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ORIGINAL PAPER

# Effect of pomegranate supplementation on the wine yeast response to acidic and osmotic stresses

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## Abstract
The aim of the present work was to verify in winemaking the anti-stress efficacy due to the integration of the grape must with two protectants: pomegranate albedo and pomegranate arils; these substances had displayed in vitro anti-stress effects. The effect of pomegranate supplementation on stress tolerance of five strains of *Saccharomyces cerevisiae*, one wild type and four descendants, against fermentation in grape must with high sugar content (30°brix) and high acidity (pH 3.00) was studied. So, micro-winemaking trials were carried out using grape must, as it is or supplemented at 2% with pomegranate albedo or with pomegranate arils, inoculated in duplicate with the yeast strains. At the end of winemaking, ethanol and acetic acid content, colour intensity, total phenolic content, and total antioxidant activity by DPPH and ABTS assays were analysed. The results shown the possibility to use pomegranate as protective agent in winemaking with high sugar content and high acidity giving wines in which the fermentable sugars will be fermented with acceptable acetic acid content, very high colour intensity values, very high total phenolic content, and very high antioxidant activity, expressed as DPPH and ABTS values.

**Keywords** Pomegranate · Protectants · *Saccharomyces cerevisiae* · Stress · Wine

## Introduction

Pomegranate (*Punica granatum* L.) is a temperate climate species native of the Central Asia; from this area, it spread to the neighbouring regions and, in the course of millennia, in other parts of the world, among them the Mediterranean Basin [1]. Pomegranate is a rich source of phenolic components [2, 3] and volatile aroma-active compounds [4]. Pomegranate contains a number of health compounds [5, 6], among which anthocyanins [7, 8]. It is considered a functional food that possess nutraceutical value due to the high antioxidant activity [9, 10].

During ethanol fermentation, *Saccharomyces cerevisiae* cells are exposed to a number of different stresses [11], such as sugar consumption, ethanol increase, temperature changes, contamination risk, phenolic compounds [12]. The hyperosmotic stress leads to a decrease in yeast viability, resulting in a decrease in the efficiency of ethanol production [13]. Stress induced changes in yeast metabolism can also lead to the production of metabolites that significantly affect wine quality [14]. Consequently, it is important to improve stress tolerance of wine yeasts [15].

The paper addresses a new way to improve the quality of wine by adding natural antioxidant rich ingredients in the must. In details, pomegranate antioxidants could be expected to act against oxidative stress. So, aim of the present work was to verify in winemaking the anti-stress efficacy due to the integration of the grape must composition with 2% of pomegranate albedo or pomegranate arils; these substances had displayed in vitro anti-stress effects [16].

## Materials and methods

Five strains of *S. cerevisiae*, obtained from the collection of the Laboratory of Microbiology (Department of Agriculture, *Mediterranea* University of Reggio Calabria, Italy), were tested in micro-winemaking trials. One strain (Sc1741) was wild type isolated in Calabria from spontaneous winemaking and the other four were its descendants, obtained at the micromanipulator by monosporal culture of the four spores contained in a single ascus.

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Calabrian black grapes of *Greco nero* cultivar were given pre-fermentative maceration to extract pigments from skins and seeds. They were destemmed, crushed and cold soaked at 0 °C for 3 days, performing a punch down twice per day.

Pomegranates were purchased at a local market in Reggio Calabria. The two protectants—pomegranate albedo, and pomegranate arils, were blended and separately added to the grape must, 2 g in 100 mL of grape must. So, the must obtained after pressing was adjusted to pH 3.00 and °Brix 30, adding sucrose and sulphuric acid. It was divided in aliquots of 100 mL and, after the addition of 2.00 g (wet weight) of each protectant, it was immediately inoculated at 5% in duplicate using 5 mL of a 2 day-preculture in grape must treated at 110 °C for 5 min, and incubated at 25 °C.

At the end of winemaking, the wines were analysed for ethanol content by ebulliometer and for acetic acid content by enzymatic kit from CDR FoodLab® (Ginestra Fiorentina, Firenze, Italy).

The absorbance at 420, 520, and 620 nm was read using an Anadeo1 spectrophotometer (Bibby Sterilin Ltd); the colour intensity was calculated with the following formula: $I = A420 + A520 + A620$ [17].

The total phenolic content was determined according to Singleton and Rossi [18].

The total antioxidant activity was expressed as percentage of inhibition of DPPH, according to Bondet et al. [19], and as a percentage of inhibition of ABTS, according to Re et al. [20].

Data were subjected to statistical analysis using StatGraphics Centurion XVI for Windows XP (StatPoint Technologies, Inc., USA) according to Fisher's least significant difference (LSD) ($p < 0.05$).

## Results and discussion

Table 1 reports the ethanol content of the wines produced using the five yeast strains.

The ethanol content ranged from a minimum value of 14.70 (strain Sc1741A-1D, pomegranate arils) to a maximum value of 17.00 vol. % (strain Sc1741A-1C, control). With the addition of pomegranate albedo or pomegranate arils the wild type and the descendant Sc1741A-1B do not exhibit significant differences compared to the control. On the contrary, three descendants exhibit significantly lower ethanol content compared to the control.

Table 2 reports the acetic acid content of the wines produced using the five yeast strains.

The acetic acid content ranged from a minimum value of 0.718 g/L (strain Sc1741A-1C, pomegranate albedo) to a maximum value of 1.202 g/L (strain Sc1741, control). With the addition of pomegranate albedo or pomegranate arils the wild type exhibit significantly lower acetic acid content compared to the control. Three descendants do not exhibit significant differences compared to the control. On the contrary, the descendant Sc1741A-1C exhibit the significantly lowest acetic acid content with the addition of pomegranate albedo.

Table 3 reports the colour intensity value of the wines produced using the five yeast strains.

The colour intensity ranged from a minimum value of 2.563 (strain Sc1741A-1B, pomegranate arils) to a maximum value of 3.036 (strain Sc1741A-1D, pomegranate albedo). With the addition of pomegranate arils, the five strains always exhibit the significantly lowest colour intensity value. On the contrary, with the addition of pomegranate

**Table 1** Ethanol content (vol%) of the wines produced using the five yeast strains in the three grape must combinations: control, addition of pomegranate albedo (2%), addition of pomegranate arils (2%)

| | Sc1741 | Sc1741A-1A | Sc1741A-1B | Sc1741A-1C | Sc1741A-1D |
|---|---|---|---|---|---|
| Control | 15.30 ± 0.00a | 15.65 ± 0.35a | 15.45 ± 0.05a | 17.00 ± 0.00a | 15.65 ± 0.05a |
| Pomegranate albedo | 15.00 ± 0.20a | 15.00 ± 0.00b | 15.70 ± 1.00a | 15.65 ± 0.05b | 15.40 ± 0.10b |
| Pomegranate arils | 15.15 ± 0.15a | 14.80 ± 0.10b | 16.15 ± 0.15a | 14.80 ± 0.10c | 14.70 ± 0.10c |

Values followed by different lowercase letters in the same column are significantly different ($P < 0.05$)

**Table 2** Acetic acid content (g/L) of the wines produced using the five yeast strains in the three grape must combinations: control, addition of pomegranate albedo (2%), addition of pomegranate arils (2%)

| | Sc1741 | Sc1741A-1A | Sc1741A-1B | Sc1741A-1C | Sc1741A-1D |
|---|---|---|---|---|---|
| Control | 1.202 ± 0.012a | 0.925 ± 0.072a | 1.114 ± 0.081a | 0.825 ± 0.007b | 0.757 ± 0.174a |
| Pomegranate albedo | 1.006 ± 0.031b | 1.036 ± 0.078a | 1.047 ± 0.028a | 0.718 ± 0.055b | 0.978 ± 0.061a |
| Pomegranate arils | 1.024 ± 0.030b | 0.922 ± 0.113a | 1.095 ± 0.048a | 1.017 ± 0.067a | 0.821 ± 0.080a |

Values followed by different lowercase letters in the same column are significantly different ($P < 0.05$)

**Table 3** Colour intensity—calculated by addition of the absorbance at 420, 520, and 620 nm, of the wines produced using the five yeast strains in the three grape must combinations: control, addition of pomegranate albedo (2%), addition of pomegranate arils (2%)

| | Sc1741 | Sc1741A-1A | Sc1741A-1B | Sc1741A-1C | Sc1741A-1D |
|---|---|---|---|---|---|
| Control | 2.812 ± 0.063a | 2.842 ± 0.031a | 2.785 ± 0.014b | 2.833 ± 0.018b | 2.939 ± 0.005a |
| Pomegranate albedo | 2.867 ± 0.011a | 2.859 ± 0.019a | 2.965 ± 0.026a | 2.969 ± 0.003a | 3.036 ± 0.060a |
| Pomegranate arils | 2.629 ± 0.043b | 2.569 ± 0.002b | 2.563 ± 0.012c | 2.572 ± 0.057c | 2.659 ± 0.056b |

Values followed by different lowercase letters in the same column are significantly different ($P < 0.05$)

**Table 4** Total phenolic content (mg/L) of the wines produced using the five yeast strains in the three grape must combinations: control, addition of pomegranate albedo (2%), addition of pomegranate arils (2%)

| | Sc1741 | Sc1741A-1A | Sc1741A-1B | Sc1741A-1C | Sc1741A-1D |
|---|---|---|---|---|---|
| Control | 3744 ± 250b | 8458 ± 511b | 8977 ± 444b | 8655 ± 309b | 9025 ± 221b |
| Pomegranate albedo | 6284 ± 92a | 13,294 ± 37a | 12,858 ± 229a | 15,223 ± 1,999a | 12,909 ± 1211a |
| Pomegranate arils | 3942 ± 30b | 7400 ± 223c | 8207 ± 22c | 8954 ± 446b | 7487 ± 1322b |

Values followed by different lowercase letters in the same column are significantly different ($P < 0.05$)

**Table 5** DPPH value, expressed as percentage of inhibition, of the wines produced using the five yeast strains in the three grape must combinations: control, addition of pomegranate albedo (2%), addition of pomegranate arils (2%)

| | Sc1741 | Sc1741A-1A | Sc1741A-1B | Sc1741A-1C | Sc1741A-1D |
|---|---|---|---|---|---|
| Control | 18.42 ± 3.06b | 30.07 ± 2.46b | 43.36 ± 6.36b | 37.87 ± 8.46b | 33.24 ± 0.58b |
| Pomegranate albedo | 31.29 ± 3.55a | 57.97 ± 19.44a | 62.95 ± 3.39a | 73.00 ± 3.51a | 76.52 ± 3.40a |
| Pomegranate arils | 15.56 ± 2.65b | 37.69 ± 3.74ab | 30.79 ± 2.28c | 29.99 ± 1.47b | 36.16 ± 1.30b |

Values followed by different lowercase letters in the same column are significantly different ($P < 0.05$)

**Table 6** ABTS value, expressed as percentage of inhibition, of the wines produced using the five yeast strains in the three grape must combinations: control, addition of pomegranate albedo (2%), addition of pomegranate arils (2%)

| | Sc1741 | Sc1741A-1A | Sc1741A-1B | Sc1741A-1C | Sc1741A-1D |
|---|---|---|---|---|---|
| Control | 3.79 ± 0.61b | 7.17 ± 1.12b | 6.52 ± 1.42c | 7.54 ± 0.69b | 5.56 ± 0.57b |
| Pomegranate albedo | 6.82 ± 0.63a | 12.49 ± 0.68a | 14.20 ± 0.36a | 15.39 ± 0.93a | 15.81 ± 1.40a |
| Pomegranate arils | 4.02 ± 0.49b | 6.29 ± 0.32b | 8.48 ± 0.47b | 7.43 ± 0.81b | 7.30 ± 0.74b |

Values followed by different lowercase letters in the same column are significantly different ($P < 0.05$)

albedo, the five strains always exhibit the highest colour intensity value.

Table 4 reports the total phenolic content of the wines produced using the five yeast strains.

The total phenolic content ranged from a minimum value of 3744 mg/L (strain Sc1741, control) to a maximum value of 15,223 mg/L (strain Sc1741A-1C, pomegranate albedo). With the addition of pomegranate albedo, the five strains always exhibit the significantly highest total phenolic content value. On the contrary, with the addition of pomegranate arils the total phenolic content values are close to the control.

Table 5 reports the DPPH value of the wines produced using the five yeast strains.

The DPPH ranged from a minimum value of 15.56% (strain Sc1741, pomegranate arils) to a maximum value of 76.52% (strain Sc1741A-1D, pomegranate albedo). With the addition of pomegranate albedo, the five strains always exhibit the significantly highest DPPH value.

Table 6 reports the ABTS value of the wines produced using the five yeast strains.

The ABTS ranged from a minimum value of 3.79% (strain Sc1741, control) to a maximum value of 15.81% (strain Sc1741A-1D, pomegranate albedo). With the addition of pomegranate albedo, the five strains always exhibit the significantly highest ABTS value.

The applicability of pomegranate fruit fermentation, based on several products (among which wine), was shown to yield health-promoting properties, as it was documented by many scientists specialized in different fields of science [21].

Phenolic compounds provide important quality attributes to red wines [22]; some work on pomegranate fermented juices alone [23–25] or blended with sweet orange (*Citrus sinensis* L.) juice [26] was performed. It is interesting to note that the total phenol content of the juice is not greatly affected during the fermentation process [27].

It was described that pomegranate juice, fermented by the wine yeast *Saccharomyces bayanus* Lalvin EC-1118, exhibits strong antioxidant activity, significantly greater than that of red wine [28]; some work on pomegranate fermented juices was performed paying attention to the antioxidant activities of pomegranate wines [29, 30].

Two pomegranate wines have been compared, one produced using juice from arils, the other produced using juice from the mixture of arils, epicarp, and mesocarp. Juicing with peel made the juice bitter and astringent, but contributed better sensory quality to wine. Peel contributed higher total polyphenols and flavonoids, but lower anthocyanins to the juice products, and caused the phenolics content to fluctuate more dramatically during making wine [31] (Wasila et al., 2013).

## Conclusion

In summary, the wines produced adding pomegranate albedo or pomegranate arils to grape must exhibited:

- very high ethanol content: this guarantees that the use of these strains as wine starters will produce wines in which the fermentable sugars will be fermented.
- acceptable acetic acid content, considering the stressful conditions employed, the pomegranate albedo reduces the acetic acid production for three out the five tested strains compared to the control.
- very high colour intensity values—with the addition of pomegranate albedo, the five strains always exhibit the highest value.
- very high total phenolic content—with the addition of pomegranate albedo, the five strains always exhibit the significantly highest total phenolic value.
- very high DPPH values—with the addition of pomegranate albedo, the five strains always exhibit the significantly highest DPPH value.
- very high ABTS values—with the addition of pomegranate albedo, the five strains always exhibit the significantly highest ABTS value.

These results shown the possibility to use pomegranate albedo as protective agent in winemaking. Consequently, phenolic compounds and antioxidant activities of pomegranate may induce an anti-stress effect on wine yeasts improving the wine quality. This research constitutes a step to control yeast stress during wine fermentation, so improving wine quality. Protectants exhibited significantly different effects on five *S. cerevisiae* strains, allowing yeasts to overcome the stressful conditions when added to grape must.

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## Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

**Human and animal rights** This article does not contain any studies with human or animal subjects.



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