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EFFECT OF VARIOUS SOURCES OF ORGANIC NITROGEN AS YEAST NUTRIENTS ON THE QUALITY AND CHEMICAL COMPOSITION OF FRUIT WINES.

I. VARIOUS NITROGEN SOURCES AND THE CHEMICAL COMPOSITION OF APPLE WINES

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Key words: apple wines, organic nitrogen, inorganic nitrogen, yeast, chemical composition of fruit wines, organoleptic quality of fruit wines.

Yeast autolysates and extracts, dry casein hydrolyzate, acidic whey, maize steep and potato juice were used as sources of organic nitrogen. All of these sources may be used as nutrients for yeasts ensuring the appearance of satisfactory ethanol content in wine in less than ten days. As compared to diammonium phosphate, the nutrients gave wines containing much more amino acids and by-products. The highest taste grades were given to wines with casein hydrolyzate and yeast autolysate. The greatest alcohol yield was obtained with casein hydrolyzate.

INTRODUCTION

In grape winemaking, nitrogen substances are regarded as one of the principal factors conditioning the taste and chemical composition of wines [9]. Grape must contains soluble nitrogen compounds in amounts exceeding those required for the development and activity of yeasts [18].

Simple proteins and peptones soluble in musts as a result of various physical factors, mainly due to reactions with tannins, precipitate forming sediments and causing turbidity. The role of peptides in wine ia still unclear [1, 6] but we know that various factors, especially the action of wine acids and elevated temperature, are responsible for the peptides' hydrolysis to amino acids. The amino acids present in must and wine are a nutrient for yeasts and other microorganisms. In the course of fermentation with yeasts, there usually appear higher alcohols and aldehydes

[2, 20]. The aldehydes are further transformed into acetals, and also combine with tannins and pigments, simultaneously reacting with amino acids, peptides and peptones giving melanoids. In some cases the transformations of amino acids lead to the appearance in wine of organic acids of the fatty acid series which undergo estrification or decarboxylation leading in turn to the formation of aldehydes (l.c. 3). The products of chemical reactions between saccharides and amino acids during natural or accelerated ageing (l.c. 3, 26) were found to include aldehydes and melanin substances as well as aromatic compounds [19]. Volatile compounds containing sulphur may appear from sulphuric amino acids not only during fermentation but also as a result of purely chemical reactions [11]. The higher alcohols are important in wine not only because of their own odour but also because they act as solvents for other odoriferous substances [25]. The issues connected with nitrogen substances in wines, their sources and directions of transformation are yet to be clarified and are the subject of the most recent studies [10, 13, 14].

The quoted information about nitrogen substances in grape musts and wines as well as the established positive effect of these substances on the wine's taste and odour indicate the advisability of enriching with them the fruit musts containing much less nitrogen fractions, the more so since the fruit musts are diluted in the technological process.

This research involved the utilization of organic nitrogen sources such as yeast autolysates ans extracts, dry casein hydrolyzate, acidic whey, maize steep and potato juice and was aimed at determining the effect of these nutrient media on the course of fermentation, the composition of by-products, and the quality of wine as compared to the effect of additions of ammonium phosphate.

EXPERIMENTS

MATERIALS

The following raw materials and materials were used in the experiments:

— pasteurized apple must from the 1981 crop;

— wine yeasts Syrena from the collection of pure cultures of the Institute of Fermentation Technology and Microbiology, Łódź Technical University;

— dry casein hydrolyzate from the Serum and Vaccine Production Plant in Warsaw;

— acidic whey from the Dairy Plant in Łowicz;

— baker's yeast autolysate from the "Polfa" pharmaceutic company in Kutno;

— yeast extract from Oxoid Ltd., England;
— maize steep from the State Farm Niechcice;
— potato juice prepared in our laboratory.

METHODS

Total and volatile acidity, extract and alcohol were determined according to methods used in winery laboratories [5]. Sugars were determined by the Luff-Schoorl method [23], and total polyphenols colorimetrically with the Folin-Ciocalteu reagent [22]. Higher alcohols were determined by gas chromatography with the use of standard solutions of the analysed compounds [8, 16, 21]; the apparatus used was a Chrom 43 gas chromatographer with flame-ionization detector, column 2.5 m long and 3.0 mm in diameter, filled with Carbovax 15 000 — 10% on Celite 545 AW, 100/120 mesh.

Total nitrogen was determined by Kjeldahl's method [4], and amino acids by ion-exchange chromatography [24] with measurements carried out on a JZC-6AM automatic amino acid analyser. Samples for the analyser were prepared by subjecting the musts or wines to a suitable treatment removing compounds accompanying the amino acids. These compounds were removed in a column filled with ion exchanger Dowex 50 W $\times$ $4H^+$ [9]. Aldehydes were determined by a method consisting in distilling them from wine at pH 9 and binding by acid sodium sulphite in the receiver [12]. Volatile esters were determined by a method consisting in distilling them from wine at pH 6.5 and saponifying them with soda lye at 50°C; the amount of lye used up in the saponification indicated the content of esters in the wine [7].

PREPARATION OF NITROGEN NUTRIENTS FOR YEASTS

Total nitrogen content was assayed in the nutrients in order to determine their doses. The nutrients were applied in two doses: the minimum, of 0.2 g/dm^3 of must, and the maximum, of 0.5 g/dm^3 of must in conversion to nitrogen in diammonium phosphate. The nitrogen content in nutrient media and nutrient doses are given in Table 1.

PREPARATION OF YEAST STARTER

To activate and suitably multiply yeasts, a pure culture was inoculated twice into sterile wort of 9°Blg an then several more times onto increasing amounts of pasteurized must so as to finally obtain a yeast starter comprising 5% of the entire must volume.

Table 1. Total nitrogen content in nutrient media and doses of nutrients per dm³ of must in conversion to nitrogen in diammonium phosphate

Nutrient	Total nitrogen (%)	Dose in cm³/dm³ or g/dm³	
		min	max
Dry casein hydrolyzate	14.74	0.28+	0.72+
Acidic whey	0.89	4.7	11.9
Yeast autolysate	0.48	8.8	22.0
Yeast extract	4.21	1.0+	2.5+
Maize steep	4.03	1.1	2.6
Potato juice	0.19	22.3	55.7
Diammonium phosphate	21.20	0.2+	0.5+

Table 2. Evaluation of the course of fermentation of apple musts with additions of various nutrient media, based on extract decrease, °Blg

Days of fermentation		0	2	3	4	5	6	9	10
		Extract °Blg							
Dry casein hydrolyzate	min	23.0	16.8	12.4	9.1	6.2	4.2	0.2	−0.6
	max	23.8	16.0	11.4	7.5	4.4	2.3	−1.0	−1
Acidic whey	min	23.2	17.0	12.9	10.0	6.9	5.0	0.6	−0.1
	max	23.5	17.0	13.0	10.0	6.9	5.1	0.8	−0.1
Baker's yeast autolysate	min	23.0	15.8	10.9	7.1	3.9	1.9	−1	−1
	max	23.0	14.8	7.2	4.0	0.6	−0.5	−1	−1
Yeast extract	min	23.0	16.5	12.6	9.2	6.5	4.2	−0.1	−1
	max	23.0	16.5	12.6	8.6	5.4	3.3	−0.5	−1
Maize steep	min	23.5	16.0	11.7	8.3	6.0	4.0	0.2	0.6
	max	23.5	14.2	9.1	5.8	3.7	2.1	−0.6	−1
Potato juice	min	23.5	16.5	11.6	8.2	5.2	3.4	−0.6	−1
	max	23.0	15.5	10.0	6.0	2.9	1.0	−1	−1
Diammonium phosphate	min	23.5	16.5	11.8	8.4	5.3	3.2	−0.5	−1
	max	23.5	15.0	10.0	6.0	2.6	0.5	−1	−1

MUST PREPARATION

Wine musts were prepared assuming the must utilization index of 0.7 dm³/dm³ of wine. The amounts of sugar and water needed to obtain 14% of alcohol in the wine were calculated according to Pijanowski [18]. The yeast starter addition amounted to 5% of must volume. Experiments were performed in series of six musts containing nutrients, with a parellel control experiment using a diammonium phosphate addition. The course of fermentation, maintained at 25°C, was controlled by measuring apparent extract.

After termination of fermentation, the young wine was siphoned off and left to age at 10-15°C. After two months of seasoning the wine was analysed (the results are given in Tables 3 and 4). Next, after filtration,

Table 3. Analysis of apple wine fermented with various nutrient media added to the musts

Nutrient / Determination	Dry casein hydrolyzate		Acidic whey		Yeast autolysate		Yeast extract		Maize steep		Potato juice		Diammonium phosphate	
	min	max	min	max	min	max	min	max	min	max	min	max	min	max
Alcohol (% vol.)	15.6	14.4	14.6	14.8	14.8	14.6	14.2	13.6	14.5	14.0	14.4	13.7	14.2	13.
Total acidity (g apple acid/dm³)	5.56	5.56	5.83	6.03	5.36	5.76	5.83	5.89	5.36	5.56	5.83	5.63	5.96	5.
Volatile acidity (g acetic acid/dm³)	0.39	0.27	0.36	0.30	0.30	0.21	0.30	0.18	0.33	0.30	0.30	0.27	0.36	0.30
Real extract (°Blg)	3.9	3.5	3.7	3.7	3.6	3.4	3.4	3.6	3.8	3.6	3.7	3.7	3.6	3.0
Sugarless extract (g/dm³)	32.16	32.08	31.03	28.63	32.96	32.18	29.5	32.5	32.23	32.72	34.36	34.33	32.41	28.30
Total sugars (g/dm³)	7.36	3.33	6.66	9.06	3.48	2.21	4.89	3.94	6.26	3.73	4.13	3.36	4.03	1.92
Reducing sugars (g/dm³)	6.98	3.18	6.43	8.96	3.07	2.06	4.63	3.68	5.58	3.55	3.18	2.35	3.53	1.58
Saccharose (g/dm³)	0.36	0.14	0.22	0.10	0.38	0.14	0.25	0.25	0.59	0.17	0.90	0.95	0.48	0.32
Aldehydes (mg/dm³)	65.29	104.10	57.99	81.93	42.68	19.62	67.41	79.37	73.39	44.35	37.48	36.69	47.78	30.72
Esters (mg/dm³)	17.6	96.8	39.6	30.8	22.0	30.8	44.0	26.4	44.0	83.6	30.8	22.0	30.8	39.6
Total polyphenols (mg/dm³)	190	202	185	212	195	230	200	218	195	238	208	230	212	152
Total protein (% N × 6.25)	0.069	0.079	0.027	0.039	0.039	0.063	0.031	0.076	0.031	0.043	0.073	0.087	0.065	0.083
Amino acids (mg/dm³)	28.38	—	37.44	—	171.24	—	59.58	—	45.46	—	54.35	—	3.88	—
Higher alcohols (mg/dm³)	725	651	710	715	711	716	655	644	711	757	706	680	680	645
Methanol (mg/dm³)	225	210	205	200	220	210	185	240	220	205	200	205	240	175
Propanol (mg/dm³)	15	20	15	15	10	20	15	10	10	10	10	10	20	50
Isobutanol (mg/dm³)	270	230	275	275	255	235	240	235	250	235	260	225	250	240
Butanol (mg/dm³)	5	6	5	5	6	6	5	4	6	7	6	5	5	5
Isoamyl alcohol (mg/dm³)	435	395	415	420	440	455	395	395	445	505	430	440	405	350

min — minimum dose of 0.2 g/dm³,
max — maximum dose of 0.5 g/dm³

Table 4. Amino acids in apple must and wine fermented with minimum doses of nutrient media (0.2 g/dm³)

Anino acids (mg/dm³) \ Must or wine	Must	Dry casein hydrolyzate	Acidic whey	Baker's yeast autolysate	Yeast extract	Maize steep	Potato juice	Diammonium phosphate
Lysine	5.98	1.16	1.02	78.99	2.48	1.17	0.88	trace
Histidine	trace	—	—	trace	trace	trace	trace	—
Arginine	—	—	—	trace	trace	—	—	—
Asparagine	632.02	2.11	6.47	11.22	0.60	5.41	7.39	0.53
Threonine	732.28	10.21	11.10	19.09	13.76	9.77	13.32	0.44
Serine								
Glutamine	29.35	2.19	2.77	15.77	6.57	7.01	8.18	0.44
Proline	59.34	—	trace	trace	trace	trace	trace	trace
Glycine	9.45	1.28	1.28	3.15	2.48	1.95	2.18	0.38
Alanine	29.55	6.49	1.51	6.85	6.16	3.03	3.47	0.27
Cysteine	trace	—	10.41	20.93	11.13	10.65	14.39	1.69
Valine	0.59	trace	0.70	1.87	0.59	1.05	1.05	—
Methionine	0.89	—	trace	2.53	1.04	0.75	trace	—
Isoleucine	1.05	0.52	0.39	2.75	0.92	1.18	0.66	trace
Leucine	1.70	1.70	0.79	5.11	4.45	1.57	1.57	0.13
Thyrosine	1.11	2.04	1.11	1.66	3.70	0.74	0.74	—
Phenylalanine	2.38	0.68	trace	1.36	0.68	1.19	0.51	—
Total	1496.70	28.38	37.44	171.24	59.58	45.96	54.35	3.88

Table 5. Taste assessment of apple wines fermented with additions of various nutrient media after two months of seasoning

Quality	Nutrient													
	dry casein hydrolyzate		acidic whey		yeast autolysate		yeast extract		maize steep		potato juice		diammonium phosphate	
	min	max	min	max	min	max	min	max	min	max	min	max	min	max
Colour (0-2 points)	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Clarity (0-2 points)	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Bouquet (0-4 points)	3.3	3.3	3.2	3.1	3.3	3.4	3.4	3.3	3.3	3.0	3.1	3.1	3.3	3.4
General taste impression (0-12 points)	9.0	9.1	8.0	8.8	8.7	8.6	8.3	8.3	7.5	7.2	7.1	7.0	8.4	8.2
Total (0-20 points)	16.3	16.4	15.2	15.9	16.0	16.0	15.7	15.6	14.8	14.2	14.2	14.1	15.7	15.6

the wine was subjected to sensory assessment, the results of which are collected in Table 5.

RESULTS AND DISCUSSION

Table 2-5 contain mean figures from two series of experiments. There were small differences in the fermentation rate depending on the kind of nutrient mediium applied. The most rapidly fermenting were musts with additions of yeast autolysate and diammonium phosphate in maximum dose: the almost complete fermentation of the musts occurred after six days. The musts with the other nutrients fermented more slowly but complete fermentation occurred by the tenth day irrespective of the kind and dose of nutrient (Table 2). The highest ethanol content, of 15.6% vol., was obtained in wine with casein hydrolyzate as nutrient. The higher ethanol yield in this experiment may be explained by the fact that this kind of nutrient reduces the demand for carbohydrates in yest biomass formation by introducing into the medium a suitable set of amino acids which may be used to form yeast protein as entire molecules, with no need for amino acids synthesis. According to the scheme of Ough et al. [17] yeasts synthesize the relevant amino acids through ketoacids originating from carbohydrates.

The differences in higher alcohols content that were found in the obtained wines may be accounted for similarly. Assuming that higher alcohols appear during the synthesis of amino acids by yeasts through transamination, the least amounts of these compounds should be expected in the musts with amino acid sets similar to those of yeast protein. Although no one determined which single amino acids are required by the yeast cells, it may be surmised that the most suitable should be the set of amino acids from yeast autolysates and extracts.

The least amounts of alcohol were found in wines with additions of yeast extract and casein hydrolyzate. Maize steep, characterized by a high leucine content [15] caused a considerable increase of isoamyl alcohol in the wines. According to Äyräpää [2], the concentration of nitrogen nutrients regulates the production of fusel oils by the yeasts, and a suitable content of leucine coupled with a deficiency of other amino acids results in an increased content of isoamyl alcohol.

The content of aldehydes was in most cases higher than in control wine, ranging from 37 to 104 mg/dm^3. The formation of aldehydes, similarly as that of higher alcohols, is connected with the transformation and synthesis of amino acids in yeast metabolism, hence the various composition of nutrient media resulted in various aldehyde amounts in the studied wines (Table 3).

There was no strict dependence between esters content and the kind of medium. The highest content of volatile esters was found in wines with the maximum dose of casein hydrolyzate (Table 3). An additional factor having bearing on the level of esters and small differences in their amounts was doubtless the fact that the analyses were performed on young wines.

In wines with greater nutrient additions there was slightly less polyphenols; this should be explained by the binding of tannins with nutrient proteins, or by the greater production of biomass which also adsorbs tannins.

The content of free amino acids in wines differed depending on the kind of nutrient. The highest amino acid content was found in wine with the yeast autolysate addition, and the lowest content in wines with organic nutrients, in wine with the casein hydrolyzate addition. The wine with the traditional nutrient, diammonium phosphate, contained the decidedly lowest amount of free amino acid (Table 4). Amino acids such as asparagine, threonine, serine, glutamine and cysteine occured in the greatest amounts in the wines, with the last-mentioned one occuring in trace amounts in the musts (Table 4). Amino acid enrichment of wines creates prospects for obtaining good flavour characteristics in the process of madeirization.

The kind of nutrient medium had no significant effect on the other studied parameters. None of the nutrients had an adverse effect on the wines' colour or clarity; both these parameters received maximum points in the sensory assessment. General taste was rated lowest in wines with additions of maize steep and potato juice, and this warrants the rejection of these nutrient media.

To sum up the sensory assessment of young wines, those with additions of casein hydrolyzate and yeast autolysate were rated best, while those with maize steep and potato juice were considered as the most inferior. The control wine with diammonium phosphate was pronounced to be medium-quality (Table 5).

CONCLUSIONS

1. All the investigated sources of organic nitrogen may be used as nutrient media for yeasts ensuring an optimum alcohol content in wine (14% vol.) after fermentation lasting no more than ten days. The highest alcohol content, 15.5%, was obtained when casein hydrolyzate was used as nutrient. Lower nutrient doses gave higher alcohol content.

2. Compared with the ammonium nutrient, the organic nutrients had no significant effect on the content of polyphenols. The lowest total polyphenols content in wines was found when higher doses of nutrients, including diammonium phosphate, were used.

3. Organic nutrient media gave a higher aldehydes content than that

in control wine with diammonium phosphate. The highest aldehyde content occurred in wine with the casein hydrolyzate addition.

4. The content of higher alcohols was in most cases greater than in the control wine.

5. No significant effect of medium kind on volatile esters content was found.

6. The attempt at amino acid enrichment of wines by using organic nutrient media proved successful, since all the wines with such nutrients contained decidedly more amino acids that the control wine. The highest content of amino acids was in wines with yeast autolysate additions. Amino acids such as asparagine, threonine, serine, alanine, glutamine and cysteine occurred in wines in significant amounts.

7. No foreign taste was found in the young wines. The highest taste assessment, on a 20-point scale, went to wines with additions of casein hydrolyzate (16.4 points) and yeast autolysate (16.0 points). The lowest-rated were wines with maize steep (14.2 points) and potato juice (14.1 points), and these disqualify the two nutrient media. The control wine with diammonium phosphate received 15.6 points.

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WPŁYW RÓŻNYCH ŹRÓDEŁ AZOTU ORGANICZNEGO JAKO POŻYWEK DLA DROŻDŻY NA JAKOŚĆ I SKŁAD CHEMICZNY WIN OWOCOWYCH. CZ. I. RÓŻNE ŹRÓDŁA AZOTU A SKŁAD CHEMICZNY WIN JABŁKOWYCH

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Streszczenie

Badano w skali laboratoryjnej wykorzystanie autolizatów i ekstraktów drożdżowych, suchego hydrolizatu kazeiny, serwatki kwasowej, namoku kukurydzianego i wód sokowych ziemniaka jako pożywek dla drożdży w procesie fermentacji moszczów jabłkowych. Próby miały na celu, oprócz dostarczenia pożywki, wzbogacenie win w aminokwasowe substraty potrzebne do formowania korzystnych cech smakowych w procesie dojrzewania.

Stosowano dawki pożywek 0,2 i 0,5 g/dm^3 w przeliczeniu na azot w $(NH_4)_2HPO_4$ prowadząc fermentację metodą tradycyjną w nastawach o poj. 3 dm^3. Próbę odniesienia stanowiły nastawy z dodatkiem fosforanu dwuamonowego.

Stwierdzono niewielkie różnice w szybkości fermentacji dla poszczególnych pożywek (tab. 2). Uzyskiwano w winach optymalną zawartość etanolu w czasie nie przekraczającym 10 dni. Zaobserwowano różne wydajności etanolu dla poszczególnych pożywek. Najwyższą zawartość etanolu (przy tym samym dodatku cukru) uzyskano w winie z hydrolizatem kazeiny jako pożywką (15,6% obj.) (tab. 3).

Pożywki organiczne dawały wyższą zawartość aldehydów i alkoholi wyższych niż w winie kontrolnym. Nie stwierdzono istotnego wpływu rodzaju pożywki na zawartość estrów lotnych i polifenoli (tab. 3). Wszystkie wina z pożywkami organicznymi zawierały zdecydowanie więcej aminokwasów niż wino kontrolne. Najwyższa ilość aminokwasów (170 mg/dm^3) wystąpiła w winach z dodatkiem autolizatu drożdżowego (tab. 4).

W znacznych ilościach stwierdzono w winach takie aminokwasy jak: asparagina, treonina, seryna, alanina, glutamina i cysteina. Aminokwasy te w winie jabłkowym kontrolnym z fosforanem dwuamonowym występowały w śladowych ilościach.

W młodych winach nie stwierdzono obcego posmaku. Najwyższą ocenę degustacyjną uzyskały wina z dodatkiem hydrolizatu kazeiny i autolizatu drożdżowego. Najniżej oceniono wino z dodatkiem namoku kukurydzianego i wód sokowych ziemniaka (tab. 5), co dyskwalifikuje te pożywki.