Sensory and antioxidant properties of beer with *Juniperus communis* L.

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ABSTRACT

*Juniperus communis* L. or common juniper is a coniferous plant distributed throughout the Arctic and temperate zone of the Northern hemisphere. Its dried astringent blue-black seed cones, known as “juniper berries” have long been used as a flavouring agent for culinary purposes and in the preparation of many alcoholic beverages. It has also been used for various medicinal purposes, and as a remedy for many health problems.

In this study it was investigated sensory and antioxidant characteristics of beers produced with juniper berries. The objective was to determine the influence of adding different content of juniper berries on sensory characteristic and antioxidant properties of the obtained beers.

The beers were produced with a traditional method of lager beer production by fermentation of wort with different proportion of crushed dried juniper berries. The basic beer parameters (alcohol, original extract, real extract, degree of fermentation and calories) were determined using the Alcolyzer Beer ME Analyzing System. The total polyphenol content of beer was determined using Folin-Ciocalteau’s phenol reagent and expressed as equivalents of gallic acid. An examination of the antioxidant power of beer was performed using DPPH, FRAP and TEAC assay. A sensorial evaluation of the obtained products has been conducted, assessing their taste, aroma, body, bitterness, freshness and general impression.

The results of sensory analysis show that beers with different proportion of juniper berries have satisfactory sensory qualities which were absolutely acceptable for testers. The juniper berries contains bitter substances and therefore the beers were characterized with a well-rounded and balanced, tasty bitterness. Phenolic compounds are generally considered as one of very important antioxidant sources in beer, and beer antioxidant activity is strongly correlated with the total phenolic content. Antioxidant activity and total polyphenol content were higher in beers with juniper berries than in control lager beer.

*Keywords: Juniperus communis; beer; antioxidant activity; sensory properties*

INTRODUCTION

*Juniperus communis* L. or common juniper is a coniferous evergreen shrub widely distributed in the Northern hemisphere. It is found across Europe, North Africa, North America, northern and western Asia and Japan. The plants bear bluish-black fruits described as berries or berry-like cones. The ripe berries should be collected in autumn and dried slowly in the shade, to avoid losing the oil [1]. The berry or fruit of this species have a aromatic, spicy aroma, and a slightly bittersweet flavour. It’s contains the following constituents: volatile oil (0.2-3.4 %) containing monoterpenes (α- and β-pinene, β-myrcen, limonen, terpinnen-4-ol and sabinene), proanthocyanidines (gallocatechin and epigallocatechin), flavonoids in small amounts, the lignan desoxypodophyllotoxin and its isomer desoxypicrodophyllotoxin, diterpene acids and sesquiterpenes (caryophyllene and cadinene) [2]. Juniper berries (the dried ripe female cone) have long been used to stimulate the appetite and for flavouring foods (sauerkraut, stuffing, vegetable pates etc.) and alcoholic and non-alcoholic beverages such as gin, beer, tea, brandy. The roasted seed is a coffee substitute. It has also been used for various medical purposes, including as an antiseptic, contraceptive, diuretic, and as a remedy for many health problems, such as urinary tract infections, diabetes, chest complaints, scrofula, rheumatism and backache. Other uses include aromatherapy, phyotherapy, perfumery and cosmetics. However, Juniper berries could cause renal irritation, and that they might also be an abortifacient. Long-term use or an overdose may cause kidney damage characterized by albuminuria or renal hematuria. Also, Juniper berry products should not be used without medical advice for more than four weeks and they are contraindicated in nephritis. Juniper berry is included in the Council of Europe’s list of substances, spices and seasonings deemed admissible for use with a possible limitation of the active compound in the final product (limitation not yet determined) [3-4].

The medieval pre-hops brewers used different herbs or mixtures of herbs (called grut) to flavor their beers. The most common of these were sweet gale, juniper, yarrow, rosemary, mugwort, and woodruff. Some herbs
had medicinal properties but some plants were poisonous. After 15th century, hops became a universal beer flavoring agent [5-6].

In this study it was investigated sensory and antioxidant properties of beers produced with three different concentrations of juniper berries. The objective was to determine the influence of adding different content of juniper berries on sensory characteristic and antioxidant properties of the obtained beers. The analysis of antioxidant characteristics was carried out by Folin-Ciocalteu, FRAP, TEAC and DPPH tests.

MATERIALS & METHODS

Plant material of *Juniperus communis* L. was collected from different shrubs growing in the south-western Serbia (altitude 1100 m). The wort, commercial beer and bottom-fermenting yeast used in this study were obtained from a local brewery. Gallic acid, Folin-Ciocalteu’s phenol reagent, ammonium hydroxide, hydrochloric acid, sodium acetate trihydrate, glacial acetic acid and sodium carbonate were purchased from Merck (Darmstadt, Germany). Ascorbic acid, 2,4,6-tripyridyl-s-triazine (TPTZ), ferric chloride hexahydrate, 2,2-diphenyl-1-picrylhydrozyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox^®), sodium dihydrogen phosphate, sodium hydrogen phosphate, sodium chloride, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate were purchased from Sigma-Aldrich (Steinheim, Germany).

Juniper berries were cut into two pieces and added to the wort in three different concentrations: 0.24, 0.48 and 0.72 g/L. The mixtures of wort and juniper berries were sterilized by autoclaving at 105°C for 8 min. The fermentation medium (2 L) was poured into 5 L sterile glass fermenters and seeded aseptically with 80 mL of a yeast suspension, corresponding to 15–20 million yeast cells per milliliter of cold aerated wort. Original gravity of the final worts was 11.91°P. Pitching was performed at 7–8°C, the temperature was allowed to rise to 9–10°C. Four days later the beer was slowly cooled to 3–4°C. Afterwards it is transferred into the maturation vessel and cooled slowly to 0°C. The fermentation process was completed after twenty five days. Alcohol, original extract, real extract, degree of fermentation and calories were determined using Alcolyzer Beer ME Analyzing System, Anton Paar GmbH – AUSTRIA.

The total phenolic content of samples was determined according to the Folin-Ciocalteu spectrophotometric method by means of a Jenway 6400 [7]. Degassed samples of beer (0.05 mL) were mixed with 0.45 mL of distilled water and 2.5 mL of 10-fold diluted Folin-Ciocalteu’s phenol reagent and allowed to react for 5 min. Two milliliters of saturated sodium carbonate (75 g/L) was added to the mixture and then shaken. After 2 h of reaction at room temperature, the absorbance at 760 nm was determined. The measurement was compared to a calibration line of prepared gallic acid (GA) solution, and the results were expressed as milligrams of gallic acid equivalents per liter of beer (mg GAE/L). All determinations were performed in triplicate.

The FRAP assay was performed according to the procedure previously described by Benzie and Strain [8]. Aqueous solutions of known ascorbic acid concentrations were used for calibration. The TEAC assay was conducted according to the procedure described by Kaneda et al [9].

The DPPH-reducing activity was estimated following the procedure described by Re at al [10], with some modifications. A solution of 14 mM of ABTS and 4.9 mM potassium persulfate was prepared in a phosphate buffer (pH 7.4), and mixed in equal volumes to produce a stable ABTS^•⁻ stock solution. The obtained dark blue-green stock solution was left in the dark at room temperature for 12-16h before use. A working ABTS^•⁻ solution was prepared by diluting ABTS^•⁻ stock solution (approximately 1/80 dilution) to an absorbance of 0.70 ± 0.02 AU at 734 nm and 30°C using phosphate buffer. After addition of 3.0 mL of working ABTS^•⁻ solution to 30 μL of degassed beer samples or Trolox standards (2.5, 1.25, 0.625, 0.3125 and 0.15625 mM Trolox solutions in phosphate buffer) the absorbance reading was taken at 30°C 6 minutes after initial mixing. Appropriate solvent blanks were run in each assay. The fractional inhibition (FI) of ABTS^•⁻ radical was calculated and plotted dose-response (DR) curves (i.e. concentration vs. fractional inhibition) for Trolox and each sample. The TEAC value was calculated using the following equation:

\[
\text{TEAC} \ (mM) = \frac{\text{Gradient of sample DR curve}}{\text{Gradient of Trolox DR curve}}
\]

A sensory evaluation of the obtained products was conducted, assessing: fragrance – a sensory attribute resulting from stimulation of the olfactory receptors in the nasal cavity by certain volatile substances (1 – unpleasant, 5 – very pleasant); taste – a sensory attribute resulting from stimulation of the gustatory receptors in the oral cavity by certain soluble substances (1 – unpleasant, 5 – very pleasant); aroma – a combination of olfactory and gustatory attributes perceived during tasting, including tactile, thermal, pain and kinesthetic effects (1 – unpleasant, 5 –very pleasant); body – the effect of the beer on the inside of the mouth, including the after-palate effect (1 - thin, 2 – watery, 3+4 - full in body, 5 - very full in body); bitterness – a taste that is sharp and acid and felt with the receptors concentrated towards the back of the tongue and throat (1 – not
present, 5 – very strong bitterness); freshness – as determined by the alcoholic strength, CO2 content and hopping level of the beer (1 – stale, 5 – fresh); general impression – the interaction of all the sensory signals (1 – very bad, 5 – very good). Sensory tests were carried out using a panel of 20 members (students from Faculty of Agriculture-University of Belgrade). A commercial lager beer was used as a reference for the assessment. The beer samples were evaluated using a 5 point scale. All samples were presented in 250 mL coded transparent drinking glasses containing 50 mL beer per glass. Sample were served at 12°C and assessed at room temperature.

The experimental results were analyzed with the student’s t-test for dependent samples.

RESULTS & DISCUSSION

The physico-chemical characteristics of the beers are shown in Table 1. Beer samples, K1, K2 and K3 were produced with 0.24, 0.48 and 0.72 g/L of juniper berries, respectively. The real degree of fermentation was higher in the control beer, which was obtained by fermentation of pure wort, probably due to the inhibitory effect of some juniper compounds on yeast cells. Consequently, the alcohol level was less in beers produced with juniper berries.

Table 1. Physico-chemical properties of beers

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cb</th>
<th>K1</th>
<th>K2</th>
<th>K3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original gravity (°Plato)</td>
<td>11.91</td>
<td>11.91</td>
<td>11.91</td>
<td>11.91</td>
</tr>
<tr>
<td>Real extract (% w/w)</td>
<td>2.80</td>
<td>3.17</td>
<td>3.80</td>
<td>3.66</td>
</tr>
<tr>
<td>Real degree of fermentation (% w/w)</td>
<td>77.60</td>
<td>74.00</td>
<td>69.69</td>
<td>70.28</td>
</tr>
<tr>
<td>Alcohol (% v/v)</td>
<td>5.00</td>
<td>4.37</td>
<td>4.23</td>
<td>4.18</td>
</tr>
<tr>
<td>Calories (kJ/100 mL)</td>
<td>148.00</td>
<td>174.60</td>
<td>180.69</td>
<td>177.02</td>
</tr>
</tbody>
</table>

Cb – control beer obtained by fermentation of pure wort; K1, K2, K3 – beers with 0.24, 0.48 and 0.72 g/L of juniper berries, respectively.

The first goal of the study has been to examine the possibility of improving the antioxidant and sensorial characteristics of beer by adding juniper berries. Phenolic compounds have important roles both in flavour and colloidal stability of beer. Also, phenolic compounds are generally considered as one of the very important antioxidant sources in beer. Total phenolic content of beer samples studied were determined by the Folin-Ciocalteau method and the results are shown in Table 2. In the beer samples with juniper, the total amount of phenolic compounds was significantly higher than in the control beer. However, mutual comparison of samples K1, K2 and K3 were shown statistically significant difference only between samples K1 and K3. The obtained results are in agreement with those reported in literature [11-13].

Table 2. Total antioxidant capacity of beers

<table>
<thead>
<tr>
<th>Sample</th>
<th>TPC (mg GAE /l)a</th>
<th>FRAP (mM AC)b</th>
<th>TEAC (mM TE)c</th>
<th>DPPH (%)d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cb</td>
<td>385.6 ± 6.6</td>
<td>3.61 ± 0.04</td>
<td>3.27 ± 0.04</td>
<td>50.59 ± 0.88</td>
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<tr>
<td>K1</td>
<td>416.2 ± 7.3</td>
<td>3.73 ± 0.07</td>
<td>3.71 ± 0.02</td>
<td>64.70 ± 1.27</td>
</tr>
<tr>
<td>K2</td>
<td>432.8 ± 5.4</td>
<td>3.84 ± 0.03</td>
<td>4.37 ± 0.02</td>
<td>69.00 ± 1.08</td>
</tr>
<tr>
<td>K3</td>
<td>450.0 ± 3.1</td>
<td>3.90 ± 0.03</td>
<td>4.45 ± 0.03</td>
<td>69.56 ± 1.40</td>
</tr>
</tbody>
</table>

Each value is the mean ± standard deviation of three replicate experiments. Bolded numbers indicate significantly different values (p < 0.05) compared with control beer.

Cb – Control beer obtained by fermentation of pure wort.

K1, K2, K3 – Beers with 0.24, 0.48 and 0.72 g/L of juniper berries, respectively.

a Total phenolic content, expressed as milligrams of gallic acid equivalents per liter of beer.
b Total antioxidant capacity expressed as mmol of ascorbic acid equivalents.
c Total antioxidant capacity expressed as mmol of Trolox equivalents.
d % of inhibited of DPPH free radical after 30 minutes.

The antioxidant capacity of beers was tested by FRAP, TEAC and DPPH assays (Table 2). These methods are included among the electron transfer-based methods, thus, similar trends were observed for the antioxidant activities measured by these assays. The DPPH and the ABTS+ radicals are the two most widely used and stable chromogen compounds to measure the antioxidant activity of beverages. Using the TEAC and DPPH assays, it was found statistically significant difference between control beer and juniper beers.
Also, for these two methods, free radical scavenging capacity of the samples K2 and K3 was not statistically different. However, the FRAP method was shown significant difference only between control beer and sample K3. For this method, difference in antioxidant capacity between the samples with juniper berries was not significant. The results obtained suggest that beer antioxidant activity is correlated with the total phenolic content.

The results of the sensory evaluation are presented in Figures 1. Obtained products had very interesting and specific sensory characteristics. Mean values of the sensorial grades for juniper beers were higher than those for control beer. Sensory analysis showed that by fermentation of wort with certain proportion of juniper berries it was possible to obtain a product with a satisfactory sensory property, which could be acceptable and even better than a common commercial lager beer. The juniper berries contain bitter substances and therefore the beers were characterized with a well-rounded and balanced, tasty bitterness. Also, these beers were characterized by a pleasant fragrance, enjoyable aroma and high smoothness of taste, with prominent freshness and good after-taste.

**CONCLUSION**

The results obtained suggest that it is possible to produce a special type of beer by fermenting wort with different proportion of juniper berries with very interesting and pleasant sensory properties. The unique flavour of these beers might be of interest to consumers. Also, the addition of juniper berries can be increased antioxidant capacity of beer. However, pregnant women and people with nephritis should not be used this product without medical advice.

**REFERENCES**


