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EFFECT OF DOSAGE AND GENERATION OF YEAST ON THE LEVEL OF PRODUCTS OF METABOLISM IN YEAST SEPARATED AFTER FERMENTATION

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Key words: yeast sediment, beer, metabolic products of yeast, physiological state of yeast.

Effect of doses and generations of pitching yeast on the quantity of metabolic products in beer separated from yeast sediment was investigated. Doses of Bratislava yeast were applied at 0.2 l; 0.5 l and 2 l per 1 hl wort. It was observed that larger doses of pitching yeast and their successive generations lead to the worsening of the physiological state of cells which is the cause of changes in the levels of products of their metabolism in young beers and in beer separated from yeast sediment. This pertains mainly to nitrogen compounds and fermentation by-products. The changes in the physiological state of yeast were accompanied by processes of desorption of bitter and colour compounds.

In the classical technology of full light beer the basic fermentation goes on for about 11 days. After pumping the beer to the storage cellar the bottom of the fermentation vat is covered with a layer of yeast, the so-called yeast sediment. From 1 hl beer we obtain about 1.5 to 2 l of the sediment with the content of dry substance between 8 and 15%. About 0.5 l beer can be separated from 1 l of the yeast sediment. Active surface of cells in 1 l sediment containing 12% d.s. equals ca 4 sq.m. The fact finds its repercussions: on account of the processes of excretion and autolysis the quantity of metabolic products in beer from the yeast sediment increases considerably. Concentration of these compounds in beer depends on the physiological state of the yeast [8, 9, 11, 12, 13, 14, 15, 16, 17, 18, 19].

The aim of the present study was to examine the effect of dosage and generation of yeast that are used in pitching for the physiological state of yeast and its consequences as levels of certain products of metabolism in beer from yeast sediment.

MATERIALS AND METHODS

The analyses were made with hopped malt wort inoculated with the Bratislava yeast at the following doses: 0.2 l; 0.5 l; 2 litres per 1 hl. The yeast was added as a sediment with 13% dry substance content. Fermentation was continued at 7 to 9.5°C. Young beer was transferred to storage cellars while the yeast sediment collected from the bottoms of fermentation vats was kept for 24 hours at 0 to 2°C. The yeast sediment beer was separated centrifugally at 2800 rpm for ten minutes. The chemical composition of the sediment beer was compared with that of the young beer. The tests were repeated in six successive experimental fermentations with the use of the yeast collected during previous experiments.

Analyses of the wort and beer were carried out after Analitica EBC [1]; Iso-compounds — after Brenner [4]; total bitter material — after De Clerck [7]. Free aminoacids were determined with gas chromatography [10]. Acetylo-lactate and diketon — after Brenner [5]. Higher aliphatic alcohols, ethyl acetate and acetylo-aldehyde — by gas chromatography [3]. Activity of proteolitic enzymes was determined after Anson method [2]. As a single unit of activity it was determined to regard the quantity of enzyme which releases in 60 minutes — during decomposition of hemoglobin under standard conditions — such a quantity of products soluble in trichloroacetic acid which, when added Folin reagent, provide the same colour that 10^{-4} mmol of tyrosine gives.

RESULTS AND DISCUSSION

Results of the analyses of the wort are given in Table 1. The physiological state of the pitching yeast for particular generations was characterized by the quantity of dead and budding cells (Table 2).

The starting yeast was collected from a propagator and contained 52% budding cells as well as 0.2% dead cells. In successive generations the physiological state of the yeast was growing worse, their vitality decreased and the number of budding cells was reduced. The phenomenon intensified along with larger dosages of pitching yeast.

After wort fermentation the harvest of the yeast sediment was larger whenever the dose of yeast had been also larger, and at the same time the quantity of dead cells increased as well (Table 3). The presence of 4% dead cells in the pitching yeast led to their triple or four-fold increase in the sediment of the next generation regardless of the applied dose. The physiological state of the yeast culture deteriorated in successive generations and this phenomenon appeared always earlier whenever the dose of the pitching yeast was greater.

The chemical compositions of the young beer and the residual beer are

Table 1. The chemical composition of pitching wort

Series	Extract %		Colour EBC	Acidity ml 1n NaOH/100 ml	pH	Bitters mg/l		Nitrogen compounds				Acetylolactate + diacetyl mg/l	Acetoin mg/l
	initial	final				total	iso-compounds	total nitrogen	formolic nitrogen	tannin nitrogen	coagulable nitrogen		
I	12.0	1.7	14	1.1	5.7	72.2	38.6	75.5	27.5	19.8	2.4	—	—
II	12.0	1.6	15	1.2	5.2	103	34.4	66.5	25.9	19.3	2.2	0.29	1.63
III	12.0	1.5	13	1.3	5.6	125	39.2	71.0	25.7	14.4	4.6	0.32	2.12
IV	11.9	1.6	17	1.2	5.6	78	28.8	69.1	22.4	19.6	2.7	0.25	1.99
V	11.7	1.7	18	1.3	5.7	88.6	28.7	74.1	25.1	18.5	4.1	0.29	2.20
VI	12.3	1.6	13	1.4	5.6	117.2	44.2	79.1	26.1	20.2	3.0	0.19	1.37

Table 2. Physiological conditions of pitching yeast

Yeast generation	Yeast dose		Quantity of yeast cells %	
	yeast sediment with 13.5% d.s. in l/hl	quantity of yeast with ca 24% d.s. in g/40 l	budding	dead
I	0.2	45	52	0.2
	0.5	112.5		
	2	450		
II	0.2	45	17	0.5
	0.5	112.5	13	0.7
	2	450	4	1.3
III	0.2	45	14	0.9
	0.5	112.5	10	1.1
	2	450	0	3.0
IV	0.2	45	10	1.4
	0.5	112.5	6	1.9
	2	450	0	4.3
V	0.2	45	7	1.9
	0.5	112.5	2	3.9
	2	450	0	15.2
VI	0.2	45	2	3.5
	0.5	112.5	0	4.3

given in Tables 4 and 5. Decomposition of intracellular glycogen in yeast as well as introduction of glucose in the chain of changes during alcohol fermentation caused an increase in the alcohol quantity in residual beer by 0.8 to 1.6%. The higher degree of rejuvenation of cells in the case of a small dose of yeast, probably on account of more intensive exchange processes between a cell and its environment, resulted in higher pH of the beer (Fig. 1).

The volume of nitrogen compounds in the residual beer grew higher with larger doses of pitching yeast and with every next generation (Fig. 2). This was caused by the yeast autolysis processes in effect of which proteins and proteolitic enzymes were released thus increasing their activity in the residual and the young beers from the fourth, fifth and sixth series of experiments (Table 4).

The large dose of pitching yeast induced higher adsorption of bitter substances from wort during fermentation. Comparison of the 0.2 l/hl in young beer from variant 3 (2 l/hl) the volume of bitter compounds was generally lower by 3 to 12 mg/l, while the volume of iso-compounds

Table 4. The chemical composition of young beer

Yeast generation	Yeast dose l/hl	Fermentation time days	Extract %		Alcohol %	Base wort %	pH	Colour EBC	Nitrogen compounds mg/100 ml			Activity of proteolitic enzymes j H/ml
			apparent	real					total nitrogen	formolic nitrogen	coagulable nitrogen	
I	0.2	8	4.6	5.9	3.0	11.9	4.2	9	52.9	11.8	1.8	—
	0.5	8	3.9	5.0	3.4	12.0	4.2	9	53.6	12.4	2.0	—
	2	8	3.5	4.0	3.7	11.8	4.2	9	53.6	12.0	2.0	—
II	0.2	10	4.6	6.0	3.1	12.1	4.1	9.5	42.1	7.8	1.9	—
	0.5	10	3.8	5.3	3.5	12.1	4.1	9.5	42.7	7.5	1.8	—
	2	10	3.1	4.8	3.8	12.2	4.2	9.5	45.5	9.2	2.3	—
III	0.2	10	4.4	5.7	3.2	12.0	4.2	9	48.1	9.6	2.4	—
	0.5	10	3.9	5.1	3.5	12.0	4.2	8.5	49.3	10.2	2.3	—
	2	10	3.4	4.8	3.7	11.9	4.2	8.5	50.3	10.8	3.5	—
IV	0.2	10	4.5	5.8	3.0	11.9	4.3		48.0	10.4	2.1	—
	0.5	10	4.3	5.5	3.1	11.8	4.4		48.6	10.8	2.0	—
	2	10	3.7	5.0	3.4	11.9	4.3		49.9	11.8	2.4	—
V	0.2	12	4.2	5.4	3.2	11.9	4.4	11	50.6	10.0	2.4	0
	0.5	12	4.0	5.0	3.4	11.9	4.4	11	51.3	10.3	2.4	0
	2	12	3.3	4.7	3.7	11.9	4.3	10.5	53.2	12.8	2.7	2
VI	0.2	14	4.1	5.7	3.3	12.2	4.4	9.0	56.1	18.0	2.0	1.6
	0.5	14	3.6	5.3	3.5	12.2	4.4	8.5	56.0	17.2	2.3	1.8

Table 3. Physiological conditions of yeast collected after fermentation of wort

Generation	Yeast inoculation*)		Yeast sediment			24% d.s. centrifuged yeast		
	quantity of yeast sediment with 13.5% d.s. in l/hl	W quantity of yeast sediment with 24% d.s. in g/40 l	sediment volume g	volume of beer after centrifuging of yeast %	quantity of dead yeast cells %	P total crop g	crop / inoculation = = growth of yeast	collected matter g/l
I	0.2	45	910	51	0.5	440	9.8	11.0
	0.5	112.5	1050	52.4	0.7	500	4.5	12.5
	2	450	1600	52.5	1.3	760	1.7	19.0
II	0.2	45	900	52	0.9	435	9.7	10.9
	0.5	112.5	1000	52	1.1	480	4.3	12.0
	2	450	1500	50	3.0	750	1.7	18.7
III	0.2	45	800	50	1.4	400	8.9	10.0
	0.5	112.5	920	51	1.9	450	4.0	11.2
	2	450	1430	49	4.3	730	1.6	18.2
IV	0.2	45	760	50	1.9	380	8.4	9.5
	0.5	112.5	850	49	3.3	430	3.8	10.7
	2	450	1410	50	15.2	710	2.6	17.7
V	0.2	45	720	50	3.5	360	8.0	9.0
	0.5	112.5	830	49	4.3	420	3.8	10.5
	2	450	1290	50	21	645	1.4	16.1
VI	0.2	45	700	51	10	340	7.6	8.5
	0.5	112.5	850	51	13	410	3.6	10.2

*) Later in the discussion on results of experimentation the yeast inoculation is given in litres of sediment pe 1 hl wort

Table 5. The chemical composition of beer separated from yeast sediment

Yeast generation	Yeast dose l/hl	Extract %		Alcohol %	Base wort %	pH	Colour EBC	Nitrogen compounds mg/100 ml			Activity of proteolitic enzymes j H/ml
		apparent	real					total nitrogen	formolic nitrogen	coagulable nitrogen	
I	0.2	1.5	3.6	4.6	12.5	4.5	10	54.3	12.5	2.5	—
	0.5	1.5	3.4	4.6	12.4	4.4	11	55.5	13.5	2.4	—
	2	1.5	3.6	4.7	12.7	4.4	12	56.8	13.0	3.8	—
II	0.2	1.3	3.4	4.6	12.5	4.8	13	44.0	8.5	2.6	—
	0.5	1.3	3.4	4.7	12.5	4.7	13.5	45.6	8.8	3.1	—
	2	1.3	3.4	4.6	12.6	4.7	14	49.8	11.2	4.1	—
III	0.2	1.8	3.8	4.2	12.4	5.1	9.5	50.2	10.7	3.2	—
	0.5	1.8	3.8	4.3	12.5	4.8	9.5	52.8	11.4	4.7	—
	2	1.8	3.8	4.4	12.7	4.7	10	55.8	12.8	6.2	—
IV	0.2	1.4	3.4	4.5	12.3	5.2		51.0	11.8	3.6	—
	0.5	1.3	3.5	4.6	12.4	5.2		53.9	12.8	4.8	—
	2	1.3	4.0	4.6	13.0	4.9		63.0	17.0	7.4	0.8
V	0.2	1.5				5.2	14	54.0	12.3	5.2	0
	0.5	1.6				5.0	14	56.9	12.9	5.4	0
	2	1.5				4.8	13	70.8	20.7	5.8	6.1
VI	0.2	1.7	3.7	4.5	12.8	5.3	9.5	67.7	22.8	2.8	2.3
	0.5	1.6	3.7	4.5	12.8	5.2	9.5	69.7	23.3	2.9	2.7

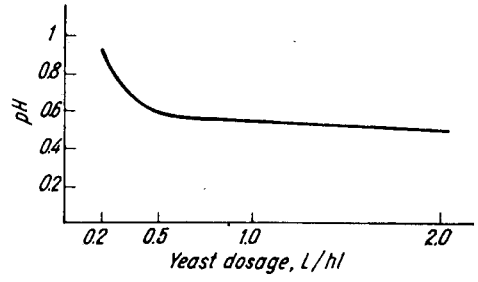

Fig. 1. Increase of pH in beer from yeast sediment in comparison with young beer in relation to yeast dosage (third generation)

was lower by 3 mg/l. A part of the yeast in the sediment does not undergo desorption from surface of the yeast cells and passes on the liquid stage. The degree of desorption of bitter substances was growing whenever the dose of pitching yeast was smaller (Fig. 3). A reverse process was observed for colour compounds.

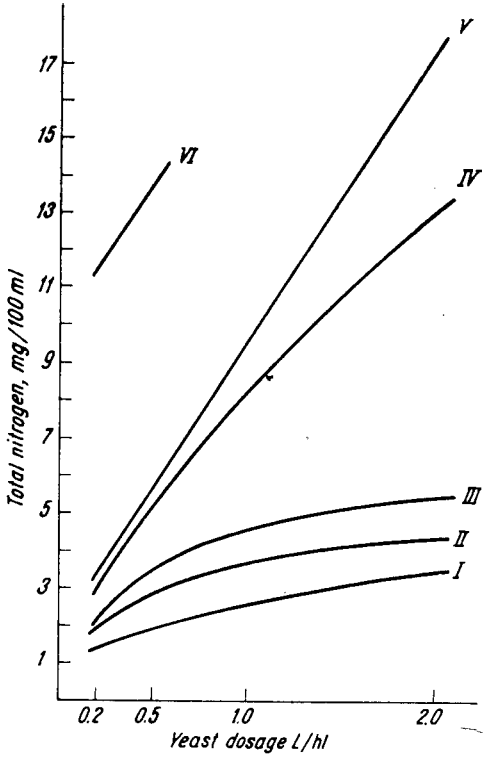

Fig. 2. Increase of total nitrogen content in yeast sediment beer in comparison with young beer for the 1 through 6 generations of yeast in relation to dosage

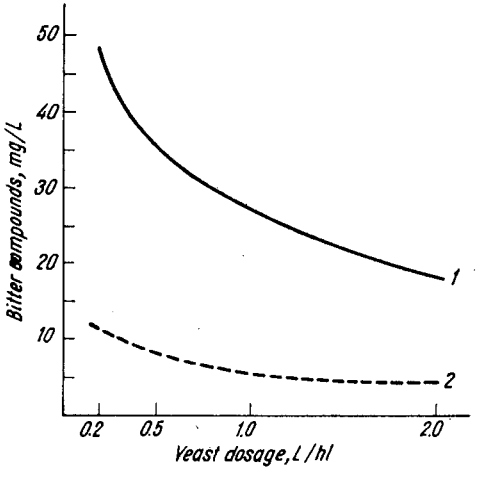

Fig. 3. Increase of bitter compounds in beer separated from sediment compared with young beer as dependent on yeast dosage (generation III); 1 — total bitter compounds, 2 — iso-compounds

Table 6. Contents of volatile products of fermentation in young beer in relation to dosage and generation of yeast (mg/l)

Yeast generation	Yeast dose 1/hl	Young beer						
		diacetyl + acetylolactate	acetoin	acetaldehyde	ethyl acetate	n-propanol	iso-butanol	amyl alcohols
I	0.2	1.2	7.0	13.6	7.6	11.2	10.3	58.2
	0.5	1.01	5.1	15.4	10.9	10.7	10.1	56.2
	2	0.81	3.0	17.2	15.4	10.9	10.7	56.8
II	0.2	0.95	4.4	10.9	10.8	11.1	11.3	57.6
	0.5	0.98	3.7	13.2	9.2	11.6	11.6	55.3
	2	0.89	3.4	16.1	13.0	8.2	10.0	52.8
III	0.2	0.86	4.4	10.1	15.0	10.2	11.8	49.7
	0.5	0.95	3.7	12.3	18.7	10.4	11.6	51.2
	2	0.80	3.1	15.9	21.5	9.9	11.0	48.0
IV	0.2	0.80	4.3	11.6	12.2	10.3	10.3	46.9
	0.5	0.63	3.4	15.7	13.9	10.0	9.9	46.4
	2	0.44	3.1	18.4	16.3	8.7	9.7	43.4
V	0.2	0.91	6.2	—	—	—	—	—
	0.5	0.81	5.5	—	—	—	—	—
	2	0.76	3.8	—	—	—	—	—
VI	0.2	0.74	5.4	13.6	23.5	8.3	9.1	46.8
	0.5	0.54	4.4	15.0	21.0	8.0	9.8	47.3

Table 7. Contents of volatile products of fermentation in beer separated from yeast sediment in relation to dosage and generation of yeast (mg/l)

Yeast generation	Yeast dose 1/hl	Diacetyl + acetylolactate	Acetoin	Acetaldehyde	Ethyl acetate	n-propanol	iso-butanol	Amyl alcohols
I	0.2	0.87	2.3	8.8	16.0	11.9	11.4	72.0
	0.5	0.61	1.7	8.8	18.2	11.9	11.2	66.4
	2	0.56	1.6	8.2	19.8	11.2	11.9	60.8
II	0.2	0.91	1.1	6.2	19.5	12.5	12.8	68.8
	0.5	0.66	1.2	7.9	16.3	12.7	12.5	64.2
	2	0.48	2.2	6.9	18.0	11.3	12.2	58.1
III	0.2	0.82	1.3	3.9	30.9	10.5	12.9	62.5
	0.5	0.80	1.2	3.2	28.3	10.4	12.5	58.6
	2	0.76	1.1	3.5	26.3	10.9	12.7	54.2
IV	0.2	0.71	1.2	2.8	28.8	11.1	14.9	58.9
	0.5	0.47	1.1	3.5	26.0	11.5	11.9	57.5
	2	0.40	1.0	5.4	23.1	11.5	12.9	51.9
V	0.2	0.57	1.4	—	—	—	—	—
	0.5	0.51	1.4	—	—	—	—	—
	2	0.62	1.1	—	—	—	—	—
VI	0.2	0.58	1.2	5.7	30.1	8.7	11.4	56.6
	0.5	0.50	1.1	4.2	27.8	9.4	10.9	54.7

During fermentation, in successive generations of yeast, the degree of utilization of aminoacids was reduced: alanine, valine, phenyloalanine and glutaminic acid. The volume of alanine, glutaminic acid, isoleucine, treonine, phenyloalanine, aspartic acid, tyrosine and lysine in the residual beer grew higher on account of deteriorating vitality of yeast and their autolysis. The concentration of volatile products of fermentation in young beer and in the residual beer changed in relation to dose and generation of yeast (Table 6, 7). The contents of acetylolactate, diacetyl and acetoine reached the highest levels in young beers from variants 1 and 2 (doses: 0.2 and 0.5 l/hl). Synthesis of acetylolactic acid takes place during the phase of intensive multiplication of yeast. For the above beer samples, as compared with variant 3, it was delayed by about two 24 hr periods thus the time of reduction of these compounds was shorter. Concentration of acetylo-aldehyde and ethyl acetate in beer grew smaller with the reduction of doses of the pitching yeast. The process of reduced secretion of acetylo-aldehyde to environment is related here to its higher utilization during synthesis of components indispensable for the cell [6].

The contents of higher aliphatic alcohols: iso-butanol, and particularly, of amyl alcohols in young beer increased with the growing process of yeast cell rejuvenation (the 0.2 l/hl dose) but here a dropping tendency was observed in the synthesis of these compounds in beer samples from successive generations.

A greater degree of equality of levels of volatile products of fermentation was observed in the residual beer as compared with the young beer. The high concentration of yeast in the sediment caused a considerable reduction of the volume of acetoin and acetylo-aldehyde in the residual beer (Fig. 4). Due to lack of nutrients the yeast re-sorbed acetylo-aldehyde. The process takes place during beer storage and maturation. The acetylo--aldehyde was also partially used in the process of synthesis of esters that led to a higher content of ethyl acetate. The contents of amyl alcohols and ethyl acetate in the residual beer increased with smaller doses of the pitching yeast (Fig. 4). Within successive generations of yeast the concentration of amyl alcohols dropped down (Fig. 5). This was due to deterioration of the physiological state of cells on account of higher pitching dosage and the successive generations of yeast which induced a slower tempo of the yeast metabolic processes.

Summing up the obtained results it can be said that reduction of the yeast dose, compared to 0.5 l and 2 l/hl, develops higher pH in the residual beer, causes a smaller increase of nitrogen compounds, and leads to a higher reduction of acetoin as well as synthesis of ethyl acetate and amyl alcohols. The cause of these changes in the chemical composition of beer is the higher degree of yeast multiplication, consequently, more active metabolism of the cells. With every successive generation of yeast the vitality of cells goes down the faster the higher was the initial dose. The

rate of metabolic processes is slowed down and that is the cause of the reduction of the degree of utilization of wort aminoacids, extension of fermentation time, reduction of synthesis of fermentation by-products. In the residual beer occurs an increase of compounds, negative from the point of view of beer's quality, such as ethyl acetate and nitrogen compounds: total, coagulable, formolic and aminoacids nitrogen, and at the same time the activity of proteolitic enzymes released during yeast autholysis becomes more intensive. Only the volume of synthesized amyl alcohols is reduced.

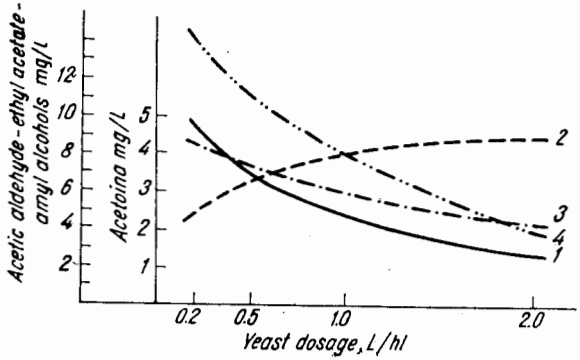

Fig. 4. Variability of contents of acetoine, acetic aldehyde and ethyl acetate in residual beer compared with young beer as dependent on yeast dose (generation I); 1 — acetoine, 2 — acetic aldehyde, 3 — ethyl acetate, 4 — amyl alcohols

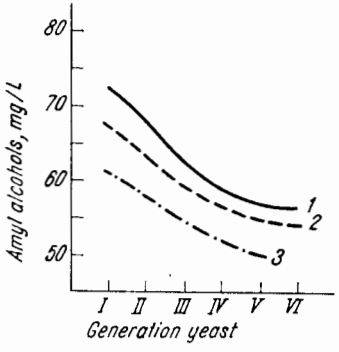

Fig. 5. Contents of amyl alcohols in residual beer depending on yeast generation; 1 — yeast dose 0.2 l/hl, 2 — yeast dose 0.5 l/hl, 3 — yeast dose 2 l/hl

The changes in the physiological state of yeast are accompanied at the same time by adsorption of wort components during fermentation and their partial release from cell surfaces at the final phase of the process. The desorption processes in the residual beer are the cause of a considerable increase in the volume of bitter and colour substances.

CONCLUSIONS

1. Increased doses of the pitching yeast and their successive generations induce a deterioration of the physiological state of yeast cells, as evidence in the lowering of cell vitality and cell metabolic activity. They cause changes in the levels of metabolic products of yeast in the young beer as well as in the residual beer separated from the sediment after wort fermentation. The changes primarily concern nitrogen compounds and fermentation by-products.

2. The changes of the physiological state of yeast are accompanied by processes of desorption of bitter compounds, whose intensity grows with the lowering of the pitching yeast dose.

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WPŁYW DAWKI I GENERACJI DROŻDŻY NA POZIOM PRODUKTÓW METABOLIZMU DROŻDŻY ODDZIELONYCH PO FERMENTACJI BRZECZKI PIWNEJ

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Streszczenie

Przeprowadzono badania nad wpływem dawki i generacji drożdży nastawnych na ilość produktów metabolizmu w piwie oddzielonym z gęstwy drożdżowej zebranej po fermentacji burzliwej brzeczki. Stosowano drożdże rasy Bratislava w daw-

kach 0,2 l; 0,5 l i 2 l/hl brzeczki chmielonej. Proces fermentacji prowadzono w temperaturze 7,0-9,5°C. Piwo z gęstwy drożdżowej oddzielano metodą wirowania i jego skład chemiczny porównywano ze składem piwa młodego. Badania powtórzono w 6 kolejnych doświadczalnych fermentacjach, używając drożdży zebranych z doświadczeń poprzednich.

Stwierdzono, że wzrost dawki drożdży nastawnych i kolejne ich generacje powodują pogorszenie stanu fizjologicznego komórek, które przejawia się obniżeniem żywotności i aktywności metabolicznej drożdży. Powodują one zmiany poziomu produktów metabolizmu drożdży w piwie młodym i w piwie oddzielonym z gęstwy drożdżowej po fermentacji. Obniżenie dawki drożdży w porównaniu z dawką 0,5 l i 2 l/hl powoduje w piwie z gęstwy stosunkowo wyższy wzrost pH, mniejszy przyrost związków azotowych, a ponadto większą redukcję acetoiny oraz syntezę octanu etylowego i alkoholi amylowych. Przyczyną tych zmian składu chemicznego jest wzrost stopnia rozmnożenia drożdży, a w związku z tym lepszy ich stan fizjologiczny, następstwem którego są aktywniej przebiegające w komórkach procesy metabolizmu.

W miarę kolejnych generacji drożdży, żywotność komórek obniża się tym szybciej im wyższa była ich dawka. Następuje zwolnienie tempa procesów metabolizmu, które jest powodem obniżenia stopnia wykorzystania aminokwasów brzeczki, zmniejszenia syntezy ubocznych produktów fermentacji i wydłużenia czasu fermentacji. W piwie oddzielonym z gęstwy drożdżowej następuje niekorzystny z punktu widzenia jakości piwa wzrost ilości octanu etylowego i związków azotowych: azotu ogólnego, koagulującego, formolowego i aminokwasów, a jednocześnie rośnie aktywność enzymów proteolitycznych uwalnianych w procesie autolizy drożdży. Zmniejsza się ilość syntetyzowanych alkoholi amylowych.

Zmianom stanu fizjologicznego drożdży towarzyszą jednocześnie zjawiska adsorpcji składników brzeczki podczas fermentacji oraz częściowego ich uwalniania z powierzchni komórek w końcowej fazie procesu. Zjawiska desorpcji w piwie oddzielonym z gęstwy drożdżowej są powodem znacznego wzrostu ilości substancji goryczkowych i barwnych.