



The effects of enzymatic pre-treatment and type of yeast on chemical properties of white wine



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ABSTRACT

The study investigated 15 variants of wine differing in their must pre-treatment (maceration by enzymes: Siha Pectinase or SihazymUni) and fermentation by different yeast strains (*S.cerevisiae* as ICV - D-47; SIHA- Cryaroma; Challenge Aroma White; and *S. bayanus* as SIHA Active Yeast). The effects of experimental conditions on chemical composition (pH, acidity, sugar), content of phenolic (LC–PDA–QTOF/MS) and volatile compounds (GC–MS), antioxidant activity (ABTS and FRAP assay), color property (CIEL*a*b*), and ethanol content were measured. For all production processes, significant changes in basic parameters and in the amount of polyphenolic compounds and antioxidant activity in wine were observed as compared to must. The wines obtained with *S. cerevisiae* were characterized by higher polyphenol content, smaller reduction of antioxidant activity and the strongest aroma. Challenge Aroma White yeast strain particularly contributed to good quality of wine. Enzymatic pre-treatment did not significantly affect the studied parameters (except pH and the content of phenolic acids).

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1. Introduction

Wine is a complex mixture of different chemical compounds that are responsible for its color, flavor, bitterness or sourness, aroma, and a positive impact on human health (Soyollkham et al., 2011; Ziyatdinova, Kozlova, & Budnikov, 2016). Phenolic and volatile compounds of grape wine are the most important factors that determine wine quality. However, the content of phenolic compounds and antioxidant activity in white wines are typically lower than in red ones (Soyollkham et al., 2011; Olejar et al., 2015).

Wine quality (nutritional and sensory) is affected by multiple factors. The content of phenolic compounds and their profile in wine depend on the growing conditions (agrotechnical processes, genetic variation, maturity, climatic and geographical conditions) (Soyollkham et al., 2011) and vinification conditions during wine-making, such as fermentation temperature, yeast strain and

application form i.e. immobilization, processing enzymes and alcohol concentration (Galanakis et al., 2012; Lima et al., 2015).

Nowadays, wine fermentation is performed under sterile conditions with wine starter cultures, and *S. cerevisiae* is used as the most common yeast strain. During alcoholic fermentation, yeasts transform grape derivatives into wine compounds by converting sugars into ethanol and other metabolites, as well as into a wide range of volatile and non-volatile end products that significantly contribute to the sensory properties of wine. The yeast may also affect the concentration and composition of wine phenolic compounds, mostly by their adsorption to the cell wall. The effects of yeasts on red wine (retention of anthocyanins and modification of antioxidant capacity) are well known but so far no studies have been conducted in white wines. Enzymatic pre-treatment also determines the quality of the final product. An addition of pectinases during grape maceration may result in important alterations of the chemical composition of grape juice, mainly related to phenolic compounds (Lima et al., 2015).

In Poland, due to prevailing climatic conditions, production of white wine is preferred. The most common winemaking cultivars

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include Solaris, Jutrzenka, Bianka, Aurora, Seyval Blanc, and Sibera (Dobrowolska-Iwanek et al., 2014). Due to their limited experience, Polish winemakers are greatly interested in how the currently available enzymes and yeasts affect the final quality of wine i.e. acidity, content of sugar or polyphenols, amount of alcohol, or fragrance. This study investigates the effects of must pre-treatment and fermentation conditions, such as the use of different new commercial enzymes (i) and two yeast species (*S. cerevisiae* and *S. bayanus*) (ii) on the final quality of white wine produced from Solaris cv. Basic chemical characteristics of the wine (pH, acidity, ethanol content, color), volatile compounds, and phenolics content were examined. The study results may be helpful in improving winemaking process and manufacturing products attractive to consumers due to their sensory quality and potential health-promoting properties.

2. Material and methods

2.1. Chemicals

2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tri(2-pyridyl)striaizine (TPTZ), and methanol were purchased from Sigma-Aldrich (Steinheim, Germany). (–)-Epicatechin, (+)-catechin, procyanidins B1, and quercetin were purchased from Extrasynthese (Lyon, France). Acetonitrile for ultra-high performance liquid chromatography (UPLC; gradient grade) and ascorbic acid were from Merck (Darmstadt, Germany). UPLC grade water, prepared by HPL SMART 1000s system (Hydrolab, Gdansk, Poland), was additionally filtered through a 0.22 µm membrane filter immediately before use.

2.2. Plant material

Fully mature Solaris cv. grapes were collected from Jaworek vineyard in Miękinia (N51° 10' 30.110", E16° 45' 6.875"), near Wrocław (Poland), in 2014.

2.3. Wine production

The berries were first destemmed and crushed with a pestle. After that, they were treated with pectolytical enzymes, either Sihazym Uni or Siha Pectinase (Eaton, Begerow, Langenlonsheim, Germany) at a dose of 0.05 g/L and 0.05 ml/L, respectively. Enzymatic treatment lasted for 1 h at ambient temperature (about 23 °C). The next step was pressing with a hydraulic press machine (TOYA, Wrocław, Poland) and supplementation with K₂S₂O₅ (POCh Gliwice, Poland) at 0.05 g/L. Must fermentation was initiated by adding SihaProferm Plus nutrient at 0.4 g/L (Eaton, Begerow, Langenlonsheim, Germany) and yeast *S. cerevisiae* Challenge Aroma White (Enartis, San Martino -Trecate, Italy), ICV-D-47 (ICV Group, Lattes, France) and SIHA Cryarome (Eaton, Begerow, Langenlonsheim, Germany) and *S. bayanus* SIHA Active Yeast 3 (Eaton, Begerow, Langenlonsheim, Germany) at 0.2 g/L. Control sample was the must without the enzymes or yeast. The fermentation of Solaris must was carried out at 20 °C for 21 days. Each variant was produced in triplicate. When finished, the wine supernatant was decanted and allowed to mature at 4 °C for four months. The samples were analyzed at each stage of the production (wine, after fermentation-F, and after storage-W).

2.4. Physicochemical analyses

Sugar content was measured by HPLC, described previously by

Nowicka, Wojdyło, and Samoticha (2016). Titratable acidity (TA) and pH were determined by titration aliquots, (Schott Titroline 7500 KF Volumetric KFTitrator; Mainz, Germany) expressed as g of tartaric acid/L. Ethanol content in wine was determined by using oscillating densimeter DMA 4500M (Anton Paar, Graz, Austria), with result as the volume percent (% vol.). The color of must and wine was determined using an A5 Chroma-Meter (Minolta CR300, Osaka, Japan), referring to color space CIEL*a*b*. All measurements were done in triplicate.

2.5. Identification and quantification of phenolic compounds by the LC–PDA–MS method

The samples preparation, was performed as described previously by Samoticha, Wojdyło, Chmielewska & Oszmianski (2016). Must and wine samples were filtered through a 0.22 µm membrane filter before analyze. The samples were analyzed by using an Acquity UPLC system (Waters, Milford, MA) with a PDA detector (Waters, Manchester, U.K.). The samples were analyzed by using an Acquity UPLC system (Waters, Milford, MA) with a Q-ToF mass spectrometer (Waters, Manchester, U.K.). An Acquity UPLC BEH C18 column (2.1 × 100 mm, 1.7 µm; Waters Corporation, Milford, USA) was used to perform the chromatographic separation of 5 µL of each sample injected into a gradient system at a flow rate of 0.42 mL/min. The column and sample managers were maintained at 30 and 10 °C, respectively. The mobile phase consisted of 4.5% formic acid in deionized water (A) and acetonitrile (B). Samples were eluted according to a linear gradient: 0–12.0 min, 1–25% B; 12.0–12.5 min, 100% B; 12.5–13.5 min, 1% B. The conditions of MS analysis were as follows: cone voltage of 35 V, capillary voltage of 2000 V, spectra rate, 3.0 Hz, source and desolvation temperature were of 100 and 250 °C, respectively, desolvation gas flow as nitrogen with rate of 300 L/h. To ensure that mass was measured accurately, leucine-enkephalin was used as the reference lock-mass compound at a concentration of 500 pg/µL. Analysis was made by ionization mode at negative [M⁻]⁻ and positive [M⁺]⁺ before and after fragmentation within mass scanning from *m/z* 100 to 1700. The data were collected by Mass-Lynx TM v 4.1 software. Quantification was achieved by injection of solutions of known concentrations ranging from 0.05 to 5 mg/mL (R² ≤ 0.998) of phenolic compounds as standards. The results were expressed as mg/L for must and wine.

2.6. Analysis of antioxidant activity

The free radical scavenging capacities were determined using the ABTS method described by Re et al. (1999), and FRAP (ferric reducing antioxidant power) method described by Benzie and Strain (1996). Spectrophotometric measurements were performed using a UV-2401 PC spectrophotometer (Shimadzu, Kyoto, Japan). All antioxidant capacity analyses were done in triplicate, and results were expressed as micromoles of Trolox per 100 ml.

2.7. Volatile compounds measurement by gas Chromatography Mass Spectrometry (GC-MS)

Extraction of wines volatiles was conducted by HS-SPME, equipped with a 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane fiber (Supelco, Bellefonte, Pa., U.S.A), with 1 cm length. Five mL of wine was placed in 40 mL glass vials with plastic screw caps and teflon coated septa (Supelco, U.K.), warmed to 60 °C. The analysis of flavour volatiles using headspace solid-phase microextraction was according to Pawliszyn (1997, pp. 20–60). The chemical composition of the volatiles, absorbed on

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