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Initial microbiological experience in small-scale fruit beer product development

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1. SUMMARY

The market share of small-scale breweries in the total Hungarian beer market was 3 percent in 2020 [1]. The goal of the law affecting the sale of small-scale beers (the so-called "Beer Act", published in Issue 275 of 2020 of the Hungarian Gazette on December 11, 2020) is to create an opportunity for small-scale breweries to gain a better market position [2]. The measure is expected to have a positive effect on the trends that have been going on in Hungary for years, such as the increase in the number of market participants, the expansion of the product range and the increase in consumer interest.

In addition to the above encouraging trends, as consumers, we find that, on average, small-scale breweries lag behind large-scale producers in terms of producing a constant high quality and in ensuring the stability and shelf life of the bottles of beers. The quality deficit mentioned is mainly due to the deficiencies in the quality management systems of small breweries, the inadequate level of expertise available and the specific sales conditions.

In this article, small-scale product development of a fruit beer representing one of the most sensitive product categories in terms of packaged product stability because of its low alcohol content and, at the same time, high sugar content is presented. Mainly the experience related to the achievement of microbiological stability is summarized in the paper, while also dealing with the development of the manufacturing environment, summarizing the most important sources of danger and possibilities for failure, and drawing the attention of existing and future manufacturers to the possibility that the certificates of conformity of manufacturers of brewing equipment do not always guarantee their proper functioning, and in many cases they may have to be reviewed and modified. The microbiological relationships mentioned in our manuscript are based on our own observations. The product-specific test methods used in the course of the project are also presented in detail.

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2. Introduction

2.1. Small-scale brewing

In legal terms, breweries that produce less than 200,000 hectoliters of beer per year has been called small-scale breweries in Hungary since 2017. Regulation before 2017 drew the line at 8,000 hectoliters, under which breweries benefited from a 50% excise tax rebate **[3]**.

2.2. Fruit beers

The category *fruit beer* includes beers that are made with some kind of fruit or a combination of several fruits. Here the word fruit is used in a culinary rather than a botanical sense: fleshy, seed-associated plant structures that are sweet or sour and can be eaten raw. These include, for example, pome fruits (apples, pears, quinces), stone fruits (cherries, plums, peaches, apricots, mangoes, etc.), as well as fruits that have "berry" in their English names (strawberries, raspberries, blueberries), currants, citrus fruits, dried fruits (dates, prunes, raisins, etc.), tropical fruits (banana, pineapple, guava, papaya, fig, pomegranate, cactus figs, etc.) **[7].**

Flavored beer: Beer for which other flavoring substances may be used instead of or in addition to hops to create a flavor effect. The detailed characteristics of these products are recorded in the product data sheet.

In the case of flavored beers, the flavoring substances are added to the wort or the beer during the brewing operations, at the latest during maturation or filtration. As a result of the flavoring substance added during maturation or filtration, the original gravity of the finished beer may not increase by more than 1/3 **[8]**.

2.3. Launching production in a small-scale brewery

Ideally, the main sub-processes of launching production in a small-scale brewery are as follows:

1. Conducting official licensing procedures
1. Construction of the production plant and auxiliary facilities
2. Product development
3. Installation of brewing technology
4. Product manufacturing
5. Product sales

It is important to emphasize that during an efficient and cost-optimized production launch, product development precedes the acquisition of brewing technology, a sub-process specifically based on the experience of the former. This sequence can be achieved with the involvement of a service organization specializing in product development, which has the professional and technological background required for the process.

2.4. Technological equipment involved in our project

For the small-scale production of fruit beer, the following main technological equipment were installed:

- Malt mill
- Mash house equipment
- Combined mashing/filtering tub
- Universal hop kettle - Whirlpool tub
- Electric control panel for the brewing process
- Wort cooling and recuperation equipment
- Heat supply equipment
- Mash house auxiliary equipment
- Fermentation area equipment
- Barrel washing and filling equipment
- Diatomaceous earth filter
- Beer pasteurization equipment
- Bottling machine
- Compressed air supply equipment
- Refrigeration technology equipment
- Brewery ancillary equipment

In the above list, equipment which is specifically used to ensure or improve the microbiological stability of beer or have an above-average effect on it are highlighted.

3. Microbiological production control

3.1. Microbiological stability of beer

The biological stability of beer is compromised by any microorganism that is able to multiply in beer, cause turbidity or form bottom sediments, and damage the beer through its metabolites. The number of these microorganisms is small, as only lactic acid bacteria and yeasts are able to grow under the given anaerobic conditions due to the alcohol content, carbon dioxide content, bitterness and low pH of beer. There is a certain period of time between the infection and turbidity caused by these microorganisms and the appearance of the bottom sediment, the length of which depends on the degree of infection, the virulence of the organisms, the quality of the beer, the access of oxygen and the storage temperature.

Microbiological stability can be ensured by the use of biologically sound adjusting yeasts with high fermentation potential, the concentrated culture of which and the thorough washing, cleaning and disinfection of the tanks, lines and equipment have been checked.

Automatic cleaning equipment deserves special attention. Sharp filtration, together with pumping with the exclusion of ambient air and the use of containers cleaned with sufficient thoroughness, allows the beer to be dispensed without pasteurization. Close microbiological control is required at each stage, such as fermentation, maturation, filtration and pumping **[4]**.

3.2. Factors influencing the microbiological stability of beer

From a microbiological point of view, beer is a relatively stable beverage. The beer parameters that contribute to this stability are as follows:

- Ethanol content (up to 10%, sometimes even higher): exposure to 5% ethanol has been shown to increase the permeability of the cell membrane and thus to interfere with the proton-driving force across the membrane (which is important for energy production). This means that most microbes do not survive or multiply in beer at this alcohol level.
- Carbon dioxide content (~0.5% v/v): dissolved CO_2 creates an anaerobic environment, preventing the growth of microorganisms that cause aerobic deterioration.
- Low pH (pH 3.8-4.7): many microorganisms are unable to grow at low pH (pH<5) because they cannot maintain intracellular pH homeostasis at these low pH values.
- Iso-alpha acids (15-100 µg/L, the concentration may be different from this): iso-alpha acids exert an antimicrobial effect by increasing the permeability of bacterial cell membranes.
- Decreased nutrient availability (most fermentable sugars are metabolized by yeast): many important nutrients, such as carbohydrates, amino acids and some vitamins B are present in very low concentrations in beer as they are consumed by the yeast during fermentation. Any increased nutrient levels (e.g., carbohydrates in low-alcohol beers) pose a risk of proliferation of microorganisms that cause spoilage.
- Low oxygen content (preferably below 0.1 µg/L): anaerobic conditions reduce the risk of potential growth of microorganisms that cause aerobic spoilage.

In modern brewing, a number of techniques are used to prevent the entry of microbiological contaminants or their survival during the brewing process, as well as during filling/packaging, in order to increase microbiological stability. Some examples:

- Boiling the mash, pasteurization, or sterile filtration before packaging.
- Well-designed brewing equipment that resists aggressive hygiene practices, such as CIP (Clean-In-Place) cleaning.
- Elimination of many traditional (and microbiologically risky) production processes (e.g., spontaneous fermentation or open fermentation vessels).

3.3. Causes of infection

Pediococcus cerevisiae in the form of mono- and diplococci, or tetracocci, clouds the beer and gives it an acidic, diacetyl taste reminiscent of butter.

Lactic acid bacteria produce lactic acid, formic acid and acetic acid. They also cause turbidity and, in part, form bottom sediment.

Wild yeasts are rare. They make the beer cloudy, form a juicy bottom sediment and also impart a mostly aromatic, distinct, partly coarsely bitter taste.

Cultured yeasts cause turbidity, bottom sediment, or only separate yeast colonies in the pumped off beer. Even if they remain only imperfectly in the beer after filtration, they can multiply after the rich oxygen uptake during pumping, especially if there is a large difference between the final degree of fermentation of the beer and the dispensed degree of fermentation **[4]**.

3.4. Spoilage microorganisms

3.4.1. Microorganisms most often associated with brewing and beer

Each raw material (e.g., malt, hops, water or additives) carries its own specific microorganisms. The proliferation of these microorganisms during one of the brewing steps results in the formation of metabolites that cause aftertastes. In the event that these microorganisms survive all steps of the brewing process, including pasteurization, if used, they may be present in the bottled beer as potential spoilage agents. Yeast used for the fermentation can also be a source of contamination.

It has been observed that during yeasting, the yeast can be contaminated with small amounts of bacteria and wild yeast. To avoid this, proper treatment of brewer's yeast is required.

Additional sources of contamination can be the brewing equipment (vessels, lines) if they are not properly cleaned and maintained. Until packaging is completed, the final steps in the manufacturing process (after fermentation) may also be prone to contamination by microorganisms that are airborne or present in the filling apparatus (e.g., due to high humidity).

Spoilage microorganisms most often found in breweries and in beer are listed in ***Figure 1***.

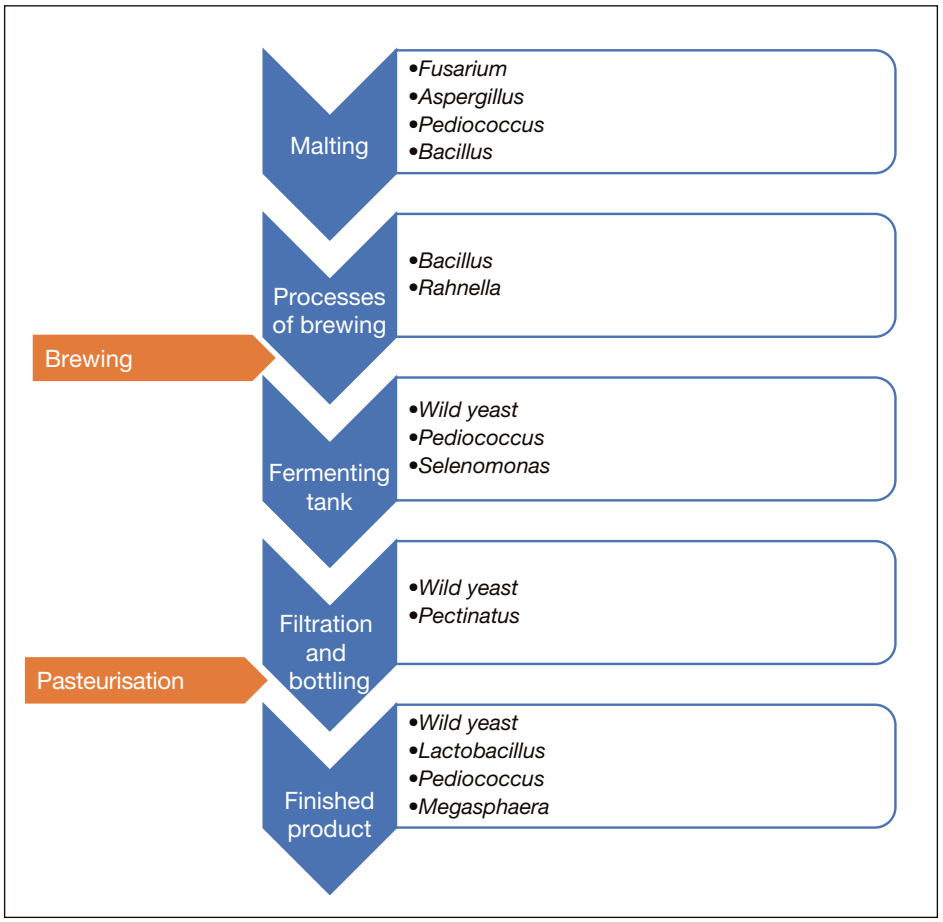

Figure 1. Most common beer spoilage microorganisms during the various steps of the brewing process and in the finished product. Orange arrows indicate the steps of the manufacturing process where the microbial load is reduced by heat treatment (wort boiling and pasteurization). ***[2]***

Contaminating bacteria in beer are mostly lactic acid bacteria belonging to the genera *Lactobacillus* and *Pediococcus* (accounting for more than 80% of the bacterial infections in beer), but other anaerobic bacteria such as *Pectinatus* and *Megasphaera* are sometimes also found in spoiled beer **[2]**.

3.4.1.1. Lactic acid bacteria **[6]**

Lactic acid bacteria are strictly fermentative, facultative anaerobic Gram-positive, non-spore-forming rods or cocci that belong to the order *Lactobacillales*. Most Gram-positive bacteria are inhibited by iso-alpha acids however some are resistant to these antibacterial compounds. The two most common lactic acid bacteria in beer are *Lactobacillus brevis* and *Pediococcus damnosus*. These bacteria produce acetic acid and lactic acid, and also compounds responsible for various aftertastes, such as diacetyl ("buttery" flavor). *Pediococcus* in particular is known to produce large amounts of vicinal diketones. *Pediococcus* also has a relatively high alcohol tolerance: it can proliferate even at ethanol concentrations above 10%. In addition, lactic acid bacteria also produce exopolysaccharides (EPS), which cause so-called silky turbidity in beer due to the increased viscosity and mucous appearance.

The most important *Lactobacillus* species are *L. brevis* and *L. lindneri*; less common are *L. rossiae*, *L. buchneri, L. coryniformis, L. casei* and *L. backii*. *L. brevis* often develops longer, parallel-walled, single or double rods with a round end (0.7 × 4 µm), with the double rods often being bent. It does not form cell chains, but extremely long rods (up to 50 µm) can sometimes be found in beer. Common characteristics of *L. brevis* are (hetero-fermentative) gas formation, fermentation of pentoses and melibioses, as well as the ability to cleave arginine. This is the most common beer spoilage bacterium, causing turbidity and sediment, while also lowering the pH perceptibly, which in turn gives beer an acidic taste. However, it does not produce diacetyl. It often appears as a secondary contaminant.

L. bucherni is able to ferment melisitose, unlike *L. brevis*. *L. lindneri* forms short, slightly irregular or coccoid cells that are arranged in longer chains. Sometimes long rods are formed. Heterofermentative species mainly ferment glucose and maltose and do not cleave arginine. Mild sedimentation and turbidity may be observed in the beer however taste defects do not usually occur. This is a typical primary contaminant that is often found in yeast factories or in the fermentation area, but can also pass through the filters, being very small cells.

L. rossiae has similar properties and is mucus-forming. Facultative heterofermentative species, such as *L. casei, L. coryniformis* and *L. plantarum* form shorter rods that are often arranged in chains. They are mostly found in weaker hop beers (e.g., wheat beer) and cause obvious taste defects due to diacetyl formation. They often appear only as secondary contaminants. The obligate homofermentative beer spoilage *L. backii* ferments mannose, mannitol and sorbitol. It also differentiated from the other species by the absence of fermentation of maltose and gluconate.

P. damnosus is characterized by the formation of tetrads. It is typically a primary contamination that often occurs in cultured yeast and unfiltered beer. The cells can also be transferred to the bottled beer through the filter. Contamination results in strong diacetyl formation (buttery taste) and a decrease in pH, and beers are often slightly turbid and exhibit noticeable sedimentation. Two other *Pediococcus* species that cause beer spoilage, *P. inopinatus* and *P. claussenii*, behave similarly, although both species are less common. The latter causes mucus formation in the beer.

3.4.1.2. Enterobacteriaceae **[6]**

Enterobacteriaceae is a facultative anaerobic Gram-negative bacterial family. The two genera commonly associated with brewing are *Citrobacter* and *Rahnella* (most likely to be introduced with the water used for brewing). These bacteria are responsible for the production of a number of compounds causing aftertastes, such as VDKs (e.g., diacetyl), 2,3-butanediol, DMS, acetaldehyde and lactic acid. These compounds are produced at the beginning of the fermentation.

3.4.1.3. Bacillus **[6]**

Gram-positive, facultative anaerobic, spore-forming bacteria. Due to spore formation, they survive heat treatment, including pasteurization. *Bacillus* also poses a risk because it can reduce nitrate to nitrite, which can lead to the formation of N-nitrosamines (classified as carcinogenic, teratogenic and mutagenic substances). Since certain *Bacillus* species are able to produce large amounts of lactic acid, they can also cause acidification. Most *Bacillus* species (but not their spores) are susceptible to the iso-alpha acids from hops.

3.4.1.4. Pectinatus **[6]**

These Gram-negative, strictly anaerobic bacteria can produce large amounts of acetic acid and acetoin, and hydrogen sulfide production (rotten egg aroma) has also been reported.

Pectinatus cerevisiiphilus and *P. frisingensis* are also strictly anaerobic, catalase-negative, Gram-negative bacteria, and have similar negative effects as the species listed so far. The cells are slender (0.8 × 4 μm), parallel-walled with a pointed end, slightly bent or serpentine or corkscrew-like, and serially flagellated on one side. Similarly to *M. cerevisiae*, they grow in the range of 15 to 40 °C (with the optimum being between 28 and 32 °C). They ferment various sugars, sugar alcohols and organic acids (mainly pyruvate and lactate). The primary metabolites are propionic acid, acetic acid, pyruvic acid, acetoin and CO_2. Beers contaminated with them (pH above 4.3, alcohol content below 5% vol.) exhibit not only serious sedimentation and turbidity problems, but also unpleasant odor and taste defects (sewage odor). Similarly to *M. cerevisiae*, these are typically secondary contaminants that occur primarily in the bottling area.

3.4.1.5. Megasphaera

Megasphaera species can appear as Gram-negative, strictly anaerobic contaminants in both wort and finished beer. They cause turbidity in beer and produce large amounts of hydrogen sulfide and a number of short-chain fatty acids ("cheesy" aroma) **[2]**.

Catalase-negative, strictly anaerobic, Gram-negative *Megasphaera cerevisiae* forms large oval or round cells (1.2 – 1.6 μm) that exist in the form of diplococci and short chains. They ferment fructose, pyruvic acid and lactic acid, in particular **[6].**

The primary metabolites are butyric acid, acetic acid, propionic acid, valeric acid, as well as CO_2 and hydrogen gas. Only a slight turbidity is exhibited by the beer; however, due to the above-mentioned metabolites, there can be significant odor and taste defects (sewage odor). The species is sensitive to alcohol (below 5% vol.) and prefers a higher pH value (above 4.4). The secondary contaminant that is present primarily in the vicinity of the bottling equipment are typically favorable to these bacterial species **[6].**

3.4.1.6. Wild yeast

Any strain of yeast, except the selected *Saccharomyces* yeast, is a contaminant. These yeast contaminants are usually referred to by brewers as wild yeast, which may be *Saccharomyces cerevisiae* or *non-Saccharomyces* strains, such as *Brettanomyces bruxellensis, Candida* or *Pichia*. Proliferation of wild yeast can carry a safety risk: the alcohol content can increase due to the metabolism of the infecting yeast. These wild yeasts are sometimes able to ferment dextrins and starch into ethanol (so-called superattenuation). Along with the production of ethanol, the CO_2-content, and thus the bottle pressure increases, which can pose a safety risk due to the bursting of the bottles. In addition, wild yeast can ruin the beer through the production of ester or phenolic aftertaste (e.g., 4-vinylguaiacol), as well as turbidity or sediment formation. It is important to note that acid washing of the yeast cells does not remove these wild yeast contaminants **[5].**

3.4.1.7. Fungi

Field infestation by *Fusarium* fungi poses a serious food safety risk to cereals. Almost all parts of the plant (germ, root, stalk, stem, leaf pod, leaf, ear and grain) can become affected. Severely infected plants produce less and lower quality crops, toxins are produced in the diseased grains, their germination vigor is reduced. The pathogens that cause the disease are different *Fusarium* species with various infectivity and toxin production, which can be greatly related to environmental factors, such as temperature and humidity. The toxins can be present throughout the entire brewing process, up to the bottled finished product. Certain *Aspergillus* species can also produce mycotoxins. Both *Fusarium* and *Aspergillus* species produce hydrophobic compounds, which are small surfactant proteins that cause foaming **[6].**

4. Cleaning, disinfection

Cleaning and disinfection of the lines, tanks and equipment is key in a brewery. All surfaces and equipment must be clean, the presence of contaminating bacteria, yeasts and fungi must be eliminated.

Examples of possible contaminants in a brewery:

- Beer left over from previous brewing
- Microbiological contaminants (yeasts, bacteria, fungi)
- Hop residues
- Calcium oxalate (beer stone that can be removed with acids)
- Lipids, proteins (removal with bases)
- Mineral deposits in the water circuit

In this regard, it is important to distinguish between cleaning agents and disinfectants.

Cleaning agents remove product residues and deposits, such as lipids and proteins. Depending on their pH value, these cleaning agents can be classified as alkaline, acidic or neutral cleaning agents. In order to further increase the cleaning capacity, additives, e.g., surfactants can be added. These are water-soluble molecules that reduce the surface tension of water, making it easier to remove contaminants.

Disinfectants are used to destroy most microbial contaminants. Here again, it is important to point out that bacterial spores are very difficult to destroy, which is why this process is called disinfection, not sterilization. Examples of disinfectants:

- Halogenated disinfectants, for example NaOCl (sodium hypochlorite). NaOCl is a commonly used product, but unstable above 40 °C (with increased risk of corrosion).
- Oxidizing agents, such as H_2O_2.
- Quaternary ammonium compounds (often called quats). Quats are cationic surfactants. Despite their good properties, quats are not used in breweries very often because they typically from foams and difficult to rinse, which endangers the quality of the beer, e.g., may impair the stability of the foam.
- Steam disinfection.
- Critical points include the so-called dead spaces (pipe ends, branches in the lines, sampling points, poor welding, etc.). Lines and tanks are best cleaned with an integrated CIP system.

5. Laboratory tests

5.1. General experience

During the microbiological testing of the sour cherry beer samples and the sour cherry concentrates, we found the following:

- Filtering of the sour cherry beer samples was not possible due to the high fiber content.
- Also, when testing the beers, in the case of the plate casting process, when covering 10 ml of the sample with a suitable layer thickness of 3 to 5 mm of PCA (for microbial count test) or DRBC (for yeast count test) culture medium in a large Petri dish (140 x 14.8 mm), the agar did not gel because of the low pH of the sample. Therefore, in these tests, the test volume was first reduced from 10 ml to 1 ml.
- Then the microbiological study of the sour cherry concentrates was started, due to the expected sterility of the raw material, using our own method for the detection of presence/absence.
- This method was further modified to include the testing of beers. As the issue was not the sterility of the beer but its practical shelf life, as a final, modified solution, the presence/absence of reproducible microorganisms was tested by an enrichment method in both cases, with the same amount of inhibitor as prescribed in the recipe of the finished beer (0.02 g/L potassium sorbate). In this way, it was practically modelled whether the microorganisms that may be present in the beer can reproduce at a high nutrient content.

5.2. Description of the test methods of sour cherry beers

5.2.1. Microbial count, plate casting, colony counting (MSZ EN ISO 4833-1:2014, accredited method)

The stock suspension and the decimal dilutions are prepared from the sample according to the international standard MSZ EN ISO 6887. Using a sterile pipette, 1 ml of the sample (for liquid samples) or stock suspension is added to two Petri dishes. The procedure is repeated with additional dilutions, if necessary. 12 to 15 ml of 44 to 47 °C PCA agar is added to each Petri dish. The Petri dishes are inverted and incubated in a thermostat at 30 °C for 72±3 hours.

5.2.2. Yeast count, surface spreading, colony counting, water activity >0.95 (MSZ EN ISO 21527-1:2013, accredited method)

The stock suspension and the decimal dilutions are prepared from the sample according to the international standard MSZ EN ISO 6887. 1 ml of the sample (for liquid samples) or stock suspension is added evenly in 3 portions to the surface of the DRBC agar filled in Petri dishes, and the sample portions are spread on the surface of the agar. The procedure is repeated with an additional degree of dilution and, if necessary, with additional dilutions. The dishes are inverted after 15 minutes and incubated in a thermostat at 25 °C for 3 to 5 days.

5.2.3. Presence/absence detection of reproducible microbes, enrichment technique (own method)

In the case of a 0.33-liter bottle of beer, the sample is divided into 3 equal portions and the sample portions are incubated for 72 hours at 30 C° in 3 x 100 ml stock broth containing 0.02 g/liter of potassium sorbate.

At the end of the culture period, 1 µl of each enriched sample portion is applied to a PCA plate, and the plates are incubated for 72 hours at 30 °C. If no increase in the colonies is observed on the plate, the result is reported as 'negative/100 ml', while if colonies do form, the result is reported as 'positive/100 ml'.

Composition of the PCA (Plate Count Agar) culture medium for microbial count determination:

- tryptone 5 g/l
- yeast extract 2.5 g/l
- glucose 1 g/l
- agar 9 g/l
- pH: 7.0±0.2 (25 °C)

5.2.4. Presence/absence detection of reproducible yeast, enrichment technique (own method)

In the case of a 0.33-liter bottle of beer, the sample is divided into 3 equal portions and the sample portions are incubated for 72 hours at 25 °C in 3 x 100 ml of malt broth containing 0.02 g/liter of potassium sorbate. At the end of the culture period, 1 µl of each enriched sample portion is applied to a DRBC agar plate, and the plates are incubated for 72 hours at 25 °C. If no increase in the colonies is observed on the plate, the result is reported as '*negative/100 ml*', while if colonies do form, the result is reported as '*positive/100 ml*'.

Composition of the DRBC (Dichloran Rose-Bengal Chloramphenicol) agar:

- enzymatically digested animal and plant tissues 5 g/l
- glucose 10 g/l
- potassium dihydrogen phosphate 1 g/l
- magnesium sulfate 0.5 g/l
- dichloran (2,6-dichloro-4-nitroaniline) 0.002 g/l
- Rose Bengal 0.025 g/l
- agar 15.0 g/l
- pH: 75.6±0.2 (25 °C)

Composition of the Takács stock broth (MSZ 3640/13-76):

- tryptone 4 g/l
- meat extract 4 g/l
- yeast extract 2 g/l
- sodium chloride 2 g/l
- disodium hydrogen phosphate 2 g/l
- pH 7.2-7.4 (25 °C)

Laboratory tests were carried out by the laboratory of EUROFINS Food Analytica Kft.

6. Relationships between the microbiological state of the finished product and the bottling machine

In all cases, the control tests applied during product development and subsequent production were extended to the examination of the microbiological condition of the technological equipment. At the same time, the possible effects of the microbiological condition of the equipment on product quality were explored.

In the course of the project, by examining the bottling machine, manufacturing defects of the machine were brought to light, which were later acknowledged and eliminated by the manufacturer based on the results of our tests.

- The manufacturer's CIP (Cleaning-In-Place) cleaning and disinfection program was set on the pneumatic branch lines of the taps with insufficient exposure time
- The foaming water supply was not connected to the CIP system
- The beer druck tank could not be cleaned adequately due to the dead spaces it contained
- The CO_2 inlet branch line of the beer druck tank was not connected to the CIP system

The results of the comprehensive microbiological testing of the bottling machine used in our project before conversion are summarized in ***Table 1,*** while the results of the microbiological testing of the beer bottled using this machine are summarized in ***Table 2***. The microbiological tests were performed by the laboratory of EUROFINS Food Analytica Kft., a testing laboratory accredited by NAH (National Accreditation Authority) under reg. no. NAH-1-1582/2021. Objectionable results are highlighted in the table in red.

Table 1. Comprehensive microbiological testing of the bottling machine before conversion

Sample ID	Sample type	Tested subunit	Tested parameter	Result	Unit
2	Water sample	Foaming water	Yeast count	15	cell/10 ml
			Microbial count	>300	pc/10 ml
5	Water sample	Beer tank leachate after rinsing with mains water	Yeast count	7	cell/10 ml
			Microbial count	$1.9*10^3$	pc/10 ml
6	Hygienic surface swab sample	Beer tank lid inside + pressure gauge nozzle	Yeast count	12	cell/100 cm^2
			Microbial count	42	pc/100 cm^2
7	Hygienic surface swab sample	Beer tank side wall	Yeast count	10	cell/100 cm^2
			Microbial count	>300	pc/100 cm^2
8	Hygienic surface swab sample	Pressure gauge foot	Yeast count	5	cell/100 cm^2
			Microbial count	26	pc/100 cm^2
9	Water sample	Pneumatic branch line leachate	Yeast count	4	cell/10 ml
			Microbial count	270	pc/10 ml

Test method – yeast count: MSZ ISO 21527-1:2013 ***[9]***
Test method – microbial count: MSZ EN ISO 4833-1:2014 ***[10]***

Table 2. Microbiological testing of small-scale fruit beer before conversion

Sample Type	Sample ID	Tested parameter	Result	Unit
Bottled beer	Fruit beer - lot 28.09.19.	Yeast count	$4.6*10^4$	cell/1 ml
		Microbial count	$4*10^4$	pc/10 ml

Test method – yeast count: MSZ ISO 21527-1:2013 [9]
Test method – microbial count: MSZ EN ISO 4833-1:2014 [10]

Prior to the conversion, the quality defects of bottled fruit beer that could be attributed to its microbiological condition were: taste defects (ester aftertaste), increase in CO_2 content and thus in bottle pressure, gushing (foaming beer squirting when the bottle is opened).

At our suggestion, the following modifications were made to the bottling machine by the manufacturer:

- Increased operating time of the CIP program on the pneumatic branch lines of the taps
- Connection of the foaming water supply to the CIP system
- Elimination of the dead spaces of the beer druck tank
- Connection of the CO_2 inlet line of the beer druck tank to the CIP system

The results of the comprehensive microbiological testing of the bottling machine used in our project after conversion are summarized in ***Table 3,*** while the results of the microbiological testing of the beer bottled using this machine are summarized in ***Table 4***.

Table 3. Comprehensive microbiological testing of the bottling machine after conversion

Sample ID	Sample type	Tested subunit	Tested parameter	Result	Unit
2	Water sample	Foaming water	Yeast count	-	cell/10 ml
			Microbial count	2	pc/10 ml
5	Water sample	Beer tank leachate after rinsing with mains water	Yeast count	-	cell/10 ml
			Microbial count	0	pc/10 ml
6	Hygienic surface swab sample	Beer tank lid inside + pressure gauge nozzle	Yeast count	-	cell/100 cm^2
			Microbial count	0	pc/100 cm^2
7	Hygienic surface swab sample	Beer tank side wall	Yeast count	-	cell/100 cm^2
			Microbial count	0	pc/100 cm^2
8	Hygienic surface swab sample	Pressure gauge foot	Yeast count	-	cell/100 cm^2
			Microbial count	0	pc/100 cm^2
9	Water sample	Pneumatic branch line leachate	Yeast count	-	cell/10 ml
			Microbial count	0	pc/10 ml

Test method – yeast count: MSZ ISO 21527-1:2013 ***[9]***
Test method – microbial count: MSZ EN ISO 4833-1:2014 ***[10]***

Table 4. Microbiological testing of small-scale fruit beer after conversion

Sample type	Sample ID	Tested parameter	Result	Unit
Bottled beer	Fruit beer - lot 29.10.20.	Yeast count	-	cell/1 ml
		Microbial count	0	pc/10 ml

Test method – yeast count: MSZ ISO 21527-1:2013 [9]
Test method – microbial count: MSZ EN ISO 4833-1:2014 [10]

Following the conversion, the hygienic condition of the bottling machine became satisfactory (***Table 3***), and the previously observed quality defects of the bottled fruit beer related to its microbiological condition were eliminated, as shown by the test results in ***Table 4***. Due to the achieved microbiological stability, the preservation of the quality of the product could be ensured.

7. References

[1] Növekedes.hu (2020): A kisüzemi sörfőzdék piaci részesedése 5-6 százalékra nőhet az új törvény hatására. (The market share of small-scale breweries could increase to 5-6 percent as a result of the new law.) https://novekedes.hu/hirek/a-kisuzemi-sorfozdek-piaci-reszesedese-5-6-szazalekra-nohet-az-uj-torveny-hatasara (Acquired: 10.16.2021.)

[2] 2020. évi CXL. törvény a kereskedelemről szóló 2005. évi CLXIV. törvény módosításáról (2020 CXL. Act CLXIV of 2005 on Trade amending the law)

[3] Council Directive 92/83/EEC of 19 October 1992 on the harmonization of the structures of excise duties on alcohol and alcoholic beverages. Section 1, Article 4.

[4] Ludwig Narziss (1981): *A sörgyártás.* (The Brewing) Mezőgazdasági Kiadó, Budapest.

[5] KULeuvenX (2021): *Beer: the science of brewing. BrewingX. KU Leuven, Leuven, Belgium*

[6] Hans Michael Eßlinger (2009): *Handbook of Brewing: Processes, Technology, Markets.* WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

[7] Beer Judge Certification Program, Inc. (BJCP) (2015): *2015 Style Guidelines*

[8] Magyar Élelmiszerkönyv Bizottság (2013): *2-702 számú irányelv (régi 2-96 számú irányelv) Sör*. Magyar Élelmiszerkönyv - Codex Alimentarius Hungaricus. Magyar Élelmiszerkönyv Bizottság, Budapest (Codex Alimentarius Hungaricus – The bier – The old directive 2-96)

[9] MSZ ISO 21527-1:2013 Élelmiszerek és takarmányok mikrobiológiája. Horizontális módszer az élesztők és a penészek számlálására. 1. rész: Telepszámlálásos technika a 0,95-nél nagyobb vízaktivitású termékekre (Microbiology of food and animal fedding stuffs. Horizontal method for teh enumeration of yeasts and moulds. Part 1: Colony count technique in products with water activity greater than 0,95)

[10] MSZ EN ISO 4833-1:2014 Az élelmiszerlánc mikrobiológiája. Horizontális módszer a mikroorganizmusok számlálására. 1. rész: Telepszámlálás 30 °C-on lemezöntéses módszerrel (ISO 4833-1:2013) (Microbiology of the food chain. Horizontal method for the enumeration of microorganisms. Part 1: Colony count at 30 degrees C by the pour plate technique (ISO 4833-1:2013)