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Yeast Selection for the Production of Grodzisk Beer

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Summary

Yeast used at present for the production of "Grodzisk" beer, is a mixture of various *Saccharomyces cerevisiae* (Hansen) yeast strains of top fermentation. Taste, flavour and technological properties, typical for the "Grodzisk" beer, were examined using a mixture of two strains: flocculating (B6/18) and a non-flocculent one (B6/10).

Introduction

Grodzisk beer is the oldest beer in Poland. The earliest known document in which the production of this beer is mentioned is a statute of the brewer's guild of 1601. Two kinds of beer, light and dark, were then made, and the best wheat malt used. Schönfeld (1938) says that the brewing industry has prospered in Grodzisk since the XIIIth century. According to historical data from about 1618, the Szadek and the Grodzisk beers were the two kinds most appreciated in Poznań in the XVth—XVIIth centuries (Majkowski 1938). Grodzisk beer is also mentioned by Warschauer (1893), Delbrück (1910), Desz (1955) Szmelich (1963) and others.

The characteristic taste and flavour of Grodzisk beer is due to a certain type of brewers yeast used, as well as the malting and brewing processes. There also was a legend about a famous Bernard's spring and the high quality of water used for the brewing of beer (Raczyński 1842, Łukaszewicz 1859).

Grodzisk yeast of exceptionally low attenuating power and early flocculating, was used in Grodzisk until end of the XIXth century (Schönfeld 1938). A natural pure culture was maintained thanks to its adaptability to a given medium (Delbrück 1910). Such a method of keeping yeast is used in England, Sweden, Denmark and Belgium (Rudin and Hough 1959; Hough 1959; Jeffery 1956; Schönfeld 1938). However, the original Grodzisk yeast did not survive until today. It was replaced by yeast of high attenuating power; high-pro-

tein wheat being at the same time replaced by low-protein, highly extractive wheat (Schönfeld 1938). The yeast used at present rapidly weakens and degenerates and must be periodically replaced, being probably maladjusted to local conditions. New seed yeast is brought now from abroad, unfortunately arriving to the Grodzisk brewery usually strongly contaminated.

To assure the production of the Grodzisk beer it was then necessary: 1) to obtain a pure culture of pitching yeast, 2) to select appropriate strains of yeast and insure the proper character of Grodzisk beer.

Experimental

Material and methods

The basic material was imported yeast, and also pitching yeast used in the production of Grodzisk beer. The yeast imported in August 1961 was designated B8/61 and that of June 1962, B6/62.

These samples of the yeast were plated on 2% wort agar in appropriate dilutions (ca 100 colonies per plate) and incubated at 25° for 48 h. The yeasts were then transferred to 15 ml of wort, 12° Blg, in test tubes, incubated for 48 h at 25° and microscopically examined; after 120 h the type of growth was observed. The isolation of pure culture was carried out by the Lindner methods (1901). The morphological and growth characteristics of cultures, thus isolated, were observed.

The fermentation ability was examined in duplicate in round flat-bottom 1 and 2 litre flasks containing 0.5 and 1 l of wheat hopped wort, 11° Blg, at room temperature, the fermenting liquid being stirred every morning and evening. The degree of fermentation was controlled on the 3rd, 5th and 12th day of fermentation. The rate of fermentation at 25° was measured according to Jakubowska (1954).

The flocculating properties of the yeast were examined in wheat wort, 12° Blg, after complete fermentation of the latter.

Sporulation was examined on agar slants, containing 0.5% of sodium acetate. The yeast was reinoculated several times: a loopfull of 48 h-yeast suspension was transferred on wort, 12° Blg, kept at 25°. Ascospores formation was examined directly, and after staining according to the Schaefer-Fulton method modified by Wirtz (acc. to Burbianka, Pliszka 1957).

The giant colonies (Lindner 1901) were obtained in duplicate on Petri dishes with hopped wheat wort, 7.5° Blg, 1.8% agar, after 40 and 60 days of incubation (Poradnik Mikrobiologa 1958). The growth on agar-wort-slants was observed after one week of incubation at 25°.

The fermentation of raffinose was examined in Einhorn tubes, according to Herzfeld's method; a 5 day yeast culture, after washing the cells with physiological solution for seeding, was used. The fermentation of other sugars was examined in Durham tubes, using a 2% solution of sugar in yeast water (Jørgensen 1956), inoculating a loop of the yeast suspension from 5-day culture on wort.

The formation of pseudomycelium was observed according to Jørgensen (1956). The formation of the yeast film was examined after 1 month of cultivation on hopped wort, 7.5° Blg, at room temperature. The behaviour of yeast on production wort was examined in 2 litre glass cylinders (Jørgensen 1956).

Beer on laboratory scale was made as follows: the hopped wheat wort, 7.5° Blg was inoculated with non-flocculating yeast for 72 h and with flocculating yeast for 120 h, and kept at room temperature. Control beer was produced in the same way, but with the use of pitching yeast. The control and valuation was based on Malcew's (1953) scale.

Results

Characteristics of imported yeast

The 72 h culture on wort at 25°: short chains or single cells; a few oval or egg-shaped cells, tapering to one end, 3—7 × 4—12 μ. In older cultures only single cells were found. In wort at room temperature —

Table I

The course of the main fermentation of Grodzisk beer, imported yeast, June 1960. Mean results of 3 fermentation tuns on the same wort. Fermentation: 62 h at 18°

Time of fermentation, hours	Temperature of beer	Fermentation		Increase of fermentation in %
		in °Blg	in %	
16°	16.0	7.80	0	0
22°	16.4	7.70	1.3	1.3
24°	16.5	7.60	2.6	1.3
2°	16.6	7.50	3.8	1.2
4°	16.8	7.40	5.1	1.3
6°	17.0	7.30	6.4	1.3
8°	17.3	7.00	10.3	3.9
10°	17.6	6.60	15.4	5.1
12°	18.0	6.15	21.2	5.8
14°	18.5	5.60	18.2	7.0
16°	19.0	5.00	35.9	7.7
18°	19.3	4.70	39.7	3.8
20°	19.6	4.50	42.3	2.6
22°	19.8	4.33	44.5	2.2
24°	19.9	4.20	46.2	1.7
2°	19.9	4.12	47.2	1.0
4°	19.9	4.06	47.9	0.7
6°	19.9	4.02	48.5	0.6
8°	19.9	3.98	49.0	0.5
10°	20.0	3.94	49.5	0.5
12°	20.0	3.90	50.0	0.5
14°	20.0	3.86	50.5	0.5
16°	30.0	3.83	50.9	0.4
18°	20.0	3.80	51.3	0.4
23°	20.0	3.77	51.7	0.5
6°	20.0	3.75	51.9	0.2

homogenous turbidity, after 40 h the appearance of weak, then stronger flocculation. In fermentation tun, ca 30 hl, almost all the yeast rises to the top during the main fermentation and forms a 10—20 cm "head"

cream coloured, and with the flavour of bread leaven and apple ester. Mixed with water, the yeast flocculated very quickly. In the Herzfeld test, it ferments 1/3 of raffinose. In production during the main fermentation the yeast ferments approximately 50% of extract during 60 hours, then the violent fermentation is inhibited and the rest of the extract slowly ferments. The course of the main fermentation is shown in Table I.

Grodzisk beer obtained on this yeast has a specific flavour and taste, contains a great amount of natural carbon dioxide and is properly foamy.

Table II
Results of examination of 6 samples of imported yeast

Mark of yeast	Date received	Infection of yeast*			Ratio of non-flocculent to flocculating yeast
		general %	<i>Lactobacillus</i>	wild yeast	
B6/58	1958	—	—	—	1.7:1
B1/60	2.2.60	2.2	+	0	3.2:1
B8/61	15.8.61	0.3	+	0	2.3:1
B2/62	3.2.62	0.3	+	0	1.8:1
B6/62	1.6.62	2.0	+	0	1.5:1
B16/62	13.6.62	10.8	+	0	2.0:1

* General infection was examined on wort-agar, on the unhopped wort with and without actidione. The presence of wild yeast was checked on hopped wort with 2% of tartaric acid.

Microbiological investigations have shown that the yeast used for production of Grodzisk beer is a mixture of flocculent and non-flocculent forms. Differences in the structure of colony margins of non-flocculent

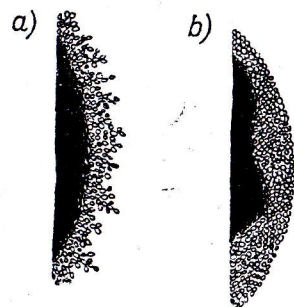


Fig. 1. Structure of yeast colonies, 48 hrs at 25°, malt wort, $\times 100$
a — non-flocculent type, b — flocculating type.

and flocculating yeast could be observed (Fig. 1). Out of 46 pure cultures (yeast B8/61), isolated by the plate method and the Lindner droplet method, 32 cultures were non-flocculent yeast, and 14 were flocculating yeasts. The ratio of the non-flocculent yeast to the flocculating yeast was then 2.3:1. The ratio of non-flocculent yeast to flocculating yeast

in 6 different imported lots was on the average of 2:1 (Table II). The imported yeasts were, as a rule, contaminated with *Lactobacillus* sp., which usually causes acidification of Grodzisk beer.

Characteristics of non-flocculent yeast

Cells of the non-flocculent yeast, during the first phase of fermentation, oval or egg-shaped, $3-5 \times 4-12 \mu$, in branched chains (Fig. 2a). After 40 h at 25° they form 1-4 spores with characteristic protoplasmic wedges (Jørgensen 1956) (Figure 2b). No pseudomycelium. On wort

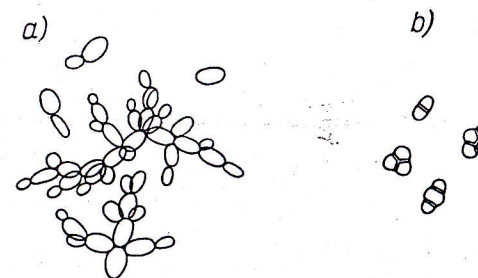
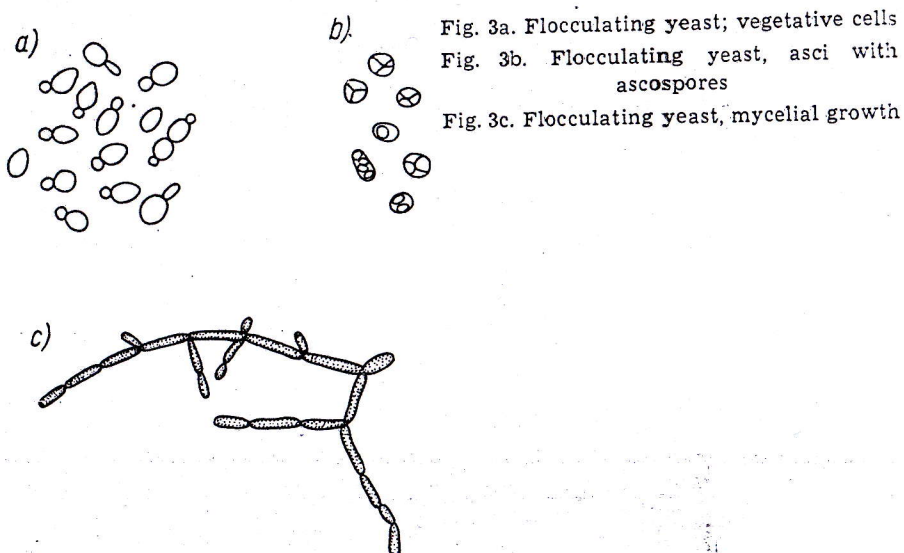


Fig. 2a. Non-flocculent yeast; vegetative cells
Fig. 2b. Non-flocculent yeast; asci with ascospores

they ferment quickly and are carried out to the surface. Clarification of the wort is very slow. Growth on the agar-slant opaque, creamy. In fermentation tun, yeast is brought to the surface of the beer, forming a thick, smeary-creamy "head". Yeast ferments glucose, galactose, saccharose, maltose and 1/3 of raffinose.

Characteristics of flocculating yeast

The cells round or egg-shaped, often tapering to one side, $3-7 \times 4-10 \mu$, single and in conglomerations; no chains (Fig. 3a). Rarely sporulating; after three weeks about 4 per cent of cells with 1-4 ascospores (Fig. 3b). Pseudomycelium (Fig. 3c). After 15-30 days at room temperature, a delicate pellicle, consisting of small islands, lightly settling at the bottom when shaken. Sediment. The growth on the agar-slant — shiny and creamy. Ferments glucose, galactose, saccharose, maltose and 1/3 of raffinose. During fermentation in 2-litre cylinders and in a tun does not rise to the surface, but settles at the bottom, forming compact sediment with small craters, which Lindner (1901) defines as "ringförmige Wulste aus denen Kohlensäurebläschen entweichen."



The following kinds of flocculating yeast may be distinguished depending on the type of flocculation:

- R1 — flocculates very quickly, forming big conglomerates, which settle down very rapidly;
- R2 — forms smaller, quickly-settling flocculi;
- R3 — forms small, slow-settling flocculi;
- R4 — forms very small, very weakly-settling flocculi.

Fermentation ability

Non-flocculent yeast on wort, 11° Blg, shows a greater fermentation ability than the flocculating yeast:

Type of yeast	Fermentation ability per cent		
	72 h	120 h	12 days
Non-flocculent	59.90—70.20	72.76—76.54	74.78—77.96
Flocculating	36.60—57.79	59.86—71.10	68.71—74.82

Non-flocculent strains are strongly differentiated in their fermentation abilities (Fig. 4). After 72 h five groups were distinguished, after 12 days only 2 groups. I — 20 cultures (74.0—76.0% attenuation; 12 cultures 75.0—75.5% attenuation); II — 12 cultures (76.5—78.0% attenuation). The flocculating yeasts are differentiated after 72 h of fermentation; after 12 days they form a uniform group (with the exception of two cultures).

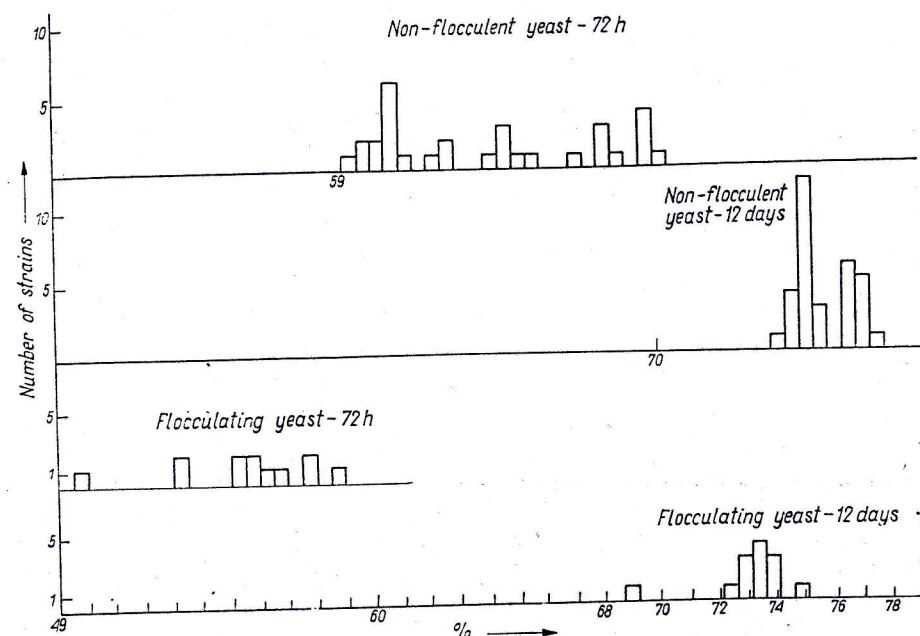


Fig. 4. Fermentation ability of yeast, in per cent

The average attenuation of the non-flocculent and flocculating yeast after 12 days is: Non-flocculent yeast, group I — 75.13%; Non-flocculent yeast, group II — 76.99%; Flocculating yeast — 73.23%.

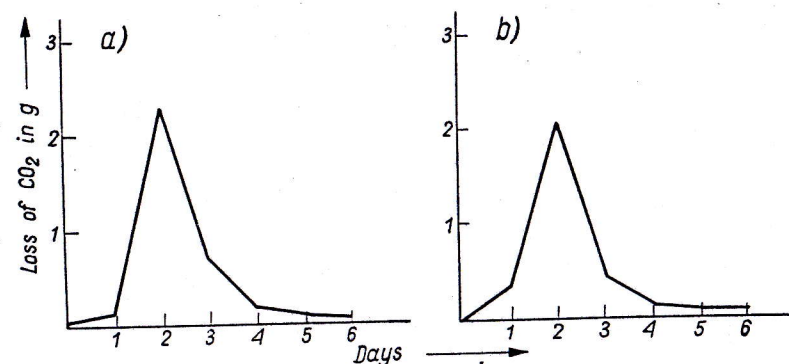


Fig. 5. Fermentation rate

a — non-flocculent yeast B6-10, b — flocculating yeast B6-18.

The rate of fermentation of wort, 7.5° Blg, measured by loss of CO₂, shows that non-flocculent yeast, B6/10, after 24 h ferments more slowly than flocculating yeast, B6/18. The situation is, however, reversed after 48 and 72 h (Figure 5).

Beer, obtained on laboratory scale, differed in flavour, depending on the type of the yeast. With non-flocculent strains, beer was less bitter and "soft", while the flocculating yeast gave beer of a bitter, almond-like taste.

Among beer from non-flocculent yeast, samples B8/22 and B8/4 produce beer with a pleasant raspberry taste, sample B8/22 gives a slightly "burned" flavour. Among beer of flocculating yeasts no essential differences in flavour were noted, with the exception of B8/2, which was yet not considered typical.

The giant colonies differed in both groups, of non-flocculent and flocculating yeast (Phot. 1a—f); 16 cultures of non-flocculent yeast were of type I (B8/4, B8/33), 4 — of type IV (B8/22) and 4 were untypical. Among flocculating yeast, 5 cultures were of one type (B8/30, B8/46).

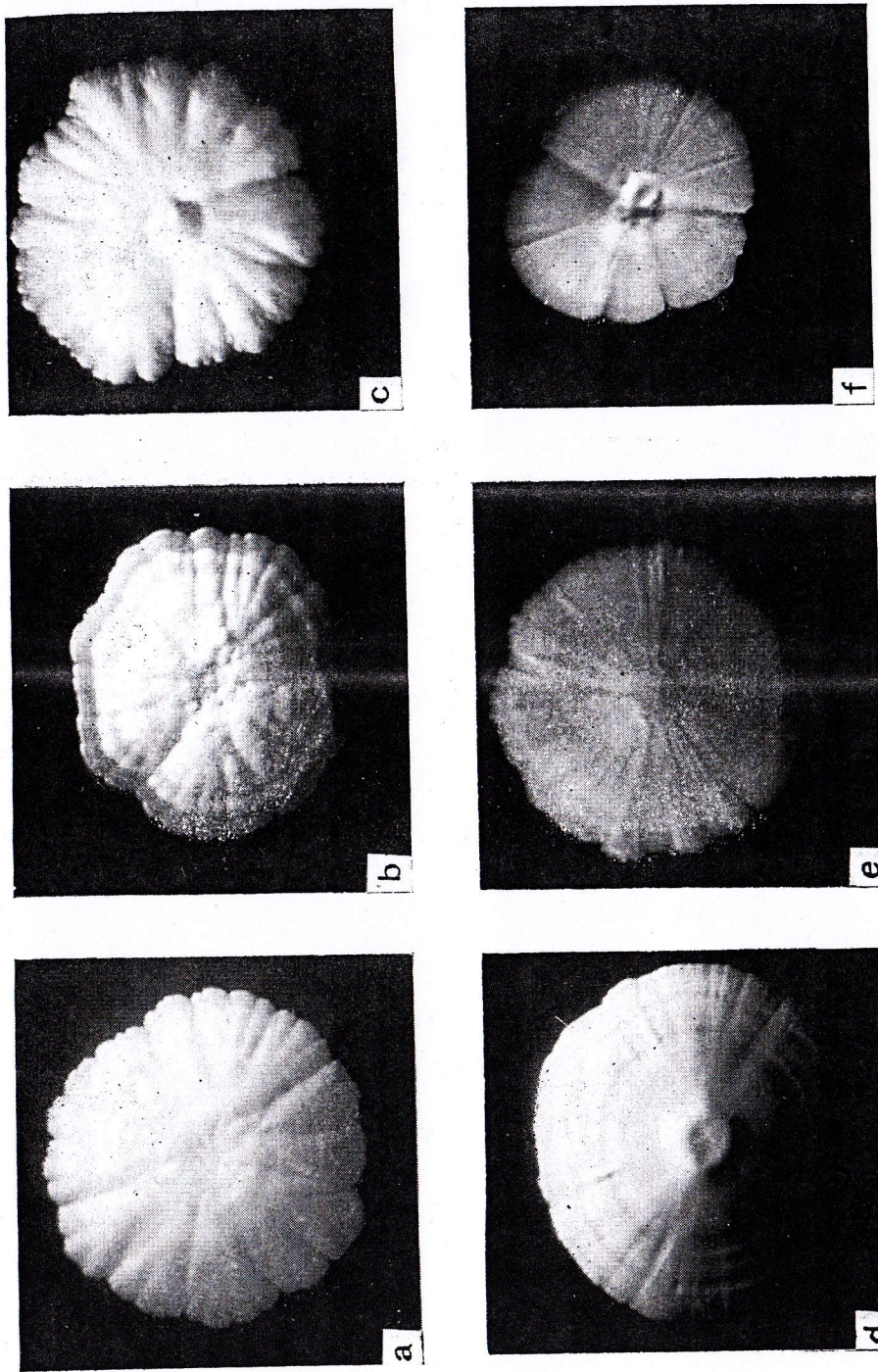
Selection of mixed yeast-population

Special attention was paid to the essential quality of Grodzisk beer, similar to the "sparkle" of champagne, caused by the suitable concentration of natural carbon dioxide. The proper saturation of Grodzisk beer with CO_2 is achieved during its lagging in bottles. Due to the presence of yeast, a secondary fermentation of residual sugar, not fermented during the main fermentation, takes place. Main fermentation of mixed yeasts population of appropriate quality did not exceed ca 50% of extract during the fermentation process. Until the moment of bottling, ca 55% of extract is fermented, the final degree of fermentation being about 75%; about 20% is supposed to ferment in the bottle. If the yeast is unsuitable, the main fermentation does not stop at 50%, but goes on to reach the final degree of fermentation, i.e. 75%; consequently the beer is not fizzy enough or even quite fully devoid of CO_2 .

Pure cultures, isolated from the Grodzisk yeast used at present, were applied, but the desired effect was not achieved. The effect was achieved, when a mixture of non-flocculent and flocculating yeast, in proper proportion, was used. Yeasts formed conglomerations, that rose to the top, the beer was clarified and the fermentation was suddenly inhibited, due to an inadequate amount of yeast in the beer. By selecting the proper kind of flocculating yeast (R1, R2, R3, R4) in the mixture, it is possible to regulate the time of "break" effect during the main fermentation.

It has been found that the ratio of non-flocculent to flocculating yeasts may vary within the range of 1.5:1 to 10:1, without the loss of the required technological properties. When the amount of non-flocculent yeast in the mixture increased over 10:1, the danger ensued that the "break" in the main fermentation may not occur.

The most suitable ratio of non-flocculent to flocculating yeasts, was 2:1 to 4:1. A greater amount of flocculating yeast improves the stability



Phot. 1. Giant colonies. Pure cultures of non-flocculent (B8—4, B8—22, B8—33) and flocculating yeast (B8—30, B8—19, B8—46)
a — B8—4, b — B8—22, c — B8—33, d — B8—19, e — B8—30, f — B8—46.

of the mixture, but has an unfavourable influence on beer quality (taste and flavour), while a greater amount of non-flocculent yeast makes the mixture less stable.

It has been found that the rates of growth of the non-flocculent yeast and the flocculating one in the mixture were different; consequently, both varieties were cultivated separately and mixed in the fermentation tuns.

It has also been found that the ratio of non-flocculent to flocculating yeast was almost constant during the main fermentation period, with a slight predominance of non-flocculent yeast. Consequently, the most suitable ratio of non-flocculent yeast to flocculating yeast in the initial mixture, should be 2:1 and not 4:1. When yeast is weakened, due to the action of acids or to long storage under water, the ratio changes and the proportion of non-flocculent yeast increases. Such yeast population loses the ability to evolve the "break" in the main fermentation.

Table III

Physico-chemical tests of Grodzisk beer produced on own yeasts, S2, and imported yeasts

	Beer No 1 yeast S2	Beer No 2 imported yeast
Alcohol in % of weight	2.22	2.23
Pseudo-extract in % of weight	1.87	1.84
Real extract in % of weight	2.89	2.86
Basic wort in °Blg	7.31	7.30
Colour in ml 0.1 N iodine	0.50—0.60	0.50—0.60
Acidity in ml 1.0 N NaOH per 100 ml	1.20	1.20
pH	4.15	4.18
CO ₂ in % of weight	0.70	0.65
Pseudo-fermentation in %	74.41	74.79
Real fermentation in %	60.47	60.82

Bottling — Sept. 9, 1962. Samples for tasting taken after one month.

Lagering — Oct. 12, 1962.

Tasted — Oct. 13, 1962.

On this basis, the composition of our own yeast used on a production scale was following:

Sign. S1: 32 strains of non-flocculent and 16 strains of flocculating yeast were isolated from imported yeast, B6/62. Separate mixtures of each type were made. This way of combining the yeast mixture for Grodzisk beer had been practised earlier with good results.

S2 — mixture consisting of two strains: non-flocculent, B6/10, and flocculating, B6/18,

- S3 — mixture of three cultures: non-flocculent, B6/10, and flocculating, B6/18 and B6/19,
 S5 — mixture of four cultures: non-flocculent, B8/40 and B8/7, and flocculating, B8/30 and B8/44,
 S6 — mixture of four yeast cultures: non flocculent, B6/10, and flocculating, B6/18, B6/19, B6/22.

Very good results were obtained with the mixture S2. This was confirmed by analysis and tasting of the beer (Table III) which is considered as being typical Grodzisk beer.

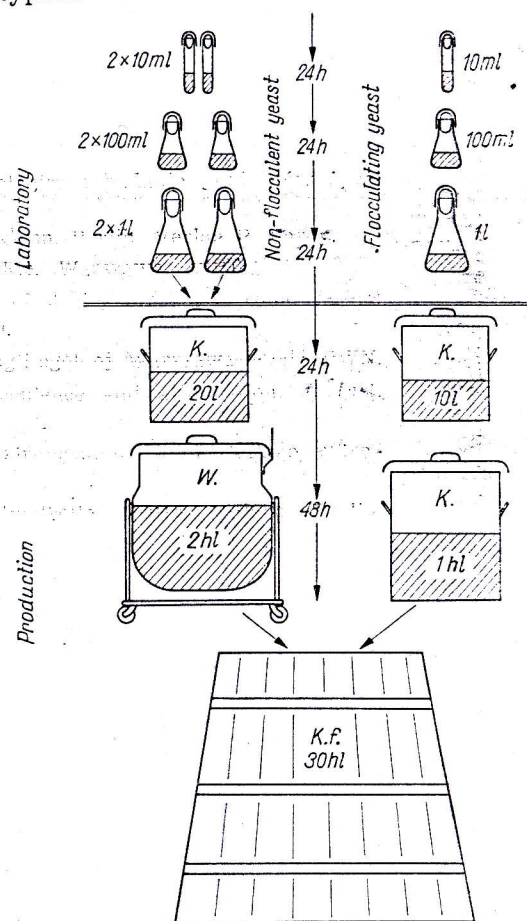


Fig. 6. Propagation of yeast mixture

On the basis of the presented results, a method of propagation of our own yeast mixture for the production of Grodzisk beer was worked out.

The pure cultures were stored in a refrigerator on agar slants under paraffin oil. A loopfull of yeasts was transferred into 10 ml of wort, and propagated according to the scheme in Figure 6.

This method permits to obtain yeast of sufficient biological purity, taking into consideration the specific conditions of the fermentation cellar (Weinfurtner 1957).

Conclusions

The yeast used for the production of Grodzisk beer consists, at the present time, of a mixture of different strains of top-fermentation yeast, belonging to *Saccharomyces cerevisiae* (Hansen).

Two kinds of yeast must be taken into consideration for this purpose: non-flocculent yeast and flocculating yeast, in a ratio 2:1, introduced according to the scheme (Fig. 6).

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