

MICROBIOLOGICAL CONTROL IN THE PROCESS OF BEER PRODUCTION KONTROLLI MIKROBIOLOGJIK NE PROCESIN E PRODHIMIT TE BIRRES

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ABSTRACT

The beer consists of a rich environment that in its different periods of fabrication, can be used as a habitat for different organisms. The microorganisms that can be grown in it are of different types, but during the fabrication process many selective factors influence upon the microflora such as: temperature, pH, the amount of oxygen, the presence of carbon dioxide, alcohol, hops etc. In spite of it, keeping under continuous control the process of beer production through microbiological analyses is indispensable to assure a fine quality of the finished product and to prevent the eventual risks of contamination.

For the selective quantification of contaminant microorganisms groups of beer, is proposed to be used cultivated mediums same as fermentation environment. It is taken a brewery as an object of study. The aim of our research is the evaluation of the contaminant microflora, analysing all the stages and the processes of beer production.

Key words: beer, process, microbiological control, contamination, microorganisms.

INTRODUCTION

The production of beer is a biotechnological process that happens under the guide of microorganisms. The preceding of fermentative processes is the result of the selective mediums and of different contaminant microorganisms that risk the quality and shelf life of the final product. It is of great importance for a microbiologist, responsible of food staff controls, to determine the periods or critical control points of beer production. Contaminated microorganisms are selected from such parameters as: pH, temperature, anaerobic conditions, the lack or the insufficiency of growth factors as well as the presence of inhibitors.

If other microorganisms will be grown in beer, different from the brewer's yeasts, we shall note the secretion of metabolics which are different from those of brewer's yeast. Microorganisms that cause beer contamination are limited in some bacterial genus, wild yeast and moulds. This is due to the fact that beer in itself is often unfavourable for the majority of microorganisms.

The content of alcohol, low pH, the presence of hops ingredients are inhibitors, and the absence of nutrients limits the development of survival cells. Off flavours and off aromas in beer usually come from wild yeast and bacteria of lactic acid. Some wild yeast produce acetaldehyde, which gives to the beer a taste of apple spoilt. Some products that come from the metabolization of contaminant microorganisms are diacetyl, dimethyl sulfide, cis-3-hexenal and organic acids. Contamination of beer can be categorised in four groups: the increase of viscosity, illness of sarcinas, sourness and haziness.

The increase of viscosity is caused by the presence of *Acetobacter*, *Lactobacillus*, and *Pediococcus cerevisiae*. The illness of sarcina is caused by the presence of *P. cerevisiae*, which gives the honey flavour.

The sourness of beer is caused by the presence of *Acetobacter spp.* This is able to oxidize ethanol into acetic acid.

The turbidity of the beer is caused by the presence of *Zymomonas* and from some wild yeast.

Wild yeasts are the most important contaminants that cause changes to beer. *Saccharomyces diastaticus* hydrolyses dextrin, causes turbidity and "attenuation" in beer. Many other yeasts, beside those of genus *Saccharomyces* are sources of unacceptable flavour, that result from the production of phenolic compounds from the decarboxylation of hydroxycinnamic acids that are present in the wort.

Lactic bacteria are the most important and representatives are those of genus *Lactobacillus* and *Pediococcus*. They provoke turbidity, precipitations and off flavours. These produce lactic acid, acetic acid, diacetyl and acetoin. Genus *Zymomonas* bears defects during the storage. Genus *Lactobacillus* is a microorganism that affects fermented beer in low temperature. Hence it represents a critical point because its elimination is very difficult.

Moulds are developed in the natural temperature. Generally those are aerobic organisms that can be developed in a wide interval of pH. The most frequent genus are: *Mucor*, *Penicillium*, *Aspergillus*, *Cladosporium*, *Geotrichum* and *Rhizopus*.

The aim of this research is the evidence of microbial contamination from qualitative and quantitative points of view, of contamination risk of the final product and of

hygienic-sanitary measures to prevent or eliminate it. The best way to make the microbiological control of beer is the elimination of the contamination sources. Technology must be respected, first of all, to obtain a safer product microbiologically, only in this way it can be assured the quality of the beer.

The nature and stages through which beer production passes, as well as the changes it undergoes as a result of microbiological contamination, needs a continuous control especially in:

The qualitative delineation of contamination that consists in the identification and isolation of determinate groups of microorganisms.

The quantitative delineation that consists in counting of special categories of contaminated microorganisms examined.

To achieve our goal, first of all, it is designed the control strategy that consists of four important steps as follows:

- The definition of critical control point
- The definition of control's frequency
- The definition of ratings kept under control
- The definition of most effective control methods

The aim of this research is the evaluation of contaminated microflora in the process of beer production by analyzing in details:

- The raw materials (water, malt, yeast and hops)
- The half ready-made products (wort, fermented beer, storage beer and filtrated beer)
- The final product
- Environment and package

MATERIAL AND METHODS

General rules for sample taking.

Samples must represent the contents of tanks or tubes where they are taken. It is of great importance for the

samples not to be subjected to a second contamination, because it brings wrong analytical results. Only training persons are allowed to take microbiological samples. The sterilization of sample equipments is also very important like the sterilisation of chemical glasses, pipettes, anse, Petri dishes, culture mediums, etc.

By cultivation in microbiology it is meant the insemination in a sterile nutrient place of any material that must be analysed later on for the presence of microorganisms in it.

Methods of cultivation of microorganisms

It should be paid great attention during the cultivation to keep uncontaminated the mediums from the environment and from the contacts of different equipment surfaces that is used during the analyses. There are used selective mediums for the qualitative definition of different groups of beer microorganisms. Those mediums are chosen to give us the opportunity to distinguish the presence of contaminants from the shape of their colonies.

The main techniques that are used for the cultivation of microorganisms are:

- Cultivation by flooding technique
- Cultivation by stripping technique
- Cultivation by sedimentation technique
- Cultivation by membrane-filter technique.

The mediums used in this case are:

- Wort Agar media is used especially for the cultivation of yeast
- PCA- universal medium contains glucose, which is used by all kinds of bacteria even by those that do not have proteolytic characteristics.
- MPA media is used to define the bacteria, this media does not have carbohydrates, but it has only protein substance. The bacteria that have proteolytic characteristics can grow in this medium.
- Capek media is used to define different moulds
- Lysine media is used for the identification of the wild yeast
- Media VRBL (violet bile agar with lactose) is used for the identification of pathogens in the water.

Moreover there are used even the direct microscopic techniques to distinguish the microorganisms.

It is also used the Gram staining technique to differentiate bacterial species into two large groups (Gram-positive and Gram-negative) based on the chemical and physical properties of their cell walls.

RESULTS AND DISCUSSIONS

Analyses are done in a brewery during the period 2006-2009.

The contaminated microorganisms of beer production process are divided according to their contaminating character in:

Absolute beer spoilage organisms grown in beer without long adaptation that give it off flavours, turbidity and precipitation. In that group are included those contaminants that change the character of the final product like: lactic and acetic bacteria.

Potential beer spoilage organisms normally do not grow in beer. However, beers with high pH, low hops concentration, low degree of fermentation, low alcohol content, with high oxygen content can be a good environment for some specified microorganisms, which can be adapted and developed in the beer after long exposure times.

Indirect beer spoilage organisms do not grow in the final product, but can develop only in some determinate stages of the process, causing off flavours and off aromas in it. Usually they occur in the pitching yeast or in the beginning of fermentation, causing quality defects.

Indicator organisms do not cause spoilage but they can be developed as a result of poor hygienic conditions during beer production. Their presence is often associated with occurrence of beer spoilage organisms. Latent organisms are uncommon during the process of beer production and they survive in some different periods of production process. We can isolate these organisms in the final beer. The typical organisms of that category are common organisms in soil and water. The revelation of such microorganisms in the final product indicates poor hygienic conditions.

Contamination microorganisms of the filling process
The filling process by itself is the most important one from microbiological point of view, due to the higher oxygen levels, higher temperatures in some cases, and also the contact of the beer with the environment and filling equipments. Filling lines create good conditions for the development of acetic bacteria, coliforms, aerobic wild yeast, as well as beer spoilage organisms oxygen-tolerant.

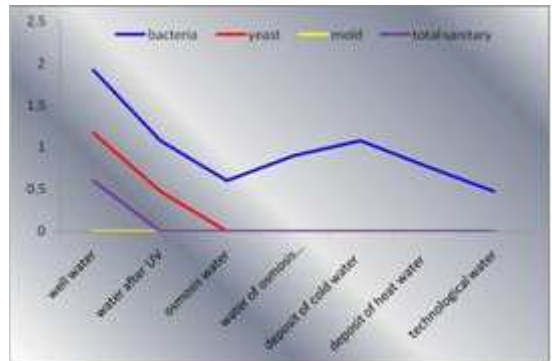
The results of microbiological analyses are shown by graphics, where it is given the relation of logarithm of (N). N is the number of microorganisms.

Regarding microbiological quality of water used in beer industry it is noticed that:

Well's water had a bacterial contamination over limits. From the quality inspection results that the total microflora of mesophilic aerobic bacteria in water is mainly represent by genus of *Brevibacterium*, *Enterobacter*, *Flavobacterium*. After the treatment of water by UV- rays and also after its treatment by reverse osmosis, the quality of water is evidently improved. Such water, after these treatments, is

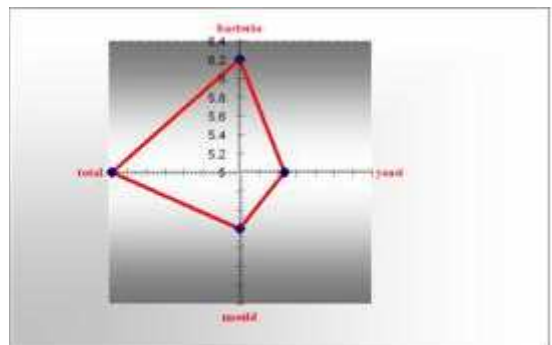
microbiologically fit to be used in all the other technological processes of beer production.

The results of microbiological analyses of the water used in a brewery are given in graphic 1.



Graphic 1. Microbiological control of water

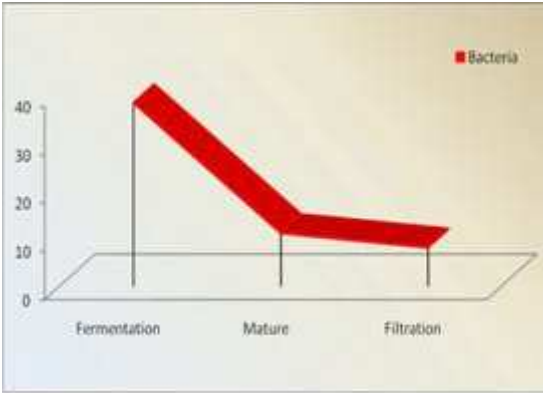
In graphic 2, there are shown the results of microbiology control of malt. The moulds developed in the malt are the typical ones that grow in the cereals. The present yeast is usually that of brewer's yeast. Based on the phenotypic characteristics and on the morphology of the development colony in the selective mediums, there are identified these moulds: *Fusarium*, *Aspergillus*, *Mucor*, *Penicillium*, *Cladiosporium*, *Geotrichum*.



Graphic 2. Microbiological results of malt

From the direct microscopic observation of the bacterial preparation grown in the WA and PCA mediums, it is noticed the presence of Gram-positive bacteria of genus *Bacillus*, and Gram-negative bacteria belonging to genus *Flavobacterium*.

From the microbiological analyses of beer in the fermentation process, in storage beer and in filtrated beer, the microbiological contamination is mainly bacterial. The brewer's yeast is considered as a contaminant in the filtrated beer.



Graphic 3. Bacterial contamination of beer from fermentation till filtration

In graphic 3, it is shown how varies the percentage of bacterial contamination in beer from the fermentation process to storage process, till the filtrated beer. All the filled tanks are subjected to analyses in such stages. Based on beer microbiological analysis, it is noticed a considerable reduction of the total microbial load from the fermented process to filtrated beer. There are some factors that influence in such a reduction like:

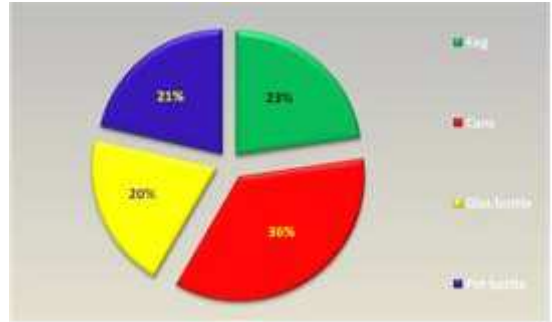
- The increasing percentage of alcohol
- The pH decreasing till 3.9-4.0, which prohibits generally the development of bacteria.
- The decrease of acidity.
- The removal of yeast and other particles.
- Temperature's decrease under 1⁰C.

From the microbiological analysis of the fermented beer, it is seen the presence of Gram-positive bacteria non-sporogene, belonging to genus *Leuconostoc*.

During the analyses of storage beer, it is cognised Gram-negative bacteria belonging to genus *Enterobacter* and *Acetobacter*.

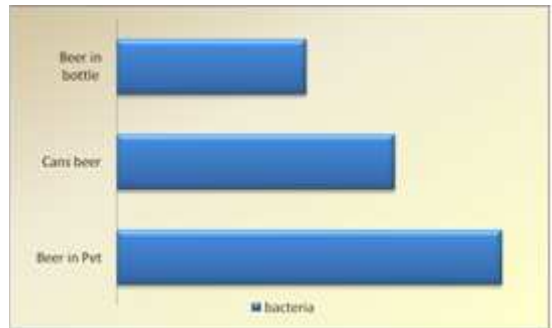
From the microbiological control of filtrated beer, it is remarked Gram-negative bacteria belonging to genus *Leuconostoc* and Gram-positive bacteria belonging to genus *Leuconostoc* and *Pediococcus*.

In graphic 4, there are shown the bacterial percentage of different packages used for beer packing. Bacterial contamination of packages is over limits, because in this case it can't be used disinfecant solutions to protect the beer from the ulterior oxidation. The contamination microorganisms of different beer packages are usually bacteria that belong to genus *Pseudomonas*, *Serratia*, *Bacillus*, *Aerobacter*.



Graphic 4. Microbiological results of different packages

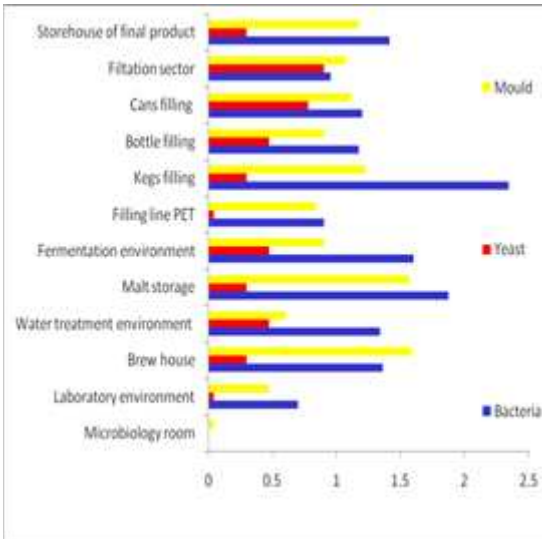
In graphic 5, it is shown the percentage of bacterial contamination in different beer's packages. Bacterial contamination is higher to PET packed beer, because in this case it is first pasteurized and then packed, being unprotected during the packing process. The bacteria of packed beers belong mainly to genus: *Aerobacter*, *Micrococcus*, *Gluconobacter*, *Micrococcus* and *Lactobacillus*.



Graphic 5. Microbiological results of different packed beers

From graphic 6, it can be observed the microbiological control of environments that have direct or indirect contact with beer. Here it is seen the presence of yeast, bacteria and moulds.

The yeasts present in the environment are wild yeast and brewer's yeast. The most frequent bacteria to the brewery environment are Gram-positive bacteria belonging to genus *Streptomyces*, *Bacillus*, *Micrococcus*, *Staphylococcus* and Gram-negative bacteria, belonging to *Flavobacterium* and *Pseudomonas*.



Graphic 6. Data of microbiological control of brewery environments

The moulds encountered in the brewery environment are mainly: *Penicillium*, *Aspergillus flavus*, *Fungi imperfect*, *Cladosporium*, *Aspergillus niger*, *Aspergillus terreus*, *Rhizopus*, *Mucor*, *Geotrichum*, *Trichotecium roseum*.

The environment with higher microbial presence, especially bacterial one, is where barrels are filled. In this place barrels are cleaned and is discharged the water of the bottle washing machine. Another environment of high contamination is brew house, where contamination is caused by the wort itself, by high temperature and by high humidity in that area.

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