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Research Article

Supplementation of beer with *Pinus sylvestris* L. shoots extracts and its effect on fermentation, phenolic content, antioxidant activity and sensory profiles

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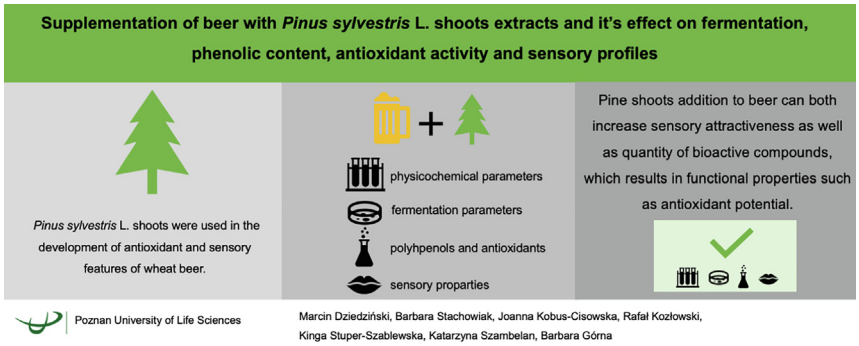
GRAPHICAL ABSTRACT



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ABSTRACT

Background: The aim of this study was to determine the role of *Pinus sylvestris* L. shoots in the development of antioxidant and sensory features of wheat beer.
Results: After storage, the alcohol content of the experimental beer was 4.04%v/v, and its bitterness was 15.83 IBU (bitterness units). Higher levels of bitterness were found compared to the control beer. Other analyzed fermentation parameters (extract, degree of fermentation) and physicochemical parameters (pH, titratable acidity, color) were similar for both types of beer. The addition of pine shoots at the brewing stage affected the profile of biologically active compounds - both polyphenolic acids and flavonols. The content of both groups of those compounds was almost 30% higher in the sample with pine shoots compared to the control sample. The sensory evaluation confirmed the high attractiveness of the beer with pine shoots. During the three-month storage period, the tested samples were microbiologically stable.bd268
Conclusions: It was concluded that pine shoots may be an attractive functional addition to flavored craft beer. It can increase both sensory attractiveness and quantity of bioactive compounds, which results in functional properties such as antioxidant potential.

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1. Introduction

Beer is one of the oldest and most popular alcoholic beverages in the world. In 2020, the global consumption of that beverage was 177.5 million kiloliters. The leader in beer consumption is China (360 million hectoliters), followed by the USA (241 million hectoliters), Brazil (138 million hectoliters), Russia (86 million hectoliters) and Germany (77 million hectoliters) [1]. In 2021, in the EU, the production of alcoholic beer amounted to 33.1 billion liters and of beer that contained less than 0.5% alcohol reached 1.7 billion liters [2]. The traditional raw materials for beer production are water, malt and hops, from which a hopped wort is prepared, which is then fermented with bottom- or top-fermenting yeast in the next stage. The addition of unconventional raw materials shapes the sensory qualities of beer, first of all, but it can also affect the content of bioactive compounds with functional effects, including antioxidants [3]. The source of such raw materials can be the forest environment, including pine species, individual elements of which, such as bark, cones, shoots, have been and are used in traditional medicine for various ailments, most often in the treatment of respiratory and dermatological diseases. The preparations obtained from them (resin, extracts, ointments, lotions, oils) show antimicrobial, antioxidant, anti-inflammatory and cytoprotective properties. They can also be used in the treatment of neurodegenerative disorders such as Alzheimer's or Parkinson's diseases, as well as in the treatment of wounds [4]. Pycnogenol, an extract from the bark of the French maritime pine, is the most powerful antioxidant known to modern medicine. It contains polyphenolic compounds (mainly procyanidins, organic acids and bioflavonoids). The quality of this extract is determined by the United States Pharmacopeia (USP 28) [5]. In turn, the extract from *Pinus sylvestris* aetheroleum is officially listed in the European Pharmacopoeia [6]. It has antibacterial, expectorant and analgesic properties and is used as an antiseptic for respiratory tract, urinary tract and kidney infections. It facilitates the dissolution of kidney stones [6]. The shoots of the Scots pine (*Pinus sylvestris* L.) are also a rich source of bioactive compounds. Among those, the most important are essential oil (0.4%) phenolic compounds (flavonoids, tannins, phenolic acids and their derivatives), vitamin C (lithium). Young shoots of *Pinus sylvestris* are used to treat respiratory diseases (e.g. asthma, cough and tracheitis). Pine shoot extract has been used as a folk remedy in the treatment of chronic inflammation, circulatory disorders and asthma, and now, it is an ingredient of pharmaceutical supplements [4].

Phenolic compounds are well-known antioxidants capable of reducing oxidative stress, which is the direct cause of most civilization diseases, such as cardiovascular diseases, cancer, and neurodegenerative diseases (Parkinson, Alzheimer). There are more than 50 components in pine shoot essential oils. Their concentration depends on the species, growing conditions, morphological part of the plant. The following components of essential oils are added in the largest amount: α-pinene, β-pinene, β-phellandrene, β-caryophyllene, camphene, α-terpineol, germacrene D, bornyl acetate, citronellol. They can determine the sensory properties of the final products [7]. Additionally, they are characterized by antiviral and antimicrobial properties. Pinens have the GRAS status, i.e. they can be used as food ingredients [8].

Beer that contains coniferous tree extracts, mainly from pine shoots, appear on the craft beer market; however, the impact of the extracts on the antioxidant activity of beer, polyphenol content and sensory properties of that beverage has not been analyzed so far. The aim of this study was to determine the role of *Pinus sylvestris* L. shoots in the development of antioxidant and sensory properties of wheat beer. To evaluate the effect on the addition of pine shoots on the characteristics of the beer, a test beer and a control beer were produced under laboratory conditions and stored for one month. Basic physicochemical properties, concentration of polyphenols and their profile, the microbiological condition of beer and its sensory quality were analyzed.

2. Materials and methods

2.1. Material

The test material was pine shoots (*Pinus sylvestris* L). Shoots collected in 2021 from the arboretum in Zielonka (Poland, 17°06′33″E, 52°06′33″N), a part of the Forest Experimental Department of Poznan University of Life Sciences. The material was air-dried at 20°C with 55% humidity and stored before usage. A ready-made brew-kit BA Hefeweizen (Browamator, Poland) was used to prepare the beer. The kit included a blend of ground malts in the following proportions: pale wheat Weyermann® – 58%, pilsner Weyermann® – 37%, carmel Carahell® – 5%; granulated aromatic hop Relax (Germany) – 30 g; dried top-fermenting *Saccharomyces cerevisiae* yeast (Safbrew™ WB-06) – 11.5 g.

2.2. Methods

2.2.1. Laboratory beer production

In the first stage, the infusion mashing with stirring was carried out under laboratory conditions. For that process, 15 l of top water and 4.3 kg of blend of malts (a weight ratio: 3.5:1) were used at the beginning. The temperature of mash was adjusted to 45°C and maintained for 10 min. Then, the temperature of mash was raised as follows: to 53°C for 15 min (for β-glucan denaturation); to 63°C for 30 min (for protein denaturation) and to 72°C up to the negative iodine test (for starch denaturation). Then, the mash-out temperature was raised to 76°C for 10 min (for enzyme denaturation). The ready mash was transferred to a plastic filter tank. The mash was left for about 30 min to create a filter bed from the malt spent grains of mainly the husk fraction. After this time, the proper filtration stage took place. After separating the first wort, the grains were washed with water at the temperature of 75°C until 20 l of liquid was obtained. The obtained wort was boiled for 80 min. Hops (15 g/l) were added after 15 min of boiling, and then, pine shoots (15 g/L) were added after 30 min. The control sample was prepared with the addition of hops, in two portions of 15 g, which were added in the same way as in the case of the pine shoot sample. The boiled wort was cooled down to the temperature of 25°C. The content of the extract was measured with the use of the Balling hydrometer by cooling down the wort to the temperature of 20°C.

Yeast was added to the cooled wort. Fermentation was carried out in a closed 30 l plastic fermentation vessel, at the temperature of 20°C for 10 d in a thermostat with a cooling system (ST700,

POL-EKO Aparatura, Poland). Next, the beer was poured into 500 ml glass bottles and kept refrigerated (4°C) for one month. They were analyzed at 3 stages of production - as wort (W), as beer after fermentation (FB), and beer after one month of storage (B1).

Explanations of sample acronyms used in the manuscript are provided below.

CW – control wort
EW – experimental wort, wort with pine shoots addition
CB – control beer after main fermentation
EB – experimental beer, beer with pine shoots after main fermentation
CB1 – control beer after one month of storage
EB1 - experimental beer, beer with pine shoots after one month of storage

2.2.2. Basic physico-chemical parameters

For the physico-chemical analysis, the samples of beer were degassed by manual shaking (5 min), filtrated through a layer of cotton wool and centrifuged (2000 × g for 15 min, 20°C).

The alcohol concentration by volume was determined after distillation (Super Dee Digital Distillator Gibertini, Italy) using automatic densitometer (DDM-2910, Rudolph Research Analytical, USA) by mechanical oscillator method. The extract content/the density in the samples was measured with the use of the Balling hydrometer at 20°C. The pH was determined using a pH-meter (Elmetron CP-411, Poland). To determine the titratable acidity, 25 ml of each sample was titrated with 0.1 N NaOH solution from the initial pH to 7.0. Total acidity was expressed in units of ml 1 M NaOH /100 ml beer. The color of beer was determined with the use of a spectrophotometer (Halo SB-10, Dynamica Scientific Ltd) at 430 nm wavelength.

The beer bitterness analysis was performed according to Analytica-EBC (2010) recommendation. An amount of 10 ml degassed beer was transferred to Falcon tubes (50 ml), and then, 0.5 ml of a hydrochloric acid solution (6 N HCl) and 20 ml of isoacetate were added. The tubes were shaken for 5 min. Next, 10 ml of the sample was placed into 15 ml Falcon tubes and centrifuged (3000 rpm, 5 min). For analysis of beer bitterness, the absorbance A275 of the isooctane layer was measured in quartz cuvettes at a wavelength of 275 nm (spectrophotometer Halo SB-10, Dynamica Scientific Ltd.) against pure isooctane. The value of bitterness is expressed in units of bitterness (IBU).

2.2.3. Microbial analysis

The pour plate method was used to determine the total count of microorganisms (nutrient agar – NA, BTL, Łódź, Poland; 30°C, 48 h), the total count of lactic acid bacteria (LAB) (de Man, Rogosa and Sharpe agar – MRS, Oxoid) under anaerobic conditions (30°C, 72 h), the total count of yeast (Yeast Extract Glucose Chloramphenicol – YGC Agar, BTL, Łódź, Poland; 25°C, 72 h).

2.2.4. Polyphenol content

Phenolic compounds in samples were analyzed after alkaline and acidic hydrolysis [9]. The analysis was performed using an Aquity H class UPLC system equipped with Waters Acquity PDA detector (Waters, USA). Chromatographic separation was performed using Acquity UPLC® BEH C18 column (100 mm × 2.1 m m, particle size 1.7 μm) (Waters, Ireland). The elution was carried out gradient using the following mobile phase composition: A: acetonitrile with 0.1% formic acid, B: 1% aqueous formic acid mixture (Ph = 2). Concentrations of phenolic compounds were determined using an internal standard at wavelengths λ = 320 nm and 280 nm. The compounds were identified based on a comparison of the retention time of the analyzed peak with the retention time of the standard and by adding a specific amount of the standard to the analyzed samples and a repeated analysis. The detection level is 1 μg/g.

2.2.5. DPPH assay

The extract's antiradical scavenging potential against DPPH radicals was analyzed. To that end, a methanolic solution of DPPH was used to evaluate the free-radical scavenging potential of the samples [10]. The degree of the solution's discoloration indicated the scavenging efficacy of the added substance. For this analysis, 1 ml of the tested solution was supplemented with 2 ml of pure methanol (Honeywell, United Kingdom), followed by 0.25 ml of 1 mM DPPH• ethanolic solution. The mixture was vortexed for ∼60 s and left for 20 min at room temperature. Absorbance was recorded at λ = 517 nm (Meterek SP 830, Taiwan). Methanol was used to prepare a reference sample and the control. To plot a calibration curve, the absorbance values were measured simultaneously for samples containing respective concentrations of Trolox (Sigma-Aldrich, Germany) as a standard (0.5, 1.0, 1.5, and 2.0 mg/ml; r2 = 0.9639). The results are expressed as % of inhibition.

2.2.6. Sensory evaluation

The sensory evaluation of beer and beer supplemented with shoots of *Pinus sylvestris* L. was carried out at sensory laboratory of Poznan University of Life Sciences. It was performed by a panel of 20 assessors (14 women and 6 men), at the age from 21 to 55, all of them were university staff members or students trained in performing sensory analysis of various alcoholic beverages (including beer). During preliminary sessions, the panelists generated 10 taste descriptors (sweet, sour, bitter, tart, fruity, yeasty, pine, malty honey, hop) and 8 aroma descriptors (citrus, malty, hoppy, yeasty, pine, caramel, foreign, fruity). The panelists were seated in separate purpose-made booths, and the environment was free of interference in terms of noise, visual stimulation and ambient odor. The samples were evaluated in duplicate and were placed in random order into standard tasting glasses filled with 50 ml of beer, and marked with a three-digit code. The beer samples were served at 12°C under white light. The panelists used an unstructured scale with boundary markings to rate the intensity of each attribute (0 = very weak, 10 = very intense), and the mean scores of attributes were submitted to quantitative descriptive analysis in order to generate the sensory profile of the two types of beer.

2.2.7. Statistical analysis

All measurements were performed using three samples (different bottles). All data were expressed as a mean ± standard deviation and subjected to one-way analysis of variance (ANOVA) using the RStudio software version 1.4 (RStudio, PBC, Delaware, USA). Statistical differences were measured at $P < 0.05$.

3. Results

3.1. Physico-chemical and microbiological parameters of beer

The results of physicochemical and microbiological tests of the wort and the produced beer are presented in Table 1. The determined base wort extract of the experimental beer (with pine shoots) and the control beer was 12% and 11.5%v/v, respectively. As a result of the wort fermentation, the actual amount of the extract in the beer decreased and was: 5.20% v/v for CB and 5.00% for SB. During storage, further, but small, consumption of the extract took place and, after a month, the parameter reached the value of 4.30% v/v for both types of beer. The content of ethanol in beer with pine shoots was higher compared to the control sample, at all controlled production stages. After one month of storage,

Table 1
Physico-chemical and microbial parameters of the prepared worts (CW, SW) and beer – young beer after main fermentation (CB, SB) and beer after a month of storage in a fridge (CB1, SB1).

Analyzed sample	Ethanol	Extract		Degree of fermentation		Acidity		Bitterness	Color	Yeast count
	% v/v	real %w/w	apparent %w/w	real %	apparent %	active pH	titratable 1 M NaOH/ 100 ml	IBU	EBC	log cfu/ml
CW	nd	11.50	nd	nd	nd	5.44 ± 0.01^{a}	0.42	17.95 ± 0.21^{a}	17.01 ± 0.19a^{bcd}	7.39
SW	nd	12.00	nd	nd	nd	5.17 ± 0.00^{b}	0.54	16.70 ± 0.43^{f}	15.00 ± 0.29^{abcd}	7.39
CB	4.27 ± 0.06^{a}	5.20 ± 0.08^{a}	4.10 ± 0.1^{bc}	54.78	64.35	4.31 ± 0.00^{c}	2.70 ± 0.02^{a}	15.39 ± 0.07^{b}	16.94 ± 0.42^{abc}	6.95
SB	4.90 ± 0.07^{de}	5.00 ± 0.11^{de}	3.70 ± 0.13 ^{fg}	58.33	69.17	4.31 ± 0.01^{d}	2.46 ± 0.03^{f}	16.39 ± 0.61 ^{g}	17.36 ± 0.09^{a}	6.74
CB1	4.81 ± 0.02^{ab}	4.30 ± 0.02^{ab}	3.10 ± 0.03 ^{cd}	62.61	73.06	4.06 ± 0.05^{e}	3.04 ± 0.01^{b}	10.37 ± 0.69^{c}	10.47 ± 0.13^{ab}	7.00
SB1	5.36 ± 0.03^{ef}	4.30 ± 0.05^{ef}	2.50 ± 0.06^{gh}	64.17	79.17	4.04 ± 0.00^{f}	3.25 ± 0.01 ^{g}	15.83 ± 0.74 ^{h}	9.77 ± 0.39^{abc}	7.30

Values are expressed as the mean (n = 3) ± standard deviation. Mean values with different letters (a, b, c, etc.) within the same column are statistically different (*P* value < 0.05).
* - original extract; nd – no data was collected at this point.

its concentration in the experimental beer was 5.36% v/v, while in the control beer - it was 4.81% v/v. Also, the actual attenuation, determined after fermentation and after one month of storage, was higher for the beer with the shoots and was 58.33% for SB and 64.17% for SB1. For the control sample, the extract was 54.78% for CB and 62.61% for CB1. The pH of the control wort was 5.44 and was higher than that of the wort that included pine shoots – 5.04. During primary fermentation and storage, the pH of both types of beer decreased, reaching approximately 4.0. The total acidity of the control wort was 0.41 and that of the wort with the shoots was 0.55. During fermentation and storage, an increase in the total acidity of both types of beer was noticed.

The bitterness in the control wort was 17.95 IBU, and it was higher than the bitterness in the wort with the pine shoots. Bitterness in the tested types of beer decreased during fermentation and storage; in the case of the control beer, it reached the final value of 10.37 IBU, while in the case of the experimental beer - it reached 15.83 IBU.

The color of the wort with pine shoots was lighter - at 15 EBC, while that of the control wort was 17 EBC. No changes in the wort color were observed after the turbulent fermentation process. In turn, after a month of storage, the color of both types of beer was definitely lighter. Once yeast was added to the wort, the yeast count was 7.39 log/ml. After fermentation, the count decreased in both types of beer, in the CB beer to 6.95 log/ml and in the SB to 6.74 log/ml. A significant increase in yeast count was found in the beer with pine shoots after one month of storage. In both types of tested beer, mesophilic bacteria and lactic acid bacteria were not found at any stage of the production process.

3.2. Polyphenols and antioxidant activity

The polyphenol content and their profile are included in Table 2. It was found that ferulic acid, caffeic acid, cinnamic acid and 4-hydroxybenzoic acid predominated among the phenolic acids found in the analyzed wort and beer samples. Naringenin was dominated among the flavonols. In the control wort, the dominant polyphenols were ferulic acid and naringenin. In contrast, the highest content of ferulic acid was noticed in beer with pine shoots. In the entire production process, the lowest level of the tested polyphenols was found for luteolin and kaempferol.

Table 2
Polyphenol content in the tested beer.

	Samples					
Compounds	CW	CB	CB1	SW	SB	SB1
Gallic acid	1.89 ± 0.04^{a}	1.83 ± 0.07^{b}	1.73 ± 0.04^{c}	11.53 ± 0.25^{bc}	11.63 ± 0.11^{bc}	12.34 ± 0.08^{ab}
2.5-Dihydroxybenzoic acid	0.67 ± 0.02^{ab}	0.89 ± 0.04^{ab}	1.31 ± 0.02^{a}	0.68 ± 0.05^{bc}	0.80 ± 0.1^{ce}	1.20 ± 0.1^{be}
4-hydroxybenzoic acid	0.23 ± 0.02^{a}	0.43 ± 0.02^{b}	1.60 ± 0.02^{ab}	50.13 ± 0.31^{ab}	57.25 ± 1.03^{ab}	59.27 ± 1.04^{ab}
Protocatechuic acid	1.30 ± 0.2^{a}	1.65 ± 0.06^{a}	1.50 ± 0.1^{c}	2.17 ± 0.12^{ac}	2.49 ± 0.2^{ac}	2.50 ± 0.04^{ac}
Caffeic acid	0.35 ± 0.03^{a}	0.45 ± 0.04^{b}	0.82 ± 0.03^{a}	50.37 ± 0.25^{ab}	81.63 ± 1.4^{ab}	85.60 ± 0.44^{ab}
Syringic acid	0.66 ± 0.02^{a}	0.75 ± 0.03^{b}	1.79 ± 0.06^{a}	6.30 ± 0.2^{ab}	7.72 ± 0.2^{ab}	10.30 ± 0.12^{ab}
P-coumaric acid	0.28 ± 0.01^{a}	0.38 ± 0.03^{ab}	0.36 ± 0.03^{bc}	18.37 ± 0.25^{ac}	20.67 ± 0.21^{ac}	26.37 ± 0.19^{ac}
Ferulic acid	56.67 ± 0.78^{a}	58.60 ± 0.7^{b}	48.37 ± 1.2^{bc}	195.47 ± 2.36^{abc}	107.41 ± 2.01^{ac}	139.33 ± 0.71^{abc}
Chlorogenic acid	3.50 ± 0.1^{a}	4.10 ± 0.1^{b}	4.37 ± 0.09^{ac}	52.67 ± 0.87^{ab}	26.45 ± 0.57^{bc}	29.10 ± 0.6^{ab}
Sinapinic acid	2.80 ± 0.1^{a}	1.74 ± 1.15^{b}	2.17 ± 0.04^{a}	4.60 ± 0.1^{ab}	3.37 ± 0.31^{b}	3.63 ± 0.25^{ab}
Cinnamic acid	10.60 ± 0.26^{a}	11.43 ± 0.32^{b}	13.57 ± 0.11^{ab}	35.73 ± 0.5^{ab}	6.14 ± 0.15^{ab}	7.37 ± 0.21^{bf}
Vanillic acid	2.20 ± 0.1^{a}	4.88 ± 0.09^{ab}	4.40 ± 0.16^{c}	1.57 ± 0.15^{bc}	6.41 ± 0.28^{ac}	6.80 ± 0.1^{ac}
Salicylic acid	0.13 ± 0.06^{a}	0.21 ± 0.04^{b}	0.13 ± 0.06^{c}	0.44 ± 0.04	0.88 ± 0.07^{abcd}	0.90 ± 0.05^{abcd}
Total phenolic acids	**81.28 ± 17.43**	**87.34 ± 17.96**	**82.12 ± 14.81**	**430.03 ± 59.33**	**332.85 ± 37.35**	**384.71 ± 45.68**
Naringenin	68.57 ± 0.83^{a}	65.00 ± 0.79^{b}	53.33 ± 0.93^{ac}	105.20 ± 1.35^{bc}	107.13 ± 0.57^{ab}	107.47 ± 0.74^{ab}
Vitexin	0.44 ± 0.02^{a}	0.50 ± 0.03^{b}	0.50 ± 0.05 ^{cd}	0.62 ± 0.04^{abc}	0.62 ± 0.03^{abc}	0.79 ± 0.07^{abc}
Rutin	2.70 ± 0.1^{a}	2.20 ± 0.09^{a}	2.41 ± 0.04^{b}	2.16 ± 0.03^{aef}	3.50 ± 0.1^{bde}	3.50 ± 0.21^{bdf}
Quercetin	1.57 ± 0.15^{a}	1.83 ± 0.09^{b}	1.13 ± 0.03^{bc}	3.60 ± 0.2^{abc}	5.30 ± 0.2^{abc}	5.36 ± 0.14^{abc}
Apigenin	0.10 ± 0^{a}	0.30 ± 0.03^{ab}	0.13 ± 0.04^{bc}	0.17 ± 0.06^{f}	0.28 ± 0.07^{ac}	0.36 ± 0.06^{acdf}
Kaempferol	0	0	0	0	0	0.02 ± 0.01^{a}
Luteolin	0	0	0	0	0.11 ± 0.02^{a}	0.17 ± 0.04^{b}
Catechine	1.11 ± 0.04^{a}	1.44 ± 0.04^{b}	0.86 ± 0.04^{bc}	1.20 ± 0.1 ^{cd}	1.25 ± 0.14 ^{cd}	2.12 ± 0.03^{acd}
Total flavonols	**74.49 ± 23.96**	**71.27 ± 22.68**	**58.36 ± 18.62**	**112.95 ± 36.82**	**118.19 ± 37.37**	**119.79 ± 37.42**

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

Antioxidant activity was determined by testing the ability to quench DPPH radicals (Fig. 1). It was found that the antiradical activity of the worts was 53.16% for the CW and 58.60% for the SW wort. It was shown that the anti-radical activity of beer was 53.16% in the case of the CW test and 64.74% for the SB1 sample. In all storage periods, samples containing the active components of pine shoots had a higher capacity but statistical analysis of the results showed that the differences were not statistically significant.

3.3. Sensory evaluation

The sensory profile was visualized in the form of a flavor and aroma profile (Fig. 2). For the control sample, the taste profile was characterized as intensely sweet and malty. The bitter and fruity tastes were at similar levels, while the sour and astringent tastes were of very low intensity. In the case of the sample with pine shoots, the profile was characterized as more complex, where

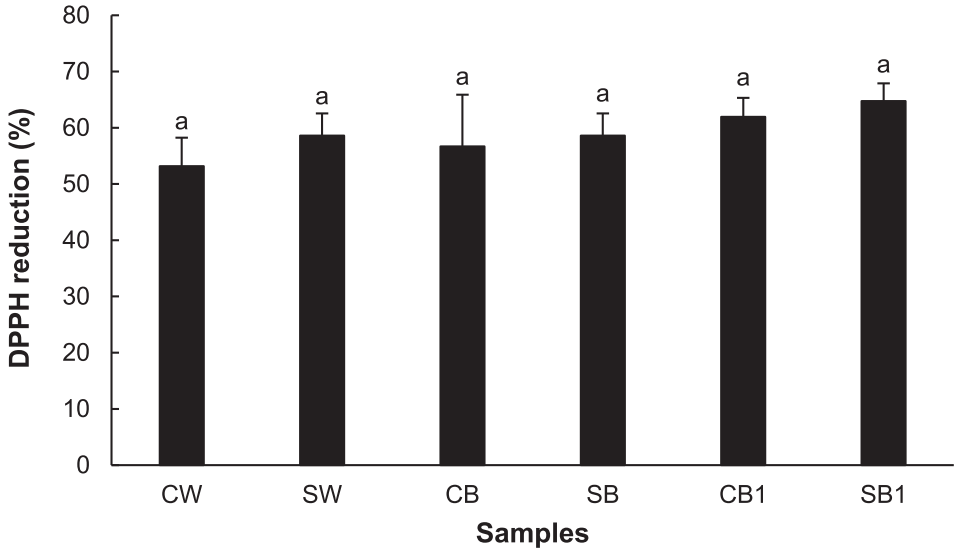

Fig. 1. DPPH scavenging effectiveness of wort and beer samples. Values are expressed as the mean (n = 3) ± standard deviation. Mean values with different the same letter (a) are not statistically different (*P* value < 0.05).

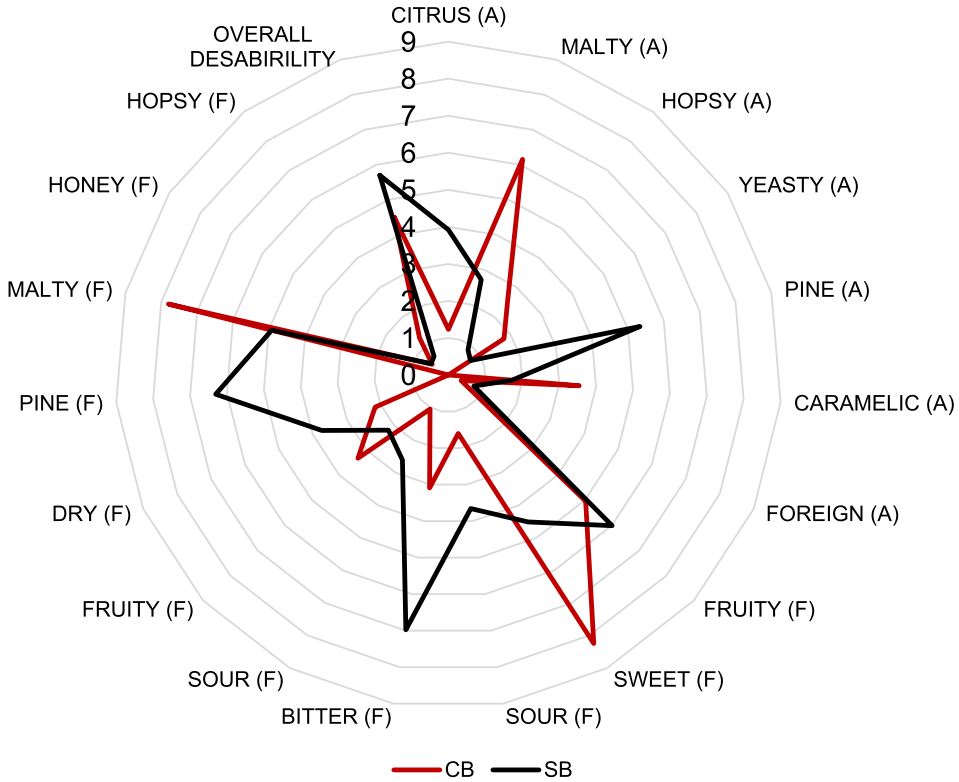

Fig. 2. Sensory profile of fresh beer samples. CB: control young beer; SB: young beer supplemented with *P. sylvestris* shoots; (F): flavour descriptor; (A): aroma descriptor.

bitter and pine flavors were also found. The level of the malt and sour tastes was determined as low.

The profile assessment of the aroma showed significant differences between the samples of the tested beer. The profile of the control sample was characterized as malty and fruity. Caramel and hop aromas were also noticeable. The level of intensity of citrus and yeasty odor descriptors was assessed as low. The experimental beer sample had an intense pine and fruit aroma. The level of intensity of citrus and malt flavor was assessed as moderate, while that of hop and yeast flavor was assessed as very low. No foreign smell was noticed in both beer samples. With regard to the assessment of overall desirability, the pine shoot sample got a higher score: 5.7, while the control sample received an average score of 4.5. However, based on statistical analysis, there were no statistically significant differences.

PCA was used to identify aroma and flavor descriptors best discriminating the two produced types of beer. The scores for each beer descriptor for the two components are presented in Fig. 3 representing the bi-plot, which globally explained 100% of the total variance. The first principal component (PC1) explained the variation across samples. Moreover, looking at the bi-plots, the differentiation of sensory profiles across samples can be noticed. There are also evident sensory variables that characterize the beer produced with the use of pine shoots, suggesting a greater complexity of the flavor profile and smaller complexity of the aroma profile, which was dominated by the pine descriptor. In the latter, a correlation could be assumed between yeasty, pine, tart and bitter flavor descriptors and pine, citrus aroma descriptors.

4. Discussion

Pine shoots are a raw material that is relatively rarely used in food production at the moment. There are attempts to use them as a food ingredient or as a raw material for the production of tinctures, as well as an additive to beer [11]. As part of the study, beer similar to the Hefe-Weizen type of that beverage, which comes from Bavaria, was produced, to which Scots pine shoots were added at the wort brewing stage. The purpose of the study was to assess the possibility to demonstrate whether the compounds in the shoots would enable the fermentation process and whether beer with new sensory properties could be obtained.

Unconventional raw materials and additives can affect not only sensory qualities or functional properties but they can also change the basic physicochemical parameters of beer or determine the course of the fermentation process. Reports on the antioxidant and antimicrobial properties of pine shoots may be important con-

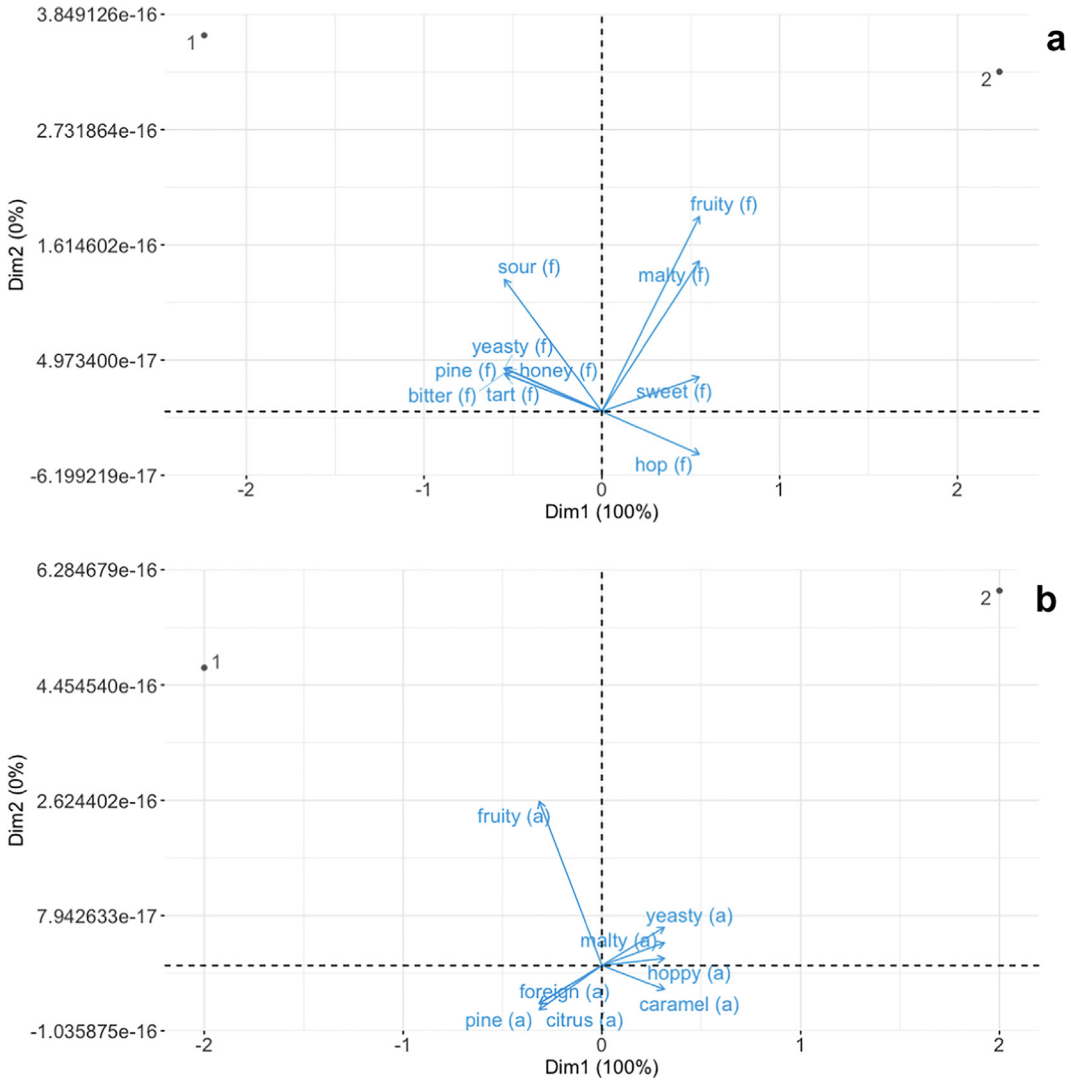

Fig. 3. Principal component analysis bi-plot of flavor (a) and aroma (b) descriptors of the control beer (1) and beer supplemented with pine shoots (2). PC1 vs PC2 accounts for 100% of the explained variation.

sidering the beer production process, during which high yeast activity is necessary [12]. Due to the potential contamination in the beer production process, microbiological quality control at every stage is an important task. The amount of brewing yeast and the total number of mesophilic microorganisms or lactic acid bacteria (LAB) is analyzed, due to their similar nutritional and environmental requirements. Their growth can cause the most undesirable consequences, such as delaying or disrupting the wort fermentation process or introducing undesirable sensory changes in beer. LAB spoil beer through acidification, haze formation, and/or diacetyl production, which gives the beer an intense aroma of artificial butter. Many strains can also produce exopolysaccharides (EPSs) in beer [13]. The presence of undesirable microorganisms, i.e. lactic acid bacteria and mesophilic bacteria, was not confirmed in the beer produced as part of the study. The only microorganisms present in the beer were yeasts. A well-known fact is that failure to adjust the inoculum to the conditions of the fermentation process prolongs the process of cell adaptation and delays proliferation and the fermentation process. Subsequently, the alcohol present in beer, as well as other changes to the parameters of the fermentation medium (e.g. lowering the pH, loss of substrates in the wort) slow down yeast metabolism and contribute to the deactivation of the weakest cells [14]. In our experiment, the largest amount of yeast in the beer wort was noticed before alcoholic fermentation started and after its completion, the number decreased in both types of beer due to the above-described regularities.

Adding pine shoots during brewing made it possible to obtain a slightly richer extract of the basic wort compared to the control wort, a higher alcohol content and actual attenuation rate in the beer after the fermentation process and after a storage period of one month. Pine shoots contain a number of soluble components that are found in the extract. Among them, there are approximately 5.15 g/100 g of soluble sugars, including glucose, fructose and sucrose. The metabolic processes of yeast are related not only to the production of ethanol but also to the production of organic acids, as a result of which the pH of beer changes (compared to the pH of the wort) [14]. Such a correlation can also be noticed in the research conducted for this thesis - the pH of the wort reached a higher value than that of the beer. Usually, the pH of beer wort varies between 5.3 and 5.5 [15]. It should also be noted that a lower value was achieved by the wort with pine shoots, due to the effect of the shoots on the pH values. In turn, the pH of wheat beer after fermentation usually reaches a value of approximately 4.3, which is also comparable to the results obtained in this study as it oscillated around 4 and 4.3 pH [16]. Both the control and test samples showed similar values of total acidity from the beginning of the performance of the tests - immediately after fermentation - until the end of the fermentation process and the completion of the tests on the samples after storage. Changes in total acidity during fermentation should be considered normal as the processes involving yeast cause an increase in total acidity, in contrast to pH - as described earlier in the case of changes in pH [17].

Beer, in studies on bitterness in beer and standards, is not classified based on that value [18]. However, in the case of light beer, the IBU level of less than 40 is considered as standard, therefore both samples - the control sample and the sample with pine shoots - should be considered valid and as meeting the standards for the level of bitterness in beer [18]. The observed discrepancy in bitterness values between wort and fresh beer may be due to the concentration of polyphenols and may be the result of the processes occurring during fermentation. The study conducted by Lazzari et al. showed a negative correlation between total flavonoid content and IBU [19]. In contrast, Kishimoto et al. noticed a decrease in IBU values during fermentation as a result of the disappearance of AA alpha-acids, e.g, as the pH decreases during fermentation, the hydrophobic components become insoluble in beer and interact with the cell walls of yeast [20].

The polyphenols and terpenes in the plant material are responsible for the typical bitter aftertaste. Therefore, their addition to food can significantly affect the sensory qualities and also the nutritional and health-promoting value [21]. With regard to the phenolic acids, chlorogenic and caffeic acids are responsible for the tangy and bitter taste [22]. In studies of polyphenol content in beer, the concentration of that compound ranged between 40 and 600 mg/l, depending on the adopted methodology and test material [23]. When comparing the values from the aforementioned study to the results obtained for the control sample in this research, it should be noted that the results are similar, as the results range from 125 to 160 mg/l, depending on the beer storage period and the fermentation process. The test sample that contained pine shoots had a significantly higher polyphenol content, of 450–600 mg/l.

The characteristics that determine the sensory quality of beer include the following: aroma, flavor, palatability, saturation, bitterness, clarity, color and the amount of the frothy foam. The most important feature is the palatability of beer, which depends on the amount of perceptible positive and negative features in taste and aroma [24]. Due to the complexity of the human sense of taste, it is difficult to determine at what level the above-mentioned quality characteristics should be present for the final effect - the high consumer acceptance - to be the best possible. The taste of beer is influenced by many factors, including the quality of malt, the strain and quality of yeast, the conditions under which the individual technological processes are performed, and the storage conditions of the finished product [25]. Factors that negatively affect the quality are as follows: too high or too low fermentation temperature, osmotic pressure, inadequate oxygenation of the wort, deficiency of nutrients in the wort, inappropriate pH, toxic agents (e.g. too high concentration of ethanol due to the inappropriate adaptation of the yeast strain to the style or disinfectants being the residue after disinfection of technical installations), and the water content in freeze-dried yeast [26]. The study showed that the control sample was a higher clarity beer and more brown compared to the sample with pine shoots, which was most likely due to the phenolic compounds in the shoots. Studies have shown that the concentration of those compounds and their transformation in beer can significantly affect its color [27]. The observed changes of color and a lighter color after storage may result from the instability of color compounds that show relative instability and are susceptible to several factors such as storage temperature, pH, oxygen, light, chemical structure, concentration and the presence of enzymes, proteins and metallic ions, as reported in studies [27]. Both samples were free of foreign flavor and smell, which is crucial in assessing the quality of beer [18]. The current development of the craft beer market indicates that consumers are looking for new flavors and aromas of beer more and more often [28]. Based on consumer preference studies, assessment of beer characteristics varies depending on whether or not the consumers have previously tried craft beer; generally, that type of beer is perceived as better quality than commercial beer due to the type of raw materials used in brewing [29].

5. Conclusions

The active compounds in pine shoots can be used as ingredients of functional beer, as they affect the composition of polyphenols and flavor. The research has confirmed that replacing half of the amount of hops indicated in the recipe with pine shoots enables fermentation and obtaining beer of good quality. Beer with pine shoots has a slightly acidic character, tested both on the pH and

total acidity scale. It is a relatively low-bitterness beer. A broad spectrum of biologically active compounds was present in the produced beer. It was shown that replacing hops with pine shoots did not reduce antioxidant properties. At the same time, it was found that the new beer was characterized by a high content of antioxidant compounds - polyphenols, among which ferulic acid, caffeic acid and naringenin predominated. The content of those compounds was statistically significantly higher in beer with pine shoots. Adding Scots pine shoots (*Pinus sylvestris* L.) at the brewing stage of wheat beer changed the sensory characteristics and did not impair the microbiological quality during storage, nor did it reduce physico-chemical quality parameters compared to the control sample.

Ethical approval

All participants in this study gave informed consent to Poznań University of Life Sciences.

Author contributions

- Study conception and design: M Dziedziński, J Kobus-Cisowska, B Stachowiak.
- Data collection: M Dziedziński; R Kozłowski; K Szambelan; B Górna; K Stuper-Szablewska.
- Analysis and interpretation of results: M Dziedziński.
- Draft manuscript preparation: M Dziedziński.
- Revision of the results and approval of the final version of the manuscript: J Kobus-Cisowska; B Stachowiak.

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Conflict of interest

The authors report no potential conflict of interest.

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