
V iniezione: 3  $\mu\text{l}$   
Fase mobile A: AcquaMQ + Acido formico 0.05%+  
5mM formiato Ammonio  
Fase mobile B: Metanolo + Acido formico 0.05%+  
5mM formiato Ammonio  
Durata corsa cromatografica: 45 minuti



## Parametri Massa

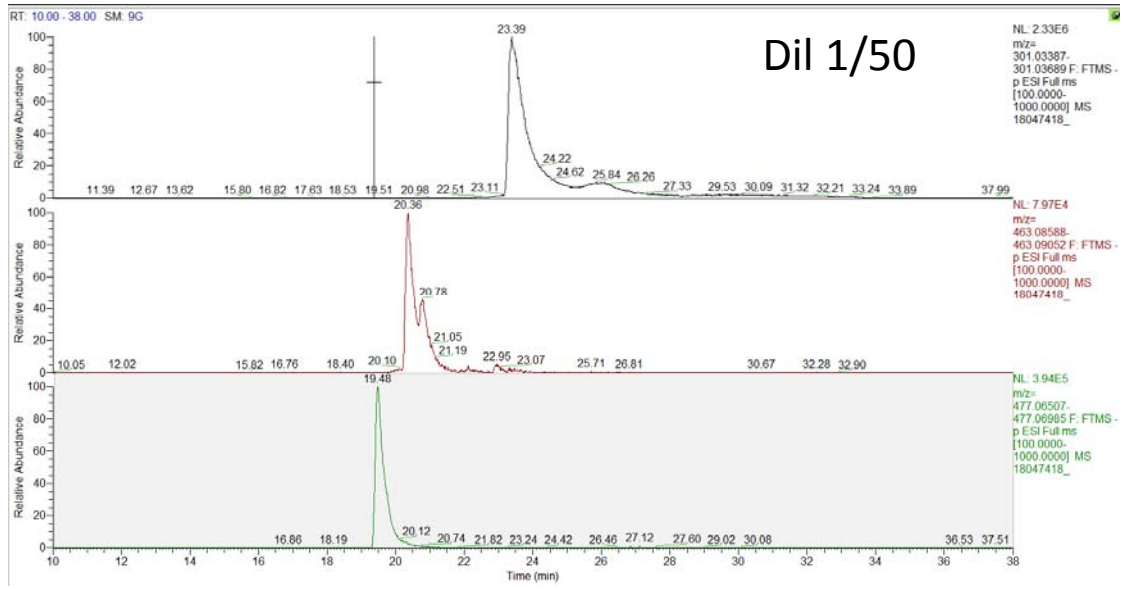
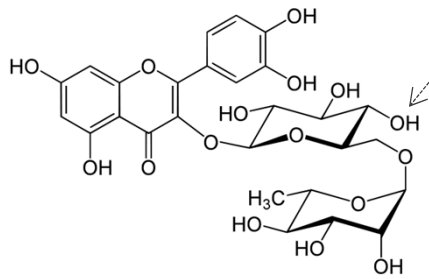
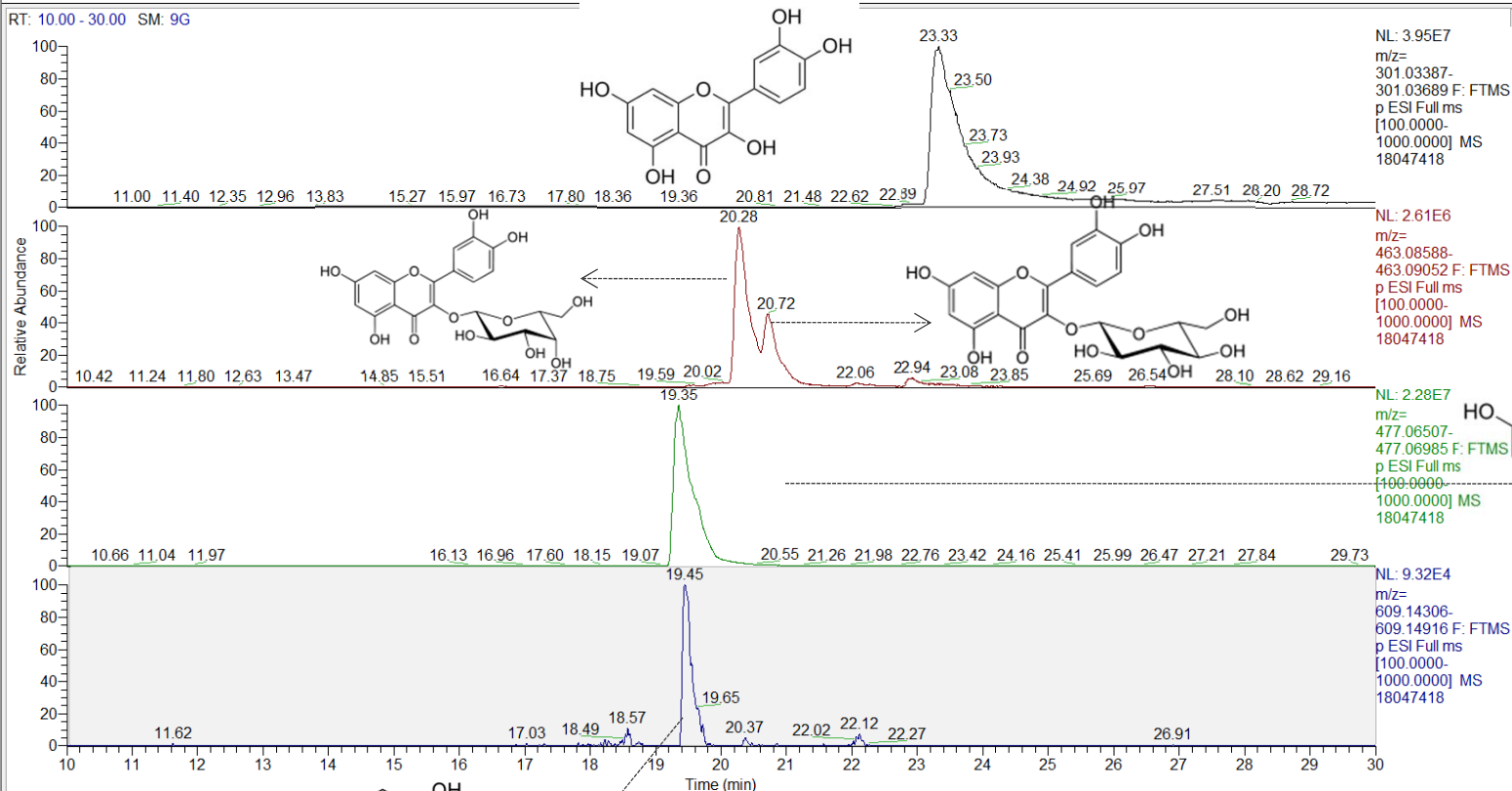
Spray voltage : 3500 V  
Sheath gas: 40 arb  
Aux gas: 5 arb  
Sweep gas: 0 arb  
Capillary temperature: 320 °C  
Heater temperature: 150 °C  
RF-lens level: 60

|           |  |
|-----------|--|
| Full Scan | Resolution 70,000 (FWHM) at m/z 200<br>Mass range 150–1000 m/z |
|-----------|--|

|       |   |
|-------|---|
| ddMS2 | Resolution 17,500 (FWHM) at m/z 200<br>Isolation windows 4.0 m/z<br>NCE: 30 |
|-------|---|

Polarità: Negativa  
Inclusion list: ON

# QUERCETINA



# Polifenoli

## Metodo Quantificazione TF 4.1

Thermo TraceFinder EFS LC

File View Tools Help

Real time status | User: AB2 |

Analysis - Data Review - ML647\_20180322\_QStab [Quan]

Batch View

Samples

Auto Samples

Reference Sample

Threshold Samples

Data Review

Sample View

Compound View

Comparative View

Report View

Local Method

Acquisition

Quantitation

Processing

Compounds

QAQC

Groups

Intel Seq

Reports

Acquisition

Analysis

Method Development

Compounds

| Flags | Compound                  | RT    | Type            |
|-------|---------------------------|-------|-----------------|
| 19    | Caffeic acid              | 4.20  | Target Compound |
| 20    | Cafutaric acid            | 1.26  | Target Compound |
| 21    | Cafutaric acid (cis)      | 1.25  | Target Compound |
| 22    | Coumaric acid             | 6.80  | Target Compound |
| 23    | Glutathione               | 1.00  | Target Compound |
| 24    | Kaempferol-3-O-glucoside  | 22.90 | Target Compound |
| 25    | Kaempferol                | 26.46 | Target Compound |
| 26    | Procyanidin B 1           | 7.50  | Target Compound |
| 27    | Procyanidin B 2           | 4.00  | Target Compound |
| 28    | Quercetin                 | 24.50 | Target Compound |
| 29    | Quercetin 3-O-galattoside | 21.10 | Target Compound |
| 30    | Quercetin 3-O-glucoside   | 21.45 | Target Compound |
| 31    | Quercetin-3-O-glucuronide | 20.50 | Target Compound |
| 32    | Resveratrol               | 21.20 | Target Compound |
| 33    | Rutin                     | 22.00 | Target Compound |

Sample Results

| Sample Type | Status  | Conn | Sample ID | Peak L | Sele | Filename | Type   | Level | Area        | RT    | Actual RT | RT Delta | Calculate |
|-------------|---------|------|-----------|--------|------|----------|--------|-------|-------------|-------|-----------|----------|-----------|
| 1           | Cal Std |      | 50ppb     | T1     |      | A        | Sample | A     | 25 1604847  | 21.45 | 21.42     | -0.03    | 0.052     |
| 2           | Cal Std |      | 250       | T1     |      | C        | Sample | C     | 26 4035392  | 21.45 | 21.37     | -0.08    | 0.232     |
| 3           | Cal Std |      | 500       | T1     |      | D        | Sample | D     | 27 7769733  | 21.45 | 21.34     | -0.11    | 0.509     |
| 4           | Cal Std |      | 1000      | T1     |      | E        | Sample | E     | 28 14476846 | 21.45 | 21.42     | -0.03    | 1.006     |
| 5           | Unknown |      |           | T1     |      | bianco2  | Sample |       | 1 343       | 21.45 | 21.53     | 0.08     | -0.067    |
| 6           | Unknown |      |           | T1     |      | bianco3  | Sample |       | 2 7129      | 21.45 | 21.37     | -0.08    | -0.066    |
| 7           | Unknown |      |           | T1     |      | CQc      | Sample |       | 3 4753693   | 21.45 | 21.44     | -0.01    | 0.286     |
| 8           | Unknown |      |           | T1     |      | CQd      | Sample |       | 4 7383485   | 21.45 | 21.44     | -0.01    | 0.481     |
| 9           | Unknown |      |           | T1     |      | 18019537 | Sample |       | 5 65819431  | 21.45 | 21.45     | 0.00     | 4.811     |
| 10          | Unknown |      | 1/50      | T1     |      | 18019537 | Sample |       | 6 1898559   | 21.45 | 21.45     | 0.00     | 0.074     |
| 11          | Unknown |      |           | T1     |      | 18019538 | Sample |       | 7 51818321  | 21.45 | 21.43     | -0.02    | 3.773     |
| 12          | Unknown |      | 1/50      | T1     |      | 18019538 | Sample |       | 8 1075719   | 21.45 | 21.46     | 0.01     | 0.013     |
| 13          | Unknown |      |           | T1     |      | 18019539 | Sample |       | 9 65025438  | 21.45 | 21.41     | -0.04    | 4.752     |
| 14          | Unknown |      | 1/50      | T1     |      | 18019539 | Sample |       | 10 1305843  | 21.45 | 21.50     | 0.05     | 0.030     |

Compound Details

Quercetin 3-O-glucoside m/z: 463.08820

RT: 21.41  
AA: 65025438  
AH: 3310901

Relative Intensity vs RT(min)

Isotope

All Isotopes

- #1: 463.08820
- #2: 464.09159
- #3: 465.09375
- #4: 466.09639
- #5: 467.09876

Scan #: 4683-4823 RT: 21.24 - 22.25 AV: 18019539

F: FTMS - p ESI Full ms [100.0000-1000.0000]

Relative Intensity vs m/z

Fragments

All Fragments

- #1: 301.03589
- #2: 300.02800
- #3: 271.02561
- #4: 178.99779
- #5: 151.00271

Minimum # of fragments needed: 1

Scan #: 4722 RT: 21.42

F: FTMS - p ESI d Full ms2 463.0898@hcd3...

Intensity vs m/z

Calibration Curve

Quercetin 3-O-glucoside

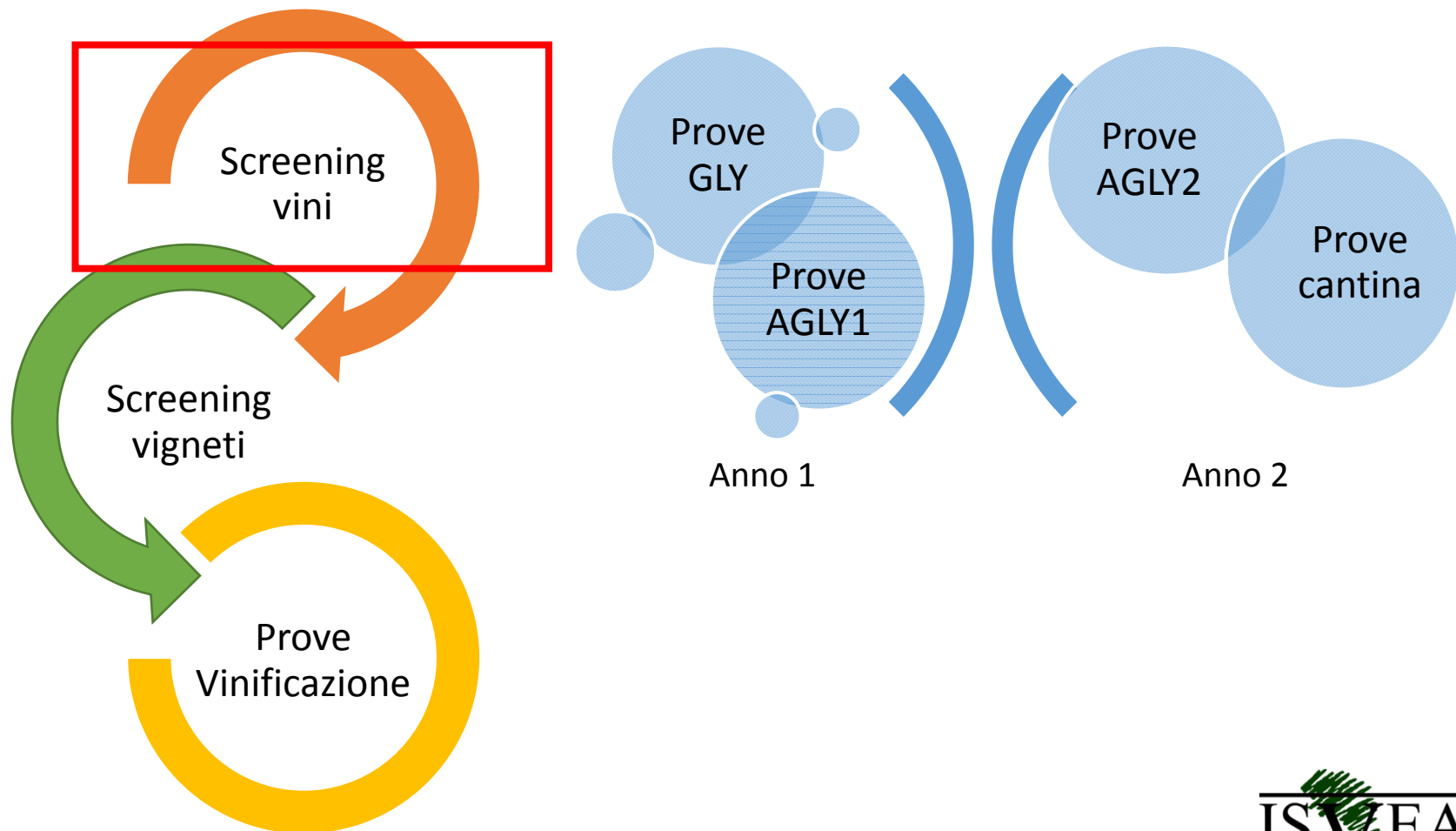
Y = 1.349e7X + 8.991e5; R<sup>2</sup>: 0.9987; Origin: Ignore; W: 1/X; Area

Area vs ppm

33 0 Isorhamnetin Target Compound External Area Linear Ignore 1/X ppm

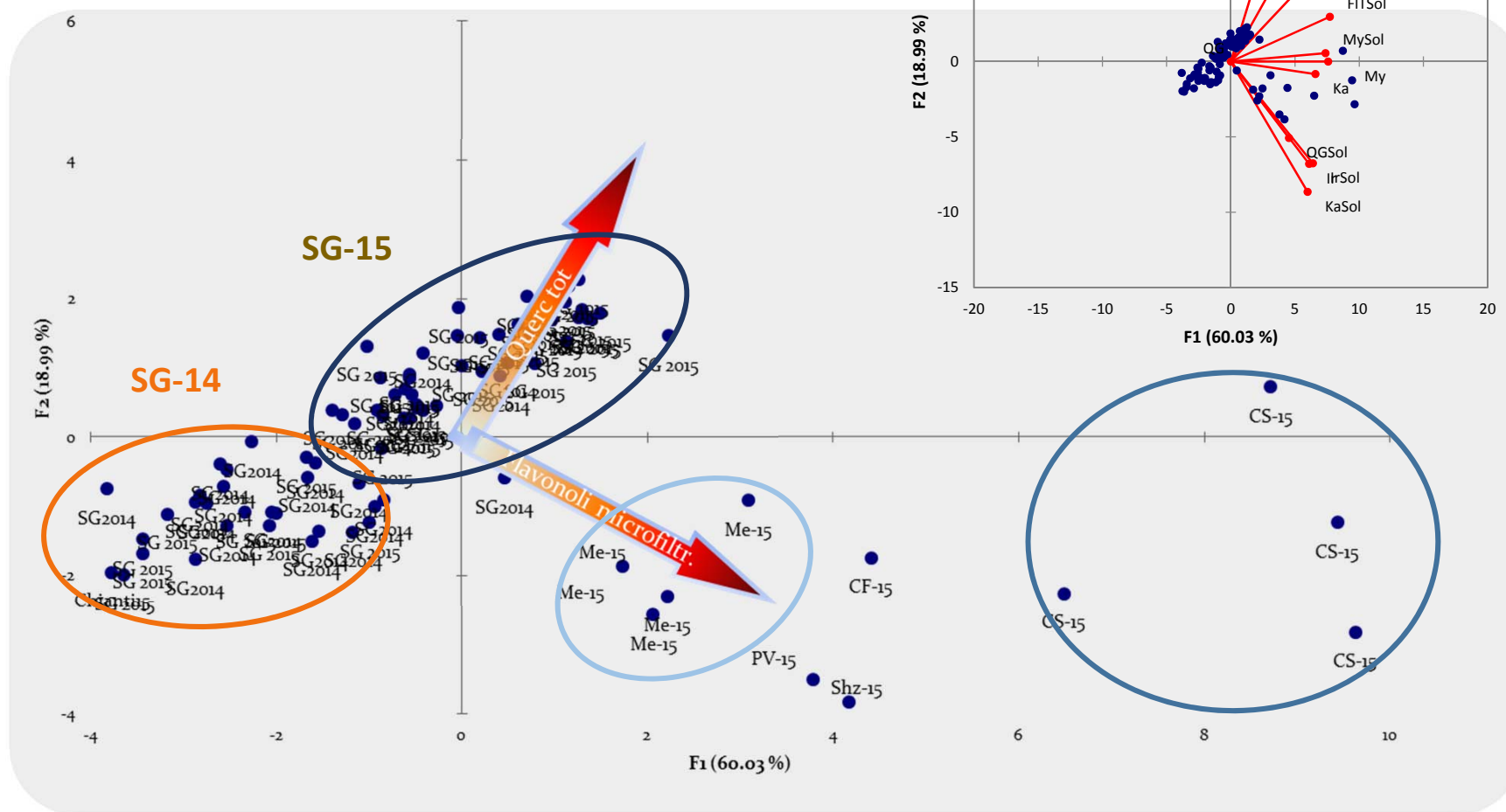


# Progetto QUESTAB

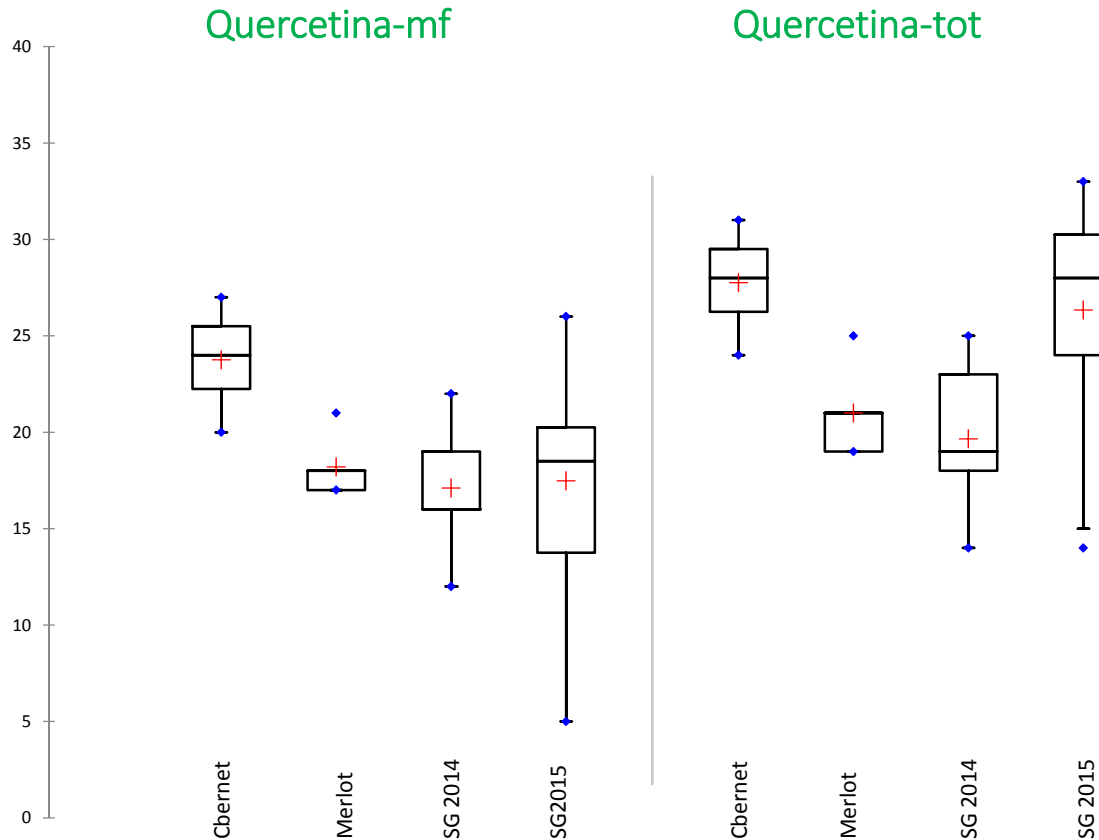


# Screening analitico vini della cantina

Flavonoli (totali e microfiltrabili)



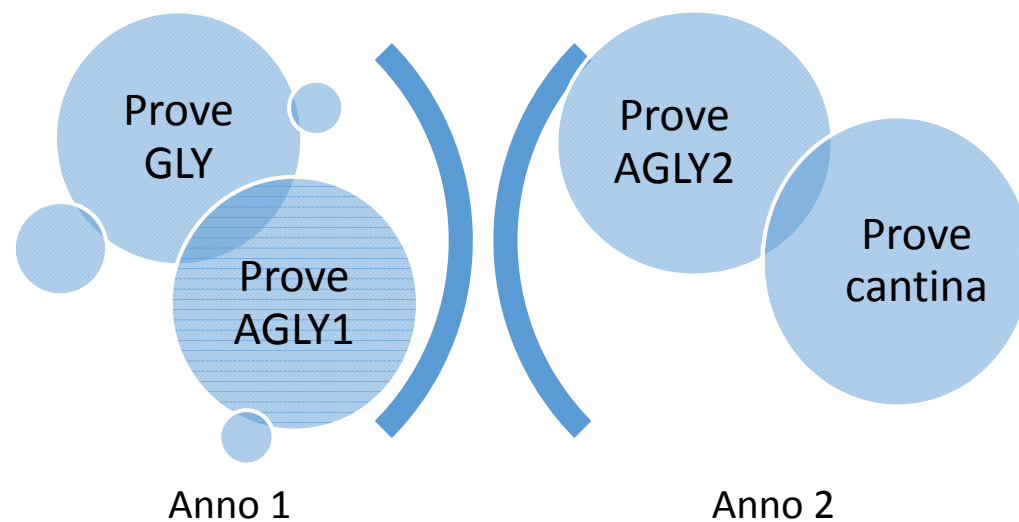
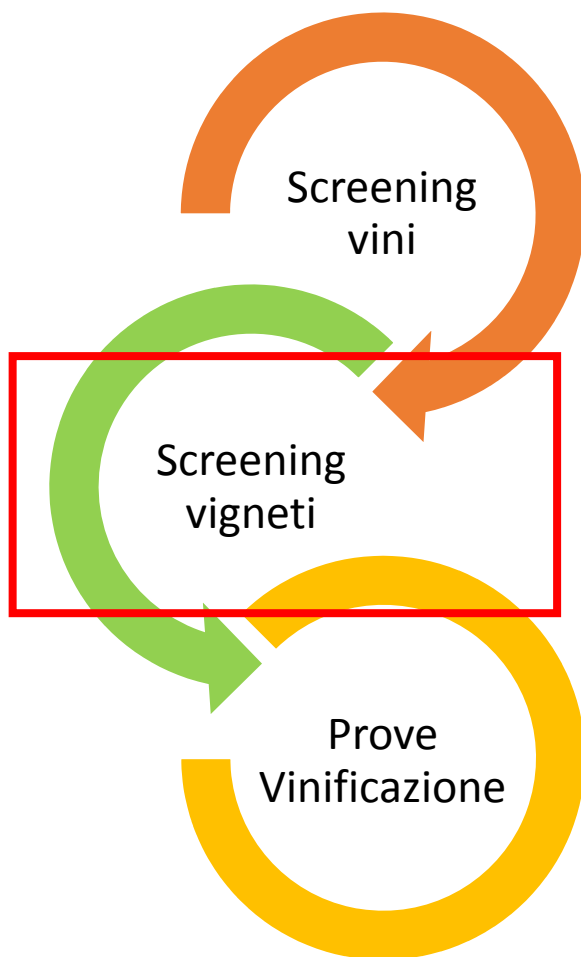
# Screening analitico vini della cantina



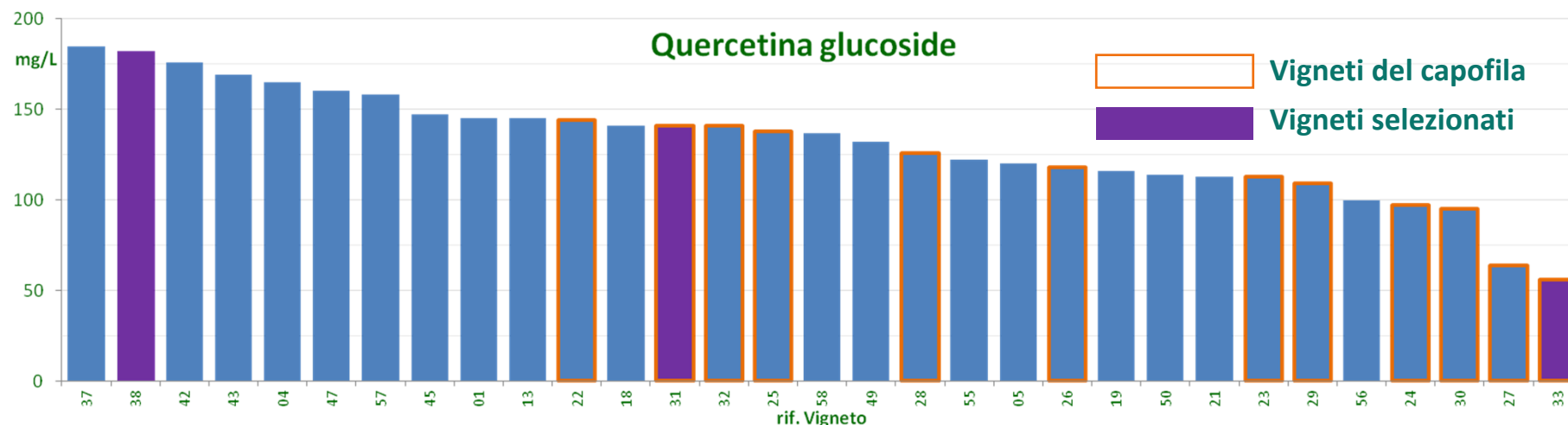
|                | Q-mf        | Q-tot       |
|----------------|-------------|-------------|
| Tutti campioni | 18<br>5-27  | 24<br>13-35 |
| Cabernet       | 24<br>20-27 | 28<br>24-31 |
| Merlot         | 18<br>17-21 | 21<br>19-25 |
| SG-2015        | 17<br>12-22 | 20<br>14-25 |
| SG-2014        | 17<br>5-26  | 26<br>14-33 |

SG 2014: mediam. 33% quercetina aglicone NON microfiltrabile;  
 SG 2015: mediam. 50% quercetina aglicone NON microfiltrabile;  
 ME 2015: mediam. 10% quercetina aglicone NON microfiltrabile;  
 CS 2015: mediam. 15% quercetina aglicone NON microfiltrabile;

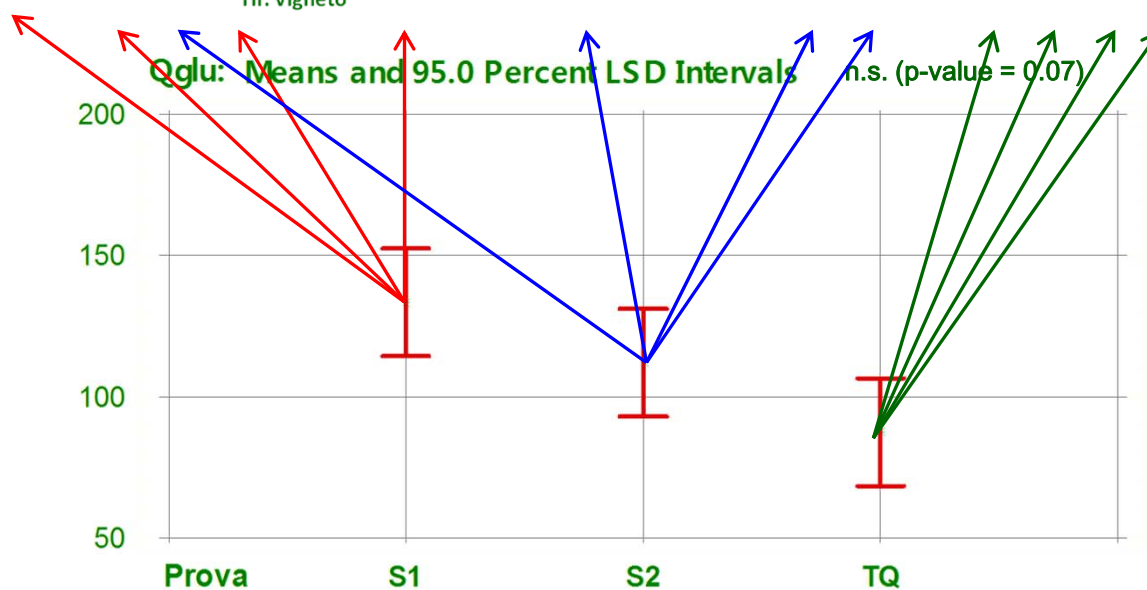
# Progetto QUESTAB



# Screening Vigneti

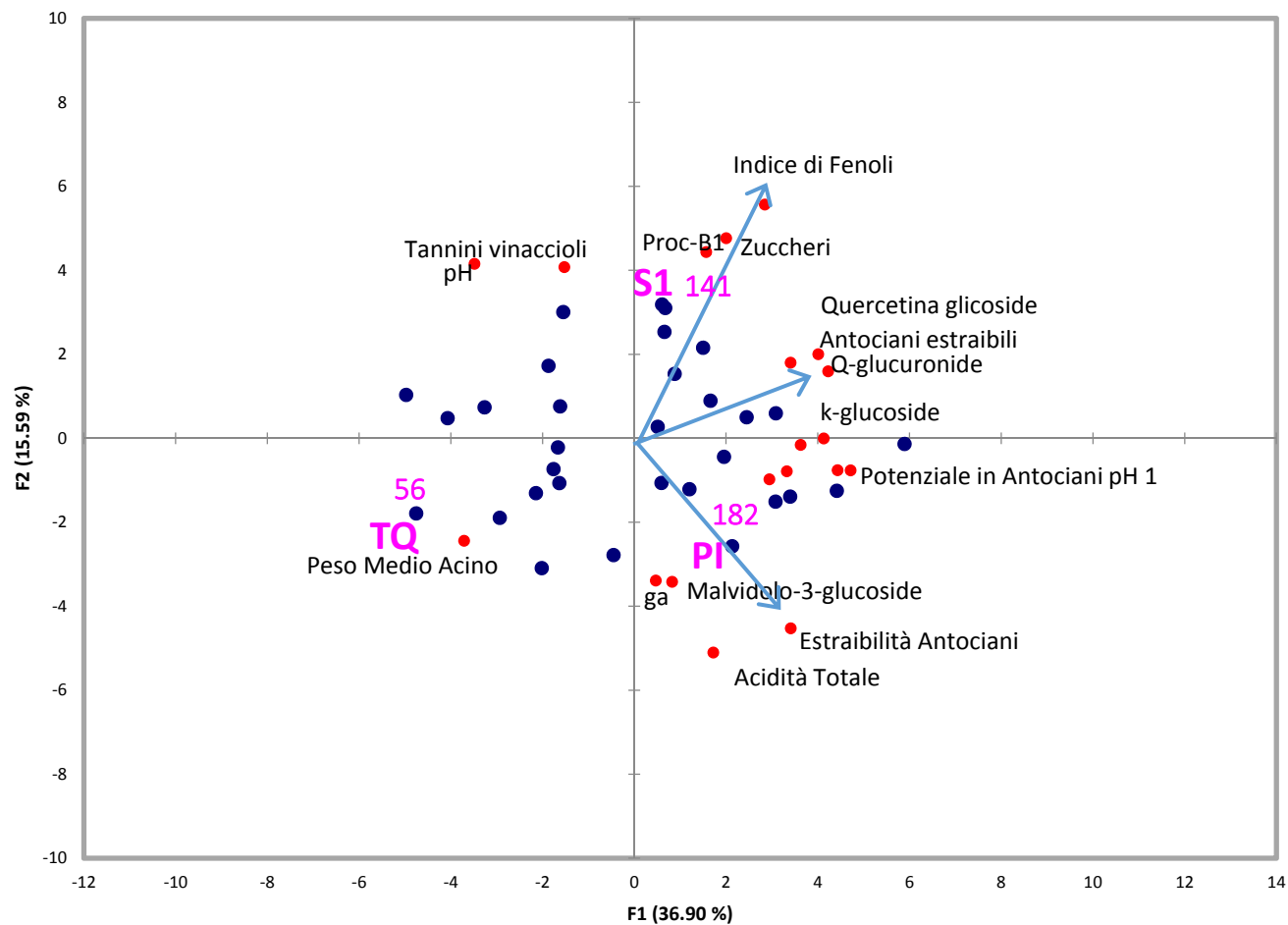


vigne capofila:  
 S1 = Sfogliatura precoce  
 S2 = Sfogliatura invaiatura  
 TQ = Testimone

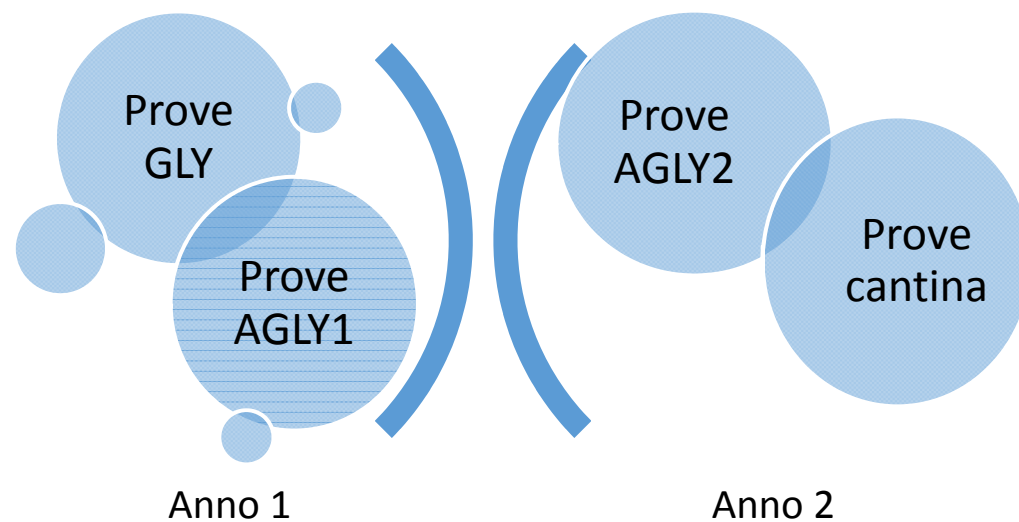
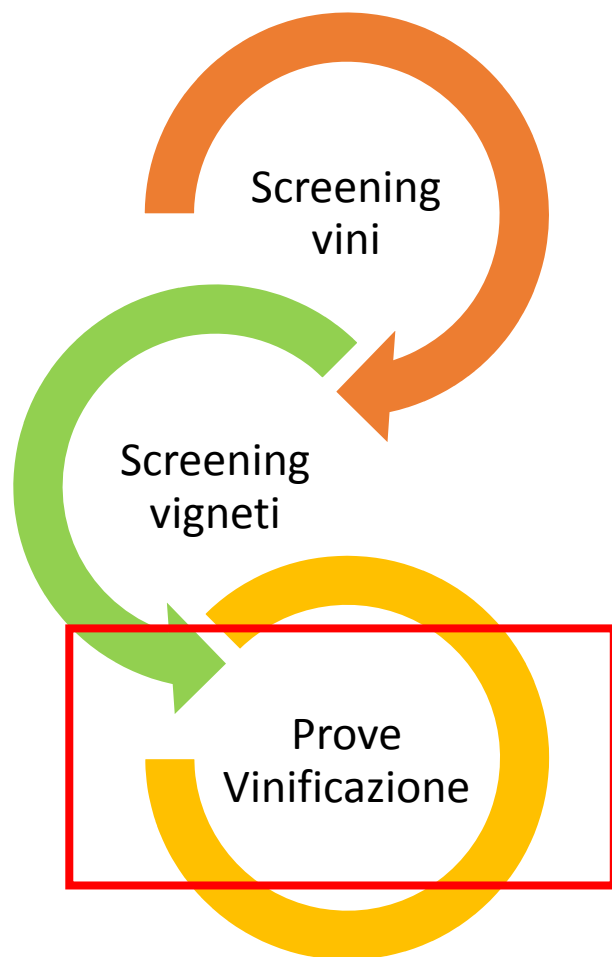


## Analisi PCA

# Screening Vigneti



# Progetto QUESTAB



# Vinificazioni sperimentali

## Vigneti

- **PI** (n°38): versante NNE, argilla/tufo, esposiz. E, quota media mt 220 slm, prod. 60 q/ha; Qg\* molto elevata
- **S1** (n°31): versante S, sabbia/limo, esposiz. SW, quota media mt 350 slm, prod. 60 q/ha; Qg\* medio-alta
- **TQ** (n°33): come S1 ma non defogliato; Qg\* molto bassa

\*) Qg verificato circa una settimana prima della vendemmia

## Obiettivi

- Descrivere l'evoluzione della quercetina in considerazione della composizione delle uve
- Descrivere gli effetti di diverse strategie di vinificazione
- Ottenere vini con diversa composizione da utilizzare in prove successive

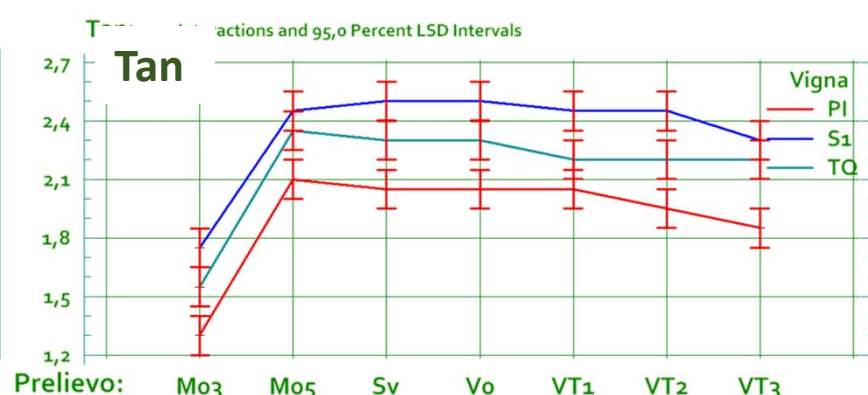
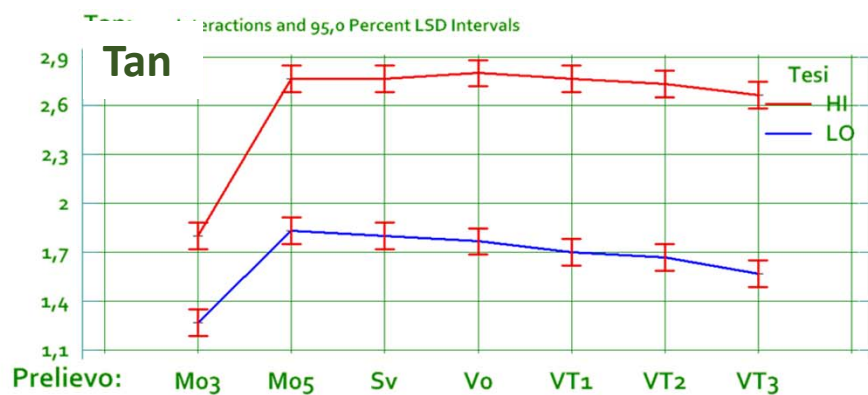
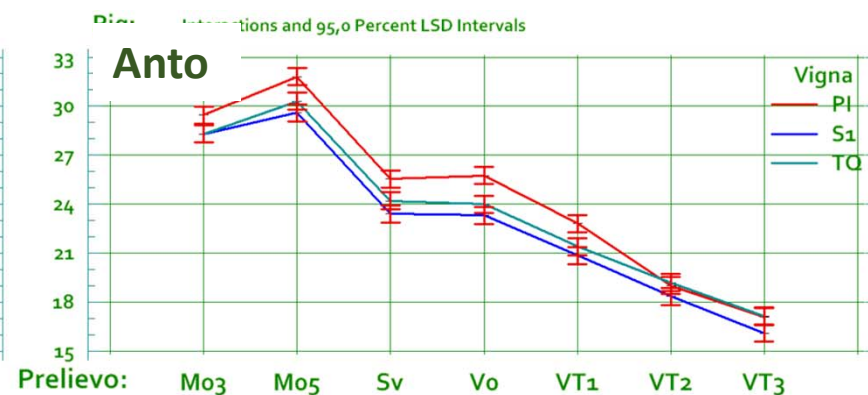
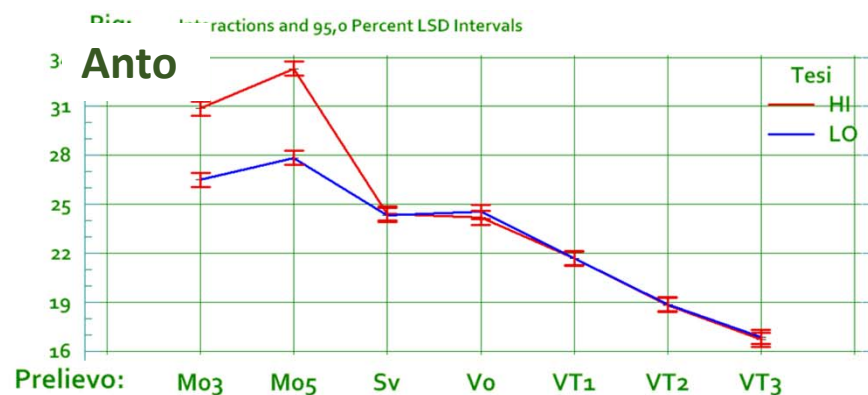


# Vinificazioni sperimentali

da ciascuna delle tre parcelle selezionate sono stati raccolti circa 2 q.li di uva suddivisi in 2 lotti vinificati secondo 2 modalità (LO ed HI)

| Variabili di processo   | prova LO | prova HI |
|-------------------------|----------|----------|
| Quantità di uva/prova   |          | Kg 85    |
| Solfitazione pigiato    |          | 30 mg/kg |
| Enzimi                  | -        | 2 g      |
| Lieviti                 |          | 30 g     |
| Coinoculo batteri (24h) |          | 2 g      |
| Nutrienti complessi     |          | 30 g     |
| T max                   | 22-25°C  | 30-32°C  |
| Durata macerazione      | 7 gg     | 14 gg    |

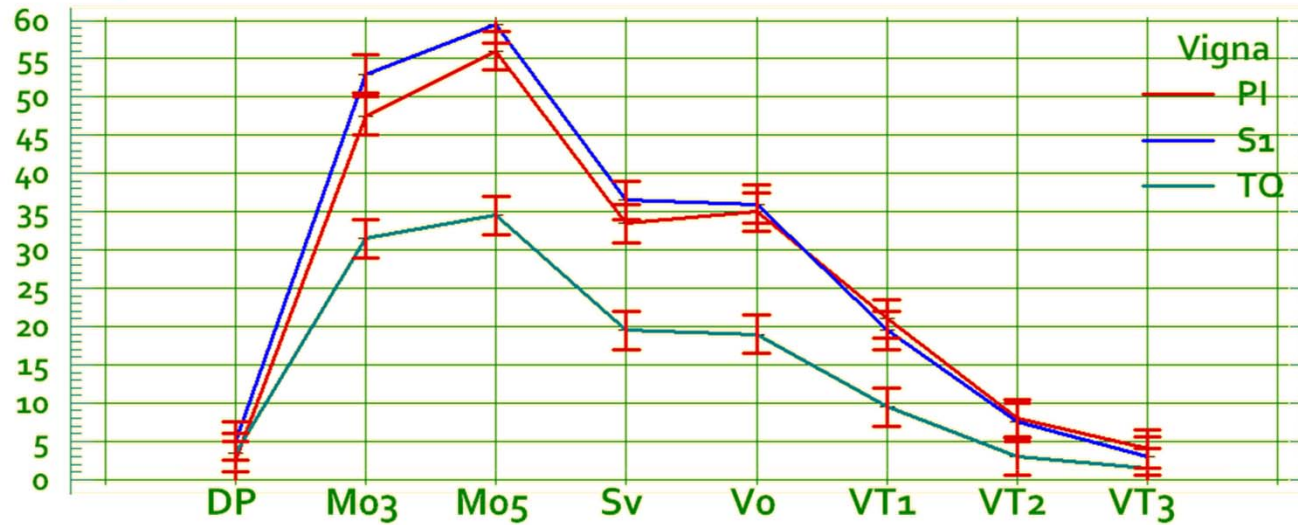
# Vinificazioni sperimentali



Effetto di tecnica di macerazione e vigneto su Tannini e Pigmenti

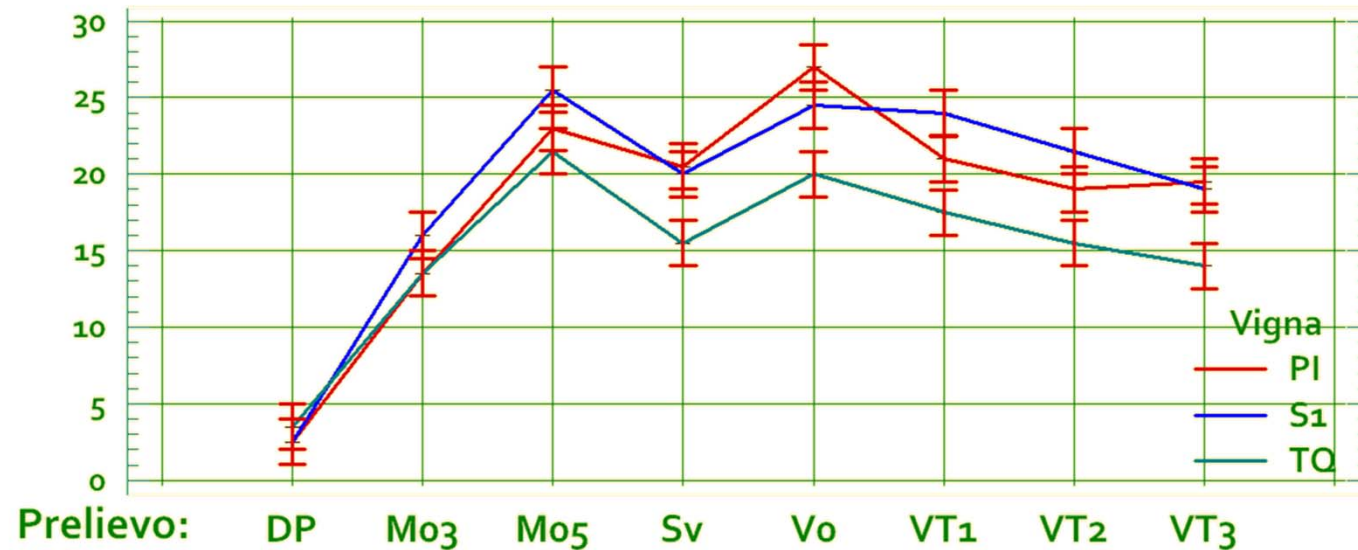
# Vinificazioni sperimentali

Qg: Interactions and 95,0 Percent LSD Intervals



Effetto del vigneto sulla quercetina

QUE: Interactions and 95,0 Percent LSD Intervals





Regione Toscana



Azienda Agricola



Unione Europea

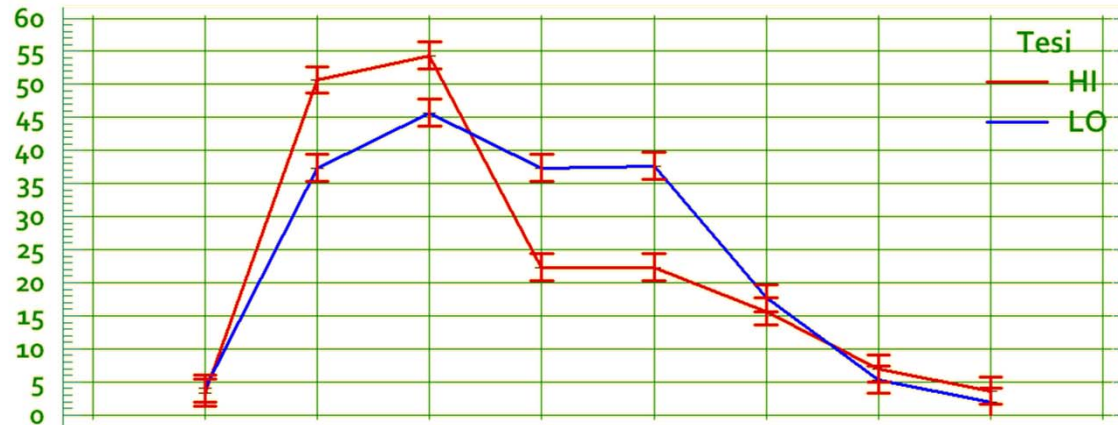


PSR  
Programma di Sviluppo Rurale



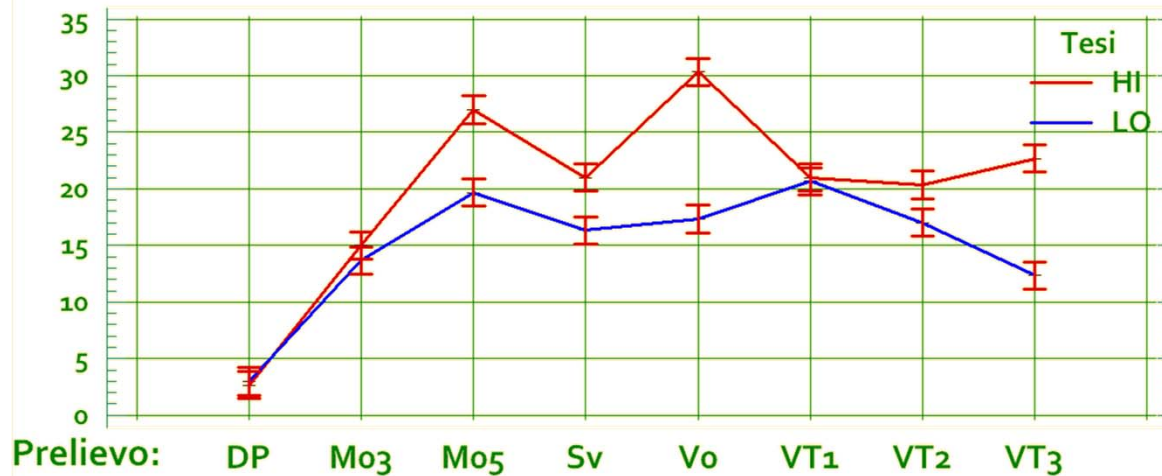
# vinificazioni sperimentali

Qg: Interactions and 95,0 Percent LSD Intervals

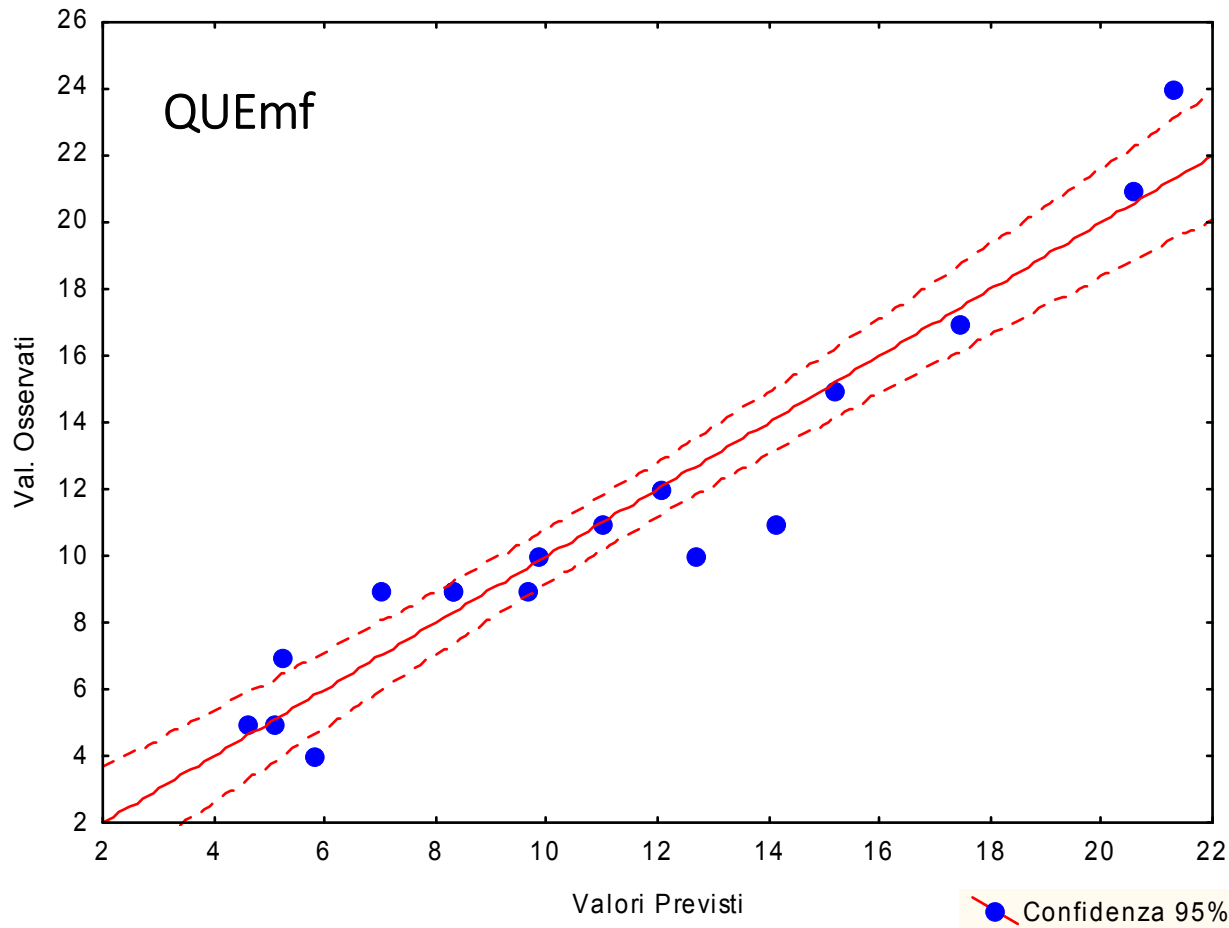


Effetto della tecnica di macerazione sulla quercetina

QUE: Interactions and 95,0 Percent LSD Intervals



# Vinificazioni sperimentali



Analisi Regressione  
Multipla

Analisi Correlazioni

|          | Que mf | Que-g mf |
|----------|--------|----------|
| A-liberi | -0,225 | 0,751    |
| T-Pigm   | 0,425  | -0,261   |
| Pigm     | 0,064  | 0,834    |
| Tann     | 0,475  | 0,213    |

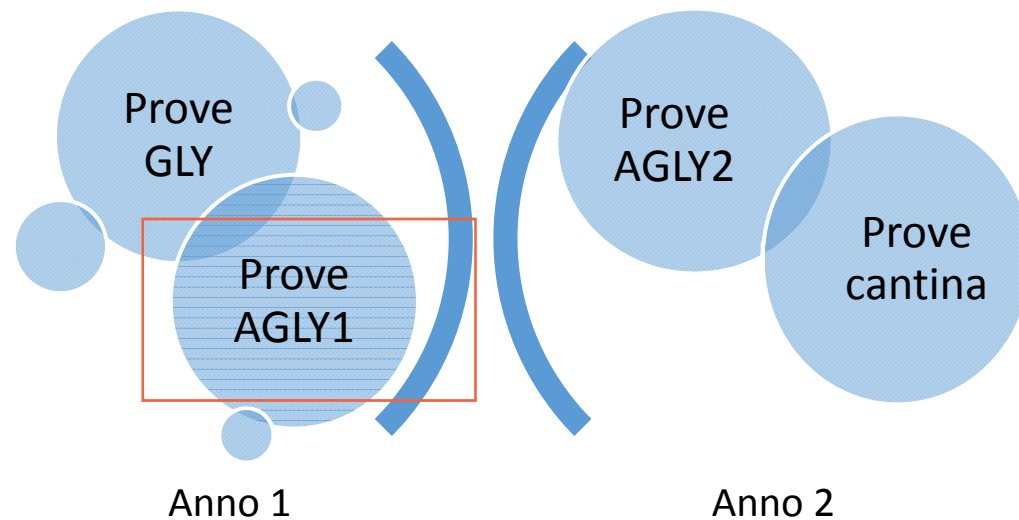
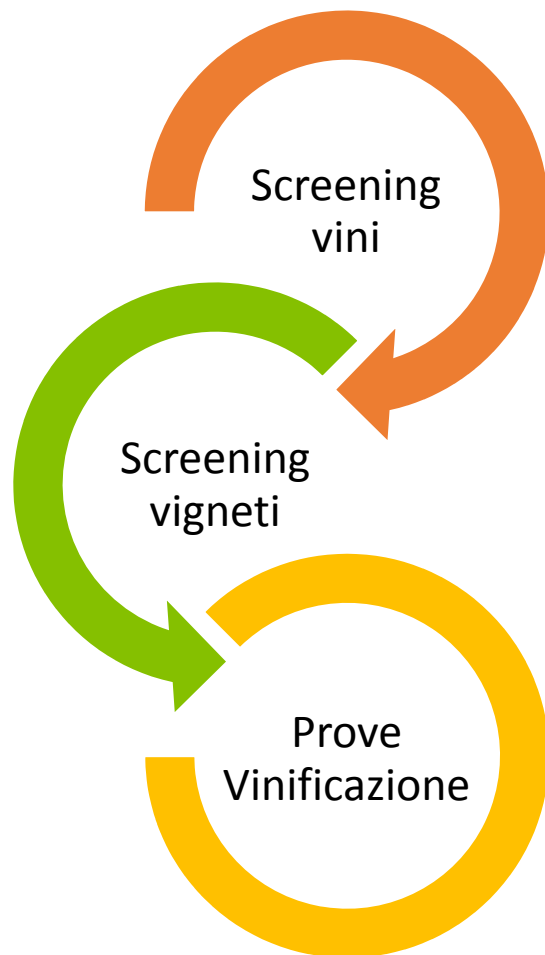
| Variabile | $\beta$ | $R^2$ | p        |
|-----------|---------|-------|----------|
| T-Pigm    | 2,7     | 0,967 | 0,000045 |
| Tannini   | -1,33   | 0,968 | 0,006614 |

# Vinificazioni sperimentali

Riassumendo.....

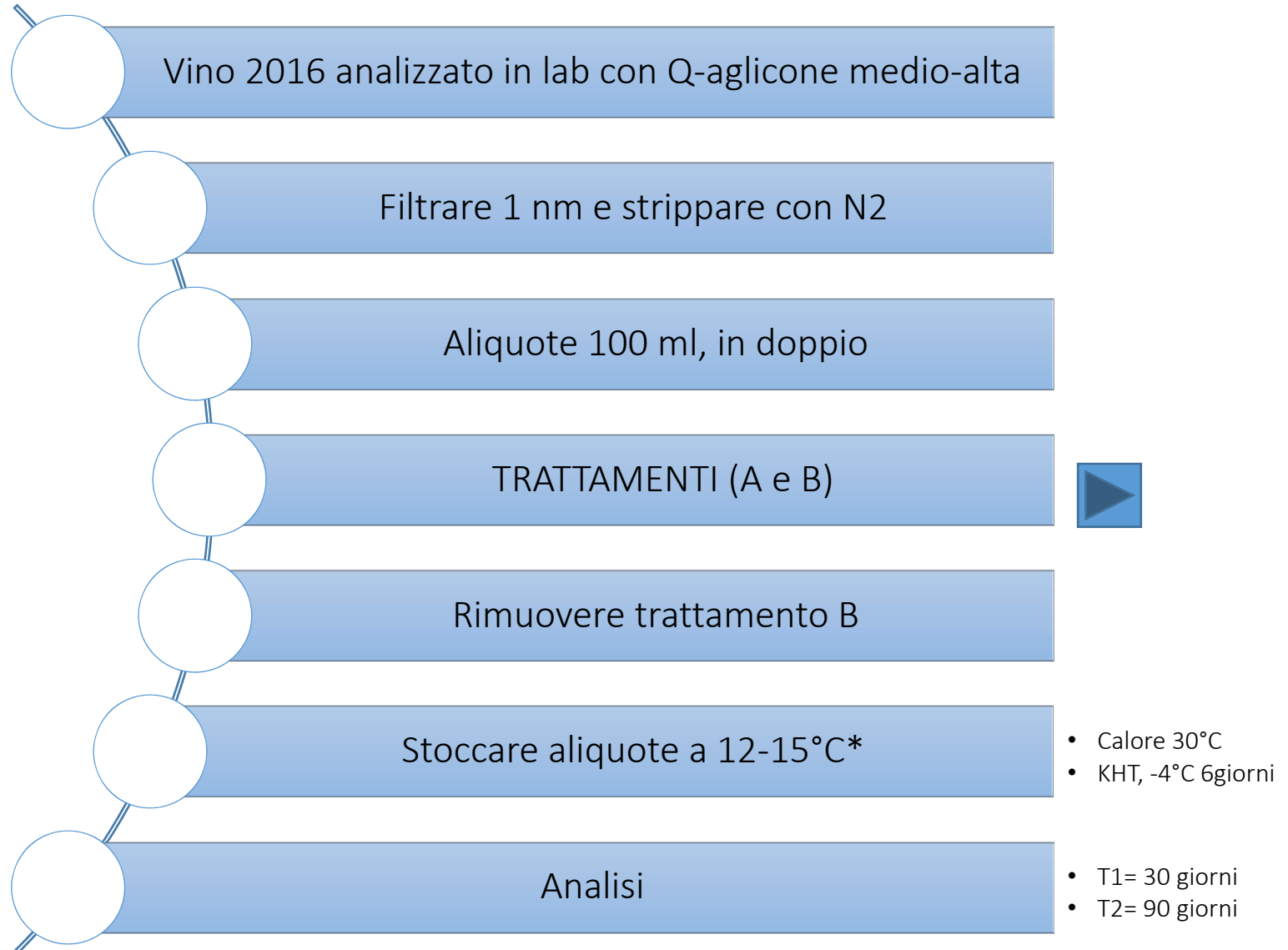
- Tecnica di vinificazione e vigneto non influiscono sul tenore di **antociani**
- Entrambi influiscono sui **tannini**
- **Quercetina-glicosilata**: no differenze tra vigneti e tesi di vinificazione al terzo travaso
- **Quercetina**: tenori di QUE diversi in funzione sia dei vigneti che della tecnica di vinificazione

# Progetto QUESTAB



# Prove AGLY 1

## Piano sperimentale





# Prove AGLY 1

## Tipo A

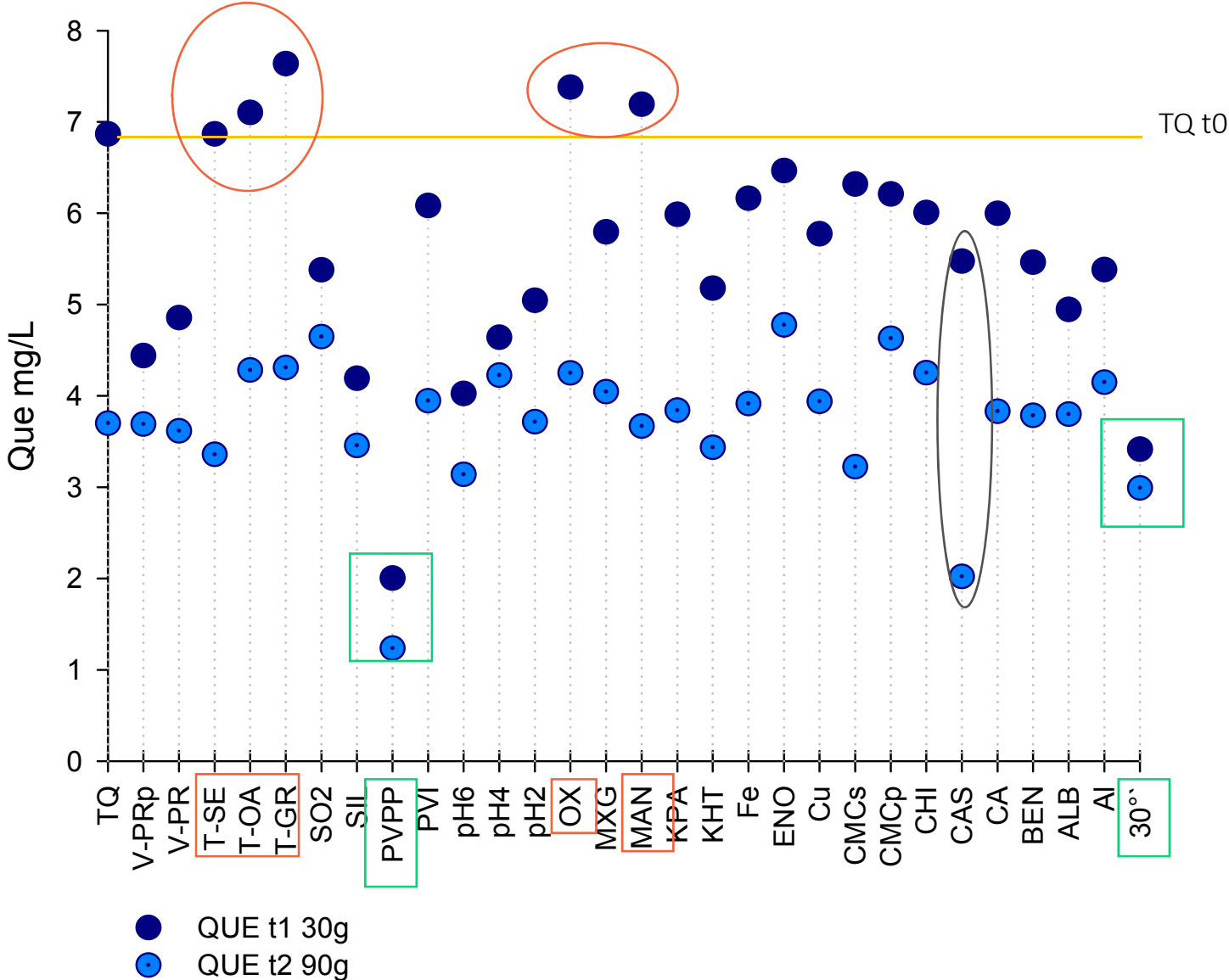
| codice | trattamento       | dose     |
|--------|-------------------|----------|
| TQ     | nessuno           |          |
| CHI    | Chitosano         | 100 g/hl |
| KPA    | Poliaspartato K   | 20 g/hl  |
| CMCs   | CMC stabilizzante | 10 g/hl  |
| T-GR   | Tannini uva       | 40 g/hl  |
| T-OA   | Tannini oak       | 40 g/hl  |
| ENO    | Enocianina        | 10ml/l   |
| CA     | Acido citrico     | 2 g/l    |
| Cu     | Cu                | 1,5 mg/l |
| Fe     | Fe                | 10mg/l   |
| Al     | Al                | 1,5 mg/l |
| 30°C   | Calore            | 30°C     |
| OX     | Ossigeno          | 8 mg/l   |

## Tipo B

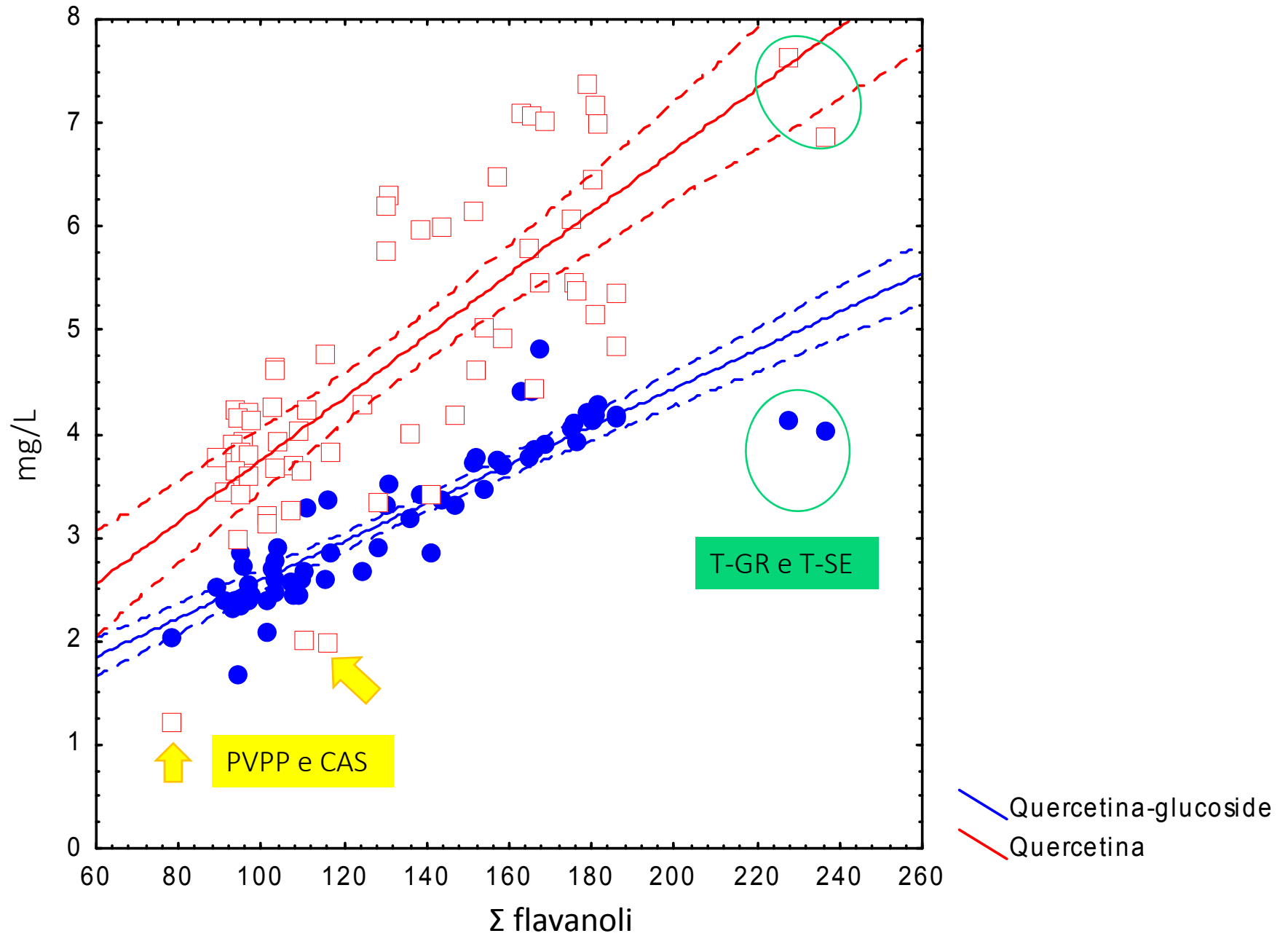
| codice | trattamento       | dose         |
|--------|-------------------|--------------|
| PVPP   | PVPP              | 100 g/hl     |
| PVI    | PVI               | 50 g/hl      |
| BEN    | Bentonite         | 100 g/hl     |
| SIL    | Silica gel        | 100 g/hl     |
| CAS    | Caseina           | 50 g/hl      |
| ALB    | Albumina          | 20 g/hl      |
| V-PR   | Vegetal proteins  | 50 g/hl      |
| KHT    | Cold KHT stabiliz | 100 mg/100ml |
| CMCp   | CMC precipitante  | 10 g/hl      |



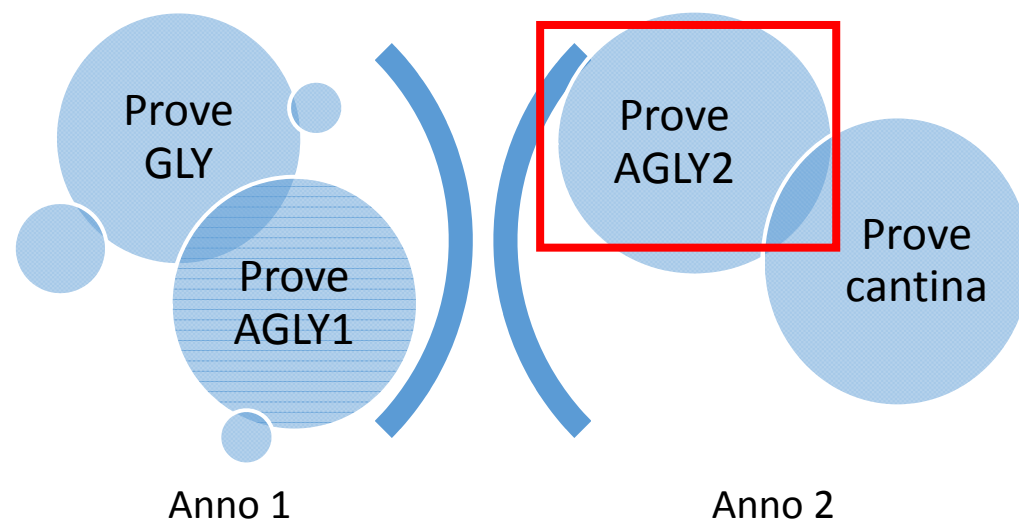
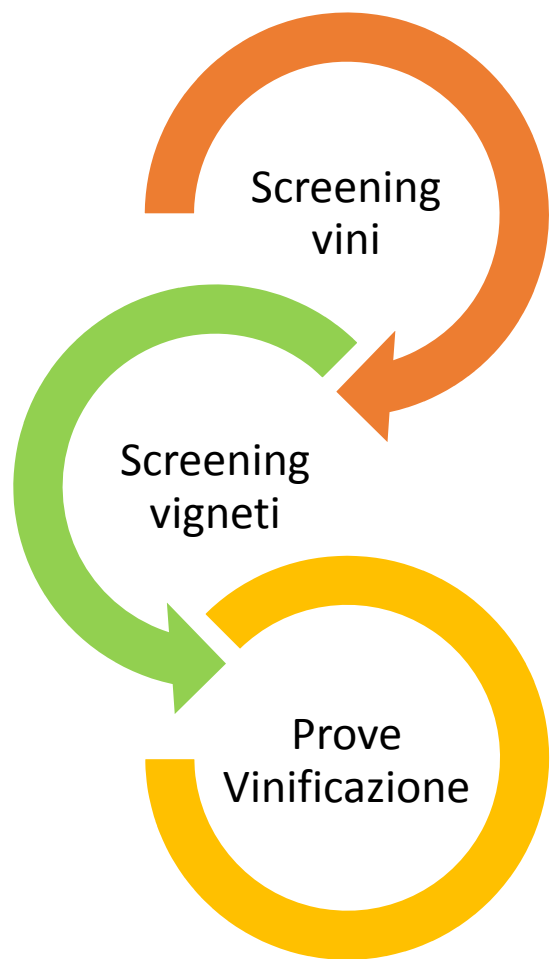
# Prove AGLY 1



# Prove AGLY 1



# Progetto QUESTAB



# Prove AGLY 2

## Piano sperimentale

| trattamento | Temperatura | Tempi (giorni) |
|-------------|-------------|----------------|
| TQ          | A           | 30             |
| CA          |             |                |
| PV          |             |                |
| OA          | H           | 60             |
| MP          | HC          |                |
| SL          |             |                |
| OASL        |             |                |

CA: caseina (30g/hl)

PV: PVPP (30g/hl)

OA: tannini quercia (20g/hl)

MP: mannoproteine (15g/hl)

SL: scorze di lievito (40g/hl)

OASL: tannini + scorze

A: ambiente

H: 5 giorni 30°C

HC: 5 giorni 30°C+ 7 giorni 18°C+ 6 giorni 4°C

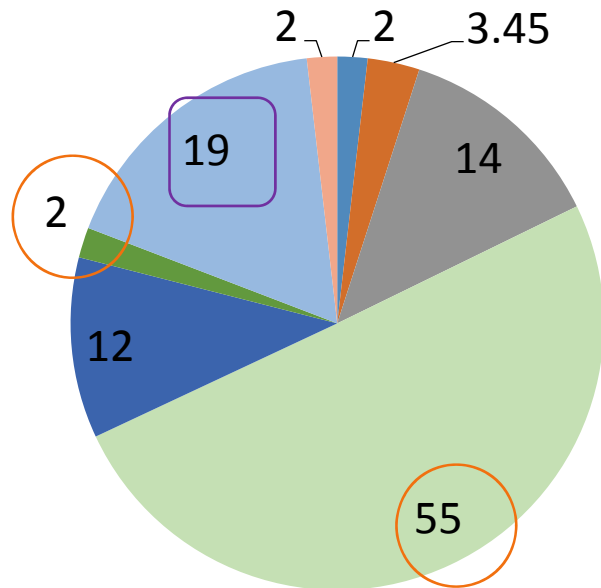


Analisi

# Prove AGLY 2

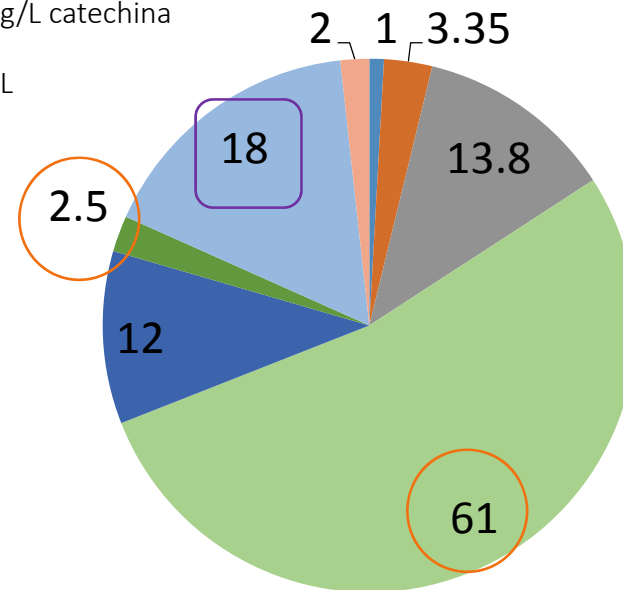
Confronto F vs G

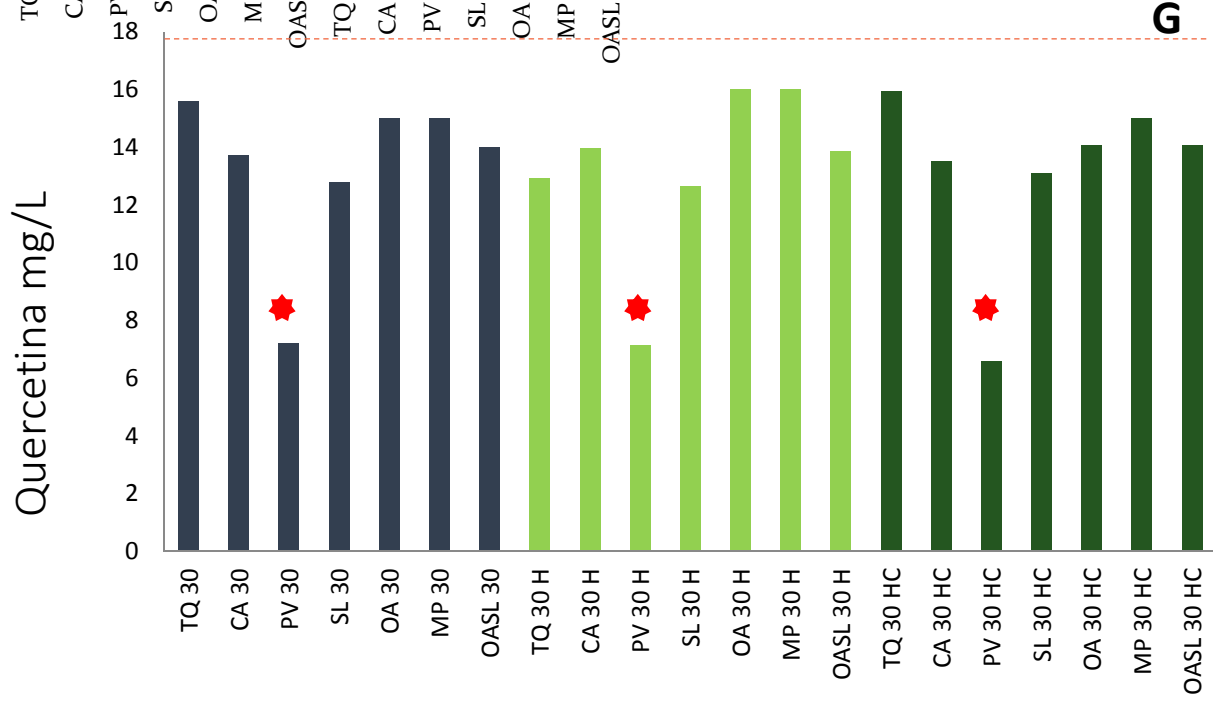
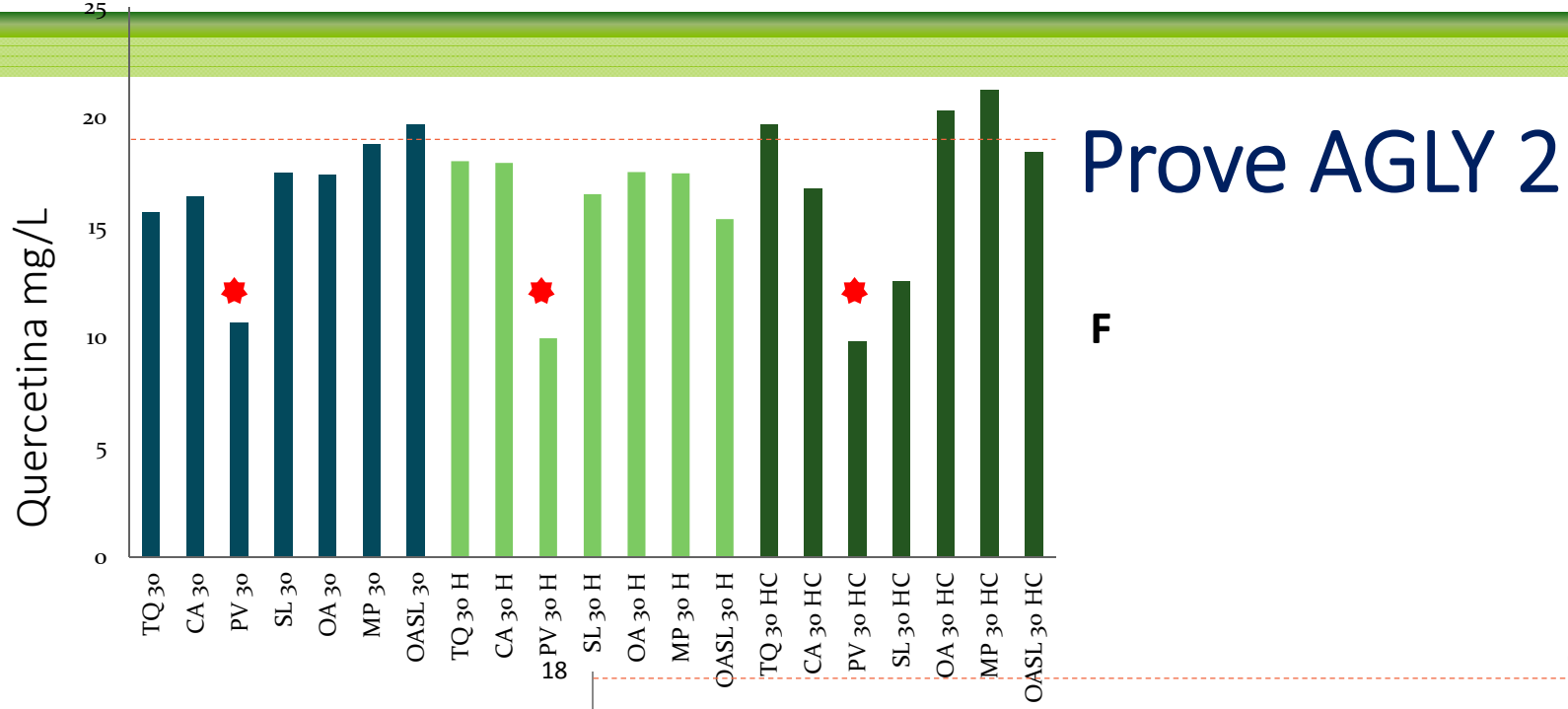
Campione F



- SO2 libera mg/L
- pH
- Titolo alcool % vol
- Fenoli tot U.A.
- pigmenti U.A.
- tannini g/L catechina
- Qmf mg/L
- Qg mg/L

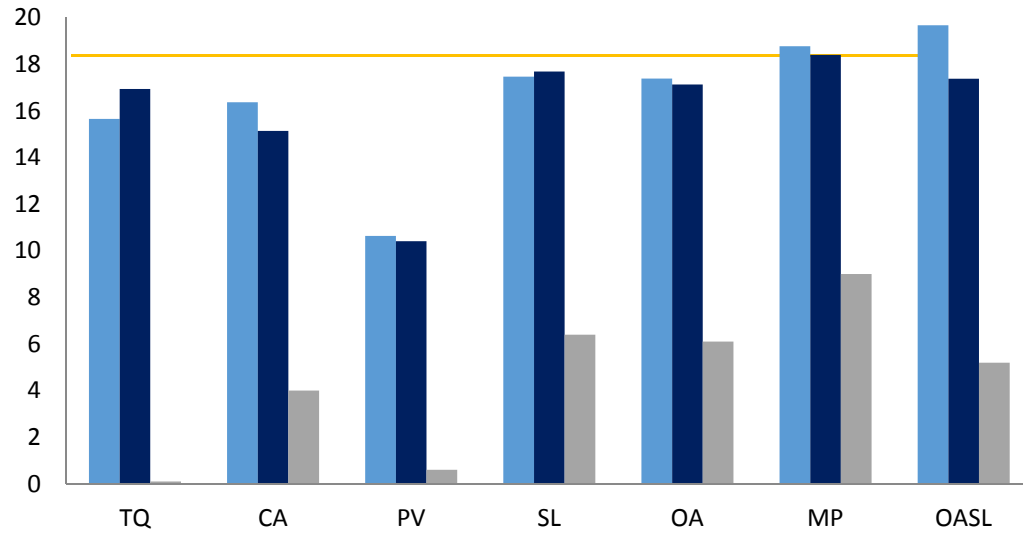
Campione G



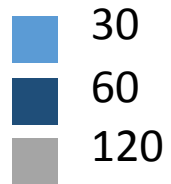


# Prove AGLY 2

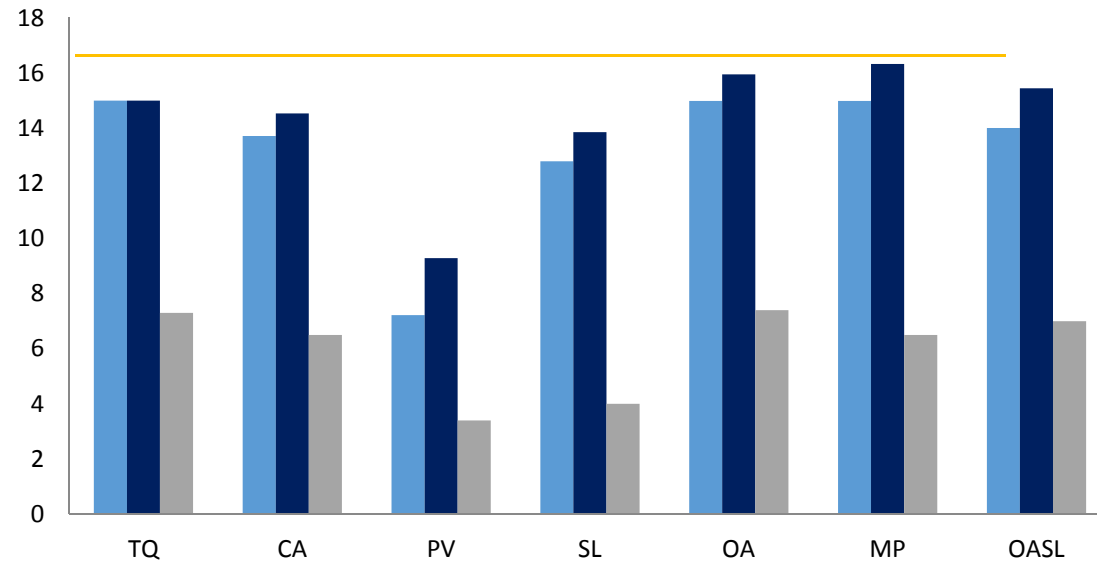
## F



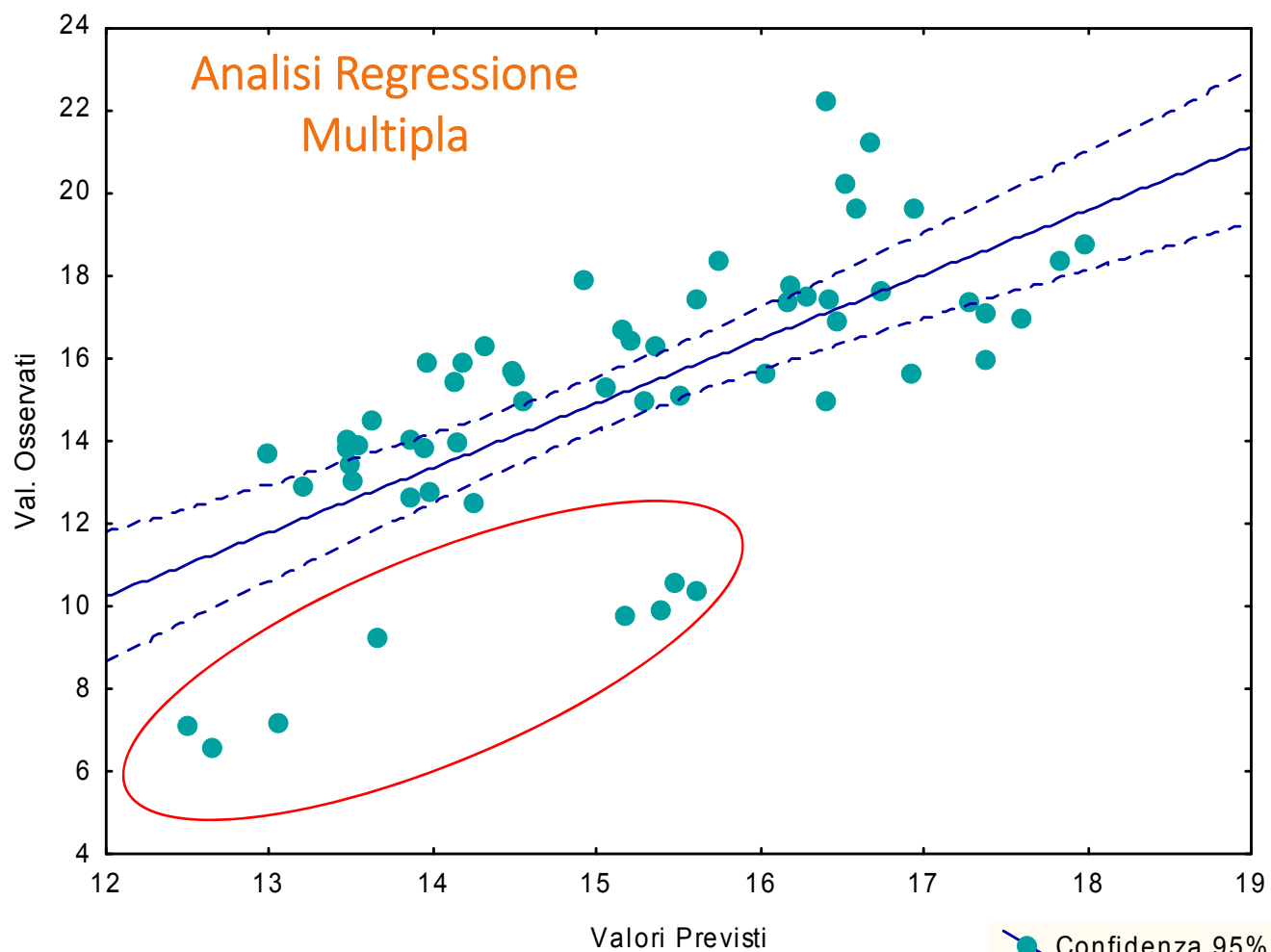
Trend temporali  
Quercetina



## G







| Variabile | $\beta$ | p      |
|-----------|---------|--------|
| T-Pigm    | 0,47    | 0,0139 |
| Catechina | 0,72    | 0,0056 |

### Analisi Correlazioni

|                    | G    | F    |
|--------------------|------|------|
| Epicate            | 0,43 |      |
| Catechina          | 0,78 | 0,60 |
| Proc-B1            |      |      |
| Proc-B2            | 0,42 |      |
| Tannini            | 0,47 |      |
| T-pigmi            | 0,44 |      |
| Fenoli tot         | 0,57 |      |
| Antociani          |      |      |
| $\Sigma$ drossic   |      |      |
| $\Sigma$ flavanoli |      | 0,43 |

p<0.01

## Prove AGLY 2

- Il PVPP favorisce la rimozione della quercetina aglicone
- Mannoproteine e tannini sembrano favorire la stabilizzazione in soluzione

