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EXTRACTS OF MEDICINAL PLANTS AS FUNCTIONAL BEER ADDITIVES

Article Highlights

- Beer enriched by extracts of medicinal plants has increased functional and new sensory features
- The enriched beer has a higher content of total phenols and antioxidant capacity
- The extract of lemon balm is best combined with a standard beer in terms of sensory acceptability

Abstract

*This paper is based on determining the level of the antioxidant activity of beer, to which sensory acceptable amounts of selected extracts of medicinal plants were added, with the aim of obtaining a beer with increased functional and new sensory features. For purposes of this study a commercial lager beer type Pils and extracts of herbal drugs: *Melissae folium*, *Thymi herba*, *Juniperi fructus*, *Urticae radix* and *Lupuli strobuli*, were used. Total phenols were analyzed by the method of Folin-Ciocalteu, and the antioxidant activity of samples was evaluated using FRAP and DPPH tests. Sensory evaluation of beer was conducted on 80 subjects, using a nine-level hedonic scale. The results showed that the content of total phenols was the highest in the beer which thyme, juniper and lemon balm were added to (384.22, 365.38 and 363.08 mg GAE/L, respectively), representing an increase of 37.09, 30.36 and 29.55%, respectively, compared to the commercial lager beer. Values of antioxidant activity were correlated with the content of total phenols. The extract of lemon balm blended in the best manner with the baseline, commercial lager beer in terms of sensory acceptability. The new beer, enriched with lemon balm, had a pleasant, appealing and harmonious flavor and aroma.*

Keywords: beer, herbal extracts, sensory evaluation, total phenols, antioxidant activity.

Beer, as a completely natural product, contains numerous beneficial ingredients. It belongs to a special group of alcoholic beverages with low alcohol content and rich in nutrients, which provides it with well-defined functional properties. It contains carbohydrates, amino acids, vitamins, organic acids, phenols compounds, bitter substances of hops, but also specific ingredients with potentially beneficial effects on a human body, which makes it unique (if consumed reasonably and moderately) [1,2].

Throughout history, almost all nations have made different types of beer, which differed in the composition of raw and flavoring materials [3].

Beer is a product that may be nicely combined with different flavors, extracts of medicinal, aromatic herbs, honey, sugar, coffee and others. It may also be combined with sweet fruit such as cherries, wild plums and raspberries. The tradition of combining beer with different supplements dates back to the earliest period, when the objective of this was to get a beverage that will be unique and distinctive, but also to mask deficiencies, caused by inadequate production conditions. A large number of plants have been added to the beer: *Artemisia vulgaris*, *Juniperus communis*, *Melissa officinalis*, *Mentha spicata*, *Origanum vulgare*, *Pimpinella anisum*, *Rosmarinus officinalis*, *Thymus serpyllum* and others. Plants that were enhancing beneficial features of beer are *Acorus cal-*

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amus, *Cinnamomum verum* and *Hypericum perforatum* [4].

Nowadays there is a multitude of new specific types of beer with the addition of extractive plants isolates. Lemon juice, raspberry syrup and selected herbal extracts are added to German wheat beer, and orange juice and coriander extract to similar Belgian types of beer. Danish Mestreechs Aajt is a sweet beer with a hint of mustard. Gingebier beer contains ginger and is produced in Denmark, England and America. The French also do not lag behind in the production of specific types of beer. Brewery Nesle in Normandy produces beer Epinoir with the addition of buckwheat, brewery Pietra produces an unusual sweet beer with a taste of chestnuts. In India, a special type of coconut beer is produced and Mexican Negra beer owes its sweet taste to a mixture of plants and bitter chocolate. The proper scale of this phenomenon may be seen on the example of the German Carlsberg brewery. Apparently, in the desire to win over the female part of population, Carlsberg has launched Karla, a beer with the addition of fruit juice, vitamins, folic acid and lecithin. As a natural product, which contributes to the improvement of general health, it can also be used as a preventative against many diseases [5].

Beer components that contribute to its functional properties are mainly antioxidants. Polyphenols from malt (70-80%) and hops (30%) are the major natural antioxidants in beer. There are 78 different identified phenols compounds including simple phenols, aromatic carboxylic and phenol carboxylic acids [6,7]. The antioxidant activity of beer depends a lot on a beer type. Dark beers seem to be considerably inferior to coffee, red wine and tea, but can be compared to rose and white wine, and orange juice [8].

Prenylflavonoids in beer that deserve special attention are xanthohumol (XH), isoxanthohumol (IX) and 8-prenylnaringenin (8-PN). They are present almost exclusively in hops and beer is practically the only foodstuff in which they can be found. The latest studies have proven that XH shows cancer chemopreventive activities, antimutagenic and anticarcinogenic properties with an exceptional broad spectrum of inhibitory mechanisms at all three stages of the carcinogenesis, initiation, promotion, and progression [6,9]. 8-PN is of special interest as the most powerful phytoestrogen found in nature [10].

The use of herbs in the prevention and treatment of various diseases dates back to ancient times. Herbal products are very interesting for the pharmaceutical and cosmetic industry, but their potential is much higher. They can be used in the food and bev-

erage industry for the production of a wide range of products with defined functional properties. Extracts of medicinal and aromatic herbs, mushrooms, wine, and others, can be an adequate solution for beer refinement [11-14]. For the purpose of these tests, we have selected four herbs that are widely used in traditional and official medicine, but also in the food industry. The ethanol-water extract of hop cones *Lupuli stroboli* has also been analyzed, in order to compare the data obtained for selected plants with the raw material, commonly used in the production of beer, which also contributes to its total antioxidant activity.

As a medicinal part of lemon balm (*Melissa officinalis* L., Lamiaceae) we have used a leaf *Melissae folium*. As active ingredients, leaves contain essential oil, tannins, flavonoids, rosmarinic acid and triterpenes. According to the Commission E monograph, lemon balm may be used for treatment of anxiety and insomnia, and acts as antispasmodic and aromatic [15,16]. The antioxidant activity of lemon balm has been evaluated by many authors [17-19]. In the food industry, lemon balm and its extracts are used as spices, natural antioxidants, and stabilizers [20,21].

As a healing part of thyme (*Thymus vulgaris* L., Lamiaceae), we have used a leaf *Thymi folium*, but also an overhead part of the flowering *Thymi herba*. Thyme contains essential oil, tannins, flavonoids and triterpenes. It is used as an expectorant, disinfectant, stomachic and externally as an antiseptic gargle for sore throat [15,16]. In the food industry, it is used as a spice and for the production of liqueurs. By assessing the antioxidant activity of the extract isolates of this valuable medicinal plant, it was found that it exerts significant antioxidant activity [22,23].

The fruit Juniperi is a healing part of *Juniperus communis* L., Cupressaceae. It contains essential oil, flavonoids, tannins, oligomeric proanthocyanidins and inverted sugar. The fruit relieves the dyspeptic complaints, and has a pronounced diuretic effect. It is used in anti-inflammatory processes of kidneys, with a decreased excretion of urine, swelling of the joint sand gout [15,16]. It has an important application in the preparation of alcoholic beverages. Studies have shown that juniper berries exert significant antioxidant activity [24,25].

As a healing part of nettle (*Urtica dioica* L., Urticaceae) we have used leaf *Urticae folium* and root *Urticae radix*. The root contains polysaccharides, lectins, sterols (β -sitosterol), lignans. The root is commonly used in prostate treatments and irritated bladder [15,16].

Cones of hop *Lupuli stroboli* represent a healing part of *Humulus lupulus* L., Cannabinaceae, and from

a technological point of view, an important raw material for beer production. It is a source of component precursors of the characteristic pleasant beer bitterness and aroma. They contain acylphloroglucinols, essential oil, flavonoids, bitter acids, tannins, resins. Hops have a soothing and sedative effect and relaxe spasms. Because of their bitter substances, they stimulate digestion [15,16]. Tests have shown that hops polyphenols exhibit significant antioxidant activity [26].

In summary, selected medicinal and aromatic plants represent a treasury of bioactive components, which makes them valuable antioxidant raw materials. *Melissae folium* and *Thymi herba* are valued medicinal, but also aromatic plants; *Juniperi fructus* is a powerfully diuretic, and *Urticae radix* has beneficial effects in a benign prostate adenoma.

The aim of this study was to investigate the level of antioxidant activity of beer, to which sensory acceptable amount of selected extracts of medicinal plants were added, in order to develop a new type of beer that will be sensory acceptable and attractive, as a new product on a market.

EXPERIMENTAL

Beer

A commercial lager beer type Pils taken from the market was used for the purpose of this experiment. The main quality parameters of beer were determined using by EBC analytical methods [27].

The plant material

For the preparation of extracts, the following dry herbal drugs were selected: balm leaf *Melissae folium*, herb thyme *Thymi herba*, juniper berries *Juniperi fructus*, nettle root *Urticae radix* and cones of hop *Lupuli stroboli*. The plant material was taken from the Institute for Medicinal Plant Research "Dr Josif Pančić" in Belgrade, and their identification was done at the Laboratory for Pharmaceutical Control Institute. Ethanol-water extracts were prepared in the pharmacopoeia single-percolation process (the ratio of plant material and the resulting extract was 1:2) [28].

Preparation of beer with plant extracts

Prepared extracts were added aseptically to commercially produce bottled Pilsner beer, and after injection of extracts, the bottles were immediately closed to mature at 10 °C for one day. The dosages of plants extracts used in the experiment are determined by sensory panel by an internal probe. From each extract, series of samples with concentrations of 0.1 to 1.0 ml/L of beer were made (a total of 50

samples), and afterwards the commission of five trained sensory valiators determined the most appropriate sensory samples. The results of determining the most appropriate dose of sensory added to commercial lager beer extracts are shown in Table 1.

Table 1. Types of prepared and analyzed beers, marks and quantities of added extracts

Extract added to beer	Mark	ml of extracts/L of beer
Standard beer	B	-
<i>Melissae folium</i>	BM	0.45
<i>Thymi herba</i>	BT	0.50
<i>Juniperi fructus</i>	BJ	0.65
<i>Urticae radix</i>	BU	0.55
<i>Lupuli stroboli</i>	BH	0.70

Sensory acceptable doses of herbal extracts were added to beer, which were far lower than the recommended therapeutic dose. For example, in the Monograph for *Melissae folium* the recommended dose for therapeutic use of liquid extract (1:1) is at 2-4 ml, 1-3 times daily. We used twice as more diluted liquid extract (1:2) and in a concentration of 0.45 mL/L of beer [16].

Standards and reagents

Folin-Ciocalteu's phenol reagent, hydrochloric acid, sodium acetate trihydrate, glacial acetic acid, and sodium carbonate (anhydrous) were purchased from Merck (Darmstadt, Germany). 2,4,6-Tripyridyl-s-triazine (TPTZ), ferric chloride hexahydrate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), were purchased from Sigma-Aldrich (Steinheim, Germany). Standard Gallic acid was purchased from Sigma-Aldrich (Steinheim, Germany). All other reagents were of analytical grade.

Determination of total phenolics

The amounts of total phenolics (TPC) in beer samples were determined according to the Folin-Ciocalteu method described by Singleton and Rossi [29]. Briefly, 0.5 mL of diluted beer was mixed with 2.5 mL of 10-fold diluted Folin-Ciocalteu's phenol reagent and allowed to react for 5 min. Two milliliters of sodium carbonate solution (75 g/L) were added to the mixture and then shaken. After 2 h of reaction at room temperature, the absorbance at 760 nm was measured. The calibration curve was prepared with gallic acid solution, and the results are expressed as mg of gallic acid equivalents per L of sample (mg GAE/L). Triplicate measurements were performed.

Determination of the antioxidant activity

FRAP test

The FRAP assay was performed according to the procedure previously described by Benzie and Strain, with some modifications [30]. The FRAP reagent solution was made by mixing acetate buffering agent (pH 3.6), TPTZ (10 mM TPTZ solution in 40 mM HCl) and $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in volume ratio 10:1:1, respectively). All samples, standards, and reagents were pre-incubated at 37 °C. An aliquot of each diluted wine sample (0.1 mL) was mixed with distilled water (0.3 mL) and FRAP reagent (3 mL). After the reaction at 37 °C for 40 min, the absorbance at 593 nm was measured. The calibration curve was prepared with Trolox solution, and the results are expressed as mmol of Trolox equivalents per L of sample (mM TE/L). Measurements were done in triplicate.

DPPH radical-scavenging activity

DPPH radical-scavenging activity of beer was estimated following the slightly modified procedure described by Kaneda *et al.* [31]. Every diluted beer sample (0.2 mL) was added to the DPPH working solution (2.8 mL) (mixture of 1.86×10^{-4} mol/L DPPH in ethanol and 0.1 M acetate buffer (pH 4.3) in volume ratio 2:1. The absorbance at 525 nm was measured after the solution had been allowed to stand in the dark for 60 min. The Trolox calibration curve was plotted as a function of the percentage of inhibition of DPPH radical. The results are expressed as mmol of Trolox equivalents per L of sample (mM TE/L). Triplicate measurements were performed.

Sensory evaluation

The sensory acceptability (degree of liking) of the beer samples was assessed using the nine-point hedonic scale (1 = dislike extremely, 9 = like extremely). The samples were evaluated by 80 panelists (consumers), males and females, 22-50 years of age, who were regular users of such products. Each assessor received six randomized, chilled refrigerated (10 °C) samples of beer (25-30 mL) in clear, tulip-shaped glasses with a volume of 100 mL. The samples were coded with three-digit random numbers and covered with watch glasses to prevent the loss of volatiles. A card containing scales of nine categories was provided, and assessors were asked to indicate their hedonic response to the samples on the appro-

priate scale. All the assessments were carried out at room temperature under white light.

Statistical analysis

Data of all measurements performed in triplicate are expressed as mean \pm standard deviation (*SD*). The experimental data were subjected to a one-way analysis of variance (ANOVA), and Tukey's test was used to detect the difference ($p \leq 0.05$) between the mean values. Statistical analyses were performed with Statistica 12 software [32].

RESULTS AND DISCUSSION

Table 2 shows the basic chemical parameters of commercial lager beer type Pils obtained from the local market.

The results are in accordance with the requirements of the Beer regulations [27].

Results of the plant extractions analysis

Plant extracts were first characterized in the terms total phenols, and then their antioxidant activity was measured by FRAP (determination of total antioxidant activity) and DPPH test.

Determination of total phenols

The content of total phenols in extracts of selected medicinal plants is shown in Table 3.

Table 3. Results of the determination of total phenols and antioxidant activity of extracts of tested plant species; different letters in same column denote a significant difference according Tukey's test, $p < 0.05$; TPC - total polyphenol content (mg gallic acid/L beer); FRAP - ferric reducing ability of the plasma; DPPH - 2,2-diphenyl-1-picrylhydrazil

Herbal extract	TPC mg GAE/L	FRAP mM TE	DPPH mM TE
<i>Melissae folium</i>	1112.3 \pm 4.52 ^a	25.63 \pm 0.50 ^a	15.11 \pm 0.09 ^a
<i>Thymi herba</i>	1301.54 \pm 5.23 ^b	26.19 \pm 0.24 ^a	16.18 \pm 0.11 ^b
<i>Juniperi fructus</i>	869.74 \pm 4.57 ^c	13.54 \pm 0.06 ^b	9.86 \pm 0.09 ^c
<i>Urticae radix</i>	612.56 \pm 2.54 ^d	9.12 \pm 0.08 ^c	6.85 \pm 0.07 ^d
<i>Lupuli stroboli</i>	542.57 \pm 6.64 ^e	8.68 \pm 0.09 ^d	6.18 \pm 0.03 ^e

The mean values of obtained results indicate that the sequence of analyzed herbal drugs extracts, by the total phenols content, is as follows: *Thymi herba* > *Melissae folium* > *Juniperi fructus* > *Urticae radix* > *Lupuli stroboli*. The results are in agreement

Table 2. Parameters of commercial lager beer type Pils; Er - real extract; Ea - apparent extract; RDF - real degree of fermentation; ADF - apparent degree of fermentation

Sample	P / °Plato	Er / mass%	Ea / mass%	RDF / %	ADF / %	Calories, kJ/100 ml	Alcohol, vol. %
Standard beer	11.61	4.17	2.39	65.54	79.42	174.99	4.89

with literature data [22,23].

Determination of antioxidant activity

Results of the determination of total antioxidant activity measured by FRAP method and the antioxidant activity analyzed by DPPH test are correlated with the content of total phenols. Extracts of *Thymi herba* and *Melissae folium* evidently have the strongest antioxidant activity: 26.19 and 25.63 mM TE, respectively (according to FRAP test), and 16.18 and 15.11 mM TE (according to DPPH test, Table 3).

According to the content of phenolic compounds, analyzed herbal extracts are significantly different at the level of statistical significance of $p < 0.05$. Given that the antioxidant activity depends directly on the content of phenolic compounds, methods for measuring the antioxidant activity of FRAP and DPPH test also showed a statistically significant difference between the samples.

Results of the analysis of beer with extracts of medicinal herbs

The results of analyzed beer samples with added sensory acceptable amount of selected extracts of medicinal plants are shown in Table 4. The beer samples were firstly evaluated in terms of total phenols levels, in the same way as the extracts, and then their antioxidant activity was measured. Finally, the sensory evaluation of analyzed beer samples was carried out in terms of acceptability and attractiveness to consumers. This was done in order to choose one beer with added sensory acceptable dose of herbal extract, which not only had improved functional properties, but also harmonious, pleasant flavor and aroma.

Table 4. Results of the determination of total phenols and antioxidant activity of standard beer and beer combined with tested plants extracts; different letters in same column denote a significant difference according Tukey's test, $p < 0.05$; TPC - total polyphenol content (mg gallic acid/L beer); FRAP - ferric reducing ability of the plasma; DPPH - 2,2-diphenyl-1-picrylhydrazil

Type of beer	TPC, mg GAE/L	FRAP, mM TE	DPPH, mM TE
B	280.26±1.14 ^a	4.15±0.02 ^a	2.54±0.02 ^a
BM	363.08±2.24 ^b	4.51±0.07 ^b	3.05±0.08 ^b
BT	384.22±3.05 ^c	4.71±0.08 ^c	3.72±0.10 ^c
BJ	365.38±2.85 ^b	4.55±0.02 ^b	3.14±0.09 ^b
BU	317.18±1.57 ^d	4.25±0.04 ^d	2.85±0.07 ^d
BH	316.67±1.76 ^d	4.27±0.07 ^d	2.83±0.03 ^d

Determination of total phenols

Table 4 shows the total phenols levels in commercial standard beer, then in beer with the addition of sensory acceptable dose of selected medical plants extracts (determined by the method of Folin-Ciocal-

teu), and finally the antioxidant activity of analyzed beer samples (measured by FRAP and DPPH test). The mean values of obtained results indicate that, by the total phenols levels, the sequence of analyzed beer samples is as follows: BT > BJ > BM > BU > BH > B.

Increase in the level of total phenols, when minimal doses of liquid herbal extracts were added (approximately 0.05%), is obvious and significant. The content of polyphenols in beer samples enriched with extracts of thyme herb, fruit berries and lemon balm leaf is 384.22, 365.38 and 363.08 mg GAE/L, respectively, as compared to standard beer, an increase of 37.09, 30.36 and 29.55%, respectively.

When added to beer, sensory acceptable doses extracts of fruit juniper and lemon balm leaf are causing almost identical effect of increase in polyphenol levels. Addition of extracts of nettle root and hop cones causes similar increase in total phenols levels (13.17 and 12.99%).

Determination of antioxidant activity

Results of the determination of total antioxidant activity (measured by FRAP method) and antioxidant activity (analyzed by DPPH test) of standard beer and beer samples combined with selected herbs, are shown in Table 4 and are correlated with total phenols levels. The highest increase in antioxidant activity (compared to standard beer) showed beer samples combined with sensory acceptable doses of extracts of herbs thyme, juniper fruit and leaf of lemon balm (4.71, 4.55 and 4.51 mM TE measured by FRAP test and 3.72, 3.14 and 3.05 of mM TE measured DPPH test, respectively).

Correlation between total phenols content and antioxidant activity of analyzed samples was statistically very significant and shown in Table 5, as well as correlation between two applied antioxidant assays.

Table 5. Correlation between total phenols content and antioxidant activity determined by different methods; r - correlation coefficient; r^2 - coefficient of determination, t - sample values applied tests, p - level of significance; TPC - total polyphenol content (mg gallic acid/L beer); FRAP - ferric reducing ability of the plasma; DPPH - 2,2-diphenyl-1-picrylhydrazil

Method	r	r^2	t	p
TPC-DPPH	0.911	0.830	4.417	0.012
TPC-FRAP	0.980	0.960	9.750	0.001
FRAP-DPPH	0.947	0.897	5.894	0.004

Results of sensory analysis of special types of beer

The results of sensory evaluation of standard beer and beer enriched with sensory acceptable doses of selected medicinal plants, obtained by the

method of scoring by male and female evaluators, are shown in Table 6.

The best sensory score was obtained by the beer enriched with extract of lemon balm. Beer with extracts of hop cones was slightly better rated than standard beer. Interestingly, beer samples with fruit extracts of juniper, thyme herb and root nettle got lower sensory score, in relation to commercial lager. Only the beer with nettle root extract obtained significantly lower sensory score in relation to commercial lager beer type Pils.

As previously stated, an ethanol-water extract of hop cones *Lupuli stroboli* was analyzed, in order to compare obtained results with the raw material commonly used in the beer production. Results showed that total polyphenol levels increased by approximately 13% and accordingly, the antioxidant capacity increased as well. In fact, recent studies have even shown that the antioxidant activities of hop polyphenols *in vitro* and *in vivo* were higher than green tea

properties. Extract of hop cones deserves special attention. In sensory acceptable dose of approximately 0.07%, its contribution to beer total phenols level, antioxidant activity and sensorial properties are much lower than previous. However, the composition of hops phenols and its functional activities in the human body are unique. XN and 8-PN are especially important, so that any enrichment of beer with them has beneficial effects.

The results of total phenols concentration, antioxidant activity, and sensory evaluation of beers enriched with herbs extracts obtained in this analysis show that beer is an alcohol beverage with a lot of potentials considering improvement in functional properties and developing new beer types. Increase in the level of total phenols, obtained when minimal doses of liquid herbal extracts were added (approximately 0.05%) was statistically significant. However, there was no significant correlation between antioxidant activity and sensory marks of enriched beers.

Table 6. Sensory evaluation of analyzed beer types; different letters in same column denote a significant difference according Wilcoxon matched pair test, p < 0.05; 1 = dislike extremely; 9 = like extremely

Type of beer	Mean	Mediana	Minimum	Maximum	Standard deviation	Standard error
B	6.5 ^a	7	2	9	1.661	0.308
BM	7.2 ^b	8	5	9	1.123	0.209
BT	5.7 ^a	6	1	9	2.034	0.378
BJ	5.8 ^{a,c}	7	2	9	2.305	0.428
BU	5.6 ^{b,c}	6	1	8	2.261	0.420
BH	6.6 ^c	7	4	9	1.152	0.214

polyphenols, at the same concentration [33]. According to the results, it can be concluded that beer enriched with extracts of medicinal and aromatic plants, represents far better source of natural antioxidants in comparison to commercial lager beer.

In this experiment, the lemon balm extract manifested the best performance in the combination with beer. This highly valued medicinal and aromatic plant contains characteristic, biologically active complexes, which, combined with beer, gives a very appealing aromatic composition. In this way, a distinctive and pleasant flavor is obtained, without impairing sensory properties of standard beer. In the same time, standard beer enriched with approximately 0.05% of liquid extract contains an increase of 29.55% of total phenols. From the functional aspect, thyme gives superior results. Beer enriched with the same content of liquid extract has 37% higher content of total phenols. The problem is that when thyme extract is added alone, beer has inferior sensorial

Finally, our results fit into the area where efforts are made so that different alcoholic and non-alcoholic beverages are enriched by the extractive isolates of medicinal and aromatic plants [11,12] in order to obtain new products with increased functional and new sensory properties.

CONCLUSION

While it is obvious that numerous medicinal and aromatic plants can be used for improving beer functional and sensorial properties, the results are not always satisfactory. The solution can be found in combining different medicinal and aromatic plants. Such possibilities are numerous. The most important thing is to choose the optimal composition of plants so that maximal functional properties could be in balance with pleasant sensorial properties. In this way, new types of beer can be produced as the products, which can fulfill two targets: beverages with improved functional properties, especially interesting in the

case of alcohol-free beers, and specialty beers with new flavor and taste.

The result of sensory evaluation showing that the lemon balm extract manifested the best performance in the combination with beer as it has been expected. This highly valued medicinal and aromatic plant contains characteristic, biologically active complexes, which combined with beer, give very appealing aromatic composition. In this way, a distinctive and pleasant flavor is obtained, without impairing sensory properties of standard beer. Although thyme is superior from the functional aspect, less harmonious effect in sensory terms is obtained.

Increase in the level of total phenols, obtained when minimal doses of liquid herbal extracts were added was statistically significant, but there was no significant correlation between antioxidant activity and sensory marks of enriched beers.

However, when it comes to achieving the final effect, possibilities for correction are great. The most important thing is to choose the medicinal and aromatic plants with optimal composition of active and sensory acceptable components.

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REFERENCES

- [1] Leskošek-Čukalović, V. Nedović, Beer as an integral part of healthy diets-current knowledge and perspective, in Proceedings of the 6th European Congress on Food, Novi Sad, Serbia, 2012, pp. 46-54
- [2] P.E. McGovern, Uncorking the past: The quest for wine, beer, other alcoholic beverages, University of California Press, Los Angeles, CA, 2009, p. 60
- [3] I.S. Hornsey, A history of beer and brewing, The Royal Society of Chemistry, Cambridge, 2003, p. 10
- [4] K. E. Behre, Veget. Hist. Archaeobot. 8 (1999) 35-48
- [5] J. Kišgeci, The world of beer and beer in the world, Aurora, Novi Sad, 2001, p. 34
- [6] C. Gerhäuser, Europ. J. of Cancer. 41 (2005) 1941-1954
- [7] S. Gorjanović, M. Novaković, N. Potkonjak, I. Leskošek-Čukalović, D. Sužnjević, J. Agric. Food. Chem. 58 (2010) 744-751
- [8] A. Lugasi, J. Hóvári, Nahrung. 47 (2003) 79-86
- [9] F. Ferk, W. Huber, M. Filipič, J. Bichler, E. Haslinger, M. Mišik, A. Nersesyan, B. Grasl-Kraupp, B. Žegura, S. Knasmüller, Mutation Research. 691 (2010) 17-22
- [10] L.R. Chadwick, D. Nikolic, J.E. Burdette, C.R. Overk, J.L. Bolton, R.B. van Breemen, R. Fröhlich, H.H. Fong, N.R. Farnsworth, G.F. Pauli, J. Nat. Prod. 67 (2004) 2024-2032
- [11] D. Stanisavljević, S. Stojčević, S. Đorđević, B. Zlatković, D. Veličković, I. Karabegović, M. Lazić, Chem. Ind. Chem. Eng. Q. 8 (2012), 411-420
- [12] I. Leskošek-Čukalović, S. Jelačić, V. Nedović, M. Ristić, Đorđević, S, Functional drinks and phytopharmaceuticals based on beer and medicinal herbs, in: Proceedings of the 31st European Brewing Convention Congress, Venice, Italy, 2007, Chapter 113, pp. 1-13
- [13] I. Leskošek-Čukalović, S. Despotović, N. Lakić, M. Nikšić, V. Nedović, V. Tešović, Food Res. Int. 43 (2010) 2262-2269
- [14] M. Veljović, S. Despotović, M. Stojanović, S. Pečić, P. Vukosavljević, M. Belović, I. Leskošek-Čukalović, Chem. Ind. Chem. Eng. Q. 21(3) (2015) 391-397
- [15] Thomson PDR Staff, PDR for Herbal Medicine, 3rd ed., Thomson Healthcare, Medical Economics, Montvale, 2004, pp. 502, 824, 477, 792, 439
- [16] M. Blumenthal, A. Goldberg, J. Brinckmann (Eds.), Herbal Medicine, Expanded Commission E Monographs, American Botanical Council, Austin, Texas, 2000
- [17] J.P. Kamdem, A. Adeniran, A.A. Boligon, C.V. Klimaczewski, O.O. Elekofehinti, W. Hassan, M. Ibrahim, E.P. Waczuk, D.F. Meinerz, M.L. Athayde, Ind. Crop. Prod. 51 (2013) 26-34
- [18] J.T. Lin, Y.C. Chen, Y.C. Lee, C. W.R. Hou, F.L. Chen, D.J. Yang, LWT-Food Sci. Technol. 49 (2012) 1-7
- [19] E. Koksal, E. Bursal, E. Dikici, F. Tozoglu, I. Gulcin, J. Med. Plants Res. 5 (2011) 217-222
- [20] I. Berasategi, I. Navarro-Blasco, C. M. Isabel, R.Y. Cavero, I. Astiasaran, D, Meat Sci. 96 (2014) 1185-1190
- [21] D. Stanojević, L. Čomić, O. Stefanović, S.S. Sukdolak, Span. J. Agric. Res. 8 (2010) 109-115
- [22] M.H. Roby, M.A. Sarhan, K.H. Selim, K.I. Khalel, Ind. Crop. Prod. 43 (2013) 827-831
- [23] R. Kaledaite, J. Bernatoniene, D. Majiene, K. Dvorackova, R. Masteikova, J. Muselik, Z. Kalveniene, J. Liobikas, A. Savickas, J. Med. Plants Res. 5 (2011) 6090-6096
- [24] N. Miceli, A. Trovato, P. Dugo, F. Cacciola, P. Donato, A. Marino, V. Bellinghieri, T. M. La Barbera, A. Guvenc, M. F. Taviano, J. Agr. Food Chem. 57 (2009) 6570-6577.
- [25] [25] M. Elmastas, I. Gulcin, S. Beydemir, O.I. Kufrevioglu, H.Y. Aboul-Enein, Anal. Lett. 39 (2006) 47-65
- [26] S. Gorjanović, T. Pastor, R. Vasić, M. Novaković, M. Simonović, S. Milić, D. Suznjević, J. Agr. Food Chem. 61 (2013) 9089-9096
- [27] Regulations on quality and other requirements for beer (SCG 36/2004)
- [28] Pharmacopoeia Jugoslavica, edition quatra (Ph. Jug. IV), Federal Institute of Public Health, Belgrade, 1984. p. 116
- [29] V. Singletton, J. Rossi, Am. J. Enol. Viticult. 16 (1965) 144-158
- [30] I.F.F. Benzie, J.J. Strain, Anal. Biochem. 239 (1996) 70-76

- [31] H. Kaneda, N. Kobayashi, S. Furusho, H. Sahara, S. Koshino, Tech. Q. - Master Brew. Assoc. Am. **32** (1995) 90-94
- [32] Statistica (Data Analysis Software System), v. 12, StatSoft, Inc., USA, 2013
- [33] X. Wang, L. Yang, X. Yang, Y. Tian, J. Sci. Food Agr. **4** (2014) 1693-1700.

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NAUČNI RAD

EKSTRAKTI LEKOVITOG BILJA KAO FUNKCIONALNI DODACI PIVU

Istraživanja u ovom radu zasnivaju se na utvrđivanju nivoa antioksidatne aktivnosti piva, kome su dodavane senzorno prihvatljive količine ekstrakata odabranog lekovitog bilja, sa ciljem dobijanja piva sa povećanim funkcionalnim i novim senzornim svojstvima. Za ova proučavanja korišćeno je domaće komercijalno svetlo Pils pivo i vodeno-etanolni ekstrakti biljnih droga: *Melissae folium*, *Thymi herba*, *Juniperi fructus*, *Urticae radix* i *Lupuli strobili*. Ukupni fenoli analizirani su metodom po Folin-Ciocalteu, a određivanje antioksidativnog kapaciteta uzoraka pomoću FRAP i DPPH testa. Senzorna ocena piva sprovedena je na 80 ispitanika pomoću hedonske skale sa 9 nivoa. Rezultati pokazuju da je sadržaj ukupnih fenola najveći u pivu kome su dodavani ekstrakti timijana, kleže i matičnjaka (384,22, 365,38 i 363,08 mg GAE/L redom), što predstavlja povećanje 37,09, 30,36 i 29,55%, redom, u odnosu na polazno standardno pivo. Vrednosti nalaza antioksidantne aktivnosti su u korelaciji sa sadržajem ukupnih polifenola (4,71, 4,55 i 4,51 mM TE merene FRAP testom i 3,72, 3,14 i 3,05 mM TE merene DPPH testom, redom). Ekstrakt lista matičnjaka najbolje se ukomponovao sa polaznim pivom sa aspekta senzorne prihvatljivosti. Novo pivo obogaćeno matičnjakom imalo je dopadljiv i harmoničan ukus i miris.

Ključne reči: pivo, biljni ekstrakti, senzorna ocena, ukupni fenoli, antioksidantna aktivnost.