
So gut kann Bier schmecken.
Bitte ein Bit
Bitburger
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Bitburger
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Research over the year and across disciplines

One of my first tasks at the beginning of the New Year is to write the foreword for the BrewingScience Yearbook, which is a review of the papers published over the previous year. It provides me with an excellent opportunity to take stock of the numerous works issued and the topics covered in the periodical at my leisure. And every year, the broad range of research carried out in the fields of science related to brewing never ceases to astound me.

Aside from the more conventional subjects of barley, malt and hops as well as of wort and beer quality, some novel areas of research emerged this year, including the implementation of artificial intelligence and machine learning in the process of kilning hops, the substitution of malt with residual ingredients from the baking industry, the impact of fermentation conditions on ethanol production using exotic “ca na” fruit, and much more.

However, a classic research topic was distinguished at the beginning of April 2023 with the Ludwig Narziss Prize in Brewing Science. At the 15th Trends in Brewing in Ghent, Belgium, this accolade for research, which was published in the journal BrewingScience in the year 2022 and has been deemed highly relevant to brewery practice, was awarded to Dr. Carsten Zufall and his co-authors Laura De Oliveira, Isabella Mendoza and Carlos de Lima of Cerveceria Polar in Venezuela. The title of their paper was “The impact of long-term pitching yeast storage on viability and fermentation performance” (BrewingScience 75 (2022), no. 9/10, pp. 92–97). The focus of this research was to identify under which lagering conditions the pitching yeast exhibited maximum viability (ratio of living/dead cells) and vitality (fermentation performance) – a very practical determination of the highest economic relevance, especially for breweries which, for whatever reason, must temporarily suspend operations for an indefinite period of time.

And now for your reading enjoyment, we present the BrewingScience Yearbook 2023.

L. Junkersfeld

(from left) Dr. L. Junkersfeld, Dr. C. Zufall and Prof. G. Aerts; Dr. C. Zufall receiving the Ludwig Narziß Award in Ghent

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A. Dymchenko, M. Geršl and T. Gregor

Brewing beer using bakery leftovers as a substitute for malt

The present study aims at the sustainable use of bakery leftovers and their application as a partial substitute for malt in beer production. We made seven samples of Lager beer where we substituted malt with up to 60 % of bread, three samples of Brown Ale with up to 50 % of bread, and four samples of Porter with up to 80 % of bread. We made a sensory evaluation of the finished beer and determined its chemical composition. Water plays an important role in beer quality, especially in the sensory profile. We did a chemical analysis of the water to compare its chemical profile with that of the brewed beer. Samples with bread that substituted malt up to 30 % showed satisfactory results in the total rating of sensory evaluation. The results of chemical measurement show that the more bread, the saltier the taste of the beer due to the increased Na and Cl- indicators. Therefore, we suggest not using more than 30 % of bread because the salty taste can be more distinct. We noticed that the quantity of K depends on the type of fermentation and the availability of bread decreases the quantity of the element. We also measured foam stability and beer colour; the results serve as indicators of beer quality. We found that the beer colour and the foam quality in top-fermented beers are not affected by bread. We calculated that 30 % substitution with bakery leftovers could save around 6 – 7.5 kg of barley per 1 hl. Brewers can collect bread leftovers from local supermarkets, cafeterias, etc.

Descriptors: beer, malt substitutes, bakery leftovers, food waste

1. Introduction

Nowadays, the world encounters the problem of a large quantity of food waste that disappears in vain, although we could use them to create new products. According to the Global Initiative on Food Loss and Waste Reduction, around 1.3 billion tonnes of food are lost or wasted annually [1]. In this research, we focused on bakery waste, which makes up 18 % of the total amount of food waste [2]. The purpose of the study was to decrease the number of bakery leftovers using beer production technology and explore this process from a scientific point of view. We collected bakery leftovers free of food-based foreign material from the local supermarkets in the Czech Republic and used them as a partial substitute for malt during beer production. The used bread did not contain seeds, herbs or other additives.

We chose beer because it is one of the most-consumed alcoholic beverages in the world. In 2019, 1.91 billion hectolitres of beer were produced worldwide [3]. In 2018, Europe produced over 405 million hectolitres of beer [4]. Bakery leftovers can partially replace barley due to similar chemical composition [5, 6]. This substitution also decreases the quantity of malt needed for beer production. Around 20 – 25 kg of barley is needed to produce 1 hl of the wort. If we recalculate the efficiency of using bread leftovers instead of barley, in a ratio of 30 : 70, we will eventually save 30 % of barley – that is 6 – 7.5 kg/hl. For this purpose, we need about 10 – 11.6 kg of fresh bread per hl that can be dried before brewing. Some eco-breweries already use up to 30 % of bread to substitute the malt; examples include Toast Ale (UK), Crumbs Brewing (UK), Instock (NL), Brussels Beer Project (BE) and Troggeling (BE). In the present study, we attempted to substitute more malt with bread and test how it would affect beer properties. Production of beer from bread leftovers has ecological savings in the category of global warming, which is the result of replacing malted barley with bread leftovers [7]. The carbon footprint of an 800 g loaf of bread ranges from 977 to 1,244 g CO_2 equivalent [8]. As every 800 g loaf of bread produces around 100 l of biogas (60 – 65 % is methane and 35 – 40 % is CO_2), it is possible to use bread to create products based on fermentation [9].

There is a report of using bread waste for ethanol production with the help of hydrolytic enzymes isolated from *Hymenobacter* sp. CKS3 strain. Waste bread hydrolysate contained 19.89 g/l of reducing sugars and was used with waste baker's yeast to obtain 1.73 % of ethanol [10]. A previous study showed that 1 g of waste bread could generate 0.332 g of glucose [11], which in beer production will be used by yeasts to produce ethanol and CO_2.

We produced bottom-fermented Czech Lager beer, which is a high-bitterness beer that has a hoppy and malty character and a fruity aroma [12]. Also, we made top-fermented beer, Brown Ale and Porter, using special malts. To evaluate beer quality, we

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conducted a sensory panel and physical measurement of foam stability and beer colour.

Beer foam forms not only by the action of carbon dioxide but also due to proteins which accumulate in the foam. Furthermore, protein characteristics and origin affect foam properties [13]. Proteins with lower surface hydrophobicity, such as Z protein [14], play an essential role in foam formation. Another investigation showed that lipid transfer protein 1 (LTP1) is also associated with foam quality [15], as it is binding lipids that harm foam stability. High-gravity brewing induces greater losses of LTP1 [16] and denaturing of LTP1 reduces its ability to bind lipids; thus, the content of this protein is crucial for foam stability [17]. The quality of the foam depends on the choice of malt as malt is the source of LTP, but this does not negate other factors that affect the foam's properties. Iso-α-acids extracted from hops are factors for light stability and beer foam as they strengthen the bubble film [18, 19]. Therefore, a small addition of hydrogenated iso-α-acid hop can optimise foam quality [15]. Non-toxic metal cations in beer have a positive effect on foam stability [20], while lipids, basic amino acids and high levels of ethanol destabilise beer foam [18]. Brewers use sorbents to stabilise the foam without affecting the beer's colour, bitterness, or sensory profile. These sorbents are activated carbon, Florisil and polytetrafluoroethylene [21]. Another option to increase foam stability is adding wheat malt to the beer as the malt is higher in protein and starch content compared with barley malt. The foam skeleton could be provided by barley malt proteins, while the low molecular weight proteins in wheat malt could be more scattered in this skeleton. In cooperation, barley malt and wheat malt can contribute to the cloudy foam [13].

We also investigated the chemical composition of the brewed beer and the water we used for beer production. The chemical composition helps to determine the number of trace elements that can affect the taste differences between conventional beer and beer made with the addition of bread.

Water is the primary ingredient of beer and contains minerals necessary for human health. In addition, these microelements influence the taste and colour of the beer. The maximum permissible level of nitrates in water is 50 mg/l; for nitrites, it is 0,5 mg/l [22]. Alkalinity is the primary buffering system in water and exists in the form of carbonate activity. The carbonate system includes carbonate ions (CO_3^{2-}-) at high pH levels, bicarbonate ions (HCO_3-) at medium pH levels and carbonic acid (H_2CO_3) at low pH levels. H_2CO_3 with dissolved carbon dioxide (CO_2) equivalent made chemical equilibrium [23]. High alkalinity gives a bitter taste to water [24] and can buffer the pH of dark beer styles, preventing them from tasting acrid [23]. Low levels of alkalinity (< 50 ppm) prevent the beer from tasting watery [23].

Malt is another ingredient in brewing; it can provide minerals such as magnesium (Mg) and potassium (K). Depending on their concentrations, they can activate or inhibit enzymes during beer production. Zinc (Zn) and iron (Fe) are the main microelements for yeast activity [25]. Calcium (Ca) removes oxalates and plays a role in yeast flocculation [26, 27]. In addition, calcium and magnesium affect the bitterness and pH of the beer. There is a report that metal ions mostly originate from malt [28].

Table 1 Parameters of Lager beer

Alcohol (% vol.)	IBU (Bitterness)	SRM (Colour)
4.5	21.19	4

Table 2 The ratio of bread to malt for Lager beer

Bread to malt ratio	Czech Pale Malt (g)	Undried bread (g)
00:100	3 124	–
10:90	2 811	520
20:80	2 499	1 040
30:70	2 187	1 561
40:60	1 874	2 081
50:50	1 562	2 603
60:40	1 249	3 123

Table 3 Hops quantity for Lager beer

Hops	Grammes	Alpha Acids (%)	Boil Time	Type
Sladek	9	7	90	Pellet
Sladek	11	7	45	Pellet
Saaz	30	4	–	Pellet

When the brew production was finished, we used the malt mash as a fertiliser for plants. In addition, some companies found more ways to use brewery by-products: Nutrinsic transforms brewery wastewater, waste beer, spent grain and yeast into fish food, Great Lakes Brewing Company uses beer by-products as fertilisers for plants and Marmite Ltd makes a Marmite spread from the brewer's yeast. In addition, brewer's spent grain, hot trub and residual brewer's yeast are used to cultivate lactic acid bacteria [29]. Spent grain is mostly used as a feedstuff for domestic animals.

Table 4 Parameters of Porter

Alcohol (% vol.)	IBU (Bitterness)	SRM (Colour)
4.5	33.31	40

Table 5 The ratio of bread to malt for Porter

Bread to malt ratio	Pale Ale (g)	Roasted chocolate wheat (g)	Brown Malt (g)	Dry bread (g)
00:100	1 328	78	156	–
30:70	859	78	156	468
50:50	547	78	156	781
80:20	–	78	156	1 249

Table 6 Hops quantity for Porter

Hops	Grammes	Alpha Acids (%)	Boil Time	Type
Chinook	11	13	70	Pellet
Chinook	9	13		Pellet

2. Material and Methods

2.1. Materials

For brewing beer, we used water from the tap. We did a chemical analysis (see section Results and Discussion) that showed compliance with parameters set by the limits for drinking water in Decree No. 252/2004 Coll. of the Czech Republic.

We produced three different beer styles with the use of bread (Tables 1 to 9) and three control samples without the use of bread.

We used bottom-fermenting yeast Saflager W 34/70 (Lager) and top-fermenting yeasts US-5 (Porter) and US Ale 01 (Brown Ale).

Bread without other food-based foreign material and additives (seeds, herbals, etc.) was collected from supermarkets. We used undried bread for Lager beer and Brown Ale because of environmental savings and the need to keep the beer light.

We converted undried bread into the dry matter following this formula:

- Bread contains approximately 40 % of water and 60 % of dry matter.
- For example, the bread-to-malt ratio is 10 : 90.
- The total quantity of malt is 3,124 g per 20 litres of beer for the resulting 4.5 % vol. alc.
- 3,124 x 90 % = 2,811.6 g (malt)
- 3,124 x 10 % = 312.4 g (dry bread)
- Dry bread / 60 % of dry matter = undried bread
- 312.4 / 60 % = 520.6 g (undried bread)

2.1.1. Lager beer

Parameters of Lager beer, the ratio of bread to malt and hops quantity are shown in tables 1 to 3.

2.1.2. Porter

Parameters of Porter, the ratio of bread to malt, and hops quantity are shown in tables 4 to 6.

2.1.3. Brown Ale

Parameters of Brown Ale, the ratio of bread to malt, and hops quantity are shown in tables 7 to 9.

Table 7 Parameters of Brown Ale

Alcohol (% vol.)	IBU (Bitterness)	SRM (Colour)
4.5	21.19	24

Table 8 The ratio of bread to malt for Brown Ale

Bread to malt ratio	Finest Pale Ale Maris Otter (g)	Red Active (g)	Crystal Mahogany (g)	Undried bread (g)
00:100	896	416	250	–
30:70	427	416	250	781
50:50	115	416	250	1 301

Table 9 Hops quantity for Brown Ale

Hops	Grammes	Alpha Acids (%)	Boil Time	Type
Bramling Cross	13	6.5	70	Pellet
Bramling Cross	20	6.5		Pellet

2.2. Workflow

One batch: Lager – 20 l, Porter – 10 l, Brown Ale – 10 l

Stirring/Mashing: Boil 6 l (11 l for Lager) of water to 40 °C. Add milled malt (%) and bread (%), mix it and boil for 10 min. Increase the temperature of the boil to 52 °C for 10 min, to 62 °C for 30 min and 72 °C for 30 min. Run an iodine test. Increase the temperature of the boil to 85 °C for 5 min.

Filtration/Lautering: Filter the mash through the sieve. Pour 10 l (16 l for Lager) of water (85 °C) into the filtrating mash and allow resting for 15 min.

Wort boiling/Hopping: Boil the wort to 100 °C. Add hops and boil for 70 min (90 min for Lager). Allow resting for 20 min.

Filtration: Filter the wort through a small sieve.

Cooling down: Cool the wort down to 18 – 20 °C (12 °C for Lager). Add yeast and do aeration.

First fermentation: Fermentation runs at 18 – 22 °C (12 °C for Lager) roughly for 14 days.

Second fermentation: Transfer beer to bottles and add glucose (2 g/l). Fermentation runs at 12 °C for 14 days (Lager, Brown Ale, Porter). Then Lager must ferment for more than 20 days at 4 °C.

2.3. Analysis

Sensory evaluation of the beer: The sensory properties of beer were evaluated in compliance with ČSN ISO 6658 [30] by ten panellists (f = 2, m = 8, range = 20 – 46 years). The beer panel was performed in a tasting room at 18 degrees Celsius. Before the tasting session, beer samples were cooled down to 7 °C. Beer (150 ml) was poured into clear colourless glasses. We used cheese and pastries as flavour neutralisers. The order of beer samples was determined to start with a control sample without the use of bread until the sample with 80 % of bread was processed. In one series, we tested seven samples of Lager beer. After a few months, we tested three samples of Brown Ale and four Porter. The panellists filled in the sensory evaluation form, which had the following fields: flavour, foreign flavour, taste (fullness and saturation), foreign taste, bitterness and overall impression. The evaluation scale was one to five, where one was Absence and five was Very strong. The overall impression was rated on a scale

Table 10 Methods of analysis

Parameters	Methods of analysis
pH (25 °C)	Electrometric method, TFA 31.3001.06 – pH CHECK
Cl-	HPLC method, Dionex ICS-2000 Ion Chromatography System (ICS-2000, Thermo Fisher Scientific, U.S.) with IonPac® AS 18 analytical (2 x 250 mm) column
SO_4^{2-}	
ANC 4.5	Titration method (0.1 N hydrochloric acid)
ANC 8.3	Titration method
HCO_3^-	Calculation method, results of (ANC4.5) and alkalinity
CO_3^{2-}	
Ca	ICP-MS Agilent Technologies 7700 (Agilent Technologies Inc., U.S.)
K	
Mg	
Na	

from one to five, with one being Excellent and five being Very bad.

Physical parameters: Measurement of beer colour by spectrophotometry: beer sample was poured into a 1 cm thick cuvette; the cuvette was rinsed several times with the same sample of beer. The measurement length is 450 nm [31] for the Standard Reference Method (SRM) and The European Brewery Convention (EBC) [32].

$$SRM = 12.7 \times D \times A_{450}$$

$$EBC = 25 \times D \times A_{450}$$

Where D is the dilution factor (D = 1 for undiluted samples, D = 2 for 1: 1 dilution, etc.), A_{450} = light absorbance at 450 nanometres.

Foam stability measurement: Pour 150 ml of beer into a clear glass; hold the glass at a 45° angle; when the glass is half full, bring the glass at a 90° angle and proceed to pour beer in the middle of the glass; foam height and its disappearance time are measured with the help of a ruler and stopwatch.

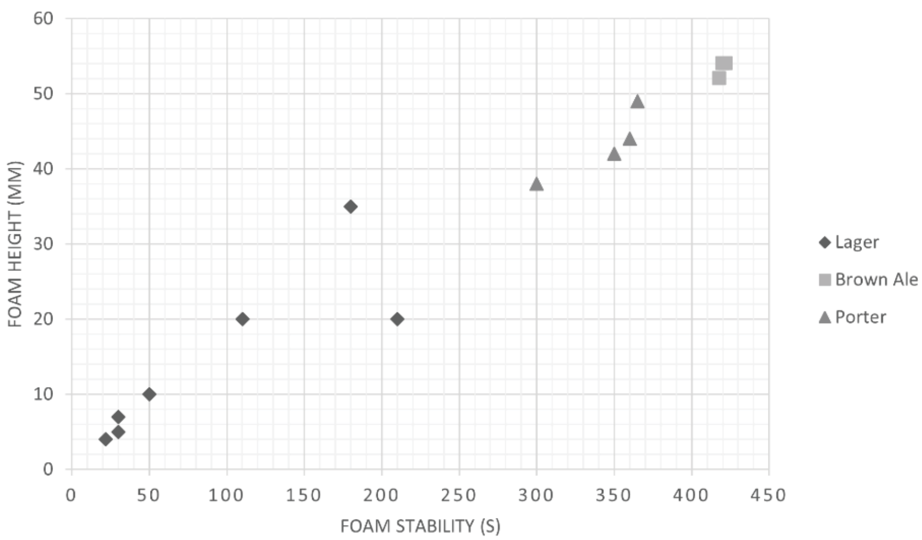

Fig. 1 Foam height and stability of Lager (0 : 100, 20 : 80, 30 : 70, 40 : 60, 50 : 50, 60 : 40), Brown Ale (00 : 100, 30 : 70, 50 : 50), Porter (0 : 100, 30 : 70, 50 : 50, 80 : 20)

Chemical analysis: We conducted a chemical analysis of beer samples and brewing water (Table 10).

3. Results and Discussion

3.1 Foam height and stability measurements

We carried out measurements of foam height and stability (Fig. 1), as both of them are indicators of beer quality. The foam is created by carbonation that makes beer so refreshing beverage. We added glucose to the bottles after draining the liquid from the fermenter to provide carbonation to the beer. Beer foam has a great effect on the flavour, expanding its spectrum. Bubbles play an important role in beer stability; the smaller the bubbles, the more stable the beer.

The Lager beer foam was mostly weak; the problem can be in bottles with caps not twisted tightly enough during the second fermentation. In addition, the Lager samples were six months old, which may have affected the quantity of foam and CO_2 in the beer. When we did a sensory evaluation of Lager after fermentation, the foam was not as strong as in top-fermented beers. The stability and height of the top-fermented beer foam showed good quality; both of them were at the level of store-bought beer. Porter and Brown Ale had a creamy wet foam and retained a foamy head for 3 – 4 min, showing robustness without disproportionation. There is a report that the dark style of beer has much more stable foam and initial foam height compared to light beer [33], which is consistent with our results.

Min. foam height of Lager (10 : 90) was 4 mm with a stability of 22 s; max. foam height of Lager (60 : 40) was 35 mm with a stability of 180 s. Min. foam height of Brown Ale (30 : 70) was 52 mm with a stability of 418 s; max. foam height of Brown Ale (00 : 100) was 54 mm with a stability of 422 s. Min. foam height of Porter (80 : 20) was 38 mm with the stability of 300 s; max. foam height of Porter (50 : 50) was 49 mm with a stability of 365 s. The addition of bread did not affect the formation of foam in top-fermented and bottom-fermented beers. There was a study where foam stability was determined in 16 samples of Lager beers (ten domestic and six from Germany, the Czech Republic, Denmark, and Holland) without the use of bread. The first sample had no foam; the other 15 samples had foam heights from 4.8 mm to 48 mm. The time for the foam to disappear was from 20 s to 300 s [34], which is similar to our results with the use of bread. However, our top-fermented beers had higher foam height and stability. We can suggest that it is due to using several types of malt except for Lager where one type of malt was used. Malt proteins play an important role in foam formation as they create foam skeletons [13]. Protein-protein linkages stabilize the foam and are responsible for mouthfeel and flavour stability. During the malting, malt proteins

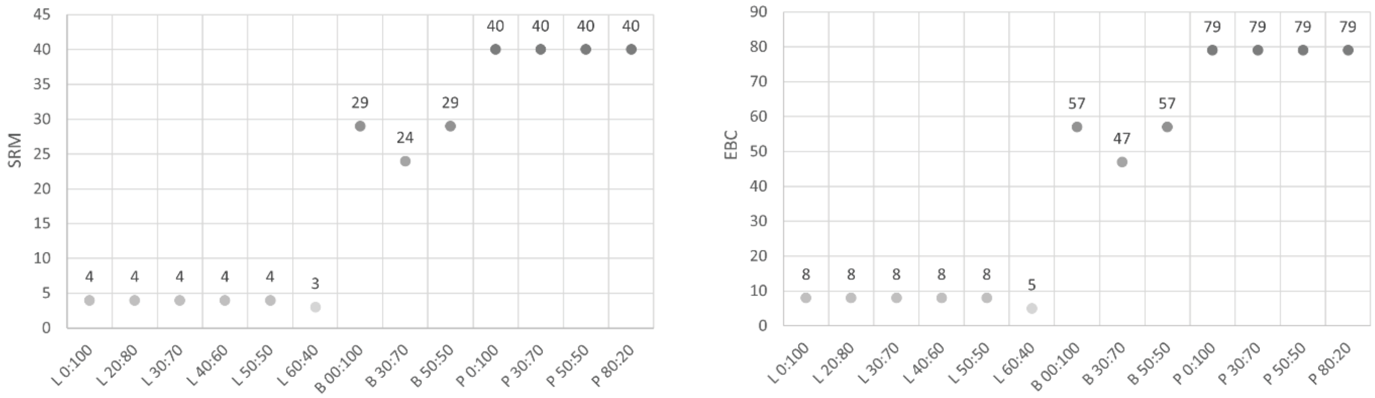

Fig. 2 SRM and EBC analysis of Lager (0 : 100, 20 : 80, 30 : 70, 40 : 60, 50 : 50, 60 : 40), Brown Ale (00 : 100, 30 : 70, 50 : 50), Porter (0 : 100, 30 : 70, 50 : 50, 80 : 20)

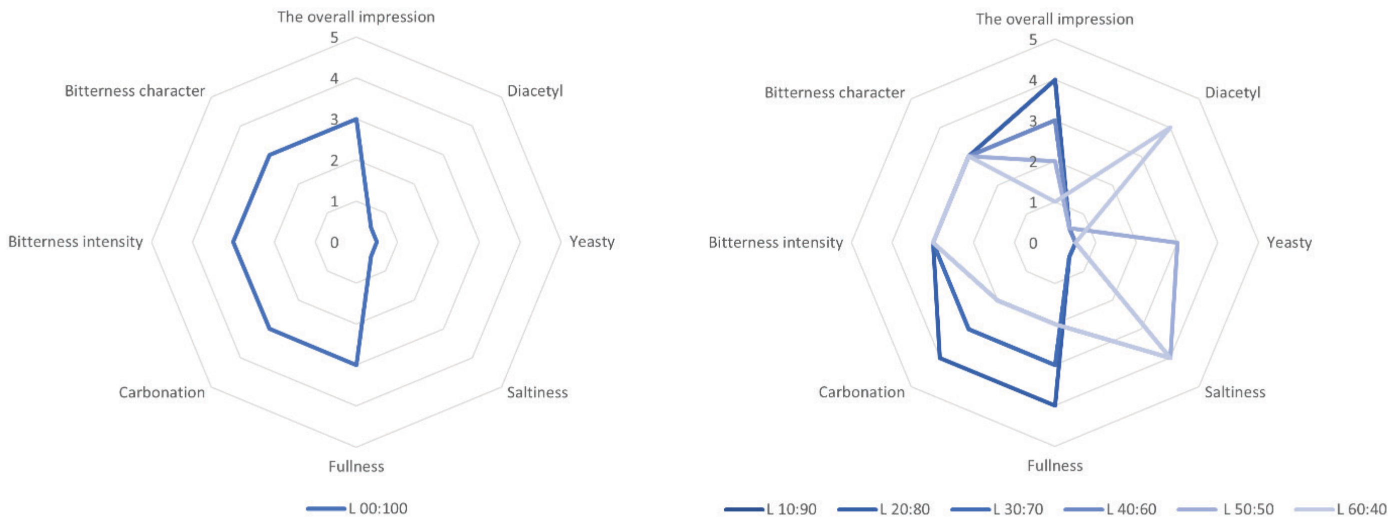

Fig. 3 Sensory profile of Lager without the use of bread (L 00 : 100) and with the use of bread (L 10 : 90, L 20 : 80, L 30 : 70, L 40 : 60, L 50 : 50, L 60 : 40)

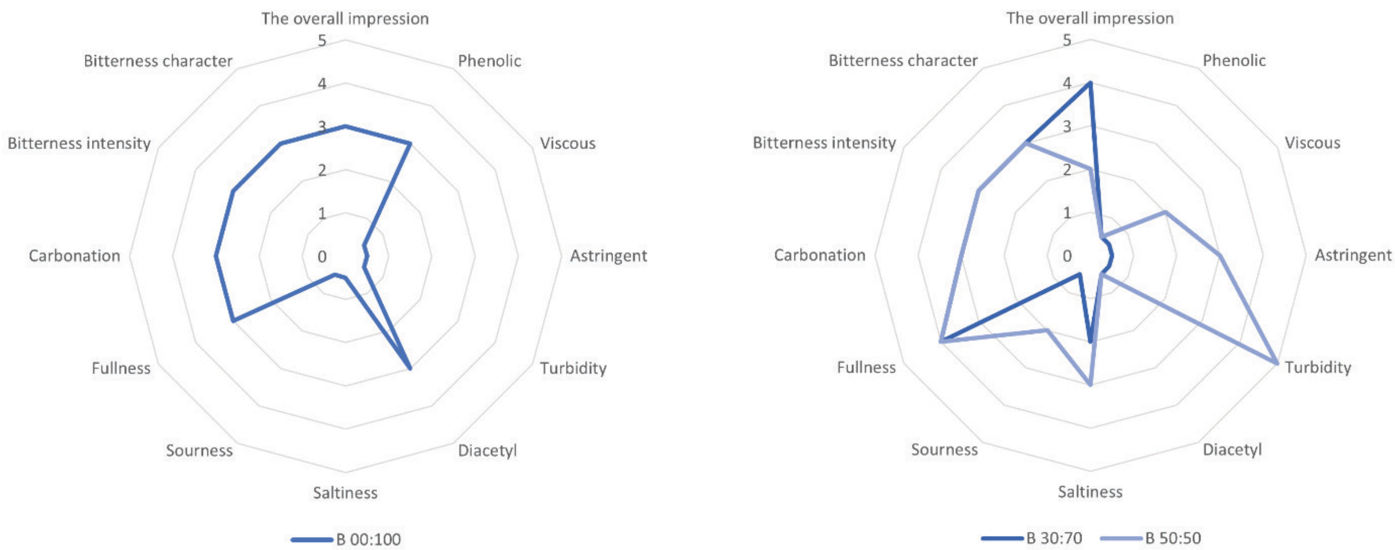

Fig. 4 Sensory profile of Brown Ale without the use of bread (B 00 : 100) and with the use of bread (B 30 : 70, B 50 : 50)

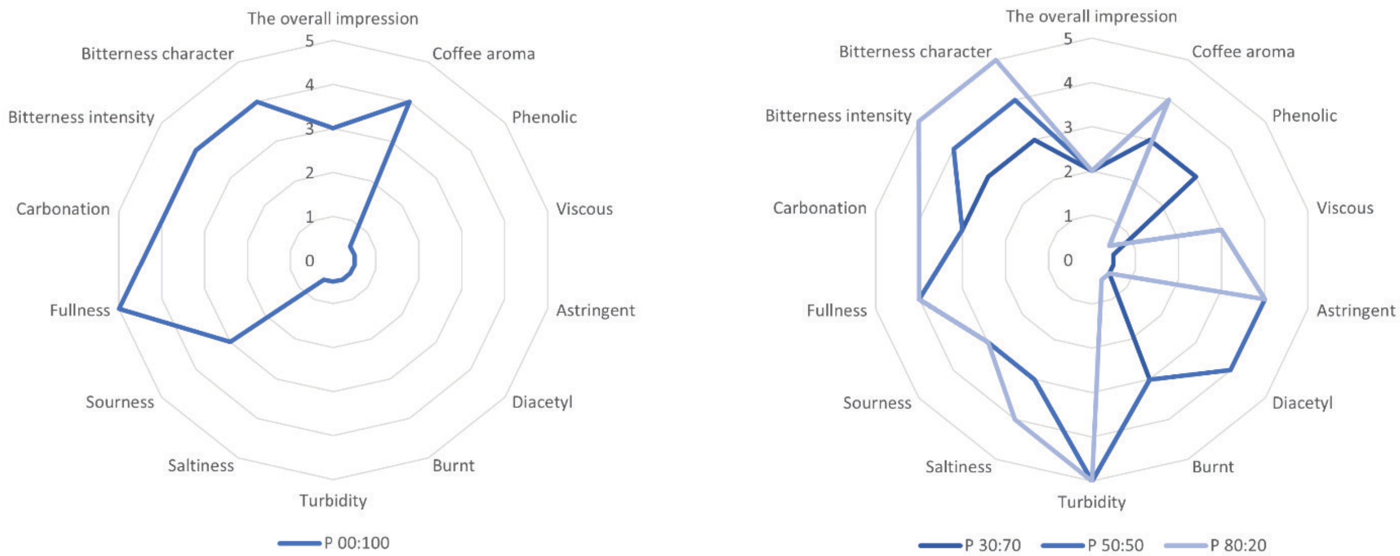

Fig. 5 Sensory profile of Porter without the use of bread (P 00 : 100) and with the use of bread (P 30 : 70, P 50 : 50, P 80 : 20)

break down into smaller peptides by proteases which contributes to foam formation. The more hydrophobic polypeptides, the bigger activity of the substances, such as hordeins that are rich in proline and glutamine content. Foams from albumins, however, are more stable than those from hordeins because albumins can withstand the presence of ethanol. For both albumins and hordeins, the foam stability can be increased by bitter acids contained in hops [35].

There is a report that a low pH level prevents the transfer of negative foam substances to the next production stages of the brewing process [21]. However, we did pH measurements just for the final beer; therefore, we cannot say there was any pH problem in the earlier brewing stages.

3.2 Beer colour measurements

The various styles of beer differ not only in the quantity of alcohol or the use of different malts and additives; indeed, colour is one of the important factors to discern beer styles. The colour of beer can come from malt, hops and other additives often used in craft beer production.

After measuring the colour of beer by SRM and EBC methodology (Fig. 2), we obtained the following results:

All samples of Lager responded to the typical colour of Lager (4 SRM, 8 EBC), except the sample with 60 % of bread, which was a little lighter (3 SRM, 5 EBC) than the other samples. The bread did not affect beer clarity.

One sample of Brown Ale (30:70) had a colour (24 SRM and 47 EBC) typical of this style. Two other samples had slightly darker colours: 29 SRM and 57 EBC. In addition, the samples featured turbidity.

Porter had intense black colour (40 SRM, 79 EBC) and it was not clear. Consequently, we suggest using less roasted chocolate wheat malt for Porter.

3.3 Sensory evaluation of the beer

Sensory analysis plays an important role in assessing the quality of beer, especially the taste component. This can help improve the recipe for subsequent brewing sessions as well as change brewing techniques or fermentation conditions if needed. The main parameters in the sensory analysis are fullness, carbonation, bitterness and aroma. It is also very important to identify unusual tastes and aromas in beer to understand the cause of the defects.

We provided the sensory evaluation and counted the average result for all beer samples. Figures 3 – 5 show the comparison of control samples of beer with samples in which bread was used. This will help determine whether the presence of bread affects the sensory profile of the beer relative to the control samples. As we can see (Fig. 3), the higher the quantity of bread, the weaker the fullness of Lager beer. There are also some taste defects such as saltiness and others. The salty taste comes from bread; the more bread is present in the beer, the saltier the bread is. The overall impression was good for beers with up to 30 % of malt substitution.

A yeasty taste may be present due to unfermented beer. The beer was not filtered after fermentation. After draining, part of the yeast entered the bottle and remained for fermentation.

Top-fermented beer is more acceptable for using bread as a substitute for malt. However, there is still an undesirable salty taste.

Diacetyl, which we detected in beer, has a buttery flavour produced by aldehydes and ketones during fermentation [22]. In addition, this off-flavour may come from bread.

Phenolic acid in wheat [5] can cause a phenolic taste in a few samples of top-fermented beers. Malt can contribute to around 95 % and 86 % of the phenolic compounds in dark and pale beers [36]. Roasted malt and high mashing temperature at low pH can induce the release of phenolic compounds [37]. Furthermore, the availability of phenolic acids can make beer astringent and bitter

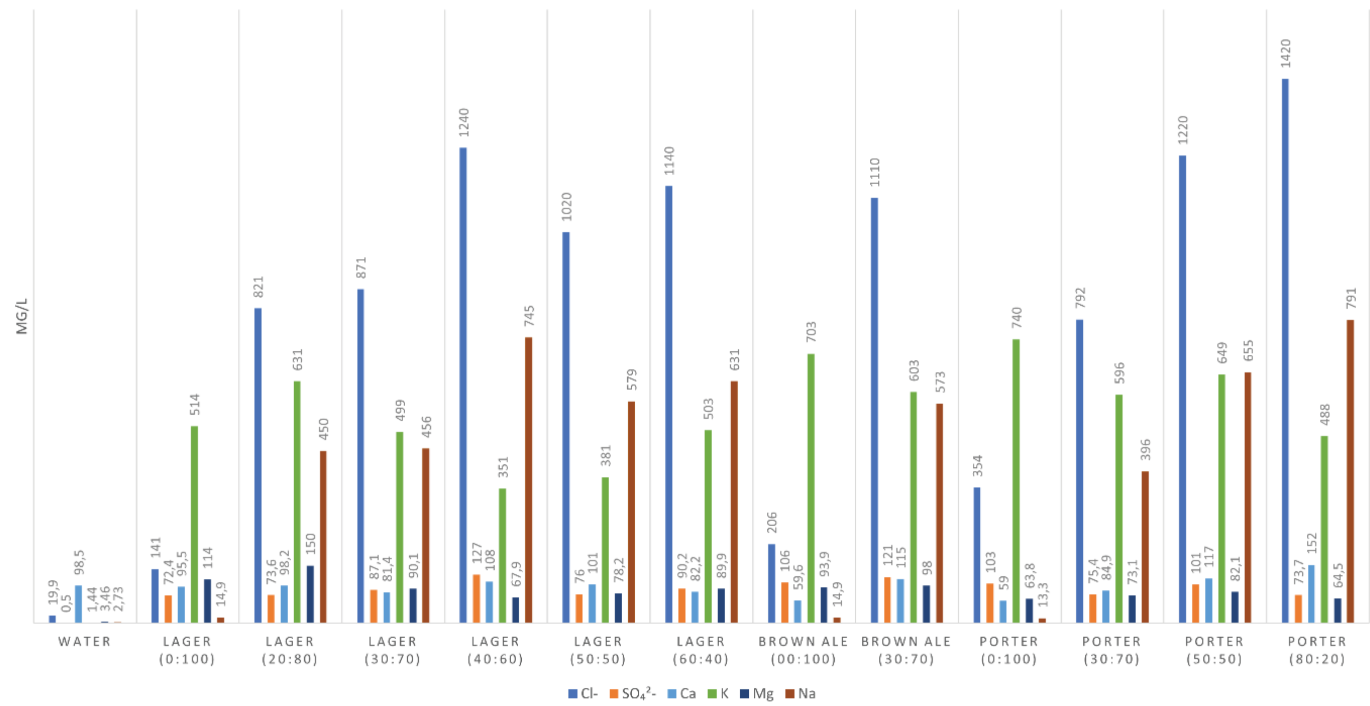

Fig. 6 **Chemical measurements of Cl-, SO_4^{2-}, Ca, K, Mg, Na in water, Lager (0 : 100, 20 : 80, 30 : 70, 40 : 60, 50 : 50, 60 : 40), Brown Ale (00 : 100, 30 : 70), Porter (0 : 100, 30 : 70, 50 : 50, 80 : 20)**

[38]. It has a medicinal off-flavour [39], which we detected in beer samples. Phenolic acids play the role of antioxidants in human health [5].

We can observe turbidity in one of the Brown Ale samples (Fig. 4), which may be caused by the presence of a large portion of bread. In addition, malt globulins can contribute to beer haze because the sulphur-containing b-globulin does not completely precipitate even if the boiling stage is extended. Protein-protein linkages form, in combination with polyphenols, haze [35] as confirmed through the Brown Ale sensory profile. A chill haze is formed when polypeptides and polyphenols are bound non-covalently. Permanent haze forms in the same manner, but covalent bonds form soon and insoluble complexes are created which will not dissolve when heated [35].

Some samples of Porter (Fig. 5) had a burnt taste because of the toasted bread used for that sort of beer. If the quantity of magnesium exceeds 80 mg/l, beer has a sour and bitter flavour [23], but in the current situation, that sour taste originated from roasted malt. We made a mistake in the roasted malt quantity for the Porter-style beer recipe. Therefore, almost all samples of Porter were evaluated as unsatisfactory.

In addition, some samples had a viscous taste that may indicate that the beer contains gram-positive bacteria *Leuconostoc mesenteroides*. It is a species of lactic acid bacteria associated with fermentation under conditions of salinity and low temperatures [40]. During growth, these bacteria produce excess turbidity and acidity [41].

Weak fullness of the Lager beer can be associated with protease, which did not degrade wheat storage proteins in bread because of high temperatures of mashing [35].

3.4 Chemical measurements

The results of chemical measurement (Fig. 6) show that the more bread, the saltier the taste of the beer due to the increased Na and Cl- indicators.

Mineral elements such as K, Mg and Ca affect beer taste, colour and durability [42]. Furthermore, Mg and Ca are vital yeast nutrients [23]. Ca prevents the thermal inactivation of α-amylase by extending the pH range of the enzyme [43]. We can see that top-fermented beer has more K (pure Brown Ale: 703 mg/l, pure Porter: 740 mg/l) compared with pure Lager (514 mg/l). We can say that the quantity of K depends on the fermentation type. The availability of bread decreases the quantity of K compared with pure beer samples. For Ca, everything is the opposite here – pure samples of top-fermented beer have a lower quantity of Ca, unlike pure Lager.

Voica et al. [44] determined the metal content (mg/l) in twenty commercial beers from Romanian markets. They obtained the following results: Ca (40 – 140 for the UK, 29.0 – 86.2 for Spain, and 3.80 – 108 for Germany), K (135 – 1100 for the UK, 22.9 – 496 for Spain, and 22.9 – 496 for Germany), Mg (60 – 200 for the UK, 42.0 – 110 for Spain, and 23.7 – 266 for Germany), and Na (21.90 – 230 for the UK, 3.95 – 103 for Spain, and 1.19 – 120 for Germany) [44]. As we can see, the beer from the UK contains a large quantity of K, so K depends on the style of the beer.

Rodrigo et al. [45] analysed mean mineral content (mg/l) in 35 Ale beers (Ca 56.1, K 474.3, Mg 84.8, Na 44.9), 7 Bitter beers (Ca 86.0, K 455.5, Mg 73.7, Na 52.7), 6 IPA beers (Ca 76.1, K 647.8, Mg 95.3, Na 51.2), 59 Lager beers (Ca 41.7, K 379.9, Mg 67.9, Na 33.0), 4 Lambic beers (Ca 39.3, K 677.4, Mg 63.6, Na 53.3), 4 Pilsner beers (Ca 25.4, K 462.6, Mg 92.7, Na 32.4) and 10 Stout/Porter beers (Ca 74.2, K 592.6, Mg 103.5, Na 51.9) [45]. Our samples have much more Ca in all types of beer. Water for brewing beer had 98.5 mg of Ca per l. Brown Ale and Porter pure samples had a lower quantity of Ca than samples with bread. The pure Lager sample had more Ca than Brown Ale and Porter. For good mash and lauter pH stability, a Ca level of 100 – 150 ppm is preferred [23].

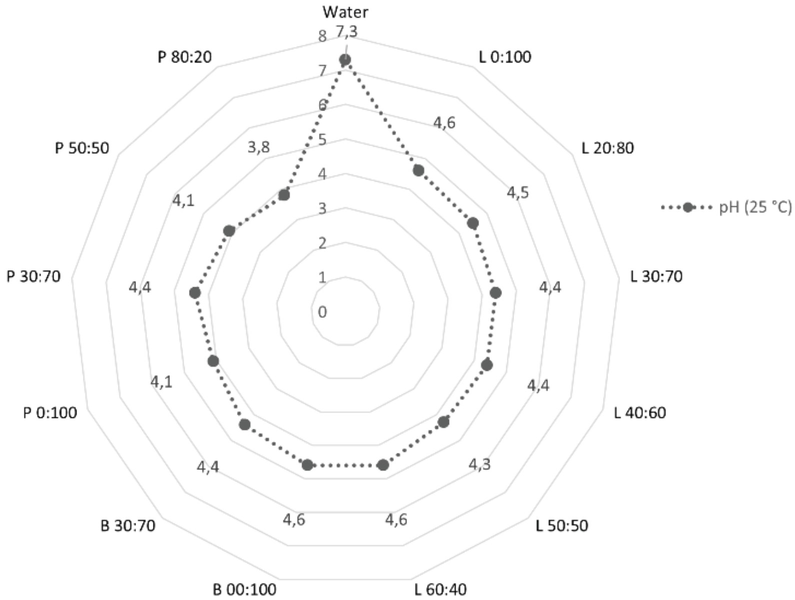

Fig. 7 **pH measurement values for water, Lager (0 : 100, 20 : 80, 30 : 70, 40 : 60, 50 : 50, 60 : 40), Brown Ale (00 : 100, 30 : 70), Porter (0 : 100, 30 : 70, 50 : 50, 80 : 20)**

Rodrigo et al. [45] also analysed 120 beers from Belgium, China, Czech Republic, Germany, Holland, Ireland, Italy, Mexico, UK and USA and had the following results: Belgium (Ca 54.8, K 504.2, Mg 83.3, Na 49.7), China (Ca 35.1, K 298.9, Mg 76.4, Na 53.2), Czech Republic (Ca 24.1, K 416.8, Mg 89.1, Na 20.8), Germany (Ca 41.7, K 450.2, Mg 79.5, Na 19.1), Holland (Ca 29.7, K 506.0, Mg 68.5, Na 20.5), Ireland (Ca 55.2, K 475.3, Mg 76.4, Na 21.2), Italy (Ca 44.0, K 412.8, Mg 72.6, Na 15.7), Mexico (Ca 50.4, K 239.8, Mg 57.3, Na 53.1), UK (Ca 61.5, K 436.5, Mg 73.6, Na 48.3) and USA (Ca 38.7, K 626.2, Mg 99.8, Na 26.8) [45]. They conclude that the geographical origin of a beer determines its mineral content. In our case, not just geographical origin is significant but also the additives such as bread in beer samples.

Styburski et al. [25] analysed mineral content in 52 beer samples imported from Asia, South America and Europe. Samples with the highest average calcium concentration were from Germany (0.31 g/l) and Armenia (0.28 g/l); beer samples from Portugal had the lowest concentration of calcium (0.05 g/l). Samples with the highest concentration of chlorine were from Ukraine (0.100 g/l ± 0.083), and the lowest concentration of chlorine was found in samples from the Czech Republic (0.023 g/l ± 0.001) and Germany (0.023 g/l ± 0.006). The highest concentration of potassium was identified for samples from Portugal (0.191 g/l ± 0.001) and the lowest for samples from the Czech Republic (0.064 g/l ± 0.005) [25]. All our beer samples had a large quantity of chlorine: 141 – 1,420 mg/l. Samples made with the use of bread had sharply high indicators compared to pure samples. Chlorine plays a role in yeast flocculation and improves clarity and colloidal stability [26].

Zambrzycka-Szelewa et al. [46] also analysed the mineral content of 11 Ale and 18 Lager beers from Poland. Bottom-fermented beers had a concentration of 10.9 ± 0.1 mg/l to 74.2 ± 1.3 mg/l for Na, 367 ± 10 mg/l to 855 ± 16 mg/l for K, 80.2 ± 1.2 mg/l to 169 ± 0 mg/l for Mg and 34.5 ± 1.0 mg/l to 117.2 ± 4.1 mg/l for Ca. Top-fermented beers had a concentration of 7.75 ± 0.04 mg/l to 74.0 ± 2.0 mg/l for Na, 428 ± 10 mg/l to 815 ± 1 mg/l for K, 64.0 ± 1.0 mg/l to 141 ± 1 mg/l for Mg and 19.1 ± 1.6 mg/l to 58.2 ± 1.5 mg/l [46] for Ca. As can be seen, the content of Mg can be decreased or increased even for the same style of beer, but with different types of malt and additives, its quantity can vary. Other indicators in our samples are much increased compared with measurements made by Zambrzycka-Szelewa et al. [46].

The relation between Cl- and SO_4^{2-}-influences the bitterness of a beer. The higher content of Cl- alleviates and softens the bitterness and supports the malty character and fullness of taste. The higher the SO_4^{2-} content (> 150 ppm), the more hops-like (bitterness and dryness) and the less malty the taste [22, 23]. To prevent the bitterness from dominating, brewers must use lower quantities of SO42- ranging from 50 to 75 ppm [23]. In the samples with bread, the content of Cl- is higher than in control samples (Fig. 6). Hence, beer has a maltier and less hoppy taste.

In the previous studies, it was observed that the mineral content of beer depends on the beer style. Top-fermented beers have higher concentrations of Ca, K and Mg than bottom-fermented beers, which may depend on the yeast type used for fermentation. Furthermore, it was noticed that the type of container for beer storage can determine the content of As ($p \leq 0.001$), Mg ($p \leq 0.01$), Na ($p \leq 0.01$) and V ($p \leq 0.01$). Metal barrels can influence Na and As concentrations in beer, while cans can affect the content of V [45]. We provided fermentation in food plastic fermenters and the final beer was stored in PET bottles.

HCO_3- and CO_3^{2}- are indicators of alkalinity. High alkalinity gives a bitter taste to water [24] and can buffer the pH of dark beer styles, preventing them from tasting acrid [23]. Low levels of alkalinity (< 50 ppm) prevent the beer from tasting watery [23]. We had high HCO_3- levels of Lager 00 : 100 (87 mg/l), 60 : 40 (64 mg/l) and Brown Ale 00 : 100 (35 mg/l) which makes them taste watery. For the other samples, the level was less than 3 mg/l and the water content was 235 mg/l. The CO_3^{2}- level was less than 3 mg/l for all samples.

We did a pH analysis for our beer samples (Fig. 7). The pH ranged from 4.3 to 4.6 for the Lager beer, from 4.4 to 4.6 for Brown Ale and from 3.8 to 4.4 for Porter. Zambrzycka-Szelewa et al., [46] analysed pH in beer from Poland; 11 Ale samples had a pH from 3.91 to 4.54 and Lager's pH ranged from 4.09 to 4.53 [46]. When compared, Poland and our beer samples have the same (low) pH in top-fermented beers and an optimal range of pH in Lager.

4. Conclusion

The present study aims at the sustainable use of bakery leftovers and their application as a partial substitute for malt in beer production. We tried to find out if bakery leftovers would affect the taste and quality of beer. We produced seven samples of Lager beer (20 l per batch), three samples of Brown Ale (10 l per batch) and four samples of Porter (10 l per batch). In addition, we had one example of each style of beer with no bread used as a control sample. Samples without any bread used and samples with bread substitute (up to 30 %) showed good results in the total rating of sensory evaluation. They almost did not have smell or taste defects. After physical measurements, we found that bread does not affect the beer's colour and the foam's quality in top-fermented beers. The Lager beer foam was mostly weak; the problem can be in bottles with caps not twisted tightly enough during the second fermentation. In addition, the Lager samples were six months old, which may have affected the quantity of foam and CO_2 in the beer.

Compared to other researchers that analysed the mineral content of different types of beer from different countries, our Na and Cl- indicators showed increased levels, hence the salty taste of beer. We suggest not using more than 30 % of bread because the salty taste can be more pronounced, which was proved by sensory analysis. We also noticed that the quantity of K depends on the type of fermentation while the availability of bread decreases the quantity.

We calculated that 30 % substitution could save around 6 – 7.5 kg of barley per hl by replacing barley with bakery leftovers. Brewers can collect leftovers from local supermarkets, cafeterias, etc. It is however important to keep bread uncontaminated by other food.

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Quality reduction of beer stored in transfer tubes of a dispensing system – an investigation with sensory and GC/MS analyses

Dispensing systems are essential in the beer supply chain and form one of the last steps to its final link – the consumer. The maintenance and hygiene of dispensing systems are crucial as they influence the beer quality directly. Dispensing systems comprise various equipment and components necessary to pour a beer in the quality desired by the brewer. The key components with the most prominent product contact are beer transfer tubes. Depending on the construction properties of a gastronomy's premises, long distances have to be covered frequently, resulting in significant filling volumes. For decades, brewers, sommeliers and connoisseurs have known that long hold-back times of beer in the beer tubes reduces quality. In such a case, discarding the whole beer tube's volume is necessary before serving the first tapped beer to meet the quality standards of brewers, gastronomers, and consumers. This study investigates sensory and analytically aroma changes of three different beer styles during storage in a beer transfer tube. For this purpose, an experimental dispensing system with six beer hoses was constructed. The beer storage in the transfer tubes was conducted at two different temperatures, 5 °C and 20 °C. After particular time intervals, the beer was poured and analysed for aging indicators, flavours by GC/MS resp and a trained tasting panel. The results show significant influences on the aroma profile after three hours of storage. The panel identified a change in the aroma profile to flavours usually associated with oxidation and aging. In the comparison of the two storage temperatures, higher temperatures significantly worsen the aroma profile. These findings were supported by an increase in aging indicators determined by GC/MS analysis.

Descriptors: dispensing systems, beer transfer tubes, beer quality, sensory analysis, GC/MS

1 Introduction

Modern dispensing systems consist of various components. The harmony of their construction provides dispensed beer with the quality desired by the brewer. One main component is the beer transfer tube that ensures the transport of the beer from the keg to the tap. For the majority of dispensing systems, they are the components with the most substantial contact with the beer. Producers mainly use polymers for manufacturing beer hoses. Following the DIN 6653-1 [1], primary materials are polyamide, polyethylene, or ethyl vinyl acetate. Depending on the construction properties of gastronomy premises, beer tubes have to cover long distances (exemplary calculations see Table 1). Thus, filling volumes of several liters quickly arise in the beer tubes. The beer itself can also function as a contaminant when it stays too long in the tubes or if insufficient cleaning occurs.

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For decades every brewer has known about the problematic hygienic situation in dispensing systems. In restaurants or pubs, beer transfer tubes are often not the main cleaning focus. The fact that dispensing systems are not sterile nor germ-free will not cause problems as long as cleaning cycles are adequate. However, microbial contamination can potentially form biofilms that create severe hygienic problems (see Fig. 1 on page 12). The reasons are either non-consciousness of this problem or economic considerations. Many components of dispensing systems are not visible due to their construction and installation in wall ducts or insulations. Furthermore, cleaning procedures are expensive and time-consuming, making a frequent execution unattractive at first glance.

Tab. 1 Exemplary beer volumes in a single beer tube with different diameters and different transfer distances; values depend on average assumptions and standard room heights

	Transfer distance	Volume in beer transfer tube / amount of beer that has to be discarded [l]	
	[m]	avg. diameter 7 mm	avg. diameter 10 mm
Keg cooler	3	0.12	0.24
Cellar cooling chamber	10	0.38	0.79
2 floors	12.5	0.48	0.98
3 floors	15	0.58	1.18

Fig. 1 Exemplary images of spoiled beer transfer tubes showing biofilm formation and fouling on the hose surface with beer contact

Another fundamental problem is the filling volume of beer that stays in the beer hose for a significant time (e. g. after the closing hour). In a poorly cleaned system, this beer interacts with the residual contamination in the beer tubes, does not meet the standards of quality anymore, and becomes unfit for consumption. In German-speaking countries, this phenomenon is also called "Nachtwächter" (night watchman) and is known to every brewer and informed beer consumer. When the gastronomy reopens, the barkeeper is expected to discard this beer volume. The image loss by selling such a beer is severe for the gastronomy and the brewery and often results in negative economic effects. The consumption of such a beer can even constitute a human health risk.

Additionally, food auditors fine gastronomies when they serve such a spoiled beer to customers. The beer does not meet the requirements of a fresh product anymore as it contains off-flavours (e.g., diacetyl or aging aromas) due to microbial spoiling and oxidation. According to European legislation (e.g., EC No 178/2002) [2], serving such a beer fulfills the combined facts of disgust and consumer deception, as a guest expects a fresh and uncontaminated product.

The microbiological influence on beer spoiling and the appearance of corresponding off-flavours were the research object in many publications [3–6]. However, the effect of quality reduction of beer stored in beer transfer tubes depends not only on spoilage by microorganisms. There is also a formation of off-flavours in new or well-cleaned systems. Different publications evidenced that the aging of beer depends on several ambient conditions [7–9]. Higher temperatures, light, and oxygen intake result in faster aging without additional microbial influence. In dispensing systems, there are often no installations of secondary cooling systems that keep the beer transfer tubes and the beer in a cold condition. The increased gas permeability of polymers and potential gas inlet at connectors can increase the oxygen concentration in the beer. Besides, there is a simultaneous CO_2 decrease due to thermodynamical laws where always an equilibrium of the partial pressure between two systems appears [10, 11]. From this oxygen intake into the beer, oxidation of the components results, which causes faster aging and the appearance of off-flavours.

This research study examined the change in the flavour profiles of beer stored in beer transfer tubes. Firstly, the construction of a specific dispensing system with six beer hoses formed the centerpiece of the experimental investigations. In the beer transfer tubes, beer was storable at two different temperatures and for particular time intervals. Additionally, an examination of the potential impact of beer styles by using three different beers showed influencing parameters as well as the intrinsic potential of the resp. beer style to mask those changes. The analysis of the flavour change occurred by three different analytical approaches. Firstly, the O_2- and CO_2-concentration analysis in a lager beer showed the thermodynamical activities at certain storing times. Secondly, a tasting panel examined the ageing-flavours of the samples via sensory analysis. Ultimately, an instrumental analysis investigated corresponding aroma components with a GC/MS-analysis. The main aim was to investigate how the beer flavour and thermodynamical properties change while being stored in beer tubes. The results display how dispensing systems and the tapping conditions in gastronomy are improvable. Herefrom, considerations on possible installations and alternative material use for beer transfer tubes are derivable.

Tab. 2 Specific properties of the selected beers

Beer style	Lager	Wheat beer	Pilsener
Original gravity [%]	11.5	12.6	11.4
ABV [%]	5.1	5.4	4.9
IBU [-]	21	14	40
Type of fermentation	Bottom fermented	Top fermented	Bottom fermented

2 Material and Methods

2.1 Beer selection

The experiments were conducted with three different beer styles that are available commercially (properties see Table 2). The style variation was necessary to highlight the impact of differently brewed beers and ingredients. Besides, this selection also enabled an immediate application of the study's results in practice. The first used style was a German Lager with the lightest aroma profile and no masking effects by other aroma components. Here, it could be assumed that any appearing off-flavours are already detectable with sensorial methods at a low threshold. The focus of the sensorial studies was on oxidation and aging aroma. The second beer style was a Bavarian wheat beer, which possessed increased masking effects due to heavy aroma components, e.g., isoamyl acetate. Following the literature, such aroma compositions can provide increased taste thresholds for off-flavours. The third beer style was a Pilsener from Northern Germany, consisting of more bitter units. In comparison to the Lager, it could be assumed that the bitter components show a different aging behaviour with accelerated effects on oxidation of the hop-derived ingredients.

During the use of all beers in the experiments, it was ensured that the beer was fresh, within the expiration date and from the same batch.

2.2 Experimental dispensing system

The experimental setup (see Fig. 2) comprised six hoses manufactured of the polymer polyethylene. Three hoses were stored cold at 5 °C, and the other three warm at 20 °C to achieve typical gastronomical environmental conditions. The beer transfer tubes were connected to a single keg and thus fed with the same beer. An additional valve at the keg enabled the pouring of fresh beer as a reference for the analysis. All beer tubes were provided with light protection to prevent the influence of ambient light on the beer. This feature is close to practical installations where beer transfer tubes usually are protected from light (e.g., insulation or cable pits). The taps were equipped with compensators, which made it possible to control the volume flow. The entire setup was cleaned before and after every trial, making microbial influence negligible.

For the sensorial analysis, the beer samples were dispensed by the tap. The beer samples for the CO_2- and O_2 measurements were directly streamed from the beer hoses via a valve positioned before the tap. This bypass avoided CO_2 loss and O_2 intake during dispensing and enabled direct flow to the measurement device.

2.3 Determination of CO_2 and O_2 content

The beer could directly flow into the measurement device via the mentioned bypass valve, enabling inline measurement. Here, the CboxQC from Anton Paar (Graz, Austria) facilitated a combined analysis of CO_2 and O_2. The entire filling volume of the respective beer transfer tube was used to ensure a stable and precise analysis. The CO_2 content in the beer was measured in g/l, while the O_2 content was determined in mg/l.

The selected measurement device enables a high standardisation as it complies with the current brewing technology methods according to ASBC, EBC and Mebak. A big advantage of the device was the easy connectivity to the experimental dispensing system. Thus, it was ensured that the beer sample passed directly into the measuring chamber and was not falsified by an intermediate transfer due to additional gas exchange. Due to the advantages listed above, further measurements of CO_2 and O_2 were not performed

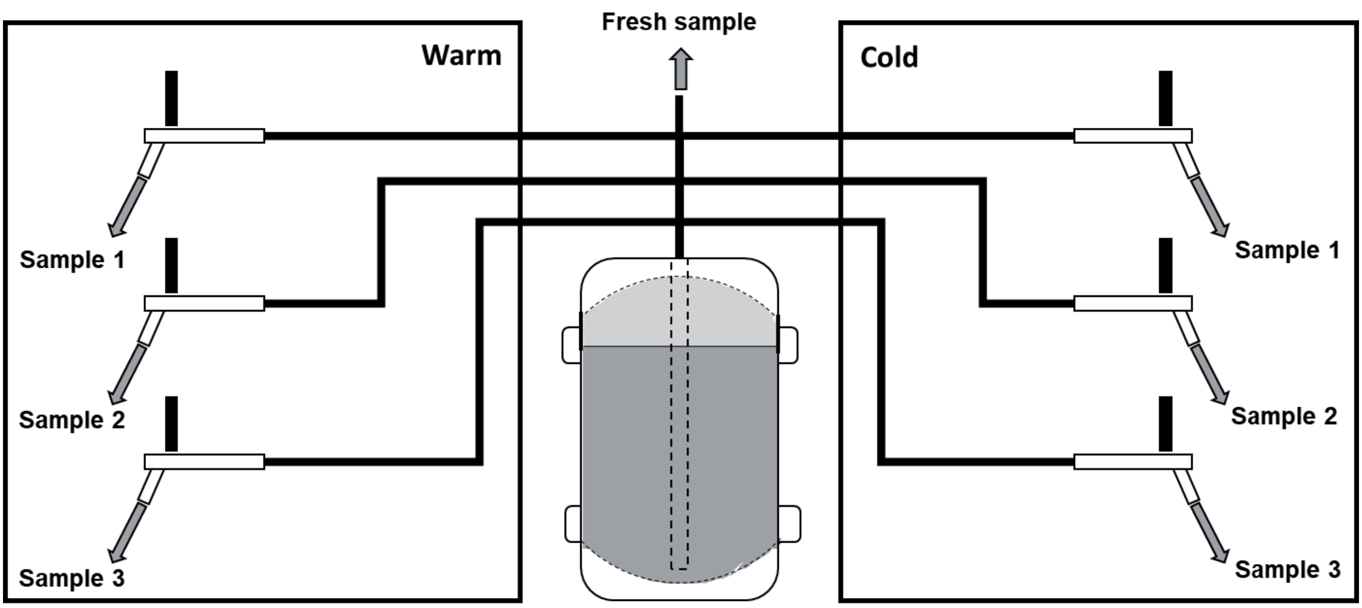

Fig. 2 Experimental dispensing system