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FACTORS AFFECTING BEER QUALITY DURING STORAGE – A REVIEW

\- Review -

Krystian Klimczak*, Monika Cioch-Skoneczny, Aleksander Poreda

Department of Fermentation Technology and Microbiology, University of Agriculture in Kraków, ul. Balicka 122, 30-149 Kraków, Poland

Abstract: Fermented beverages such as beer are known for their relatively long shelf life. However, the main factor limiting their shelf life is the qualitative changes that occur during storage. From the moment the beer is produced, its characteristics, such as taste, aroma, and colloidal stability undergo continuous change. The intensity of these changes depends on the type of beer, storage conditions, and length of storage. While some degree of ageing can have a positive influence on sensory characteristics of a beer, beer stalling is seen as a significant problem. As it is currently understood, beer ageing is mainly caused by the formation of stalling aldehydes. At the same time, compounds which bestow the beer its flavour, such as esters, terpenes, and iso-α-acids undergo qualitative and quantitative changes. As a result, aroma discriminants such as freshness, fruitiness or florality are often lost over time. In their place, aromas described as ribes, cardboard, bread-like, honey-like or sherry-like appear. The article aims to present the changes in beer sensorial, physicochemical, and microbiological characteristics during storage and the factors that affect beer quality during ageing The article also describes the variables which according to the current literature, may alter the flavour stability of a beer.

Keywords: beer, staling, stability

INTRODUCTION

Beer is one of the oldest, and most widespread alcoholic beverage in the world. In many regions, its production and consumption are of great economic, cultural, and social importance. Beer's position on the market has led producers to continually seek out new products to gain consumer interest. In recent years, many not widely known beer styles and types have gained popularity. Brewers can use special malts, different varieties of hops, the addition of fruit and other botanicals (including functional additives), and different strains and species of yeast to interest the consumer. Traditionally, beer contains 3-6% ABV (alcohol by volume), but beers with much higher as well as lower alcohol content can be found on the market (Marcos et al., 2021). It is the latter that has been gaining popularity in recent years. This is related, among other things, to growing health awareness of consumers. Thanks to this trend, alcohol-free, reduced, and low-alcoholic beers have emerged in recent years. Unfortunately, the changes in the brewing market So far, the naming of beers based on their alcohol content have not been consistently distinguished in individual countries. The scale of this problem can be illustrated, for example, by the differences between the legal frameworks of the United Kingdom and Finland. While in the UK, alcohol-free beer must contain less than 0.05% ABV, in the case of Finland such beers can have up to 2.8% ABV (Okaru & Lachenmeier, 2022).

Nowadays, food safety is not simply an expected issue – it is a requirement from consumers and legislators (Lipp, 2021). In that respect, beer is widely regarded as a microbiologically stable product. Factors such as very low oxygen content, low pH, the presence of hop-derived compounds (such as iso-α-acids), carbon dioxide and alcohol, along with the absence of nutrients significantly limit the growth of unfavourable microflora in the product (Oldham & Held, 2023). The processes to which beer is often subjected, such as sterilization and pasteurization are also important. This does not mean that unfavourable microflora can't develop in all types of a beer – but the survival of food-borne pathogens under these conditions is made much more difficult (Menz et al., 2011).

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* Corresponding author.
E-Mail address: krystian.klimczak.sd@student.urk.edu.pl

For most standard beers on the market, microbial contamination is a relatively unlikely event. However, it should be borne in mind that the situation may be different for reduced, or non-alcoholic beers. The absence of one of the microbial growth hurdles may make them more susceptible to microbial contamination. At the same time, among other factors, when fruit additives are used, nutrients are supplied to the finished product that can be utilized by microorganisms (Moreno et al., 2022).

However, the transformations that affect all beers are the qualitative changes that occur during storage. From the moment beer is produced, it undergoes a continuous process of changes in, among other things, aroma, flavour and colloidal stability. These changes can vary in intensity depending on the type of beer, storage conditions and length of storage. Understanding the factors that drive these changes can enable brewers to prolong the flavour stability of beer. While some styles can benefit from prolonged maturation and storage (such as porters and stouts), many of the more common styles on the market (such as lagers or IPAs) tend to deteriorate with prolonged storage. Finding factors that can extend the stability of a beer will therefore be of great benefit to both brewers and consumers. It cannot be overlooked that food waste is currently a crucial issue. In 2021, more than 58 million tonnes of food waste were generated in the European Union alone (Food Waste and Food Waste Prevention - Estimates, 2023). Beer ageing is a significant problem because it adversely affects consumer perception of the product, hurts the brand image, and can contribute to resource wastage. The aim of this paper is to review the physicochemical changes occurring during beer storage. In addition, an attempt will be made to describe the factors influencing stability of the product. This information can be used by brewers to understand factors driving beer flavour deterioration.

CHANGES IN BEER DURING THE STORAGE

Beer is an extremely complex mixture of chemical compounds derived from the raw materials used, those formed during wort production and by-products of yeast metabolism. Although it consists almost solely of water, alcohol, and residual extract, there are hundreds of other of substances in its composition. These substances are present in much lower concentrations than those listed, but they have a key influence on the sensory characteristics of the beverage produced (Steiner et al., 2010). These compounds are often not in chemical equilibrium or are relatively unstable. For this reason, spontaneous reactions will occur during storage which mainly change the organoleptic characteristics of the beer. The transformations occurring during beer storage will be discussed below.

Changes in taste and aroma

Some of the most noticeable changes that occur during the storage of beer are changes in its sensory characteristics. During storage, aroma discriminants such as freshness, fruitiness or florality are often lost over time. In their place, aromas described as ribes, cardboard, bread-like, honey-like or sherry-like appear (Boccorh & Paterson, 2002). It is important to note that the sensory changes depend on the type of beer, whether it is a *lager* or an *ale*. This is related to their initial sensory characteristics, thus among others - volatile compounds composition. In a study by Schubert et al. (2022) fresh ales contained higher concentrations of aldehydes (Schubert et al., 2022).

For many years, researchers have tried to determine what phenomena are behind the appearance of these undesirable sensory changes. Current literature indicates that the ageing process of beer appears to be significantly related to aldehydes produced in Maillard reactions, Stecker degradation, and lipid oxidation (Baert et al., 2012). Some of the more important compounds responsible for beer flavour deterioration are given in Table 1. Although these compounds are often found below their sensory threshold, the concentration above or below sensory threshold does not mean the compound will affect beers' sensory characteristics. Synergistic, antagonistic or additive effects can alter sensory thresholds of compounds (Gernat et al., 2020; Stevens, 1996). Dark beers and beers with a high alcohol content may be especially prone to the formation of elevated 2-furfuryl ethyl ether concentrations. This compound, in amounts exceeding the sensory threshold, can impart a solvent-like, stale flavour (de Almeida et al., 2012). Low pH may promote the appearance of this compound (Vanderhaegen et al., 2004).

Table 1. Selected compounds associated with beer staling(Aguiar et al., 2022; Gijs et al., 2000; Saison, De Schutter, Uyttenhove, et al., 2009; Soares da Costa et al., 2004; Wietstock, 2017)

Compound	Aroma	Sensory threshold [ppb]	Concentration in fresh beer [ppb]	Concentration in aged beer [ppb]
Strecker aldehydes				
2-methylpropanal	Grainy, varnish, fruity	86	17.2[a,b]	215.8[a] 57.2[b]
2-methylbutanal	Almond, apple-like, malty	45	6.5[a,b] 3.2-5-6[d] 3.4[g]	21.2[a] 10[b] 6.5-8.1[d] 4.6[g]
3-methylbutanal	Malty, cherry, almond, chocolade	56	16.6[a,b] 7.1-12[d] 7.2[g]	50.0[a] 25.3[b] 9.7-14.9[d] 7.9[g]
phenylacetaldehyde	Hyacinth, flowery, roses	105	24.7[a,b] 5.3-10.4[d] 19.2[g]	51.6[a] 31.9[b] 7-15.4[d] 29.5[g]
benzaldehyde	Almond, cherry stone	515	1.4[a,b] 0.9-1.1[d] 5.7[g]	1.5[a] 1.7[b] 1.1-1.9[d] 5.1[g]
methional	Cooked potatoes, worty	4.2	4.2[a,b] 11-41[d]	15.4[a] 8.5[b] 26-66[d]
Fatty acid oxidation				
hexanal	Bitter, winey	88	0.8 [a,b]	1.2[a] 0.7[b]
trans-2-nonenal	Cardboard, papery, cucumber	0.03	0.033 [a,b] 0.08[g]	0.090[a] 0.046[b] 0.10[g]
Maillard reaction				
2-Furfural	Caramel, bready, cooked meat	15,157	5.1[a,b] 2.5-5.7[d] 48.1[g]	221.4[a] 17.5[b] 32.4-47.8[d] 258.5[g]
5-hydroxymethylfurfural	Bready, caramel	35,784	1800[f]	4230[f]
acetylfuran	Nutty, almond, burnt	513	3.4-3.6[d] 8.4[g]	3.6-4[d] 14[g]
furfuryl ethyl ether	Solvent	11	2.7[g]	16.1[g]
γ-nonalactone	Coconut, vanilla, glue, rancid	607	15.1-16[d]	20-22.3[d]
Carotenoid degradation				
β-damascenone	Coconut, tobacco, red fruits	203	6-25[c] 1.0[g]	14-210[c] 2.0[g]
Polysulfides				
dimethyl trisulfide	Onion, rotting fruit, red cabbage, sulfury	0.027	0.006-0.019[e]	0.098-0.179[e]

The same superscripts in table cells refer to data obtained from the same study
a – study by (Schubert et al., 2022) fresh blonde ale; beer after 24 weeks at room temperature,
b – study by (Schubert et al., 2022) fresh blonde ale; beer after 24 weeks at 4°C,
c – study by (Chevance et al., 2002), fresh; artificially aged beer (40°C for 5 days),
d – study by (Herrmann et al., 2010), fresh; artificially aged beer (1 day shaking, 4 days at 40°C),
e – study by (Gijs et al., 2000), fresh; force aged beer (40°C for 5 days),
f – study by (Bravo et al., 2001), beer kept at 0°C; beer kept at 28°C,
g – study by (Vanderhaegen et al., 2003), fresh; beer stored for 6 months at 20°C

During storage, levels of dimethyl sulfide (DMS) can also elevate (Baldus & Methner, 2019). In addition to the appearance of compounds with unfavourable sensory properties, those found in beer undergo transformation and/or degradation. Terpenes found in beer are susceptible to oxidation processes (Almeida et al., 2015). Myrcene is known to be absorbed on cap crown liners of a bottles (Kemp et al., 2021). It can also be adsorbed on the yeast walls if yeast cells are present in beer (Haslbeck et al., 2017). Esters found above their chemical equilibrium will hydrolyse. In turn, the esterification of ethanol with organic acids present in the beer will occur simultaneously. In particular, for some highly hopped beers this can have a detrimental effect on their aroma. In contrast, the concentration of terpene alcohols appears to be relatively stable during storage (Preedy, 2009; Schubert et al., 2022). The instability of esters and terpenes, with concurrent relatively high stability of terpene alcohols is confirmed by (Guan et al., 2019). In the case of polyfunctional thiols (which are currently suspected to be an important determinants of the tropical aroma of some beers), their concentration appears to depend on storage time. In a study by Tran et al. (2015), the content of these compounds increased up to 3 months of storage, while after 1 year of storage, the beers lost most of the evaluated polyfunctional thiols (Tran et al., 2015). Beer bitterness also undergoes quantitative and qualitative changes during storage. This is primarily related to the transformation of hop acids (α-, β- and iso-α-acids), which undergo oxidative degradation during beer storage. During the boiling of wort, α-acids from hops are isomerized to iso and trans-forms. Typically, the ratio of cis:trans-izo-α-acids is 70:30%. Whereas iso-forms are mostly stable, trans-forms are far more susceptible to degradation (Preedy, 2009; Stewart et al., 2017; Titus et al., 2021). The transformations of trans-iso-α-acids are largely responsible for the quantitative and qualitative declines in bitterness during beer storage (Cooman et al., 2000). Intelmann et al. (2011) report that the proton-catalysed cyclization of these compounds produces conglomerates with harsh and lingering bitterness (Intelmann et al., 2011). In beers subjected to dry hopping, a significant part of their bitterness may be derived from humulinones extracted from hops (Parkin & Shellhammer, 2017). Ferreira and Colin (2021) report of a relatively high instability of humulinones in dry-hopped Belgian beer. In the author's study, after a 2-year storage period at 20°C, a 25% decrease in iso-α-acids content was observed, but the losses of humulinones amounted to 91% (Ferreira & Collin, 2021). Alpha acids are also susceptible to photodegradation (range of light λ= 350-500 nm), resulting in a development of so-called sunstruck flavour caused by the formation of 3-methyl-2-butene-1-thiol (MBT). Due to the very low sensory threshold of this compound, this aroma is formed with a minimal loss of iso-α-acids (Caballero et al., 2012; De Keukeleire et al., 2008).

Physicochemical changes

Other parameters of beer can also undergo changes during the storage. The most visible change is the colour of beer, which gets darker. The degree of colour change depends, among other things, on factors such as pH, redox capacity, temperature, and oxygen availability in the beer matrix (Savel et al., 2010). The latter two are likely the main causes of polyphenol oxidation, which leads to the formation of compounds that give the beer a deeper colour. Polyphenols contribute to beer's bitterness, colour, body, and astringency (Oladokun et al., 2016; Preedy, 2009). Structural rearrangements of flavan-3-ol monomers can result in brown-yellowish compounds. (+)-Catechin is suspected to be an important precursor of these reactions. Another potential cause are the coloured molecules derived mainly from the Maillard reactions (Callemien & Collin, 2007, 2009). The initial products of the Maillard reactions have no colour, but as the reactions proceed further, they result in larger molecules, which begin to bestow the colour (Bamforth & Lewis, 2007; Nooshkam et al., 2019). Dark beers and beers with a higher alcohol content show a higher levels of Maillard reactions products (Vanderhaegen et al., 2007). This may be related to the higher Free Amino Nitrogen (FAN), and polyphenol content of such beers (Brey et al., 2002; Cooper et al., 1998; Rivero et al., 2005; Younis & Stewart, 1999). In addition, according to the current knowledge, polyphenols are responsible for the formation of chill and permanent haze. Probably as a result of oxidative and acid-catalysed polymerization, polyphenol particles bind into larger clusters. Due to the interactions with proteins, a haze may be formed (Habschied et al., 2021). A summary of changes is given in a Table 2.

Table 2. Summary of changes occurring during storage period

Type of changes	Process	References
Changes affecting taste and aroma	Maillard reactions, strecker degradation, lipid oxidation, carbohydrate degradation	(Baert et al., 2012; Bravo et al., 2008; Dale et al., 1977; Gibson et al., 2018)
	Increase in DMS levels	(Baldus & Methner, 2019; Grigsby & Palamand, 1977)
	Terpene oxidation and/or adsorption onto cap crown liners or yeasts	(Almeida et al., 2015; Guan et al., 2019; Haslbeck et al., 2017; Kemp et al., 2021)
	Changes in polyfunctional thiol content and composition	(Cordente et al., 2015; Nizet et al., 2014; Tran et al., 2015)
	Hydrolysis of esters occurring above chemical equilibrium, and subsequent formation of ethyl esters	(Kemp et al., 2021; Preedy, 2009; Rettberg et al., 2020)
	Degradation of iso-α-acids (mainly trans-iso-α-acids)	(Ferreira & Collin, 2021; Intelmann et al., 2011)
	Losses of humulinones (in dry hopped beers)	(Ferreira & Collin, 2021; Gahr et al., 2020)
	Iso-α-acid photooxidation	(Caballero et al., 2012; De Keukeleire et al., 2008)
	The concentration of terpene alcohols can fluctuate depending on the compound	(Guan et al., 2019; Schubert et al., 2022)
Physicochemical changes	Haze formation	(Habschied et al., 2021; Wang & Ye, 2021)
	Darkening of a beer	(Bamforth & Lewis, 2007; Callemien & Collin, 2007, 2009)

Growth of microflora

As mentioned previously, beer is regarded as intrinsically microbiologically safe beverage. Its microbiological safety can be enhanced further by treatments such as pasteurization and sterilization (Hammond et al., 1999). The fact of microbiological safety of beer is attributed in part to the significant antimicrobial activity of hop constituents and is mainly observed against gram (+) bacteria. The individual components of hops have different antimicrobial activity, where β-acids show greater activity than α-acids (Srinivasan et al., 2004). However, β-acids pass into beer only in trace amounts (Cortese et al., 2020). Important food pathogens such as *Listeria monocytogenes* or *Staphylococcus aureus* do not show growth even in non-alcoholic beer. This is related to the hop resins which significantly inhibit the growth of gram (+) bacteria. Gram (-) foodborne pathogens such as *Escherichia coli* or *Salmonella typhi* also do not show growth in beer, although, in the authors' study, *S. typhi* could survive for more than 30 days in a beer with medium ABV levels (2.5-3.6 %) when stored at 4°C (Menz et al., 2011). Most authors confirm that foodborne pathogens are unable to grow in beers of typical alcohol content (Kim et al., 2014; Menz, Vriesekoop, et al., 2010; Munford et al., 2017). However, these bacteria can survive in such an environment or remain in spore form by which they are relatively often detected in beers found on the market (Jeon et al., 2015; López et al., 2021; Munford et al., 2017). However, attention should be paid to emerging bacterial strains that show the ability to grow in beer. An example of a pathogenic strain showing the ability to grow in beer is the recently discovered *Bacillus cereus* strain 3012 (Wang et al., 2017).

Although there seems to be no direct threat from pathogenic bacteria, microorganisms which are able to grow in beer can significantly alter the sensory qualities and physicochemical parameters of the beer. Bacterial infections can cause various types of turbidity, development of films on the surface of the beverage, foreign aromas and aftertastes. Examples of such microorganisms include but are not limited to, lactic acid bacteria, *Pediococcus damnosus*, *Pectinatus cerevisiiphilus*, *Megasphaera cerevisiae* and *Staphylococcus xylosus* (Menz, Andrighetto, et al., 2010; Rodríguez-Saavedra et al., 2020; Sakamoto & Konings, 2003; Yu et al., 2019).

A significant threat to beer quality is the development of wild yeasts (non-*Saccharomyces* species). In the craft beers studied by Lopez et al. (2021), wild yeasts were detected in 100% of samples of beer below 5% ABV (López et al., 2021). *Candida pelliculosa* yeast species can be found on production surfaces in breweries. This yeast can grow in beer and form a biofilm (Timke et al., 2008). An important threat is *S. cerevisiae* var. *diastaticus*, which shows the ability to degrade sugars not fermented by brewer's yeast. In the beers studied by Štulíková et al. (2021), storing beer infected with this yeast for 14 weeks at 23°C resulted in a decrease in extract content, with an increase in alcohol content and in carbonation (almost twofold increase of CO_2 content). When beverages were stored at a lower temperature (8°C), a much lower severity of these defects was observed. In extreme cases, infection with these yeasts can cause gushing to occur (Krogerus & Gibson, 2020; Štulíková et al., 2021). Yeasts of the genus *Dekkera* - *D. anomala, D. bruxellensis* and *B. custersianus* are also known to grow in beer (Shimotsu et al., 2015).

As alcohol is one of the microbial hurdles present in beer, beers containing low levels of ethanol, or no-alcohol beers are more vulnerable to various types of microbial contamination. Menz et al. (2011) found that *E. coli* O157:H7 and *Salmonella typhimurium* can grow in non-alcoholic beer. However, the growth was arrested when the pH of the beer was lowered from 4.3 to 4.0 (Menz et al., 2011). Similar results regarding the importance of pH levels were obtained by Munford et al. (2017). The authors did not observe the growth of sporeforming bacteria in non-alcoholic beer, which was attributed to the low pH of these beers (below 4.5) (Munford et al., 2017). The relatively low susceptibility of alcohol-reduced beers to LAB infections can be inferred from a study by Sohrabvandi et al. (2010), where the authors used the addition of probiotic cultures to low and non-alcoholic beers. A very large decrease in the viability of the cultures was observed. The authors suggest that beer is not a suitable medium for such cultures (Sohrabvandi et al., 2010). Nevertheless, the important role of alcohol in ensuring the microbiological safety of beer must be kept in mind. Quain (2021), based on the measurement of absorbance at λ=660 nm, determined that, in draught beers, the growth of microorganisms in non-alcoholic ($\leq$ 0.05% ABV) and low-alcohol ($\leq$ 1.2% ABV) beers was 2-5 times higher than in control beers (4.5% ABV). The degree of absorbance rise correlated with the content of fermentable sugars in these beers (Quain, 2021). L'Anthoën and Ingledew (1996) reports of increased temperature resistance of microorganisms in reduced alcohol beer (0.5%), compared to regular beer (5%). In the authors' study, the bacteria *Pediococcus acidilactici*, *Lactobacillus delbrueckii*, *E. coli* O157:H7 and *S. typhimurium* showed 3-17x higher temperature resistance in non-alcoholic beer (L'Anthoën & Ingledew, 1996). If arrested fermentation methods are used to produce alcohol-reduced beer, a significant number of fermentable sugars can remain in the product which may increase the susceptibility of such beverage to infection (Sohrabvandi et al., 2010).

FACTORS AFFECTING FLAVOUR STABILITY

In recent decades, brewing science has made considerable progress in understanding the factors that affect stability of a product. However, due to the vastness of the physicochemical reactions occurring during ageing and the number of interactions between beer components, many questions remain unanswered. Based on the current state of knowledge, it can be concluded that almost every factor occurring during production process has an impact on the final stability of the product. In the following paragraphs, literature reports on factors that can affect the storage stability of the final product will be presented. A summary of the changes which will be presented below is given in Figure 1.

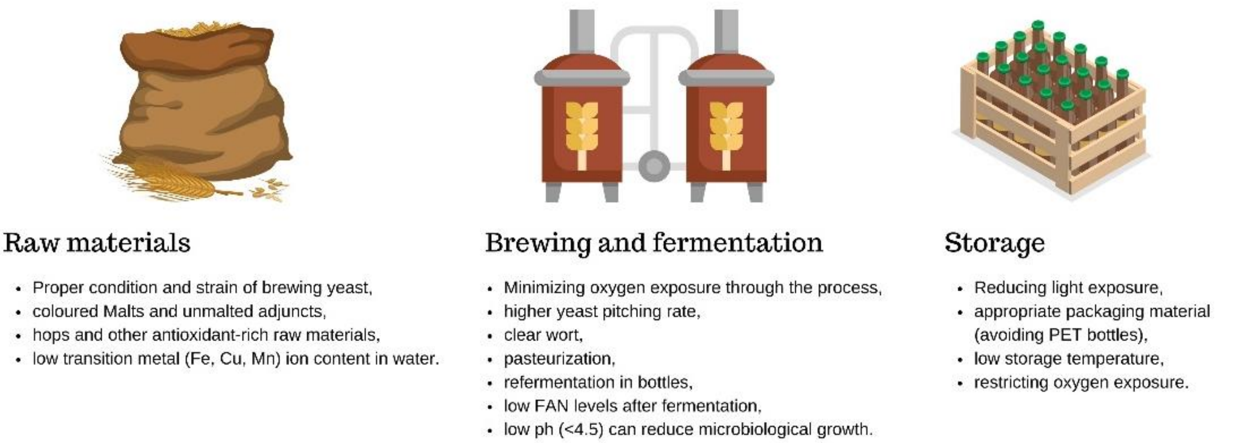

Figure 1. Overview of factors promoting beer flavour stability.

Raw materials

The selection of raw materials for the production of wort is a base for all brewing recipes. As it has a very significant impact on the final characteristics of the product, it naturally will affect the stability of the beer during storage. Guido et al. (2007) point to the important role of malt-derived polyphenols in improving beer flavour stability. Highly polymerized phenolic compounds and/or insoluble bound polyphenols have an important function in reducing oxidation reactions during malting and mashing stages. In contrast, the fraction remaining in the wort after wort production seems to have no such effect, and even function as pro-oxidant (Guido et al., 2007). However, Mikyška et al. (2002) report that malt polyphenols can have a positive effect on a beer's stability (Mikyška et al., 2002).

An important issue affecting the final sensory and physicochemical characteristics of the beer, along with the fermentation yield, is the Free Amino Nitrogen (FAN) content. For a standard wort with an extract of 10-12°P, it is assumed that the FAN content should be around 130 mg/L. Below this value, yeast growth is nitrogen-dependent (Hill & Stewart, 2019). In some cases, it is possible to not reach the required FAN levels (for example, when large proportions of unmalted adjuncts are used). Some breweries may use the so-called protein rests, which involve holding the mash at 45 - 50°C to increase the FAN level due to the activity of endogenous malt proteinases (Bamforth, 2009; Farber & Barth, 2019). However, excessive increases in FAN concentration can have adverse effects on the storage stability of the beer. High levels of FAN are associated with significant levels of stalling aldehydes (Maia et al., 2023). Lehnhardt et al. (2020) reports of elevated Strecker aldehydes content caused by high soluble nitrogen levels (Lehnhardt et al., 2020). For this reason, the use of unmalted adjuncts may be beneficial to reduce FAN content and increase flavour stability, by reducing the amount of Maillard reaction precursors (Kunz et al., 2011). However, too high proportion of unmalted adjuncts can provide too little FAN and low amounts of soluble proteins which results in emptier-bodied drink. It can also have an adverse effect on foam characteristics (Bogdan & Kordialik-Bogacka, 2017).

The currently available literature points out that various types of malts and unmalted raw materials can affect the storage stability of beer differently. According to Hellwig & Hentle (2020) the type of malt (light, dark, caramel, colour) is not a determinant of the content of Maillard reaction precursors (Hellwig & Henle, 2020). However, Shopska et al. (2021) report that malts subjected to higher heat treatment (which are characterized by darker colour) have a higher content of Maillard compounds with antioxidant activities, and thus higher antioxidant activity (Shopska et al., 2021). The higher antioxidant activity of beers with coloured malts is also reported by the (Liguori et al., 2021). In a study by Maia et al. (2023) addition of unmalted rice or maize at 50% of grist allowed to reduce total metal ion content, colour, thiobarbituric cid index, and stalling aldehyde content (Maia et al., 2023). Tsuji and Mizuno (2010) report fewer stalling compounds in beer made from raw materials other than malt (Tsuji & Mizuno, 2010). The use of LOX-less (lipoxygenase-less) malt may allow to obtain a wort with a lower nonenal potential. LOX-less malt lacks genes encoding lipoxygenase enzyme responsible for catalysing oxygenation of some unsaturated fatty acids. In the finished forced-aged beers obtained during the study, significantly lower concentrations of trans-2-nonenal and γ-nonlactone were determined than in the control sample brewed with traditional malt. In addition, the beers were characterized by significantly better results of sensory evaluation in the 'freshness' category (Yu et al., 2014). Additionally, adequate malting conditions can significantly reduce the nonenal potential of a malt (Guido et al., 2005)

As well as providing aroma and flavour compounds, hops are also an important source of polyphenols in beer production. It can provide up to 20-30% of these compounds to the wort, while the rest comes from malt (Mikyška et al., 2011). Current literature indicates that hop-derived polyphenols can have a positive effect on the storage stability of beer. This is confirmed by Mikyška et al. (2011), where the authors used different doses of hops (25-50-100%) in the production process. Higher doses of this raw material significantly reduced the formation of undesirable carbonyl compounds. The authors attribute this partly to the significant content of antioxidants in hops (Mikyška et al., 2011). The important role of hops in its raw form in terms of beer storage stability is also confirmed by the authors' previous research. Using the pelleted hops instead of hop CO_2 extract significantly increased the beverage storage stability (Mikyška et al., 2002). Drexler et al. (2010) reports that hops harvested at a later date of technological suitability (21 day difference between the harvest date) may provide better aroma stability during storage in dry-hopped beers. However, this improvement was only observed for the first 45 days of storage, after which the differences between the beers subsided (Drexler, 2010). The positive effect of hops on oxidative stability was also reported by Hrabia et al. (2022). Beers subjected to dry hopping showed a greater lag time (the time after which free radical reactions initiate) (Hrabia et al., 2022). Mikyška et al. (2022) report that aromatic hops, with a high polyphenol to α-acid ratio, can reduce sensory ageing to a greater extent than bittering hops (Mikyška et al., 2022). A similar effect was not observed by Jaskula et al. (2007), where no significant differences in bitterness stability were observed between hop types when early kettle hopping was used. Similarly, no effect of dry hopping on bitterness stability was observed. However, late hopping during the whirlpool phase resulted in the stabilization of iso-α-acids. Almost full stabilization of cis isomers was observed, with a partial stabilization of trans isomers. In terms of bitterness stability, the use of a pre-isomerized extract of tetrahydroiso-α-acids gave the best results, where the authors observed no change in their concentration even when prolonged forced ageing was used (Jaskula et al., 2007). Hops, particularly those sprayed with copper-based fungicides, can be a significant source of Cu ions. In addition, they can introduce significant amounts of manganese ions. In the authors' study, a 296-511% increase in Mn content was observed compared to the non-dry-hopped sample. In the case of Cu ions, it was 105% (Chrisfield et al., 2020). The significant content of metal ions in hops is also reported by Wietstock et al. (2015).

Conditions of the fermentation process

The state of the yeast and the conditions under which fermentation takes place are important factors in determining the storage stability of the beer. Kaneda et al. (1992) report that lower dissolved oxygen concentrations, higher yeast pitching rate, and clearer wort lead to higher sulfide production, which in the authors' study translated into higher beer storage stability. The strain of the yeast itself appears to also be of significant importance, which is confirmed by the results of the aforementioned study (Kaneda et al., 1992). These reports are supported by a study by Jenkins et al. (2021), where the authors studied 11 *S. cerevisiae* strains. The authors indicate that the application of yeast strain with high resistance to multiple oxidative stresses correlates with the production of beer with higher oxidative stability (Jenkins et al., 2021). Yeast strains which produce higher concentrations of sulfide may allow to obtain beers with higher stability. Sulfide production is probably strain-dependent (Saison et al., 2009). On the other hand, Murmann et al. (2023) report that higher FAN utilization may be a more important factor than sulfide production. In the authors' study, beer obtained using a strain that produced higher levels of sulfide scored lower in the general evaluation of sensory characteristics after storage (16 weeks at 35°C) than the beer obtained with a strain which utilized higher amounts of FAN. Beer produced with a high-sulfide-producing strain of *S. cerevisiae* had more fruity/vinous characteristics, which authors attribute to Maillard reactions (Murmann et al., 2023). Yeast can also complex Fe^{2+}, Mn^{2+}, and Cu^{2+} ions. However, in the authors study, the content of these ions in the wort increased towards the end of fermentation, suggesting yeast autolysis or secretion of these ions by yeast cells (Mochaba et al., 1996). Guido (2004) reports that pitching yeast with higher viability can result in higher sensory stability.

Metal Ions

Beer can also be seen as an aqueous solution of organic and inorganic substances. Ions of the latter are the important components of such solution. They are transferred with the water used in the production of beer, derived from raw materials, or equipment, or are intentionally added during the production process. Some of the ions (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, Mn^{2+}, Zn^{2+}) in the adequate levels are required for the correct course of mashing and fermentation, and/or directly affect the sensory characteristics of the beer. On the other hand, the presence of other ions, especially in excessive levels can cause sensory defects (Fe^{2+}, Fe^{3+} ions - metallic taste above 1 mg/L). However, it has long been known that transition metal ions are largely responsible for the formation of stalling aromas in beer. In the presence of Fe and Cu ions, stable oxygen molecules present in beer capture electrons and form superoxide anions. In a series of reactions reactive oxygen species are formed (O_2^-, $OOH^\bullet$, H_2O_2, $OH^\bullet$) which react with the organic compounds present in the beer. As a result, sensory characteristics of a beer can be altered (Preedy, 2009). In the case of Fe, concentrations of 50 ppb are sufficient to affect the sensory characteristics of the finished product. Zufall and Tyrell (2008) report that Mn ions can also act in a pro-oxidative manner. Manganese ions are required for the proper yeast growth. To determine the effect of Cu^{2+}, Fe^{2+} and Mn^{2+} ions, the authors added specific amounts of these ions during the beer production process. A significant decrease in Fe ions was observed during fermentation. In contrast, Cu content decreased during boiling and trub removal. The addition of Mn to the wort correlated with a higher Mn content in the beer. Accelerated spoilage was observed in beers to which Mn was added, which was manifested by the appearance of a sherry aroma after 4 weeks of storage. The effect of Mn ions was concluded to be detrimental to the storage stability of the beer, but lower than that of Cu and Fe ions (Zufall & Tyrell, 2008). According to Jenkins et al. (2018), the addition of even 10 ppb of transition metals can affect the oxidative stability of beer. In the authors' study, the addition of Cu caused the greatest reduction in lag time among assessed metals (Fe, Cu, Mn). The addition of Fe caused a reduction in lag time and an increase in T450 value (an indication of how much staling may occur in a particular beer). In line with the results obtained by Zufall and Tyrell (2008), the addition of Mn generally resulted in the greatest increase in T450 value. The effect of Mn ions seems to be detrimental (Jenkins et al., 2018). Application of selected metal chelators to the mashing process can reduce the content of transition metal ions. Mertens et al. (2021) report that additives such as green tea extract, tannic acid or pomegranate extract can reduce Fe content. Interestingly, in the study, addition of ethylenediaminetetraacetic acid (EDTA), which is known as a potent chelator, counterintuitively increased the Fe content of the resulting wort. Authors also observed that acidification of wort (from pH 6 to 5 using HCl) caused significantly higher levels of Fe, Mn, and Zn in resulting wort (230, 320 and 150%, respectively) (Mertens et al., 2021). Transition metal ions can also be partially removed by bitter resins derived from hops. Wietstock et al. (2016) report that in a buffered model system, hop acids showed the ability to complex Cu^{2+}, Fe^{2+} and Fe^{3+} ions. There was no observed complexation of Ca^{2+}, K^{+}, Mg^{2+}, Mn^{2+} and Zn^{2+}. The ability to complex ions depended on the pH of the medium and the acid tested (α-, iso-α, β-acids) (Wietstock et al., 2016). Maillard compounds and polyphenols can also exhibit chelating properties for Fe ions (Mondaca-Navarro et al., 2017; Mussche & Pauw, 1999).

Antioxidants

In brewing, sulfide, especially when it is present in excessive levels, is usually associated with defects, as it bestows a beer with a fetid smell of rotten eggs. Sulfide is a product of natural yeast metabolism. Typically, the concentration of this compound in beer is below 10 ppm, and higher levels of sulfide are produced under low yeast growth conditions (Lea & Piggott, 2003; Stewart et al., 2017). On the other hand, sulfide is also an important compound with the ability to prolong the storage stability of a beer. For some time, it was believed that sulfide action was related to the binding of carbonyls via adduct formation. However, it's activity is probably linked to antioxidant properties (Bushnell et al., 2003; Kaneda et al., 1996). Lund et al. (2015) report that protein-thiols could play secondary roles as antioxidants, mainly by quenching 1-hydroxyethyl radicals. However, theirs reactivity is lower than that of sulfide. Authors report thiols probably compete with hop-delivered bitter acids, as those also react with 1-hydroxyethyl radicals (Lund et al., 2015). The content of thiol groups is strongly correlated with sulfide content and beer oxidative stability. The content of thiol groups is not correlated with protein content, suggesting that these groups may be present in peptides and smaller molecules (Lund & Andersen, 2011). The lesser degree of thiol antioxidant reactivity compared to bitter acids is confirmed by the study of Andersen et al. (2017). In the study, authors performed a forced ageing of pilsner beer (IBU 35, 4.6% ABV). Thiols were found to react with around 9% of 1-hydroxyethyl radicals, while bitter acids accounted for 88%. The authors noted that the effect of polyphenols was not significant, probably due to the low reaction rates and low concentrations of these compounds in the beer tested (Andersen et al., 2017).

The positive effects of polyphenols on the storage stability of beer have been reported by many authors. In a study by De Francesco et al. (2020) the addition of phenolic-rich extracts significantly improved colloidal stability, colour formation and foam quality. It also increased flavour stability and had a protective effect on beer quality (De Francesco et al., 2020). The positive effect of individual polyphenols or beer additives containing high amounts of them, such as soybean extract, dried red raspberries or green tea, among others, on the storage stability of beer is reported by many authors (Bamforth & Parsons, 1985; Chen et al., 2023; Walters et al., 1997a; Yang et al., 2023; Yin et al., 2021). These additives are likely to increase the antioxidant activity of the beer. Another raw material that may increase the antioxidant activity of beer is spent hops (Jaskula-Goiris et al., 2014). Hop antioxidants suppress the formation of carbonyl compounds during the storage (Mikyška et al., 2011). However, some additives, such as olive leaves can harm the colloidal stability of a beverage (Guglielmotti et al., 2020). Although in the studies of the above-mentioned authors, the addition of polyphenols, or polyphenol-rich materials had a positive effect on storage stability, not all sources confirm such results. In the study by Walters et al. (1997) the addition of catechins (+) and ferulic acid had no effect on the rate of formation of stalling aldehydes (Walters et al., 1997b). The addition of tannins may also reduce the development of a sunstruck flavour (Munoz-Insa et al., 2015).

Technological operations

Pasteurization is a commonly used process in industrial beer production. Current knowledge indicates that this procedure can have a positive effect on the storage stability of beer. In a study by Lund et al. (2012), pasteurization increased the storage stability of beer as determined by electron spin resonance spectroscopy. In unpasteurized beer, a faster rate of radical formation and greater protein degradation were observed, probably due to the enzymatic activity of yeast residues. The authors suggest that higher protein content may have a positive effect on the storage stability of beer by binding prooxidative metals or by acting as a catalyst for the removal of H_2O_2 formed during oxidative reactions in beer (Lund et al., 2012). The positive effect of pasteurization is also reported by Hoff et al. (2013), where the authors subjected unfiltered beer to accelerated ageing (40°C, 41 days). The pasteurization process negatively affected the volatile compound composition of the beer, but after the storage period studied, the differences between the samples disappeared. During storage, pasteurized beers gave a lower rate of radical formation (determined using ESR spectroscopy). No differences were observed in the content of pro-oxidative metals (iron and copper) between the beers (Hoff et al., 2013). The positive effect of pasteurization on long-term storage stability is also reported by Liu et al. (2018). The staling grade of a fresh pasteurized beer was similar to that of draft beer after 1 month of storage. The ageing rate of pasteurized beer was slower than that of fresh beer (Liu et al., 2014). However, attention should be paid to the parameters of the pasteurisation. Cao et al. (2011) studied the deterioration of the beer sensory characteristics during 6-month storage of samples subjected to increasing PU (Pasteurization Units) – 2,8,14. With increasing PU, an intensification of colour, and a decrease in both polyphenol and ester content were observed, with a concomitant increase in undesirable compounds such as acetaldehyde or DMS. The unfavourable effect of higher PU values was also confirmed by sensory analysis, where beers with higher PU were rated worse than those with lower (Cao et al., 2011). Another interesting way to extend the shelf life of beer is the use of additives. The addition of chitooligosaccharide can limit the growth of LAB. In addition, these compounds can limit flavour deterioration by inhibiting the formation of stalling compounds, and increasing beer antioxidant activity (Yang et al., 2017; Zhao et al., 2016)

A refermentation in bottles – a procedure which is often used to carbonate the beer in homebrewing, and sometimes in the craft industry, can prolong beverage stability (Štulíková et al., 2020). Ferreira et al. (2019) report that the use of bottle conditioning reduced the release of the trans-2-nonenal from Schiff bases and resulted in the release of terpenols and phenols which positively affected the beers aroma. During the sensory evaluation, the intensity of attributes usually associated with beer ageing was judged to be absent or low (Ferreira et al., 2019). This is confirmed by the study by Saison et al. (2010), where the effect of refermentation on aged beer was studied; the yeast's have significant ability to reduce the compounds responsible for stale aroma. Cardboard, Ribes, Maillard and Madeira aromas were greatly reduced as a result of this process. A significant decrease in the content of (E)-2-nonenal, Strecker aldehydes, 5-hydroxymethylfurfural and diacetyl was observed (Saison et al., 2010). The ability of yeast to reduce stalling aromas/indicators has also been confirmed by other authors (Carneiro et al., 2006; Wang et al., 2006).

Storage temperature

Studies by many authors indicate that one of the key factors in extending the shelf life of beer is to store it at as low temperature as possible, preferably refrigerated. Valentoni et al. (2022) studied the effect of temperature on the storage of refermented craft beer for 13 weeks. When stored at 45°C, a reduction in IBU, a deepening of colour and an increase in the concentration of the beer's ageing products: furan compounds and aldehydes were observed. No differences were found between the 22°C and 6°C samples, and these beers showed less ageing signs. During sensory analysis, beers stored at 45°C were characterized by higher ripe fruit, cardboard, sweet and paint flavour, while freshness was lower than in beers stored at 22°C. This trend was evident up to 6°C, at which temperature the beer had the highest freshness (Valentoni et al., 2022). Walters et al. (1997) report that when beer was stored at 40°C for 156 days, a 71% loss of iso-α-acids was observed, while temperatures of 0 and 20°C were sufficient to arrest these losses (Walters et al., 1997b). Heuberger et al. (2012) report that when beer was stored at room temperature for 14 weeks, greater changes in flavonoids, purine bases and peptide concentrations were observed compared to a sample stored at 4°C (Heuberger et al., 2012). The lower temperature (4°C compared to 22.5°C) also reduces the losses of esters (Heuberger et al., 2016). For highly hopped ales, the lower storage temperature (~3°C) helps to retain a higher intensity of 'citrous', and 'tropical' qualities compared to storage at a higher temperature (20°C). When stored at a higher temperature, the malty, sweet, and alcoholic qualities are brought out (Kemp et al., 2021; Schubert et al., 2022). Barnette & Shellhammer (2019) reports that the loss of hoppy, fruity and citrousy characteristics is not reflected in a loss of terpene alcohols concentrations. The authors suggests the changes in concentrations of other compounds, such as polyfunctional thiols, aldehydes or lipid oxidation products might be responsible for such changes (Barnette & Shellhammer, 2019). In general, research by many authors indicates that keeping beer as cold as possible (~4°C) is the most beneficial way to prolong its shelf life in terms of aroma (He et al., 2012; Rodriguez-Bencomo et al., 2012). A similarly beneficial effect of low temperature on the stability of bitter substances is reported by (Gahr et al., 2020).

Restricting oxygen exposure

As mentioned earlier, because oxygen takes a part in radical reactions, its availability in the finished beer has a detrimental effect on the stability of the sensory characteristics. Wietstock et al. (2016) report that oxygen significantly promotes the formation of Stecker aldehydes during wort production. In the authors' study, the content of Stecker aldehydes in beer during storage was closely related to the available oxygen. The application of oxygen barrier liner crown corks significantly reduced the content of these compounds. The additional use of 10 mg/L EDTA effectively diminished the content of Stecker aldehydes (Wietstock et al., 2016). The increase in the content of 2-furaldehyde is oxygen dependent (Carneiro et al., 2006). However, the content of the some of the most prominent staling compounds, such as trans-nonenal might be not affected by oxygen levels. Lermusieau et al. (1999) reports the major source of this compound is the non-oxidative degradation of its precursors, synthesized before fermentation (Lermusieau et al., 1999). The negative effect of oxygen at most stages of beer production and storage is confirmed in the literature (Bamforth et al., 1993). The oxygen content in the beer headspace before pasteurization is correlated to the degree of stalling that would take place. Hempel et al. (2013), suggests the maximal oxygen content in bottle headspace of 1% (Hempel et al., 2013). However, the commonly used brewing procedure of oxygenating the wort before yeast inoculation, at a dose of 8 mg O_2/L of wort, does not seem to harm the storage stability of the beer, probably due to the rapid utilization of oxygen by the yeast (Depraetere et al., 2008). Kucharczyk and Tuszyński (2017) reports of no changes in the content of higher alcohols, esters and vicinal diketones when the aeration in the range of 7-12 mg O_2/L of wort was used.

Other factors

A characteristic feature of beer which significantly limits the growth of unfavourable microflora is its relatively low pH. The pH of most beers is in the range of 3.4-4.8, with most beers having a pH in the upper range (Preedy, 2009). The low pH of 3.3-3.9 is mostly found in sour beers (Ciosek et al., 2020). Beers with a low pH may be more prone to develop an oxidized taste during storage (Kaneda et al., 1997). Guyot-Declerck et al. (2005) report that 'cardboard' aroma (trans-2-nonenal) and cooked vegetables (methional) are less perceptible in beers with higher pH (4.6 vs 4.2). In contrast, coconut aroma (γ-nonalactone) may be enhanced. François et al. (2006) report that higher pH also reduces perceptible astringency in beer (François et al., 2006). Higher pH slows down the degradation rate of trans-iso-α-acids and leads to lower furfural concentrations. However, it can result in higher levels of Strecker aldehydes when FAN content is significant. In addition, it can increase the concentration of lipid oxidation aldehydes (Jaskula-Goiris et al., 2011). Gijs et al. (2002) report lower concentrations of β-damascenone in higher pH beers, indicating that the appearance of this compound is related to the acid-hydrolysis of its precursors. The concentration of methional, in contrast to the Guyot-Declerck (2005) study, was higher at higher pH (Gijs et al., 2002). Therefore, the effect of pH level on the stability of sensory attributes is ambiguous. However, based on the information presented above, it is known that lower pH can significantly reduce the risk of microflora development, particularly in non-alcoholic beers, or those with reduced alcohol content. Siebert (2009) reports that the maximum intensity of protein-polyphenol haze formation occurs at pH ~4 (Siebert, 2009).

Another important factor of beer stability is the type of container used. Gagula et al. (2020) report that when PET bottles are used, the composition of volatile compounds in beer changes significantly after only 1 month of storage. In the case of glass bottles and cans, after 6 months of storage, the authors estimated changes in volatile compounds to be minimal (Gagula et al., 2020). Similarly, inadequate barrier properties of PET bottles have been reported by (Lorencová et al., 2019). Containers that do not transmit light <500 nm (brown containers, cans) prevent the formation of sunstruck flavour caused by the formation of MBT. Beers with higher colour are greatly more resistant to development of this flavour. Removing riboflavin from beer can also prevent the formation of MBT (De Keukeleire et al., 2008; Duyvis et al., 2000; Sakuma et al., 1991).

CONCLUSIONS

Currently available literature clearly shows that to slow down detrimental sensory changes which take place during beer ageing, a few actions can be taken. To ensure the highest stability of a beer, it should be kept at low, preferably refrigeration temperatures. Additionally, several brewing practices can significantly reduce the potential for stalling during aging. Reducing oxygen exposure during wort production, limiting prooxidative metal ions content in brewing water, controlling free amino content levels, and appropriate raw materials can prolong beer stability.

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