EFFECTS OF DRY LAGER BREWING *SACCHAROMYCES CEREVISIAE* STRAIN ON THE FERMENTATION PROCESS AND BEER QUALITY

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Abstract: *This paper describes the use of the dry lager brewing Saccharomyces cerevisiae (Saflager S-189) yeast strain in a beer factory from the moment of inoculation until the secondary fermentation stage. The research follows the physico-chemical and microbiological parameters of the inoculum, primary fermentation, secondary fermentation and unfiltered beer. Special attention was paid to the way the yeast was prepared for inoculation. In the laboratory measurements made on wort and beer during the fermentation process, different devices existing in the laboratory factory from S.C. Bermas S.A. Suceava were used, such as: saccharimeters, thermometers, oxygen content, microscop, Thoma camera, carbon dioxide, refractometer for beer analysis, viscosimeter, spectrophotometer e.g. The yeast presents good flocculation ability, a quick adaptation to the medium conditions (wort composition) and the beer obtained with this strain yeast presents good physical-chemical parameters and organoleptic ones.*

Keywords: *yeast, fermentative process*, *beer quality*

1. Introduction

To make beer four ingredients are necessary: water, malt, yeast and hop. Therefore, brewer's yeast is a principal element in beer production [1]. Especially, ale beers are made using *Saccharomyces cerevisiae* strains and lager beers are made using *Saccharomyces pastorianus* (formerly called *Saccharomyces carlsbergensis* then as *Saccharomyces uvarum*) yeast strains [2]. Beer quality is strongly influenced by the biochemical performance of the yeast during fermentation [3]. The selection of a certain yeast strain for beer production is made taking into account the main specific characters of yeast: final fermentation grade and fermentation rate, the ability of yeast to metabolize all of the available substances, the flocculation and sedimentation capacity, the range and the amounts of secondary products resulted from secondary fermentation with implications in beer aroma and flavor, its resistance to degeneration and contamination e.g. [4].

Before yeast is added, the breweries must provide a suitable growth medium for it. Yeast fermentation performance and beer quality is influence especially by the wort composition, oxygen prior to pitching, fermentation temperature, size and shape of the fermentor vessels e.g [5]. The fermentation environment must be highly aerobic (that's the way the wort is first aerated) [6] and the fermentation tanks must be cooled and maintained at 8°C to 15°C for lagers and 15°C to 22°C for ales. During the fermentation process different factors that affects beer yeast activity like oxygen concentration, osmotic potential, pH, ethanol concentration, nutrient availability and temperature are in continues fluctuations [7].

Because yeast growth conditions are never constant, the brewers must always control the fermentation process (i.e. temperature, pH, CO2, extract decomposition, ethanol content e.g.) to avoid a stressed yeast which may lose its ability to replicate, become unable to ferment or die. Yeast quality is measured by its viability (the percentage of live cells within a population) and/or vitality (metabolically active yeast).

At the end of the fermentation process when yeast fermented all the fermentable sugars from the medium, its flocculations occurs [8] and the beer is considered to be fully attenuated. Once the yeasts have flocculated, yeasts are collected and removed from the beer [9]. Beer flavor, clarity, and overall quality depend on the moment when yeast flocculate. Even if the yeast cells are removed after their sedimentation, the beer will still contain some yeast cells that will led to undesirable flavor compound. Therefore, it is essential to have a secondary fermentation to eliminate the remaining yeasts and haze-forming proteins, to produce beer with a mature or finished flavor and some amount of CO2 gas formation.

The aim of this paper is to evaluate the quality of a lager brewing *Saccharomyces cerevisiae* strain quantitatively and qualitatively with the highlight of the technological parameters that changes during the fermentation process with implications for the evolution of the fermentative process and the quality of the beer obtained.

2. Materials and methods

2.1. Yeast and wort. All experiments were carried out with an industrial dry lager brewing strain of *Saccharomyces cerevisiae* (Saflager S-189) (Fermentis, Belgium) (humidity 3.5 to 6; dry matter 94 to 96.5%) on eighteen malt hopped wort at S.C. Bermas S.A. Suceava. The malt hopped wort quality parameters (mean and standard deviation) used in this study are shown in Table 1.

Table 1
Physico-chemical and microbiological properties of cooled hopped wort

Characteristics	Avarage value	Standard deviation
Original extract, °P	10.2	0.15
pH	5.2	0.1
Acidity, mL NaOH 1n	1.7	0.16
Turbidity, EBC	69.5	1.5
Color, EBC	8.2	0.2
Final fermentation grade, %	88.4	1.6
Live cell/g	none	

2.2. Propagation and fermentation conditions. For yeast propagation, 40 kg of *Saccharomyces cerevisiae* (Saflager S-189) strain were rehydrated in a fermentor vessel with a capacity of 200 hl with approximately 150 l of sterile malt hopped wort at 23°C temperature. Obtained cream was spilled over 40 hL wort to a temperature of 23°C in a cooling tank with internal cooling coils. The yeast mixture obtained was left to grow for 3 h in the wort medium for its regeneration and for the initiation of the fermentation process. Finally the process continues by pouring a quantity of 100 hl of cooled hopped wort at 8.0÷8.5°C temperature above the yeast inoculum. Thus, the amount of yeast inoculum that was obtained is of 140 hL with the following parameters: apparent extract 10°P, temperature 12°C and the number of viable cells/mL of 32.5.

2.3. Fermentation analysis. The number of yeast cells was counted using a cytometry camera Thoma. The yeast cell viability was assessed using the methylene blue staining method [10]. The physico-chemical parameters of yeast inoculum, wort and beer quality were determined according to the Romanian standard methods or by using the technological instructions from S.C. Bermas

S.A. Suceava: wort apparent extract using a saccharimeter device (technological instructions), wort temperature using a termometre device (technological instructions), oxygen content (EBC method), original (SR 13355-5:2005), density (SR 13355-5:2005), alcohol concentration (SR 13355-3:2005), pH (SR 6182-14:2009), color (SR 13355-7:2005), acidity (SR 13355-6:2000) bitterness value (SR 13355-9:2003), viscosity (EBC method), carbon dioxide (SR 13355-8:2003) and fermentation grade for sale using a saccharimeter device (technological instructions).

2.4. Data analysis. All analytical determinations were performed at least in triplicate. The values of different parameters were expressed as the mean ± standard deviation to a confidence interval for mean of 95%. All data was processed with Microsoft Excel 2003. Also, STATISTICA 6.0 software was used for graphical representation.

3. Results and discussion

Physical and chemical analysis. The yeast inoculum parameters (temperature, apparent extract, yeast cell number, oxygen content, pH) were determined during 28 h time period. The results obtained are shown in Table 2.

Table 2
The yeast inoculum parameters

Time (h)	Parameters (average value ± standard deviation)				
	Temperature (°C)	Apparent extract (°P)	Yeast cells number (x 10^6 cel/mL)	Oxygen content (mg/L)	pH
0	13.00 ± 0.01	10.00 ± 0.01	35.00 ± 0.20	9.60 ± 0.10	5.20 ± 0.20
4	13.50 ± 0.05	9.40 ± 0.02	-	-	-
17	14.00 ± 0.02	8.10 ± 0.03	57.50 ± 0.50	0.60 ± 0.10	5.00 ± 0.10
24	12.50 ± 0.05	7.20 ± 0.02	85.00 ± 0.50	-	-
28	12.00 ± 0.02	6.60 ± 0.02	85.40 ± 0.40	-	-

From the Figure 1 we can see an increase of the yeast cell number correlated with a decrease of the apparent extract.

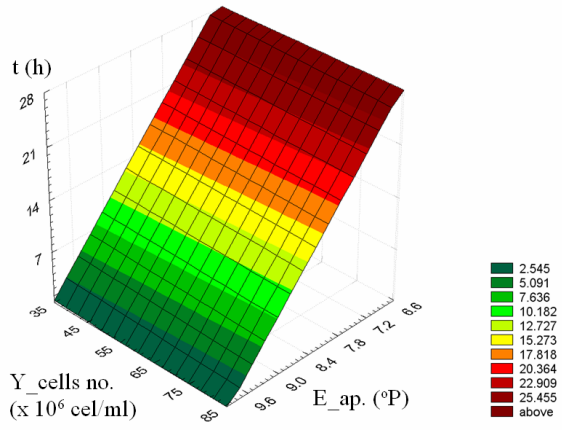

Figure 1. Time (t) variation of the yeast cells number (Y_cell no.) and of the apparent extract (E_ap.).

The yeast population in this phase increased almost 2.5 times from the starting inoculum quantity. This fact is explainable because in this phase yeast uses the amino acids as a source of nitrogen and glucose and the fermentable sugars as a source of energy and carbon [18] leading to a decrease of the apparent extract with almost 34%. According to the recommendations made by the yeast producers the yeast inoculum temperature did not exceed 14°C. In the last hours of yeast inoculum fermentation the temperature was decreased until 12°C to avoid a decrease to high of the apparent extract (producer's recommendation in this phase for apparent extract values was that it should varied only between 6-7°P). The pH of the hopped wort is favorable to yeast

growth (5.2 to 5.0) and its decrease from 5.2 to 5.0 it can be explained by the amino acids yeast metabolization and the formation in the wort medium of the organic and volatile fixed acids [19]. Oxygen consumption rate is very high (about 16 times in the first 17 hours) due to its extensive utilization by the yeast cells [6]. The amount of yeast inoculum obtained (140 hL) with the parameters mentioned above was transfuse on a 130 hL of cooled hopped wort. Over this another quantity of cooled hopped wort of 130 hL was added. Both quantities of cooled hopped wort had the same technological parameters (temperature 8°C and original extract 10.2°P). The parameters evolution during the fermentation process is shown in Table 3.

Table 3
The tank fermentation parameters

Time (h)	Parameters (average value ± standard deviation)			
	Temperature (°C)	Apparent extract (°P)	Yeast cells number (x 10^6 cel/mL)	Oxygen content (mg/L)
0	10.00 ± 0.01	8.00 ± 0.01	25.00 ± 0.10	0.53 ± 0.01
7	10.80 ± 0.02	7.70 ± 0.03	35.00 ± 0.15	-
11	11.50 ± 0.05	7.50 ± 0.05	47.00 ± 0.20	-
24	11.50 ± 0.05	6.80 ± 0.04	55.40 ± 0.20	-
31	12.00 ± 0.02	6.20 ± 0.02	84.50 ± 0.50	-
35	12.00 ± 0.02	5.80 ± 0.03	-	-
48	12.00 ± 0.02	5.00 ± 0.04	93.70 ± 0.30	0.45 ± 0.01
59	12.00 ± 0.02	4.10 ± 0.01	-	-
72	10.00 ± 0.01	3.50 ± 0.05	41.25 ± 0.25	-
83	9.50 ± 0.05	3.10 ± 0.03	-	-
96	9.50 ± 0.05	2.80 ± 0.02	27.50 ± 0.02	-
107	9.50 ± 0.05	2.60 ± 0.04	23.50 ± 0.03	-
120	9.50 ± 0.05	2.40 ± 0.02	18.75 ± 0.25	-
133	7.00 ± 0.04	2.40 ± 0.02	-	-
144	5.50 ± 0.05	2.30 ± 0.01	12.50 ± 0.02	-
148	5.00 ± 0.02	2.30 ± 0.01	7.50 ± 0.01	0.43 ± 0.01

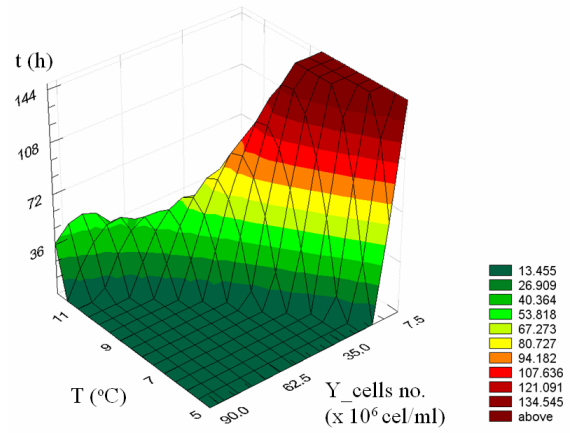

Figure 2. Time (t) variation of the yeast cells number (Y_cells no.) and of the temperature (T).

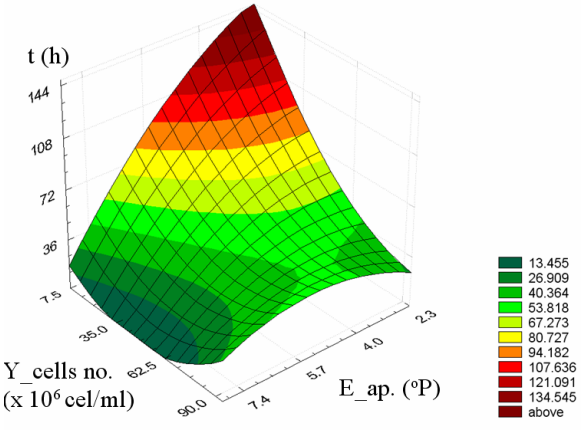

Figure 3. Time (t) variation of the apparent extract (E_ap) and of the yeast cells number (Y_cells no.).

This phase is characterized by an intense fermentative metabolism of yeast with the production of ethanol, CO2 and other fermentation products. The yeast population from the beginning of fermentation process increases from 25 x 10^6 cel/mL to 93.70 x 10^6 cel/mL at 48 h of fermentation while the apparent extract decreases from 8°P to 4,1°P as is shown in Figure 2. When the wort temperature increases until 12°C the fermentation tank is cooled gradually until it reached 5°C at the end of the fermentation process. As Figure 3 shows we can observe a slower decrease of the apparent extract and of the yeast population leading to the end of the fermentation process to 2.3°P and 7.5 x 10^6 cel/mL the moment when primary fermentation is considered complete. The fermentation process continues during the secondary fermentation in tanks held at lower temperatures for a longer time.The secondary fermentation beer parameters evolution is given in Table 4.

Table 4
The secondary fermentation beer parameters

Parameters	Value
Apparent extract (°P) at the beginning of secondary fermentation	2.30 ± 0.12*
Yeast cell number (mil cel/mL) at the beginning of secondary fermentation	7.00 ± 1.00*
Tank pressure (barr)	0 to 0,9
Temperature (°C)	5.8 to 2
Apparent extract (°P) after 14 days of fermentation	1.80 ± 0.10*

* Average value ± standard deviation

The secondary fermentation process was held for 30 days after which the laboratory tests were performed on unfiltered beer quality parameters as it shown in Table 5.

From it we can noticed that the secondary fermentation process has normal regarding the decrease of extract from the beginning of the process until the end and thus we can explained why the fermentation grade for sale determined in the beer factory is very close to the fermentation grade for sale determined in the laboratory.

This shows that in the secondary fermentation tank, an optimal amount of yeast cells passed, allowing the fermentation process to continue to take place and therefore to the decrease of beer apparent extract during the secondary fermentation from 2.3° P (at the beginning of the process) to 1.8°P after 14 days of fermentation and 1.7°P when the fermentation ends. The diagram of the secondary fermentation respectively the correlation temperature / pressure on the tank was respected fact that contributed to the good beer quality parameters like carbon dioxide content of 0.56 g/100 mL beer and to the very good organoleptic qualities like taste and smell nice, clean with a bitterness tint, persistent foam e.g.

Table 5
The unfiltered beer parameters

Parameters	Average value	Standard deviation
Original extract (°P)	10.20	0.08
Apparent extract (°P)	1.70	0.09
Density (g/mL)	1.0008	-
Alcohol concentration % (v/v)	4.60	0.30
Acidity (mL NaOH 1n/100 mL beer)	1.80	0.07
pH	4.20	0.18
Color (EBC)	8.00	0.80
Carbon dioxide (g/100 mL beer)	0.56	0.04
Bitterness value (BE)	20.00	0.60
Viscosity (cP)	1.35	0.03
Fermentation grade for sale (%)	83.30	1.76
Yeast cell number (x 10^3 cel/mL) at the end of the secondary fermentation	500	132.34

4. Conclusions

The dry lager brewing *Saccharomyces cerevisiae* (Saflager S-189) yeast strain used

in our research has been quickly adapted to the environmental conditions (wort composition) its growth is continuous during all the fermentation phases form the starting inoculum until the secondary fermentation stage. The yeast presents good flocculation ability because at the end of the primary fermentation phase the yeast cell population was 7.5 x 10^6 cel/mL enough for the sugars metabolisation during the secondary fermentation phase. The diagram of primary and secondary fermentation was well conducted, the fermentation grade for sale determined in the beer factory being very close to the fermentation grade for sale determined in the laboratory target that must be achieved by the brewers. Regarding the beer physical-chemical parameters and also the organolpetic ones the yeast strain used improved them.

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6. References

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