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# THE INFLUENCE OF MICROORGANISMS ON BEER QUALITY

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**Abstract**. The paper investigated the influence of microorganisms on the quality of Oettinger and Timișoreana beer, in the context of improving the manufacturing methodology of the finished product. Two types of yeast were used for fermentation: Oettinger and SAB-5. At the end of the fermentation process, the microbiological sample was selected to determine the anaerobic bacteria harmful to the beer. Various methods of seeding and sampling were analyzed, such as seeding on several types of media: Wort, Endo and NBB-A, media used to determine the types of microorganisms present at each stage of production. As the level of free amino acids (FAN) is a significant indicator of the completion of the fermentation process, the influence of FAN on the dissociation of yeast and diacetyl content was also analyzed. Subsequently, at the end of the fermentation process, microbiological growths were determined.

**Keywords:** *OT-Oettinger and TM-Timișoreana beer, microorganisms, yeast, culture media, bacteria Pectinatus, FAN - free amino acids.*

**Rezumat.** Lucrarea a investigat influența microorganismelor asupra calității berii Oettinger și Timișoreana, în contextul îmbunătățirii metodologiei de fabricație a produsului finit. Pentru fermentare au fost folosite două tipuri de drojdie: Oettinger și SAB-5. La sfârșitul procesului de fermentație, proba microbiologică a fost selectată pentru a determina bacteriile anaerobe dăunătoare berii. Au fost analizate diferite metode de însămânțare și prelevare, precum însămânțarea pe mai multe tipuri de medii: Must, Endo și NBB-A, medii utilizate pentru determinarea tipurilor de microorganisme prezente în fiecare etapă de producție. Deoarece nivelul de aminoacizi liberi (FAN) este un indicator semnificativ al finalizării procesului de fermentație, a fost analizată și influența FAN asupra disocierii conținutului de drojdie și diacetil. Ulterior, la sfârşitul procesului de fermentație, au fost determinate creşteri microbiologice.

**Cuvinte cheie:** *bere OT-Oettinger și TM-Timișoreana, microorganisme, drojdie, medii de cultură, bacterii Pectinatus, FAN - aminoacizi liberi.*

**Introduction**

Beer is a finished product obtained by a biotechnological process in which yeast is used to convert fermentable carbohydrates from the wort into ethyl alcohol, carbon dioxide and other secondary compounds. Beer yeast belongs to the Ascosporogene group, the Saccharomycetaceae family, the genus Saccharomyces cerevisiae [1]. In 1970 Lodder describes Saccharomyces cerevisiae cells as being spheroidal, sublimed, ovoid, ellipsoidal or cylindrical to elongation, which appear alone in pairs, occasionally in short chains or groups. Cells can be grouped into three classes by size. A large type, 4,5-10,5 x 7,0-21,0 μm (microns); a small cell type ranging from 2,5-7,0 x 4,5-11 μm and an intermediate cell group measuring 3,5- 8,0 × 5,0-11,0 μm. Some yeast may form filaments which can be up to 30 mm long. The yeast cells of beer fall into either of these categories; however, they tend to be quite large cells, a consequence of polyploidy. A critical parameter in brewing is the correct management of yeast between fermentation, as the characteristic flavour of any beer is largely determined by the yeast strain used and the composition of the wort [2].

The most commonly used beer yeast belongs to the genus Saccharomyces cerevisiae due to some essential characteristics for the brewing process, such as high efficiency ethanol production, the metabolism of sugars using the preferential fermentation path based on the presence of Crabtree (glucose breathing suppression) and its ability to tolerate many environmental stress (primarily the presence of ethanol). The quality of any beer is mostly determined by the used yeast strain and, as a result, currently are being analyzed new yeast strains in order to obtain innovative beers.

Beer yeasts are classified in two categories Ale and Large yeasts also known as high fermentation and low fermentation yeasts [3]. In the production of "ale", the yeasts of which belong to the species Saccharomyces cerevisiae traditionally lead to "peak fermentation", where the yeasts accumulate on the surface of the fermentation wort and the temperature at which they ferments is 14 to 25 °C [4,5].

The fermentation process of beer is influenced by yeasts whose objective is to metabolize sugars in ethanol, carbon dioxide and a variety of secondary metabolites that greatly influence chemical composition, colour and sensory quality. Minor metabolites, which are produced by the yeast of beer and which influence the quality of the beer are: esters, higher alcohols and acids which contribute positively to the flavour. These myriads of minor components characterize a brand of beer and make it identifiable for a drinker. The chosen yeast must also control the removal of undesirable aromatic components from raw materials or from fermentation. Much of this aroma improvement is taking place at maturation [5,6].

In brewing, the most commonly used input products are Saccharomyces cerevisiae (ale beer) and Saccharomyces pastorianus (lager beer). Their widespread use is primarily due to the repression of glucose respiration (Crabtree-positive) and the preference for the fermentative pathway, the efficient production and tolerance of large amounts of ethanol, the production of desired flavours, and the absence of toxin production [6,7]. In Figure 1. is shown the metabolic activity of Saccharomyces that influences the quality of beer. This simplified scheme summarizes the main metabolic pathways related to the modulation of beer flavour by Saccharomyces [8].

The aroma profiles of beer can be attributed mainly to biochemical activities during fermentation in the yeast cell, in which the sugars in the must are converted into ethanol and volatile compounds, such as alcohols and higher esters, which are intermediates and secondary products of yeast metabolism. These volatile compounds are different from the

aromatic compounds present in malt and hops and have a significant impact on the aroma and taste of beer. Ethanol and $CO_2$ are the primary by-products formed during fermentation, other yeast-derived active aromatic compounds are carbonyls (aldehydes / ketones), higher / fusible alcohols, esters, fatty acids, organic acids and sulphur compounds. The two main classes of nutrients that influence the performance of brewer's yeast are carbohydrates and nitrogen compounds [5,9].

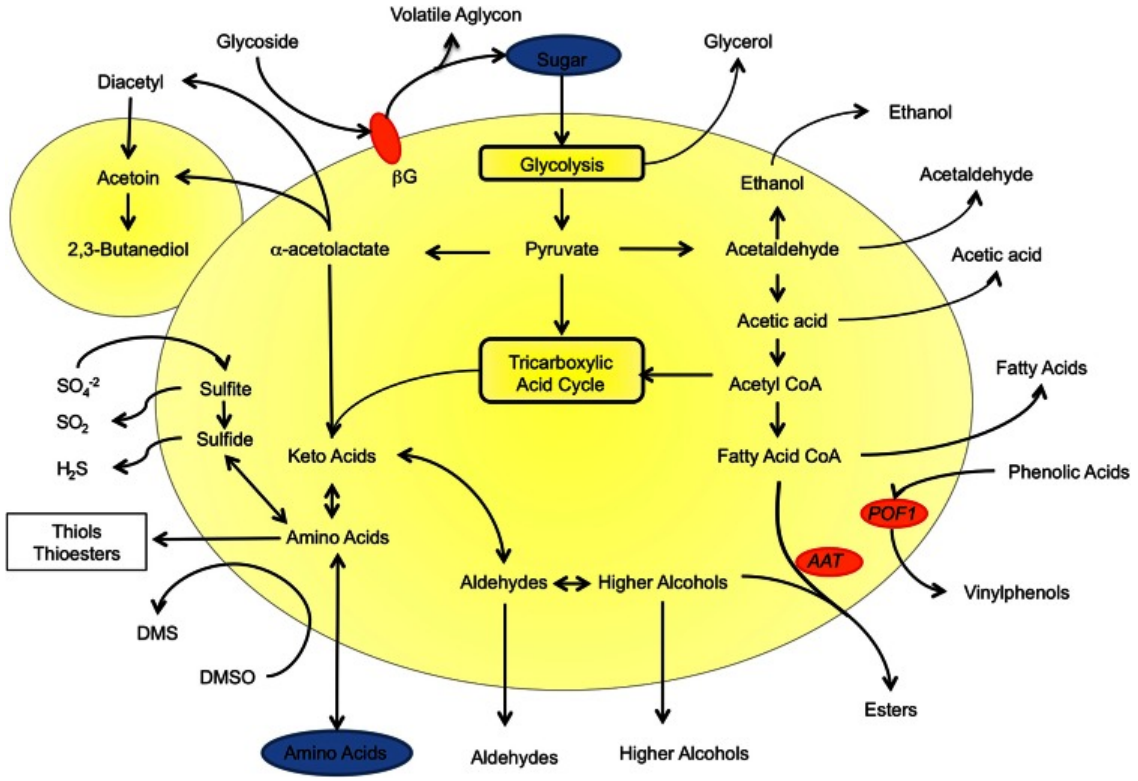


**Figure 1.** Overview of Saccharomyces metabolic activities that influence beer quality [8].

Yeast strains and many carbohydrates during brewing process (glucose, sucrose, fructose, maltose, galactose, refiner, and maltotriosis) can be used and the main characteristic which distinguishes "ale" and "lager" yeasts is the capacity of "lager" yeast for fermentation of melibiose. The generalized pattern of sugar absorption begins with the absorption of the simplest sugars (glucose and fructose), followed in the increasing order of complexity of disaccharides (maltose) and trizaccharides. As regards nitrogen compounds, the main function of malt is to provide yeast-like nitrogen sources, which are amino acids, ammonium ion, and some di- and tri-peptides [5]. The majority of the must-free amino nitrogen (FAN) is used by the yeast to form the proteins needed to increase yeast. However, the level and composition of FAN in the must has a significant influence on higher alcohol, ester, vicinal dictation and $H_2S$ formation due to the function of amino acid metabolism in the formation of these aromatic compounds [10].

Fermentation conditions are ideal for bacteria to grow, and contamination can delay or extend fermentation and cause various flavours and odours. Typically, specific gravity, pH and flavour are checked during preparation, and microbiological analysis is performed only if problems occur during fermentation [9].

**Methodology**

In this paper, beer production schemes and micro-organisms that may develop in some technological processes were analysed. Micro-organisms may be beneficial to production in

some cases, and in others may cause damage to the finished product or performance which will pose a risk to human health, which would run counter to the quality requirements of all producers and required by consumers.

The analyses have been carried out on the basis of two types of beer Oettinger and Timişoreana. Their basic parameters are: alcohol, pH, quantity of $CO_2$ and quantity of $O_2$, antibacterial compounds resulting from the addition of boiling hops. Beer wort is a by-product that can be used by microorganisms as a substrate for development. Beer on the other hand is less beneficial for the development of microorganisms. Because of the high alcohol content, the low oxygen content, the high content of $CO_2$, the izo-α-acid and β-acid content, which are antiseptic substances. All microbiological analyzes was carried out under the "Olympus" type of microscope.

Thus, the latest generation equipment from the economic entity was used in the research process, which allows to analyse and research of all aspects of the finished product in detail, the experimental results obtained being subject to further analysis.

**Results and discussions**

Initial parameters were determined at the beginning of the experiments on the determination of micro-organisms in beer to ascertain whether conditions were favourable for the development of certain micro-organisms. The tables below show the general parameters of the beers examined, the fermentation process that was carried out at the first boiled must (Table 1) and further after the second must (Table 2).

*Table 1*

**General parameters of bottled Oettinger and Timisoreana beer**

| ***Physico - chemical indices*** | ***Timişoreana*** | ***Oettinger*** | ***Permissible limits after MEBAK and SM 143:2001 Beer. National assortments [11 - 15]*** |
|---|---|---|---|
| Ethyl alcohol, % vol, la 20 °C, | 4.90 ± 0,42 | 5.24 ± 0.13 | 5.1 ÷ 5.7 |
| Bitter, IUB | 21 ± 0.78 | 17 ± 0.59 | 18 ÷ 22 |
| pH-log$H^+$ | 4.39 ± 0.18 | 4.41 ± 0.17 | 4.0 ÷ 4.6 |
| $Ca^+$,mg/l | 29 ± 0.59 | 28 ± 0.59 | 40 ÷ 80 |
| Density, | 1.00907 ± 0.006 | 1.00751± 0.006 | - |
| Extract, P P | 11.4 ± 0.42 | 12.23 ± 0.17 | 12.0 ÷ 12.4 |
| Concentration of $CO_2$, w/w | 0.53 ± 0.13 | 0.56 ± 0.13 | 0.49 ÷ 0.57 |
| Concentration of $O_2$ | 0.084 ± 0.051 | 0.054 ± 0.042 | - |

The general parameters obtained are within the permissible norms according to the MEBAK standard and SM 143:2001 Beer. National assortments [11 - 15].

In this paper the values of FAN (FAN- free amino nitrogen) and diacetyl were determined. The sum of the nitrogen compounds bioavailable in must is represented by free amine nitrogen. Excessive FAN content can lead to problems with taste and microbiological stability of beer.

*Table 2*

**General parameters of bottled Oettinger and Timisoreana beer**

| ***Physico - chemical indices*** | ***Timişoreana*** | ***Oettinger*** | ***Permissible limits after MEBAK and SM 143:2001 Beer. National assortments [11 - 15]*** |
|---|---|---|---|
| Ethyl alcohol, % vol, la 20 °C, | 4.95 ± 0,17 | 5.19 ± 0.16 | 4.7 ÷ 5.3 |
| Bitter, IUB | 20 ± 0.59 | 19 ± 0.68 | 16 ÷ 24 |
| pH-log$H^+$ | 4.39 ± 0.17 | 4.45 ± 0.15 | 3.9 ÷ 4.6 |
| $Ca^+$,mg/l | 29 ± 0.59 | 30 ± 0.59 | 35 ÷ 50 |
| Density, | 1.00527 ± 0.004 | 1.00823± 0.006 | - |
| Extract, P P | 11.19 ± 0.17 | 12.31 ± 0.17 | 10.85 ÷ 11.35 |
| Concentration of $CO_2$, w/w | 0.56 ± 0.13 | 0.57 ± 0.14 | 0.51 ÷ 0.59 |
| Concentration of $O_2$ | 0.089 ± 0.042 | 0.077 ± 0.069 | - |

Brewer's yeast and wild yeast ferment excess amino acids in long-chain alcohols (propanol, iso-butanol). FAN levels are also a good indication of the completion of fermentation. FAN monitoring with the DR6000 will help to overturn tanks more quickly once FAN levels are sufficiently low. The typical FAN content is 200 - 250 mg / L in must and 10 - 120 mg / L in beer.

The concentration of FAN (Table 3 and 4) also directly influences the dissociability of diacetyl. Thus to analyse this interdependency, the determination of FAN in the must was carried out and how the dissociation of diacetyl from the yeasts took place in the fermentation process.

*Table 3*

**Values of FAN at first fermented wort at temperature of 17 °C.**

| **Date of the experience** | **Value of FAN** | | **The admissible value of FAN in wort after *MEBAK* [11].** | **Measure-ment unit** |
|---|---|---|---|---|
| | **Oettinger wort** | **Timişoreana wort** | | |
| 19.10.2021 | 217± 0,5 | | 200 ÷ 250 | mg/L |

*Table 4*

**Values of FAN second fermented wort at temperature of 17˚C.**

| **Date of the experience** | **Value of FAN** | | **The admissible value of FAN in wort after *MEBAK* [11].** | **Measure-ment unit** |
|---|---|---|---|---|
| | **Oettinger wort** | **Timişoreana wort** | | |
| 19.10.2021 | 224± 0,5 | | 200 ÷ 250 | mg/L |

The general parameters obtained are within the permissible norms according to the MEBAK standard [11].

Concentration of diacetyl is important to be determined because during the fermentation of the yeast, 2-acetolactate and 2-acetohydroxybutylate appear during fermentation. By oxidation, they are transformed into vice, diacetyl and 2,3-pentanedione dicetones. However, diacetyl may also appear as a characteristic metabolic product of microorganisms. The beer produces an altered flavour when content of vicious dicetone is too high. This often results in a candy flavour of burned sugar and butter or an oily sensation in the mouth that is unpleasant for the consumer. The target value for blond beer is less than 0.05 mg / kg.

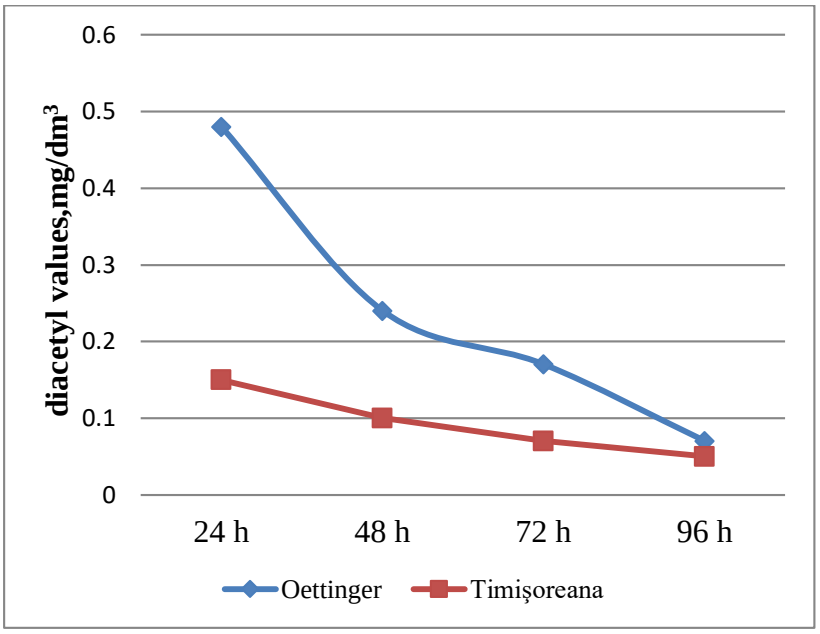


**Figure 2.** The quantity of diacetyl formed during fermentation of the first wort, at which the FAN value is 217 mg/dm$^3$.

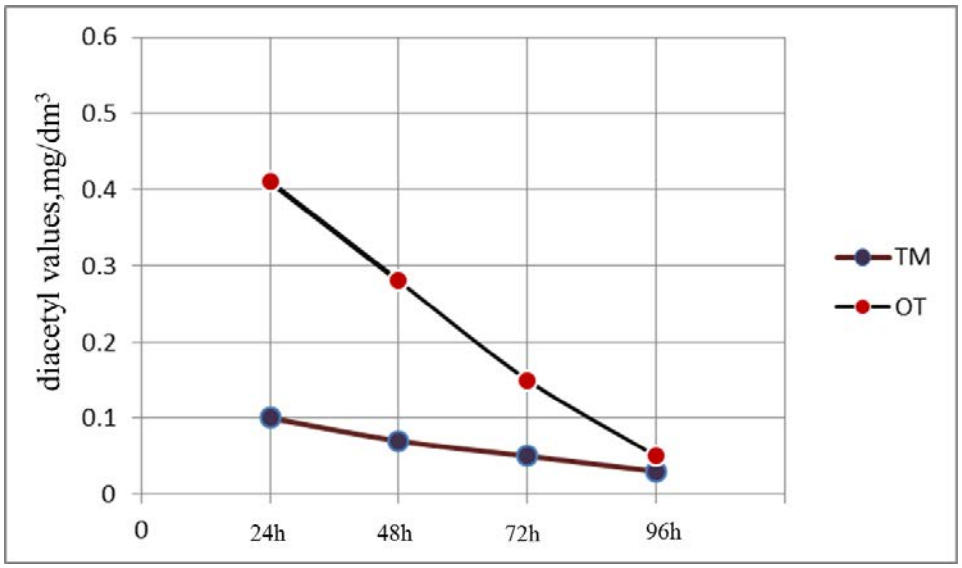


**Figure 3.** The quantity of diacetyl formed during fermentation of the second wort, at which the FAN value is 224 mg/dm$^3$.

Figure 2 and 3 represent the decrease amount of diacetyl according to the FAN concentration in the beer wort. The value of diacetyl is an indicator of the finishing fermentation process. At the end of fermentation, the yeast enters what is known as the stationary phase. This phase takes place when the beer goes through a maturation process to develop the right balance of flavours. One of the key elements of maturation is the reduction of di-acetyl. Not only does yeast produce the di-acetyl precursor, it also consumes the produced di-acetyl and reduces it enzymatically. The yeast reabsorbs di-acetyl from the medium and converts it into acetoin and later into 2,3-butane-diol, both with high flavour thresholds (difficult to detect), so as a result neither contributes much in terms of flavour [16,17]. But the amount of diacetyl is influenced by several parameters such as: $Ca^{2+}$ ions, of $Zn^{2+}$, sulphur ions and the initial amount of free amino nitrogen of the must. Also the level of di-acetyl is influenced by: the temperature at which the fermentation takes place, by the bacterial contamination, by the yeast strain, by the level of aeration [18,19].

To control the aroma and quality of beer, breweries rely on several tests to prove and maintain consistency. Brewers' associations and government agencies have established guidelines for testing specific parameters that are important for determining and controlling beer quality. We emphasize a key parameter for brewing - Free Amino Nitrogen (FAN). FAN testing is part of the quality control analysis of standard brewing, because they allow the estimation of protein content and serve as an indicator of beer quality [20,21].

The general FAN content of the wort may affect the absorption rate of the valine and thus the production of diacetyl. The larger is initial FAN content both the maximum concentration of diacetyl during fermentation will decrease [11].

Experience has shown that a small decrease in the quantity of FAN will lead to a reduction in the amount of diacetyl initial during fermentation, as faster absorption of preferred amino acids of yeasts takes place, resulting in higher demand for valine and its increased absorption due to lower competition for interactions. Figures 4 and 5 show the type of yeast used for the fermentation of the wort for the beers analysed.

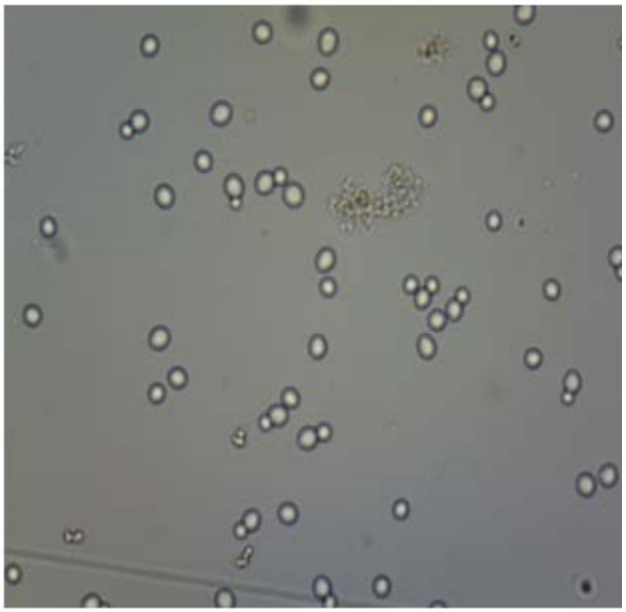

**Figure 4.** Oettinger yeast used for the fermentation of Oettinger beer.

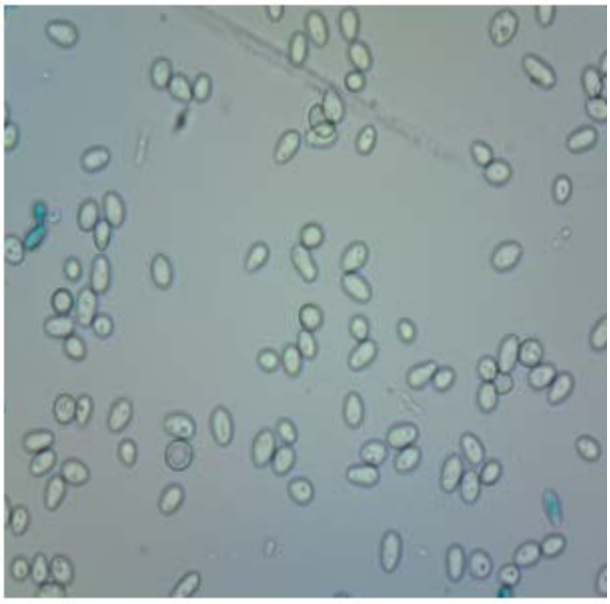

**Figure 5.** SABB-5 yeast used for the fermentation of Timișoreana beer.

Determination of microbiological increases at the end of the fermentation process.

The possibility of infecting beer with microorganisms is possible at any stage of production. Thus, to ensure that a quality finished product is produced, it is necessary to keep all production processes under control.

Two types of high fermentation yeasts (Oettinger and SAB-5) were used in the fermentation process, which at the end of the fermentation process with the help of cooling jackets fitted to the cubic-tapered tanks were subjected to cooling and sedimentation at the bottom of the tanks to collect them. After the yeast collection has taken place, the microbiological sample for the determination of bacteria and the presence of yeasts has been selected. The results of the seeding are represented in figure 6. Here we can see the presence of yeast colonies [19, 22].

Microscopic findings have shown that the colonies of yeast obtained from the sowing of the fermented must are of culture. Visual analysis of this conclusion can be sufficient, because the yeast colonies were white, consistent and glossy. Wild yeast colonies are usually small in diameter and have a white to transparent colour, appear to be aqueous [23, 24].

In Figure 6 (b) is microscopically represented a colony of yeast raised on the Wort medium. In this picture we can see large and medium diameter yeast cells, their cell wall is smooth and cell organs are not seen inside the cells. Thus, we can see that the yeasts presented are pure culture yeast and that they are young yeasts. Old yeasts are yeasts whose cell wall is rough, cells are large and cell organs are observed inside the cell. These yeasts look like they would crack soon. The expression of young yeasts takes into account that yeasts are used in the first stages, and are the first generations of yeasts used in the manufacturing process [25, 26].

a) b)

**Figure 6.** Wort sowing results after fermentation process: a) cultural characteristics; b) morphological characteristics.

Microbiological inoculations were also performed to determine the anaerobic bacteria harmful to beer. The determination of anaerobic bacteria harmful to beer is performed on NBB-A medium (Figure 7, 8) and the use of Anaerolult to achieve an anaerobic environment, by the absorption of oxygen by Anaerocult and the elimination of carbon dioxide, the ideal environment for the development of Pectinatus bacteria.

Today most breweries are trying to trace Pectinatus bacteria, because beer as a finished product is not favorable for the development of many other micro organizations such as L. Patentis, L. lindneri, Ped. Damnosus and others. The ideal growth environment for Pectinatus bacteria is in the filling unit and on the filling unit. Oxygen is not needed for the development

of these micro-organisms, on the contrary, it requires a $CO_2$. Pectinatus are relatively acidic and tolerant to ethanol [22 - 24].

**Figure 7.** Bacterial growth on the anaerobic environment of the OT beer.

**Figure 8.** Results of increases on the NBB-A anaerobically environment of TM beer.

From the results obtained after inoculation on NBB-A medium under anaerobic conditions for TM beer, no increase was registered, that indicates that the beer is not infected with microorganisms harmful to beer. Thus the product obtained will not present any risk of damage to the appearance and gustatory qualities.

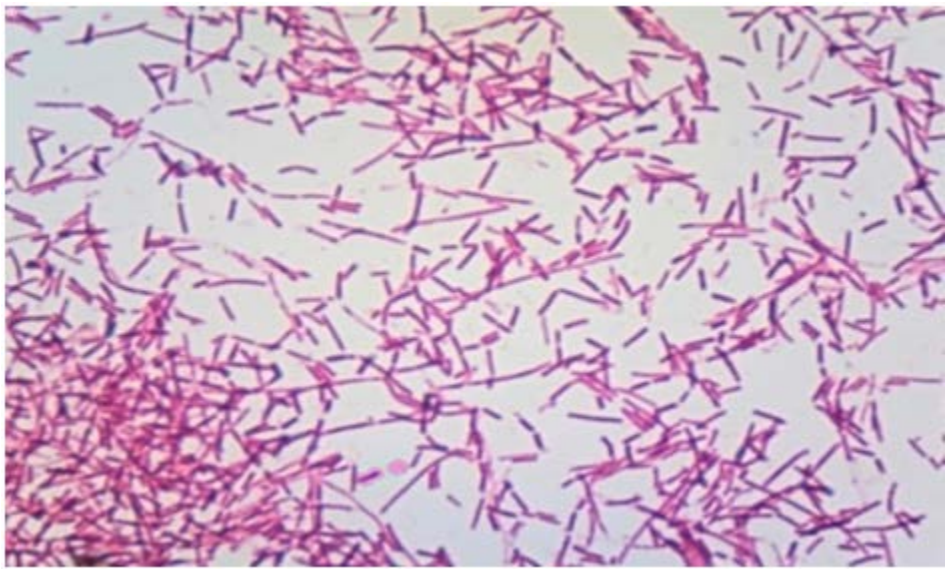

**Figure 9.** Microscopic results of colonies raised under anaerobic conditions.

The second seed was Oettinger beer. Several colonies have been grown after seeding and thermostats of the samples for 10 days. Thus, for the identification of the types of microorganisms that have developed, it was necessary to perform staining according to Gram. Following the test, it was identified that the grown microorganisms are Gram-negative (Fig.9). Following the study, we determined that Pectinatus bacteria are Gram negative bacteria but also that they appear individually, in pairs or in short chains.

**Conclusion**

The concentration of diacetyl in the fermentation process must reach values lower than 0.1 mg/l, otherwise the beer will have a buttery taste and an oily consistency. Following the determinations of the diacetyl concentration, the values were lower than 0.1, so the fermentation process was finished.

The FAN values were analysed and identified in the first and second fermentation must, where the determined values must be within the permissible limit of 200-250 mg/l, which was demonstrated by obtaining values of 217 and 224 mg./l for the first must of fermentation and respectively for the second must.

The importance of determining the FAN indicator lies in the fact that this index is perceived as a good parameter for anticipating the healthy development of yeast, viability, vitality and fermentation efficiency, which results in a higher quality of beer.

In order to determine the microorganisms, general parameters were determined in the Oettinger and Timişoreana beers, which fall within the admissible values according to the MEBAK standard.

Following the microbiological control, the presence of culture yeasts in the fermentation process was determined, which is not a problem, because the beer filtration process is planned in such way as to retain the tube as well as microorganisms such as yeasts. The amount of yeast added has to provide 12-15mil cells / ml must. The yeast we add has to ensure the start of the fermentation of the whole mass after 12-16 hours at 6ºC. In some cases the amount of yeast must be higher (in the case of higher density musts), and in that environment a higher number of cells is required to be added.

Moreover, the presence of Pectinatus bacteria in the finished product was also analysed. Following the anaerobic incubations in Oettinger, there was ascertained that the number of colonies increased, but in Timişoreana they were not observed. Due to the fact that there was no acidification or disturbance in the storage of Oettinger beer for a long period of time, we can conclude that the grown colonies were not Pectinatus bacteria.

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