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ADDITION OF PINE SHOOTS ON THE DIFFERENT STAGES OF BEER PRODUCTION PROCESS – INFLUENCE ON THE PHYSICOCHEMICAL AND SENSORY ANALYSIS

– Research paper –

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Abstract: In recent years, growing consumer interest in craft beers has encouraged the industry to search for new raw materials that can enhance beer's sensory profile. The aim of the study was to investigate the effect of pine shoots addition on the physicochemical and sensory profile of beer. The shoots were added at the different stages of wort production (at the beginning and end of the boiling), and during the fermentation, in two doses (15 and 30 g/L). As a control sample, beer produced without the addition of pine shoots was used. The worts and resulting beers were analyzed regarding their extract and alcohol content, pH, titratable acidity, color, metal ion and carbohydrate content. Additionally, the resulting beers underwent sensory evaluation. Introducing pine shoots at different stages of beer production significantly affected some of the physicochemical parameters of resulting beers. Regarding the sensory characteristics, beers produced with a lower dose of shoots (15 g/L) were preferred. Among the variants, beers produced with the addition of shoots during the fermentation, and at the beginning and end of boiling were rated the best.

Keywords: craft beer; pine shoots; sensory analysis; beer flavor.

INTRODUCTION

The craft beer revolution has changed the beer market as it was known before. Thanks to the changes brought out by this movement, the market for innovative craft beers has experienced dynamic growth (Garavaglia and Swinnen, 2017). Many of the breweries have aligned with this trend. Such breweries often focus heavily on producing beers with unique sensory characteristics. Innovations in beer production often involve the use of new malts, hop varieties, yeast cultures, and the addition of fruits, vegetables, and other ingredients. These materials are used to enhance the sensory properties of the product, or to offer new, distinct styles of beer. Nowadays, beer consumers are increasingly interested in products with unique flavor profiles (Da Costa Jardim et al., 2018; Jaeger et al., 2020), lower alcohol content (Anderson, 2023), and often, ingredients that exhibit health-promoting properties (Habschied et al., 2020). To meet these expectations, manufacturers expand their offer with non-alcoholic, low-alcoholic, gluten-free, and isotonic versions of beer (Kozłowski et al., 2021). They also use a wide array of plants, herbs, spices, fruit or concentrates to attract consumer interest.

Scotch pine (*Pinus sylvestris* L.) primarily associated with timber and horticulture, exhibits numerous health-promoting properties. Almost every part of this tree (needles, shoots, bark, cones and resin) is rich in various bioactive substances, such as: polyphenols (including flavanols and flavonoids), vitamins and terpenes (Dziedziński et al., 2021; Mármol et al., 2019; Nisca et al., 2021). These substances are believed to positively influence bodily functions. The health benefits of the pine, and pine products have been known for thousands of years. Pine species have been used in traditional medicine of many cultures, such as Egyptian and Al-Andalus (Casas-Agustench et al., 2011), or in cultures of European continent (Papp et al., 2022). Pine has many health benefits, including a positive effect on the respiratory system, antiseptic properties, and medicinal uses for kidney and bladder issues, rheumatic conditions, and diseases of the mucous membranes. Additionally, pine shoots are traditionally used as an expectorant (Njoku et al., 2022).

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Although initially this material may not appear to be associated with brewing, it has been traditionally used in Nordic farmhouse brewing. Along with other conifers, such as juniper or spruce, it is used as a flavoring agent in the production of Kornøl and Stjørdalsøl. The addition of these materials imparts characteristic bitterness, and resinous notes (Garshol, 2020). Resinous, pine-like, and woody flavors are often sought after in specialty beers. The traditional way to introduce these flavors is to use hops with these flavor characteristics. Due to the health benefits of pine shoots, and their potential to impart desired sensory characteristics to the product, they could potentially serve as a valuable ingredient in specialty beer production. Essential oil of pine shoots contains high quantities of terpene hydrocarbons, such as α- and β-pinene, myrcene, and terpenes, such as limonene, α- and γ-terpinene and terpineol. These substances are also found in high quantities in hops (Rutnik and Košir, 2022; Matłok et al., 2020).

The aim of the study was to investigate the influence of pine shoots addition on the physicochemical and sensory profile of beer. The shoots were introduced at different stages of wort preparation (at the beginning and end of boiling), and during the fermentation, in two doses (15 and 30 g/L). As a control sample, beer without addition of pine shoots was used. *S. cerevisiae* US-05 yeasts were used for fermentation. The resulting beers were analyzed regarding their real extract and alcohol content, pH, titratable acidity, color, as well as the content of fermentable sugars and metal ions. Additionally, the beers were subjected to sensory evaluation.

MATERIALS AND METHODS

Materials

The research material consisted of worts prepared from dry light malt extract (SLADOVNA, Bruntál, Czech Republic), pine shoots (*Pinus sylvestris* L. - Małopolska voivodeship, Poland) and Cascade hops (AlePiwo, Rzeszów, Poland). The worts were fermented using *Saccharomyces cerevisiae* Safale US-05 (Fermentis, Rue Gabriel Péri, France) yeast.

Preparation of worts

The worts for the experiment were prepared using light dry malt extract. For this purpose, an appropriate amount of malt extract was added to boiling water to achieve a wort with an extract of 11.5-12.5 °Plato after boiling. After the addition of the dry malt extract, Cascade hops were added in an amount sufficient to obtain wort bitterness of 20 IBU. All wort samples were boiled for one hour, cooled in a water bath to room temperature, and decanted from the sediment. The wort samples (300 mL) were transferred to fermentation vessels and inoculated with *Saccharomyces cerevisiae* SafAle US-05 yeast. Scotch pine shoots (*Pinus sylvestris* L.) were added at distinct stages of wort production process, in two doses (15 and 30 g/L), resulting in two distinct variants for each stage of pine shoot addition. In the produced variants, the pine shoots were added:

I - at the start of the boil (60 minutes of boiling; 15 g/L), II - at the start of the boil (60 minutes; 30 g/L), III - after the boil (added during cooling; 15 g/L), IV - after the boil (added during cooling; 30 g/L), V - half the dose at the start of the boil (7.5 g/L) and half at the end of the boil (7.5 g/L), VI - half the dose at the start of the boil (15 g/L) and half at the end of the boil (15 g/L), VII - at the fermentation stage (15 g/L), VIII - at the fermentation stage (30 g/L), IX - control sample (without pine shoot addition).

The fermentation process was carried out for 19 days at 20°C (each sample in triplicate).

Methods

Determination of fermentation dynamics

The fermentation rate was determined on the basis of a sample mass loss weighted every 24 h with 0.01 g accuracy. Results from three independent repetitions were presented as a percentage loss of the fermentation media mass.

Determination of alcohol, real and apparent extract content, real and apparent attenuation, caloric content

The analysis was performed according to Ciosek et al. (Ciosek et al., 2020). A density meter DMA 4500 M with Alcolyzer Beer Analyzing System from Anton Paar was used to determine the alcohol content, real and apparent extract content, real and apparent attenuation, and caloric content. Before measurement, samples were degassed and centrifuged (3000g × 5 min).

Determination of titratable acidity

The potentiometric method was applied to determine titratable acidity, titrating a sample with 0.1M NaOH solution to obtain pH = 8.

Determination of color

The color of the filtered samples was determined spectrophotometrically (Beckman DU-650 UV-Vis) at a wavelength of 430 nm (according to

Analytica EBC Methods 8.5 and Analytica EBC Methods 9.6) (European Brewery Convention, 2007).

Determination of pH

The determination consisted of direct measurement of the samples using a pH meter (Elmetron CP-505).

Determination of carbohydrate content

The content of carbohydrates (glucose, sucrose and maltose) was determined using Maltose/Sucrose/D-Glucose Assay Kit (Neogen, Bray, Ireland) according to the manufacturer's instructions.

Determination of metal ions

The analysis was performed according to Ciosek et al. (Ciosek et al., 2020). The metal content in the wort was measured using atomic absorption spectrometry with a flame atomization technique (Varian AA240FS) and an automatic sample dispensing system (SIPS-20, Agilent, Santa Clara, CA, USA). The gas flow rates were set at 14 dm³/min for air and 3.5 dm³/min for acetylene. Prior to analysis, samples of wort and beer (3 mL) were placed in sealed pressure vessels, with 5 mL of 65% nitric acid added, and underwent wet mineralization in a Mars Xpress microwave oven (1200 W, 170 °C, 15 min) (CEM Corp., Matthews, NC, USA). After mineralization, the absorbance of the samples was measured at specific wavelengths (422.7 nm for Ca^{2+}, 330.2 nm for Na^{+}, 213.9 nm for Zn^{2+} and 202.6 nm for Mg^{2+}). Metal ions were determined using a single sample aspiration in Fast Sequential mode. Standard solutions for Ca, Na, Zn and Mg (50, 50, 5, and 10 mg/L, respectively) were prepared from 1000 mg/L stock solution (Merck, Billerica, MA, USA).

Sensory analysis

A sensory analysis was carried out by a panel of 10 experienced panelists familiar with beer sensory evaluation methods and the goals of the analysis. Each panelist assessed the quality of various aspects of each beer—overall aroma, color, clarity, and taste—using a 5-point scale. On this scale, 1 indicated the lowest, unacceptable quality, while 5 represented the highest, most desirable quality.

In addition, a detailed aroma assessment was performed, focusing on specific aroma qualities. Panelists were asked to identify the presence and intensity of particular aromas in each beer sample. If an aroma was detected, its intensity was rated on a scale from 1 to 5, where 1 signified a faint aroma and 5 a very strong one. If an aroma was absent, its intensity was marked as 0. The aromas assessed included floral, fruity, banana, green apple, herbal, malt, almond, bitter, bready, acetic, pine, solvent, caramel, yeasty, acidic, buttery, and acidic.

The individual scores for all quality parameters are presented as the arithmetic mean of all evaluations, rounded to one decimal place. Additionally, a 'total' score was calculated by summing the arithmetic means of overall aroma, color, clarity, and taste.

Statistical analysis

The results have been presented as the arithmetic mean of three repetitions, standard deviation included. Additionally, repeated measures ANOVA and a Tukey (HSD) multiple range tests at the significance level of $\alpha = 0.05$ were performed.

RESULTS AND DISCUSSION

Physicochemical parameters of worts

The data in Table 1 present the physicochemical characteristics of the produced worts. The wort extract ranged from 11.8 to 12.2 [% w/w]. The addition of pine shoots at various stages of wort production led to a slight increase in extract levels in the samples compared to the control. The pH of the produced worts was slightly higher (6.18-6.32) than the typical, and suggested range (5.0-6.0) (Esslinger, 2009). This might have been caused by a relatively high pH of used brewing water (7.6), and the fact that dry malt extract has been used to produce wort. pH control is essential to ensure the stability and desired organoleptic properties of the beer. Higher wort pH can lead to unexpected changes in beer quality due to non-optimal pH ranges for mash enzymes. It may also cause higher utilization of α-acids from hops (Palmer, 2017; Bastgen et al., 2020). Additionally, with higher pH values, the bitterness of the beer is more pronounced and less pleasant (Habschied et al., 2021). The titratable acidity of the samples ranged from 0.99 to 1.10 mL 1M NaOH/100 mL of wort (Table 1). It was higher in samples to which the pine shoots were added, especially in higher quantities. The highest acidity was found in variant II. This suggests, that prolonged boiling of pine shoots may release acidic compounds. Addition of pine shoots have affected the color of a beer, however to a very small degree. The difference between the lightest, and the darkest sample was just 1.2 EBC (Table 1).

Table 1. Physicochemical parameters of worts (shown as a mean of three repetitions ± standard deviation).

Variant	Wort extract [% w/w]	Caloric content [kcal/100ml]	Titratable Acidity [mL 1M NaOH/100 mL of wort]	Color [EBC]	pH
I	12.2 b (±0.06)	44.7 b (±0.24)	1.04 b (±0.00)	15.7 a (±0.01)	6.31 d (±0.01)
II	11.9 a (±0.03)	43.4 a (±0.10)	1.10 c (±0.00)	15.0 c (±0.01)	6.21 abc(±0.01)
III	11.8 a (±0.06)	43.3 a (±0.23)	1.00 a (±0.00)	15.6 b (±0.01)	6.29 bcd (±0.01)
IV	11.9 a ±0.06)	43.6 a (±0.23)	1.06 b (±0.00)	15.5 d (±0.05)	6.18 a (±0.01)
V	12.0 a (±0.06)	43.8 a (±0.23)	1.00 a (±0.00)	16.2 e (±0.05)	6.32 cd (±0.01)
VI	12.0 a (±0.05)	43.8 a (±0.18)	1.06 b (±0.02)	15.7 ab (±0.05)	6.20 ab (±0.1)
VII-IX	11.9 a (±0.02)	43.7 a (±0.06)	0.99 a (±0.00)	15.7 a (±0.01)	6.26 abcd (±0.01)

The respective variants (I-IX) differ in the phase when the pine shoots were added. For corresponding variants, the pine shoots were added: **I** - start of boiling (15 g/L), **II** - start of boiling (30 g/L), **III** - end of boiling (15 g/L), **IV** - end of boiling (30 g/L), **V** - start and end of boiling (15 g/L), **VI** - start and end of boiling (30 g/L), **VII-IX** - control.
a- – mean values marked with different letters (for individual parameters) show statistical differences according to Tukey's HSD test ($p<0.05$)

Sugars

All worts were characterized by a similar level of glucose. The differences appeared in the case of sucrose and maltose (Table 2). According to Palmer (Palmer, 2017), the share of analyzed sugars in the wort should be 10-15% glucose, 1-2% sucrose, and 50-60% maltose. However, other authors, such as Navarro et al. (Navarro et al., 2012) list lower values for maltose, i.e. 46%, which is more in line with results obtained in a study. The proportion of all analyzed sugars in the wort, considering wort extracts (Table 1) is lower than the literature data suggests. The most probable explanation for this deviation is the raw material used for the wort preparation, dry malt extract. The malt extract used might have been intended for the production of beers with lower attenuation degree, which are known to be fuller in flavor. Worts for such beers are characterized by a higher content of unfermentable sugars, such as dextrins, oligosaccharides and non-starch polysaccharides (Collins and Wilhelmson, 2005; Garrett, 2012; Kunz et al., 2012; Li et al., 2020). The addition of pine shoots probably did not significantly affect the wort fermentable sugar levels. According to Jyske et al. (Jyske et al., 2020) pine shoots have relatively low content of fermentable sugars. The main sugars found in this material are sucrose (1.5 g/100 g of dry weight) and fructose (1.6 g/100 g of dry weight)

Table 2. Sugar profile and metal ions in worts (shown as a mean of three repetitions ± standard deviation).

Variant	Glucose [g/L]	Sucrose [g/L]	Maltose [g/L]	Ca^{2+} [mg/L]	Na^{+} [mg/L]	Mg^{2+} [mg/L]	Zn^{2+} [mg/L]
I	9.58 (±0.41)	5.03 c (±0.20)	57.83 b (±1.17)	33.9 ab (±0.12)	35.6 a (±1.27)	75.9 b (±0.08)	1.53 c (±0.12)
II	9.76 (±0.22)	3.58 b (±0.22)	54.6 ad (±1.93)	30.4 d (±0.48)	33.8 a (±1.72)	76.9 d (±0.33)	1.43 abc (±0.05)
III	9.49 (±0.31)	3.68 bc (±0.03)	55.4 ab (±0.92)	25.6 c (±0.22)	34.9 a (±0.64)	71.4 a (±0.50)	1.23 a (±0.08)
IV	9.58 (±0.16)	4.19 a (±0.09)	50.6 c (±0.33)	25.4 c (±1.41)	32.1 a (±1.15)	70.1 c (±0.49)	1.28 a (±0.08)
V	9.22 (±0.01)	4.17 a (±0.41)	56.5 ab (±1.01)	34.4 ab (±1.56)	33.7 ab (±1.19)	75.8 b (±0.30)	1.51 bc (±0.01)
VI	9.55 (±0.23)	4.19 a (±0.1)	52.4 cd (±0.02)	33.0 b (±0.86)	28.4 ab (±1.9)	78.0 e (±0.11)	1.41 abc (±0.06)
VII-IX	9.71 (±0.04)	3.75 bc (±0.16)	56.3 ab (±0.05)	31.5 ad (±0.04)	29 ab (±0.86)	71.5 a (±0.25)	1.33 ab (±0.01)

The respective variants (I-IX) differ in the phase when the pine sprouts were added. For corresponding variants, the pine shoots were added: **I** - start of boiling (15 g/L), **II** - start of boiling (30 g/L), **III** - end of boiling (15 g/L), **IV** - end of boiling (30 g/L), **V** - start and end of boiling (15 g/L), **VI** - start and end of boiling (30 g/L), **VII-IX** - control. a-c – mean values marked with different letters (for individual parameters) show statistical differences according to Tukey's HSD test ($p<0.05$)

Metal ion content

The worts contained appropriate levels of metal ions (Preedy, 2011). Evaluated ions are important for the quality of the produced beer, as they take a part in yeast metabolism, and/or significantly influence the beer's flavour. Calcium is known to be a secondary messenger in growth and stress response modulation. It is also reported to shield the structural membrane phospholipids and play a part in yeast flocculation (Lodolo et al., 2008; Stewart, 2016). Interestingly, addition of pine shoots at the end of boiling has significantly reduced the Ca levels of resulting wort (Table 2). This may be explained by the initial complexation of Ca ions into compounds which are precipitated. Prolonged boiling might degrade these compounds, releasing previously bound Ca ions.

Magnesium is a key structural element in most biological structures, and acts as an essential enzyme cofactor (Birch and Walker, 2000; Udeh and Kgatla, 2013). The addition of pine shoots has increased the content of Mg in all of the samples (except samples III-IV) over the control sample. A similar mechanism as for Ca ions might occur. Higher doses of pine shoots resulted in an increased magnesium content in the worts, which is a desirable effect, as magnesium is essential for the proper functioning of yeast cells.

Sodium plays a part in the functioning of specific macromolecules. However, yeast cells actively keep their intracellular concentration low, due to high toxicity of this metal (Amparo and Joaquín, 2007). High levels of monovalent cations, such as Na or K may antagonize the uptake of other cations, such as Ca and Mg (Jones and Gadd, 1990). The addition of pine shoots increased the content of Na ions in worts. Interestingly, higher doses of pine shoots resulted in a reduction of Na ions compared to lower doses, although the levels remained higher than in the control sample.

Proper zinc levels of wort are especially important, as Zn is an essential element in nearly 300 yeast enzymes. As such, it governs processes such as cellular growth, metabolism and fermentation (De Nicola and Walker, 2009). In some cases, the worts might have insufficient levels of this metal. The fermentation problems which might be caused by zinc deficiency are especially problematic in industrial environments. Therefore, supplementation of this metal is often suggested (Nobis et al., 2023). According to the literature, the wort zinc levels should be in the range of 0.15 – 0.5 mg/L, or at least 1 mg/L, depending on the source (Bromberg et al., 1997; Poreda et al., 2015). Therefore, all worts had sufficient Zn levels (in the range of 1.23-1.53 mg/L). A similar relationship to the Na ions was observed for Zn ions. Higher doses of material resulted in lower concentrations of ions in worts, but still above control sample levels.

Overall, the addition of pine shoots has significantly affected the content of evaluated metal ions in worts. However, looking purely at these values, the changes below 10 mg/L for Ca, Na, and Mg are rather insignificant for the beer quality. Pine spouts contain relatively low concentration of magnesium and calcium, lower than hops (Lafontaine & Shellhammer, 2018; Jyske et al., 2020).

Fermentation process

Figure 1 illustrates the fermentation process of samples with the addition of pine shoots, introduced at various stages of wort preparation. The primary fermentation started already on the first day and lasted for six days. On the seventh day, the weight loss of the samples began to stabilize, indicating that the yeast had consumed most of the sugars present in the wort. A similarity was observed in the fermentation process between the variants with pine shoots and the control sample. In the wort to which pine shoots were added during fermentation (15 g/L and 30 g/L), a slightly more intense fermentation process was observed compared to the other samples. A higher dose of pine shoots at the fermentation stage resulted in slightly higher attenuation (Figure 1 and Table 3). Plant constituents are known to have antimicrobial properties. Among many compounds, pine contain pinosylvin, which in concentrations of 300 μg/g can arrest *S. cerevisiae* growth (Plumed-Ferrer et al., 2013). However, the addition of pine shoots did not affect fermentation efficiency.

Physicochemical parameters of beers

The produced beers had the alcohol content in the range from 4.91 to 5.04% v/v (Table 3). The highest ethanol content was found in beers with pine shoots added during wort fermentation (5.01, 5.04% v/v, addition of 30 and 15 g/L of pine shoots, respectively), possibly due to fermentable compounds in the shoots that yeast converted into ethanol. The real extract contains organic compounds such as carbohydrates, proteins, beta-glucans, organic acids and polyphenols. It also contains minerals. All of these compound's groups contribute to the flavor and aroma of the beer (Olšovská et al., 2015). In the case of the real extract, no significant differences were found between the beers (Table 3). The results for alcohol and extract content suggests, that the addition of pine did not have a detrimental effect on the fermentation efficiency, as suggested earlier. Antimicrobial agents found in pine may be available in too low concentrations in wort to have an effect (Plumed-Ferrer et al., 2013).

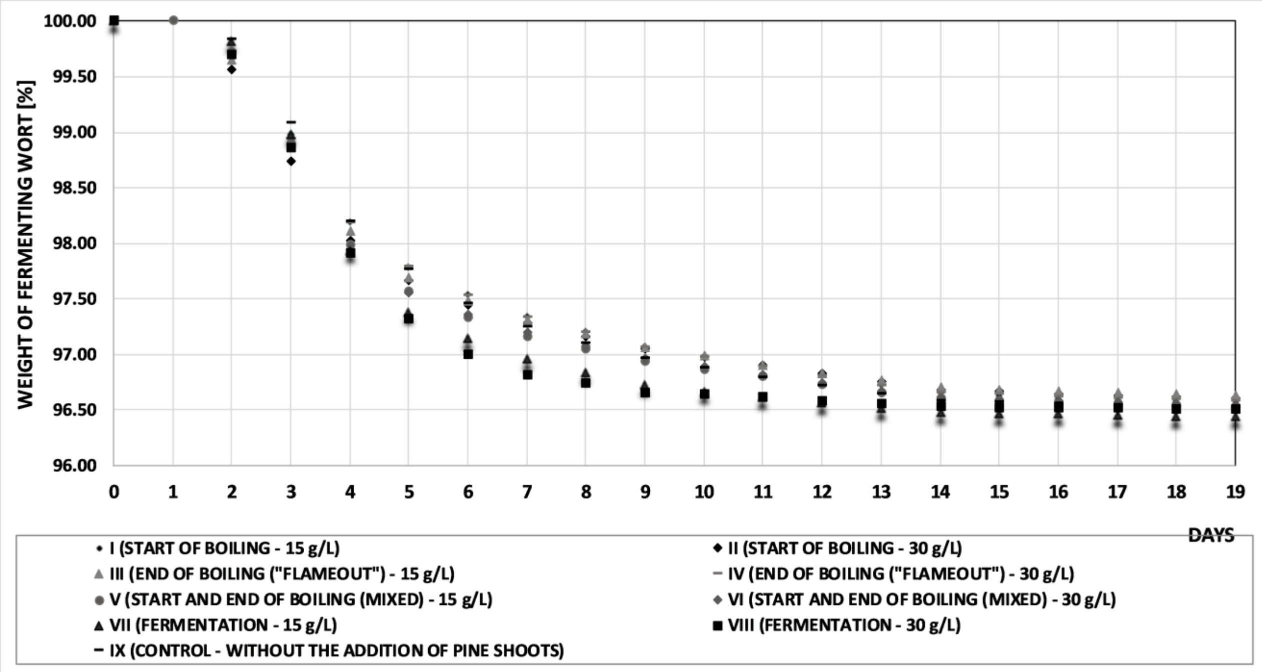

Figure 1. Course of the fermentation of worts (shown as a mean of three repetitions ± standard deviation) Individual measurements are characterized by a standard deviation below 0.5%

The apparent degree of fermentation (ADF) ranged from 77.5 to 79.3%. These values are close to those provided by the manufacturer, with average attenuation for the SafAle US-05 strain expected to be 78–82% (Fermentis). Many factors affect wort attenuation, including wort composition, oxygenation, pH, temperature, and fermentation time. It is worth noting that the maltose level in the analyzed wort was relatively low, which may have led to a slightly lower degree of attenuation (Table 2). However, it is probable that the produced worts had high content of maltotriose, which resulted in relatively high content of alcohol. Typically, this sugar constitutes 15-20% of all the wort sugars (Palmer, 2017). Although *S. cerevisiae* US-05 is an *Ale* strain, it can utilize maltotriose (Heitmann et al., 2017).

Color is an important quality aspect of beer, playing a key role in shaping consumer preferences. The color of beer is primarily influenced by the raw materials used in wort production and processes such as drying or roasting, which lead to Maillard reactions (Michalska and Zielinski, 2007). The resulting beers had a light color (12.5–13.6 EBC), (Table 3). The addition of pine shoots has slightly altered the color. This was probably due to the presence of resins, essential oils (e.g., α-pinene and β-pinene), and flavonoids in the plant material (Papp et al., 2022). The only sample with a statistically significant darker color was sample V. Color intensity could depend on factors such as the stage when the pine shoots were added, freshness of the shoots (fresh, stored, dried), and the beer's pH. The pH of the environment can influence specific substances that impart color (Schweiggert, 2023).

pH has a significant impact on beer quality, influencing both the quality and stability of the beverage. It affects the flavor profile, appearance, clarity, foam stability, bitterness perception, and fermentation process. Factors like the mashing method, malt quality, and water pH are critical in determining the final pH of the beer (Garrett, 2012). During fermentation, wort pH decreases due to the production of organic acids and the consumption of buffering nitrogen compounds (Esslinger, 2009). pH decrease was observed in all analyzed variants, with the pH of beers ranging from 4.37 to 4.56 (Table 3), typical for beers (Krogerus and Gibson, 2013). The variants to which the pine shoots were added at the hot side of the process were characterized by a lower pH level. However, these results were not statistically significant. The titratable acidity was also at a similar level (2.57–3.79 mL 1M NaOH/ 100 mL of beer), (Table 3), with a similar trend of acidity being higher in the samples with earlier shoots adittion During fermentation, titratable acidity increases due to the production of weak acids by yeast. Brewing yeast *S. cerevisiae* produces several organic acids during fermentation, including acetic, pyruvic, and succinic acids (Yoshida and Yokoyama, 2012; Li and Liu, 2015).

Sugar profile

The beers after fermentation contained low concentration of residual sugars (around 1 g/L, expressed as a sum of glucose, sucrose and maltose) (Table 4).

Table 3. Physicochemical parameters of obtained beers (shown as a mean of three repetitions ± standard deviation).

Variant	Alcohol [%vol]	Alcohol [% w/w]	Real Extract [°P]	RDF [% w/w]	Apparent Extract [°P]	ADF [% w/w]	Caloric Content [kJ/100 mL]	pH	Titratable Acidity [mL 1M NaOH/100 mL of Beer]	Color [EBC]
I	5.03 c (±0.01)	3.94 cd (±0.02)	4.56 (±0.07)	64.1 (±0.35)	2.74 (±0.07)	77.5 (±0.45)	43.9 b (±0.25)	4.46 (±0.04)	3.79 (±0.50)	13.3 cd (±0.08)
II	4.91 a (±0.02)	3.84 a (±0.02)	4.40 (±0.05)	64.4 (±0.33)	2.62 (±0.05)	77.9 (±0.42)	42.6 a (±0.10)	4.42 (±0.03)	3.29 (±0.36)	12.5 a (±0.08)
III	4.92 a (±0.02)	3.85 ab (±0.02)	4.35 (±0.05)	64.7 (±0.21)	2.56 (±0.14)	78.3 (±0.27)	42.5 a (±0.24)	4.41 (±0.01)	3.43 (±0.51)	12.9 abc (±0.16)
IV	4.96 abc (±0.03)	3.88 abcd (±0.03)	4.37 (±0.11)	64.7 (±0.73)	2.58 (±0.13)	78.3 (±0.93)	42.8 a (±0.24)	4.37 (±0.01)	3.77 (±0.20)	12.9 bc (±0.14)
V	4.96 abc (±0.06)	3.88 abcd (±0.05)	4.36 (±0.02)	64.8 (±0.29)	2.57 (±0.03)	78.4 (±0.35)	42.8 a (±0.36)	4.49 (±0.04)	2.89 (±0.27)	13.6 d (±0.32)
VI	4.94 ab (±0.03)	3.87 abc (±0.02)	4.38 (±0.02)	64.6 (±0.23)	2.59 (±0.03)	78.2 (±0.28)	42.7 a (±0.11)	4.42 (±0.02)	2.76 (±0.39)	12.6 ab (±0.09)
VII	5.04 c (±0.02)	3.95 d (±0.02)	4.31 (±0.02)	65.5 (±0.06)	2.48 (±0.01)	79.3 (±0.06)	43.0 a (±0.18)	4.50 (±0.06)	2.60 (±0.14)	13.0 bc (±0.05)
VIII	5.01 bc (±0.02)	3.92 bcd (±0.02)	4.33 (±0.02)	65.2 (±0.13)	2.51 (±0.02)	79.0 (±0.15)	42.9 a (±0.06)	4.56 (±0.03)	2.57 (±0.13)	12.8 ab (±0.15)
IX	4.98 abc (±0.03)	3.90 abcd (±0.03)	4.41 (±0.13)	64.6 (±0.78)	2.61 (±0.14)	78.2 (±0.98)	43.1 a (±0.35)	4.53 (±0.10)	2.63 (±0.06)	12.9 abc (±0.14)

The respective variants (I-IX) differ in the phase when the pine shoots were added. For corresponding variants, the pine shoots were added:
I - start of boiling (15 g/L), **II** - start of boiling (30 g/L), **III** - end of boiling (15 g/L), **IV** - end of boiling (30 g/L), **V** – start and end of boiling (15 g/L), **VI** - start and end of boiling (30 g/L), **VII** - fermentation (15 g/L), **VIII** - fermentation (30 g/L), **IX** - control.
a-d – mean values marked with different letters (for individual parameters) show statistical differences according to Tukey's HSD test ($p<0.05$)

Table 4. Sugar profile and metal ions in beers (shown as a mean of three repetitions ± standard deviation).

Variant	Glucose [g/L]	Sucrose [g/L]	Maltose [g/L]	Ca^{2+} [mg/L]	Na^{+} [mg/L]	Mg^{2+} [mg/L]	Zn^{2+} [mg/L]
I	0.07 bc (±0.00)	0.01 b (±0.01)	0.80 abc (±0.35)	42.0 b (±1.13)	48.5 e (±0.10)	78.6 d (±0.02)	1.47 d (±0.08)
II	0.06 ab (±0.00)	0.04 a (±0.00)	1.13 a (±0.02)	41.5 b (±1.39)	41.1 d (±0.23)	78.8 d (±0.80)	0.69 a (±0.03)
III	0.06 ab (±0.00)	0.03 ab (±0.01)	1.07 a (±0.14)	38.2 a (±0.66)	40.2 d (±0.5)	75.4 c (±0.98)	0.91 b (±0.07)
IV	0.05 a (±0.00)	0.04 ab (±0.00)	0.73 abc (±0.35)	36.5 a (±0.75)	35.5 c (±0.95)	73.6 bc (±0.52)	0.69 a (±0.07)
V	0.06 a (±0.00)	0.03 ab (±0.01)	0.78 abc (±0.17)	36.7 a (±0.57)	37.2 c (±0.89)	69.3 f (±0.14)	0.86 b (±0.04)
VI	0.06 ab (±0.00)	0.05 a (±0.00)	0.94 ac (±0.04)	47.0 d (±0.55)	26.9 b (±0.69)	71.9 b (±0.6)	0.91 b (±0.05)
VII	0.07 cd (±0.00)	0.08 c (±0.02)	0.47 bc (±0.01)	42.8 b (±0.91)	23.9 a (±0.46)	66.4 a (±0.31)	0.71 a (±0.07)
VIII	0.08 d (±0.00)	0.10 c (±0.01)	0.37 b (±0.03)	25.7 c (±0.67)	22.7 a (±1.23)	66.1 a (±1.32)	0.65 a (±0.01)
IX	0.06 ab (±0.01)	0.09 c (±0.01)	0.83 abc (±0.06)	26.1 c (±0.1)	27.4 b (±1.19)	62.4 e (±0.42)	0.29 c (±0.02)

The respective variants (I-IX) differ in the phase when the pine shoots were added. For corresponding variants, the pine shoots were added:
I - start of boiling (15 g/L), **II** - start of boiling (30 g/L), **III** - end of boiling (15 g/L), **IV** - end of boiling (30 g/L), **V** - start and end of boiling (15 g/L), **VI** - start and end of boiling (30 g/L), **VII** - fermentation (15 g/L), **VIII** - fermentation (15 g/L), **IX** - control.
a-f – mean values marked with different letters (for individual parameters) show statistical differences according to Tukey's HSD test ($p<0.05$)

Although the majority of residual extract is usually composed of unfermentable sugars, some small quantities of sugars such as maltose or glucose can be found in finished beer (Preedy, 2011; Li et al., 2020). Overall, there are small differences between the samples, regarding their sugar content. This is in line with the results of the fermentation course (Figure 1) and alcohol content of finished beers (Table 3). Some of the results between the samples are statistically significant; however, the overall concentrations of sugars in the finished beers were small.

Metal ions

Ions are also known to influence the taste of the resulting beer. The recommended levels of calcium in beer are in the range 20-150 mg/L. The effect of calcium on taste alone is reported to be rather low. Excess levels (200 mg/L+) can introduce mineral taste. Excess levels of magnesium (above 30 mg/L) may introduce sour taste. Sodium levels in the range of 75 – 150 mg/L contribute to beer's flavor fullness and accentuate sweetness (Preedy, 2011; Kaminski and Palmer, 2013).

Most of the beers had higher levels of Ca, than corresponding worts (Tables 2 and 4). The only exceptions were samples XVIII and IX. The increase in the Ca ion content might have been caused by the secretion of these ions by the yeast cells. During the initial stages of cellular growth, yeast can accumulate metal ions (such as Ca, Zn, Mg) from the environment. These ions can be released back into the wort in the later stages of the fermentation process (Mochaba et al., 1996; Poreda et al., 2009).

The beers differed in the content of Na. It is probable that the addition of pine shoots on the hot side of the brewing process (beers I-V) introduced a significant amount of sodium. The only exception was sample VI, which had similar sodium levels to the samples where pine shoots were added during the fermentation, or a control sample. It is possible, that Na ions were released by the yeast cells during the fermentation process (Ariño et al., 2010).

In the case of Mg, the control sample was characterized by the lowest content of this metal. Interestingly, the samples to which the pine shoots were added at the beginning of the boiling (I-II), or at the end (III-IV), were characterized by a higher content of Mg than corresponding wort samples.

The beers differed significantly regarding their zinc content. The highest content of this metal was found in the sample I, and the lowest, in the sample IX. The fact that the highest zinc utilization was found in sample to which the pine shoots were not added, suggests that phytochemicals extracted from pine shoots might have negatively affect zinc utilization by yeast. Considering the initial wort zinc levels (Table 2), and the fact that not all zinc was utilized, the wort had sufficient zinc levels. Different zinc utilization did not negatively affect fermentation course (Figure 1), therefore different zinc levels were probably not related to different yeast propagation efficiency in beers.

Sensory analysis

The obtained beers were evaluated in terms of aroma, taste, color and clarity. Figures 2 and 3 show the aroma profile of the beers. In the control sample (without the addition of pine shoots), typical beer aromas were detected (malty, floral, fruity, and bitter). Cascade hops were used to produce the wort. These hops are characterized by citrus, fruity and floral aromas. The aromatic compounds such as sesquiterpenes derived from the hops have a decisive influence on the beer's aroma. Among the key components are myrcene (mossy and musky aroma), limonene (tropical fruit and citrus aroma), geraniol, humulene and linalool (floral aroma). Sesquiterpenes can also impart a resinous smell, as well as bitter and spicy flavors (Lutosławski et al., 2018). A very similar aroma to the control sample was observed in the beers with the addition of pine shoots introduced at the beginning and end of wort boiling (30 g/L), as well as during fermentation (30 g/L), (Figure 2). The introduction of pine shoots, containing essential oils rich in various chemical compounds (e.g., α-pinene, β-pinene), at the fermentation stage imparted a characteristic resinous aroma to the beverages (Figure 2), (Leszczyński and Chrapek, 2014). Similar results regarding the resinous aroma were observed when pine shoots were incorporated into the brewing process by Dziedziński et al. (Dziedziński et al., 2023). The beers to which pine shoots were added at the beginning (30 g/L) and the end (30 g/L) of wort boiling exhibited a slight green apple aroma, which is considered a defect. This was likely caused by an elevated acetaldehyde content in these beverages. Acetaldehyde is a direct precursor of ethanol. It is formed during the initial stages of fermentation, and its content decreases over time (Verbelen et al., 2009; Olaniran et al., 2017). Small amounts of acetaldehyde can have a beneficial effect on the flavor and aroma properties of beer. However, at higher concentrations (above 3-4 g/L), it contributes to a grassy, apple-like taste (Esslinger, 2009). All beers were characterized by floral aromas, but these notes were most noticeable in beverages to which pine shoots were added during wort fermentation (Figure 2).

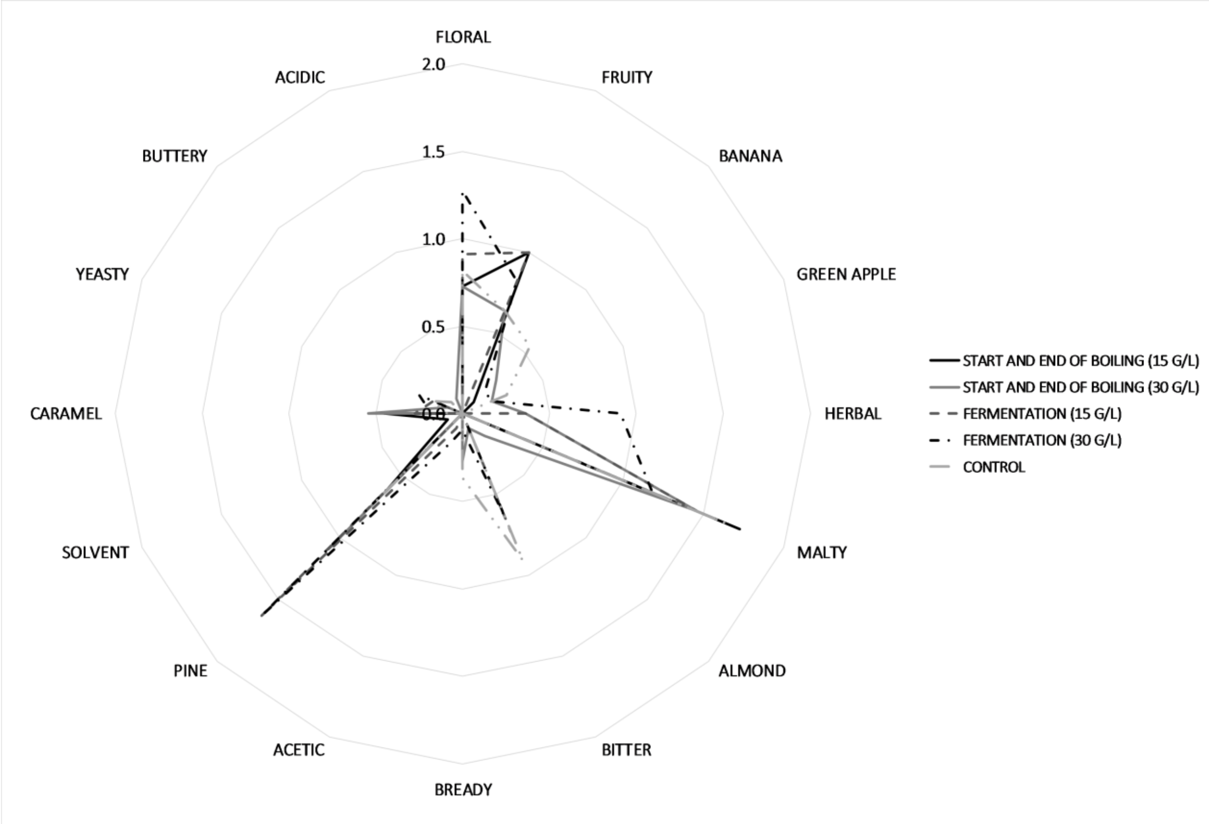

Figure 2. Analysis of beer aroma

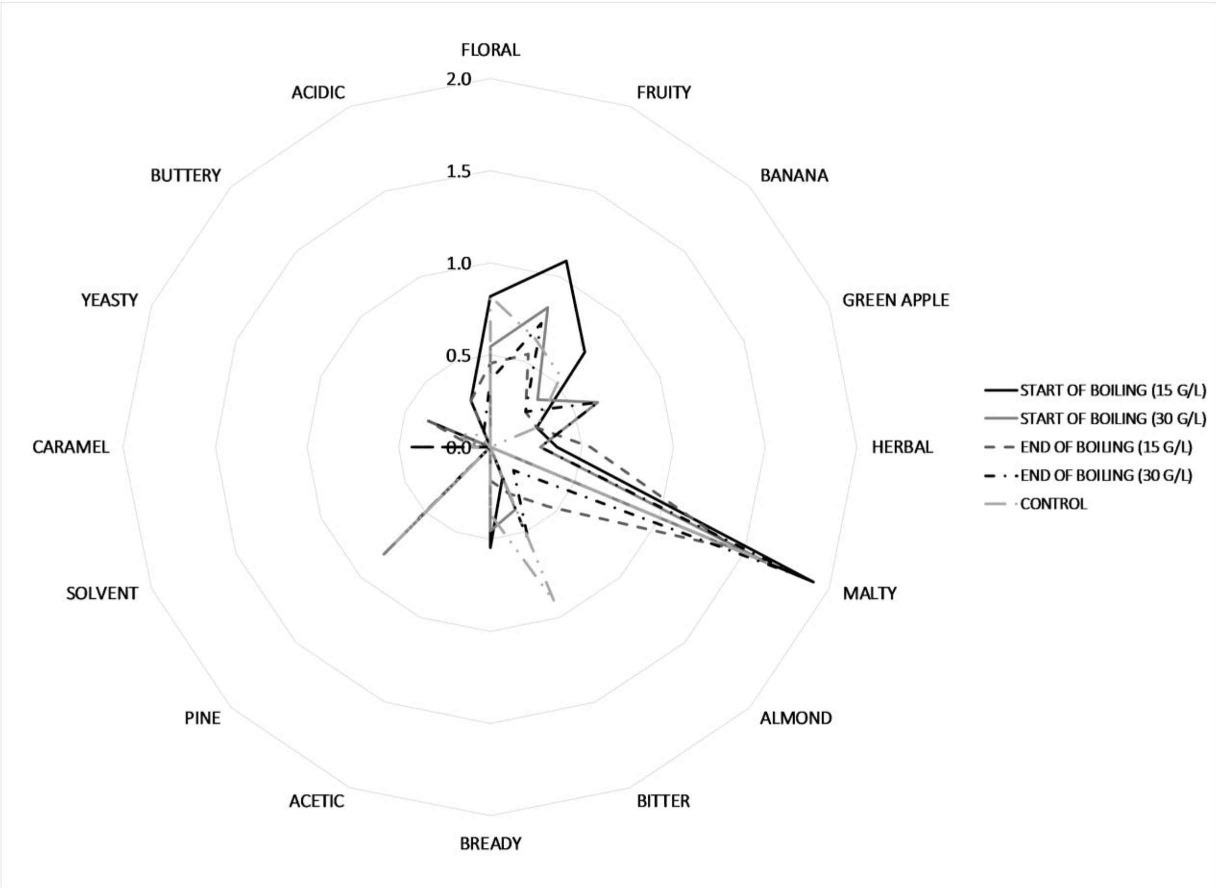

Figure 3. Analysis of beer aroma

Beers with pine shoots added at the beginning and end of wort boiling had the lowest aroma intensity compared to other variants (Figure 4). Beverages with pine shoots added during fermentation (30 g/L) and at the beginning of wort boiling (15 g/L) were rated as the most aromatic of all variants. In terms of color, the least desirable shade for consumers was found in beers to which pine shoots were added at the end of wort boiling. The highest scores were given to beers with pine shoots added during fermentation (30 g/L) and at both the beginning and end of wort boiling (15 g/L). Similar results were obtained when evaluating the taste of the beverages (Figure 4).

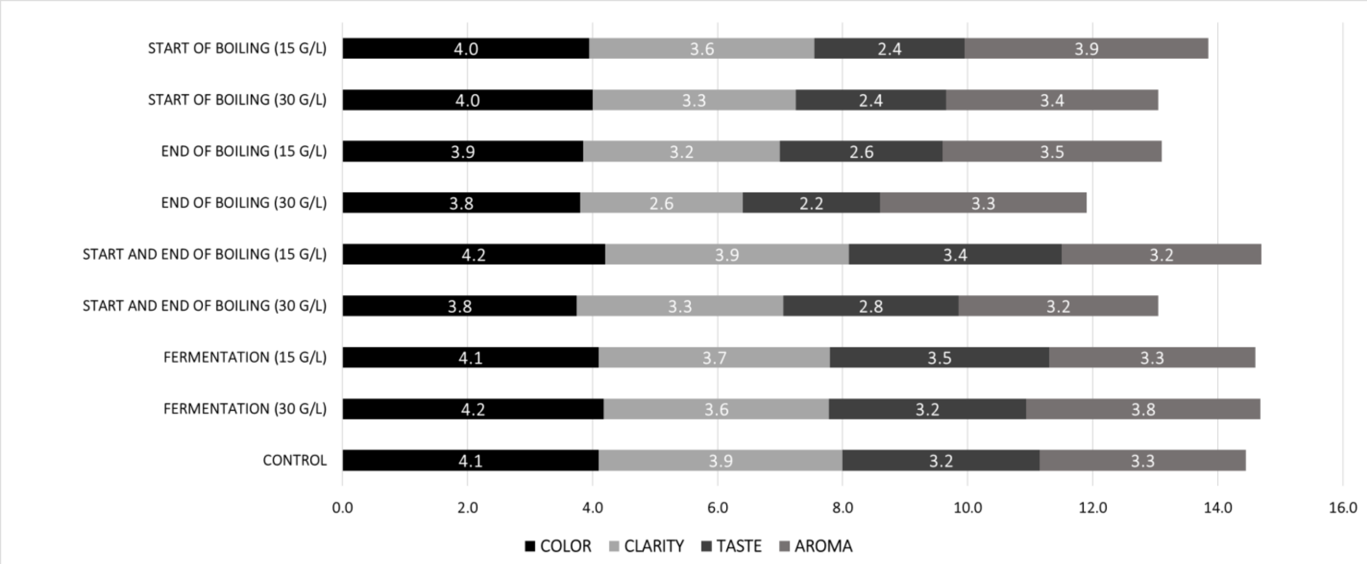

Figure 4. Sensory characteristics of evaluated beers.

CONCLUSIONS

The addition of pine shoots at different stages of beer production significantly affected certain physicochemical parameters of the beers (alcohol content, color). Introducing pine shoots at the fermentation stage (particularly at a dose of 30 g/L) imparted a characteristic resinous aroma to the beer, while preserving fruity and floral notes typical of Cascade hops and those formed during fermentation. Beverages with pine shoots added at the fermentation stage and at the beginning of wort boiling (15 g/L) were rated as more aromatic and flavorful. Pine shoots can be a valuable addition in specialty beer production, providing a unique aroma and taste when applied at appropriate doses and stages.

DECLARATIONS

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