

Vybrané vlastnosti ovocného piva vyrobeného z netradičních druhů ovoce

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Ovocné pivo – charakteristika, legislativní předpisy, sortiment.
Popište suroviny pro výrobu a technologické postupy.
Faktory ovlivňující vybrané vlastnosti piva

II. Praktická část:
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Proveďte základní provozní analýzy.
Výsledky výroby a analýz popište a diskutujte, vyvoďte závěry.

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- [1] Nardini, M., & Garaguso, I. (2020). Characterization of bioactive compounds and antioxidant activity of fruit beers. *Food Chemistry*, 305. <https://doi.org/10.1016/j.foodchem.2019.125437>
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ABSTRAKT

Netradiční přísady, jako je máslová dýně, nabízejí v pivovarnictví nové možnosti, i když jejich vliv vyžaduje charakterizaci. Tato diplomová práce zkoumala, jak přísady máslové dýně (0,0, 5,0 a 12,50 % hm. přidané do varu) ovlivňují konečné vlastnosti svrchně kvašeného piva; vyšší koncentrace se ukázala jako nevhodná pro analýzu. Hotová piva byla podrobena fyzikálně-chemickému (hustota, pH, TSS, etanol), reologickému a senzoričkému hodnocení. Zvyšování koncentrace máslové dýně až na 12,50 % hm. snižovalo konečný obsah ethyl alkoholu a postupně zvyšovalo konečné pH, což odpovídalo zásaditosti příměsi. Reologická analýza naznačila potenciální dilatantní chování, zejména u vzorků s přidaným množstvím máslové dýně, což lze pravděpodobně přičíst vyšší koncentraci pevných látek jak z přísady, tak z nefiltrovaného stavu piva. Senzorická analýza ukázala, že barva se mezi kontrolními pivy a pivy s obsahem dýně významně lišila ($p < 0,05$). Tato zjištění prokazují pozoruhodný vliv máslové dýně na chemismus a vzhled piva v rámci typických přísad a poskytují základní údaje pro pivovary.

Klíčová slova: máslová dýně, řemeslné pivo, chemie piva, reologická analýza

ABSTRACT

Non-traditional adjuncts like butternut squash offer novel possibilities in brewing, though their impact requires characterisation. This master's thesis investigated how butternut squash additions (0.0, 5.0, and 12.50 % w/w added to the boil) influence the final properties of a top-fermented ale; a higher concentration proved unsuitable for analysis. Finished beers underwent physicochemical (density, pH, TSS, ethanol), rheological, and sensory evaluation. Increasing squash concentration up to 12.50 % w/w decreased final ethyl alcohol content and progressively increased final pH, consistent with the adjunct's alkalinity. Rheological analysis suggested potential dilatant behaviour, particularly with butternut squash addition, likely attributable to the higher solids concentration from both the adjunct and the beer's unfiltered state. Sensory analysis revealed that colour was significantly different ($p < 0.05$) between the control and the squash-containing beers. These findings demonstrate butternut squash's notable impact on beer chemistry and appearance within typical addition rates, providing baseline data for brewers.

Keywords: butternut squash, craft beer, beer chemistry, rheological analysis

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This achievement is as much yours as it is mine.

*"A fine beer may be judged with only one sip, but it's better to be thoroughly sure."
- Czech Proverb*

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INTRODUCTION

Beer is one of the most consumed beverages in the world, ranked third overall after water and tea, and the most consumed among alcoholic beverages (Hornsey, 2016). The history of brewing starts at the beginning of urbanisation and civilisation in the Neolithic period (Eßlinger, 2009). Throughout this long period, different types of alcoholic beverages from a whole range of products were labelled as beer. However, nowadays, some of them are labelled as wine or as a distilled beverage (Poelmans and Swinnen, 2011).

Archaeological findings support the theory that beer is an accidental discovery in making bread (Novák Večerníček, 2015). Hence, since beer manufacturing is more complicated than the fermentation of fruit or honey, it is highly likely that beer is not the first or oldest alcoholic beverage made on Earth (Stewart et al., 2018). The incredible thing is that the brewing process has not changed that much throughout history. However, some critical technical advances helped us find more sophisticated and efficient ways to manufacture beer (Guido, 2013).

The first mention of fermented beverages made from barley was discovered in Mesopotamia and Egypt, where many different recipes came from. During that time, most beers were homemade, even if there was proof that some breweries were uncovered by archaeologists in ancient Syria. By the time of the Roman Empire, both beer and wine had already permeated the people living around the Mediterranean Sea. Moreover, its consumption was part of citizens' daily lives. It was the Roman Empire and its expansion all around Europe that helped beer expand (Mosher, 2017).

During the last few decades, the craft and experimental beer production surge has led to the manufacturing of both traditional and new beer types. Consumers' demand for a broader range of beer types also helped this latest surge of beer types. Because of the expansion, a new beer classification was to be made available. This extended classification was first proposed in 2022 by Bouchez and De Vuyst. The basic, four-production-type categorisation was not enough anymore. However, it was still the foundation for the new scheme, which considers three defining attributes of beer production: fermentation type, microorganisms implicated and acidification principles. Lactic acid and acetic acid bacteria were also included in this classification (Croonenberghs et al., 2024).

I THEORETICAL PART

1 FRUIT BEER

Fruit beers are a type of flavoured beer, into which fall a wide range of very different beers that have been flavoured at the brewery using an ingredient different from or as well as hops (Buglass, 2011). As craft beer and breweries became popular in recent years, fruit beer regained people's attention. It became a popular beverage choice for those who do not like bitter options.

Fruit beers can be defined as beer flavoured with fruits rather than alcoholic drinks made from fruits (Pérez-Alva et al., 2023). Thanks to this definition, separating alcoholic beverages made from pressed fruits, such as wines and ciders, is straightforward and easy to understand (Croonenberghs et al., 2024). Flavouring the beer can be carried out in different stages during the brewing process. The most favourite options are the boiling stage or, more likely, performed during the fermentation or maturation (Buglass, 2011; Zhao et al., 2023).

In 2024, Croonenberghs, with his colleagues, was the first to separate fruit beer into distinguishing categories, ranging from non-sweet fruit to sweet ones produced without the co-fermentation step.

Non-sweet fruit beers result from the co-fermentation process of beer/wort with any fruit that produces a slightly sweet to non-sweet alcoholic beer. As fruits contain additional saccharides and carry microorganisms residing on their skin, the results of the secondary co-fermentation process are less sweet or even sour beer compared with fruit beers without the co-fermentation step, while still maintaining most of the fruity flavours (Croonenberghs et al., 2024).

Traditional non-sweet fruit beers are from Belgium, such as the Belgian Oude Kriek beer, which is produced by adding fresh sour cherries to one-year-old lambic beer. The lambic sour beer used for production is a typical old type of spontaneously fermented beer typical for the areas around Brussels (Croonenberghs et al., 2024; Pérez-Alva et al., 2023).

Sweet fruit beers lack the co-fermentation step and result from mixing any beer with any fruit or fruit juice (Croonenberghs et al., 2024).

Before this categorisation was published, scientists mostly separated fruit beer into categories based on which form of fruit was added. Beer with fruits added directly, addition

of fruit extracts and beer with fruit-flavoured additives (Zhao et al., 2023). These categories can still be used as they are not based on the same parameters as the categories made by Croonenberghs et al. (2024).

1.1 Legislation

Fruit beer in the Czech legislation is explained as: „*ochuceným pivem je definováno pivo vyrobené s přidavkem látek určených k aromatizaci, potravin a surovin s vlastním aromatem, lihovin nebo ostatních alkoholických nápojů uvedených v § 21; obsah alkoholu pocházejícího z lihovin a ostatních alkoholických nápojů přitom nesmí překročit obsah alkoholu v původním pivu*“ in 248/2018 Sb. (Česko, 2018).

In Germany, the biggest beer producer in the world, the purity law is still in effect. This law prohibits the usage of ingredients other than water, hops, barley and yeast. The *Reinheitsgebot* was first introduced in 1516, and today, it is the oldest law concerning food production in the world. Over the centuries, the purity law underwent several amendments to accommodate new ingredients. It was in 1906 when yeast was added as a standard ingredient for brewing for the first time (Zýbrt, 2005). *Vorläufiges Deutsches Biergesetz*, the provisional beer law, was drafted in 1993, allowing more variety in malted grains, hops, and stabilising agents to be used for production (Germany, 1993).

In 2016, the European Union published Article 26(2) of Regulation (EU) NO 1151/2012 of the European Parliament and of the Council on quality schemes for agricultural products and foodstuffs as regards a name of traditional speciality guaranteed. For this regulation, Belgium submitted multiple names of lambic-style beers to be registered and protected per Article 25(2) of Regulation (EU) NO 1151/2012. Some of the beer type is obtained by adding cherries, cherry juice or cherry juice concentrates, like *Vielle Kriek*, where the fruit must have to represent between 10 and 25 % w/w of the final product (European Commission, 2016).

1.2 Assortment of fruit beer

With the recent rise of craft beer, fruit beer has also become very popular. Furthermore, the range of fruit beers on the market is increasing yearly. The oldest fruit beers are produced in Belgium, where in the 16th century, brewers added cherries to the beer. This gave birth to

Lambic-style beers. In the Czech Republic, fruit beers started to appear in the 21st century with the boom of craft breweries (Březinová, 2021; De Roos and De Vuyst, 2018).

Lindemans is one of the oldest producers of Lambic-style beer from Belgian breweries. Old Kriek Cuvée René, with cherry flavour and sour-sweet balance, is one of their best beers. Cherries are added to the 6-month-old Lambic beer, which restarts the fermentation process, after which the beer matures for 8 to 12 months before it is ready to be put on the market (Lindemans, 2025; Boulton, 2013).

In Italy, it has become popular to produce a new type of beer known as Italian Grape Ale in recent years. This beer is often said to be between beer and wine. IGAs are made by utilising pale base malts with grape addition ranging between 5 and 40 % w/w added in different fermentation stages (Mastrangelo et al., 2023; Siesto et al., 2023).

2 RAW MATERIALS

There are four main ingredients when we talk about the brewing industry. Water is the most important, with the highest percentage in the final product. Then, there are malt or malt additives, such as hops and yeast. In this part, these four ingredients were looked at more closely.

2.1 Water

Water is the most significant ingredient in the brewing industry. Beer contains between 90 and 98 % of this ingredient (Boulton, 2013). It has the highest total volume, making the ingredients very hard to ship from place to place, so breweries source water locally (Mosher and Trantham, 2017). Water contains essential minerals that can impact the final taste of beer. The ionic composition of the water used significantly affects the brewing operations and processes. In the past, this was determined by the development of brewing styles (Boulton, 2013; Palmer and Kaminsky, 2013).

It was determined that soft water is better for lagers and other bottom-fermented beers than hard water. On the other hand, hard water can bring a better taste and result in brewing ALEs and other top-fermented beers (Boulton, 2013). Softer water contains a low concentration of dissolved salts; hard water, on the other hand, contains high concentrations, mainly of calcium bicarbonate or calcium sulphate (Punčochářová et al., 2019). For example, regions with hard water, such as London or Dublin, are known for their darker beers, as high levels of bicarbonates tend to make the pH of the water too high. These levels of pH then affect both mash efficiency and yeast metabolism. Adding highly roasted grain to this water helps lower the pH to the optimum acidic volumes (Goyal et al., 2023; Mosher and Trantham, 2017; Palmer and Kaminsky, 2013).

Most water must be treated before usage in any food production, and brewing is no exception. Water treatments aim to remove undesirable suspended solids and dissolved components, as they can help introduce spoilage microorganisms or produce effects that directly and badly affect the brewing process (Boulton, 2013). The addition of sodium chloride is sometimes done to enhance “palate-fullness”; potassium chloride can be added at low concentrations to help achieve a less sour flavour (Punčochářová et al., 2019). If water

salinity is too high, reverse osmosis can reduce water hardness and remove harmful ions (Zhao et al., 2010).

The volume of water required is many times higher than the volume of beer produced; to produce one litre of beer, depending on the size and technical condition of the brewery, from 7 to 12 litres of water is used (Chládek, 2007). With modern technology, the current ratio is more about 1 litre of beer to 4-5 litres of water.

Water in a brewery can be separated into three main distribution categories. The biggest group is brewing water, which is used as an ingredient in brewing. The second group is made with washing water and water used for sterilisation. That water must be without any microorganisms or chemical contaminants. The last group is created with operational water (Basařová et al., 2021; Fillaudeau et al., 2006).

2.2 Grains and malt

The production of malt usually does not happen inside the brewery itself. Most of the breweries buy finished malt from the malting plant. The maltster provides the brewery with a large selection of malts that can be used in brewing.

Malting describes how various plants' seeds or grains can germinate under controlled conditions. The most common grain used is barley. However, it is not the only grain which can be malted. Maise, oats, rye, sorghum, rice and others may also be used (Boulton, 2013).

Barley is superior to other cereals thanks to its unmalted seeds' higher starch-to-protein ratio and lower moisture content, with 60-68 % w/w amount of starch in dry matter. Starch is a naturally occurring substance consisting of amylose and amylopectin molecules. Amylose fraction is a linear chain with the glucosyl units connected by α -(1-4)-linkages (Li and Zhu, 2018). Amylopectin is, on the other hand, a highly branched structure composed of more than a thousand linear α -(1-4)-D-glucan unit chains and around 5 % of α -(1-6)-glucosidic bonds (Punia, 2020). Barley also contains complex carbohydrates (80 %), β -glucans (3.7-7.7 %), proteins (11.5-14.2 %), lipids (4.7-6.8 %) and ash (1.8-2.4 %) (Li et al., 2024b).

Malt is usually made from spring barley, *Hordeum vulgare* L., a member of the grass family *Poaceae*. Malt substitutes are used mainly for brewing a special beer or for economic

reasons. Rice is primarily used for making gluten-free beer (Basařová et al., 2021; Kok et al., 2019).

Malt substitutes are divided according to the processing method into starchy (indirectly processable) and sugary (directly processable). Starchy malt substitutes include unsweetened cereals (maize, rice, wheat, sorghum or oats), starchy leaches, syrups, concentrates, and special malt substitutes. Sugar substitutes include crystal, raw, inverted or starch sugar (Basařová et al., 2021).

2.3 Hops and hops extract

At the beginning of brewing, hops were not used at all. Instead, other additives are often found in historical recipes, which were used to flavour the final product. Various plants, fruits, vegetables and spices were used for this purpose. While hops were used in brewing as early as 800 years ago, it was not until the 13th century that hops became a consistent ingredient in flavouring beer (Mosher and Trantham, 2017).

Hops are dried flowers of the hop plant *Humulus lupulus* L. These flowers, which are climbing, herbaceous perennial flowers in the family Cannabaceae (Mosher and Trantham, 2017; Steward, 2016), are rich in the volatile compounds that give beer its characteristic odour (Coultate, 2002). Today, up to 98 % of the *H. lupulus* crops produced worldwide are used in the brewing industry (Korpelainen and Pietiläinen, 2024). Hop cones are now used only in a few breweries where beer is still brewed in the most traditional way. Most breweries use processed hops as hop extract or pellets prepared from ground flowers (Barth, 2013; Priest and Campbell, 1996).

Even now, hops are an irreplaceable raw material for beer production as they give the product unmistakable bitterness and aroma, which separates beer from other alcoholic or non-alcoholic beverages. Substances unique to hops are hop resins, essential oils, and polyphenols. The holders of hop bitterness are resins (10-30 % w/w of dry matter of hops, according to Sun et al. (2022), which can be separated into soft (9-27.5 % w/w) and hard (1.5-2.5 % w/w) resins. Soft resins are used in brewing beer as they contain bitter acids (Korpelainen and Pietiläinen, 2024; Sun et al., 2022). Resins are strongly influenced by the isomerisation products of α -acids and β -acids (Basařová et al., 2021; Coultate, 2002). The most common α -acids are the humulone, cohumulone and adhumulone, in the β -acids it's

the lupulone, colupulone and adlupulone (Barth, 2013; Steward, 2016). β -acids are not easily isomerised, and they generally become destroyed during the beer brewing process. These acids are also hydrophobic and not soluble in water (Korpelainen and Pietiläinen, 2024).

The α -acids contribute most significantly to the beer's bitterness after conversion during wort boiling to soluble iso- α -acids. Mainly isohumulone. Some or all parts of the kettle hops can be replaced by pre-isomerised hop extract, which could be added to the beer after fermentation instead of using hops during the wort boiling. The essential oils from the hops are a complex mixture of terpene-related compounds and form only a tiny portion of the dried hop (0.5-3.0 % w/w) (Korpelainen and Pietiläinen, 2024). These compounds can impart the aroma of hops (Ingledew and Hysert, 1994; Roberts, 2016; Steward, 2016). The levels of the aromatic hop oils and other biochemicals then depend on several factors: variety, ripening stage, weather conditions, soil composition and storage (Alonso-Esteban et al., 2019; Sanz et al., 2019).

Different varieties of hops are specially cultivated to have higher levels of lighter compounds, thus intended for the aroma (Mosher and Trantham, 2017).

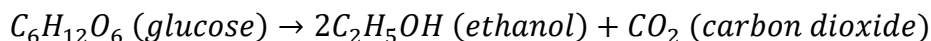
Hops substitutes are substances used instead of hops to impact the bitterness and flavour of the beer. It is used primarily when climatic and economic considerations prohibit conventional brewing materials, like in Africa, where malted barley is used instead of malted sorghum. The hops substitutes include seeds from *Garcinia kola* and extracts from bitter leaf *Vernonia amygdalina* (International Food Information Service, 2009).

2.4 Yeast

Yeast is a single-celled eukaryotic organism which is technically a fungus. Most yeasts reproduce asexually by mitosis. However, the yeasts used most for brewing beer are reproduced by an asymmetric process called budding (Mosher and Trantham, 2017). This yeast can also occasionally produce four sexual ascospores in a sac called an ascus (Ingledew and Hysert, 1994). On a nutritional level, yeasts are less demanding than other microorganisms, such as lactic acid bacteria. Their growth is supported by the existence of basic compounds: fermentable sugars, amino acids, vitamins, minerals and oxygen. Yeasts can be separated into two large groups: *Saccharomyces* and non-*Saccharomyces*, where

Saccharomyces cerevisiae is the most studied species and the most utilised in the fermentation of beers (Basařová et al., 2021; Maicas, 2020).

From a biochemical point of view, fermentation is carried out by yeasts when pyruvate generated from glucose is broken down into ethanol and carbon dioxide (Maicas, 2020). The scheme of the chemical equation to produce ethanol is as follows:



Let us look at the yeast from a morphological view. Yeast presents a high morphological divergence, with the most common shapes being round, ellipsoidal and oval (Maicas, 2020), with a size between 3 and 15 μm depending on the genus and cultivation conditions (Kopecká et al., 2012).

From a taxonomic point of view, brewing yeast strains are part of the genus *Saccharomyces* and of the species *cerevisiae* or others, depending on the type of beer. The top-fermenting yeast is *Saccharomyces cerevisiae*, and the bottom-fermenting yeast is *Saccharomyces pastorianus* (Piggott, 2012). The latter yeast is a hybrid yeast that combines phenotypes of both its parental species, *Saccharomyces cerevisiae*, which has high fermentation capacity, and *Saccharomyces eubayanus*, which is more tolerant to colder temperatures (Thesseling et al., 2019).

For the top-fermenting yeast, the strain must be able to work at warmer temperatures, at about 16-22 °C. Once the fermentation is done, the yeast flocculates at the top of the vessel.

For the bottom-fermenting yeast, the yeast strains ferment at lower temperatures, at about 6-16 °C. Furthermore, once the fermentation is finished, they will settle at the bottom of the fermentation vessel (Iorizzo et al., 2021; Piggott, 2012).

A third category of yeast can be used in the brewing industry: non-*Saccharomyces* yeast, which can develop beers with a broad range of flavours. Non-*Saccharomyces* yeast has lower fermentative power and ethanol production. However, they possess characteristics absent in *Saccharomyces* yeast, such as metabolising desirable aroma-active substances, such as monoterpenes, phenols, acids and fruity esters (Iorizzo et al., 2021; Maicas, 2020).

For example, *Brettanomyces* yeast species are commonly present in the later stages of Belgian lambic beer fermentation (Thesseling et al., 2019).

2.5 Fruit component

Fruit is the most crucial part of the production of fruit beer. As a plant product, fruit is rich in flavour and nutrients, which is why it has been used as an ingredient for beer for centuries. The best-known example of fruit beer is Belgium's lambic beer, which became popular in the 16th century (Witrick et al., 2017). It is also the earliest example of fruit beer globally (Park et al., 2019).

All kinds of fruits can be added to beer, such as drupes, kernel fruits, tropical fruits or berries (Zhao et al., 2023). These fruits can also be added in multiple forms; whole fruit, fruit juice or puree is the favourite (Shopska et al., 2024).

Fruits introduce new flavours and increase the concentration of bioactive compounds. In some examples, adding fruit changes basic physicochemical parameters such as pH, colour and alcohol content. Antioxidant activity can also improve (Castro Marin et al., 2021; Kawa-Rygielska et al., 2019). A study by Nardini and Garaguso (2020) determined that cherry beers had the highest values of antioxidant activity, total polyphenols and flavonoid content, closely followed by grape, plum and orange beers.

3 BREWING PROCESS

The process of making beer is built on historical experience. Through time, the process changes minimally, even if technology changes drastically. Here is a short understanding of each critical step in beer production.

3.1 Malting

Malting is the first step in brewing and converts barley grains into fermentable malt (Cribb, 2005). The steps of malting are steeping, germination and kilning.

The first part of the process mimics what would typically occur in nature if the barley were left in the field. The grain is steeped in cold water, which is drained occasionally so as not to asphyxiate it. At least two to three wet and dry periods are needed so the moisture of the grain is raised from storage levels to approximately 45 % to 46 % (Mosher and Trantham, 2017); some can also say up to 48 %. This process should occur between 40 and 60 hours at a 5-20 °C temperature. Bacterial contamination can also be problematic, so the steeping liquid should be frequently changed (Ingledew and Hysert, 1994).

After the moisture is raised, the grains are laid out as a shallow bed to let them start growing and producing roots and embryonic shoots. The passing of chilled wet air helps to keep moisture and cool grains (Steward et al., 2018). During this time, occasional turning and mixing are also needed as they help prevent the rootlets from moving together (Briggs et al., 2004). Slowly revolving drums can turn over the growing malt (American Society of Heating, Refrigerating and Air-Conditioning Engineers, Inc., 2022). Each cone of barley should germinate at the same rate, with 97 % or more sprouting in 72 hours at 18 °C (Ingledew and Hysert, 1994).

The grain embryo then produces a plant hormone, gibberellin, which activates the aleurone layer of the grain. This layer produces unique enzyme proteins (Steward et al., 2018). Usually, it is the α -amylase which is produced. Amylases are extremely important as they liquefy the starch to dextrin and convert it to fermentable sugars like maltose and glucose. Other enzymes, which are synthesised during germination, carry out protein degradation, dissolution of the cell wall of the endosperm, liberation of inorganic phosphate and the degradation of maltose and maltotriose to glucose (Ingledew and Hysert, 1994; Parés Viader et al., 2021). These enzymes, together with the ones already in the grain, begin to

break down the food reserves of the grain, and if this continues, new barley plants will be formed. However, thanks to heating, **kilning**, and the grain with hot, dry air (49-66 °C), the germination of the shoots and roots stops, and moisture is removed from the green malt. Firstly, moisture is removed down to 8-14 %; then, the heat is increased for the reduction of moisture down to 4 % w/w. (American Society of Heating, Refrigerating and Air-Conditioning Engineers, Inc., 2022; Steward et al., 2018). Enzymes are not extensively destroyed in green malt, the name of immaturity, but some are partially degraded (Ingledew and Hysert, 1994).

The white malt is kilned using low temperatures, so the enzyme survival is considerable. If the malt is darker and coloured, they are kilned at higher temperatures, so the enzyme survival is lower. This malt is called caramel or crystal malt and is made by kilning wet malt under steam pressure (Arendt and Zannini, 2013). In highly dark cases, special malts are heated in a roasting drum; the finished product contains no active enzymes (Briggs et al., 2004). This product is sometimes called ‘chocolate malt’ (Arendt and Zannini, 2013).

Roots are removed mechanically, and the finished product is ready for use (Steward et al., 2018). Culms are usually used for cattle feed.

3.2 Milling

Milling is the most common step at the start of brewing for most breweries. Malted grain, imported from malting plants, must be milled first to produce a range of smaller particles called grist (Steward et al., 2018).

This mechanical process helps expose the endosperm full of white starch (Mosher and Trantham, 2017). Exposure of the endosperm is necessary for the physico-chemical and enzymatic reactions in the process (Basařová et al., 2021). Another purpose of milling is to make the malt easier to wet during the next mashing stage and to extract the soluble components faster.

More types of mills are used for malt, such as roller mills that produce coarse grist for use with mash tuns or lauter tuns (Stewart, 2016). The rolls are arranged in pairs with adjustable gaps between them to allow the crushing of different types of grain. The grains are broken by a combination of crushing, shear and cutting. In more complex types of roller

mills, there can be more than one pair of rollers (Boulton, 2013). Another type of mill is the hammer mill, which produces much finer grist thanks to more severe grain crushing (Steward et al., 2018), containing a chamber into which the grains are introduced at a controlled rate via a rotary valve. Hammers inside this chamber impact against the grains and, in doing so, crush them. Impact mills consist of disc and hammer mills, and rather than relying on grinding, cutting and shear forces, these mills disrupt and degrade grains by violent contact with hard surfaces (Boulton, 2013).

Most mills are designed to crush dry grains (Mosher and Trantham, 2017); in some cases, the grains must be moistened before milling to make the husk more flexible to keep the interior dry and, therefore, brittle (Boulton, 2013).

Several larger breweries prefer to mill the malt grains into a fine powder, resulting in a more effective conversion of starch to sugar. On the other hand, smaller breweries mostly prefer coarse milling, where the grains are cracked, but the hulls are left intact. That way, they can act as a filter bed for the wort (Burglass, 2011).

3.3 Mashing

Mashing, the next step in this production, is the process of mixing the crushed malt and, if used, cereal adjuncts with controlled amounts of water at a chosen temperature (Briggs et al., 2004). It is the process of turning the malt, the grist, into a sweetened liquid (Ruiz-Medina and Fernández-de Córdova, 2015). The mixture is then allowed to stand while the enzymes degrade the proteins and starch to yield the soluble malt extract, which is called wort, at this stage, unhopped (Stewart, 2017). The starches are hydrolysed into sugars, and complex proteins are broken down into simpler proteins, polypeptides and amino acids (American Society of Heating, Refrigerating and Air-Conditioning Engineers, Inc., 2022).

The main reason for mashing is to provide enzymatic access to starch in the malt for maximal extract recovery. One of the main complexes of polysaccharides to break down is β -glucans. The activity of β -glucanase is known to occur optimally between 45 and 50 °C, while proteolytic enzyme activity occurs around 50 °C (Burglass, 2011).

Mashing is complete when the starches are converted to iodine-negative sugars and dextrins; the temperature is raised to stop the amylolytic action. Wort is then separated from

the mash solids using a lauter tub or other equipment (American Society of Heating, Refrigerating and Air-Conditioning Engineers, Inc., 2022).

According to the brewery's beer type, there are different mashing techniques for which different equipment and materials are available to the brewer (Stewart, 2017).

3.3.1 Decoction

A decoction is typically a European mashing system and is most used for brewing lager beer (Stewart, 2017). The system uses a series of different temperatures, where the mash is held for a while, then the temperature is increased and held again. According to Ingledew and Hysert (1994), these temperature rests are designed to allow adequate time for converting starches into various sugars, including dextrins and those that are fermentable, concluding with a final temperature rise.

Decoction historically refers, in some places also traditionally, to heating the mash mixture by removing a portion of the overall mash, heating it to a boiling point, and returning it to the main mash, where it increases the temperature. A decoction can have multiple runs, achieving a different rest temperature by varying the amount of heated mash (Burglass, 2011).

In European breweries which still use the decoction regime, double decoction is used the most. The process starts with mixing the milled malt with liquid to make wort at about 40-50 °C. A part of the wort, where the amount is determined by the recipe (usually about one-third of the wort) (Goyal et al., 2023), is separated from the wort and heated up to the first sugar-making temperature, which lies between 62 °C and 65 °C, after reaching this temperature, it holds for about 10-20 minutes.

Then, part of the wort is heated again to the second sugar-making temperature between 72 °C and 74 °C, where the temperature is held for another 10-15 minutes. The next step is heating the wort to a boil for another 10-20 minutes. After the boiling, the boiled part is mixed slowly with the rest of the wort, which was separated at the beginning. After mixing the wort together for about 5-10 minutes, where the temperature after the mixing is usually at the first sugar-making temperature, the wort is separated again. The cycle repeats, but starts with heating up to the second sugar-making temperature. After the second cycle is finished, the wort is prepared for the next stage in the brewing process.

This type of mashing has a historical reason. In the past, it was hard to control the brewing process temperature, so by removing a part of the mash and heating it before returning it to the more significant part of the mash to increase the temperature, brewers were able to hit multiple temperature zones, which are required for the ingredients used or for the beer styles that are being made (Burglass, 2011; Holbrook, 2020).

3.3.2 Infusion

Ingledew and Hysert (1994) state that infusion mashing typically occurs in a single container. The process involves an initial mashing period of about 20 minutes at 53-68 °C, followed by a 30-minute rest to facilitate conversion. The temperature is then increased to 75 °C before runoff begins. This method can be performed as either a single or multi-step infusion, also called step-mashing (Holbrook, 2020).

Single infusion mashing is a practice of mashing in malt with temperature-controlled water without additional heating. It usually occurs in a combined mash-lauter vessel, where mashing and the next step, lautering, happen. Suppose the temperature is too cool at the end of mashing-in, and the malt starch may not be sufficiently gelatinised. On the other hand, if the temperature is too hot at the end of the process, critical amylase enzymes may be denatured or rapidly inactivated during the mash rest. That can result in a wort with a very low fermentability. Typical mash rest in the infusion process is between 60 and 70°. This temperature range should sufficiently ensure the broadest scope of amylase enzyme activity that mashing at one temperature can provide. Single infusion is the standard mashing regimen used in the United Kingdom and the USA (Holbrook, 2020; Kinčl, 2022).

3.4 Lautering and wort separation

The next step in the beer-making process is lautering and, subsequently, wort separation. It starts when the mashing process is complete, and the wort that has now been produced from the grains must be separated from the insoluble material. The general purpose of this step is to separate the particles from the liquid part. And to recover as much residual extract as possible from the grains while maintaining the desired wort quality (Holbrook, 2020).

Vorlaufing, the first step of lautering, is to recirculate the wort over the top of the mash bed. This is done for two reasons: one is to set the grain bed and to establish a workable differential pressure across the mash bed, which will lead to a smooth and efficient run-off

without destroying the grain bed or sticking it to the false bottom, which would lead to an increasingly tricky run-off. This also helps with clarifying the wort. The second reason is establishing the grain bed as a filter bed to collect the wort in the vessel (Basařová et al., 2021; Holbrook, 2020).

Once the wort collection begins, in the wort kettle or a separate lautering wort buffer tank, the first running of the wort will be of the highest density, usually measured in degrees Plato. As the run-off progresses, the volume of the liquid in the tank and above the mash bed will decrease, eventually exposing the filter bed. When this happens, applying sparging liquor to the top of the bed should begin (Briggs et al., 2004; Holbrook, 2020).

In most breweries, the lautering happens in a Lauter Tun vessel. This vessel is equipped with a slotted floor or set of screens, called a false bottom, on which the mash bed sits (Barth, 2013). This setup allows spent grain solids to be held back while the sweet wort is drawn down and collected on the hard bottom of the vessel, which is later used in the next step. Vorlaufing happens before the actual collection of the wort. After this step, the actual wort collection can start based on the time limit or getting the desired wort quality (Holbrook, 2020).

The first running starts at a lower speed so as not to cause a significant pressure difference between the top pressure on the mash bed and the pressure at the bottom of the vessel. As run-off continues, ensuring the mash bed's top is not completely dry will be important, as it could collapse and destroy the filtering bed. At this point, it is time to introduce the sparging water at a maximum temperature of 78 °C to the top of the mash bed (Holbrook, 2020). Adding hot sparging water can help heat up the grain bed, which reduces wort viscosity when combined with the gradual weakening of wort concentration. (Briggs et al., 2004; Holbrook, 2020).

Grain removal should commence immediately after the end of the run-off so as not to let it dry. In a small Mash Tun or Lauter Tun, the removal can be done manually from the door at the bottom or top of the vessel. Manual removal would be impractical if the Lauter Tun were on the bigger side. That is where the 'plow' blade is usually incorporated as part of the raking equipment.

3.5 Hopping and wort boiling

After the grains are removed, the wort is heated until boiling. Hops can be added in many forms. Whole-leaf hops are now used only in some cases, mainly if a special beer is made. Instead of whole-leaf hops, the most popular option is to use pellet hops. Another form used in modern breweries is hop extracts, and hop powder is often used (Priest and Campbell, 2003). Wort boiling is also subsequently applied for wort sterilisation, removing unwanted volatile compounds and the precipitation of protein and polyphenol complexes (Kosseva and Webb, 2020).

The hop dose depends on the desired bitterness of the finished beer. Later, the hop is added, the more the beer will be bitter. Hopping happens in one, two or three doses. One dose is used for beer with less bitterness, where the first dose occurs at the start of hopping or 10 minutes after the start. Two or three doses are needed if the final product must be more bitter. If there is only one dose, the whole dosage is added at the start or 10 to 15 minutes after the start of the hopping. If there are two doses, one is added at the start of hopping, which is the bigger part of all the added hops (around 70-80 %). The second dose is then added between 15-30 minutes after the start of hopping, where the number of added hops is between 20 and 30 %. If there are three doses, the first one (50 %) is added at the beginning, the second (35 %) after 50 to 60 minutes and lastly, the third (15 %) 10 to 15 minutes before the end of hopping (Basařová et al., 2021; Kinčl, 2022).

3.6 Trub separation

Following the hop addition phase in brewing, it becomes necessary to separate the liquid wort from solid hop debris, known as trub (Stewart, 2017). The most common method for achieving this separation utilises a specialised tank called a whirlpool. Wort is introduced into this vessel tangentially at a high velocity, causing it to rotate rapidly. According to Goyal et al. (2023) and Mosher and Trantham (2017), the resulting centrifugal effect forces the denser trub particles to accumulate in a cone at the tank's centre.

However, Stewart (2017) notes that techniques like sedimentation or filtration can also be used as alternatives to whirlpooling for removing these residues.

3.7 Cooling and pitching

When the wort is separated from the residues, it is time to cool it down so it can be pitched with yeast (Kinčl, 2022).

Modern breweries rely primarily on plate heat exchangers for cooling wort, replacing the easily contaminated open systems previously used. Kinčl (2022) highlights the benefits of these exchangers, such as their high cooling efficiency, adaptability, ease of expanding capacity, and compact nature compared to open coolers. The system passes wort and a cooling medium (commonly water) on opposite sides of internal plates; this separation prevents mixing and potential spoilage of the wort while allowing effective heat exchange.

Pitching temperatures and fermentation times vary based on beer style. Ales (top-fermented) generally require pitching between 15-22 °C (18-22 °C per Stewart, 2017), while lagers (bottom-fermented) are pitched cooler, between 6-12 °C (8-15 °C per Stewart, 2017). Consequently, ale fermentation typically lasts 5-7 days, whereas lager fermentation takes longer, around 8-14 days (Yeoman et al., 2021). Yeast multiplication during this stage can lead to a fourfold increase in cell count (Bryce and Morris, 2000). Prior to pitching, Briggs et al. (2004) emphasise the need for swift, aseptic cooling of the wort to arrest chemical changes and minimise contamination risks.

3.8 Fermentation

There exist two main types of fermentation systems in the beer industry. Top fermentation is used in American kinds of beer, like ALEs or IPAs, and bottom fermentation is mainly used in Europe for lagers, the most popular type of beer in the world. We use different types of fermenting vessels and different yeasts for each system.

Beer fermentation has multiple stages, which correspond with the yeast reproduction stages. We can observe these stages on the surface of an open vessel. Inside cylindroconical vessels, the fermentation is faster as the conditions are easier to manage.

1 – Lag phase

2 – Active phase

3 – Stationary phase

4 – Conditioning phase

The lag phase is the first stage, where the foam forms on the surface thanks to carbon dioxide production. This step usually happens in the first 24 hours; the pH and extract value decrease, and the temperature rises.

The active phase starts between 24 and 40 hours after the start of the fermentation. It is the time of the highest carbon dioxide production, as the yeast is at the growth peak. This phase can be observed due to the formation of the foam on the top of the surface. In some literature, this foam is called krausen (from the German word *kräusen*, which means to curl) (Wang et al., 2021). The pH and extract value are still decreasing, and the temperature is rising.

The stationary phase happens between the third and fifth day of fermentation. The foam rings, which were now nicely white, turned brown due to sludge carried out from the fermentation medium. pH and extract value are still decreasing, and the temperature rises to the maximum. At this temperature, the fermentation continues for up to two days before the wort must be cooled down by 1 °C per day.

The conditioning phase is a stage in fermentation where yeast clumps and sediments occur. The foam rings are collapsing, and only a few centimetres of foam remain on the surface. This foam, often called a ‘blanket’, must be collected in time to prevent its dispersion in the wort. The ‘blanket’ contains polyphenols, bitter substances, polysaccharides and dead yeast cells (Basařová et al., 2021; Kinčl, 2022).

3.8.1 Bottom fermentation

If we talk about bottom-fermented beer, the more traditional fermentation method is used. Of course, even in bottom-fermented beers, like lagers, we can use a cylindroconical vessel (par 3.8.2) as it is much faster than the traditional method. Traditionally, bottom-fermented beer is fermented in a single vessel, open to the atmosphere, to facilitate yeast removal.

In medieval times, these vessels were made from wood; gradually, breweries moved from wood to concrete and steel with various coatings to stainless steel, which is used now (Kinčl, 2022). These open vessels are usually smaller than cylindroconical vessels, at about 80 to 1000 hl. Vessels are also usually grouped together in fermenting rooms, and to lower

the risk of microbial infection, the room's surfaces must be smooth and easy to clean. Drainage has to be possible, and these drains must be constructed to avoid foul odours (Briggs et al., 2004).

Some examples of bottom-fermented beers are pale lager, which has a very light golden colour with a light flavour. On the other hand, Dark lager has an intense colour and is generally bitter with some sweet variants. Pilsner is a typical beer brewed in the Czech Republic. Bocks are German beers with heavy, malty flavours that can make them nutty and sweet. Marzen, also known as “march beer”, is a golden to deep amber-coloured lager with a bitter taste (Goyal et al., 2023).

3.8.2 Top fermentation

Top fermented beers are mostly fermented in cylindroconical vessels, which were the work of Leopold Nathan, who, in 1908, made the first ever cylindroconical vessel. These vessels or tanks can range in size from 100 to 6000 hl. These vessels cannot be entirely filled for the first fermentation, as a considerable amount of foam is created thanks to the evolution of carbon dioxide during the fermentation. This could cause pressure-release valves to block (Briggs et al., 2004).

Some examples of top-fermented beers are pale ales, which are full of flavours but not too heavy. Porter and Stout are fermented at a high temperature and can be easily identified by their colour and a hint of sweetness. These beers are almost black, and this colour is developed thanks to roasted barley or malt. Wheat beer is another type of top-fermented beer that is typically very light in colour and has a less bitter taste. Brown ales are brown to deep copper and can taste almost like chocolate or caramel (Goyal et al., 2023).

3.9 Maturation

Maturation is the longest part of the brewing process in which clarification takes place, the beer's final taste is formed, and it is saturated with carbon dioxide (Kinčl, 2022). The maturation technique varies from brewery to brewery. In the case of lagers, beer is transferred from an open vessel to a separate tank for maturation before the end of the first fermentation, which happens at a temperature of 10-14 °C. The beer must be cooled down to achieve a temperature between 4-6 °C. This cooling cannot be done rapidly as it could

give the product shock and induce the yeast to excrete protease enzymes that could be detrimental to foam stability (Briggs et al., 2004).

3.10 Methods of fruit incorporation into the brewing process

As shown above, beer brewing is a complex and lengthy process of multiple steps, where fruit components can be added at any stage. The chosen addition time then determines what properties are wanted (Egushova et al., 2024).

In most studies, fruit was added during the fermentation as the sugars in fruit provide nutrients for yeast and can affect yeast metabolism by altering osmotic pressure (Zhao et al., 2023). Adding fresh fruits during fermentation can cause a significant increase in bioactive compounds such as flavonoids and phenolic acids (Salaňă et al., 2020). Gorzelany et al. (2022) also decided to enrich wheat beers with fruit during fermentation and studied the change.

Fruit may be incorporated early in the brewing process, such as during mashing or boiling, primarily to contribute fermentable sugars for the yeast to consume. However, this early addition typically yields minimal fruit flavour or aroma in the finished beer because the volatile compounds responsible for these characteristics tend to be driven off by heat or during fermentation. Consequently, brewers usually avoid using expensive or flavour-forward fruits, like berries, during these initial stages (Cunha et al., 2023; Moreno et al., 2022).

Fruit can also be introduced toward the end of the brewing process, during maturation or as a final addition. One consequence of adding fruit during maturation is a potential decrease in the beer's final ethanol level because of the added liquid volume (Moreno et al., 2022). However, late additions can also contribute positively to flavour; research by Georgieva et al. (2021) showed that blueberry additions at the maturation stage promoted the formation of esters, key aroma compounds in beer.

4 SELECTED FACTORS INFLUENCING BEER PROPERTIES

Multiple factors can influence the beer quality and its properties. From the malt to the quality and composition of water, the ingredients used play a significant role in the quality of the final product. Another important factor is the brewing process itself.

4.1 pH

pH is an important criterion, together with total acidity, for brewers due to its influence on sensory attributes and biological and chemical stability. Generally, common beer has a low pH of around 3.8-4.7. Such a low value influences ageing and stability by affecting resistance to microbial spoilage, foam and flavour stability, palate smoothness, and drinkability. Craft beer usually has a slightly higher pH, between 4.27 and 4.92. These values were also substantiated in a study by Granato et al. 2011, who reported pH values from 4.13 to 4.97 (Granato et al., 2011; Silva et al., 2022).

From a sensory view, if the pH of the beer decreases below 4.0, the sharp acid and bitter taste increase rapidly in intensity. Conversely, pH above 4.0 increases mouth-coating, with higher scores for toasted characters (Guyot-Declerck et al., 2005).

A study by Guyot-Declerck et al. found that pH variation did not significantly modify the aroma perception of fresh beer. It was also found that during ageing, the pH increases (Guyot-Declerck et al., 2005). Ritter et al. did a study on the impact of fruits on the pH during the brewing process, with multiple types of fruit used with a pH between 3.05 and 5.13 to assess different situations. During the initial growth of the yeast cell during fermentation, all samples showed the same phenomenon of a decrease in pH. Depending on the pH of the additional ingredient, a particular trend was set for further development. Fruits with a lower pH than the beer resulted in a lower final pH and vice versa (Ritter et al., 2016).

4.2 Colour

Colour is one of the most significant attributes of beer, as it creates the first impression and perception for the consumers. The colour of beer contributes to the style and branding of the beer as it is linked to its composition (Ualema et al., 2024). The colour change brought by different raw ingredients like malt and fruit can be easily identified by consumers (Deng et al., 2020).

The most influential processes are kilning and roasting grains into malt. Coloured malts are produced as a source of extra colour and distinct types of flavour; these malts are used in small quantities (Guido and Mareira, 2013; Kok et al., 2019).

There are many speciality malt products with different uses; most of these malts also introduce intriguing aromas to the final product. Some of these types can be seen in **Table 1** (Guido and Mareira, 2013).

Table 1 Speciality malt products

Product type	Beer style	Colour Range (EBC units)	Moist ure (%)	Descriptive
Roasted barley product		1440-1800	< 3.5	Astringent, burnt, smoky
<i>Pale malt products</i>				
Amber malt		48-96	< 3.5	Dry, baked
Chocolate malt		1200-1440	< 3.0	Mocha, treacle, chocolate
Black malt	Stout	1440-1800	< 3.0	Smoky, coffee
<i>Green malt products</i>				
Cara malt		25-40	< 7.5	Sweet, caramel
Crystal malt		90-360	≈ 3.5	Malty, caramel, toffee-like
Dark crystal malt		120-150	< 3.5	Burnt toffee, caramel
Caramel malt	Lagers, ales	260-320	< 3.5	Burnt toffee, caramel
<i>Coloured kilned malts</i>				
Munich malt	Dark lager bock	42278	≈ 3.8	Grainy, malty (marked)
Vienna malt	Vienna Marzen	45937	≈ 4.5	Grainy, malty (subtle)

(source: Guido and Mareira, 2013)

Specific malt products are used in different styles of beer. For example, **Black malt** is mainly used in *Stouts*, **Chocolate malt** in *Porters*, **Amber malt** is used primarily in *Indian Pale Ales* and **Caramel malt**, or Caramalt as it is often called, is mainly used in *Lagers* (MacLeod and Evans, 2016).

Introducing light-coloured fruit will not cause a significant colour change in the beer. Conversely, dark fruits can make change very visible. The main reason for the colour change is the natural pigments that the fruits contain. These pigments are not metabolised by yeast after the fruit addition, allowing the fruit beer to retain its typical colour (Zhao et al., 2023). Jahn et al. 2020 analysed the antioxidant content of aronia-infused beer, adding aronia berries at different times during the boiling stage. It was discovered that prolonged exposure of the berries to the boiling wort results in the thermal degradation of anthocyanins from the berries. However, adding the berries in the fermentation stage did not have the desired results. The study concluded that the optimal time for adding Aronia berries was around 45 minutes before the end of the boiling step if the colouration of the beer was the desired factor (Jahn et al., 2020). Anthocyanins in these berries are water-soluble natural pigments, and most chromogenic fruit substances are associated with them (Deng et al., 2020).

In addition to water-soluble pigments, fat-soluble pigments can also influence the colour of the beer. In a 2018 study by Cho et al., the influence of adding persimmon fruit containing carotenoid pigment to beer was studied. Since carotenoids are fat-soluble, the fruits that contain this pigment may transfer relatively little colour to the beer. It can only enhance the redness and yellowness of the light-coloured beer (Cho et al., 2018).

Colour change can also happen later in the brewing process, thanks to reactions occurring during fermentation and storage. Castro Martin et al. in 2021 analysed fruit beer made with Cv. Lambrusco Grapes Must, in which it was concluded that after primary fermentation, a slight decrease in colour was primarily observed by the absorption of coloured compounds by proteins and polysaccharides of the yeast cell wall. Later, during storage, a slight increase in the colour was observed, which could be attributed to chemical oxidation (Castro Martin et al., 2021).

4.3 Turbidity/haziness

Clarity is one of the main appealing characteristics of beer, and most beers worldwide are served “bright”. Beer is considered clear and brilliant if no haze forms when chilled to 4 °C or below. Haze formation in alcoholic beverages is a common problem and can result in considerable economic losses, as consumers often perceive haziness as a defect and a potential health threat. The composition of hazes can vary, and they can be microbiological in origin, inorganic, carbohydrate-based or formed by interactions between polyphenols and proteins, which produce complexes and are held together by hydrogen bonding (Mastanjevic et al., 2018; Miedl et al., 2005).

Haze originating from microbial sources, like wild yeasts or bacteria, is relatively straightforward to prevent or mitigate through strict hygiene practices during brewing and storage. In contrast, non-biological haze presents a greater challenge because it stems from interactions between inherent beer components, specifically large molecules such as dextrans, β -glucans, proteins, and polyphenols. Proteins (constituting 40-75 % of organic haze material) and polyphenols are the primary culprits in forming this type of cloudiness. Over time, factors like light exposure and physical agitation during storage and shipping can alter these macromolecules, leading to the development of visible turbidity (Steiner et al., 2010; Wang and Ye, 2021).

Non-biological haze is then classified as either chill haze or permanent haze. Chill haze appears only at low temperatures (around 0 °C) and dissolves again when the beer warms to roughly 20 °C. It involves small particles (0.1-1.0 μm) formed by weak bonds (hydrogen/hydrophobic) between large molecules; these particles tend to grow larger in aged beer due to polyphenol changes. Permanent haze, in contrast, persists at room temperature (≥ 20 °C), although Basařová et al. (2021) note it may dissolve between 40-70 °C. This type features larger particles (1-10 μm), requires oxidation and covalent bond formation, and its development is linked to the specific raw materials used (Basařová et al., 2021; Kahle et al., 2020; Olšovská et al., 2021). Importantly, chill haze often serves as the foundation for permanent haze, which develops through further oxidative polymerisation of these initial haze complexes (Wang and Ye, 2021).

II PRACTICAL PART

5 MAIN OBJECTIVES OF THIS STUDY

In the practical part of this study, four types of model beer samples were manufactured with different amounts of fruit components added. The study focused on determining the effect of fruit addition on selected properties of the final product. The selected properties analysed were density and ethyl alcohol content, pH, colour, turbidity, total soluble solids, rheological and organoleptic properties. Samples were manufactured with fruit additions of 0, 5.0, 12.50 and 25.0 % w/w.

The theoretical part contained the following:

- Basic information about fruit beers, the legislation of these types of beer
- Description of raw materials used in the beer industry, technological equipment and technological procedures used during brewing.
- Selected factors influencing beer properties

The practical part contained:

- Production of model beer samples with different amounts of added fruit component
- Selected analyses of model samples were measured four weeks after production due to beer maturation.

6 MATERIALS AND METHODOLOGY OF THE WORK

6.1 Used materials and equipment

Czech Pilsner malt and hop Ekuanot from Pivotáka s.r.o. based in Olomouc, Czech Republic, were used to produce model samples. The used yeast was SafAle US-05 from the company Fermentis from Lille, France. Butternut squash, which was used, was from BAKKER s.r.o., based in Divišov, Czech Republic. The water was from the water supply.

Basic laboratory equipment was used, including a manual grain grinder from Pivotáka s.r.o., for production, based in Olomouc, Czech Republic. Another equipment used was a refrigerator from SK Design s.r.o., which is based in Želechovice and Dřevnicí in the Czech Republic. The vacuum sealer was from Henkelman, based in 's-Hertogenbosch in the Netherlands. Beer PET bottles used for maturation and storage were from Pivotáka s.r.o. based in Olomouc, Czech Republic.

6.2 Production of model fruit beer samples

A total of 4 beer samples were made with varying amounts of butternut squash added. The selected amounts of added butternut squash were 0, 5.0, 12.50 and 25.0 % w/w. The raw materials used and the utilised quantities are shown in **Table 2**.

Table 2 Raw materials used and their quantities

Malt	Hop	Yeast	Butternut squash	Water	Dextrose
0.5 kg	10.12 g	1.6 g	0/100/250/500 g	2.5 l	6 g

All samples were manufactured using the same protocol; the only difference was the amount of butternut squash added. The first step was to prepare the butternut squash a day before.

The butternut squash was baked and covered with a sugar coating. Firstly, the squash was cut into four pieces, and the seeds were separated. Then, the squash pieces were coated with 50 to 80 g of sugar a piece and baked at 200 °C for 70 minutes or until they were soft. The skin was separated after the squash pieces were removed from the oven (MONTYCON gastro, s.r.o., Ostrava, Czech Republic) and let aside for an hour to cool down to 20 ± 2 °C.

The squash was then vacuum sealed (Henkelman, 's-Hertogenbosch, The Netherlands) and stored in $2-8\text{ }^{\circ}\text{C}$ in the refrigerator to be used later in production.

To produce the beer samples, the infusion mashing technique was used; temperatures and times of stages can be seen in **Table 3**. After the mashing process was completed, lautering took place using a sieve. First running was separated, and sparging water at a temperature of $70 \pm 2\text{ }^{\circ}\text{C}$ was added to the sieve for the second running. The first and second runs of lautering were collected and added to the kettle for the next step in the process. During wort boiling, there were three stages of hop addition and one stage of fruit addition. The total time of wort brewing was 60 minutes. Hops were added at the start in the amount of 1.98 g, and then after 30 minutes, the same amount was added. Lastly, 15 minutes before the end of the wort boiling, another 1.32 g of hops were added. The fruit component was added with the second addition of hops 30 minutes into the wort boiling, in the amount previously determined in **Table 2**.

Table 3 Mashing scheme

Step	Temperature ($^{\circ}\text{C}$)	Time (min)
Pre-heating /malt resting	38	5
Mashing	68	60

For fermentation, 1.6 g of the top fermentation yeast, SafAle US-05, was used. Fermentation took 9 days at a temperature of $20 \pm 2\text{ }^{\circ}\text{C}$; on the 5th day, the dry hopping took place with the addition of 4,84 g of Ekuanot hops. After that, beer was transferred into sterilised brown PET bottles with a volume of 1 L. Dextrose addition followed, and the bottles were sealed. Beer was then stored at a temperature of $4 \pm 2\text{ }^{\circ}\text{C}$ for 3 weeks to mature. The photographic documentation of the samples' production is presented in Appendix I.

6.2.1 Sample collection

The collection of samples was taken according to the scheme (**Table 4**). The model samples were then analysed in different production stages to better understand the occurring changes.

In total, nine samples were analysed according to the sampling scheme presented in **Table 4**.

Table 4 Scheme of sample collection

Sample collection	Description
Young beer (Day 0)	Samples taken after brewing, before the addition of yeast
After fermentation (Day 9)	Samples taken after 9 days of fermenting at a temperature of 20 °C
After maturation (Day 21)	Samples taken after 3 weeks of maturation at a temperature of 2 °C

6.2.2 Determination of basic physico-chemical parameters

Specific density and alcohol content (% v/v) were determined on the Anton Paar Density Meter DMA 4500M with module Alcolyzer Beer ME (Anton Paar GmbH, Austria). Based on the determined values, other parameters such as alcohol content in mass per cent, real extract, apparent extract, degree of fermentation, and energy value were calculated. The degree of apparent and real fermentation (or apparent and real attenuation) had to be calculated (Equations 1 and 2) from the data we obtained on Alcolyzer Beer ME. Analyses were performed in duplicate.

$$AA = ADF = 100 \cdot \left(1 - \frac{Ea}{p}\right) = 100 \cdot \left(\frac{p-Ea}{p}\right) \quad (1)$$

where AA is the apparent attenuation (% w/w), ADF is the degree of apparent fermentation (% w/w), Ea is the apparent extract (% w/w) and p is the wort extract (°Plato or % w/w).

$$RA = RDF = 100 \cdot \left(1 - \frac{Er}{p}\right) = 100 \cdot \left(\frac{p-Er}{p}\right) \quad (2)$$

where RA is the real attenuation (% w/w), RDF is the degree of real fermentation (% w/w), Er is the real extract (% w/w) and p is the wort extract (°Plato or % w/w) (Kinčl, 2022; Thesseling et al., 2019).

6.2.3 Total soluble solids (TSS) determination

Refractometry measures a substance's refractive index based on the bending of a ray of light when passing from one medium into another (Castritius et al., 2010). The refraction index is not determined directly but instead utilises a scale based on the percentage of sucrose by weight (°Brix). In particular, 1 °Brix is 1 g of sucrose in 100 g of solution (1 °Brix = 1 %

w/w sugar), making it the easiest way to measure sugar levels in food and beverages (Jaywant et al., 2022).

The Brix value was measured using a digital refractometer Kern ORF 45BE (Kern and Sohn GmbH, Germany).

Measurement was performed after calibration with distilled water. Analyses were performed in triplicate.

6.2.4 Determination of pH value

The pH measurement is widely used as a rapid and accurate measure of the acidity and alkalinity of aqueous or other liquid solutions, which is determined by the concentration of the hydroxyl (OH^-) and hydronium (H_3O^+) ions. The chemical definition of pH is “the negative logarithm of the hydronium concentration” and corresponds to Equation 3 (Karastogianni et al., 2016; Zeece, 2020).

$$pH = -\text{Log}[\text{H}_3\text{O}^+] \quad (3)$$

The pH value was measured on the instrument pH meter HI99161 (Hanna Instruments, Czech Republic) with a glass tip electrode.

Measurement was performed by inserting the electrode into a beaker with a sample temperature of 20 ± 2 °C. The value of the pH was then shown on the display. Between different samples, the electrode was washed with distilled water. Analyses were performed in triplicate.

6.2.5 Turbidity analysis

Turbidity is the „reduction of the transparency of a liquid caused by undissolved substances (Kahle et al., 2020).

The turbidity measurement was done on the ISO Turbidimeter HI88713 (Hanna Instruments, Czech Republic), which works according to the ISO 7027 Water Quality – Determination of Turbidity. The instrument utilised an infrared LED lamp (860 nm), and the method used in this determination was nephelometry, which measures the diffuse radiation in the water of low turbidity, like drinking water. This method presents the values in nephelometric turbidity units, NTU (ISO, 2016; Rejdlová, 2024).

The optical system of the turbidimeter consists of an LED light and two detectors for scattered and transmitted light.

The measurement started by filling the cylindrical cuvette with 10 ml of the sample and closing the cuvette with a cap. The cuvette had to be cleaned to make sure no impurities on the cuvette disturbed the results before being inserted into the instrument at the given location. Analyses were performed in triplicate.

6.2.6 Instrumental analysis of colour

The colour was determined by the system that uses the three-dimensional CIELAB space. This space expresses the colour using three numerical values: value L^* , value a^* and value b^* . The L^* value axis represents the luminosity of the sample and ranges from 0 (black) to 100 (white); the higher the value, the stronger the lightness of the sample. The a^* value axis represents the green (-) and red (+) colour spectrum. The b^* value axis represents the blue (-) and yellow (+) spectrum of colours (**Figure 1**). (Rejdlová et al., 2023; Zhao et al., 2023). The spectrophotometer UltraScan PRO (HunterLab, USA) was used for the analysis.

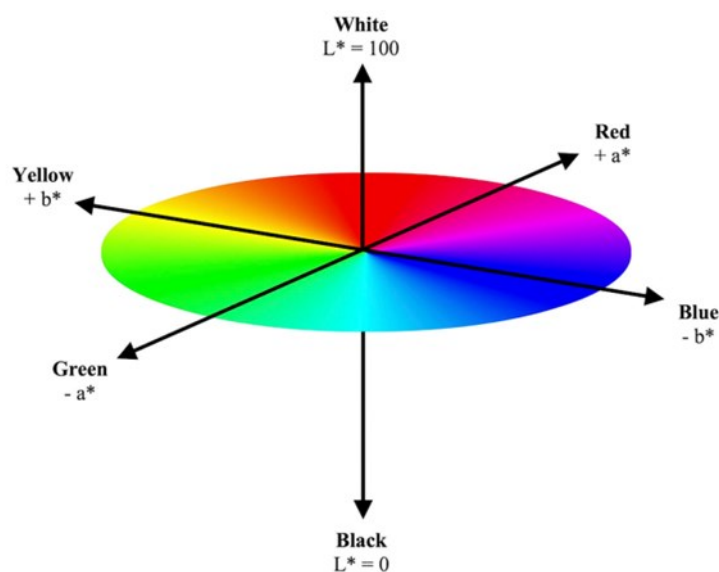


Figure 1 Model illustration of three-dimensional CIE Lab colour space

(source: Belasco et al., 2020)

Before the measurement, the instrument had to be calibrated according to the manufacturer's instructions. Part of the sample was used to fill the cuvette or cell (10 mm), then cleaned to ensure no impurities on the cuvette disturbed the results and put inside the

instrument at the given location. The sample was analysed under D65 (daylight) illumination and at a 10° angle. Analyses were performed in triplicate.

6.2.7 Determination of the rheological properties

Samples' flow properties were determined on a Kinexus Pro+ rheometer (NETZSCH-Gerätebau GmbH, Selb, Germany). The system with a concentric cylinder geometry and a 9.15 mm gap was set. The amount of sample used was 15.0 ml for each measurement at a temperature of 20 ± 1 °C. Shear viscosity (Pa·s) was measured as a function of shear rate (s^{-1}) in the range 0 to 450 s^{-1} to determine the steady-state rheological characteristics. Nonlinear regression analysis using the Power Law model (Equation 4), Bingham model (Equation 5) and Herschel-Bulkley model (Equation 6) was used to process the flow curves obtained.

$$\tau = K\dot{\gamma}^n \quad (4)$$

where τ is the shear stress (Pa), K is the flow consistency index (Pa·s), $\dot{\gamma}$ is the shear rate (s^{-1}), and n is the Power Law index (-).

$$\tau = \tau_y + \mu_p \dot{\gamma} \quad (5)$$

where τ is the shear stress (Pa), τ_y is the yield point (Pa), μ_p is the plastic viscosity (Pa·s), $\dot{\gamma}$ is the shear rate (s^{-1}).

$$\tau = \tau_0 + K\dot{\gamma}^n \quad (6)$$

where τ is the shear stress (Pa), τ_0 is the yield stress (Pa), K is the flow consistency index (Pa·s), $\dot{\gamma}$ is the shear rate (s^{-1}), and n is the flow index (-).

The Bingham Plastic and Power Law models are the most widely used rheological models. The Herschel-Bulkley model was later proposed to overcome the earlier models' inherent drawbacks (Khataniar et al., 1994). **Figure 2** shows possible behaviours of liquids. This behaviour is determined by value n , representing the flow index; $n = 1$ means that the liquid is Newtonian, $n < 1$ means the liquid shows pseudoplastic behaviour, and if $n > 1$, the liquid is dilatant (Burhan et al., 2023). Analyses were performed in triplicate.

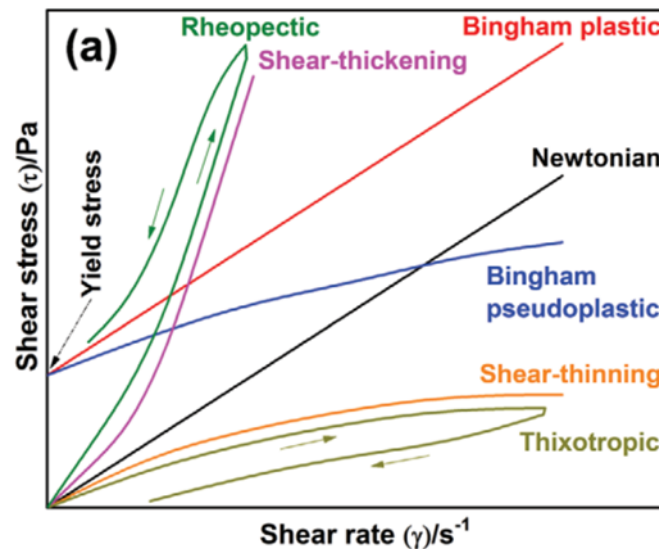


Figure 2 Typical rheological curves for different types of fluids

(source: Li et al., 2021)

6.3 Sensory analysis

The sensory analysis was realised in a sensory analysis laboratory, on the same day the other analyses were performed, per ISO 6658 (ISO, 2017). Beer samples were served simultaneously during analysis at a temperature of 20 ± 2 °C in glasses, while water and toast bread were provided as neutralisers. The assessors consisted of 7 men and 5 women between the ages of 23 and 30. The samples were evaluated using an intensity scale and a preference test. Each beer sample was presented under a three-digit code so the assessors would not know which beer sample they were tasting. The sensory evaluation protocol can be found in Appendix II.

As part of the sensory analysis, the samples were evaluated for the following seven attributes using a 5-point intensity scale and one 9-point scale:

- Off-flavours (1 – Just recognisable, 3 – Moderate, 5 – Very strong)
- Bitterness (1 – Just recognisable, 3 – Moderate, 5 – Very strong)
- Colour (1 – Just recognisable, 3 – Moderate, 5 – Very strong)
- Level of Carbonisation (1 – Just recognisable, 3 – Moderate, 5 – Very strong)
- Sweetness intensity (1 – Just recognisable, 3 – Moderate, 5 – Very strong)

- Fullness (1 – Just recognisable, 3 – Moderate, 5 – Very strong)
- Overall rating (1 – Extraordinary good, 9 – Extremely bad)

The assessors were also asked to rate the samples according to their preferences. With a hedonic scale:

- 1 – most preferred, 3 – least preferred

6.4 Statistical analysis

The data presented are the means $\pm$ standard deviation. All measurements were performed at least in triplicate. The examined parameters were compared using a nonparametric analysis of variance (Kruskal-Wallis test) followed by Dunn's multiple comparisons as a post *hoc* test, with 95 % reliability. Moreover, the sensory properties of beer samples were verified by Kruskal-Wallis and Neményi tests. The significance level used in the test was 0.05. Statistical analysis was performed using GraphPad Prism 10.4.2 (GraphPad Software, Boston, United States). The nonparametric method (Kruskal-Wallis test) was chosen as the sample pool is significantly smaller than those needed for parametric methods like the one-way ANOVA test.

7 RESULTS AND DISCUSSION

With four samples manufactured, only three samples (0, 5.0, 12.50 % w/w) were analysed, as the sample with 25.0 % w/w addition of fruit was unsuitable for further measurements.

7.1 Basic physico-chemical parameters

7.1.1 Density

Density results can be seen in **Table 6**, which shows a significantly higher density of the samples on day 0, before fermentation, than on day 21, after maturation. Before fermentation, density was in the range of 1.072-1.087 g/cm³, with the highest density detected for the sample with 5 % w/w addition of fruit component. After maturation, density was measured within the range of 1.016-1.023 g/cm³. The decrease in density of the samples during fermentation is due to the conversion of fermentable carbohydrates to ethyl alcohol, CO₂ and other sensory active substances, and it is not influenced at all by the fruit addition (Pal et al., 2021).

Table 5 Changes in density before fermentation (Day 0) and after maturation (Day 21)

Sample	Density (g/cm ³)	
	Day 0	Day 21
0 %	1.072 ± 0.001 ^a	1.020 ± 0.001 ^b
5 %	1.086 ± 0.001 ^a	1.023 ± 0.001 ^b
12.50 %	1.072 ± 0.001 ^a	1.016 ± 0.001 ^b

Results are expressed as mean ± standard deviation. Different letters in the same row are significantly different ($p < 0.05$).

A study by Paucar-Menacho et al. (2025) observed that the wort density before fermentation was higher than that of the final product. Another study by Patrascu et al. (2018) compared the quality parameters of fruit beers on the market. In this study, it was reported that the density of the final product was also between 1.01 and 1.03 g/cm³. Similar density values were reported in the study by Pereira et al. (2020), where the density of wheat beers with added orange peel was around 1.02 g/cm³ in all tested samples. Furthermore, commercial red fruit beers were analysed in a study by Pérez-Alva et al. (2023). Fruits used

in these beers were cherry and different kinds of berries. The study determined that the commercial beer's final density was in the same range, 1.00-1.04 g/cm³. A Buonocore et al. (2024) study evaluated the fermentation phases of different types of beer. During these observations, density was measured before and after the fermentation. It was discovered that the same phenomenon happened with our samples, which had a higher density before fermentation. This study measured the original density before fermentation at 1.036-1.073 g/cm³. After fermentation, density was measured at about 1.010-1.018 g/cm³ (Buonocore et al., 2024). Another study was performed by Pirrone et al. (2025), who observed how non-conventional yeasts can influence the sensory quality of craft beer. The study measured density at $1.08 \pm 0.10 - 1.012 \pm 0.08$ g/cm³ (Pirrone et al., 2025). This also corresponds with the narrative that fruit addition does not affect the density level.

7.1.2 Other physico-chemical parameters

The presence of butternut squash significantly influenced the ethyl alcohol content of the sample. A higher amount of added fruit significantly lowered the ethyl alcohol content; the sample without fruit addition had an ethyl alcohol content of 8.02 ± 0.01 % v/v, while the sample with 12.50 % fruit addition had an ethyl alcohol content of 5.86 ± 0.01 % v/v, which is a significant decrease. This and other parameters can be found in **Table 6**. This observation does not correspond with other studies' results, where the addition of fruit happens before the fermentation, and could be a reason for further testing.

In **Figure 3**, the levels of TSS in beer samples correspond with the final ethyl alcohol content. Even before fermentation (day 0), the sample with 0 % fruit addition had a higher TSS value than the other samples. That means that this sample could convert more sugar into ethyl alcohol.

Table 6 Basic physico-chemical parameters of the samples after maturation (Day 21)

Sample	Ethyl alcohol		Extract		Attenuation		Wort extract
	% v/v	% w/w	<i>Apparent</i> (% w/w)	<i>Real</i> (% w/w)	<i>Apparent</i> (%)	<i>Real</i> (%)	°Plato
0 %	8.02 ± 0.01 ^a	6.17 ± 0.01 ^a	7.13 ± 0.01 ^a	9.83 ± 0.01 ^a	66.34 ± 0.08 ^a	53.60 ± 0.08 ^a	21.19 ± 0.01 ^a
5 %	6.84 ± 0.01 ^a	5.29 ± 0.01 ^a	5.64 ± 0.01 ^a	8.01 ± 0.01 ^a	68.53 ± 0.05 ^a	55.33 ± 0.03 ^a	17.92 ± 0.03 ^a
12.50 %	5.86 ± 0.01 ^a	4.55 ± 0.01 ^a	4.59 ± 0.01 ^a	6.66 ± 0.01 ^a	70.05 ± 0.01 ^a	56.53 ± 0.03 ^a	15.31 ± 0.03 ^a

Results are expressed as mean ± standard deviation. Different letters in the same column are significantly different ($p < 0.05$).

A study by Egushova et al. observed lower alcohol content in beer samples with the highest percentage of cherry juice. The ethyl alcohol content in samples with 30 % cherry juice was measured at 3.9 ± 0.2 % v/v; in samples with 10 % cherry juice, the ethyl alcohol content was measured at 4.3 ± 0.2 % v/v (Egushova et al., 2024). A study by Moreno et al. observed that the beer with added peach juice had lower alcohol content than the beer without the addition (Moreno et al., 2022). In these cases, it was determined that the lower alcohol content of the final beer is due to adding fruit juices at the end of the process, after fermentation. This means that the final alcohol content is diluted by the additional juices (Egushova et al., 2024; Moreno et al., 2022).

A study comparing different beers with the addition of mango fruit concluded that most of the mango beers (various styles of fruit added to each sample) had similar or higher alcohol content than that of the control beer without any addition; the control sample had alcohol content measured at 4.16 ± 0.02 % v/v while mango beers had alcohol content measured between 4.13 ± 0.02 and 4.63 ± 0.02 % v/v (Gasiński et al., 2020). Cho et al. (2017), who enhanced beer with persimmon fruit during fermentation, did not observe significant changes in alcohol content with different amounts of persimmon fruit addition (Cho et al., 2017). Martínez et al. (2017) also added different amounts of persimmon juice to the beer samples before fermentation; the results show that a higher amount of juice means

a higher alcohol content of the sample (Martínez et al., 2017). In a study by Castro Marin et al. (2021), beer samples were enhanced with Lambrusco grape must during brewing. Results confirmed that adding must from Lambrusco grapes enriched beers in multiple parameters, including alcohol content, as samples with 20 % of grape must had the highest alcohol content among all samples at 6.12 %. In comparison, the control sample had the lowest alcohol content at 4.70 % (Castro Marin et al., 2021). These studies conclude that with the addition of fruit during the brewing process and, more importantly, before fermentation, the alcohol content is higher, the higher the amount of fruit added. This is thanks to the additional fermentable sugars in the wort (Castro Marin et al., 2021; Martínez et al., 2017).

7.2 Total soluble solids

The changes in TSS values are presented in **Figure 3**. The initial values of the samples ranged from 15.4 ± 0.1 to 21.4 ± 0.1 °Brix. During fermentation, the decrease of TSS was constant until the start of maturation on day 9. From this point, a slight increase in TSS can be observed until the end of maturation on day 21.

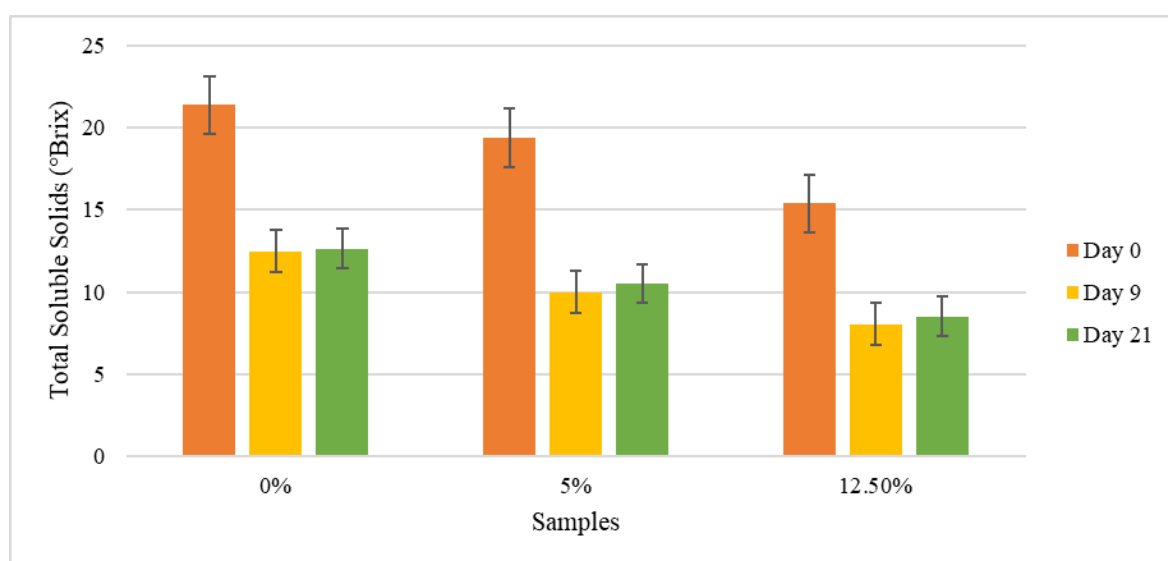


Figure 3 Changes in levels of total soluble solids in samples before fermentation (Day 0), after fermentation (Day 9) and after maturation (Day 21)

The final TSS value of the control sample (0 %) was 12.6 ± 0.1 °Brix. A decrease in TSS was observed in both samples with butternut squash addition; the higher the amount of fruit added, the higher the reduction in TSS. With a 10.5 ± 0.1 °Brix value of TSS in the 5 % sample and an 8.5 ± 0.1 °Brix value of TSS in the 12.50 % sample. Looking at **Figure 3**, it is easy to see that the initial levels of TSS copy the final curve. The control sample has the

highest TSS value at 21.4 ± 0.1 °Brix, while the lowest level of TSS was determined at 15.4 ± 0.1 °Brix for the sample with 12.50 % butternut squash. The amount of fermentable sugar converted to ethyl alcohol is very similar between the samples. This confirms that fruit addition did not influence the changes in TSS values in the samples. One of the reasons could be that butternut squash does not contain high amounts of fermentable sugar, which the yeast could use. The other reason could be that butternut squash has higher water content, thus diluting the sugars in the wort and reducing the total soluble solids content of the sample. That could also be the reason why the addition did not influence the ethyl alcohol content as was predicted (Armesto et al., 2020).

In a study by Martínez et al. 2017, a control sample had a final TSS value determined at 12.9 ± 0.22 °Brix, while the sample with the highest amount of persimmon added had the final TSS value measured at 6.8 ± 0.19 °Brix (Martínez et al., 2017). In a study by Pal et al. (2021), it was determined that the addition of star fruit decreases the TSS of the final beer. The control sample had a final TSS value observed at 8.50 ± 0.05 °Brix, whereas the sample with 10 % of star fruit addition had a final TSS determined at 7.00 ± 0.13 °Brix (Pal et al., 2021). However, in both studies, the initial TSS level was not considered.

7.3 pH

The changes in pH of the samples can be observed in **Figure 4**. Before the fermentation, the pH values of the samples were significantly higher than those after the maturation. The pH value was between 5.69 ± 0.01 and 5.73 ± 0.01 before the fermentation. And the values after maturation were between 6.11 ± 0.04 and 6.25 ± 0.0 . The significant increase in value may be due to the fact that butternut squash is an alkaline food with a higher pH than beer (Cho et al., 2018). The butternut squash pH value was approximately 5.8 to 6.12 for some varieties, and its high water content could also dilute the more acidic components of the malt and further contribute to a higher overall pH of the samples (Armesto et al., 2020).

Generally, the pH of beer is between 4.3 and 4.6 (Patrascu et al., 2018); some studies show a larger span of values between 4.0 and 5.5 for beer (Li et al., 2016). Most of the studies on fruit beers showed a significant decrease in pH with the addition of fruit to beer. Yin et al. (2021) added dried red raspberries to the beer in different stages of the brewing process. A decline in pH was observed, with the lowest pH being 4.1, with dried raspberries added at the beginning of boiling (Yin et al., 2021). In another study by Deng et al. (2020), the pH of

the beer decreased significantly with the addition of omija fruit (also called magnolia berry). The control beer in this study had a pH of 4.77, and the beers with the addition of omija fruit had a pH between 4.05 and 4.36 (Deng et al., 2020).

In some studies, the opposite effect was observed. A study by Cho et al. (2018) found that the pH of the beer slightly increased after introducing persimmon fruits into the beer. The higher the concentration of fruits, the higher the pH, where the control sample of beer had a pH of 4.20 and the beer with the highest concentration of fruit had a pH of 4.78 (Cho et al., 2018).

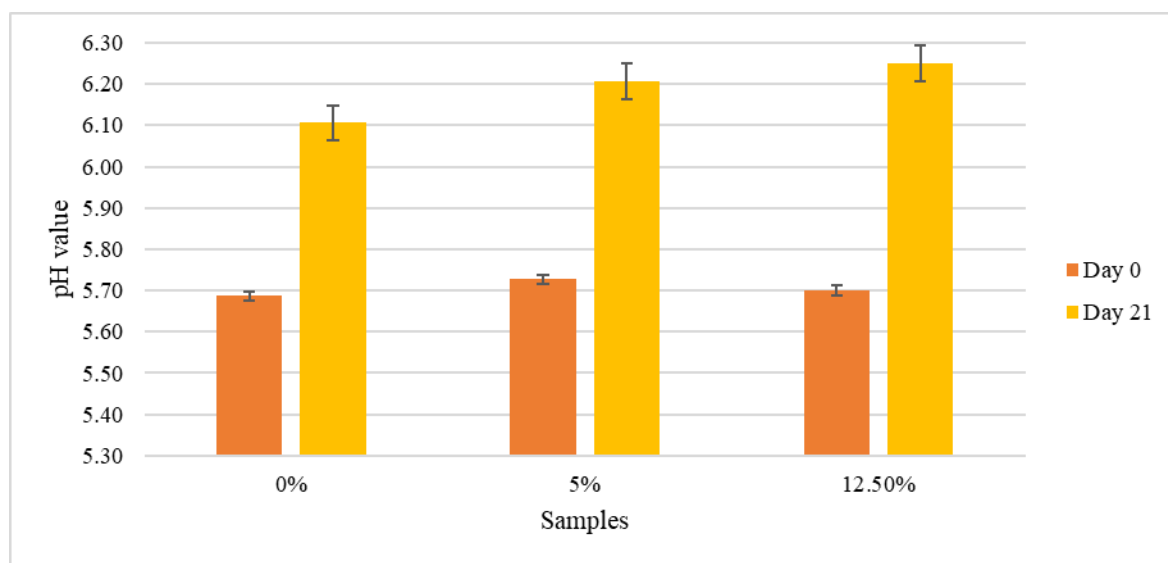


Figure 4 Changes in pH in samples before fermentation (Day 0) and after maturation (Day 21)

7.4 Turbidity

Turbidity values are depicted in **Table 7**. The highest turbidity value was detected in the sample containing 5.0 % w/w of butternut squash before fermentation. After maturation, the sample with a 0 % w/w addition of fruit had the highest turbidity value at 22.20 ± 5.27 NTU. Turbidity values of beer decreased along fermentation and maturation, probably owing to precipitation on the bottom of the fermentation tank. The higher the amount of fruit addition, the lower the turbidity value (Martínez et al., 2017).

Table 7 Turbidity in samples before fermentation (Day 0) and after maturation (Day 21)

Sample	Turbidity (NTU)	
	Day 0	Day 21
0 %	871.67 ± 2.52^{ab}	22.20 ± 5.27^a
5 %	1028.00 ± 5.27^a	20.43 ± 2.57^a
12.50 %	463.67 ± 2.57^b	12.80 ± 2.09^a

Results are expressed as mean $\pm$ standard deviation. Different letters in the same column are significantly different ($p < 0.05$).

The same phenomenon was observed in a study by Martínez et al. 2017, where it was discovered that the higher the amount of added persimmon fruit, the lower the turbidity value in the sample. There could be multiple reasons why the turbidity values decreased. One could be that the fruit molecules dragged the haze particles to the bottom through sedimentation (Martínez et al., 2017). Another reason could be due to the higher pH value of the beer and slightly higher ethyl alcohol content. Storage at a low temperature also helps lower the haze particles in beverages (Olšovská et al., 2021; Siebert, 2006).

7.5 Instrumental analysis of colour

CIELab colour values of the beer samples were slightly affected by adding butternut squash (**Table 8**). The lightness (L^*) value in the control sample was lower before fermentation than in the beer sample with fruit addition. After maturation, on day 21, it had the opposite effect, and the L^* value was higher in the control beer than in other samples. The lightness value ranged from 80.89 ± 0.01 to 83.74 ± 0.01 before fermentation and 87.19 ± 0.01 to 89.80 ± 0.01 after maturation. On the contrary, the redness (a^*) value significantly increased with the amount of fruit added to the analysis after maturation. However, the samples' yellowness (b^*) after maturation behaves differently. The sample with the highest amount of fruit added has the lowest yellowness value at 27.89 ± 0.01 .

As we compared the samples before fermentation and after maturation, it was easy to see that the samples after maturation have slightly lighter colours than before fermentation. This change often occurs in commercial beers (Barczi et al., 2022).

Table 8 Colour values of the beer samples before fermentation (Day 0) and after maturation (Day 21)

Sample	L*	a*	b*
	Before fermentation (Day 0)		
0 %	80.89 ± 0.01 ^{a, A}	-1.22 ± 0.01 ^{a, A}	41.62 ± 0.02 ^{a, A}
5 %	81.62 ± 0.01 ^{ab, A}	-1.22 ± 0.01 ^{ab, A}	38.12 ± 0.01 ^{ab, A}
12.50 %	83.74 ± 0.01 ^{b, A}	-1.88 ± 0.01 ^{b, A}	33.19 ± 0.01 ^{b, A}
	After maturation (Day 21)		
0 %	89.80 ± 0.01 ^{a, B}	-1.36 ± 0.01 ^{a, B}	36.71 ± 0.01 ^{a, B}
5 %	85.44 ± 0.01 ^{b, B}	0.97 ± 0.01 ^{ab, B}	31.17 ± 0.02 ^{ab, B}
12.50 %	87.19 ± 0.01 ^{ab, B}	1.22 ± 0.01 ^{b, B}	27.89 ± 0.01 ^{b, B}

Results are expressed as mean ± standard deviation. Different letters in the same column are significantly different, different capital letters for the same sample, but different fermentation times are significantly different ($p < 0.05$).

Since butternut squash is rich in carotenoids and fat-soluble pigments, different proportions of squash are added as a natural colouring agent to enhance the redness of the light-coloured beers. A similar phenomenon was also observed in the study by Cho et al. (2018). Colour changes were less significant than adding fruits containing water-soluble pigments, like berries containing anthocyanins (Zhao et al., 2023).

7.6 Rheological analysis

To evaluate the flow properties of the beer samples, the obtained data were fitted into three models (Power Law model, Bingham model and Herschel-Bulkley model, respectively), which can be seen in **Table 9**. The R^2 coefficient (coefficient of determination) determines how „well“ the data fit the regression model. Lower values of the coefficient of determination mean a greater distance of the data from the given model (Kouřilová et al., 2022). With this information, we can determine that the best-suited model for these samples is the Herschel-Bulkley model, as the coefficient of determination ranges from 0.991 to 0.998. For other models, the R^2 coefficient ranges from 0.809 to 0.927 (Power Law model) and 0.869 to 0.961 (Bingham model). The reason for this difference could be that the Power

Law model does not include a yield stress, which is apparent from other models (Tropea et al., 2007)

Power Law and Herschel-Bulkley model give two different results, but thanks to the coefficient of determination, we can lean towards the results from the Herschel-Bulkley model; the results from said model determine that the samples behave like dilatant fluids.

Table 9 Rheological parameters of the tested samples obtained by the three models after maturation (Day 21)

Sample	Model	R^2	n	K	τ	κ
		-	-	$\text{Pa} \cdot \text{s}^{0.682}$	Pa	$\text{Pa} \cdot \text{s}$
0 %	Power law	0.810	0.682	0.024		
	Bingham	0.892			0.177	0.004
	Herschel-Bulkley	0.991	1.570	0.000	0.272	
5 %	Power law	0.804	0.726	0.017		
	Bingham	0.869			0.149	0.003
	Herschel-Bulkley	0.995	1.700	0.000	0.248	
12.50 %	Power law	0.927	0.776	0.014		
	Bingham	0.961			0.122	0.004
	Herschel-Bulkley	0.998	1.330	0.001	0.185	

Dilatant behaviour could happen thanks to high solids concentration in the samples. This high concentration could be thanks to the non-filtered state of the beer sample and also thanks to the addition of butternut squash, which adds more solids into the samples (Trávníček et al., 2015; Trilling-Haasler et al., 2024).

The highest viscosity was measured for the control sample, while the lowest was for the sample with the highest amount of fruit addition, as seen in **Figure 5**. This corresponds with other studies, which determined that a higher amount of soluble solid content means higher viscosity (Ibarz and Pagán, 1987; Diamante and Umemoto, 2011).

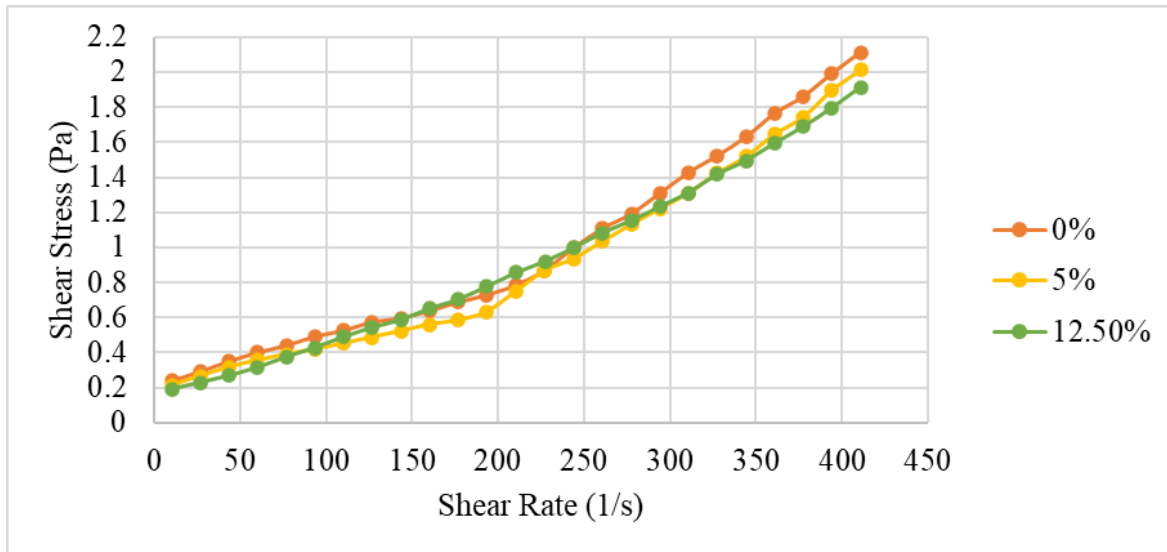


Figure 5 Rheological behaviour of the samples

In **Figure 6**, which shows samples' viscosity dependence on shear rate, from the curve, we could deduce that the correct behaviour should be pseudoplastic, as with higher shear rate, the viscosity of the samples decreases. A fluid with a yield stress always appears pseudoplastic with apparent viscosity against shear rate. As the beer samples exhibit yield-stress in both the Bingham and Herschel-Bulkley models and fit those models the best, we can conclude that after the samples are flowing, their behaviour will be that of pseudoplastic liquids (Nguyen and Boger, 1992).

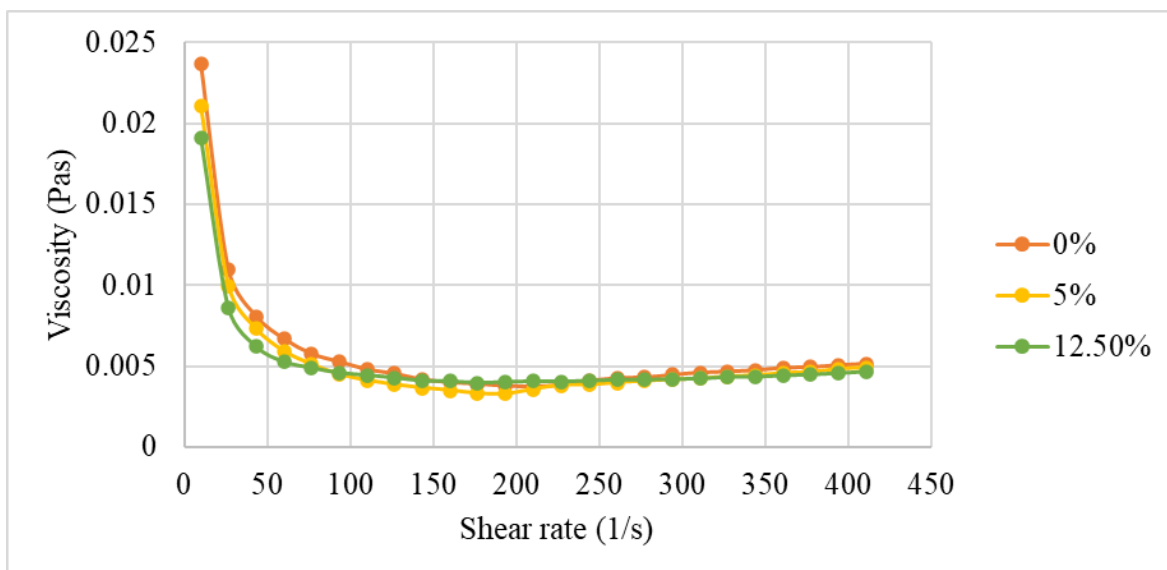


Figure 6 Samples' viscosity dependence on shear rate

Figure 7 shows the flow curves of the samples as affected by the different percentages of added fruit components in the Power Law model. This figure shows us that the curves are non-linear, which tells us that the liquid is non-Newtonian; furthermore, the curves bend downwards, which means that the liquid in all fruit concentrations has a pseudoplastic character. This trend can also be seen in **Table 9**, where for the Power Law model, the n value is lower than 1, which is typical for pseudoplastic behaviour. The n value increases with higher fruit addition; the control sample (0 %) showed an n value of 0.682, while the sample with the most butternut squash addition showed an n value of 0.776. Furthermore, with the behaviour of the individual samples, we can read the sample's viscosity, where the 12.50 % sample had higher viscosity than the control sample. The 5 % fruit addition sample had lower viscosity than the control sample. With knowledge that the 12.50 % fruit addition sample should have lower viscosity than that of the control sample. The discrepancy in these results may be due to the low value of the coefficient of determination for the Power Law model.

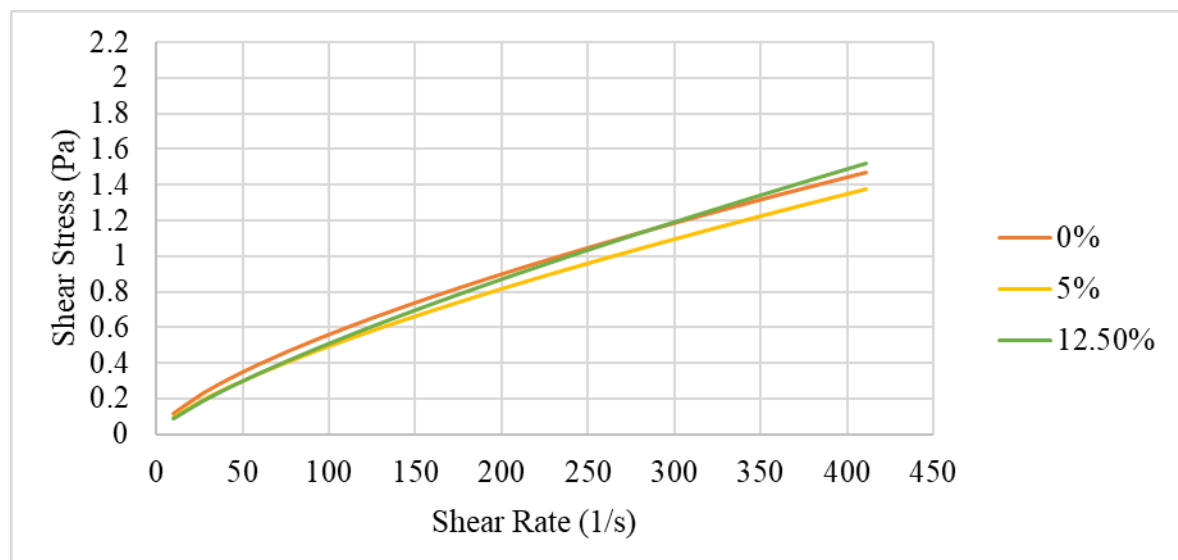


Figure 7 Flow curves (Power Law model) of the samples after maturation (Day 21)

Figure 8 shows the flow curves of the sample as affected by the different percentages of added fruit components in the Bingham model. The curves are primarily linear, which is typical for Bingham plastic behaviour. **Figure 8** also shows us that the control sample has the highest viscosity, closely followed by the sample with the most fruit added. The sample with the 5 % fruit addition has the lowest viscosity. Yield stress of the sample was between 0.122 and 0.177 Pa according to **Table 9**.

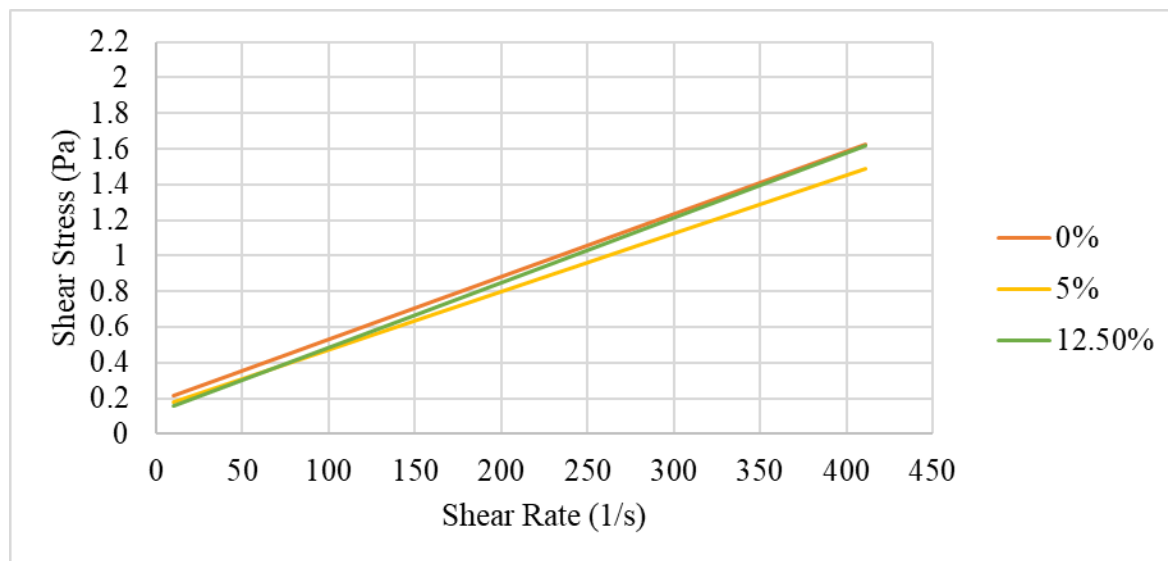


Figure 8 Flow curves (Bingham model) of the samples after maturation (Day 21)

Figure 9 shows non-linear curves of the sample, which can be interpreted as non-Newtonian behaviour. The curves bend towards the top, which tells us the samples have dilatant behaviour. Furthermore, in **Table 9**, all samples have the n value > 1 , which can be interpreted as shear-thickening, dilatant behaviour, where the n value is between 1.570 and 1.700. We can also see that the control sample has the highest viscosity, closely followed by the 5 % fruit addition sample. The lowest viscosity is that of the sample with the highest addition of fruit. This also corresponds with **Figure 3**, as it was determined that the higher the amount of soluble solids, the higher the viscosity. In this model, yield stress was even higher than that calculated in the Bingham model; the values were between 0.186 and 0.272 Pa according to **Table 9**.

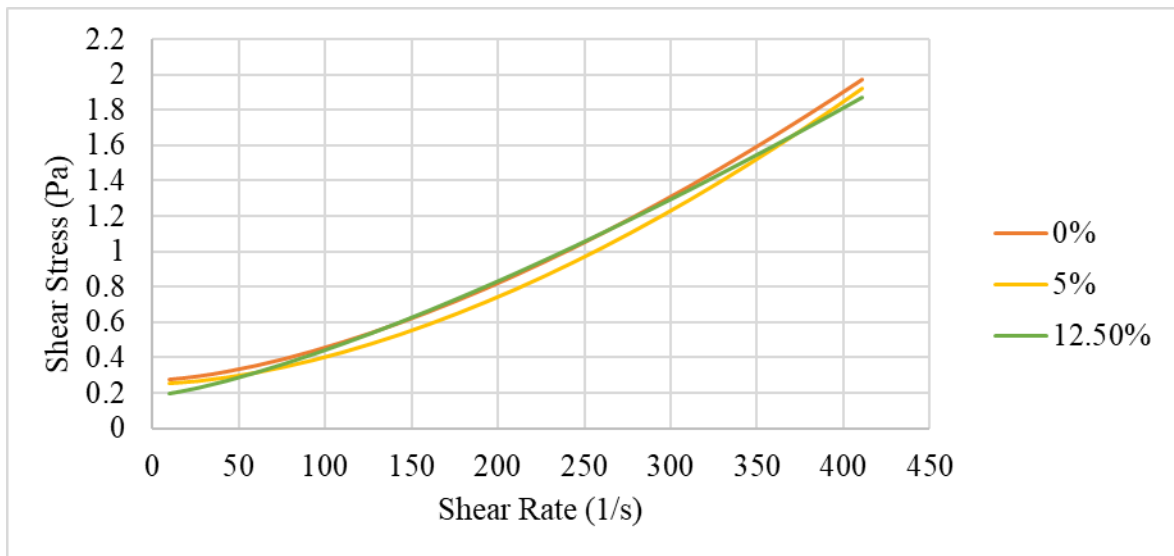


Figure 9 Flow curves (Herschel-Bulkley model) of the samples after maturation (Day 21)

7.7 Sensory analysis

Sensory evaluation was conducted on the control (0.0 % w/w), 5.0 % w/w, and 12.50 % w/w butternut squash beers, assessing attributes such as off-flavours, bitterness, colour, carbonation, sweetness, fullness, overall rating, and preference. Significance testing was performed using Kruskal-Wallis (attributes) or Friedman (preference) tests, followed by Nemenyi post-hoc tests where appropriate ($\alpha = 0.05$).

Statistically significant findings were observed for:

- **Colour:** A significant overall difference was found (Kruskal-Wallis, $p < 0.05$). The Nemenyi test showed that the 5.0 % w/w and 12.50 % w/w squash beers were perceived as significantly different in colour from the control (0.0 % w/w).
- **Preference:** A significant overall difference in preference ratings was detected (Friedman, $p < 0.05$). The Nemenyi test revealed significant differences between the 12.50 % w/w sample and the 5.0 % w/w and 0.0 % w/w samples. Specifically, the 12.50 % w/w sample was significantly less preferred than the 5.0 % w/w and the control sample.

No statistically significant differences ($p > 0.05$) were found between the samples for the other evaluated attributes: off-flavours, bitterness, carbonation level, sweetness, fullness, or overall rating, although trends may have been observed.

Analysis of the sensory median scores (**Figure 10**) indicates apparent effects of butternut squash addition on the beer sample's profile. Notably, perceived **off-flavour** increased with butternut squash addition. This is likely attributable to the introduction of earthy or vegetal notes characteristic of butternut squash, which may be perceived as atypical or undesirable in this beer style.

Concurrently, perceived **sweetness** exhibited a dose-dependent decrease; this sensory finding aligns well with instrumental measurements, specifically the lower Total Soluble Solids (TSS) observed post-maturation in the 12.50 % w/w sample, suggesting a less sweet product. Furthermore, perceived **carbonisation levels** showed a similar inverse relationship with squash concentration, decreasing from the control sample (0 % w/w) to the 12.50 % w/w sample. This reduction in perceived fizziness might be influenced by factors associated with the altered composition, such as the lower TSS, potentially impacting CO₂ solubility. Interestingly, perceived **bitterness** slightly decreased at the highest addition level.

Another significant finding is the impact on the **overall rating**, where a higher score indicates lower preference. The 12.50 % w/w sample received the worst rating, compared to the control and 5 % w/w samples. This demonstrates the negative impact of the highest butternut squash concentration on overall sensory quality. This result is consistent with findings from the separate preference test, which also identified the 12.50 % w/w sample as being the least preferred by assessors. The increased off-flavour and markedly reduced sweetness likely drove this negative perception.

Comparing these results with other studies using fruit adjuncts, some differences emerge. Studies on persimmon (Cho et al., 2018) and on loquat (Li et al., 2024a) beers reported improved overall quality, which was driven by desirable fruity and floral flavour notes. Similarly, additions of Lambrusco Grape Must introduced distinct fruity and grape aromas (Castro Marin et al., 2021). In contrast, the addition of butternut squash did not improve preference and significantly worsened it at the 12.50 % w/w sample. A primary distinction is in sweetness perception, as a loquat juice notably increased perceived sweetness (Li et al., 2024a), whereas butternut squash significantly decreased it. While it was determined that both butternut squash and loquat juice reduced perceived bitterness, the Lambrusco Grape Must significantly increased perceived acidity and astringency (Castro Marin et al., 2021). The negative preference for the butternut squash beer suggests its

specific combination of reduced sweetness and increased off-notes was less favourable than in other fruit beers.

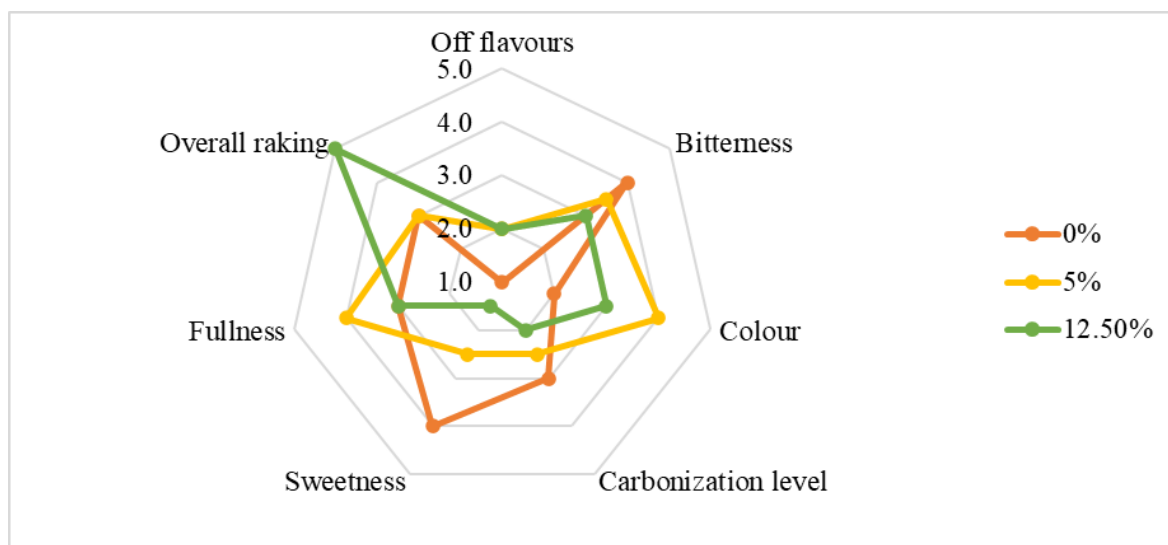


Figure 10 Results of sensory analysis of samples (0.0, 5.0 and 12.50 % w/w) on different attributes

The whole sensory analysis dataset and calculations can be found in Appendix III.

CONCLUSION

This thesis aimed to investigate the impact of incorporating different levels of butternut squash (0.0, 5.0, and 12.50 % w/w added to the boil) on the final physicochemical, rheological, and sensory properties of a top-fermented ale.

The primary findings of this research were as follows:

- Increasing squash concentration up to 12.50 % w/w resulted in a progressive decrease in final ethyl alcohol content compared to the control sample.
- Final beer pH increased progressively with higher squash additions within the tested range, consistent with the adjuncts' inherent alkalinity.
- Squash addition appeared to aid clarification, with the 5.0 % and 12.50 % w/w samples exhibiting lower final turbidity than the control beer sample.
- While instrumental colour measurements were not significantly different, sensory analysis revealed a significant perceived colour difference ($p < 0.05$) between the control and the squash-containing beers.
- The adjunct influenced the beer's rheological properties, with viscosity varying between samples (control being highest), suggesting potential impacts on mouthfeel.

These results demonstrate that brewers utilising butternut squash must consider its notable effects on fermentation performance (yield) and final beer chemistry (pH), as well as its contribution to visual characteristics and potentially mouthfeel. The findings provide valuable baseline data for recipe formulation using this non-traditional adjunct up to 12.50 % w/w.

Limitations of this study include the focus on boil additions only and the use of a single yeast strain and base recipe. The inability to analyse the 25% w/w sample also restricts conclusions about higher addition rates.

Future research is recommended to explore the precise mechanisms influencing ethyl alcohol yield, investigate the effects of different addition timings (mash, fermentation), and conduct detailed sensory profiling to better understand the flavour and aroma contributions

of butternut squash. Different squash varieties or processing methods could also yield varying results.

In conclusion, this thesis provides foundational insights into the practical effects of using butternut squash as a brewing adjunct, highlighting key considerations for brewers seeking to innovate with non-traditional ingredients.

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LIST OF ABBREVIATIONS

ANOVA Analysis of variance

EBC European Brewery Convention

IGA Italian grape ale

NTU Nephelometric turbidity units

TSS Total soluble solids

APPENDICES

Appendix P I: Photodocumentary of the sample production

Appendix P II: Sensory analysis questionnaire

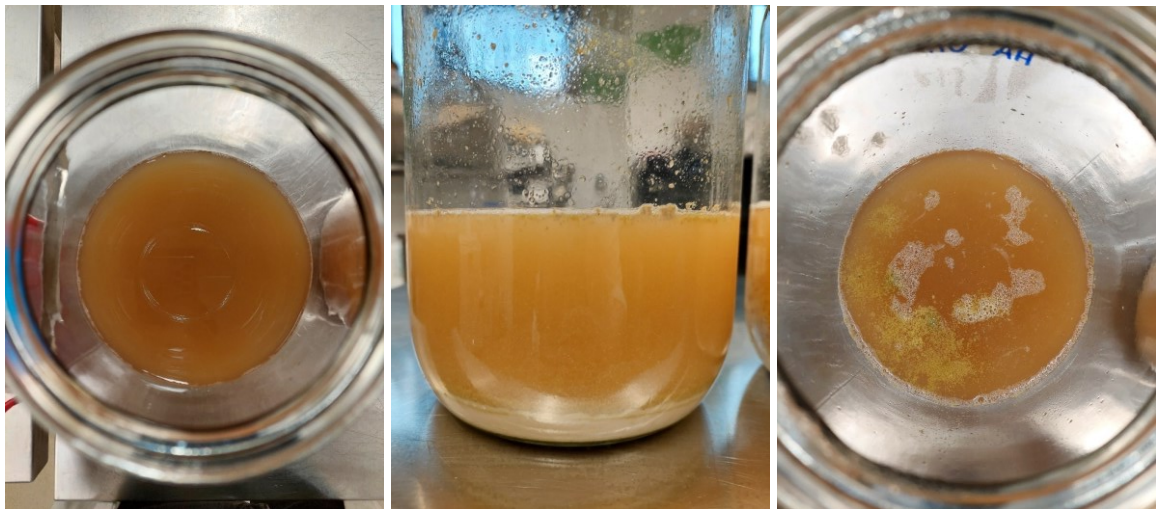
Appendix P III: Sensory analysis dataset and calculations

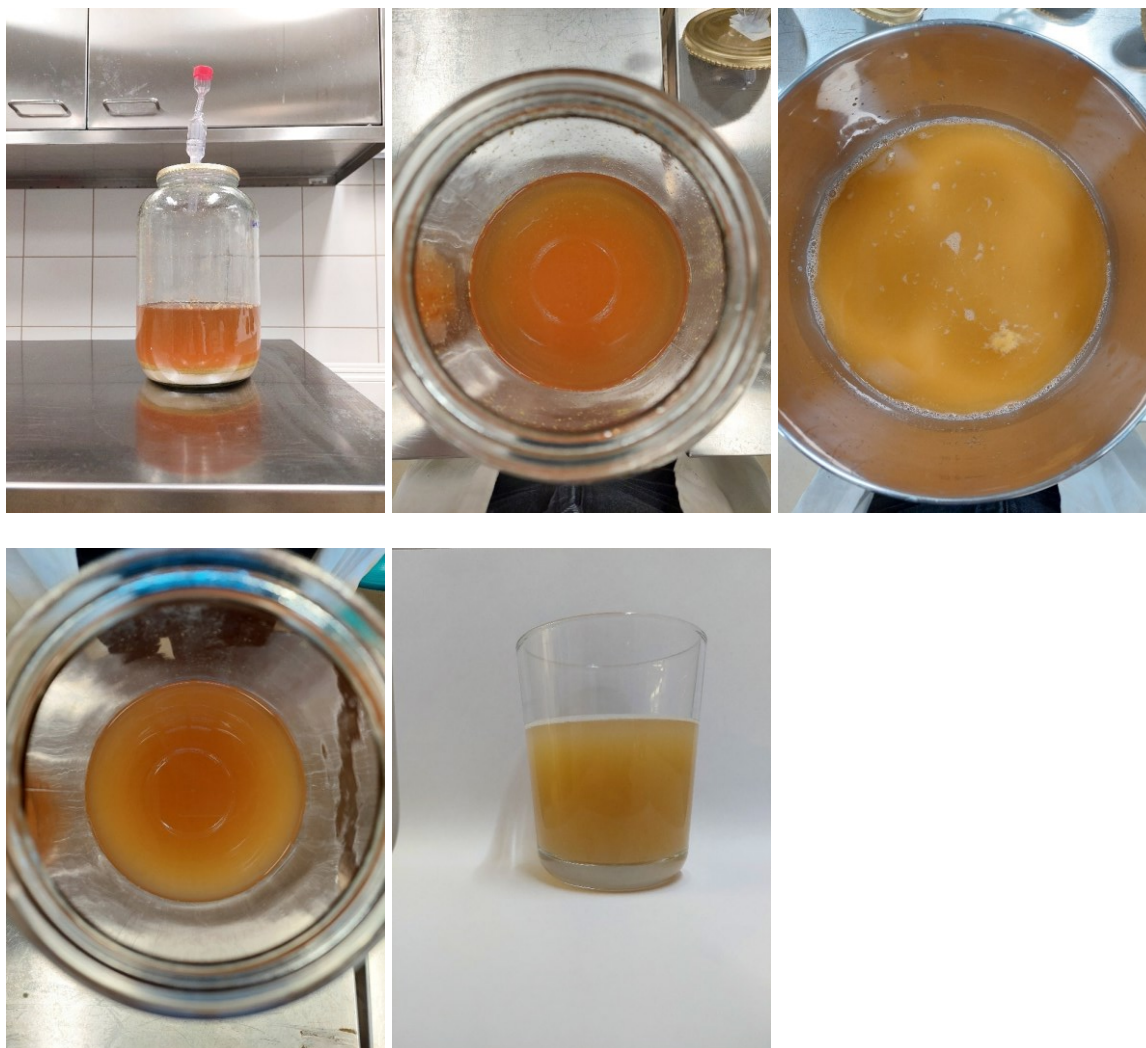
APPENDIX P I: PHOTODOCUMENTARY OF THE SAMPLE PRODUCTION

Brewing:

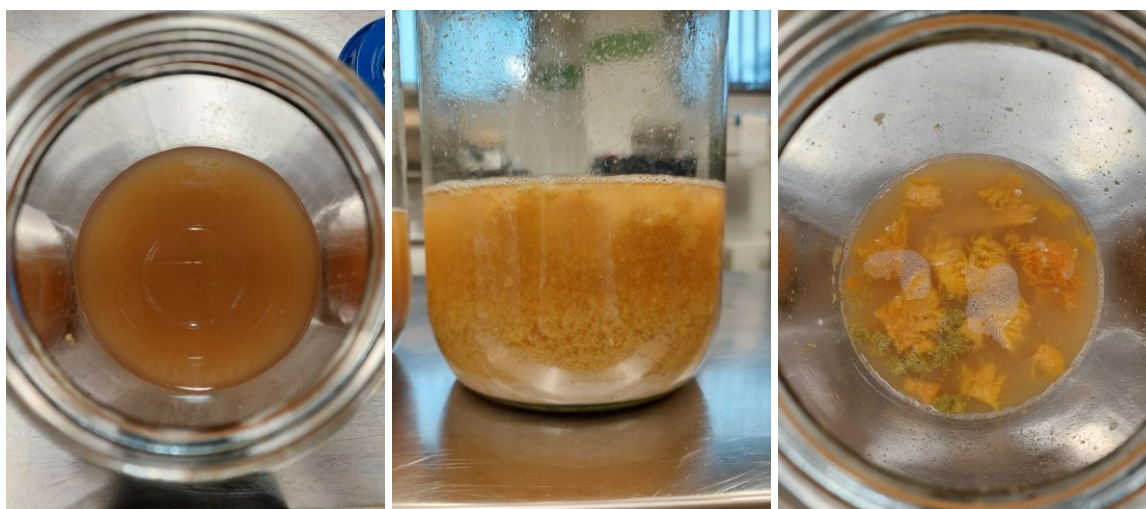


0% w/w sample (before fermentation, dry hopping, after fermentation, after maturation)



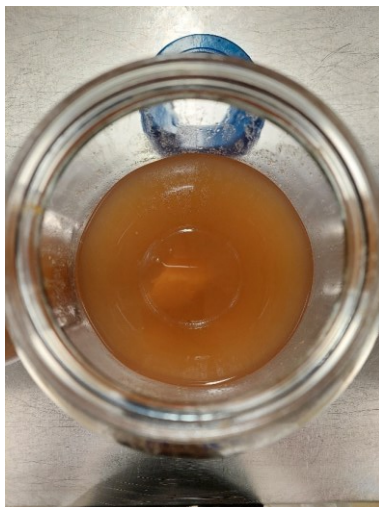


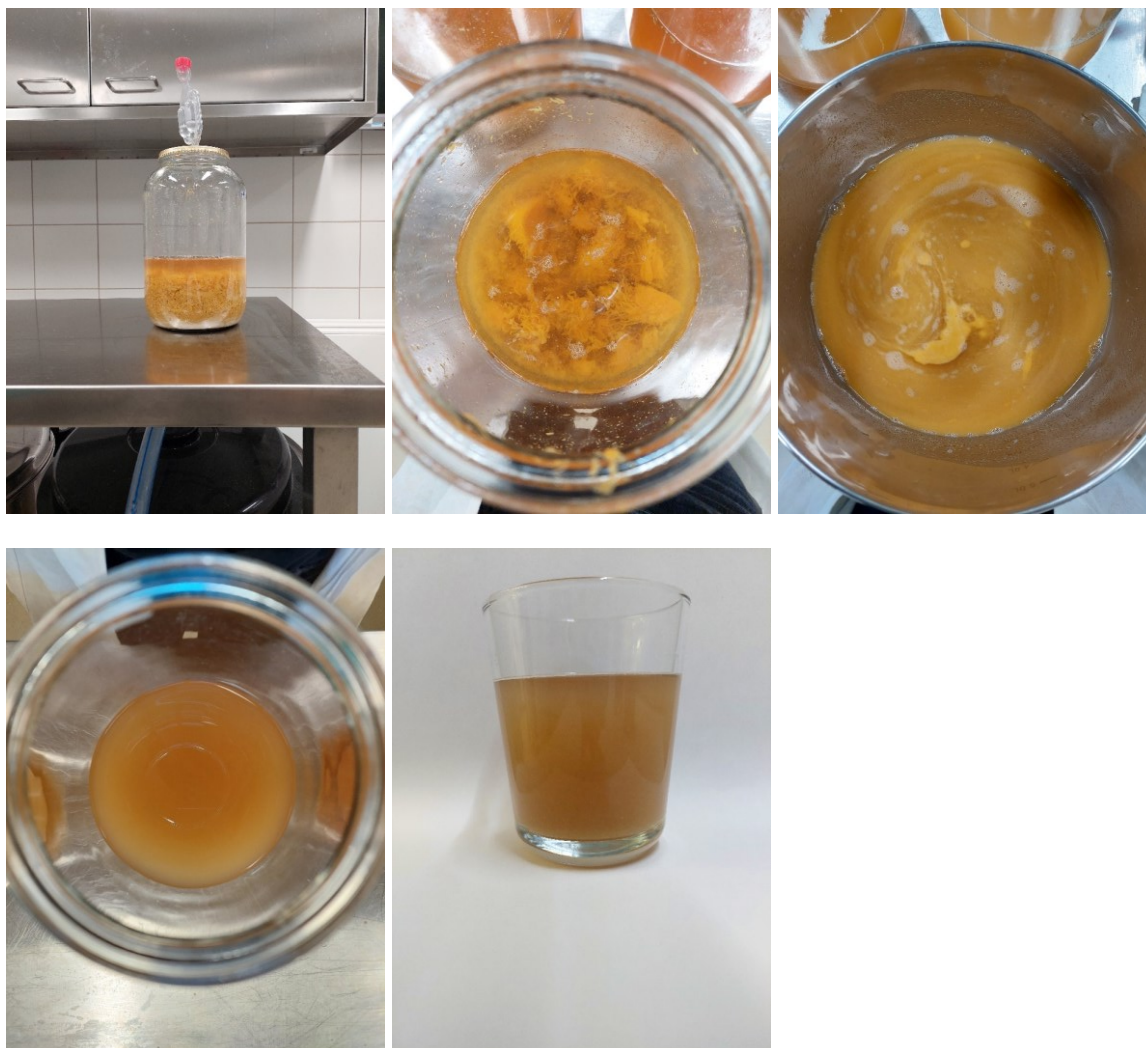
5% w/w sample (before fermentation, dry hopping, after fermentation, after maturation)





12.50% w/w sample (before fermentation, dry hopping, after fermentation, after maturation)





25% w/w sample 12.50% w/w sample (before fermentation, dry hopping, after fermentation, after maturation)





APPENDIX P II: SENSORY ANALYSIS QUESTIONNAIRE

Protokol: Senzorické hodnocení dýňového piva

Jméno a příjmení:

Datum:

Senzorické posouzení piva dle stupnice

Předložené vzorky zhodnoťte pomocí přiložené stupnice a do tabulky запиšte příslušný stupeň. Stupnice pro hodnocení se nachází na konci dotazníku v příloze.

Zakalení není považováno za závadu.

Označení vzorku	Off flavours	Hořkost	Barva	Úroveň karbonizace	Intenzita sladké chutě	Plnost	Celkový dojem
A							
B							
C							

Zhodnocení preference piva

Ochutnejte předložené vzorky a následně je seřaďte podle vlastních preferencí. Výsledky запиšte do tabulky, kde 1 je vámi nejlépe hodnocený výrobek a 3 je nejméně preferovaný výrobek.

1	2	3

Stupnice pro hodnocení piva:

BARVA (color)

1 – VELICE SVĚTLÁ – velmi světlé pivo (very light)

3 – PLNÁ – tradiční barva piva (very traditional color of beer, yellowy-orange)

5 – EXTRA TMAVÁ – černé pivo (very dark, almost black)

OFF FLAVOURS

1 – MINIMÁLNÍ – tak tak zřetitelné (just recognizable)

3 – MÍRNÁ – mírná (moderate)

5 – VELMI SILNÁ – velmi silná nepříjemná chuť (very strong)

ÚROVEŇ KARBONIZACE (carbonization level)

1 – MINIMÁLNÍ – tak tak zřetitelné (just recognizable)

3 – MÍRNÁ – mírná (moderate)

5 – VELMI SILNÁ – velmi silná úroveň karbonizace (very strong)

HOŘKOST (bitterness intensity)

1 – MINIMÁLNÍ – tak tak zřetitelné (just recognizable)

3 – MÍRNÁ – mírná, příjemná úroveň hořkosti (moderate)

5 – VELMI SILNÁ – až moc hořké (very strong)

PLNOST (fullness)

1 – MINIMÁLNÍ – tak tak zřetitelná (just recognizable)

3 – MÍRNÁ – mírná, příjemná úroveň, tak akorát (moderate)

5 – VELMI SILNÁ – velice plné pivo (very strong)

INTENZITA SLADKÉ CHUTĚ (sweetness intensity)

1 – MINIMÁLNÍ – tak tak zřetitelná (just recognizable)

3 – MÍRNÁ – mírná, příjemná úroveň (moderate)

5 – VELMI SILNÁ – velice sladké pivo (very strong)

CELKOVÝ DOJEM (overall rating)

1 – MIMOŘÁDNĚ DOBRÉ (extraordinarily good)

9 – EXTRÉMĚ ŠPATNÉ (extremely)

APPENDIX P III: SENSORY ANALYSIS DATASET AND CALCULATIONS

Sample	Parameter	Accesorres											
		1	2	3	4	5	6	7	8	9	10	11	12
A (12.50%)	Off flavours	3	2	1	4	1	3	1	2	2	1	4	2
	Bitterness	1	3	3	4	4	3	2	1	4	3	5	3
	Colour	3	4	4	3	3	4	5	2	3	4	3	3
	Carbonization level	1	2	3	2	2	3	2	1	2	2	3	1
	Sweetness	1	1	3	1	2	1	2	2	1	1	2	2
	Fullness	3	4	3	3	4	3	5	5	3	3	2	3
	Overall raking	7	5	4	7	5	4	6	6	7	4	5	7
B (5%)	Off flavours	3	2	2	1	2	2	3	2	1	2	1	3
	Bitterness	5	4	2	3	4	3	3	3	5	4	2	5
	Colour	5	4	5	4	3	5	4	5	3	4	3	5
	Carbonization level	3	2	3	4	2	2	1	4	5	2	2	3
	Sweetness	1	2	3	4	3	5	1	1	3	2	4	2
	Fullness	5	4	4	2	4	3	5	3	4	4	3	4
	Overall raking	3	3	3	2	7	5	2	4	3	3	4	4
C (0%)	Off flavours	1	2	1	3	1	3	1	1	2	2	1	1
	Bitterness	5	4	3	4	2	5	4	2	3	2	4	5
	Colour	1	3	2	1	2	2	1	1	3	2	2	1
	Carbonization level	5	1	3	1	3	4	3	2	2	1	3	3
	Sweetness	5	2	3	4	4	5	3	4	3	4	4	2
	Fullness	1	3	3	4	4	3	2	4	4	2	1	5
	Overall raking	1	4	3	5	2	4	1	2	4	3	3	4

Colour

		Scale					
		1	2	3	4	5	Σ
Sample	A	0	1	6	4	1	
	B	0	0	3	4	5	
	C	5	5	2	0	0	
	nK	5	6	11	8	6	250
	NK	5	11	22	30	36	
	RK	0	8.5	17	26.5	33.5	
	TA	0	8.5	102	106	33.5	
	TB	0	0	51	106	167.5	
	TC	0	42.5	34	0	0	

Tested hypothesis H: $H: A \equiv B \equiv C$

One that is statistically different from the others in colour does not exist between samples.

Alternative hypothesis A: $A: \text{non } H$

At least one exists between samples that is statistically different from the others.

Test criterium Q_{KW} :

$$Q_{KW} = \frac{12}{n \cdot (n + 1)} \cdot \sum_{i=1}^R \frac{T_r^2}{n_r} - 3 \cdot (n + 1)$$

$$Q_{KW} = \frac{12}{n \cdot (n + 1)} \cdot \left(\frac{T_A^2}{n_A} + \frac{T_B^2}{n_B} + \frac{T_C^2}{n_C} \right) - 3 \cdot (n + 1)$$

$$Q_{KW} = \frac{12}{36 \cdot (36 + 1)} \cdot \left(\frac{37^2}{12} + \frac{40^2}{12} + \frac{22^2}{12} \right) - 3 \cdot (36 + 1)$$

$$Q_{KW} = 19.37$$

Corrected test criterium Q^*_{KW} :

$$Q^*_{KW} = \frac{Q_{KW}}{1 - \frac{\sum(n_k^3 - n_k)}{n^3 - n}}$$

$$Q^*_{KW} = \frac{19.37}{1 - \frac{(5^3 - 5) + (6^3 - 6) + (11^3 - 11) + (8^3 - 8) + (6^3 - 6)}{36^3 - 36}}$$

$$Q^*_{KW} = 20.40$$

Critical area:

$$20.40 \geq 5.99147$$

With a 95% probability, there is a statistically significant difference between samples in colour.

Némenyi test:

Tested hypothesis H :

$$H: I \equiv J$$

Alternative hypothesis A :

$$A: I \not\equiv J$$

Critical area:

$$|T_i - T_j| \geq q_{1-\alpha}(R; n)$$

$$|T_i - T_j| \geq q_{0.95}(3; 12)$$

$$|T_i - T_j| \geq 120.9$$

	473	815
815	74.5	
269	173.5	248

Off flavours

		Scale					
		1	2	3	4	5	Σ
Sample	A	4	4	2	2	0	
	B	3	6	3	0	0	
	C	7	3	2	0	0	
	nK	14	13	7	2	0	
	NK	14	27	34	36	36	
	RK	0	21	31	35.5	36.5	
	TA	0	84	62	71	0	217
	TB	0	126	93	0	0	219
	TC	0	63	62	0	0	125

Test criterium Q_{kw}:

$$Q_{KW} = -27.91$$

Corrected test criterium Q*_{kw}:

$$Q_{KW}^* = -31.46$$

Critical area:

$$-31.46 \geq 5.99147$$

With a 95% probability, there is no statistically important difference between samples.

Bitterness

		Scale					
		1	2	3	4	5	Σ
Sample	A	2	1	5	3	1	
	B	0	2	4	3	3	
	C	0	3	2	4	3	
	nK	2	6	11	10	7	
	NK	2	8	19	29	36	
	RK	0	5.5	14	24.5	33	
	TA	0	5.5	70	73.5	33	182
	TB	0	11	56	73.5	99	239.5
	TC	0	16.5	28	98	99	241.5

Test criterium Qkw:

$$Q_{KW} = 0.72$$

Corrected test criterium Q*kw:

$$Q_{KW}^* = 0.76$$

Critical area:

$$0.76 \geq 5.99147$$

With a 95% probability, there is no statistically important difference between samples in their bitterness.

Carbonisation level

		Scale					
		1	2	3	4	5	Σ
Sample	A	3	6	3	0	0	
	B	1	5	3	2	1	
	C	3	2	5	1	1	
	nK	7	13	11	3	2	
	NK	7	20	31	34	36	
	RK	0	14	26	33	35.5	
	TA	0	84	78	0	0	162
	TB	0	70	78	66	35.5	249.5
	TC	0	28	130	33	35.5	226.5

Test criterium Q_{kw}:

$$Q_{KW} = -6.05$$

Corrected test criterium Q*_{kw}:

$$Q_{KW}^* = -6.59$$

Critical area:

$$-6.59 \geq 5.99147$$

With 95% probability there is no statistically important difference between samples in carbonization level.

Sweetness

		Scale					
		1	2	3	4	5	Σ
Sample	A	6	5	1	0	0	
	B	3	3	3	2	1	
	C	0	2	3	5	2	
	nK	9	10	7	7	3	
	NK	9	19	26	33	36	
	RK	0	14.5	23	30	35	
	TA	0	72.5	23	0	0	95.5
	TB	0	43.5	69	60	35	207.5
	TC	0	29	69	150	70	318

Test criterium Q_{kw}:

$$Q_{KW} = 4.09$$

Corrected test criterium Q*_{kw}:

$$Q_{KW}^* = 4.31$$

Critical area:

$$4.31 \geq 5.99147$$

With 95% probability, there is no statistically important difference between samples in sweetness.

Fullness

		Scale					
		1	2	3	4	5	Σ
Sample	A	0	1	7	2	2	
	B	0	1	2	3	2	
	C	2	2	3	4	1	
	nK	2	4	12	9	5	
	NK	2	6	18	27	32	
	RK	0	4.5	12.5	23	30	
	TA	0	4.5	87.5	46	60	198
	TB	0	4.5	25	69	60	158.5
	TC	0	9	37.5	92	30	168.5

Test criterium Q_{kw}:

$$Q_{KW} = 0.69$$

Corrected test criterium Q*_{kw}:

$$Q_{KW}^* = 0.76$$

Critical area:

$$0.76 \geq 5.99147$$

With 95% probability there is no statistically important difference between samples in fullness.

Overall ranking

		Scale									
		1	2	3	4	5	6	7	8	9	Σ
Sample	A	0	0	0	3	3	2	4	0	0	
	B	0	2	5	3	1	0	1	0	0	
	C	2	2	3	4	1	0	0	0	0	
	nK	2	4	8	10	5	2	5	0	0	
	NK	2	6	14	24	29	2	7	7	7	
	RK	0	4.5	10.5	19.5	27	0	5	7.5	7.5	
	TA	0	0	0	58.5	81	0	20	0	0	159.5
	TB	0	9	52.5	58.5	27	0	5	0	0	152
	TC	0	9	31.5	78	27	0	0	0	0	145.5

Test criterium Qkw:

$$Q_{KW} = -58.66$$

Corrected test criterium Q*kw:

$$Q_{KW}^* = -61.03$$

Critical area:

$$-61.03 \geq 5.99147$$

With 95% probability, there is no statistically important difference between samples in overall ranking.

Preference

		Preference		
		A	B	C
Assessors	1	3	2	1
	2	3	1	2
	3	3	1	2
	4	3	1	2
	5	2	3	1
	6	3	2	1
	7	2	3	1
	8	2	1	3
	9	3	1	2
	10	3	2	1
	11	3	1	2
	12	3	2	1
	Ti	33	20	19
	Ti2	1089	400	361
	Σ	1850		

Tested hypothesis H: $H: A \equiv B \equiv C$

Between samples, one is statistically different from the other in preference.

Alternative hypothesis A: $A: non H$

At least one exists between samples that is statistically different from the others.

Test criterium FR:

$$FR = \frac{12}{n \cdot R \cdot (R + 1)} \cdot \sum_{i=1}^R T_i^2 - 3 \cdot n \cdot (R + 1)$$

$$FR = \frac{12}{12 \cdot 3 \cdot (3 + 1)} \cdot 1850 - 3 \cdot 12 \cdot (3 + 1)$$

$$FR = 142.13$$

Critical area W_α :

$$FR \geq Q_{1-\alpha}(R; n)$$

$$142.13 \geq Q_{\hat{e},95}(3; 12)$$

$$142.13 \geq 6.17$$

With a 95% probability, there is a statistically significant difference between samples in preference.

Némenyi test:

Tested hypothesis H:

$$H: I \equiv J$$

Alternative hypothesis A:

$$A: I \not\equiv J$$

Critical area:

$$|T_i - T_j| \geq q_{0.95}(3; 5)$$

$$|T_i - T_j| \geq 11.5$$

	473	815
815	13	
269	14	1