A C T A A L I M E N T A R I A P O L O N I C A
Vol. XVII (XLI) No. 1 1991

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DESORPTION OF POLYPHENOLS DURING AUTOLYSIS OF SEDIMENTARY WINE YEASTS

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Key words: after-fermentation yeasts, autolysis, desorption of polyphenols

It was found that during controlled autolysis of sedimentary wine yeasts following fermentation of apple and black-currant musts, the polyphenols adsorbed on yeast cells are desorbed. 6-8% of total polyphenols in the must were retained by the yeasts, and of this amount 13.4-18.2% passed on to the autolysate. The polyphenols in the autolysate were catechin monomers and polymers; no phenolic acids were found to accompany them.

INTRODUCTION

The adsorption of polyphenolic compounds by yeast cells during fermentation of musts is a commonly known phenomenon, described in numerous works in the 1960s [1-10]. However, the reverse process — polyphenols desorption from yeast cells — is inadequately researched. Maschelein [6] was the only scientist to suggest that polyphenol compounds adsorbed on yeasts may be liberated during autolysis.

At present, when sedimentary yeasts autolysates are becoming valuable additives in wine production, used particularly to enhance fermentation [11] and to improve maderization of apple wines [12], a study of this desorption seems advisable.

In this research we investigated the quantitative aspect of desorption, recording the amounts of polyphenols starting from the musts up to the autolysate. We also identified the polyphenols isolated from the autolysates.

EXPERIMENTAL

MATERIAL

1. Sedimentary wine yeasts from fermented apple and black-currant musts.
2. Standard compounds: chlorogenic, gallic, caffeic, p-coumaric and protocatechuic acids obtained from Koch-Light Laboratories Ltd.; (—) epicatechin and (+) catechin obtained from Fluka AG., Buchs, Switzerland.

METHODS

Total polyphenols content was determined by the colorimetric method with the Folin-Ciocalteu reagent [13]. Monomer polyphenols were identified by thin-layer chromoatography on Kisegel 60 Merck silica gel [14]. Polyphenols were extracted from wines and autolysates with ethyl acetate according to the method of Dimair and Polster [15]. Also used in identifying polyphenols was fluorescence in UV light and standards of pure compounds. Amino acid nitrogen was determined colorimetrically with ninhydrin [16]. Dry mass of autolysates was determined by heat drying in a laboratory drier [17].

AUTOLYSATES

The studied autolysates were obtained from sedimentary yeasts after laboratory-scale fermentation of apple and black-currant musts. The apple musts were fermented with *Saccharomyces cerevisiae* yeasts of the „Syrena" kind. 0.7dm^3 of apple must was required to produce 1 dm^3 of wine. The black-currant must was diluted to three times its volume and fermented with S. (—) *cerevisiae* yeasts of the „Burgund" kind. The stock wines were sweetened with saccharose to adjust ethanol content in wine to 14% by volume. The starter of yeast addition was 5%; the nitrogen source for the yeasts was diammonium phosphate (0.3 g/dm^3). Fermentation was maintained at 24°C. After fermentation, the yeast sediment was centrifuged off and washed five times in water, following which it was diluted with water (1:1 ratio) and autolysed at 51°C for 48 h.

CALCULATION AND PRESENTATION OF RESULTS

The fermentation of 2-3 dm^3 stock wines was carried out in glass flasks. The investigated components were determined three times in three parallel experiments. The results collected in the tables are arithmetical means from the performed measurements.

RESULTS AND DISCUSSION

One of the findings of previous research [18] was that the autolysates of sedimentary wine yeasts greatly improve the quality of apple Madeiras. The chemical composition and organoleptic properties of Madeirized apple wines indicate that in addition to the already studied amino acids, the autolysate of sedimentary yeasts also contained polyphenols. In this research we decided to investigate these polyphenols.

A characteristic of the autolysates and the quantities of polyphenols that were determined are given in Tables 2 and 3.

Table 1. Chemical composition of experimental musts

Characteristic	Apple must	Black-currant must
Total extract content (g/dm^3)	137.60	195.30
Sugar-free extract (g/dm^3)	28.80	86.50
Reducing sugars (g/dm^3)	96.40	92.80
Total content of sugars after inversion (g/dm^3)	109.40	109.50
Saccharose (g/dm^3)	12.40	15.90
Total acids content g malic acid/dm^3)	7.60	30.80
Total content of volatile acids g acetic acid/dm^3)	0.10	0.13
Total polyphenols content (g/dm^3)	0.415	5.800

Table 2. Characteristic of autolysates from sedimentary wine yeasts

Kind of autolysate	α-amino acid nitrogen (g/dm^3)	Polyphenols (g/dm^3)	Dry mass (%)
Following fermentation of apple must	0.84	0.463	10.1
Following fermentation of black-currant must	1.31	4.560	11.4

Table 3. Polyphenols contents after fermentation of apple and black-currant musts calculated for 5 dm^3 stock wines yielding 0.52 dm^3 of autolysate of the composition given in Table 2

	Polyphenols content (mg/dm^3) in	
	apple must brew	black-currant brew
Initial must	1500	7270
Wine	1370	4000
Adsorption on yeasts and other losses	120	3270
Autolysate	24	435
Recovery of polyphenols in autolysate 3 (%)	18.8	13.4

Similar amounts of yeast residues and autolysates were obtained from the various musts and for both kinds of yeasts. On average, each 5 dm^3 brew yielded 0.52 dm^3 of autolysate with a dry mass content of 10.1-14.4% (Table 2). The total polyphenols content in the autolysates was directly proportional to this content in the initial musts. As can be seen in Table 3, of all the polyphenols introduced into the brew with the must, 6-10% was adsorbed on the yeasts. In this figure, however, we must also include other losses of polyphenols such as those due to oxidation and precipitation from the solution in the from of flobafenes. Quoting a work by Flanzy and Andre, Jakubowska et al. [19] report that there remains 1 g of sedimentary yeasts per every liter of produced wine. Berg and Akiyoshi [1] found that during fermentation of grape musts, the content of polyphenols dropped from 1240 to 1020 mg/dm^3 (i.e. by 17.7%).

Of the polyphenols retained by the yeasts, 13.4% were recovered from autolysates from yeasts after fermentation of black-currant musts, and 18.8% from autolysates from yeasts after fermentation of apple musts (Table 3).

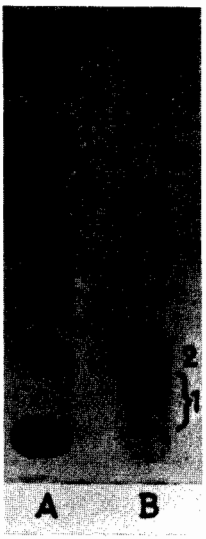

Fig. 1. Thin layer chromatogram of polyphenols extracted with ethyl acetate from apple wines and autolysates from sedimentary yeasts developed in the system chloroform: ethyl acetate: formic acid (5:4:1); A — Wine: 1 — (-) epicatechin, 2 — chlorogenic acid, 3 — caffeic acid, 4 — o-coumaric acid, 5 — ferulic acid, n — unidentified compounds; B — Autolysate: 1 — polymer compounds, 2 — (-) epicatechin

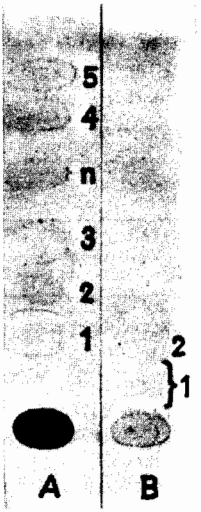

Fig. 2. Chromatogram of polyphenols extracted with ethyl acetate from black-currant wines and autolysates from sedimentary yeasts developed in the system chloroform: ethyl acetate: formic acid (5:4:1); A — Wine: 1 — (+) catechin or (-) epicatechin, 2 — quercetin, 3 — caffeic acid, 4 — o-coumaric acid, 5 — ferulic acid, n — unidentified compounds; B — Autolysate: 1 — polymer compounds, 2 — (+) catechin or (-) epicatechin

In addition to total polyphenols content, we also studied polyphenol monomers in the wines and autolysates. In wines we found the previously identified [14] catechins, and chlorogenic, o-coumaric and caffeic acids (Fig. 1, 2). Only one catechin monomer was identified in the autolysates along with polymer compounds which in the separation technique we employed produced spots on the start and striae just off the start [15].

In apple wines and autolysates of sedimentary yeasts obtained after the fermentation of these wines this catechin compound was (-) epicatechin, identified as the principal component of extracts obtained from cider with ethyl acetate [20]; this compound is also easily determined by high pressure liquid chromatography [21].

The compounds identified in the autolysates and the known mechanisms of reactions between polyphenols and proteins suggest that a major role in adsorption on yeast cells is played by the molecular mass of polyphenols [22]. Nevertheless, according to Milisavljevic [4], the loss of leucoanthocyanogens due to contact with yeasts amounted to 25% and depended in part on the yeast strain. Moreover, the other substances removed with the yeasts were enotannins, catechins and protocatechuic acid. Chevalier et al. [5] argued that yeasts behave like dispergated insolluble proteins and adsorb tannins from the fermenting must in proportion to the number of yeast cells and tannins content.

CONCLUSIONS

1. In the course of autolysis of sedimentary wine yeasts obtained after fermentation of apple and black-currant musts the polyphenols adsorbed on yeast cells undergo desorption.

2. Similar amounts of yeast residue and autolysates were obtained from the two different musts and for the two yeast kinds that were used. Every 5 dm^3 stock wines yielded an average of 0.52 dm^3 of autolysate with a dry mass content of 10.1-11.4%.

3. Of all the polyphenols introduced into the stock wines by the must, 6-8% are adsorbed on the surface of yeast cells, and of these 13.4-18.8% passed on to the autolysate.

4. The polyphenols identified in the autolysates were catechin monomers and polimers.

5. No phenolic acids were found in the autolysates.

6. The presence of polyphenols in autolysates from sedimentary wine yeasts is significant when these autolysates are used in Madeirization of wines [12].

This research was performed as part of the program CPBP.04.11 financed by the Ministry of National Education.

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Manuscript received: June, 1989
Authors address: 90-924 Łódź, ul. Stefanowskiego 4/10.

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DESORPCJA POLIFENOLI PODCZAS AUTOLIZY OSADOWYCH DROŻDŻY WINIARSKICH

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Streszczenie

Badano polifenole w autolizach z osadowych drożdży winiarskich. Drożdże osadowe otrzymywano w wyniku fermentacji moszczów jabłkowych za pomocą drożdży *S. cerevisiae* rasy Syrena oraz moszczów z czarnej porzeczki drożdżami rasy Burgund. Fermentację prowadzono w warunkach laboratoryjnych w butlach szklanych; objętości nastawów wynosiły od 2 do 3 dm^3, temperatura fermentacji 24°C.

Moszcze o składzie podanym w tabeli 1, dosładzano sacharozą w celu uzyskania w winie ok. 14% obj. etanolu. Zużywano na 1 dm^3 wina jabłkowego 0,7 dm^3 moszczu, natomiast moszcz z czarnej porzeczki rozcieńczono w stosunku 1:2.

Po fermentacji osad drożdży odwirowywano, przemywano 5-krotnie wodą, następnie rozcieńczono wodą w stosunku 1:1 i poddawano autolizie w temp. 51°C w czasie 48h.

Dla obydwu ras drożdży i różnych moszczów uzyskiwano podobne ilości gęstwy drożdży i autolizatów. Średnio z 5 dm^3 nastawu uzyskiwano 0,52 dm^3 autolizatu o składzie podanym w tabeli 2.

Zawartość polifenoli ogółem w autolizatach była wprost proporcjonalna do ich zawartości w moszczach (tab. 1, 2). Z ogólnej ilości polifenoli wprowadzonych do nastawu z moszczem 6-10% ulegało adsorpcji na drożdżach, z tego w autolizacie odzyskiwano 13,4-18,8%. W autolizatach były monomery katechin i polimery. Kwasów fenolowych nie stwierdzono w autolizatach (rys. 1, 2).