

Research Article

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Antioxidant, sensory, and functional properties of low-alcoholic IPA beer with *Pinus sylvestris* L. shoots addition fermented using unconventional yeast

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Abstract: The study investigated the potential use of pine (*Pinus sylvestris* L.) shoots and standard and unconventional yeast strains for the production of low-alcohol IPA beer. For this purpose, control worts without added shoots, and worts enriched with pine shoots at 10 g/L were prepared. Worts were inoculated with yeast: *Saccharomyces cerevisiae* var. *chevalieri* SafBrew™ LA-01, *Saccharomyces cerevisiae* var. *boulardii*, *Pichia kluyveri* NEER™, and *Saccharomyces cerevisiae* Safale US-05. The final beer was tested for yeast cell number, basic physicochemical properties, effects on cell line and antioxidant properties. Pine shoots and the tested yeast strains were shown to be suitable for developing low-alcohol beers with potential functional effects. Pine shoots have no negative impact on the beer-making process and may increase the antioxidant potential of beer. Beers supplemented with pine shoots were shown to increase the ability to quench DPPH free radicals *in vitro*, while all low-alcohol beers tested were found to have the ability to reduce nitrite production by lipopolysaccharide-induced RAW264.7. Depending on the yeast used, pine shoots affect the flavour and aroma profile differently, possibly

masking foreign aftertastes and odours resulting from the use of unconventional yeast.

Keywords: *Pinus sylvestris* L., low-alcoholic beer, *Saccharomyces cerevisiae* var. *chevalieri* SafBrew™ LA-01, *Saccharomyces cerevisiae* var. *boulardii*, *Pichia kluyveri* NEER™, *Saccharomyces cerevisiae* Safale US-05

1 Introduction

Beer is one of the most widely consumed alcohols in the world. It is a fermented alcoholic beverage produced with water, malted barley, and *Saccharomyces cerevisiae* yeast [1]. The most popular lager beer's distinctive features are its light, clear colour, carbonation, presence of foam, and ethanol content of 4.5–5.5% [1]. Since the beginning of the twenty-first century, however, there has been a clear diversification in the beer market and changes in consumer preferences [2]. Currently, the Beer Judge Certification Program distinguishes more than 80 different styles of beers, which vary significantly in terms of physicochemical and sensory parameters [3]. In addition to the differentiation among styles, there are also new assortment groups, classifying beers by alcohol by volume (ABV), i.e. alcohol-free ($\leq 0.5\%$ ABV) and low-alcohol ($\leq 3.5\%$ ABV) beers [4]. Beer contains B vitamins, minerals, polyphenols, fibre, and prebiotics. However, excessive alcohol consumption has negative health and social consequences, which is why the range of products with reduced ethanol content is developing rapidly [5]. Low-alcohol beer can be seen as a functional beverage, i.e. a beverage in which herbal ingredients, amino acids, vitamins, minerals, and ingredients derived from vegetables or fruit are found that enhance the nutritional value of this group of beverages [6]. The use of herbs and additives in beer production is a well-known practice and is aimed at enhancing flavour and aroma [6].

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Nowadays, many plant raw materials are used at various stages of the production of this beverage to provide health-promoting effects. Xu et al. used okra (*Abelmoschus esculentus* L.) pulp as an additive for wheat beer [7]. The study revealed that incorporating okra resulted in heightened cloudiness, improved foam texture, and enhanced stability in the beers. Furthermore, the beers exhibited elevated levels of terpenes, specifically styrene, which contribute to the characteristic flavour of hazy wheat beer [7]. Ducruet et al. added dried goji berries (*Lycium chinense*) to ale-type beers and assessed the effect on the composition and sensory properties [8]. They found that independently adding 50 g/L of goji berries at the wort boiling stage increased the antioxidant potential of the beer [8]. Besides the addition of plant-based raw materials, unconventional yeast strains have also been increasingly used. Such yeasts include a wide range of strains of *Saccharomyces cerevisiae*, wild strains of *Saccharomyces eubayanus*, in addition to a wide array of various species comprising *Mrakia gelida*, *Torulaspora delbrueckii*, and *Lachancea thermotolerans* [9]. Compared to conventional brewer's yeasts, such strains offer a number of functional advantages, including the possibility of obtaining aromatic low-alcohol beers, reduced caloricity, reduced acidity, the possibility of improving existing beer styles, creating new styles, or faithfully recreating traditional or ancient beer styles [9]. Previous studies have shown that the consumption of alcohol-free beers has a positive effect on human health through the supplementation of biologically active polyphenols and phenolic acids and an increase in the diversity of the intestinal microflora in beneficial bacteria, whereas the presence of alcohol in standard beers impairs this effect [10]. In the present study, the use of cell line research, which is an unusual method for beer analysis, has allowed a preliminary evaluation of the functional effect of hitherto unexplored low-alcohol beers and the possibility of their positive protective effect on the human digestive system [11].

The aim of this study was to investigate the possibility of using unconventional yeast i.e. *Saccharomyces cerevisiae* var. *chevalieri* SafBrew™ LA-01, *Saccharomyces cerevisiae* var. *boulardii*, and *Pichia kluyveri* NEER™ pine shoots (*Pinus sylvestris* L.) to obtain low-alcohol IPA beer with *Pinus sylvestris* shoots addition. The study was carried out to assess the impact of these factors on the brewing process, basic physicochemical properties of the final product, antioxidant activity, and cell lines cultures. It is also aimed to evaluate the effect of pine shoots on the flavour and aroma profile of the beer.

2 Materials and methods

2.1 Material

The experimental samples comprised of air-dried pine shoots (*Pinus sylvestris* L.) harvested in 2021 from the arboretum located in Zielonka, Poland (17°06'33"E, 52°06'33"N). These shoots were maintained at a temperature of 20°C with a relative humidity of 55%. Material also comprised hopped malt extract Coopers Brew A IPA (Coopers Brewery Limited, Australia) and four different strains of yeast: *Saccharomyces cerevisiae* Safale US-05 (Lesaffre, France), *Saccharomyces cerevisiae* var. *chevalieri* SafBrew™ LA-01 (Lesaffre, France), *Saccharomyces cerevisiae* var. *boulardii* (BART Sp. z o.o., Poland), and *Pichia kluyveri* NEER™ (Chr. Hansen Poland Sp. z o.o., Poland).

2.2 Methods

2.2.1 Beer production

In the first stage, hopped malt extract was mixed with an adequate quantity of water and transferred to 1 L glass flasks. Half of the worts were pitched with *Pinus sylvestris* L. shoots in a concentration of 10 g/L (S) and the remaining half were without shoots as controls (C). Worts were pasteurised and cooled to the temperature of 25°C. Prepared worts were inoculated with 10×10^6 yeast cells per millilitre and fermented for 10 days at 20°C and bottled. Then, they were fermented in brown glass bottles for 14 days. After fermentation, the beer was stored at 4°C for 14 days.

2.2.2 Basic physicochemical parameters

To conduct the physicochemical analysis, the beer samples were subjected to degassing through manual agitation for a duration of 5 min. Subsequently, the degassed samples were filtered using a layer of cotton wool and then centrifuged at a speed of 448 RCF for 15 min at a temperature of 20°C (Thermo Fisher Scientific, United Kingdom).

The alcohol content by volume was determined following a distillation process using the Super Dee Digital Distillator (Gibertini, Italy). An automatic densitometer (DDM-2910, Rudolph Research Analytical, USA) employing the mechanical oscillator method was utilised for this

analysis. The extract content in the samples was measured at 20°C using the Balling hydrometer. The measurement of pH was conducted utilizing a CP-411 pH-meter (Elmetron, Poland) based on the PN-A-79093-4:2000 standard.

To assess the bitterness of the beer, the recommended protocol outlined in Analytica-EBC was followed [12]. Initially, 10 mL of degassed beer sample was moved into 50 mL Falcon tubes, to which 0.5 mL of a 6 N hydrochloric acid solution and 20 mL of iso-octane were added. The mixture was vigorously shaken for a duration of 5 min. Subsequently, 10 mL of the resulting sample was transferred into 15 mL Falcon tubes and subjected to centrifugation (Thermo Fisher Scientific, United Kingdom) at a speed of 1,008 RCF for 5 min. For the bitterness analysis, the absorbance of the isooctane layer was measured against pure isooctane utilizing a Halo SB-10 spectrophotometer (Dynamica Scientific Ltd) at a wavelength of 275 nm. The level of bitterness was quantified using international bitterness units (IBU).

2.2.3 Microbiological analysis

To ascertain the overall yeast count, the pour plate technique was employed [13]. All the plates were incubated at 25°C for 72 h. Results showing 30–300 colony-forming units (CFU) were used to analysis.

2.2.4 Polyphenol content

The determination of the total polyphenolic index (TPI) involved the utilisation of Folin–Ciocalteu reagent (FCR), following a procedure outlined by Penkina et al. [23]. The measurement involved assessing the colour change of the radical, transitioning from light blue to dark blue, after a 30 min incubation period at 760 nm using a Shimadzu UVmini-1240 UV-Vis spectrophotometer (Kyoto, Japan). The quantification of total polyphenols (TP) involved the creation of a calibration curve using gallic acid ($3\text{--}20\text{ mg L}^{-1}$, $R^2 = 0.9961$). Finally, the TPI was calculated and reported as milligrams of gallic acid equivalent (GAE) per litre of beer.

2.2.5 Free radical scavenging activity

The assessment of antioxidant activity was conducted using a DPPH radical, following a modified method described by Balik et al. [24]. A stock solution was prepared by dissolving 24 mg of DPPH in 100 mL of methanol. The DPPH stock solution was then filtered using methanol, resulting in a usable

mixture with an absorbance of approximately 0.973 at 517 nm. In a test tube, 3 mL of the prepared DPPH solution was combined with 100 μL of beer, while another tube contained a solution of 3 mL of DPPH in 100 μL of methanol as a control. Subsequently, the tubes were placed in complete darkness for a duration of 30 min. The absorbance was measured at 517 nm. To calculate the percentage of antioxidant activity or RSA, the following formula was applied [19]:

$$\begin{aligned} &\% \text{ of free radical scavenging activity} \\ &= [(A_c - A_s) \div A_c] \times 100, \end{aligned}$$

where A_c is the absorbance of the control reaction; and A_s is the absorbance of the testing specimen.

2.2.6 Effect on induced nitric oxide (NO) production in RAW264.7 cell line

The RAW264.7 macrophage-like cell line was seeded at a density of 35,000 cells per well in 96-well plates and incubated for 24 h to evaluate the effect of beer samples on induced NO production. After the incubation period, the cell culture medium was removed, and the cells were then pre-treated with either beer samples or the control medium alone. After a 2 h interval, the cells were stimulated with 1 mg mL^{-1} of lipopolysaccharide (LPS) for 22 h [14]. To measure NO production, the level of nitrites, a stable end-product of NO metabolism, was determined [15]. Nitrites present in the cell culture medium were detected by combining 75 mL of the medium with an equal volume of Griess reagent, consisting of 1% sulphanilamide and 0.1% *N*-(1-naphthyl)ethylenediamine in 2% H_3PO_4 . The plate was then incubated for 10 min in the dark at room temperature, and the absorbance was measured at 560 nm using a microplate reader (BioTek, Winooski, VT, USA). The absorbance values were standardised in relation to the non-pretreated cells that were stimulated with LPS (positive control), serving as the reference value of 100%. The outcomes are presented as relative percentages of the positive control, reflecting the extent of the response compared to the cells that were fully stimulated.

2.2.7 Measurement of cytotoxic potential of beer samples and beer digest on cancerous Caco-2/HT-29 intestinal epithelial cells

To assess the potential cytotoxic effects of beer and *in vitro*-digested beer, an MTT assay was conducted on a Caco2/HT-29 co-culture. To simulate gastrointestinal conditions, an harmonised static protocol, as described by Minekus et al.

was employed for *in vitro* gastrointestinal digestion [16]. Prior to the cell assays, the *in vitro*-digested beer samples were thawed at room temperature and diluted 12 times in a culture medium [17]. Prior to incubation for 24 h, the Caco2/HT-29 co-culture was subjected to pre-treatment with beer, *in vitro*-digested beer, or the complete medium as the control. After the incubation period, the medium was removed, and the cells were exposed to a 0.5 mg mL^{-1} stock solution of MTT for 3 h. Afterward, dimethyl sulfoxide was introduced to facilitate the dissolution of the formazan crystals. Absorbance values were measured at 570 nm using a microplate reader (Biotek, Winooski, VT, USA). The results are expressed as a percentage of the absorbance value of the control group (medium only), with the control group set to 100%. This allows for comparison and determination of the relative impact of the beer and *in vitro*-digested beer on cell viability.

2.2.8 Sensory evaluation

The sensory evaluation of the beer samples took place at the sensory laboratory of Poznań University of Life Sciences, facilitated by a professional panel consisting of 20 judges (14 women and 6 men). These judges, aged between 21 and 55, possessed extensive training in the sensory analysis of beer. To establish a comprehensive understanding of the beer's characteristics, the panel conducted preliminary sessions where they identified 8 aroma descriptors (citrus, fruity, malty, caramel, hoppy, piney, yeasty, and foreign) as well as 10 taste descriptors (hoppy, malty, yeasty, honey-like, piney, fruity, tart, bitter, sour, and sweet). During the sensory evaluation, the judges were comfortably situated in individual specialised booths, ensuring an environment devoid of disturbances such as noise, visual distractions, and extraneous

odours. The beer samples, each marked with a unique three-digit code, were presented in standard tasting glasses containing 50 mL of beer. The samples were randomised to prevent any order bias. The beer was served at a temperature of 12°C , under white lighting conditions. To assess the sensory attributes, the judges employed an unstructured scale with clearly defined boundaries, allowing them to rate the intensity of each attribute on a scale from 0 (very weak) to 10 (very intense). The mean scores of these attributes were collected and subjected to quantitative descriptive analysis, enabling the generation of a sensory profile for both types of beers.

2.2.9 Statistical analysis

A total of three samples from distinct bottles were utilised for all measurements. The data obtained were presented as the mean value $\pm$ standard deviation and subjected to statistical analysis using one-way analysis of variance through the implementation of RStudio software version 1.4 (RStudio, PBC, Delaware, USA). Statistical significance was determined at a threshold of $p < 0.05$, indicating the presence of notable differences.

3 Results

The physicochemical parameters of the beer varied depending on the yeast strain used and the addition of pine shoots (Table 1). The ethanol content (% v/v) of the beer ranged from 0.98 ± 0.02 in the SC sample to 2.26 ± 0.11 in the LC sample. Beers without shoots had a slightly higher ethanol content than these supplemented, although the differences between the

Table 1: Physicochemical and microbial parameters of the prepared beer

Analysed sample	Ethanol (% v/v)	Extract		Acidity (PH)	Bitterness (IBU)	Yeast count (log CFU/mL)
		Real (% w/w)	Apparent (% w/w)			
CC	2.16 ± 0.03^{ab}	3.00 ± 0.00^{ab}	2.10 ± 0.00^{ab}	4.26 ± 0.08^c	28.99 ± 0.48^{ab}	1.60×10^3
CP	2.06 ± 0.01^{ab}	3.10 ± 0.00^{ab}	2.47 ± 0.29^{ab}	4.23 ± 0.06^{bc}	27.79 ± 0.41^{ab}	2.42×10^6
SC	0.98 ± 0.02^b	3.17 ± 0.29^{ab}	2.87 ± 0.06^{ab}	3.97 ± 0.03^{bc}	27.76 ± 0.07^{ab}	5.60×10^6
SP	1.56 ± 0.02^{ab}	3.77 ± 0.06^b	3.00 ± 0.00^b	4.27 ± 0.03^d	28.73 ± 0.12^{ab}	7.20×10^5
LC	2.26 ± 0.11^a	2.67 ± 0.06^a	1.97 ± 0.06^a	4.37 ± 0.02^b	24.95 ± 0.33^a	1.12×10^6
LP	2.18 ± 0.02^a	2.80 ± 0.00^a	2.10 ± 0.00^{ab}	4.74 ± 0.01^a	30.19 ± 0.14^b	5.00×10^6
NC	1.49 ± 0.01^{ab}	3.57 ± 0.06^{ab}	2.80 ± 0.00^{ab}	4.18 ± 0.03^c	28.62 ± 0.67^{ab}	1.80×10^6
NP	1.34 ± 0.03^{ab}	3.47 ± 0.06^{ab}	2.70 ± 0.17^{ab}	4.22 ± 0.09^c	29.95 ± 0.29^b	1.98×10^6

Values are expressed as the mean value ($n = 3$) $\pm$ standard deviation. Mean values with different letters (a, b, c, etc.) within the same column are statistically different (p value < 0.05).

sample beers were not statistically significant. There was less variation between beers for real and apparent extracts. The acidity of the beers described in terms of pH ranged between 3.97 ± 0.03 for the SC sample and 4.37 ± 0.02 for the LC sample. The beers with added shoots had higher pH. Bitterness as described in terms of IBU was not widely varied, except in the LC beer where IBU was 24.95 ± 0.33 . Due to the lack of a pasteurisation process, all beer samples contained yeast. The CC beer had the lowest log CFU mL⁻¹ content (1.60×10^3), while the SC beer had the highest (5.60×10^6).

3.1 Physicochemical and microbiological parameters of beer

3.1.1 Polyphenol content and free radical scavenging activity (RSA)

The analysed beers contained polyphenols as measured with the FC method and the ability to quench DPPH free radicals (Table 2). The content of TP ranged from 222.42 ± 12.5 mg GAE L⁻¹ for the CP beer to 294.72 ± 27.05 mg GAE L⁻¹ for the SC beer, while free RSA (% RSA) ranged from 31.72 ± 6.52 for the CP beer to 52.91 ± 1.40 for the CC beer, but the differences were not statistically significant. No correlation was observed between polyphenol content and % RSA. For free radical quenching, beers supplemented with pine shoots showed greater efficacy, while for polyphenol content, such a relationship did not exist for all beers and most differences between samples were not statistically significant.

Table 2: Polyphenol content and free RSA of beer samples

Analysed sample	TPI mg GAE L ⁻¹	DPPH % RSA
CP	222.42 ± 12.51^b	31.72 ± 6.52^{ab}
CC	222.58 ± 37.65^b	52.91 ± 1.40^a
SC	294.72 ± 27.05^a	44.98 ± 2.76^{ab}
SP	277.85 ± 15.06^{ab}	46.56 ± 3.32^{ab}
LC	221.39 ± 11.16^b	52.09 ± 5.08^{ab}
LP	274.59 ± 21.48^{ab}	79.67 ± 1.53^c
NC	270.08 ± 29.84^{ab}	39.23 ± 2.66^{ab}
NP	244.80 ± 2.51^{ab}	42.98 ± 6.28^{ab}

Values are expressed as the mean value ($n = 3$) $\pm$ standard deviation. Mean values with different letters (a, b, c, etc.) within the same column are statistically different (p value < 0.05). Abbreviations: GAE – gallic acid equivalent; DPPH – 2,2-diphenyl-1-picrylhydrazyl; RSA – radical scavenging activity.

3.2 Cell line assays

3.2.1 Effect on induced NO production in RAW264.7 cell line

The pre-treatment of LPS-stimulated RAW264.7 cells with beer samples reduced the induced NO production (Figure 1). The lowest % NO compared to the control was shown for NP, which meant that this sample had the highest protective effect. There was a significant difference in NP activity compared to the LP sample, which showed the lowest protective effect, but no statistically significant differences were observed between the other samples.

Figure 2 presents the results for the effect of beer samples and *in vitro*-digested beer on the viability of the Caco2/HT-29 co-culture. Depending on the sample, cell viability ranged from $64.54\% \pm 6.16$ for the LP beer sample to $100.28\% \pm 0.18$ for the NC *in vitro*-digested beer sample. In most samples, *in vitro*-digested samples resulted in greater cell viability.

3.3 Sensory properties

Sensory testing of taste (Figure 3) and aroma (Figure 4) showed variations in profiles depending on the addition of pine shoots and the yeast strain used. The flavour defined as piney was only noticeable in beers supplemented with pine shoots. Apart from this descriptor, no particular correlations were noted in the taste profiles. The CC sample had the highest bitterness and hop flavour sensation, while the LC beer was an outlier in terms of flavour described as foreign, malty and yeasty.

In terms of aroma, higher variation was observed than in the taste profile (Figure 4).

As in the flavour profile, the pine aroma was only perceived in the pine-supplemented beers. In terms of aroma, the beers with added shoots also had an aroma described as citrus. A foreign aroma was perceived in the NP, SC, and NC beers.

4 Discussion

The study indicated the potential for the use of unconventional yeast strains and pine shoots in the production of low-alcohol functional beer. This study is of particular relevance as far as the needs of today's consumers are concerned, as they often seek to reduce alcohol in beer and are

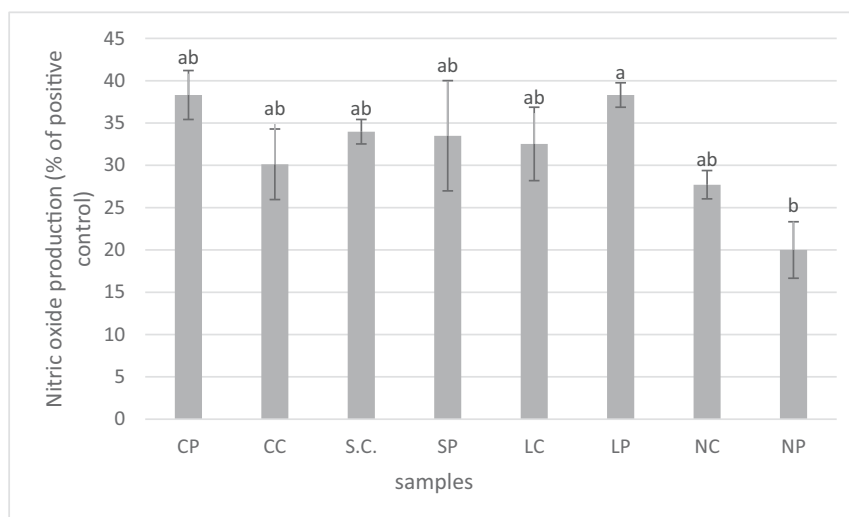


Figure 1: Effect of beer samples on nitrite production by LPS-induced RAW264.7. Values are expressed as the mean value ($n = 3$) $\pm$ standard deviation. Mean values with different letters (a, b, c, etc.) are statistically different (p value < 0.05).

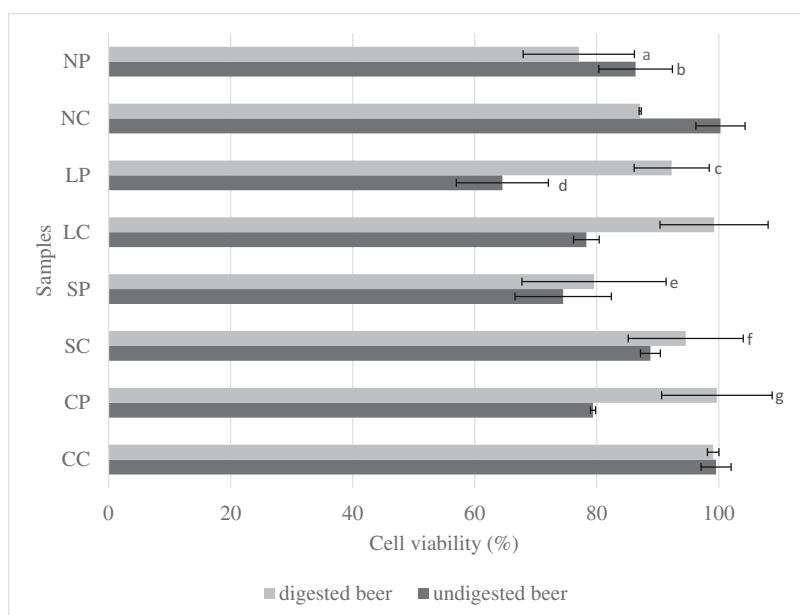


Figure 2: Effect of *in vitro* digested beer and undigested beer samples against induced cytotoxicity of Caco2/HT-29 coculture. Values are expressed as the mean value ($n = 3$) $\pm$ standard deviation. Mean values with different letters (a, b, c, etc.) are statistically different, those without any letter do not differ statistically (p value < 0.05).

looking for new original functional products [6]. The yeast used in the study, such as *Saccharomyces cerevisiae* var. *chevalieri* SafBrew™ LA-01, *Saccharomyces cerevisiae* var. *boulardii*, *Pichia kluyveri* NEER™, and *Saccharomyces cerevisiae* Safale US-05, had good technological and sensory properties. The *Saccharomyces cerevisiae* var. *chevalieri* SafBrew™ LA-01 yeast has not been described in detail in the literature so far. In one of the few studies on the use of this yeast, Simões *et al.* indicated that this yeast showed

high potential for the production of lager beer, and beers made with it had high sensory acceptability and contained volatile compounds in desirable concentrations [18]. In this study, it was confirmed that IPA beers fermented with unconventional yeasts with pine shoots of *Pinus sylvestris* addition showed typical physicochemical characteristics and balanced taste and aroma. The *Saccharomyces cerevisiae* var. *boulardii* and *Pichia kluyveri* NEER™ are considered as useful in the production of low-alcohol beer, while

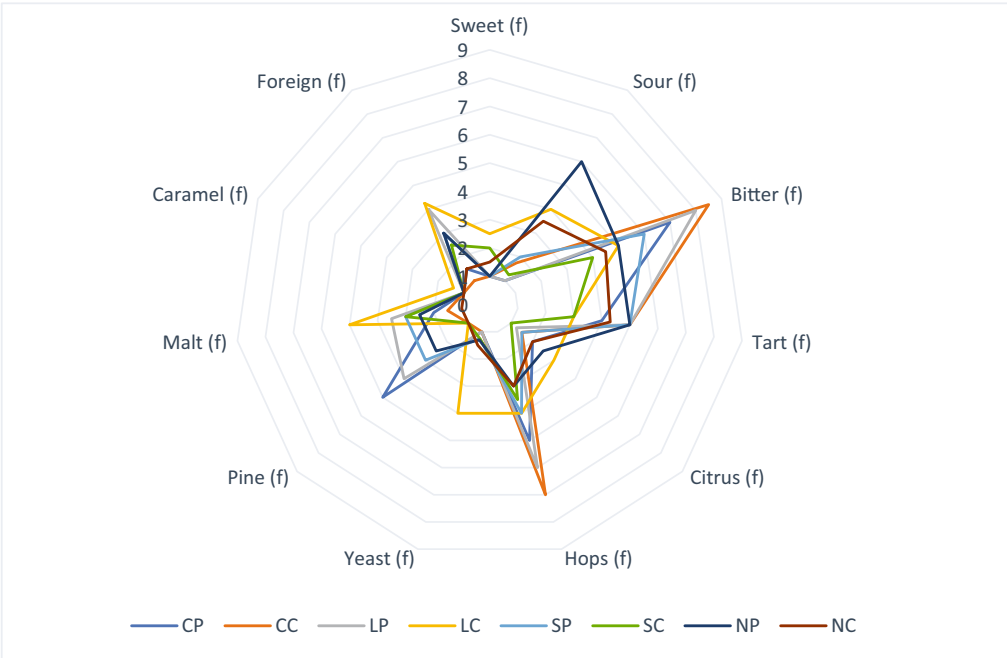


Figure 3: Flavour profile of tested beer samples. Intensities of the specific attributes are according to the compiled flavour classes, where 0 is not perceivable, 10 is very strong, and (f) stands for flavour.

in the current study, it was showed that this yeast modified the aroma of control beers and supplemented beers [19]. This may be due to the higher alcohol content in the control beers and consequently a lower content of residual sugars. Moreover, in the beers with the *Pinus sylvestris* shoots addition, the taste was found as more bitter, which

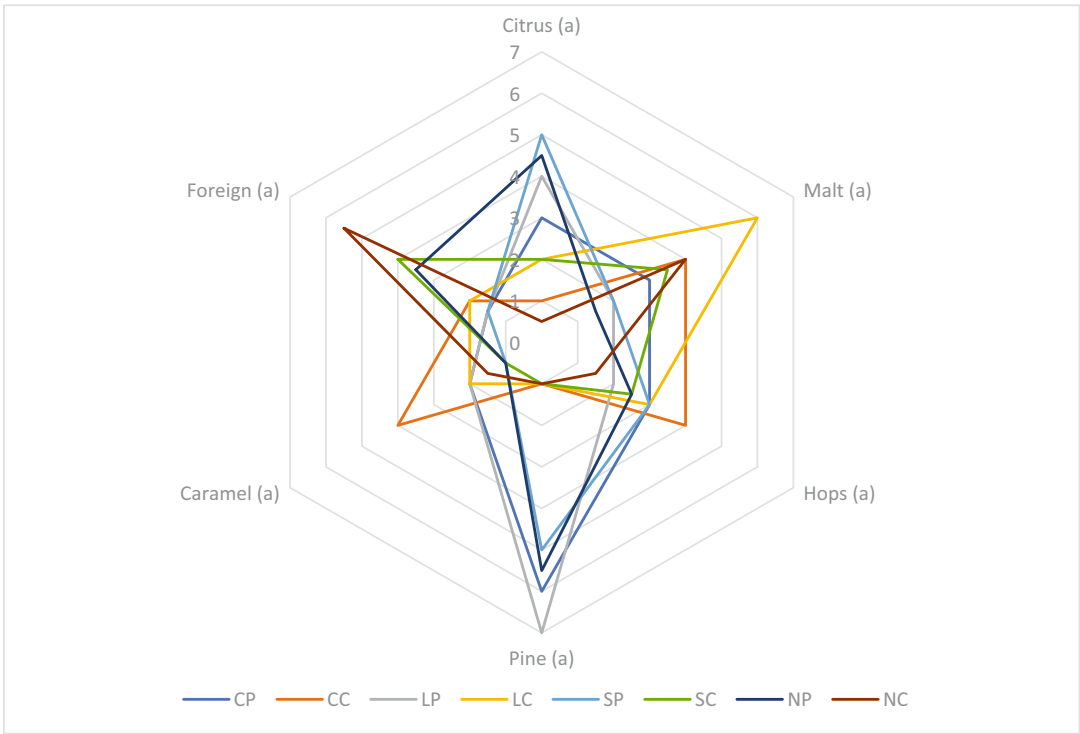


Figure 4: Aroma profile of tested beer samples. Intensities of the specific attributes are according to the compiled aroma classes, where 0 is not perceivable, 10 is very strong, and (a) stands for aroma.

may be due to the presence of compounds such as alkaloids and tannins. The addition of shoots to the wort could influence the yeast fermentation activity [19]. The technological challenge with *S. boulardii* is that these microorganisms can convert fermentable wort sugars into ethanol even at 2°C, which makes it very difficult to produce commercial low-alcohol beers containing live probiotic cultures and fermentable sugars [20]. It can be observed in the current study that ethanol content in beers fermented with *S. boulardii* is in the range of 0.98–1.56% v/v.

Functional beers are often enriched with the use of plant-based raw materials and their extracts. The literature indicates the use of, inter alia: *Coriandrum sativum*, *Brassica nigra*, *Artemisia vulgaris*, *Juniperus communis*, *Melissa officinalis*, *Mentha spicata*, *Origanum vulgare*, *Pimpinella anisum*, *Rosmarinus officinalis*, *Thymus serpyllum* [21]. Commercial beers are available on the market that have been produced using pine shoots, e.g. “Miłosław Sosnowe APA” (Fortuna Brewery, Poland), Forest IPA (Nepomucen Brewery, Poland), and Pine Shoot NEIPA (Austmann Bryggeri, Norway), but so far these have only been beers with standard or high alcohol content (4.8–7.8% ABV). No information was found in the literature on the possible use of pine shoots, while a few studies have used needle extract [22,23]. In the study, the authors noted that the addition of pine needle (*Pinus sylvestris* L.) aqueous extract increased the antioxidant capacity of the beer and could serve as a partial substitute for hops due to similar levels of bitterness and positive effects on sensory properties [22,23]. In the current study, pine shoots showed effects on IBU, and most tested beers supplemented with pine shoots were perceived as more bitter; however, in general, for IPA beers, the addition of pine shoots showed a positive effect on organoleptic properties. Perhaps this is since IPA-style beers are characterised by a higher bitterness expected by the consumer. Parts of other coniferous trees are also used in beer production. In a study by Balík *et al.*, knots, aqueous extracts, or alcoholic extracts of spruce knots (*Picea abies*) were added to the wort at various stages of boiling, and then the content of lignans was measured in pilsner beers [24]. It was shown that the highest content of lignans was found in the sample where the sawdust was in the wort for 65–75 min of boiling, while the lowest was observed when alcohol extract was added, confirming the validity of using shoots as opposed to extracts in the current study [24]. In another study, juniper berries (*Juniperus communis* L.) were added to the wort at different concentrations, i.e. 0.24, 0.48, and 0.72 g L⁻¹ [25]. In contrast to pine shoots, juniper berries negatively affected yeast activity and reduced fermentation, while the enriched beers showed higher polyphenol content and oxygen radical absorbance capacity measured *in vitro* in contrast to the control sample [25].

Compared to supplementation with pine shoots, beers made with juniper berries had a higher polyphenol content and a greater free RSA [25]. When it comes to cell line research, beer is not a common object of study. This may be related to the fact that it is only in recent years that an increased interest in low-alcohol and alcohol-free beer has been noted [26]. According to Kokole *et al.* alcohol-free beer accounted for 3.8% of total beer volume in 2019 [26]. However, as the literature indicates, studies using cell lines may be unreliable at low ethanol concentrations, i.e. below 2.5% [27]. All of the beers in the study had ethanol concentrations ranging from 0.98 ± 0.02 to 2.26 ± 0.11, which is comparable to the alcohol content of functional drinks such as kefir or kombucha [28,29]. In the current study, it was decided that intestinal epithelial cell lines and macrophage lines would be used that can tolerate the ethanol concentrations present in the investigated beers as they can be a good indicator in the study of potential cytotoxic and anti-inflammatory effects [27,30]. A study by Di Domenico *et al.* analysed the effects of brewing fractions from the mashing, filtration, and boiling process with the addition of hops (solution after hopping) of “La Meridionale” beer from Birrificio Bari (Italy), with the addition of Gargano’s IGP orange, coriander, and borage on D-dSC stem cells and Caco-2 intestinal epithelial lines [31]. The findings of the investigation demonstrate that at low concentrations, beer fractions elicit a notable enhancement in cell proliferation. Conversely, when administered at higher doses, these fractions exhibit an inhibitory effect on cell proliferation [31]. The authors conclude that this effect may be due to the higher sugar content of the beer fractions as they are not yet fermented, which may explain the effect seen in the present study, given that low-alcohol beers are richer in sugar. In current study, *in vitro* digested beer in most cases showed higher cell viability. One possible explanation for the observed results is the good tolerance yeast on low pH of stomach and the presence of nutrient compounds in beer, which could have become more available and easier to assimilate after digestion process. Beer is known to be rich in various compounds with potential health benefits, including amino acids, prebiotics, minerals, and B vitamins, which could have contributed to the increased cell viability observed [32]. The results also suggest a potential variability in the effects of different beer samples on cell viability, this may be due to differences in the composition of the beer, which might include variation in the quantity and types of nutrient compounds present, their availability, or their interactions with other beer components [33]. In an experiment where oxidative stress was induced with H₂O₂, the results indicate that the fractions counteract the oxidative effects in D-dSC and Caco-2 cells, which is analogous to the results obtained in the current study on nitrite production

by LPS-induced RAW264.7, where a 60–80% reduction in NO production was observed.

5 Conclusion

The presented study showed that pine shoots at a concentration of 10 g L^{-1} of wort did not adversely affect the activity of the yeasts, namely, *Saccharomyces cerevisiae* Safale US-05, *Saccharomyces cerevisiae* var. *chevalieri* SafBrew™ LA-01, *Saccharomyces cerevisiae* var. *boulardii*, and *Pichia kluyveri* NEER™, and the use of these yeasts and pine shoots makes it possible to obtain low-alcohol beer. Pine shoots do not adversely affect the physicochemical parameters of beer, while they can positively affect the oxygen radical absorbance capacity, as observed in the case of the study on antiradical activity with DPPH, and in the study on nitrite production by LPS-induced RAW264.7. Depending on the yeast strain used, they can affect the perceived taste and aroma in different ways, for example by masking the foreign aroma of a low-alcohol beer made with *Pichia kluyveri* NEER™ yeast.

Abbreviations

CC	control beer/ <i>Saccharomyces cerevisiae</i> Safale US-05
CP	beer with pine shoots/ <i>Saccharomyces cerevisiae</i> Safale US-05
LC	control beer/ <i>Saccharomyces cerevisiae</i> var. <i>chevalieri</i> SafBrew™ LA-01
LP	beer with pine shoots/ <i>Saccharomyces cerevisiae</i> var. <i>chevalieri</i> SafBrew™ LA-01
SC	control beer/ <i>Saccharomyces cerevisiae</i> var. <i>boulardii</i>
SP	beer with pine shoots/ <i>Saccharomyces cerevisiae</i> var. <i>boulardii</i>
NC	control beer/ <i>Pichia kluyveri</i> NEER™
NP	beer with pine shoots/ <i>Pichia kluyveri</i> NEER™

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