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Alert for microbreweries – a case study on unpasteurized and unfiltered beer

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Abstract

Beer stability, influenced by physical, chemical, and biological factors, poses a considerable challenge for breweries. Biological stability, as a major concern, is effectively managed through practices such as good hygiene, pasteurization, and filtration. Microbiological contamination, often recognizable by increased turbidity or off-flavours, is influenced by temperature, with optimal conditions promoting the growth of specific microorganisms. Lower temperatures slow down flavour changes and chemical reactions in beer, impacting its non-biological stability. This case study explores the impact of storage temperature on autolysis off-flavour development in 10 different unfiltered and unpasteurized beers. The results showed that autolysis off-flavour occurred at both low and higher storage temperatures and that its dependence on the temperature was minimal. This unexpected fact was discovered mainly due to uncovered significant contamination of the commercial beers tested (wild yeasts, lactic acid bacteria, enterococci and coliform bacteria). For this reason, the concentration of cells in the beer appeared as a more influential factor in the development of off-flavours, including autolysis, than the storage temperature. The partial results need to be studied in more detail, nevertheless, we felt it was important to publish the revelation of a strong contamination of beers purchased from regular stores for our experiments. At this point it can already be concluded that the low storage temperature did not help to prevent the development of the contamination and the associated sensory deterioration of the beers.

Keywords: storage temperature; yeast autolysis; beer stability

1 Introduction

It is known that beer stability is influenced by many factors, namely: physical, chemical and biological. Biological factors are undoubtedly the biggest threat for the most of the breweries (Liu et al., 2014; Stewart, 2004). Biological factors must be carefully controlled and can be influenced by good hygiene, pasteurization and filtration routines (Aguiar et al., 2022). Biological stability of beer depends on chemical composition of the beer, such as nutrients, alcohol content or concentration of bitter acids (Kulka, 1953). Also physical factors, especially storage temperature, play an important role in the biological stability of beer (Stewart, 2016).

Microbiological contamination, which is caused by microbes such as wild yeasts, bacteria and molds, is usually recognizable by increased turbidity of the beer or by off-flavours (Vaughan et al., 2005). There are two types of microbial contamination – primary and secondary (Boulton & Quain, 2001). In the case of primary contamination, the entire batch is contaminated, usually during fermentation or lagering. Secondary contamination only occurs in beer in a single container, so only one bottle or can is contaminated, not the whole batch. Contamination can be limited with pasteurization process (Aguiar et al., 2022). The effect of temperature on the development of contamination is significant. Optimum growth temperature, together with other factors (such as the chemical composition of the beer), can promote the growth of specific species of microorganisms. Most of the beer contaminants are mesophiles (Vaughan et al., 2005).

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A temperature also effects the non-biological stability of beer. In lower temperatures the level of flavour changes and chemical reaction in beer are slowed down (Jaskula-Goiris et al., 2019; Kaneda et al., 1995).

When all the nutrients are depleted or the alcohol concentration is high, the yeasts undergoes an irreversible process of autolysis (Alexandre & Guilloux-Benatier, 2006; Xu et al., 2014). In beer this happens at the beginning of the post fermentation. This cellular death mechanism is triggered by specific enzymes to eliminate damaged cells from the yeast culture (Vosti & Joslyn, 1954). During autolysis, huge amounts of intracellular material are released into the medium. These are mainly proteins, fatty acids and polysaccharides of various sizes (Alexandre & Guilloux-Benatier, 2006). Hydrolytic enzymes are released from the vacuoles after one months of soaking in the beer. These enzymes degrade the organelles and form holes in the cell membrane. Together with the shrinkage of the cell wall, this leads to the release of intracellular material. This process intensifies over time, leading to an increasing release of substances into the extracellular space (Xu et al., 2014).

Cytoplasmic compounds have damaging influence on the flavour and colloidal stability of beer. Even just 5% autolyzed yeast will seriously affect the sensory quality of the beer, not to mention increase the nutrient content for potential microbiological contamination (Wang et al., 2014). Therefore it is important to regulate yeast autolysis in the brewing industry.

From the point of view of durability it is best to filter and pasteurize the beer and store it at a low temperature (recommended 0–4 °C) (Paternoster et al., 2020), yet the possibility of chill haze occurrence must be kept in mind (Bamforth, 1999). With the boom of microbreweries, which often do not have the technical capacity to pasteurize or filter beer, unfiltered and unpasteurized beers are increasingly appearing on store shelves. Even more emphasis must therefore be placed on the storage temperatures of these beers.

The primary aim of this study was to monitor the effect of storage temperature on yeast autolysis. However, during the course of the experiments, microbial contamination was detected in a number of commercial samples. This finding significantly affected the results and the original objective as well. Therefore, this work was finally designed as a preliminary study to draw the attention of microbreweries in particular to this worrying finding.

2 Materials and Methods

2.1 Samples

Samples of beer were obtained from 5 different mini breweries (1–5). In total 10 different beers were examined – 1 lager (L) and 1 ale (A) from each brewery. All samples are summarized in Table 1. In total 20 bottles of each beer were gathered. Half of the samples (10 bottles of each beer) was stored in dark at a room temperature (at 26 °C which is typical for summer months) and the other half in a fridge (at 8 °C which is the highest recommended temperature for beer storage). Each of the ten weeks the beers were tested by the following methods.

Table 1 *Sample list with input data*

Sample	Beer type	Original Extract [°Plato]	Alc. [% v/v]	Weeks to Best before date
1L	Lager	10	4.0%	8
1A	Ale	10	4.1%	8
2L	Lager	11	4.5%	7
2A	Ale	13	5.0%	7
3L	Lager	10	4.2%	8
3A	Ale	12	5.3%	8
4L	Lager	10	4.0%	7
4A	Ale	10	4.2%	7
5L	Lager	12	5.1%	22
5A	Ale	12	5.1%	24

2.2 Yeast Counting

Yeast count and the ratio of dead cells were determined every week for 10 weeks. After mixing, 100 ml of the sample was taken and degassed on a shaker. The sample was then centrifuged at 4500 g. 1 ml of the pellet was transferred to an Eppendorf tube and centrifuged again at 9000 g. Excess water was aspirated from the centrifuged sample and the pellet was resuspended in 1 ml of distilled water. The sample was transferred to a test tube and made up to 10 ml with distilled water. In the diluted sample the yeast count was determined by Bürker chamber microscopy at 400 × magnification. The ratio of dead yeast cells in the sample was also determined by staining with methylene blue (concentration 0.5 M).

2.3 Determination of Contamination

In the fifth week of the experiment, microbial contamination was determined in the samples stored at 26 °C. 10 ml of each sample was filtrated through sterile nitrocellulose 0.45 µm filters which were then placed on selective agar plate.

Non-Saccharomyces yeasts were determined on the wort agar with iodoacetic acid (186 mg/l) and total count of wild yeasts were determined on the wort agar with copper sulphate (312 mg/l). These agar plates were cultivated at 28 °C for 3 days. Then colony-forming units were counted (Matoulkova et al., 2013).

MRS Agar (Merck) with addition of actidion (25 mg/l) and β-phenylethanol (3 ml/l) was used to determine lactic acid bacteria. Petri dishes were cultivated in anaerobic conditions at 28 °C for 6 days. Then colony-forming units were counted (Matoulková & Kubizniaková, 2015).

Coliform bacteria were determined on the Chromocult Coliform Agar (Merck), where coliform bacteria are stained in dark purple and the *Enterobacteriaceae* family is light. Petri dishes were cultivated at 37 °C for 2 days. Consequently, the colony-forming units were counted (Kubizniaková et al., 2020).

Samples of each beer were also spread on plate with WLN agar (Oxoid). Agar plates were cultivated at 26 °C for 3 days. Following that, the appearance of the colony-forming units was assessed in terms of homogeneity (Matoulkova et al., 2013).

2.4 Chemical Analysis

In addition to the microbiological data, the values of standard beer parameters such as actual residual extract, actual attenuation and alcohol are also important parameters that give information about the beer ageing process or the potential development of microbial contamination. Hence, they were weekly measured using an Anton Paar Alcolyzer Beer ME and Anton Paar DMA 4500 M.

2.5 Sensory Evaluation

To support the analytical and microbiological data, an indicative tasting of the samples was performed every week. The sensory evaluation was carried out by a brewer, an experienced evaluator who is a long-time member of the RIBM sensory panel.

3 Results and Discussion

The data from the first and the last week of the experiment are shown in Table 2. It can be seen that either the gravity, alcohol or attenuation practically did not change in both types of the beers stored at 8 °C. However, the alcohol content of beers stored at 26 °C increased in most

Table 2 *Analytical data measured on the first and the last week of the experiment*

	First week					Last week				
Sample	Extract [°Plato]	Actual Attenuation [%]	Alc. [% v/v]	Cell Conc. [cell/ml]	Dead Cell Ratio [%]	Extract [°Plato]	Actual Attenuation [%]	Alc. [% v/v]	Cell Conc. [cell/ml]	Dead Cell Ratio [%]
1L – 8 °C	4.6	56.0	3.9	7750	33	4.6	56.4	3.9	13000	69
1L – 26 °C	4.6	56.0	3.9	7750	33	4.4	57.9	4.0	58600	31
1A – 8 °C	3.5	67.5	4.8	1625	20	3.5	67.5	4.8	6875	93
1A – 26 °C	3.5	67.5	4.8	1625	20	2.9	73.1	5.1	54675	33
2L – 8 °C	4.2	63.3	4.7	34750	2	4.1	63.9	4.8	453750	84
2L – 26 °C	4.2	63.3	4.7	34750	2	3.7	67.2	5.0	3182000	85
2A – 8 °C	5.0	63.6	5.8	275000	12	5.0	63.6	5.9	87500	89
2A – 26 °C	5.0	63.6	5.8	275000	12	4.0	71.0	6.6	3778500	60
3L – 8 °C	3.9	63.0	4.4	70000	13	3.9	63.2	4.4	55000	53
3L – 26 °C	3.9	63.0	4.4	70000	13	3.9	63.4	4.4	22500	88
3A – 8 °C	4.6	62.8	5.1	4375	26	4.6	62.8	5.1	15750	99
3A – 26 °C	4.6	62.8	5.1	4375	26	4.6	62.8	5.1	9500	95
4L – 8 °C	4.0	60.6	4.1	8375	18	4.0	60.9	4.1	69500	60
4L – 26 °C	4.0	60.6	4.1	8375	18	3.9	61.9	4.2	467500	94
4A – 8 °C	4.1	59.5	4.0	108750	23	4.1	60.2	4.1	10875	95
4A – 26 °C	4.1	59.5	4.0	108750	23	3.9	61.7	4.1	353500	83
5L – 8 °C	4.4	62.6	4.9	800	25	4.5	62.1	4.9	1000	75
5L – 26 °C	4.4	62.6	4.9	800	25	4.5	62.7	5.0	2750	67
5A – 8 °C	4.0	67.9	5.6	375	33	4.0	67.2	5.4	2925	88
5A – 26 °C	4.0	67.9	5.6	375	33	4.0	67.9	5.6	375	75

cases. The highest increase in alcohol was observed in a sample 2A by as much as 0.71%. The changes in alcohol values together with a slight decrease in gravity and increased attenuation indicate that samples 1L, 2L, 1A, 2A and 4A were microbiologically active.

Most samples showed an increase in cell concentration as well as ratio of dead cells at both storage temperatures. The massive increase in cells, particularly in samples 2L (8 and 26 °C), 4L (8 and 26 °C), 2A (26 °C) and 4A (26 °C), suggests along with other aforementioned data the development of wild yeast contamination has occurred (Juvonen et al., 2011).

The dead cell ratio has almost always risen above 70% in the last week. The ratio of dead cells was mostly higher when samples were stored at 8 °C. This statement refers to all samples of ales and two lagers (1L and 5L). The other two lagers (3L and 4L) had a higher percentage of dead cells at 26 °C and the one (2L) showed the value more or less the same for both temperatures.

A rather higher percentage of dead cells at 8 °C could be explained by the development of microbial contamination, which is able to survive in a warmer environment.

Results from the continuously monitored shift in the parameters of beers stored at different temperatures showed 3 scenarios that are presented below. Each scenario is shown in different figures (Figure 1, Figure 2 and Figure 3).

The first scenario of the evolution of the parameters of beer is shown in Figure 1, in this case it is lager 1L (Figure 1a and 1b) and ale 1A (Figure 1c and 1d). It can be seen that the initial concentration of cells in the beer was quite high. The cell concentration then increased over time, indicating the development of microbial contamination. At the same time, the proportion of dead cells was already relatively high in the first week of the experiment (more than 30%). Dead cells, which subsequently autolysed, increased the nutrient content of the beer for potential contaminating microflora (Wang et al., 2014). The presence of contaminating microflora can also be indirectly observed by an increase in alcohol content and a change in attenuation (Juvonen et al., 2011). All these aspects are more evident in beers stored at 26 °C. The effect of temperature is therefore a clear indication of the acceleration of all processes and changes in the finished beer. At the same time, the contaminating microflora benefit more from the higher temperature (Axelsson, 2004; Suzuki, 2015). The beers stored at 26 °C showed an autolysing flavour in the fourth week of the experiment (indicated by the red box). However, the autolysis off-flavour in such contaminated beers may have been 'overwhelmed' by another off-flavour associated with the contamination.

The second scenario is represented by the beers 3L and 3A in Figure 2. It is a type of beer with high initial concentration of yeast cells but without any indicators of microbial contamination. The high cell concentration allowed to manifest the autolysis off-flavour in the fifth week of the experiment in case of beers stored at 26 °C (highlighted in red) and in the fourth week in case of the beers stored at 8 °C (high-

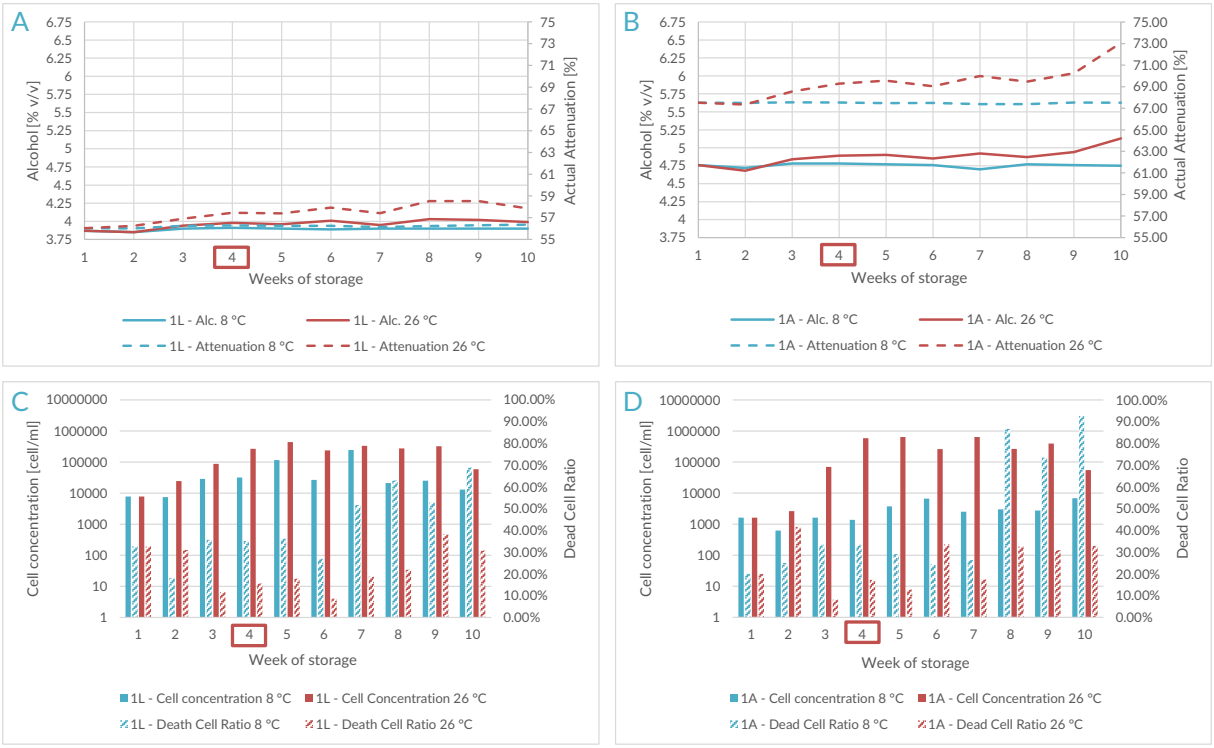

Figure 1 *Changes in alcohol content and attenuation during 10 weeks of experiment in beers 1L and 1A (A, B). Changes of cell concentration and dead cell ratio during 10 weeks of experiment in beers 1L and 1A (C, D).*

lighted in blue). Absence of contamination also contributed to the development of autolysis off-flavour in means of not overwhelming it. Very low or zero level of contamination can be deducted from zero changes in the attenuation and alcohol content during the whole duration of the experiment.

no changes in the alcohol content nor attenuation, thus it is expected that the beers were without any contamination.

Microbial contamination was also determined on the fifth week of the experiment using selective agar media and filtration method of beer sampling. The results are

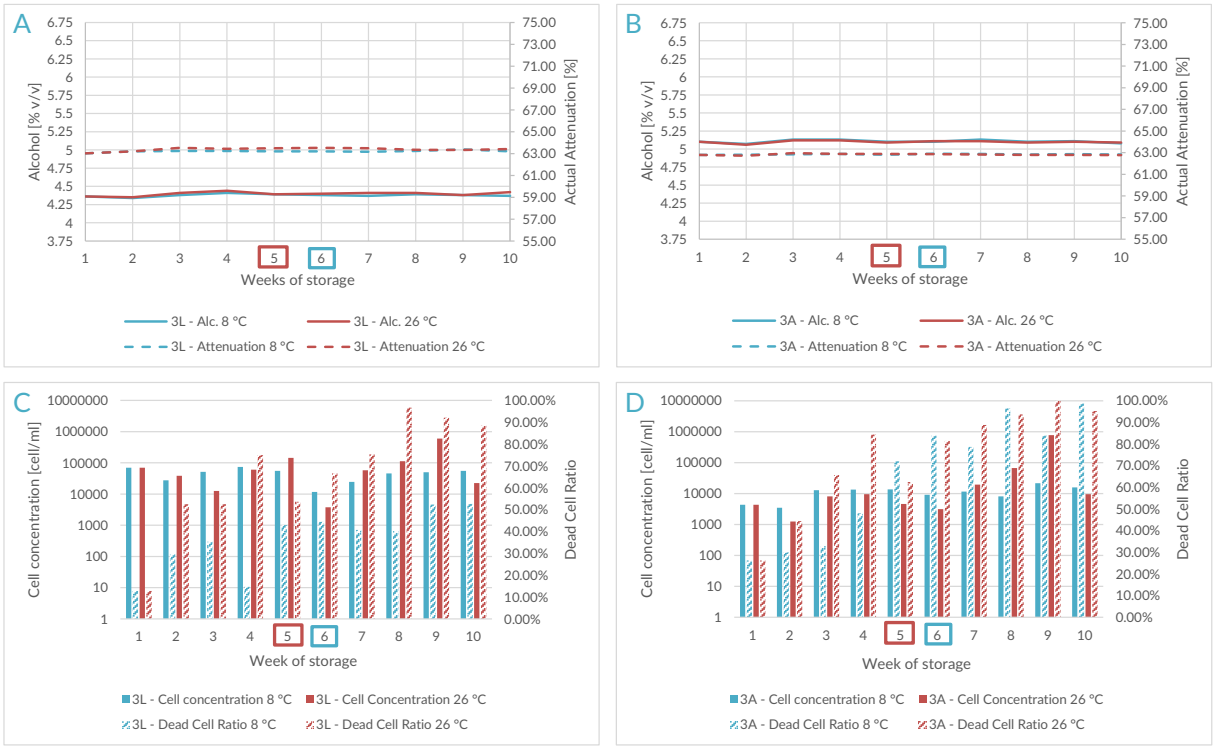

Figure 2 *Changes in alcohol content and attenuation during 10 weeks of experiment in beers 3L and 3A (A, B). Changes of cell concentration and dead cell ratio during 10 weeks of experiment in beers 3L and 3A (C, D).*

The third scenario is shown in Figure 3. In this case it is beer 5L and beer 5A. The concentration of cells had been very low from the beginning. Therefore, even in the last week of the experiment the autolysis off-flavour was not notable even when 100% cells were dead. There were also

shown in Table 3. Each determination was always carried out in 10 ml of beer. In some samples, the number of colony forming units (CFU) was greater than 300, this is listed as UC in the table. In one beer (1L) even the presence of *E. coli* was detected.

Table 3 *Results of contamination determination in 10 ml of beer on selective agar plates (samples were stored 5 weeks at 26 °C)*

Sample	*Enterobacteriaceae*/ Coliform Bacteria [CFU/10 ml]	Non-Saccharomyces [CFU/10 ml]	Wild Yeasts [CFU/10 ml]	WLN Agar	Lactic Acid Bacteria [CFU/10 ml]
1L	UC/UC (E.coli)	240	UC	Homogeneus	UC
1A	UC/UC	UC	250	Homogeneus	UC
2L	120/1	UC	UC	Inhomogeneus	UC
2A	UC/UC	UC	UC	Homogeneus	UC
3L	160/2	280	500	Homogeneus	20
3A	UC/UC	23	205	0	10
4L	UC/UC	UC	UC	Inhomogeneus	40
4A	UC/11	4	39	Inhomogeneus	50
5L	3/0	40	18	0	0
5A	5/0	25	38	0	0

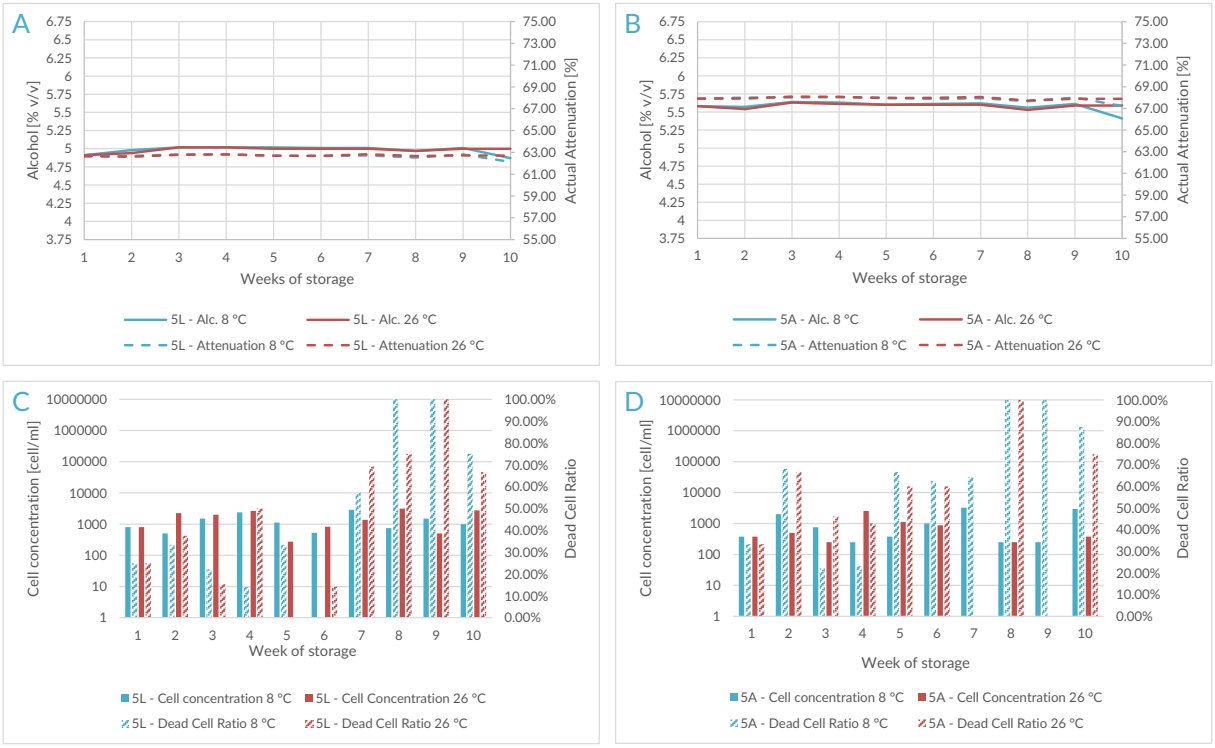

Figure 3 *Changes in alcohol content and attenuation during 10 weeks of experiment in beers 5L and 5A (A, B). Changes of cell concentration and dead cell ratio during 10 weeks of experiment in beers 5L and 5A (C, D).*

The original intention of this study was to observe how unfiltered and unpasteurized beers from microbreweries purchased in a regular retailer behave during storage in a commercial chain. The breweries have no chance to influence the storage conditions when the final product leaves the brewery. This study was designed to give them an idea of the condition in which the beer can leave the shop and reach the customer before the expiry date.

Thus two types of the most commonly produced beers, i.e. lagers and ales, were tested. These commercial samples were stored at two temperatures, which were carefully chosen. There is no doubt that the beer should be stored at a low temperature (Aguiar et al., 2022) and therefore a value of 8 °C has been chosen, which is slightly higher than the highest recommended temperature for storing beer (Baert et al., 2012). On the other hand, beers in chain stores are often placed in a warehouse without controlled temperature, so the second chosen temperature was 26 °C, which is a representative temperature for summer months in the Czech Republic. The aim of the study was to demonstrate, how autolysis of the present yeasts causes a change/damage of the sensory properties of beers.

The preliminary results showed a high proportion of dead cells and an emerging autolysis off-flavour at both tested temperatures. The monitored parameters reached non-standard values and it was not possible to give the brewers a reliable insight into the development of autolysis off-flavour. For example, even at the low temperature of 8 °C, high cell concentrations and dead cell ratios were found in some samples and the observed autolysis off-flavour could not be detected due to other off-flavours. All these indicators led to a serious revelation, namely that the beers were entering the market chains microbiologically unclean. The presence of wild yeasts along with lactic acid bacteria is alarming, but even enterococci and coliforms including *E. coli* in one sample were found in a random selection of 10 beers from 5 Czech microbreweries. Table 3 shows the microbiological condition of the beer samples in fifth week of storage at 26 °C. Some beers (1L, 1A, 2L, 2A, 4L) showed massive development of contamination, which may be related to the high yeast cell input. Contaminating microorganisms may use autolysed cells as a substrate. Such massive contamination affects the sensory quality of the beer. In the case of beers 3L, 3A and 4A, contamination did develop, but not to such an extent. It can therefore be assumed that the sensory stability of the beer was not significantly affected in terms of microbial contamination. In beers 5L and 5A the contamination hardly developed, which is probably related to the very low input cell count considering non-pasteurized and non-filtered beer. The results of yeast homogeneity determination on WLN soil indicate a relatively frequent cross-contamination (3 beers out of 10).

4 Conclusion

This study investigated the effect of beer storage temperature on autolysis off-flavour on 10 different beers purchased from a common shop. It was found that the death and development of autolysis off-flavour occurred at both low (8 °C) and higher (26 °C) storage temperatures. Autolysis off-flavour development occurred with a difference of one week. However, a reliable study of the development of autolysis off-flavour had to be postponed because of the serious contamination of the samples. A partial conclusion of this preliminary study is that the development of this undesirable flavour is much more affected by the number of cells in the beer, which could be influenced by filtering the beer or simply by the correct removal of the yeast during the lagering. A low cell concentration also indirectly reduced the risk of microbial contamination and the development of other off-flavours. The presented results indicate that in case of strong contamination of beers leaving the brewery, even low storage temperature does not prevent its massive development.

The findings of this preliminary study will be further examined and described in more detail including a statistical data evaluation. First of all, the research will focus on the problem of contamination of beers available in a common store. The second part will devote to the development of the autolysis off-flavour in unpasteurised and unfiltered beers.

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