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THE EFFECT OF BEER WORT FERMENTATION ON LEVELS OF THE YEAST METABOLISM PRODUCTS

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Key words: beer wort, yeast metablism products, autolysis of yeast.

The pressure fermentation as well as the classic fermentation at at 7° to 9.5°C and at the lowered temperature of 6° to 8.2°C were used. The physiological parameters of the test variants' yeast as compared to the control test revealed substantial worsening. The content of dead yeast cells in the sediment after pressure fermentation grew higher and their autolysis resulted in a considerable quantitative increase in nitrogen compounds. Depletion of the yeast sediment during fermentation tends to reduce this negative phenomenon. The beer from yeast sediment revealed growing quantities of esters, alcohols and products of desorption.

Excretion and autolysis result from metabolism in a yeast cell during alcohol fermentation. Devreux recorded considerable differences in the contents of aminoacids, peptides, nucleoides, colour compounds as well as the activity levels of invertase and glucosidases, depending on the level in the beer storing tank from which the sample was taken, and the distance from the yeast layer deposited at the bottom [9]. Concentration of these compounds depends on storing conditions and produces changes in the physicochemical and organoleptic properties of beer.

The aim of the study was to examine the effect of fermentation methods, temperature and processing time on levels of selected products of metabolism of yeast in beer separated from yeast sediment.

MATERIALS AND METHODS

The hop-malt filtered wort inoculated with the Bratislava strain at 0.5 l/hl was used in the experiment. The yeast was supplemented as a 13% dry- substance yeast cake. The fermentation process was conducted in 205 cm tall and 36 l capacity vertical fermentation columns with conical bottoms. The tests were carried out under the conditions of the

traditional fermentation process at a lowered temperature of 6° to 8.5°C, aver. temp. — 7,2°C (Variant 1), and at 7° to 9.5°C, aver. 8.5°C (Control variant 2), as well as under pressure fermentation of 0.7 to 1.8° atm. at 11° to 16°C. In the first pressure test the yeast collected in the conical parts of the fermentation columns was separated after 3 days of fermentation (Variant 3), while in the other test it was separated after completion of the process, that is, after four days (Variant 4).

The young beer, cooled down to 5°C, was pumped to the storing tank to stay there for 10 days at 0° to 2°C and 0.8 atm. The yeast sediment of the fermentation period, recovered from the fermentation column's conical section was stored for 24 hrs at 0° to 2°C. The chemical composition of the beer centrifuged from the yeast sediment (2800 rpm over 10 min) was compared to the chemical composition of young beer.

The analyses of wort and beer were performed according to Analitica EBC [1]. The contents of iso-compounds, according to Brenner [6], bitters according to De Clerck [9], polyphenols according to Bishop [5], were established. Free aminoacids were determined by gas chromatography [10]; acetolactate and diketones — according to Brenner [7]. Higher aliphatic alcohols, ethyl acetate, acetate aldehyde were determined by gas chromatography [2].

RESULTS AND INTERPRETATION

THE YOUNG BEER

The chemical composition of the starting wort is given in Tables 1 and 2. The velocity of the wort fermentation process, according to the three assumed technological variants, showed considerable differentiations. The weakening of the yeast metabolism intensity due to the lowering of the fermentation temperature as compared to the control test, led to a lower utilization of aminoacids and low-molecule peptides of wort by ca. 11% (Tables 4 and 5). On the other hand, a longer contact of the beer with yeast resulted in a higher adsorption of colour substances and isocompounds by 8% from the wort. The accelerated metabolism processes, effected by the higher temperature of the wort during the pressure fermentation against the control beer, led to a higher utilization of aminoacid nitrogen. At the same time a considerable reduction in quantity of iso-compounds and poliphenols occurred in effect of more intensive adsorption. The separation of yeast taking place 24 hrs earlier, as compared to the Variant 4 beer, reduced the adsorption degree, or in other words, the losses in iso-compounds, by 15%.

The differentiated rate of metabolic changes in yeast cells during fermentation was reflected primarily in the quantity of volatile products in the young beer (Table 6). The lowering of the fermentation temperature

Table 1. Chemical composition of wort

Extract %		Colour EBC	Acidity ml NaOH/100 ml	pH	Hop bitter substance mg/l		Polyphenols mg/l	Nitragen compounds mg/100 ml			
initial	final				total	iso-compounds		total nitrogen	formol nitrogen	cannin nitrogen	coagulating nitrogen
12.2	1.8	15	1.5	5.6	88.2	30.3	147	107.7	42.8	19.8	4.1

Table 2. Aminoacid composition of wort mg/100 ml

Alanine	22.8
Valine	18.2
Glycine	2.8
Iso-leucine	11.0
Leucine	30.4
Proline	69.7
Treonine	8.3
Serine	20.2
Hydroxyproline	1.5
Phenylalanine	19.0
Aspartic acid	20.4
Glutamin acid	11.9
Tyrosine	4.7
Lysine	18.3
Total content	259.2

was the cause of lower concentrations of the following elements in beer: ethyl acetate, isobutanol, amyl alcohols and the higher contents of acetic aldehyde and diketones. On the other hand, the higher intensity of the intra-cell yeast transformations during the pressure fermentation led to a substantial increase of the contents of ethyl acetate, iso-butanol, amyl alcohols, acetate aldehyde and diketones in beer.

THE YEAST SEDIMENT

The analyses of the yeast sediment and the physiological parameters of the yeast after fermentation provide evidence that longer fermentation time due to the lowered temperature leads to only a minor reduction in cells' vitality and to a lower yeast crop (Table 3). The pressure fermentation, on the other hand, with its higher temperature and increased pressure of carbon dioxide led to a pronounced cutting of their vitality. The quantity of dead yeast cells in the slip following pressure fermentation was more than three times as high as in the traditional process (10% and 7% vs. 3%), while at the same time the yeast separation after 72 hrs reduced the concentration of dead cells considerably (to 6.9%).

Table 3. Parameters of the physiological state of yeast collected after wort fermentation

Sample No.	Sample description	Yeast slip		Separated yeast ca. 24% d.m.			
		slip quantity g	beer quantity %	P crop total g	increment $\frac{P}{W}$	cell population% budding	dead
1	Standard fermentation — long secondary fermentation of extract	600	50	300	3.6	1	3.7
2	Standard fermentation — control	630	51	310	3.7	6	3.0
3	Pressure fermentation — separation of yeast after 72 hours	650	52	250 × 65*)	3.7	0	6.9
4	Pressure fermentation — separation of yeast after completion of the process (4 times 24 hrs)	650	52	315	3.7	0	10.7

*) The residue of yeast collected from the fermentation tank bottom after four days of fermentation

THE BEER FROM YEAST SEDIMENT

Secretion, exchange, desorption and autolysis — showing different levels of intensity in the yeast sediment account for further differentiations in the chemical composition of beer separated from it. Decomposition of glycogen in yeast cells and inclusion of glucose into the chain of alcohol fermentation effected a higher level of ethyl alcohol in the beer from yeast sediment (0.8% to 2%) (Table 4). This occurred with highest intensity in the beer from yeast cake following pressure fermentation. Colouring the yeast with Lugols solution revealed that during the phase of intensive fermentation the quantity of cells containing glycogen was higher than 94%. On the last day of pressure fermentation the level of cells containing glycogen in the yeast sediment was ca. 14%. Intensity of metabolic processes in the yeast cells deposited as cake at the bottom of fermenting columns depends on conditions around them [3]. Higher temperature in the course of the pressure fermentation intensified metabolic processes while higher pressure accelerated secretion, exchange, autolysis and desorption. In effect of these processes there was a higher content of alcohol, higher pH, greater quantities of nitrogen, bitter, and colour compounds as well as more polyphenols, as compared to corresponding levels in the beer from Variant 1 and Variant 2 [11, 12]. It pertains to the Variant 4 beer in particular. Separation of yeast 24 hrs earlier —

Table 4. Chemical composition of new beer and beer separated from yeast sediment

Sample No.		Variant	Fermentation time	Extract, %		Alcohol %	Basic wort %	Colour EBC	pH	Polyphenol mg/l	Bitter compounds mg/l		Nitrogen compounds		
				apparent	real						iso-compound	total	total Na	formolic Na	coagulating Na
1	New beer	Extended secondary fermentation of extract	18	2.3	4.2	4.2	12.3	12	4.4	156	15.6	53	84.2	26.2	4.5
2		Control sample	13	2.4	4.3	4.0	12.2	13	4.4	150	17.0	56	79.2	23.5	4.7
3		Pressure fermentation, separation of yeast after 72 hours	4	2.4	4.3	4.1	12.3	14	4.3	145	15.1	55	78.6	22.0	3.1
4		Pressure fermentation	4	2.4	4.2	4.1	12.2	13	4.3	146	13.1	54	76.0	21.4	3.4
1	Slip beer	Extended secondory fermentation of extract		1.5	3.8	5.0	13.6	15	5.4	179	28.2	73	95.4	32.3	5.7
2		Control sample		1.5	4.3	5.4	15.3	20	5.1	175	36.8	77	88.4	33.1	5.3
3		Pressure fermentation, separation of yeast after 72 hours		1.6	4.4	5.8	15.9	20	5.4	190	48.2	88	87.6	31.4	7.1
4		Pressure fermentation		1.6	4.3	6.0	16.0	22	6.2	192	50.1	89	93.0	34.7	7.8

Table 5. Contents of fermentation by-products in young beer and in beer separated from yeast sediment

Sample No.	Variant	Young beer							Beer from yeast slip						
		diacetyl	acetoin	acetic aldehyde	ethyl acetate	n-propanol	iso-butanol	amyl alcohols	diacetyl	acetoin	acetic aldehyde	ethyl acetate	n-propanol	iso-butanol	amyl alcohols
1	Extended secondary fermentation	0.32	2.9	19.0	15.0	9.8	6.6	48.7	0.19	1.02	9.5	25.3	10.6	8.0	53.1
2	Control sample	0.24	2.3	14.3	16.2	10.0	7.7	51.6	0.13	1.20	7.9	21.4	10.9	8.9	55.9
3	Pressure fermentation, separation of yeast after 72 hours	0.45	2.1	10.6	19.6	9.5	8.6	53.8	0.40	1.30	17.1	23.1	10.8	10.4	59.7
4	Pressure fermentation	0.54	2.7	18.0	21.0	9.9	9.3	57.3	0.42	1.1	16.0	25.1	12.0	10.8	62.4

Table 6. Contents of aminoacids in young beer and in beer separated from yeast sediment (mg/100 ml)

Sample No.		Variant	Alanine	Valine	Glycine	Isoleucine	Leucine	Proline	Treonine	Serine	Hydroxyproline	Fenylalanine	Aspartic acid	Glutamic acid	Tyrosine	Lysine	Total aminoacids	
																	without proline	total
1	Young beer	Extended secondary fermentation	15.7	10.8	5.8	3.8	7.3	44.0	0.5	11.1	0.2	7.4	5.4	6.4	6.6	4.7	85.7	129.7
2		Control sample	15.5	10.3	6.0	4.5	6.2	44.5	0.3	11.0	0.2	7.0	5.1	5.9	6.7	3.2	81.9	126.4
3		Pressure fermentation, separation of yeast	15.7	10.1	5.9	3.4	4.6	46.1	0.2	12.9	0.1	6.0	3.9	5.9	6.6	2.1	77.4	123.5
4		Pressure fermentation	15.0	10.4	5.8	4.2	4.5	44.9	0.4	12.7	0.1	5.9	3.9	5.7	7.1	2.1	77.8	122.7
1	Sediment beer	Extended secondary fermentation	16.1	9.6	6.9	4.2	7.5	39.6	0.7	11.7	0.2	8.5	8.2	11.0	7.9	5.1	97.6	137.2
2		Control sample	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
3		Pressure fermentation, separation of yeast	18.8	11.9	6.2	4.4	4.7	45.6	0.6	13.1	0.2	5.8	4.2	7.3	5.6	2.3	85.1	130.7
4		Pressure fermentation	19.7	11.2	6.7	5.4	4.9	46.2	0.7	13.9	0.2	6.1	4.5	8.8	8.3	2.6	93.0	139.2

Variant 3 — reduced the negative effect of the adverse conditions. This is best evidenced by lower increase of pH and of the contents of nitrogen, bitters and colour substances as well as polyphenols (Tables 4 and 5). Compared to the young beer, the higher contents of nitrogen compounds related to quantities of total, coagulating and formolic nitrogen as well as aminoacids. This is due to yeast autolysis, in the first place. The autolysis during the pressure fermentation tests caused an increase in the contents of glutamic acid, alanine, iso-leucine, and lysine (Table 5). Increment in aminoacids in Test 4, compared to the young beer, was 13%. An earlier separation of yeast in Variant 3 resulted in only a 6% increase in aminoacids due to a lower concentration of dead cells in the yeast sediment similarly to the beer from yeast sediment in Variant 1. In comparison with the control test the lower temperature and longer fermentation time induced in the beer from yeast sediment a higher concentration of products of secretion, exchange and autolysis, higher pH and higher contents of total nitrogen and aminoacids. Inhibition of desorption was observed on account of lower increase in bitter and colour compounds.

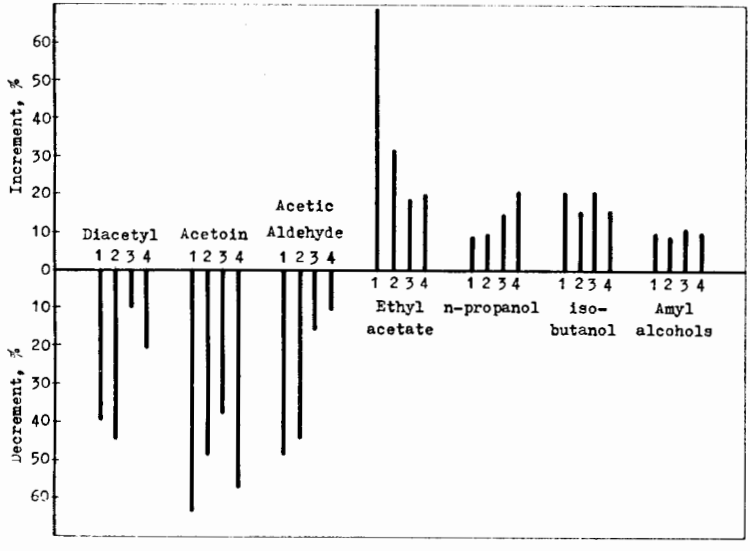

Fig. Effects of conditions and methods of fermentation on content changes in beer fermentation volatile products. The yeast slip beer is compared to new beer; 1 — extended secondary fermentation, 2 — control sample, 3 — pressure fermentation, yeast separation, 4 — pressure fermentation

Lessening of the yeast metabolic activity after fermentation was reflected in the quantity of volatile products of fermentation in the separated beer (Table 6). Percentage of change in these contents in both

the beer from yeast sediment and the young beers is given in Fig.

Lessening of the yeast metabolic activity after pressure fermentation — Variants 3 and 4, as compared to the traditional fermentation — was revealed in slowed down transformation of diacetyl by 20%, acetic aldehyde by 25 to 39% and in slower synthesis of ethyl acetate by 13 to 51% [4, 13]. Inhibition of synthesis of acetyl-lactate was also observed. The prolonged secondary fermentation of beer extended the time of retention of the yeast sediment at the bottom of the fermenting column and effected a substantial increase in the concentration of ethyl acetate [3] and in amyl alcohol, as in the control test.

FINAL PRODUCT

The chemical composition of the market-ready beer showed no basic differences of parameters as compared to that of the young beer. It was noticed, however, that the quantities of diketones and acetic aldehyde were reduced in all samples of beer (Table 7). They were below threshold of detectability in all cases except the acetic aldehyde in Variant 1 beer. Comparison of the young beer and the secondary-fermentation beer in terms of the contents of the investigated esther and aldehyde (Table 5) as well as higher aliphatic alcohols retained the same levels, which accounts for a slower rate of metabolism during storing.

In the other beers the quantities of ethyl acetate and amyl alcohols went up. In the control sample the content of ethyl acetate grew by 23%; amyl alcohols — 4%. For Variant 3 these were, respectively, 16% and

Table 7. Contents of volatile products of fermentation in beer (mg/l)

Sample No.	Variant	Diacetyl	Acetion	Acetic aldehyde	Ethyl acetate	n-propanol	Isobutanol	Amyl alcohols
1	Extended secondary fermentation	0.08	1.65	18.6	15.1	10.8	6.7	50.9
2	Control sample	0.00	2.04	8.6	20.0	9.3	7.9	53.9
3	Pressure fermentation, yeast separation	0.00	1.67	8.8	22.8	8.9	8.4	58.6
4	Pressure fermentation	0.00	1.24	10.0	25.4	10.2	9.6	61.2

8.5%, while for Variant 4 — 20% and 7%. The best organoleptic properties were found in Variant 2 — traditional fermentation, and in Sample 3 — pressure fermentation modified by an earlier separation of the yeast sediment from the fermentation column (Table 8). Somewhat altered

sensoric properties were observed in the beer from pressure fermentation — Variant 4.

The beer secondarily fermented over an extended period of time was assessed as inferior in terms of bitterness, taste and flavour. Summing up, it can be said the physiological parameters of yest, differentiated by application of different fermentation methods and their modification, affect the levels of products of metabolism in young beer, in the yeast sediment beer, and in the market-ready product. In the course of the pressure fermentation, which involved a higher temperature of the process, metabolism and adsorption of wort components were much more intensive than during the traditional fermentation. The nitrogen components in wort are better utilized while at the same time the synthesis of fermentation volatile products increases, mainly, higher aliphatic alcohols, acetic aldehyde and ethyl acetate. The worsening of physiological parameters and reduction of life of yeast after pressure fermentation cause an increase in pH, in contents of nitrogen compounds and ethyl alcohol as well as intensification of desorption of bitters, polyphenols and colour compounds in the beer separated from yeast sediment.

Table 8. Organopletic assessment of experimentally obtained beers; points

Sample No.	Variant	Foaming	Clarity	Colour	Saturation	Bitterness	Taste and arome	Total score
1	Extended secondary fermentation	9.0	8.3	4.0	8.8	17.8	27.7	75.6
2	Control sample	9.0	9.0	4.0	8.5	19.0	30.8	80.3
3	Pressure fermentation; yeast separation	9.0	8.6	4.0	8.8	19.2	30.3	79.9
4	Pressure fermentation	8.6	8.7	4.0	8.6	18.9	29.2	78.0

Application of lower temperature of the process that extends secondary fermentation of wort, as compared to the traditional method, induces a general reduction in the metabolic activity of yeast. It is evidenced by lower assimilation of nitrogen compounds and synthesis of volatile products of fermentation and also by poorer desorption. These processes, when put together, affect negatively the organoleptic properties of beer.

CONCLUSIONS

Conditions of wort fermentation modify the physiological parameters of yeast, which produce changes in concentration of products of metabolism excreted to beer surrounding the cells.

1. The extended fermentation time, regardless of the low temperature of wort, reduces the vitality of yeast, which leads to a considerable increase of concentration of nitrogen compounds and ethyl acetate as well as to changes in pH of the beer from yeast sediment.

2. The pressure fermentation process alters substantially the physiological parameters of yeast which affect the composition of metabolic products in beer separated from the yeast sediment. Such a beer contains higher quantities of nitrogen compounds, ethyl alcohol, acetic aldehyde, ethyl acetate, and amyl alcohols. Intensive desorption is the cause of a pronounced increase of the quantities of polyphenols, bitters and colour compounds. The process of yeast autolysis, inducing higher contents of nitrogen compounds, can be inhibited by removing the yeast sediment from the bottom of the fermentation columns during wort fermentation, as already applied in the Nathan system, for example.

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WPŁYW WARUNKÓW FERMENTACJI BRZECZKI PIWNEJ NA POZIOM PRODUKTÓW METABOLIZMU DROŻDŻY

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Streszczenie

Przeprowadzono badania nad wpływem metody fermentacji, temperatury i czasu trwania procesu na stan fizjologiczny drożdży i związany z nim poziom niektórych produktów przemiany materii drożdży w piwie oddzielonym z gęstwy drożdżowej. Stosowano brzeczkę słodową filtrowaną, którą szczepiono drożdżami Bratislava w dawce 0,5 l gęstwy/hl. Badania wykonano w układzie warunków fermentacji

klasycznej w temperaturze 7-9,5°C średnio 8,5°C i w temperaturze obniżonej 6-8,5°C średnio 7,2°C oraz fermentacji ciśnieniowej w temperaturze 11-16°C pod ciśnieniem 0,7-1,8 atn. Z pierwszej próby ciśnieniowej oddzielano gęstwę drożdżową po 3 dobach fermentacji, natomiast z drugiej próby po zakończeniu procesu, tzn. po 4 dniach. Młode piwo leżakowano w temperaturze 0-2°C i ciśnieniu 0,8 atn w ciągu 10 dni. Gęstwę drożdżową zebraną po fermentacji przechowywano w temperaturze 0-2°C w ciągu 1 doby, po czym oddzielano piwo metodą wirowania. Skład chemiczny piwa z gęstwy porównywano ze składem piwa młodego. Ponadto przedstawiono charakterystykę fizykochemiczną i organoleptyczną produktu końcowego.

Stwierdzono, że podczas fermentacji ciśnieniowej wskutek wyższej temperatury procesu, przemiany metaboliczne i zjawiska adsorpcji składników brzeczki przebiegają znacznie intensywniej niż w czasie fermentacji prowadzonej metodą klasyczną. Wyższy jest stopień wykorzystania składników azotowych brzeczki, a jednocześnie zwiększa się synteza lotnych produktów fermentacji, w tym głównie alkoholi amylowych, aldehydu octowego i octanu etylowego. Pogorszenie stanu fizjologicznego, obniżenie żywotności drożdży po fermentacji ciśnieniowej jest przyczyną znacznego wzrostu pH i ilości związków azotowych i alkoholu etylowego oraz nasilenia się zjawisk desorpcji składników goryczkowych, polifenoli i substancji barwnych w piwie z gęstwy drożdżowej. Niekorzystne zmiany w składzie chemicznym tego piwa mogą być częściowo zmniejszone przez oddzielenie drożdży osadzonych na dnie fermentorów jeszcze w czasie trwania procesu fermentacji, a nie dopiero po jej zakończeniu.

Stosowanie niższych temperatur procesu, powodujące przedłużone deformowanie brzeczki w porównaniu z fermentacją klasyczną, powoduje zmniejszenie ogólnej aktywności metabolicznej drożdży wyrażające się mniejszą asymilacją aminokwasów i syntezą lotnych produktów fermentacji. W piwie oddzielonym z gęstwy drożdżowej, wskutek procesów autolizy drożdży i zjawisk desorpcji następuje wzrost ilości substancji azotowych, goryczkowych, barwnych, polifenoli, octanu etylowego oraz wartości pH.