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TECHNOLOGICAL SUITABILITY OF MONOSPOROUS POPULATIONS AND HYBRIDS OF BREWERS' YEAST. PART I. LABORATORY-SCALE TESTS

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Key words: fermentation by-products, beer flavour, yeast properties brewers' yeast hybrids,

The production properties of mixed brewers' — bakers' yeast hybrids which in laboratory and large-laboratory scale conditions produce beer of a good flavour and low content of by-products were determined. The different varieties of beer were qualified on the basis of a conventional set of points correlating flavour properties, the attenuation level and the content of by-products in experimental beer.

Factor determining industrial suitability of brewers' strains include not only production of ethyl alcohol and CO_2 but also capabilities for producing acids, esters, higher alcohols and ketones in harmonized quantities providing the characteristic beer flavour and aroma. Individual varieties of beer differ considerably in their content of alcohol fermentation by-products. These differences depend not only on composition of raw materials, fermentation method and storage time but also largely on yeast properties.

The aim of the present study was to characterize technological features of mixed brewers' strains obtained from combined monosporus populations of either brewers' or bakers' yeast. Control materials consisted of initial populations of yeast varieties used in the domestic brewing industry and of their monosporus versions isolated from sporogenous yeast populations [12].

EXPERIMENTAL PART

Initial biological material consisted of strains listed in Table 1.

At particular stages of the investigation the strains were used on the following scale:

— laboratory-scale (Fig. 1)

— large laboratory-scale (Fig. 2)

The tests and analyses were performed at Experimental Laboratory, of the Institute of Fermentation Industry, located in a brewery of Biskupiec, Poland.

Table 1. Yeast strains selected for analyses

a) initial populations

Parent yeast		Monospore populations	
No.	origin	brewers'	bakers' yeast
3	IPF	3-5 3-15	
15	ŁOCK*)	15-5 15-11	
16	IPF	16-5 16-6	
28	IPF-Biskupiec	28-9 28-13	
		28-19 28-X	
Ja-64	ŁOCK*)		11D 14D 14D

b) mixed strains used in analyses

No.	Combined monospore populations	Number of isolated zygotes	Number of analyzed mixed strains
M 5	16-5×15-11	3	M5
M6	16-5×28-9	10	M6-1
			M6-2
			M6-3
			M6-4
M10	16-6×15-11	10	M10-1
			M10-2
			M10-3
			M10-4
			M10-5
M16	28-13× 3-5	1	M16
M21	28- X×15-11	7	M21
M23	11D× 3-5	3	M23
M26	14C× 3-15	4	M26-1
			M26-2
M27	14D× 3-15	6	M27-2
			M27-4

*) ŁOCK = Center for Pure Cultures in Łódź (Łódź Technical University)

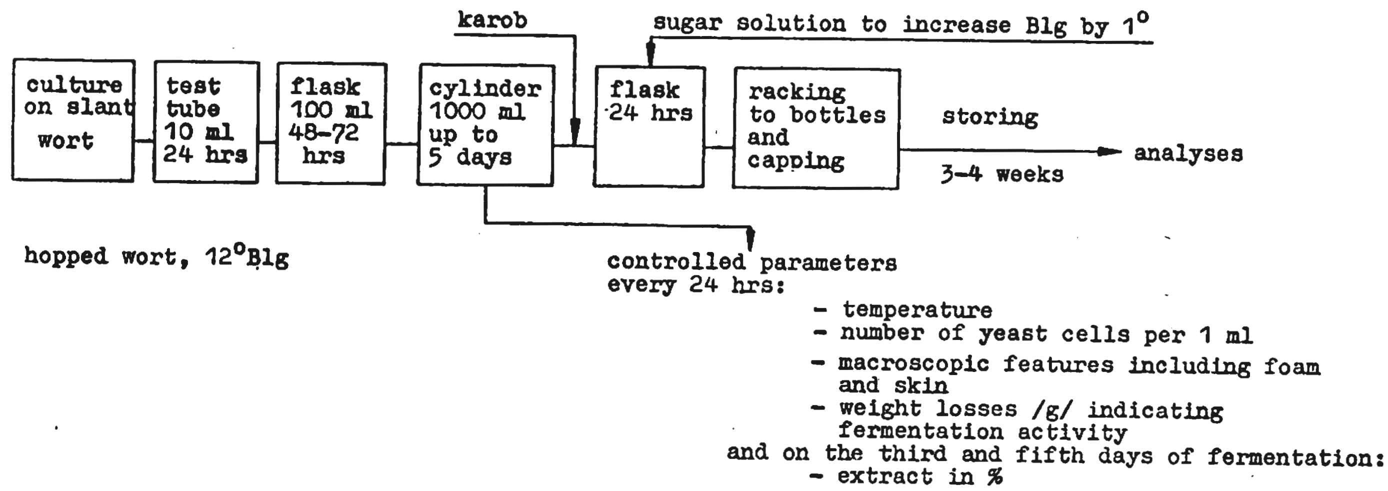

Fig. 1. Laboratory — scale beer production

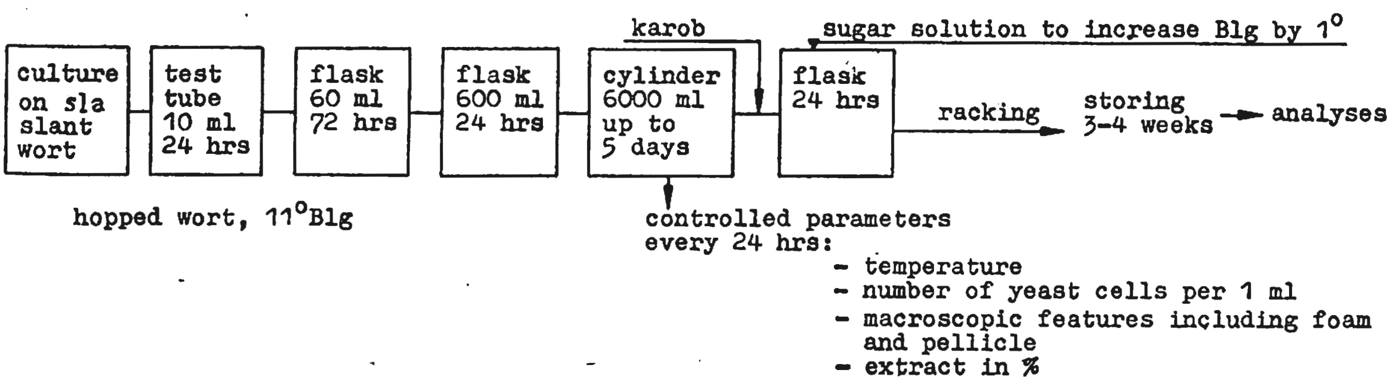

Fig. 2. Large laboratory — scale beer production

ANALYTICAL METHODS

a) in all trials during the fermentation process routine determinations included the number of cells, temperature extract in % — in selected trials macroscopic characteristics of the fermenting liquid were controlled with taking into account the formation of foam, pellicle and quantity of releases CO_2 (g) as an assessment of fermentation activity and yeast crop — sediment of cells,

b) beers obtained in the above mentioned process were analyzed according to a Polish standard (PN-65/A-79093). In addition, levels of diacetyl and acetoine were determined after Brenner et al. [6] and fusel oil alcohols after Boruff using methods applied in Experimental Laboratory, Institute of Fermentation Industry in Biskupiec, Poland.

c) organoleptic evoluation of beers was conducted according to a 100-point scale.

RESULTS

Resulting beers were not typical of the bottom or top fermentation processes. In the technological process top fermenting brewers' yeast was used together with barley wort. Data from the fundamental analyses of these beers were compared with the standard for light 12°Blg beers, and results of special analyses — with the data in literature concerning beer production [2].

Introductory assessment of industrial suitability of the obtained hybrids was performed during laboratory fermentation tests. Comparison involved monosporus populations and initial technological strains. The suitability of strains was assessed on the base of the degree of attenuation of wort and degustation appraisal of the beers. Results of the experiments are given in Tables 2, 3, 4 and 5.

It was proved that the analyzed hybrids are fit for technological purposes since they promise obtaining of good quality beers. Among the three compared groups of strains, the hybrids were characterized by the best fervementation capability. If we compare the value of extract (Ep) on the third day of fermentation (Table 2), we can observe that the mixed strains had Ep lower by 70% vs. the initial strains, and the monosporous population, respectively, showed Ep lower by 18%. It is characteristic that four monosporous populations (15-5; 15-11; 28-13; 28-19) fermented equally well as hybrid strains did.

This is confirmed by the results of determination of attenuation of wort performed on finished beer (Table 3). In comparison with the group of parent strains, monosporous populations were characterized by an 8%

Table 2. Extract of lab beers; analyzed on the third day of fermentation

Initial strains	Extract; % (g/g)	Monospore populations	Extract; % (g/g)	Hybrid strains	Extract; % (g/g)
3	7.68	3-5	6.91	brewers'	
		3-15	5.90	M5	1.87
15	4.07	15-5	1.87	M6-3	1.05
		15-11	1.87	M6-4	1.03
16	3.25	16-5	3.80	M10-2	1.85
		16-6	3.80	M10-5	1.92
				average	1.54
28	6.28	28-9	5.58	brewer's and bakers' yeast	
		28-13	1.70	M23	1.13
		28-19	1.16		
Ja-64	2.97	11D	2.99	M26-1	1.59
		14D	3.12	M26-2	1.57
		14D	3.12	M27-2	1.08
				M27-4	1.08
				average	1.29
average	4.85	average	3.49		

Table 3. Fermentation rate of lab-scale beers

Strains	Fermentation rate; %
Parent yeast	
3	63.0
15	57.0
16	63.0
28	63.0
Ja-64	64.0
average	62.0
Monospore	
3-5	59.0
3-15	61.0
15-5	69.0
15-11	69.0
16-5	63.0
16-6	67.0
28-9	72.0
28-13	79.0
28-19	75.0
11D	64.0
14C	65.0
14D′	64.0
average	67.0
Brewers' hybrids	
M5	67.0
M6-3	73.0
M6-4	76.0
M10-2	78.0
M10-5	69.0
average	72.6
Brewers' bakers' Yeast hybrids	
M23	75.0
M26-1	75.0
M26-2	76.0
M27-2	73.0
M27-4	76.0
average	75.0

higher attenuation while the group of mixed strains differed in the following way:

— for hybrid of brewer's and baker's yeast — 20%
— for brewers' hybrids — 16%

The content of by-products in finished beer (Table 4) was also clearly differentiated depending on the kind of applied initial material. Beer produced with the use of monosporous populations contained two times less diacetyl than beer that produced with brewer's — baker's hybrid yeast, which released in beer three times less diacetyl in comparison with the parent strains' productivity.

Levels of acetoine in beer obtained with the use of brewer's and baker's yeast hybrids were 20% lower than in those obtained with the parent strains. On the other hand, monosporous populations and brewers' hybrids produced in beer 10% more acetoine than the initial strains.

Table 4. Contents of by-products in beer from lab-scale production

Strains	Diacetyl; mg/l	Acetoine; mg/l	Higher alcohols; mg/l
Parent yeast			
3	0.27	1.55	—
15	0.20	1.77	30
16	0.22	2.18	71
28	0.42	1.62	—
Ja-64	0.15	2.33	38
average	0.25	1.89	46
Monospore			
3-5	0.13	2.71	27
3-15	0.18	1.64	56
15-5	0.09	2.56	67
15-11	0.18	1.90	80
16-5	0.04	2.37	95
16-6	0.07	2.18	95
28-9	0.13	1.69	95
28-13	0.04	1.78	79
28-19	0.06	1.50	127
11D	0.20	2.13	31
14C	0.11	2.44	—
14D	0,24	2,09	31
average	0.12	2.08	71
Brewings' hybrids			
M5	0.04	1.42	62
M6-3	0.09	1.73	200
M6-4	0.29	2.84	108
M10-2	0.18	2.80	106
M10-5	0.06	1.53	80
average	0.13	2.06	111
Brewings' and bakers' hybrids			
M23	0.06	1.96	98
M26-1	0.06	1.33	93
M26-2	0.08	1.35	97
M27-2	0.09	1.65	97
M27-4	0.13	1.33	95
average	0.08	1.52	96

The increased content of higher alcohols in beers produced both with the monosporous as well as the hybrid strains, if we compare it with the levels of such compounds in beers based on the initial strains, is probably related to a higher degree of wort attenuation by these strains.

Beers obtained on the laboratory scale were regarded as good in organoleptic criteria. (Table 5). Organoleptic evaluation proved they were

Table 5. Selected organoleptic features of beers from lab-scale production

Strains	Score for flavour and odour	Sum total	Bitter taste
Parent yeast			
3	27.0	76.0	18.2
15	27.8	76.4	19.0
16	28.2	79.8	20.5
28	29.0	78.0	19.0
Ja-64	28.5	79.0	20.0
average	28.1	77.8	19.1
Monospore			
3-5	29.2	78.0	19.7
3-15	28.2	79.0	19.0
15-5	27.7	75.4	18.3
15-11	26.0	72.8	18.0
16-5	27.2	75.6	18.2
16-6	26.0	73.8	18.0
28-9	27.4	74.2	17.2
28-13	26.8	75.8	18.0
28-19	27.3	74.8	18.0
11D	31.0	84.0	21.0
14C	27.0	77.5	18.5
14D	28.7	79.8	20.0
average	27.7	76.7	18.7
Brewers' hybrids			
M5	27.0	74.1	17.4
M6-3	27.6	76 8	18.2
M6-4	27.8	73.8	17.6
M10-2	28.6	73.6	17.8
M10-5	27.0	74.4	17 6
average	27.6	74.5	17.7
Brewers' and bakers' hybrids			
M23	27.3	75.8	18.0
M26-1	27.8	75.4	17.2
M26-2	27.4	75.8	17.6
M27-2	27.6	72.4	17.4
M27-4	27.2	71.8	17.2
average	27.5	74.2	17.5

Table 6. Wort fermentation with selected hybrids in large lab-scale production and some characteristics of finished beer obtained with such strains

Strains	Extract in succesive days of fermentation; % (g/g)					Fermentation rate of finished beer; %	Organoleptic evaluation (selected scores)		
	1	2	3	4	5		flavour odour	bitter taste	sum total
M23	7.53	6.20	5.23	4.29	3.45	62	27.0	18.6	77.0
M26-1	5.13	3.75	3.07	3.16	—	61	28.2	19.0	78.8
M27-2	4.51	3.27	2.94	2.94	—	64	27.2	19.0	76.6
M27-4	5.78	3.60	3.14	3.12	—	64	27.6	19.2	77.8

properly saturated with CO_2 and had rich foaming. Discrepancies in the assessment of flavour and aroma were not very significant (2.6 to 3.1 points). Higher scores were given to those varieties of yeast which provided to beer higher levels of by-products. The hybrids strains were in the group of those fermenting either well or very well. To have a better evaluation of the hybrids and to select the best variety of yeast for further analyses a system of classification was established.

According to the system, rejected strains were those which:

— produced more diacetyl in beer than 0.2 mg/l,

— produced more acetoine in beer than 2.0 mg/l,

— had poor fermentation (attenuation of wort) — extract on the third day of fermentation was above 2%.

The features taken into account were as follows: (scale of 1 to 5 points in scoring):

— the lowest value of apparent extract (5 points),

— the best degustation appraisal (5 points),

— the lowest content of by-products (5 points).

Using the above criteria, the analysts gave the highest scores to the hybrids:

M27-2 21 points
M27-4 20 ,,
M26-1 20 ,,

which belonged to the group of brewer's and baker's yeast hybrids.

These three strains were selected for further tests and for control strain the M23 was chosen which scored the lowest number of points (17). The selected strains were used in the large laboratory-scale tests. Selected results are given in Table 6.

The hybrids, except the control M23 strain, revealed good fermentation activity and beers produced with them were considered good in terms of flavour and aroma. Beers from the large laboratory-scale tests were characterized by low levels of fusel-oil alcohols. Two mixed strains (M23, M27-4) produced a higher than normal level of acetoine while the beer produced with M26-1 strain featured 60% higher contents of diacetyl (Table 7).

Table 7. Contents of byproducts in beers from large lab-scale production

Strain	Diacetyl; mg/l	Acetoine; mg/l	Higher alcohols; mg/l
M-23	0.11	3.17	10
M26-1	0.32	1.21	33
M27-2	0.13	1.26	56
M27-4	0.10	2.30	34

Eliminated from further tests were strains producing in beer a) more diacetyl than 0.2 mg/l, and b) more acetoine than 2.0 mg/l.

The best of the analyzed strains was one of the hybrids (M27-2) and it was further used in tests on a pilot plant scale.

The presented data show that hybridization of strains can be applied to improve characteristics of brewers yeast. A number of hybrids were obtained which revealed very good fermentation activity producing beers with harmonized flavour and low levels of by-products.

DISCUSSION OF RESULTS

Technological appraisal of the obtained brewers' hybrids was primarily based on evaluation of the composition of byproducts developed in beers by the strains under study. These components have a decisive effect on beer flavour. The content of fusel-oil alcohols in beer may range between 20 to 100 mg/l; higher levels pertain to beers of top fermentation type. Those values depend on yeast strains [15] and on fermentation conditions as well as storing condition [2, 9].

In the present study the content of higher alcohols in beers produced with different yeast strains ranged from 30 to 200 mg/l. Concerning carbonyl compounds, attention was focused on levels of diacetyl and acetoine. They do not affect negatively beer flavour if their quantities do not exceed 0.2 mg/ and 3.0 mg/l, respectively.

The content of such compounds in beer depends on properties of yeast [8], composition of wort and fermentation conditions [1, 7, 10, 11, 14, 16].

The level of diacetyl in the discussed beers was between 0.04 mg/l and 0.3 mg/l; acetoine — 1.3 to 2.8 mg/l.

Results given in the paper prove that improvement of brewer's yeast by combining sexually active spore populations is possible. The resulting hybrids possessed good capacity for attenuation of wort, better than that of the initial strains. Beers produced with such hybrids had harmonized flavours and low content of by-products.

CONCLUSIONS

1. In the course of qualifying strains with optimal features combining for abilities to cope with complex requirements of beer brewing technology, it is helpful to establish a conventional scoring table embracing the interdependence of the organoleptic evaluation, degree of attenuation of sugars and the content of by-products.

2. The content of fusel-oil alcohols produced by yeast hybrids in the experimental beers was considerably lower than in typical top fermentation beers, staying within limits permissible for bottom fermentation beers.

3. Levels of acetoine and diacetyl in the experimental beers based on hybrids were from 0.04 mg/l to 0.3 mg/l (diacetyl) and from 1.3 to 2.5 mg/l (acetoine).

4. High degustation scores were given to beers with varying content of by-products. This indicates the subjectiveness of this assessment.

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PRZYDATNOŚĆ TECHNOLOGICZNA POPULACJI MONOSPOROWYCH I MIESZAŃCÓW DROŻDŻY PIWOWARSKICH. CZ. I. PRÓBY W SKALI LABORATORYJNEJ I WIELKOLABORATORYJNEJ

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Streszczenie

Przeprowadzono charakterystykę porównawczą mieszańców drożdży piwowarsko-piekarskich, szczepów wyjściowych (tab. 1) oraz odmian monosporowych otrzymanych z tych szczepów. Spośród wymienionych grup drożdży mieszańce posiadały najlepszą zdolność fermentacyjną (tab. 2) uwzględniając wartość E_p w trzecim dniu

fermentacji. Równie dobrze jak mieszańce fermentowały populacje monosporowe (tab. 3). Różnice w zdolnościach fermentacyjnych poszczególnych odmian, w tym także różnych mieszańców, zależały od wyjściowego materiału biologicznego, z którego były konstruowane odpowiednie hybrydy. W piwach gotowych uwidoczniły się wyraźne różnice w zawartości produktów ubocznych (tab. 4) również uzależnione od rodzaju krzyżówek i od odmian badanych drożdży. Ogólnie można było stwierdzić, że poddane kontroli mieszańce można było zaliczyć do odmian dobrze i bardzo dobrze fermentujących dających piwa o dobrej ocenie organoleptycznej (tab. 5).

Na podstawie umowy zaproponowano system kwalifikacyjny obejmujący współzależność oceny degustacyjnej, stopnia odfermentowania i zawartości produktów ubocznych i wytypowano do dalszych badań 3 hybrydy, które uzyskały najwyższą liczbę punktów. Próby fermentacyjne wykonane w skali wielkolaboratoryjnej (rys. 3) objęły także oceną mieszańca M-23, który uzyskał najniższą notę w ocenie kwalifikacyjnej. Wyniki badań porównawczych są przytoczone w tab. 6. Jak z nich wynika, poza szczepem M-23, umieszańce posiadały dobrą aktywność fermentacyjną, a piwa uzyskane przy ich użyciu posiadały dobre cechy degustacyjne. Zawartość produktów ubocznych w tych piwach była wyraźnie zróżnicowana (tab. 7). Zasługuje na podkreślenie, że wysokie oceny degustacyjne piw nie korelowały z wyrównaną zawartością w nich produktów ubocznych.

Na podstawie tych wyników wytypowano do badań w skali ćwierćtechnicznej mieszańca M-27. Uznano również, że przyjęty w pracy umowny system kwalifikacyjny może być polecany do typowania szczepów dla technologii piwowarskiej.