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New Type of Beer – Beer with Improved Functionality and Defined Pharmacodynamic Properties

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Summary

The paper highlights the facts about the possibilities of improving the functionality of beer with extracts of thyme (*Thymus vulgaris*), lemon balm (*Melissa officinalis*) and mushroom *Ganoderma lucidum*. It briefly summarizes the most important data about possible positive action of moderate beer consumption and the benefits of beer as a base for developing a variety of products with enhanced functionality. It gives an overview about the mentioned herbs and the mushroom, their use in traditional medicine, chemical composition, pharmacodynamic properties and possible benefits from the brewing point of view. Procedures for extraction of biological material, experimental results of antimicrobial properties, antioxidant capacity and sensory evaluation of beer enriched with these extracts are given. Experimental results indicate that commercially produced and bottled pils beer enriched with tinctures of *Thymus vulgaris* and *Melissa officinalis* shows improved antimicrobial and antioxidative properties. *Ganoderma* is particularly important because of its unique functional properties and sensory compatibility with beer. Products obtained like this could fulfill several goals: developing novel beer types, developing products with health-promoting properties that meet market needs and eventually gain new beer consumers. Alcohol content of such products depends on the type and alcohol content of initial beer.

Key words: beer, lemon balm, thyme, *Ganoderma*, antimicrobial, antioxidative, sensorial properties

Introduction

Beer is the oldest and one of the most used alcoholic beverages in the world. Its huge popularity is gained not only by low cost and easy access, but by its immense properties and possible health benefits. Researchers confirm that drinking beer in moderation has beneficial effects on numerous aspects of health such as: reducing the risk of cardiovascular disease, blood cholesterol levels, diabetes, osteoporosis, dementia, and it can be part of a healthy 'mass reduction' and valuable source of vitamins, minerals and antioxidants (*1*). Beer as a truly natural drink contains hundreds of different compounds (vitamins, minerals, amino acids, carbohydrates, organic acids, polyphenols) necessary for good body functioning (*2*). Some main components of beer are shown in Table 1 (*3*). Numerous studies indicate that if consumed in moderate and responsible manner, beer can act positively on the overall health condition.

There is evidence that moderate beer consumption can provide a significant increase of vitamin B6, B12, A and folate intake in case of females and vitamin B2, B6, niacin and folate in case of men (*4*). Beer is often quoted as a valuable source of magnesium, potassium, phosphorus and silicon (*5,6*). All these biotics are of great importance for appropriate body functioning. Potassium helps in elimination of sodium and chlorides from the body, while magnesium is necessary for appropriate brain, muscle and coronary artery functioning. It has been successfully used in prevention and treatment of arrhythmia and coronary diseases (*7*). Furthermore, magnesium contributes to calcium metabolism, and good bone mineral

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Table 1. Relative composition of beer (*3*)

Substance	Concentration	Substance	Concentration
water	90–94 %	potassium	200–450 mg/L
ethanol	3–5 % (by volume)	sodium	20–350 mg/L
carbohydrates	1–6 % *m/V*	calcium	25–120 mg/L
folic acid (B9)	0.04–0.60 mg/L	magnesium	50–90 mg/L
pyridoxine (B6)	0.07–1.70 mg/L	iron	0.01–0.30 mg/L
biotin (B8)	0.015 mg/L	zinc	0.01–1.48 mg/L
riboflavin (B2)	0.02–0.10 mg/L	cobalt	0.01–0.11 mg/L
pantothenic acid (B5)	0.04–2.0 mg/L	chromium	0.04 mg/L
thiamin (B1)	0.08 mg/L	manganese	0.03–0.20 mg/L
cyanocobalamin (B12)	0.03 mg/L	silica	40–120 mg/L
niacin (B3)	0.3–5.0 mg/L	total nitrogen content	300–1000 mg/L

density (BMD) (*8–10*). One litre of beer can be a source of approx. 25–30 and even 40 % of the recommended daily amount of magnesium and similar quantity of potassium and phosphorus (*10,11*). Beer becomes more and more interesting as a source of silicon as well. Recent studies have shown that supplemental silicon has both anti-resorptive and anabolic action on human osteoblast-like cells, and not only inhibits bone loss but also increases BMD (*12,13*).

Beer contains important dietary fibres as well. Their content in beer is between 0.4 and 6.2 g/L, which is 2–16 % of recommended daily intake (*14,15*). Moderate consumption of beer can result in increasing HDL cholesterol and suppressing LDL cholesterol and platelet aggregation in the similar way as wine and other alcoholic beverages (*16*). The obtained results indicate that homocystein levels, responsible for damaging arteries and inducing atherosclerosis, do not increase among moderate beer drinkers (*17,18*). This may be due to the fact that beer contains a large quantity of vitamin B6 and folic acid. In the case of diabetics, the evidence from observational studies suggests that drinking beer in moderation (6–48 g alcohol/day) can be associated with increasing insulin sensitivity and a highly significant 30 % reduced risk of type 2 diabetes (also called adult onset or non-insulin-dependent diabetes) (*19–21*). Furthermore, insulin levels could be reduced, which is good for non-diabetics because it decreases the chance of developing atherosclerosis, acting as blood thinner and reducing the risk of coronary thrombosis (*22*). It seems that the risk of developing kidney stones is also reduced as beer helps in flushing the kidneys (*23*). Positive effect on the immune system of healthy adults is mentioned as well (*24*). There is also a question of cancer diseases. The use of alcoholic beverages has been related to increased risks of several cancers in humans. Threats of oral, laryngeal, esophageal and liver cancer are elevated among drinkers, usually in proportion to the consumed quantity (*25*). However, there is evidence that some compounds in beer, mainly micronutrient fractions from hops, could prevent colonic carcinogenesis (*26*). The most important biologically active compounds found in hops are polyphenols, especially xanthohumol (XH) and 8-prenylnaringenin (8-PN) (*27,28*). Several studies have revealed that XH strongly suppresses one of the key enzymes in cancer development and stimulates detoxication enzymes to repress carcinogenesis (*29–32*). 8-PN is one of the most powerful phyto-oestrogens found in nature. It was considered that their action is negligible because their concentrations in beer are generally very low, but the latest studies show that moderate beer consumption can provide these compounds in quantities within the range of biological activity (*27,28*). Considering all these facts, it is obvious that beer has great potential as a beverage with pharmacodynamic action. Our aim is to improve this property with natural materials such as medicinal herbs, namely lemon balm and thyme and mushroom *Ganoderma*.

Numerous herbs have a long tradition in folk medicine. For ages they have been used for curing and medical purposes. Throughout the history, a lot of them were used in the process of brewing as well, for flavouring and masking beer deficiencies. With the introduction of hops, this tradition has come to an end.

Lemon balm (*Melissa officinalis*) contains numerous bioactive compounds: essential oils, phenolic compounds, flavonoids and tannins. More than 190 components of the essential oils have been described. About 50–70 % of these oils are citral, citronellol, geraniol, caryophyllen, and nerol. Traditionally, lemon balm was used in treatment of insomnia, anxiety, depression, migraine, tension headache, digestion-improving agents, anorexia, colic, and Graves' disease and other thyroid conditions (*33*).

Thyme (*Thymus vulgaris* L.) contains 1.2–2.5 % essential oils, tannins, flavonoids, triterpens, phytosterols and saponins. Essential oils contain primarily the isomeric monoterpen thymol. These substances are shown to be cancer chemoprotective (*33*). Both herbs are on the GRAS (generally recognized as safe) list of the World Health Organization (WHO) and the European Scientific Cooperative on Phytotherapy (ESCOP) (*34,35*).

Ganoderma lucidum is a woody mushroom often called 'Elixir of Life', 'Food for Gods' and 'Mushroom of the Universe'. It is well known by several different names in the world like: *Ganoderma* in China, *Reishi* in Japan and *Young Zhi* in Korea. *Ganoderma* contains numerous natural bioactive components, polysaccharides, ergosterols, proteins, ganoderic acids, unsaturated fatty acids, vitamins and minerals. In numerous studies it was confirmed that *Ganoderma lucidum* possesses anti-cancer properties. It

mainly comes from polysaccharides β-glucans, located in mycelia, the fruiting body of fungi and spores. Triterpenoids isolated from the mushroom have antioxidative, immunomodulating and antitumour effect. Major triterpenoides isolated from *Ganoderma* mushroom are ganoderic acids R, T, U, V, W, X, Y and Z; lucidimol A and B; ganodermanondiol; ganoderiol F; and ganodermanontriol (*36*). It has been proven that these terpenoids have numerous positive actions on the human body, carcinostatic effects on cancer cells, and many of them also possess anti-angiogenic activity (*37*,*38*).

The purpose of this study is to assess the potential effects of the given herbs and mushroom for the creation of beer with enhanced functionality. Their sensory profiles, antioxidant potential and antimicrobial activities were assessed.

Materials and Methods

Raw materials

As a requirement for this experiment dry plant herbs *Thymus vulgaris* L. and *Melissa officinalis* were used for the preparation of tinctures. The tinctures were prepared at the Institute for Medicinal Plant Research Dr Josif Pančić, Belgrade, Serbia, using the conditions prescribed according to European Pharmacopoeia (*34*). Dry herbs were cut into pieces and mixed thoroughly with 70 % (by volume) ethanol solvent in a drug/solvent ratio of 1:5. Extraction was done in a percolator at a temperature of 21 °C for 24 h. The residue was pressed out and the liquid combined with the percolate.

Mushroom *Ganoderma lucidum* tissue was cut into pieces and mixed with an alcoholic solution of 70 % (by volume) ethanol. Extraction was performed as a daily mixing in a magnetic stirrer for 10 min and then allowed to stand in a dry and dark place at room temperature. Extraction period was 21 days. After the extraction, the solution was filtered and concentrated in a vacuum to 1/5 of its initial content.

Bottled pils beer was used for the experiment. The recommended daily doses of tinctures (1–1.5 mL) were added to 0.5 L of beer in a laminar hood. After inoculations, the beer samples were closed hermetically and left to mature at 4 °C for 7 days.

Antimicrobial activity

Screening beer for antibacterial activity was done using the disk diffusion method. Antimicrobial assays were performed on six organisms from the American Type of Culture Collection (ATCC): *Staphylococcus aureus* ATCC 6538, *Kocuria rhizophila* (*Micrococcus luteus*) ATCC 9341, *Escherichia coli* O157:H7 ATCC 35150, *Listeria monocytogenes* ATCC 19115, *Geobacillus stearothermophilus* ATCC 7953 and *Candida albicans* ATCC 24433. The cultures were adjusted to approx. 10^5 CFU/mL with sterile saline solution. Petri dishes were inoculated with 0.2 mL of microorganism suspension and overlaid with 20 mL of the medium. For cultivating the bacteria, Trypticase soy agar with 0.6 % yeast extract (TSA-YE) was used. Three filter disks (Whatman®, Sigma-Aldrich, St. Louis, MO, USA, and Schleicher&Schuell, Dassel, Germany, 6 mm in diameter) were placed on each agar and diffusion method was performed by adding 10 μL of appropriate suspension on each disk. Blind probe contained only 70 % C_2H_5OH. Bacteria were incubated at 37 °C for 24 h and after the incubation period, the zone of inhibition was measured. Small sectors from the zone of inhibition were taken and inoculated in TSA-YE for cultivated microorganisms. Sectors in TSA-YE were incubated at 37 °C for 24 h to see if the effect of beer was microbicidal or microbistatic (*39*). Studies were performed in triplicate, and mean value was calculated. Antimicrobial assay was performed on the above strains using beer with lemon balm, beer with thyme, beer with lemon balm and *Ganoderma lucidum* and beer with thyme and *Ganoderma lucidum.*

Extract analysis

Ganoderma extract was analyzed by liquid chromatography-mass spectrometry (LC/MS) method. LC/MS analysis was performed on an Agilent MSD TOF coupled to an Agilent 1200 series HPLC (Karlsruhe, Germany), using Zorbax Eclipse XDB-C18 column (RR, 30×2.1 mm, i.d. 3.5 μm). Mobile phase A was 0.2 % formic acid in water, and mobile phase B was acetonitrile. The injection volume was 5 μL, and elution was performed at 0.7 mL/min with gradient program (0–1.5 min 5 % B, 1.5–10 min 5–95 % B, 10–15 min 95 % B, 15–16 min 99–5 % B). Mass spectra were acquired using an Agilent electrospray ionization-mass spectrometric detection (ESI-MSD) time-of-flight (TOF). Capillary voltage was 4000 V, fragmentor voltage 140 V, nebulizer pressure 310 kPa (45 psig), drying gas 12 L/min, gas temperature 350 °C, mass range 100–1500 *m/z*, negative and positive ionization mode. Processing of the data was done with the software Molecular Feature Extractor and Mass Profiler (Agilent Technologies Inc, Santa Clara, CA, USA).

Tinctures of *Thymus vulgaris* L. and *Melissa officinalis* were characterized by the combination of gas chromatography and mass spectrometry (GC/MS). GC/MS analyses were carried out on a Hewlett Packard, HP G1800C (Palo Alto, CA, USA) gas chromatograph equipped with a split/splitless injector and Agilent HP-5MS capillary column. Individual constituents were identified by comparing their retention times with those of analytical standards of available terpenoids, but also by computer searching, matching mass spectral data with those held in reference libraries (Wiley 275/NIST).

Antioxidant activity

Free radical scavenging ability was done using the DPPH method. This method is based on the reduction of a stable DPPH (2,2-diphenyl-1-picrylhydrazyl) radical by antioxidants in a methanolic solution. In the presence of antioxidants, the purple colour of the DPPH radical solution changes to a bright yellow and the intensity of this change can be monitored spectrophotometrically. The samples were analyzed according to the method reported by Brand-Williams *et al.* (*40*). Briefly, methanol DPPH solution with buffer at pH=4.3, c(DPPH)=0.184 μM, was added to different volumes of diluted sample and the free radical scavenging capacity of the sample was evaluated by measuring the absorbance at 525 nm immediately after the addition of DPPH (t=0) and after 15 min. The results were expressed as the percentage of reduc-

tion ('inhibition') of the DPPH (Q), which is defined by the following equation:

$$Q=100\cdot(A_0-A_s)/A_0 \qquad /1/$$

where A_0 is the initial absorbance and A_s is the value of absorbance after the reaction reached steady state. All determinations were performed in triplicate. Antioxidant activity was investigated in beer with thyme and beer with lemon balm, while beer with *Ganoderma* showed inconclusive results and they need to be done over again.

Sensorial evaluation

The consumer acceptance test was carried out by 100 untrained consumers with the following average profile: 95 % were 20–25 years of age, 29 % were female, 72 % with the consumption frequency of one or more beers per week and 27 % were non-drinkers of beer. The beer samples were evaluated using a 5-point scale. Consumers did not have any formal training or experience in the description of beer flavour. Aroma, taste, body, bitterness, liveliness and overall impression were tested. Three beer types were evaluated: beer with *Melissa officinalis* (BM), beer with *Thymus vulgaris* (BT) and beer with *Ganoderma lucidum* (BG) *vs.* initial beer (IB).

Statistical analysis

The experimental results were analyzed with the statistical package Statistica v. 6.1 (*41*). The data analysis of the sensory properties was performed using Friedman ANOVA and Wilcoxon matched pairs test.

Results and Discussion

Results of LC/MS analysis of *Ganoderma lucidum*, *Thymus vulgaris* and *Melissa oficinalis* extracts are given in Table 2. LC/MS analysis has verified that *Ganoderma* extract contains biologically active terpenoids (ganoderic acid A, B, C, D, H and J) essential for its expected pharmacodynamic action. The composition of the tested plant extracts, thyme and lemon balm, shows the presence of their constituents (carvacrol, neral, thymol, geranial), given in Table 2.

Antimicrobial activity

The antimicrobial activity of beer and beer with extracts against standard microorganisms (expressed as zones of growth) is given in Table 3. Blind probe showed that 70 % ethanol had no antimicrobial influence on the investigated microorganisms. The results indicate that initial beer (IB) even stimulates the growth of *S. aureus* ATCC 6538 and *E. coli* O157:H7 ATCC 35150. IB showed light microbistatic effect against *K. rhizophila* ATCC 9341 and *L. monocytogenes* ATCC 19115, while in the case of *G. stearothermophilus* ATCC 7953 and *C. albicans* ATCC 24433, it showed no activity. Beer with *Thymus vulgaris* (BT) extract had microbistatic activity against *K. rhizophila* ATCC 934, *L. monocytogenes* ATCC 19115 and *G. stearothermophilus* ATCC 795, whereas towards *S. aureus* ATCC 6538, *E. coli* O157:H7 ATCC 35150 and *C. albicans* ATCC 24433, it showed no activity. Beer enriched with *Melissa oficinalis* (BM) did not indicate any activity against *K. rhizophila* ATCC 9341, *E. coli* O157:H7 ATCC 35150, *G. stearothermophilus* ATCC 7953 or *C. albicans* ATCC 24433, but it showed microbistatic activity against *L. monocyto-*

Table 2. Relative content of constituents in *Ganoderma lucidum*, thyme and lemon balm extracts

	Constituents	RT/MS	Bruto molecular formulae	RRI
Ganoderma lucidum	ganoderic acid E	4.575	$C_{37}H_{36}O_2$, $C_{30}H_{40}O_7$	512.2
	ganoderic acid G	4.721	$C_{30}H_{44}O_8$	532.3
	ganoderic acid B, A	4.821	$C_{30}H_{44}O_7$	516.3
	ganoderic acid C, D, J	5.209	$C_{30}H_{42}O_7$	514.2
	ganoderic acid H	5.549	$C_{32}H_{44}O_9$	572.2
	ganoderic acid F	6.163	$C_{32}H_{42}O_9$	570.2
	ganodermanontriol, lucidumol A	7.389	$C_{30}H_{48}O_4$	472.3
Melissa oficinalis	coumaran	16.842	$C_9H_6O_2$	1223.0
	geranial	17.174	$C_{10}H_{16}O$	1238.3
	neral	17.685	$C_{10}H_{16}O$	1268.1
	thymol	18.698	$C_{10}H_{14}O$	1299.8
	carvacrol	19.765	$C_{11}H_{14}O$	1312.5
Thymus vulgaris	thymol	19.505	$C_{10}H_{14}O$	1292.1
	carvacrol	19.774	$C_{11}H_{14}O$	1300.0
	vinylguaiacol	20.087	$C_9H_{10}O_2$	1309.5
	syringol	21.369	$C_8H_{10}O_3$	1349.4
	eugenol	21.495	$C_{10}H_{12}O_2$	1353.0

RT/MS – identification by GC retention times (RT) and mass spectrometry (MS) of authentic compounds, RRI – relative retention index

Table 3. Zones of growth (in mm) for selected beers

Microorganism	IB	BT	BM	BTG	BMG
S. aureus ATCC 6538	10.00*	–	8.50*	–	–
K. rhizophila ATCC 9341	1.50†	2.00†	–	–	10.00†
E. coli O157:H7 ATCC 35150	3.00*	–	–	–	–
L. monocytogenes ATCC 19115	3.33†	5.00†	6.60†	4.33†	8.00†
G. stearothermophilus ATCC 7953	–	10.00†	–	3.00†	–
C. albicans ATCC 24433	–	–	–	–	–

Values for the zones of growth inhibition are presented as mean values; IB – initial beer, BT – beer with thyme, BM – beer with lemon balm, BTG – beer with thyme and *Ganoderma*, BMG-beer with lemon balm and *Ganoderma*; * stimulation, † inhibition, – no activity

genes ATCC 19115 and stimulation in the case of *S. aureus* ATCC 6538. Results of antimicrobial activity of beer samples with both thyme and *Ganoderma* (BTG) and beer with lemon balm and *Ganoderma* (BMG) showed no activity against *S. aureus* ATCC 6538, *E. coli* O157:H7 ATCC 35150 and *C. albicans* ATCC 24433, while in the case of *L. monocytogenes* ATCC 19115, both BTG and BMG exerted inhibitory effect. Compared to IB, the highest antibacterial activity was expressed by BMG when applied on *K. rhizophila* ATCC 9341 and BT on *G. stearothermophilus* ATCC 7953 strain. IB showed even upgrowth on *S. aureus* ATCC 6538 and *E. coli* O157:H7 ATCC 35150, but all other samples showed no activity. There was no stimulation, except in the case of BM on *S. aureus* ATCC 6538, which means that all other beers slowed down the upgrowth of the above strains.

Antioxidant activity

Antioxidant activity was determined in IB, BT and BM using DPPH method by calculating the IC_{50}. Antiradical activity was determined as the quantity of antioxidant required to reduce the initial DPPH concentration by 50 %. For each tested antioxidant concentration, graphs were plotted and presented (Figs. 1 and 2). The analyzed beer showed evident antioxidant activity. DPPH radical scavenging was concentration-dependent, with IC_{50}=7.2 μL/mL for IB, IC_{50}=3.3 μL/mL for BT and IC_{50}=5.4 μL/mL for BM. The lower number signifies the higher antioxidant potential in beer. DPPH analysis suggests that IB has significantly lower antioxidant potential than BM and BT.

Sensory evaluation

The results of sensorial evaluation of the tested samples are presented by means of statistical computation. As it was expected, different tasters judged the beer samples and analyzed sensorial parameters in relatively different ways. Grades were in the range from 1.0 to 5.0. The lowest grade (1.0) was given for the bitterness of IB, and 1.5 for the aroma of BM and BT. The lowest score for all other sensorial parameters in all tested beer samples was 2.0. Maximum grade for all tested parameters and beer samples was 5.0. The spider diagrams of the obtained sensorial properties and overall impression of the tested beer samples are given in Figs. 3–5.

Tasters characterized IB as refreshing with an emphasized aroma. They agreed that the body was poor

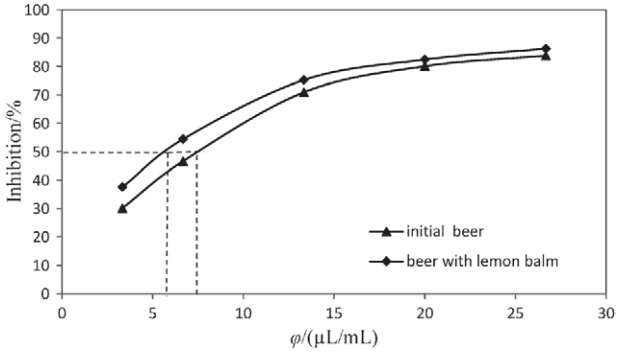

Fig. 1. Inhibitory effect of initial beer and beer with lemon balm on the DPPH radical

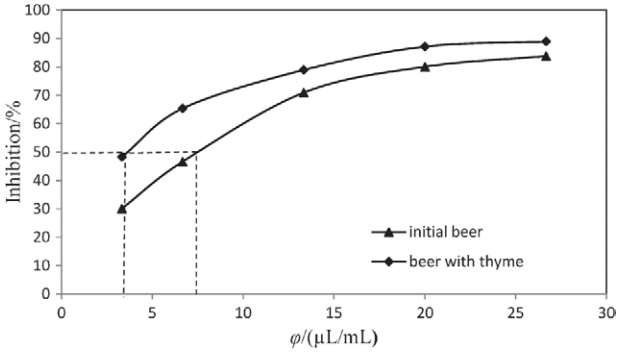

Fig. 2. Inhibitory effect of initial beer and beer with thyme on the DPPH radical

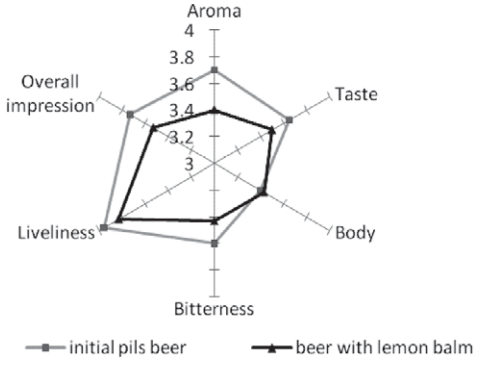

Fig. 3. Mean values of sensory parameters of initial beer and beer with lemon balm

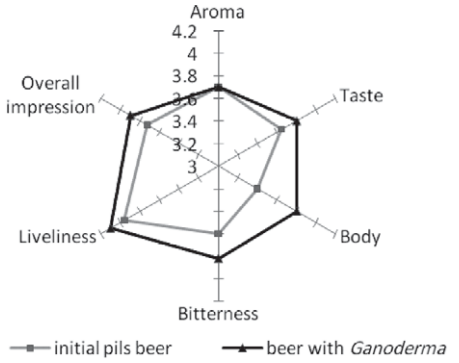

Fig. 4. Mean values of sensory parameters of initial beer and beer with *Ganoderma*

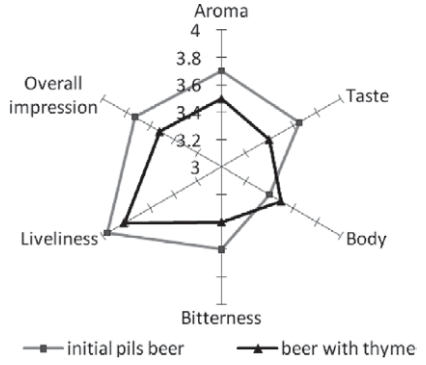

Fig. 5. Mean values of sensory parameters of initial beer and beer with thyme

and bitterness was overpowering. Compared with IB, they found that BT was very specific beer with unusual but pleasant aroma and taste. It was sweeter than other beers and a bit spicy. BM was weak in bitterness and poor in body, while BG was characterized as great, refreshing with pleasant bitterness. It was definitely well accepted. According to the mean grades, it was evaluated similarly or better than the initial commercially produced pils beer. On the other hand, BM and BT showed good results in liveliness and body. However, more reliable data can be obtained only by further statistical analysis. According to Friedman ANOVA, tasters evaluated the analyzed beers as statistically significantly different in liveliness (χ^2=10.961; p=0.012), and statistically highly significant difference in flavour (χ^2=23.634; p<0.01), taste (χ^2=16.724; p<0.01), fullness (χ^2=20.813; p<0.01), bitterness (χ^2=32.287; p<0.01) and overall impression (χ^2=25.848; p<0.01). We found differences in sensory properties mainly as the result of differences between the beers with added medicinal herbs, and not differences between the initial beer and beer with medicinal herbs. According to the results of the Wilcoxon matched pairs test (Table 4), IB has a statistically significantly better aroma than BM, and significantly better aroma and taste than BT.

BG, compared to IB, is statistically significantly better in the average score for bitterness and even better for body. The general impression is that BG has the same sensory quality as IB, whereas BM and BT are statistically significantly inferior to IB.

Male tasters assessed that the difference between the four analyzed types of beer was statistically highly significant according to all sensory parameters, as well as in the general impression. On the contrary, female tasters concluded that the analyzed beers were statistically not significantly different in body or liveliness, but statistically significantly different in taste and overall impression, while the difference in aroma and bitterness was statistically highly significant (Table 5).

Male tasters rated BG statistically significantly even better than IB in all tested sensory characteristics and overall impression, except liveliness, for which the average grade was statistically significantly better. The tasters from this group ranked IB statistically significantly better than BM in overall impression and statistically even better in liveliness (Table 6). Women tasters specified that the aroma of IB was statistically significantly better than the aroma of BG, and statistically even better than BM. Furthermore, BT was marked with significantly lower average grade compared to the IB in terms of aroma, taste and bitterness (Table 6). It is noticeable that the two main groups of tasters mainly disagreed in evaluation of analyzed beers. Male tasters preferred full-bodied beers with distinguished bitterness, opposed to female tasters, who preferred more refreshing, light and drinkable beers. Both groups showed their interest in these types of beverages as a valuable source of nutrients, but their main concern was alcohol content.

Table 4. Results of Wilcoxon matched pairs test – comparison of sensory properties of initial beer to the beer with lemon balm, beer with *Ganoderma* and beer with thyme

Sensorial properties and overall impression	Beer with lemon balm		Beer with *Ganoderma*		Beer with thyme	
	z	p	z	p	z	p
aroma	3.834	**0.000**	0.334	0.739	2.082	**0.037**
taste	1.886	0.059	1.504	0.133	2.456	**0.014**
body	0.281	0.779	3.476	**0.001**	0.494	0.621
bitterness	1.601	0.109	1.975	**0.048**	1.330	0.184
liveliness	1.352	0.176	1.188	0.235	1.452	0.146
overall impression	2.562	**0.011**	1.813	0.070	2.119	**0.034**

p<0.05 statistically significantly different, p<0.01 statistically highly significant difference

Table 5. Results of Friedman ANOVA test according to the gender of tasters

Sensorial properties and overall impression	Male		Female	
	χ^2	p	χ^2	p
aroma	17.070	**0.001**	15.673	**0.000**
taste	16.506	**0.001**	7.831	**0.049**
body	30.361	**0.000**	3.707	0.294
bitterness	24.070	**0.000**	18.221	**0.000**
liveliness	17.962	**0.000**	5.768	0.123
overall impression	28.134	**0.000**	11.003	**0.012**

p<0.05 statistically significantly different, p<0.01 statistically highly significant difference

Table 6. Results of Wilcoxon matched pairs test for sensorial properties of initial beer, compared to the beer with lemon balm, beer with *Ganoderma* and beer with thyme according to the gender of tasters

Gender	Sensorial properties and overall impression	Beer with lemon balm		Beer with *Ganoderma*		Beer with thyme	
		z	p	z	p	z	p
Male	aroma	1.894	0.058	2.984	**0.003**	0.817	0.414
	taste	1.329	0.184	3.733	**0.000**	1.224	0.221
	body	1.213	0.225	3.771	**0.000**	0.373	0.709
	bitterness	0.130	0.897	3.711	**0.000**	0.614	0.539
	liveliness	2.642	**0.008**	2.294	**0.022**	0.257	0.797
	overall impression	2.222	**0.026**	3.650	**0.000**	1.049	0.294
Female	aroma	3.358	**0.001**	1.976	**0.048**	1.985	**0.047**
	taste	1.385	0.166	0.125	0.900	2.090	**0.037**
	body	0.292	0.770	1.802	0.072	0.359	0.720
	bitterness	1.855	0.064	0.111	0.912	1.998	**0.046**
	liveliness	0.114	0.909	0.164	0.870	1.589	0.112
	overall impression	1.612	0.107	0.087	0.930	1.848	0.065

p<0.05 statistically significantly different, p<0.01 statistically highly significant difference

Conclusions

The purpose of this study was to determine some functional properties and sensorial acceptability of new types of special beers with improved health benefits. Experimental results indicate that commercially produced and bottled pils beer enriched with tinctures of *Thymus vulgaris* (thyme), *Melissa officinalis* (lemon balm) and *Ganoderma lucidum* show improved antimicrobial and antioxidative properties and are sensorially acceptable. Beer enriched with the tincture of *Thymus vulgaris* has evident microbistatic potential against *L. monocytogenes* ATCC 19115. The same beer enriched with the tincture of *Melissa officinalis* showes significant microbistatic potential against *G. stearothermophilus* ATCC 7953. Antioxidant capacity of the beer enriched with lemon balm and especially with thyme was obviously improved. In the case of beer with thyme, antioxidant capacity was twice as high as in initial pils beer. *Ganoderma* obviously has great potential of sensory compatibility with beer. The evaluation of bitterness is especially promising, as *Ganoderma* is well known for its bitter taste, mostly because of its terpenoids. Well accepted bitter taste of beer with *Ganoderma* suggests that higher doses of the extract can be taken into account. Sensory results of beer with thyme and lemon balm were rather disappointing. Our previous results with the same herbs, but in lower doses, were much more promising (*33*). It is obvious that the doses of extracts and the combination of herbs are of great importance. A lot of medicinal herbs can be used for flavouring, adjustment of sensory properties and affinities of different target groups of consumers.

The obtained results indicate that the herbs with long tradition in folk medicine can be very interesting raw materials for brewing industry. A variety of different beers with defined functional properties can be produced, created for special purposes and target groups. Alcohol content of such products depends on the type of initial beer, meaning that using alcohol-free beers even functional drinks can be created. Such products could fulfill several goals: developing novel beer products, developing products with health-promoting properties that meet the market needs and eventually gain new beer consumers.

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