EXPERIMENTS CONCERNING PHYSICO-CHEMICAL AND MICROBIOLOGICAL CONTROL OF BAKERY YEAST INDUSTRIAL PRODUCTION

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Abstract: *Biotechnological characteristics of bakery yeast can be affected by deficiencies attributed to raw materials (especially molasses), technological process and certain microbiological causes. Compliance with process parameters and standards of hygiene throughout the manufacturing process of the yeast, using appropriate raw materials and taking appropriate action to prevent contamination of yeast lead to sensory and biotechnological qualities of yeasts that are suitable for use in bakery and confectionery industry . During the process of yeast manufacturing, concomitant with the multiplication of the cells that belong to the pure culture, other microorganisms can develop in the different phases of technological flow, thus increasing the contaminations degree of the finished product and causing a reduction of biotechnological qualities of yeast. So as to prevent the multiplication of contaminated microorganisms, it is imposed a severe microbiological control on the production stages through the study of hygiene degree and the detection of contaminants that can proceed from various sources. This paper aims to determine the biotechnological qualities and the extent of contamination of bakery yeast for four industrial batches of finished product. The importance of monitoring physical, chemical and microbiological parameters in each technological phase of the industrial production of bakery yeast was highlighted.*

Keywords: *monitoring, biotechnological qualities, storage-ability*

1. Introduction

Yeast is used in bakery industry as biological loosener and flavor-maker in bread. Yeast is used for centuries, but industrial-scale production began in 1850. Today the total amount of yeast produced annually is in the tens of millions of tons, and the benefits are estimated at several billion dollars [1, 2].

Technology of baking yeast in our country is to multiply a pure culture of yeast using diluted leaven and five successive stages of multiplication, in the laboratory and industrial system respectively: Phase I called yeast inoculums, Phase II - yeast pre-culture, phase III - yeast culture, phase IV - yeast starter, phase V - commercial yeast.

To obtain a quality yeast biomass, every stage of the technological process is monitored from physico-chemical and microbiological points of view. During the process of making bakery yeast, simultaneously with the multiplying yeast cells can develop other microorganisms that increase the degree of contamination of the final product and reduces yeast biotechnological qualities and storage-ability [3-6].

The paper presents a monitoring of physico-chemical and microbiological

parameters for the industrial production of bakery yeast for four batches, from receipt of raw materials and ending with finished product, highlighting relevant aspects for obtaining bakery yeasts with constant biotechnological qualities [7 - 10].

2. Experimental

Monitoring the technological process of obtaining bakery yeast was performed in S.C. ROMPAK S.R.L. Paşcani in April 2012.

Pure culture used in the project was a strain of *Saccharomyces cerevisae* obtained from the Center for Biotechnology Pak, a part of Pakmaya yeast factory from Izmit, Turkey.

Physicochemical control for all phases of fermentation and for the final product was performed both in the Fermentation Department's Laboratory and in the Central Laboratory of S.C. ROMPAK S.R.L. Paşcani.

The monitored physico-chemical parameters and the apparatus used are shown in Table 1.

Table 1
Physico-chemical parameters and apparatus

Physico-chemical characteristics	Apparatus
Mash concentration (°Bllg)	Thermosacharometer
pH	pHmeter
Temperature	Electrical thermometers
Pressure	Manometer
Molasses and air flow rate	Flow-meter
Alcohol determination	Alcoholmeters
Yeast concentration determination	Centrifuge
Percentage of budding and dead cells determination	Microscope
Yeast biomass determination 27% (kg)	Vacuum pump, analytical balance
Fermentative capacity (cm^3 CO2/h)	Fermentograph SJA
Yeast dry substance	Thermo balance

Monitoring frequency of Bllg level, pH and yeast milk and diluted molasses' temperature is that of every two hours, starting at zero fermentation hours, while molasses and air flow is monitored hourly. Alcohol determination from fermenting leaven is done hourly, starting from the first hour of fermentation by two methods: physical (with alcoholmeter) and chemical method. Determination of yeast concentration is the amount of yeast in suspension volume, percentage having a significant role because it provides an important clue to the normal multiplication of yeast cells and the total quantity of yeast. Determination of yeast for phases II and III is at the end of the fermentation process and for phases IV and V of the monitoring frequency is two hours from the first hour of fermentation. Study of biomass accumulation during yeast multiplying is a current analysis and consists in determining the amount of biomass by centrifuging a sample of leaven, the result expressed in g yeast / L leaven.

The analysis was performed for all phases of the manufacturing process, being determined the initial concentration of inoculums in the growing medium and the final accumulation of biomass in fermented leaven. Fermentative ability of yeast biomass was estimated using fermentograph at the end of each technological stage, respectively the finished product.

Physiological state of the yeast cells at different stages of the growing period was assessed by determining the percentage of budding cells and dead cells. Microbiological control at each fermentation stage and the final product

was performed daily in the microbiology laboratory. Samples were collected from a sterile environment (beginning of fermentation) and from the yeast suspension (end of fermentation) and the number of CFU of total bacteria, coliforms bacteria, atypical yeasts, and *Escherichia coli* were determined. The same analyzes were performed for bakery yeast - finished product (500g block). The method used is the cultural, using culture mediums of European standards: PCA - nurturing environment for all bacteria, TBX - medium for growing Escherichia coli; VRBL - nurturing environment for coliforms bacteria; Lysine - medium for cultivation of yeasts and moulds.

Table 2
Monitored microbiological characteristics and utilized apparatus

Microbiological charactaristics	Apparatus
Coliforms bacteria ufc/g Total bacteria ufc/g Atypical yeasts ufc/g Escherichia coli ufc/g	Microbiological hood, sterile pipette, Petri plate, colonies counter numărător colonii, thermostat

3. Results and Discussions

3.1.Physico-chemical control at manufacturing stages

The results for main parameters' monitoring at each manufacturing stage are shown in tables 3, 4, 5, and 6.

Table 3
Technological parameters per manufacturing phase – batch 111

Multiplication phase	Mash concentration [°Bllg]	Temperature [°C]	pH	Alcohol content [%]	Multiplication period [h]
Phase I	10÷11	32÷34	5.6÷4.8	0÷2.85	24
Phase II	8.9÷3.7	33÷34	5.4÷4.7	0.05÷2.75	13
Phase III	2.7÷4.7	33÷34	4.6÷5.2	0.12÷2.9	16
Phase IV	1.7÷10.3	31÷35	4.17÷6.24	0.59÷0.15	19
Phase V	2.2÷10.5	30÷32	3.24÷7.53	0.09÷0	16

Table 4
Technological parameters per manufacturing phase – batch 168

Multiplication phase	Mash concentration [°Bllg]	Temperature [°C]	pH	Alcohol content [%]	Multiplication period [h]
Phase I	10÷11	32÷34	5.6÷4.9	0÷3.5	24
Phase II	9.2÷3.8	32÷34	5.4÷4.7	0÷2.7	13
Phase III	2.9÷5.1	33÷34	4.55÷5.3	0.21÷2.3	16
Phase IV	1.8÷10.5	31÷35	4.29÷6.08	0.72÷0.26	19
Phase V	2.2÷11.3	31÷32	3.34÷7.83	0.04÷0	16

Table 5
Technological parameters per manufacturing phase – batch 172

Multiplication phase	Mash concentration [°Bllg]	Temperature [°C]	pH	Alcohol content [%]	Multiplication period [h]
Phase I	10÷11	32÷34	5.6÷4.9	0÷3.4	24
Phase II	9.8÷3.9	33÷34	5.7÷4.75	0÷2.65	13
Phase III	2.6÷4.8	33÷34	5.2÷4.6	0.14÷2.5	16
Phase IV	1.7÷10.9	30.7÷35	4.23÷6.5	0.62÷0.24	19
Phase V	2÷10.8	30.5÷32	3.39÷7.67	0.08÷0	16

Table 6
Technological parameters per manufacturing phase – batch 183

Multiplication phase	Mash concentration [°Bllg]	Temperature [°C]	pH	Alcohol content [%]	Multiplication period [h]
Phase I	10÷11	32÷34	5.6÷4.7	0÷2.95	24
Phase II	9.2÷3.5	33÷34	5.5÷4.6	0÷2.45	13
Phase III	2.8÷5.3	33÷34	4.6÷5.4	0.20÷2.7	16
Phase IV	1.9÷10.8	31÷34.5	4.16÷6.25	0.41÷0.22	19
Phase V	2÷11.4	30÷32	3÷7.7	0.08÷0	16

The company's central laboratory makes very important determination, namely, the accumulation of biomass per multiplying phase of yeast, an indicator which shows if the process was conducted in pre-established technological parameters.
The data presented in Figure 1., for example, show that the amount of yeast biomass accumulated in the culture medium increased with 864% (from 25.7 g / L to 222.21 g / L) in only 64 hours (2 days and 16 hours).

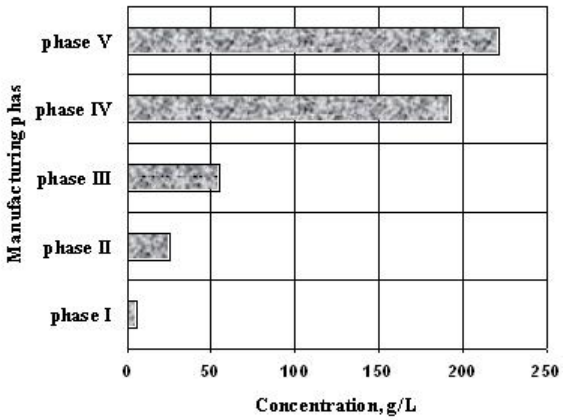

Figure 1. Biomass accumulation per manufacturing phases

A very important biotechnological feature of bakery yeast - end product is the fermentation power, which is determined in the factoring by using a SJA fermentograph. This method is based on the fact that yeast fermentation power is the time required by certain amount of yeast to develop 450 cm^3 CO_2, under conditions determined by fermentograph apparatus. All four batches analyzed showed a fermentation power corresponding to the quality management systems limits implemented in society (table7).

Table 7
Bakery yeast's fermentation power at different stages of technological process

Yeast batch	Fermentation power [cm^3 CO_2 / h]	Manufacturing phase				Yeast block
		II	III	IV	V	
111		540	660	680	700	790
168		690	660	710	690	760
172		670	660	700	680	750
183		620	650	680	660	740

3.2. Microbiological control for manufacturing phases

A major source of contamination can be pure laboratory culture or culture resulting from Phase II, under the conditions that the regime of nutrient medium sterilization, hygienic conditions at sowing and cultivation are not complied with, or when there is no efficient air filtering.
Microbiological control was determined using counting chambers for yeast cell concentration and percentage of cells autolysates, by suspending the cells in citrate methylene blue. In general, according to the microbiological control conducted, the culture of yeast used was appropriate from microbiological point of view, without contamination by foreign microorganisms and having a high percentage of viable cells. For each stage of the technological multiplication process, a microbiological control was achieved for

the four physico-chemically monitored batches. Results of the microbiological examination conducted are summarized in Tables 8, 9, 10 and 11.

Table 8
Results of microbiological control – batch 111

Multiplication phase	Total bacteria [CFU/g]	Total coliforms bacteria [CFU /g]	*Escherichia coli* [CFU /g]	Atypical yeast [CFU /g]
Phase II	-	-	-	-
Phase III	-	-	-	-
Phase IV	2.90×10^2	-	-	-
Starter	6.40×10^2	1	-	-
Phase V	3.70×10^2	-	-	-
Yeast milk	1.04×10^3	4.10 x 10	7.00	-
Yeast block	4.10×10^3	5.60×10^2	3.00 x 10	

Table 9
Results of microbiological control – batch 168

Multiplication phase	Total bacteria [CFU /g]	Total coliforms bacteria [CFU /g]	*Escherichia coli* [CFU /g]	Atypical yeast [CFU /g]
Phase II	-	-	-	-
Phase III	-	-	-	-
Phase IV	1.10×10^2	-	-	-
Starter	2.00×10^2	1.00	-	-
Phase V	4.00×10^2	-	-	-
Yeast milk	3.00×10^2	-	-	-
Yeast block	8.20×10^3	1.00 x 10	-	-

Table 10
Results of microbiological control – batch 172

Multiplication phase	Total bacteria [CFU /g]	Total coliforms bacteria [CFU /g]	*Escherichia coli* [CFU /g]	Atypical yeast [CFU /g]
Phase II	-	-	-	-
Phase III	3.00×10^2	-	-	-
Phase IV	9.00	-	-	-
Starter	1.00×10^2	1.00	-	-
Phase V	2.00 x 10	-	-	-
Yeast milk	3.00 x 10	-	-	-
Yeast block	1.95×10^4	5.00	-	-

Table 11
Results of microbiological control – batch 183

Multiplication phase	Total bacteria [CFU /g]	Total coliforms bacteria [CFU /g]	*Escherichia coli* [CFU /g]	Atypical yeast [CFU /g]
Phases II and III	-	-	-	-
Phase IV	1.60×10^2	-	-	-
Starter	2.50×10^2	1.00	-	-
Phase V	8.70×10^2	-	-	-
Yeast milk	2.00 x 10	-	-	-
Yeast block	7.40×10^3	-	-	-

It is observed that, in general, in the first three phases of multiplication no contamination occurs (except batch No.172 - phase three, having a bacterial count of 3.00 x10^2 CFU / g

The degree of bacterial contamination is reduced, taking into account the fact that in the yeast block a 2 ‰ contamination (two bacterial cells allowed tp 1000 yeast cells) is accepted (Auerman, 1995).

Yeast comes in blocks (500 g), which retain its technological properties up to 40 days, depending on the quality of yeast and storage conditions (optimum at 0 ÷ 0÷40C, relative air humidity 65 ÷ 70%).

Yeasts with a high degree of purity do not contain putrefaction bacteria or atypical yeasts, and can be stored up to 3 months; good quality yeasts must not contain bacteria of putrefaction in excess of 0.1 ÷ 0.2% (1 g yeast containing 5•109÷1010 cells, of which may be allowed 100 to 1000 bacterial contaminants).

4. Conclusions

Bakery yeast is a cell biomass of the species *Saccharomyces cerevisiae*, with particular importance for the bakery industry. The industrial manufacturing of bakery yeast requires a physical, chemical and microbiological rigorous control, covering all phases of production.

To obtain quality yeast is necessary to use high quality raw materials with appropriate physico-chemical and microbiological characteristics, in accordance with a strict observance of technological process and good working and hygienic practices (GMP and GHP).

Following measurements it was found that *Saccharomyces cerevisiae* yeast culture is used as inoculum is a microbiologically appropriate culture.

By studying the biomass accumulation during multiplication of yeast in each manufacturing phase, it was observed a good multiplication of cells, which reach values of $10^8 \div 10^{10}/cm^3$ environment. Main biotechnological property for evaluating bakery yeast quality is the leavening power/ability.

Bakery yeast - finished product is the result of efforts made to avoid contamination with foreign microorganisms, the quality of which is conditioned by the degree of microbiological purity posed by delivery in bakery industry; out of the four analyzed batches, one batch presented contamination microorganisms (bacteria) in phase III of multiplication, microorganisms that can cause a decrease of biotechnological properties during storage.

5. References

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