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DEGRADATION PRODUCTS OF NUCLEIC ACIDS IN WINES FERMENTED WITH DRIED AUTOLYSATE OF SEDIMENTED WINE YEAST

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The study aimed at determining and comparing the levels of degradation products of nucleic acids and fermentation yield of apple wines fermented with stimulators obtained from sedimented wine yeast. It was demonstrated that increasing doses of yeast autolysate added to wine pitching resulted in an elevated concentration of purine bases (adenine and guanine), pyrimidine bases (thymine, cytosine, uracil), and uric acid.

Compared to the control sample fermented only with diammonium phosphate, the samples fermented with autolysate applied at a dose of 4.5 g/L pitching (100 mg/L when converted into α-amino acid nitrogen) were characterised by twice as high concentrations of purine and pyrimidine bases and uric acid. The total content of those compounds did not exceed 11.4 mg/L of wine.

INTRODUCTION

Apart from carbon, nitrogen is the second indispensable dietary component assimilated by yeast [Pretorius, 2000]. The appropriate concentration of nitrogen in cytoplasm provides regular transport, nitrogen catabolism and proteolytic mechanisms [Beltran *et al.*, 2004]. An ammonia ion present in the medium lowers transport efficiency of nitrogen sources detrimental to a cell on the pathway of "nitrogen catabolic repression" which is determined by the presence of three proteins: GLN3p, URE2p and GAP1p. The GLN3p and URE2p proteins are responsible for the transcription of a number of genes which, in turn, affect the alternative pathways of nitrogen assimilation [Magasanik, 1992]. In addition, it has been demonstrated that the GLN3p protein activates their transcription when the preferred nitrogen sources are unavailable, and that the URE2p protein inhibits the assimilation of unnecessary sources of nitrogen [Magasanik, 2002; ter Schure *et al.*, 2000]. The GAP1p protein constitutes a basic amino acid permease and takes part in the transfer of all amino acids, except for proline, through cytoplasmatic membranes [Ribereau-Gayon *et al.*, 2000]. Proline may be transported to the cell's interior through either basic amino acid permease or specific permease, the latter being a product of a PUT4 gene [Salmon & Barre, 1998]. During alcoholic fermentation, amino acids contained in must are metabolized with a different intensity.

Henschke & Jiranek [1995] have demonstrated that the concentrations of arginine, phenylalanine, serine, isoleucine, histidine and methionine are subject to a substantial decrease (by 70–95%) at a simultaneous increase in the contents of alanine, glycine and lysine. In addition, Lehtonen [1996] have demonstrated that in the course of fermentation yeast do not utilize proline as a source of nitrogen and its content in grape wines constitutes from 35% to 85% of the total amino acid pool. Compared to grape musts that contain 0.7% of nitrogen compounds, the apple musts are characterised by a low concentration of those compounds, *i.e.* 0.3%, of which as little as 2% is constituted by proline, whereas asparaginate constitutes as much as 60% of the total amino acid pool [Elkins *et al.*, 1996]. The supplementation of grape musts with nitrogen compounds is a routine treatment in winemaking [Marks *et al.*, 2003]. Most commonly applied supplement is diammonium phosphate. It has been demonstrated that the appropriate nitrogen concentration in the medium positively affects the kinetics and yield of the fermentation process. In addition, grape must supplementation with nitrogen has been reported to stimulate the utilization of fructose present in the medium by yeast and to affect the formation of volatile components of wine aroma during fermentation [Berthels *et al.*, 2004]. Investigations of Bely *et al.* [2003] have shown that the appropriate nitrogen concentration of the medium contributed to a lowered level of volatile acidity.

Due to a low nitrogen content, apple musts need to be supplemented with nitrogen compounds. Commonly, use is made of nitrogen culture medium in the form of ammonium sulphate or phosphate applied at a dose of 0.1–0.4 g /L, however the winemaking practice tends to reduce its addition due to the potential formation of carcinogenic ethyl carbamate [Wzorek & Pogorzelski, 1998].

In accordance with Article 15 of the Act of the 22nd January of 2004 on the production and bottling of wine products, turnover of those products and organization of the wine market [Law gazette No.34, item 292, No. 96, item 959 and No. 173, item 1808], one or a few of the following substances can be used for pitchings of fermented wine beverages: prep-

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arations obtained from cell walls of yeast at a dose not higher than 40 g per hectoliter; ammonium phosphate or diammonium orthophosphate at a dose not higher than 0.4 g/L; ammonium sulphate or ammonium disulphate at a dose not higher than 0.3 g/L.

Nitrogen culture media can also be applied in the form of mixtures, *e.g.* of diammonium phosphate(V) and ammonium sulphate(VI) or ammonium sulphate(VI) alone. European and French norms specify that the maximum dose of ammonium sulphate(VI) cannot exceed 0.3 g/L, which when converted into nitrogen accounts for 65 mg/L [Bely *et al.*, 2003]. As an additional source of nitrogen use can be made of yeast autolysates that, in addition to proteins and amino acids, contain vitamins and mineral compounds stimulating the course of the fermentation process [Wzorek & Pogorzelski, 1998; Minarik & Jungowa, 1987].

Apart from benefits resulting from the use of yeast autolysates that enable accelerating fermentation and shortening its time span, their application as a source of valuable nitrogen is likely to cause increased concentrations of degradation products of nucleic acids: purine and pyrimidine bases and uric acid, in the finished wine. It is of significance due to the fact that the excess of purines in a human diet results in the accumulation of uric acid salts in joints and urinary systems, which may lead to such pathological states as podagra or arthritis [Edozien, 1970]. A daily dose of nucleic acids in a human diet should not exceed 2 g, which corresponds to *ca.* 50 g of dry matter of yeast.

The study was aimed at determining the effect of nitrogen stimulators of fermentation, namely yeast autolysate and diammonium phosphate, on the content of degradation products of nucleic acids in wines.

MATERIAL AND METHODS

Pitchings of apple wines were prepared from a commercial apple concentrate, which was diluted with water to obtain apple must of 10°Bx. Must consumption for pitchings reached 70%. The pitchings were sweetened with saccharose to a level that enabled obtaining wine proof of 14% vol. alcohol. Fermentation was carried out with *Saccharomyces cerevisiae* yeast of Syrena species that were obtained from the Pure Culture Collection of the Institute of Fermentation Technology and Microbiology, Technical University of Łódź, ŁOCK 105. The yeast were added in the form of seed yeast at a dose of 10% of pitching volume. The fermentation process was carried out at a temperature of 25°C.

The contents of purine bases, pyrimidine bases and uric acid were determined with the HPLC method according to Ruther & Baltes [1995] and Zhang *et al.* [1984], using a Perkin Elmer chromatograph model 4,6^{H}250 Binary LC Pump equipped in an Adsorbosphere column C_{18} 250 × 4.6 mm (5 μ) (Alltech). Separation was run under following conditions: temperature 20°C, eluent: 30 mmol/L solution of sodium 1-heptansulphonate in phosphoric acid (pH 2.3) and acetonitrile (92:8 v/v), isocratic flow rate of 0.35 mL/min.

Detection was carried out at λ=254 nm. A sample to be analysed was prepared as follows: 0.05 g of autolysate in 10 mL of water or 10 mL of wine were mixed with 2 mL of 60% perchloric acid. The suspension was heated for 10 min at a temperature of 100°C, then cooled and centrifuged (1600 g, 15 min). After separation of the precipitate, 20 μL of the filtered sample were injected onto the chromatographic column.

Total nitrogen was determined with the Kjeldahl's method [Analytica-EBC, 1998]. The concentration of α-amino acid nitrogen was assayed with the ninhydrine method using glycine as a standard (Sigma G-7126) [Analytica-EBC, 1998]. The determination of the proximate chemical composition of apple wines obtained, including: alcohol, total extract and sugar-free extract, total sugars, total and volatile acidity, and their sensory assessment was carried out following Polish Standards [PN-90/A-79120/04, PN-90/A-79120/05, PN-90/A-79120/06, PN-90/A-79120/007, PN-90/A-79120/08, PN-90/A-79120/02]. Dry matter content was determined with the gravimetric method.

Preparation of yeast autolysate. In order to obtain yeast autolysate, use was made of commercial wine post-fermentation yeast sediment.

The wine yeast slurry obtained after fermentation (20 L, dry matter content of 16.5%) was rinsed 5 times with tap water (1:1). Each portion was centrifuged (4000 rpm, 10 min, a temperature of 5°C). Yeast washed and centrifuged this way were again mixed with tap water (1:1), and the obtained suspension was subjected to the autolysis process at a temperature of 50°C for 48 h. Next, the autolysate was centrifuged and the obtained eluate was concentrated under vacuum to an extract of 11.4°Bx, which was then spray-dried to obtain a dry preparation to be used as a stimulator of the fermentation process. The preparation was determined for the contents of total nitrogen, α-amino acid nitrogen, and dry matter. It was applied as an additive to pitchings at concentrations of: 3.4 g (sample I) and 4.5 g (sample II) of autolysate/L, which when converted into α-amino acid nitrogen accounted for 75 and 100 mg/L pitching, respectively. The pitching supplemented with a 0.3 g/L dose of diammonium phosphate only served as a control.

Results compiled in tables are means of three technological series with standard deviation considered.

RESULTS AND DISCUSSION

So far it has been demonstrated that, compared to a control sample fermented only with diammonium phosphate, the addition of autolysate at a dose of 10 mg/L of apple wine pitching enables obtaining 14% vol. of alcohol in wine, at concurrent shortening of the fermentation period by *ca.* 6 days, *i.e.* from 15 days for a control sample to 9 days for a sample with autolysate addition of 4.5 g/L [Pogorzelski *et al.*, 2000].

The wine industry tends to increase the cost-efficiency of wine production trough shortening the period of fermentation, which is linked with the application of appropriate stimulators of that process. Apart from yeast autolysates [Minarik & Jungowa, 1987; Pogorzelski & Masior, 1986], preparations of *Botrytis cinerea* mould are known to have served this purpose as well [Masior & Czyżycki, 1963]. The application of yeast autolysates as stimulators of the fermentation process not only shortens its time span but also affords the possibility of utilizing post-fermentation yeast, which is of significance in the reduction and treatment of sewage generated.

TABLE 1. Contents of purine bases, pyrimidine bases and uric acid in yeast autolysate.

Components	Content (mg/100 g)
Adenine	46.0±1.35
Guanine	32.1±1.31
Cytosine	28.3±1.10
Thymine	22.5±0.90
Uracil	16.2±0.65
Uric acid	52.1±2.00
Total	197.2

The presented research was aimed at determining and comparing the contents of degradation products of nucleic acids in apple wines fermented with the addition of diammonium phosphate.

Total nitrogen content of apple must accounted for 221 mg/L, including 40% of α-amino acid nitrogen. The autolysate applied contained 89.4% of dry matter, 11.7% of total nitrogen converted into dry matter, and 3.8% of α-amino acid nitrogen. The yeast preparation obtained was determined for the concentrations of purine bases, pyrimidine bases and uric acid (Table 1).

The highest concentration was reported for uric acid (52 mg/100 g), which constituted 26% of the total pool of all compounds determined (a sum of: adenine, guanine, thymine, cytosine, uracil, and uric acid). The lowest concentration, *i.e.* 16 mg/100 g, was noted for uracil.

The concentration of degradation products of nucleic acids was also assayed in apple wines. The concentrations of particular compounds were demonstrated to increase along with an increasing dose of yeast autolysate added to the pitching. Compared to the control sample fermented only with diammonium phosphate, the samples fermented with autolysate added at a dose of 100 mg/L of pitching converted into α-amino acid nitrogen were characterised by twofold higher concentrations of purine and pyrimidine bases as well as uric acid. Their total level did not exceed 11.4 mg/L of wine (Table 2).

Both the pitchings and apple wines were also determined for the contents of total nitrogen and α-amino acid nitrogen (Table 3). The highest concentrations of those compounds were found in wines fermented with autolysate added at a dose of 4.5 g/L of pitching. Compared to the initial content of total nitrogen in the pitchings, its concentrations after the fermentation process were observed to decrease by 30, 50 and 47%, respectively (Table 3). Perez-Coello *et al.* [1999] have demonstrated that during the fermentation process yeast utilized from 140 to 243 mg of nitrogen contained in the medium.

TABLE 2. Contents of purine bases, pyrimidine bases and uric acid in apple wines supplemented with yeast autolysate (mg/L).

Components	Control sample	Sample I	Sample II
Adenine	1.4±0.07	2.5±0.13	2.8±0.15
Guanine	0.9±0.05	1.6±0.08	1.8±0.09
Cytosine	0.8±0.04	1.5±0.07	1.6±0.07
Thymine	0.6±0.03	1.2±0.08	1.3±0.06
Uracil	0.4±0.02	0.9±0.05	0.9±0.04
Uric acid	1.5±0.08	2.5±0.13	3.0±0.16
Total	5.8	10.2	11.4

The yeast *Saccharomyces cerevisiae* are capable of using various sources of nitrogen for their growth, however not all their forms facilitate that growth to the same extent. The young apple wines obtained were subjected to a 2-month seasoning process, and then evaluated for their organoleptic quality according to the Polish Standard [PN-90/79120/02]. The organoleptic assessment indicated that the addition of autolysate did not have any significant effect on the sensory quality of wines. The sensory evaluation demonstrated that the discussed apple wines obtained notes indicative of their good quality. The samples analysed were also shown to meet requirements for the proximate chemical composition, *i.e.* alcohol, total and volatile acidity, and sugar-free extract (Table 4).

TABLE 3. Contents of total nitrogen and α-amino acid nitrogen (mg/L).

Nitrogen content	Control sample	Sample I	Sample II
Total nitrogen of pitching	225.0±11.3	580.0±29.0	685.0±34.3
Tototal nitrogen of wine	170.0±8.4	297.0±14.7	336.0±16.8
α-Amino acid nitrogen of wine	25.5±1.3	56.4±2.8	70.6±3.5

TABLE 4. Chemical composition of apple wines.

Wine component	Control sample	Sample I	Sample II
Alcohol (% vol.)	14.2	14.2	14.3
Total extract (g/L)	40.5	36.4	38.5
Total sugars (g/L)	9.5	10.4	9.2
Sugar-free extract (g/L)	31.1	26.0	29.3
Total acidity as malic acid (g/L)	3.7	4.0	4.0
Volatile acidity as acetic acid (g/L)	0.4	0.4	0.5
Sensory evaluation – 5-point scale	4.17	4.12	4.15

CONCLUSIONS

Compared to a control sample fermented only with diammonium phosphate, the addition of autolysate at a dose of 10 mg/L of apple wine pitching enables obtaining 14% vol. of alcohol in wine with simultaneous shortening of the fermentation process from 15 to 9 days. It was demonstrated that concentrations of purine and pyrimidine bases as well as uric acid increased with increasing doses of yeast autolysate added to the pitchings, and their concentrations were twofold higher in wines fermented with autolysate added at a dose of 4.5 g/L of pitching, compared to the control sample. The total level of purine and pyrimidine bases and uric acid did not exceed 11.4 mg/L of wine, which – considering recommendations for moderate consumption of wines – does not pose a risk of their excessive intake.

The results obtained indicate that the dried yeast autolysate applied at doses used in this study may find its application in the winemaking technology as an alternative source of nitrogen compounds.

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PRODUKTY DEGRADACJI KWASÓW NUKLEINOWYCH W WINACH FERMENTOWANYCH Z UDZIAŁEM SUSZONEGO AUTOLIZATU Z OSADOWYCH DROŻDŻY WINIARSKICH

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Badano i porównano poziom produktów degradacji kwasów nukleinowych oraz wydajność fermentacji win jabłkowych fermentowanych z udziałem stymulatorów uzyskanych z osadowych drożdży winiarskich. Wykazano, że wraz ze wzrostem ilości dodawanego do nastawu autolizatu drożdżowego zwiększały się stężenia zasad purynowych (adeniny i guaniny), pirymidynowych (tyminy, cytozyny, uracylu) oraz kwasu moczowego.

W porównaniu z próbą kontrolną fermentowaną jedynie z fosforanem dwuamonu, próby do fermentacji których dodano autolizat w ilości 4,5 g/L nastawu (100 mg/L w przeliczeniu na azot α-aminokwasowy) zawierały dwukrotnie wyższe stężenia zasad purynowych, pirymidynowych oraz kwasu moczowego. Sumaryczny poziom tych związków nie przekraczał 11,4 mg/L wina.